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Ovarian Androgen Production in Postmenopausal Women

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Context: Several studies previously reported that the postmenopausal ovary produces androgens. However, these findings have recently been questioned in a group of women with adrenal insufficiency.

Objective: We sought to use contemporary assay methodologies to investigate whether the postmenopausal ovary is hormonally active and contributes to the circulating pool of androgens.

Design and Patients: Serum was collected from the ovarian veins of 13 postmenopausal women undergoing total abdominal hysterectomy and bilateral oophorectomy, with sufficient quantities obtained to allow for measurement of several hormones. Serum was also analyzed from peripheral blood collected preoperatively, intraoperatively, and postoperatively.

Setting: The study took place at the Los Angeles County Women's and Children's Hospital, University of Southern California Keck School of Medicine.

Main Outcome Measures: Testosterone (T), androstenedione (A), dehydroepiandrosterone (DHEA), estrone (E1), and estradiol (E2) were measured by RIA with preceding organic solvent extraction and Celite column chromatography.

Results: Statistically significant gradients were seen between the ovarian venous and peripheral samples for T, A, DHEA, E1, and E2. Postoperative levels of T and E1, but not A, DHEA, or E2, were statistically significantly lower than preoperative levels. A gradient for T between the ovarian venous and peripheral blood was present in four of five women who were menopausal for more than 10 yr.

Conclusions: The postmenopausal ovary is hormonally active, contributing significantly to the circulating pool of T. Furthermore, this contribution appears to persist in women as long as 10 yr beyond the menopause. **(*J Clin Endocrinol Metab* 92: 3040–3043, 2007)**

THE POSTMENOPAUSAL OVARY has long been thought to be a significant source of circulating androgens, namely testosterone (T) and androstenedione (A). Judd *et al.* (1) were the first to demonstrate a decline in peripheral concentrations of T and A in postmenopausal women after bilateral oophorectomy. These investigators subsequently compared concentrations of T and A between the ovarian veins and peripheral circulation in postmenopausal women and were the first to observe concentration gradients of these androgens (2). Their findings were later supported by other reports (3–8).

More recently, however, Couzinet *et al.* (9) challenged the role of the postmenopausal ovary in androgen production by evaluating T and A levels in postmenopausal women with adrenal insufficiency. Despite limitations in their study design, the authors presented strong evidence that the postmenopausal ovary does not contribute significantly to circulating androgen levels.

Our objective in the present study was to investigate, using improved ovarian venous sampling techniques and highly sensitive and specific RIA, the magnitude of androgen production by postmenopausal ovaries and to resolve the apparent discrepancies in the existing literature.

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Abbreviations: A, Androstenedione; BMI, body mass index; E1, estrone; E2, estradiol; T, testosterone.

Subjects and Methods

Subjects

Thirteen postmenopausal women undergoing total abdominal hysterectomy and bilateral salpingooophorectomy for various indications were prospectively recruited. Table 1 details the age, body mass index (BMI), preoperative serum FSH level, and surgical indication for each subject. The mean age (±SD) of the subjects was 57 ± 8 yr (range, 45–72 yr). Postmenopausal status was confirmed by preoperative FSH levels of more than 40 U/liter (mean ± SD, 50 ± 13 U/liter) and/or amenorrhea of more than 12 months (mean ± SD duration, 67 ± 47 months). The mean BMI (±SD) of the subjects was 32 ± 7 kg/m^2 (range, 21–45 kg/m^2). Patients were excluded from participation if they had poorly controlled diabetes, a history of hyperandrogenism, had recently used medications known to alter androgen production and/or metabolism such as cytochrome P450 modulators, or had any history of hormonal therapy within 6 wk of surgery.

Protocol

Institutional Review Board approval and participant informed consent was obtained for this study. Peripheral blood samples were collected from participants preoperatively, either at the visit before or on the morning of surgery, as well as postoperatively (mean ± SD number of days after surgery, 55 ± 39 d). At the time of oophorectomy, blood was obtained from both the right and left ovarian veins using a technique that ensured adequate collection of blood (5–10 ml/side). After transection of the infundibulopelvic ligament, the clamp securing the medial aspect of the pedicle was released and the blood was collected in a sterile tube (Fig. 1). Simultaneously, an intraoperative peripheral blood sample was collected. All samples were allowed to clot and centrifuged, and the serum was stored at −20 C.

TABLE 1. Descriptive characteristics and surgical findings of postmenopausal women undergoing total abdominal hysterectomy and bilateral salpingooophorectomy

Age (yr)	BMI (kg/m^2)	FSH (U/liter)	Surgical/pathological findings
51	30	56.9	Fibroids
56	40	41.1	Fibroids, endometrioma
52	45	57.4	Ovarian dermoid cyst
72	24		CIN III, fibroids
54	30	41.3	Bilateral benign ovarian Brenner tumors, dermoid cyst
55	29	45	Ovarian mucinous cystadenoma, LMP; bilateral endometriomas
55	35	26.7	Fibroids, endometrial polyp
69	21	37.6	CIN III, fibroids
48	35	51.3	Fibroids, endometrioma
66	34	55.1	1A1 squamous cell cervical cancer
45	36	76	Fibroids
62	22	60	CIN II
52	30	57	Ovarian serous cystadenoma

CIN, Cervical intraepithelial neoplasia; LMP, low malignant potential.

Hormone assays

T, A, dehydroepiandrosterone (DHEA), estrone (E1), and estradiol (E2) were measured in all samples by RIA, after hexane:ethyl acetate (3:2) extraction and Celite column partition chromatography, as described previously (10–12). A, DHEA, and T were eluted in 0, 15, and 40% toluene in isooctane and E1 and E2 in 15 and 40% ethyl acetate in isooctane, respectively. The RIA for each analyte used an iodinated radioligand in conjunction with a highly specific antiserum. After a 16- to 18-h incubation period, a second antibody was used to separate antibody-bound from unbound steroid. The sensitivities of the T, A, DHEA, E1, and E2 RIAs were 2 ng/dl, 0.035 ng/ml, 0.04 ng/ml, 5 pg/ml, and 4 pg/ml, respectively. The intraassay and interassay coefficients of variation ranged from 6–9 and 12–14%, respectively, at low, medium, and high levels of the five different steroid hormones in quality control samples.

Statistical analysis

Comparisons of ovarian venous *vs.* intraoperative peripheral samples and of preoperative *vs.* postoperative samples were made using the Wilcoxon signed-rank test. Correlations were performed using the Spearman correlation test. Differences were considered statistically significant if $P < 0.05$.

Results

The ovarian venous samples contained statistically significantly higher levels of T, A, DHEA, E1, and E2 than found in the peripheral circulation ($P < 0.05$) (Table 2). No statistically significant differences in the ovarian venous hormone concentrations from the right and left ovarian veins were seen, and the averaged values were used for analysis. Hormone concentrations between ovarian venous and intraoperative peripheral samples ranged from a 24-fold difference for T down to a 2-fold difference for DHEA.

Postoperative serum levels of T and E1, but not of A, DHEA, or E2, decreased significantly compared with preoperative levels ($P < 0.05$) (Table 3). Despite the statistically significant difference between ovarian venous and peripheral serum concentrations for all of the hormones studied, the significant postoperative decline seen only in T and E1 emphasizes the existence of multiple factors regulating circulating steroid hormone concentrations.

In four of the five patients with more than 10 yr of amenorrhea (mean ± SD, 64 ± 8 yr of age), gradients between ovarian venous and intraoperative peripheral T levels were still present (Table 4). However, neither a correlation between age and preoperative T levels nor one between the duration of menopause and preoperative T levels was seen. Additionally, we found no correlation between age and preoperative A or DHEA levels.

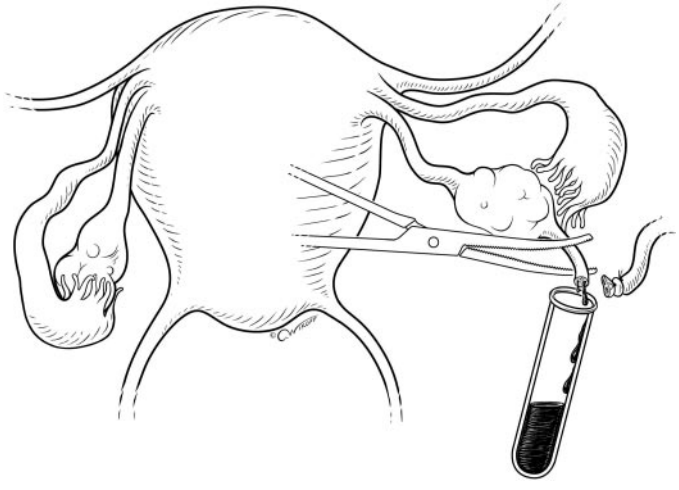

FIG. 1. Sampling technique of ovarian venous effluent at the time of total abdominal hysterectomy and bilateral salpingooophorectomy.

Discussion

Several early studies demonstrated that the postmenopausal ovary is a source of androgens and, to a lesser extent, estrogens (1–8). A weakness of these studies, as noted by some of the authors themselves, included suboptimal techniques for collecting ovarian venous blood, occasionally resulting in inadequate amounts of serum for analysis. Additionally, the use of assays less sensitive than those currently available is another limiting factor of these early studies. Even so, the demonstrations of androgen production by the postmenopausal ovary were convincing. However, these findings were recently questioned as the result of a study evaluating the ovarian contribution of T and A in a group of postmenopausal women with Addison's disease (9). Using improved sampling techniques and contemporary assay methodologies, we sought to demonstrate that the postmenopausal ovary is hormonally active and to substantiate findings from previous studies that first established the postmenopausal ovary as a source of androgen production.

TABLE 2. Mean (range) concentrations of T, A, DHEA, E1, and E2 from ovarian venous and intraoperative peripheral serum

	Ovarian	Peripheral	Fold difference	*P* value
T (ng/ml)	7.2 (0.2–62.0)	0.3 (0.2–0.8)	24	<0.05
A (ng/ml)	4.3 (0.0–21.7)	1.1 (0.6–1.6)	4	<0.05
DHEA (ng/ml)	8.8 (3.6–33.8)	5.2 (2.0–9.0)	2	<0.05
E1 (pg/ml)	150 (24–1276)	50 (24–88)	3	<0.05
E2 (pg/ml)	99 (5–834)	15 (4–32)	7	<0.05

Fold difference = ovarian venous/peripheral.

By demonstrating statistically significant gradients between ovarian venous and peripheral levels of T, A, and DHEA, along with a statistically significant decline in postoperative values of T after oophorectomy, we have confirmed that the postmenopausal ovary secretes androgens. Specifically, we found 24-fold and 4-fold higher concentrations of T and A, respectively, in the ovarian venous *vs.* peripheral circulation (Table 2) and decreases in T of 42% from the preoperative to postoperative time period (Table 3). A nonsignificant 17% decrease in postoperative A levels was observed. This relative lack of change is possibly attributable to postoperative compensatory production by the adrenal gland. DHEA demonstrated a 2-fold difference between the ovarian venous and peripheral serum, although a nonsignificant decline of only 18% was seen in postoperative DHEA levels. The lack of statistical significance could also be attributed to a type II error.

Of note, nine of our subjects were obese, a condition known to alter SHBG and other binding globulin concentrations. However, given the relatively short duration of the study and the fact that each patient served as her own control, the presence of obesity is unlikely to alter our overall conclusions. Furthermore, no correlations between BMI and the various steroid concentrations were detected (data not shown).

Given our results, we approximate that the postmenopausal ovary secretes 25 μg/d of T (bilateral production, ~50 μg/d). This is based on a gradient for T between the ovarian venous and peripheral serum of 6.9 ng/ml. Given that approximately 5 ml blood was collected from the ovarian vein over 2 min, we calculated an ovarian production rate of T of 1.04 μg/h. Our findings are comparable to the calculated ovarian contribution of 37 μg/d reported by Aiman *et al.* (5) and 60 μg/d reported by Adashi (13). Using similar calculations, we determined that the postmenopausal ovary directly contributes 11.5 μg/d of A (bilateral production ~ 23 μg/day) to circulating concentrations, an amount less than the 46 μg/d suggested by Aiman *et al.* (5) and the 300 μg/d suggested by Adashi (13).

Our findings support those of previous studies in which significant gradients between ovarian venous and peripheral concentrations, as well as between preoperative and postoperative concentrations, were demonstrated (1–8). However, other studies have suggested limited steroid production by the postmenopausal ovary (9, 14). Couzinet *et al.* (9) compared postmenopausal women with adrenal insufficiency to women having had bilateral oophorectomy. They found that levels of T and A were undetectable in subjects with adrenal insufficiency despite having intact ovaries. The authors concluded that the adrenal gland, not the ovary, is responsible for postmenopausal androgen production. The study design was confounded, however, in that subjects with adrenal insufficiency were taking glucocorticoids, therapy that has previously been shown to alter ovarian steroid production (15).

In vitro data evaluating the presence of steroidogenic enzymes in the postmenopausal ovary exist, both supporting and refuting the possibility of continued androgen production (16, 17). Most recently, Havelock *et al.* (16) demonstrated the presence of all enzymes necessary for steroid production in postmenopausal ovarian stroma. The authors noted that previous contradictory findings may have resulted from the well-known heterogeneous distribution of secondary interstitial cells in the stroma or from phenotypic transformation of *in vitro* cultured stromal cells.

Our findings also substantiate postmenopausal ovarian production of both E1 and E2. A 3-fold difference for E1 and 7-fold difference for E2 were seen between the ovarian venous and peripheral hormone concentrations (Table 2). Despite this evidence of ovarian production, only postoperative E1 values declined significantly with a mean decrease of 26% (Table 3). A nonsignificant decline of 8% was seen in postoperative E2 levels. This lack of statistical significance for E2 may be the result of a small sample size. Additionally, it could be due to compensatory peripheral conversion of androgens to estrogens, although one would expect to see a nonsignificant decline in postoperative E1 levels as well if such were the case. This finding further underscores the influence of multiple factors involved in the regulation of peripheral steroid hormone concentrations.

Judd *et al.* (2) showed a 2-fold difference between the ovarian venous and peripheral circulation for both E1 and E2, whereas Aiman *et al.* (5) demonstrated a 1.4-fold and 4-fold difference between the ovarian venous and peripheral

TABLE 3. Mean (range) concentrations of T, A, DHEA, E1, and E2 from preoperative and postoperative serum

	Preoperative	Postoperative	Percent difference (%)	*P* value
T (ng/ml)	2.6 (1.0–6.0)	1.5 (0.6–2.1)	42	<0.05
A (ng/ml)	0.6 (0.2–0.9)	0.5 (0.2–1.2)	17	NS
DHEA (ng/ml)	2.2 (0.6–5.4)	1.8 (0.7–3.1)	18	NS
E1 (pg/ml)	46.0 (25.0–119.0)	33.9 (17.0–61.0)	26	<0.05
E2 (pg/ml)	15.9 (3.0–32.0)	14.7 (4.0–62.0)	8	NS

Percent difference = (postoperative − preoperative)/preoperative. NS, Not significant.

TABLE 4. Ovarian venous and intraoperative peripheral concentrations of T in subjects 10 or more years menopausal

Age (yr)	Ovarian T (ng/ml)	Peripheral T (ng/ml)
52	0.4	0.2
62	0.8	0.2
66	0.2	0.2
69	1.0	0.3
72	13.0	0.4

circulation for E1 and E2, respectively. *In vitro* data from Dennefors *et al.* (18) demonstrated E2 production from hilus strips of postmenopausal ovaries, providing additional evidence to the biological plausibility of estrogen production by the postmenopausal ovary.

In four of our five subjects who were postmenopausal for 10 or more years, a gradient for T between the ovarian venous and peripheral circulation was still present (Table 3). Results from the Rancho Bernardo Study demonstrating higher concentrations of total T in older postmenopausal women compared with younger postmenopausal women support the idea of hormonally active ovaries years beyond the menopause (8). Similarly, Davison *et al.* (19) demonstrated that the higher T levels seen in women with ovaries *vs.* those without ovaries persisted beyond 65 yr of age. Thus, our findings, along with those of others, support the idea that not only is the postmenopausal ovary still active in androgen production but also that this production seems to persist even into the late menopause.

In conclusion, using improved sampling techniques and contemporary, highly sensitive and specific RIA methodology, we have demonstrated production of androgens and estrogens by the postmenopausal ovary. Furthermore, we have shown that the ovarian contribution of androgens is sufficient to result in significant decreases in postoperative concentrations of T after bilateral oophorectomy. Lastly, we have demonstrated that this ovarian production of androgens appears to persist even 10 yr beyond the onset of menopause. We conclude that the postmenopausal ovary remains hormonally active, secreting significant amounts of androgens and estrogens. Given recent findings that ovarian preservation is beneficial to the overall health and longevity of postmenopausal women (20) and that oophorectomy before the age of 45 may actually be detrimental (21), we suggest that ovarian preservation be considered in appropriately selected women who may benefit from the effects of endogenous hormone production.

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