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The source(s) of estrogen production in hirsute women with polycystic ovarian disease as determined by simultaneous adrenal and ovarian venous catheterization

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To determine the significant source(s) of estrogen production in women with polycystic ovarian disease (POD), 12 women underwent selective adrenal and ovarian vein catheterization, with simultaneous peripheral blood samplings for determination of cortisol, androstenedione (Δ^4A), testosterone, estrone (E_1), and estradiol (E_2). Ovarian vein E_2 gradients were observed in 11 of the 12 patients with a mean of 13.4, whereas adrenal blood samples did not demonstrate significant E_2 gradients. Seven of 8 patients exhibited ovarian secretion of E_1, with a mean gradient of 13.6 times that of peripheral blood, whereas 4 of the 8 adrenal samples showed E_1 gradients. The mean value was 1.4 times peripheral levels. No significant correlations were found between peripheral E_1 levels and body weight or degree of adiposity, nor was there a relationship between obesity and E_1/Δ^4A molar ratio in peripheral blood. The subjects with the highest ovarian Δ^4A levels had a significant correlation between peripheral Δ^4A and E_1. Therefore, our data indicate a significant contribution of ovarian E_1 secretion to the peripheral E_1 pool in addition to the extraglandular conversion of Δ^4A to E_1. There was general lack of correlation between peripheral E_1 concentrations and plasma E_2, and these relationships versus body size suggest that the major source of E_2 in women with POD was ovarian secretion. Fertil Steril 49:56, 1988

Elevated blood levels of a variety of androgens have been demonstrated[1,2] in patients with polycystic ovarian disease (POD); however, attempts to define whether these elevated levels arise from adrenal or ovarian sources has been subject to controversy. In a previous publication,[3] we tried to localize the major sites of androgen production in POD by direct sampling of adrenal and ovarian effluents. The data strongly suggest the ovaries as the chief source of androgen overproduction.[3]

Despite excessive androgen production, most women with POD show no evidence of estrogen deficiency. In this syndrome, estrogen metabolism is characterized by predominance of circulating estrone (E_1) compared with estradiol (E_2).[1,2] This finding resembles that which occurs in postmenopausal women in whom the bulk of estrogens are not secreted, but arise indirectly by extraglandular peripheral conversion of androstenedione (Δ^4A).[4,5] In addition, kinetic studies suggested that virtually all of the estrogens formed in women with POD are derived from extraglandular conversion of Δ^4A, and that elevated production rates of Δ^4A in POD provide an abundant source of precursor to be converted to estrogen, primarily E_1.[4,5]

In the current study, we wished to obtain more

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Table 1 Clinical Data of 12 Hirsute Patients with Polycystic Ovary Disease

Patient	Age	Weight	Ideal body weight	Duration of symptoms	Menses[a]	Hirsutism[b]	Ovaries[c]	
							Size	Cyst
	yr	*kg*	*%*	*yr*				
1	18	68	119	7	O	2+	**	*
2	24	67	131	14	O	2+	**	**
3	26	42	80	10	A	2+	*	*
4	15	36	100	7	A	3+	***	**
5	17	54	103	4	A	2+	*	*
6	21	65	122	10	A	2 to 3+	**	*
7	28	83	155	18	O + A	3+	**	**
8	16	63	118	4	O	3+	**	**
9	19	64	130	6	O	3+	*	*
10	20	92	177	8	O	2+	**	*
11	24	126	220	10	A	3+	***	**
12	26	123	190	12	O + A	3+	**	**

[a] O, oligomennorrhea; A, amenorrhea.
[b] 1+, mild facial and lower abdominal; 2+, moderate; 3+, marked hirsutism in androgen-sensitive areas (face, chest, abdomen, and thighs).
[c] Evaluated by pneumogynecography and/or ovarian biopsy: N, normal; * to ***, enlargement; 0, cysts absent; * to **, cysts present.

detailed data on estrogen secretion in women with polycystic ovaries. We measured E_2 and E_1 gradients in ovarian and adrenal venous effluents obtained via simultaneous catheterization of these tissues. Our studies provide evidence of direct estrogen secretion, primarily by the ovaries in women with POD.

MATERIALS AND METHODS

Patients

Twelve hirsute women with polycystic ovaries, proven by pneumogynecography and/or ovarian biopsy, were studied. Their clinical data are presented in Table 1. Patients 2, 6, 7, and 9 to 12 were considered obese, exceeding ideal body weights by 20%; however, only patients 7 and 10 to 12 exhibited major obesity. Hirsutism was moderate or marked, and menstrual abnormalities were present in all patients. These women also had confirmatory biochemical findings of elevated androgens and elevated LH/FSH ratios, as noted in Table 2. All patients exhibited significantly increased peripheral blood levels of testosterone (T), free T, and Δ^4A.

All patients were hospitalized for evaluation of hirsutism, during which time selective venous catheterizations were conducted. These studies were approved by the Human Investigations Committee of the Department of Medicine (University of Sao Paulo Medical School). Informed written consent

Table 2 Basal Plasma Hormone Data from 12 Hirsute Women with Polycystic Ovarian Disease

Patient	LH	FSH	LH/FSH	E_2	E_1	PRL	Testosterone		
							Total	Free	Free concentration
	mIU/ml			*pg/ml*		*mg/ml*	*ng/ml*	*%*	*pg/ml*
1	20	4	5.0	75	53		0.850	2.85	24.05
2	18	7	2.6	73	50	8	1.160	2.21	25.64
3	19	6	3.0	87	156	13	0.450	2.19	9.74
4	55	4	13.7	56	120	32	0.845	2.87	25.25
5	20	7	2.9	41	58	3	0.280	3.08	8.62
6	57	4	14.2	90	310	3	0.724	2.25	16.29
7	22	6	3.7	75	100	17	1.500	2.47	37.05
8	19	8	2.4	76	79	10	1.280	2.66	34.05
9	32	8	4.0	58	104		1.205	3.11	37.47
10	33	14	2.5	59	96	25	0.308	3.36	10.35
11	37	11	5.2	37	90	5	1.660	2.56	42.50
12	27	9	3.0	27	160	13	1.360	2.30	31.28

was obtained from the patients or their parents. Venous catheterizations were performed percutaneously between 8:00 A.M. and 9:00 A.M., after a 12-hour fast, via the right femoral vein. The catheters were placed in the left adrenal and left ovarian veins and wedged in as far as possible under fluoroscopic visualization. Adrenal and ovarian venograms were performed to assure correct catheter placement. Blood samplings were obtained simultaneously from the adrenal and ovarian veins and from the peripheral vein (brachial vein). Two samples were drawn over an interval of 15 minutes. The mean steroid concentrations obtained from the samples were used in the subsequent calculations.

E_2 and E_1 were measured by specific radioimmunoassay (RIA) performed after separation on 5×1 cm Sephadex L-20 columns (Pharmacia Fine Chemicals, AB Uppsala, Sweden). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) were measured using specific RIA kits. Plasma (unbound) T was measured using the technique of equilibrium dialysis of undiluted plasma.[3,6]

RESULTS

As indicated in Tables 1 and 2, all patients had elevated or high-normal plasma LH levels for the follicular phase of the menstrual cycle. Although there was no relationship noted between the size of the patient's ovaries and plasma LH, the two patients with the highest LH values (patients 4 and 11) had the largest ovaries in our series. FSH levels were low-normal in most of the patients compared with concentrations observed in the follicular phase. The plasma LH/FSH ratio was high in all 12 subjects, as previously reported in POD.[1]

Serum PRL levels were in the normal range in 8 of 10 patients measured. In two women (patients 4 and 10), levels were slightly elevated. Plasma T was increased in 7 of the 12 patients; however, the percent free (unbound) T was increased in all 12 women, resulting in elevated free T concentrations in all 12 POD patients.

Serum E_2 concentrations were within the normal range of the follicular phase of cycling women in 9 of the 12 subjects. In 2 patients (3 and 6), the E_2 values were elevated slightly and, in one woman (patient 12), it was below normal range. E_1 values were higher than normal for cycling women during the follicular phase in 9 of the 12 POD subjects, and the E_1/E_2 ratio was greater than unity in 10 of 12 women.

Catheterization Data

The plasma concentrations of the several steroids in peripheral, adrenal, and ovarian venous effluents drawn simultaneously are presented in Table 3. Basal peripheral cortisol (F) values ranged from 5.4 to 22.1 μg/dl, with a mean value of 10.9 μg/dl, suggesting that the patients undergoing retrograde catheterization were not under stress. Figure 1 shows the logarithmic plot of the ratios of the

Table 3 Peripheral Adrenal and Ovarian Venous Effluents Concentrations of Cortisol (F), Testosterone (T), Androstenedione (Δ), Estradiol (E_2) and Estrone (E_1) in 12 Hirsute Patients with Polycystic Ovarian Disease[a]

Patient	Sample	F	T	Δ	E_2	E_1
		μg/dl	*ng/ml*	*ng/ml*	*pg/ml*	*pg/ml*
1	PV	5.4	1.13	2.13	75.1	52.8
	LAV	47.7	2.87	14.28	80.1	60.1
	LOV	4.8	1.26	2.09	96.5	125.0
2	PV	9.5	0.81	1.26	73.3	50.0
	LAV	90.4	3.21	14.58	78.6	
	LOV	7.2	0.99	1.57	154.5	
3	PV	13.2	0.98	1.41	86.9	156.5
	LAV	141.0	2.44	20.34	98.5	282.9
	LOV	11.8	0.59	1.99	84.2	187.7
4	PV	9.0	0.84	5.92	56.4	120.3
	LAV	21.8	1.55	17.00	71.3	120.7
	LOV	9.8	16.45	188.31	4645.4	5580.0
5	PV	8.1	0.24	2.57	41.1	58.4
	LAV	16.3	0.34	8.35	25.0	39.4
	LOV	6.3	3.52	43.38	1273.6	1025.6
6	PV	12.3	0.72	1.69	90.2	310.4
	LAV	38.9	1.28	5.40	70.0	400.0
	LOV	10.5	7.27	15.47	700.2	6210.0
7	PV	6.9	1.08	1.62	74.7	100.4
	LAV	21.9	1.51	6.07	49.9	105.0
	LOV	4.9	24.9	133.87	440.6	2000.0
8	PV	14.4	1.20	2.54	75.6	78.9
	LAV	56.4	3.04	28.15	88.0	150.0
	LOV	13.4	2.32	24.50	484.3	147.4
9	PV	11.2	1.07	4.72	56.5	104.1
	LAV	99.2	5.06	31.62	79.7	
	LOV	10.0	2.88	10.36	213.7	
10	PV	11.5	0.38	3.90	59.1	95.8
	LAV	178.4	1.70	28.00	55.3	
	LOV	12.9	3.23	11.87	192.4	
11	PV	22.1	3.25	4.69	37.2	90.0
	LAV	164.2	11.61	63.21	39.0	
	LOV	17.2	9.32	23.90	80.0	
12	PV	7.2	1.36	11.38	27.3	159.8
	LAV	77.9	5.13	103.64	63.2	373.6
	LOV	6.0	4.49	76.50	364.8	353.4

[a] Samples were obtained simultaneously from the left adrenal (LAV), left ovarian (LOV), and peripheral vein (PV).

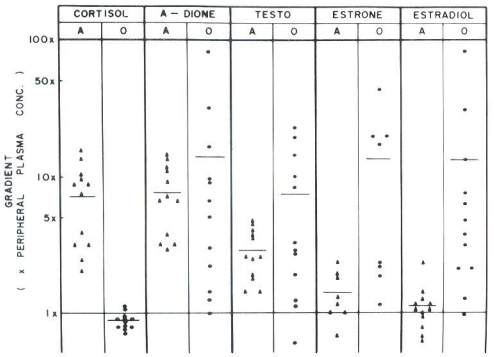

Figure 1 Steroid gradients from left adrenal and ovarian venous effluents in the hirsute patients with POD. Gradients (steroid concentration in the effluent/peripheral blood) are plotted on a logarithmic scale.

steroid concentrations measured in the adrenal and ovarian venous effluents to the corresponding peripheral values drawn simultaneously. A ratio significantly greater than 1 is indicative of secretion of the steroid from that gland.

Ovarian and adrenal venous F gradients were determined as reference values for the various steroids and to test whether adrenal venous effluents admixed with ovarian vein samples, since F is a unique secretory product of the adrenal gland. In none of the ovarian vein samples was there a F gradient; by contrast, significant F gradients were observed in all adrenal vein samples with a mean 7.1-fold greater than peripheral plasma levels. Patients 4 to 7 exhibited small gradients of F in the adrenal venous effluents, suggesting that these patients were not in a state of active adrenal secretion at the time of their catheterization. The adrenal T, Δ^4A, and E_1 (but not E_2) gradients were similarly minimal in these patients, in contrast to those patients with much larger adrenal F gradients.

T and Δ^4A gradients were observed in all 12 adrenal effluents. Ovarian T gradients were observed in 10 of the 12 women, and Δ^4A gradients in 11 of 12 samples. Ovarian vein gradients of E_2 were noted in 11 of the 12 patients, with a mean gradient of 13.4-fold greater than peripheral blood concentrations. By marked contrast, only 3 of the 12 adrenal vein samples demonstrated a significant E_2 gradient. Of 8 patients in whom measurements of E_1 were made in adrenal and ovarian effluents, 7 exhibited ovarian secretion of E_1, whereas 4 of the 8 adrenal samples showed no gradients. The mean adrenal E_1 gradient was only 1.4.

We next assessed for possible correlations between plasma androgens, estrogens, gonadotropins, and obesity in our POD patients. No significant correlations were found between gonadotropin levels and serum estrogens or androgens. There was a significant correlation between LH/FSH ratio and peripheral E_1 concentration ($r = 0.642$; $P = <0.03$). There was no significant correlation between plasma Δ^4A and plasma E_1, nor between plasma E_1 and E_2 concentrations. In contrast to data from other laboratories, we found no correlation between plasma E_1 and body weight, percent excess body weight, or degree of adiposity (defined as the difference between actual body weight and ideal body weight). Further, we found no correlation between the degree of adiposity and the E_1/Δ^4A molar ratio in peripheral blood. Peripheral E_2 concentrations did seem to show a negative correlation compared with body weight ($r = -0.548$; $P < 0.05$), as noted in Figure 2. Peripheral E_2 similarly correlated negatively with percent above ideal body weight ($r = -0.503$; $P < 0.05$) and degree of adiposity ($r = -0.621$; $P < 0.03$), previously not found by others.

Evaluating the steroid concentrations in the glandular effluents, no correlations were found between Δ^4A and E_1, nor E_1 versus E_2 levels in either adrenal or ovarian venous effluents. However, there was a significant positive relationship between plasma LH and ovarian E_1 levels ($r = 0.93$; $P < 0.05$), as shown in Figure 3.

DISCUSSION

As previously reported, the source of excess androgens in our patients seemed to be ovarian overproduction.[2] In the syndrome of POD, estrogen metabolism is characterized by predominance of circulating E_1 and reversal of the E_2/E_1 ratio in peripheral blood. In our patients, 10 of the 12 women exhibited plasma concentrations of E_1 ex-

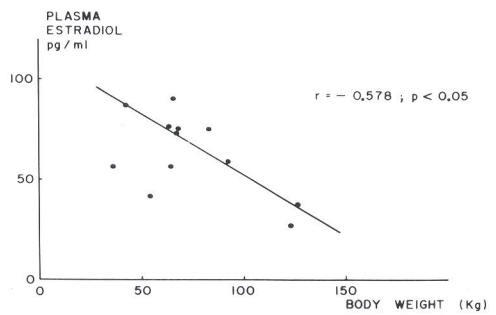

Figure 2 Negative correlation between body weight and peripheral plasma E_2 concentrations in 12 patients with POD.

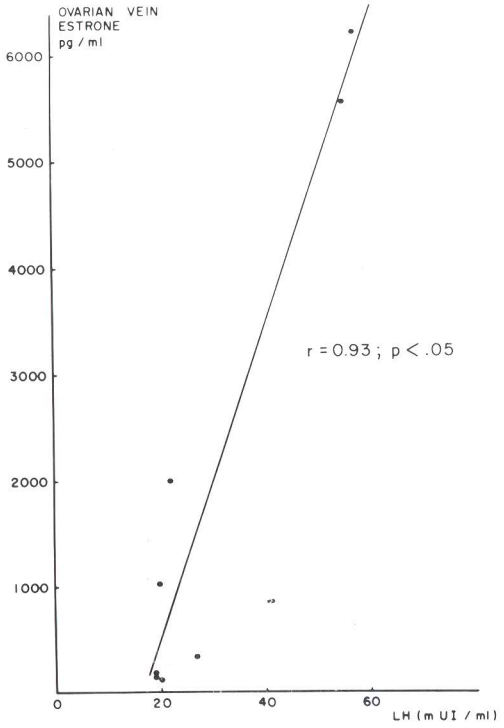

Figure 3 Correlation of peripheral LH and ovarian vein E_1 concentrations in eight patients with POD (see text for details).

ceeding that of E_2, the latter being within the normal range of the early follicular phase of cycling women. This finding resembles that observed in postmenopausal women in whom the bulk of estrogen is not secreted as E_2, but arises indirectly by extraglandular peripheral conversion of Δ^4A.[4,5] Previous kinetic studies of androgen-estrogen conversion also have suggested that, in women with POD, all of the estrogens arise from peripheral aromatization of androgens.[5] Although the conversion rate from Δ^4A to E_1 is normal (1.2 go to 1.7%) in women with POD, the elevated production rates of Δ^4A apparently provides an abundant source of precursor for peripheral aromatization to estrogens, primarily E_1. This relatively high basal estrogen level could result in a functional derangement of the hypothalamic-pituitary system, resulting in elevated concentrations of LH and low FSH in peripheral blood.[5,7] High levels of LH could stimulate the ovary to secrete increasing amounts of Δ^4A, so that this condition is aggravated. Indeed, our patients presented LH concentrations at the upper limit or above normal, while FSH levels were low-normal in the great majority of subjects, confirming the data of others.[1]

If circulating Δ^4A is the major determinant of plasma E_1 levels, there should be some correlation between these two variables in the peripheral blood, as described by Marshall et al.[8] in postmenopausal women (however, not confirmed by Vermeulen and Verdonck[9]). We have not observed a significant correlation between plasma, Δ^4A, and E_1 in our patients with POD, suggesting other major source(s) for plasma E_1 (i.e., direct secretion from ovaries and/or adrenal).

Because E_1 concentrations in ovarian venous blood were greater than the corresponding levels in peripheral blood in seven of the eight cases and in adrenal gradients in four of the eight subjects, our studies suggest that this estrogen is secreted in women with POD, and is not formed exclusively from peripheral aromatization of circulating androgens. These studies confirm previous findings in women with idiopathic hirsutism, in whom similar estrogen gradients in adrenal and ovarian veins were noted.[10] The poor parallelism between ovarian and peripheral E_1 concentrations is not unexpected since the concentration of a steroid in the peripheral circulation is inversely related to its metabolism, as defined by the metabolic clearance rate, and directly related to its rate of production (glandular secretion plus peripheral conversion).[11] The interesting significant correlation between peripheral LH and ovarian E_1 secretion supports the suggestion that ovarian secretion contributes to circulating E_1 in patients with POD.

Regarding E_2 secretion, our catheterization data indicate an ovarian origin for this steroid in 11 of 12 patients. In only 1 of our subjects (patient 3) was the adrenal vein E_2 concentration higher than the corresponding ovarian effluent. This subject was 1 of the 3 patients with high adrenal cortisol gradients, suggesting that blood was drawn during a spike of adrenocortico-tropic hormone secretion and that, perhaps at peaks of adrenal secretion, some E_2 is secreted here as well. These data support previous results of Baird et al.[12] and Kirschner et al.[10] that E_2 is not normally secreted in significant amounts by the adrenals.

It has been postulated that there exists a defect in the aromatase system in ovaries of women with POD, which may be related to the relatively low local concentration of FSH in the granulosa cell.[1] Thus, when the LH/FSH ratio is elevated, as in POD, excessive androgen production and secretion due to chronic stimulation of theca tissues by LH may result and, despite abundant precursor availability, the granulosa cells are incapable of forming adequate amounts of estrogens because of the

FSH-dependent aromatase deficiency.[1] Our findings of E_1 and E_2 secretion by the polycystic ovary (also observed by Kirschner et al.[10] and Laatikainen et al.[13] suggest that there is no lack of aromatization capacity in the polycystic ovarian tissue, although a mild enzymatic defect can be ruled out. In addition, we recognize, however, that in POD there is overgrowth of theca cells, thus analysis of ovarian effluents may not adequately reflect granulosa cell contributions.

It has been reported that the peripheral conversion of Δ^4A to E_1 is strongly related to body weight, and the extent of peripheral aromatization of circulating Δ^4A to E_1 is correlated positively to adipose tissue mass.[14,15] Unfortunately, we could find no such correlation between plasma E_1 and body weight or degree of adiposity in the current study. Perhaps the sample size here was too small to confirm the previous studies in this area, and we did not directly measure peripheral conversion of Δ^4A to E_1 in our patients.

In conclusion, the catheterization data strongly suggests an ovarian source of estrogen production in patients with POD, as we had previously indicated for T and Δ^4A.[2] Our catheterization data do not fit the concept that the majority of estrogen production in women with POD is derived from peripheral aromatization of androgens, as suggested by Siiteri and MacDonald.[5] We suggest that there is continued ovarian secretion of E_2 and E_1 in this abnormality, and superimposed upon these basal estrogen levels is the E_1 derived from peripheral aromatization of circulating androgens, such as Δ^4A.

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