Sex-Specific Differences in Cortisol Production Rates in Humans

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Production rates of cortisol were determined in healthy men (n = 7) and in healthy women during the follicular phase of their menstrual cycle (n = 7) using the stable-isotope dilution technique and analysis by gas chromatography/mass spectrometry (GC/MS). 1α, 2α-D-Cortisol was infused for 10 hours (116 ± 6μg/h; 8 AM to 6 PM). Blood samples obtained at 20-minute intervals during the last 4 hours (2 PM to 6 PM) were pooled and used for analysis. Estimated production rates of cortisol were 0.94 ± 0.15 mg/h and 0.38 ± 0.14 mg/h in healthy men and women, respectively. Even when corrected for body-surface area, production rates of cortisol in men (0.48 ± 0.09 mg/m² · h) were higher ($P < .001$) than in women (0.22 ± 0.08 mg/m² · h). An increased production rate of cortisol was seen in 12 patients with Cushing's syndrome, although in four of nine female patients, it was within the range considered normal for healthy men. It is concluded that women have a lower production of cortisol than men and that this sex-specific difference is of clinical relevance in patients with endogenous hypercortisolism.

URINARY EXCRETION of cortisol, the most important adrenal steroid hormone, is higher in men than in women.[1] This sex-specific difference has been ascribed to differences in serum cortisol binding and/or renal cortisol handling,[1] to a preponderance of 11-oxoreductase relative to 11-dehydrogenase activity in males,[2] or simply to differences in body-surface area.[3] Surprisingly, the question whether cortisol production rates are different in healthy men and women has not yet been studied systematically. Using stable-isotope dilution and mass spectrometry, Esteban et al[4] recently determined that the human adrenal produces approximately 6 μg cortisol/d · m², which is less than previously[5] assumed. In the present study, we used the same technology to study the possibility of a sex-dependent difference in cortisol production rates in healthy volunteers and in patients with Cushing's syndrome.

MATERIALS AND METHODS

Experimental Protocol

Healthy volunteers. Seven healthy, non-obese men aged 23 to 36 years and seven healthy non-obese women aged 20 to 34 years (in the follicular phase of the menstrual cycle) who had been carefully informed about the aims and the possible risks of the study gave their written consent to participate in this investigation. On the day of the experiments, an indwelling catheter was inserted into an antecubital vein and a constant (40 mL/h) intravenous infusion of 1α, 2α-D-cortisol (2.0 mg in 500 mL 0.9% saline also containing 2 mL of the individual's own blood) was started at 8 AM. At the beginning and end of each infusion, a sample of the infusate from the end of the infusion line was obtained to determine losses by adsoption. Hence, individual infusion rates (116 ± 6 μg/h) were determined retrospectively. After an equilibration period of 6 hours (at 2 PM), a second indwelling catheter was inserted into the contralateral arm and blood samples were obtained from 2 PM until 6 PM at 20-minute intervals. These blood samples were subsequently pooled and used for analysis. In three male and two female subjects, additional plasma samples were obtained 20, 40, 60, 80, and 100 minutes after the end of the infusion of 1α, 2α-D-cortisol.

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Patients with Cushing's syndrome. Using an identical protocol, endogenous cortisol production rates were also determined in 12 patients with Cushing's syndrome (three men and nine women). The patients' hypercortisolism was due either to a corticotrophin (ACTH)-producing pituitary adenoma (Cushing's disease, n = 7), or to a cortisol-producing adrenal adenoma (n = 3) or carcinoma (n = 1). One patient suffered from an ACTH-producing thoracic carcinoid tumor.

Materials

All organic solvents were of high-performance liquid chromatography (HPLC) grade and purchased from Baker Chemicals, Phillipsburg, NJ. Nonactive cortisol (F; 11β,17,21-trihydroxy-4-pregnene-3-20-dione) was obtained from Sigma, St Louis, MO, whereas radioactive [^{3}H]1,2,6,7-cortisol (speficic activity, 60 Ci/mmol) and stable-labeled 1α, 2α-D-cortisol (isotopic enrichment, 99.0%) were purchased from Amersham, Amersham, UK, and from CIL, Andover, MA, respectively.

Sample Preparation and Analysis by Gas Chromatography/Mass Spectrometry

Plasma samples (5.0 mL) supplemented with 50,000 dpm of ^{3}H-cortisol for later control of recovery were extracted with ethylacetate and separated by thin-layer chromatography (benzene-acetone, 50:50). The zone containing cortisol was eluted (2 × 2.5 mL methanol) and supplemented with tetrahydrocortisone (3α,17, 21-trihydroxy-5β-pregnane-11,20,dione) as an internal standard for gas chromatography/mass spectrometry (GC/MS) analysis. Subsequently, derivatisation was performed using methoxyamine and trimethylsililation for the oxo and the hydroxy groups, respectively. Analysis by GC/MS (Hewlett-Packard HP5995; Hewlett Packard, Palo Alto, CA) equipped with a 25-m CB5-fused silica column, was made using selective ion monitoring (SIM) mode and electric ionization (resolution, 800). The tracer ions were m/e 609 for the internal standard tetrahydrocortisone (M+), and m/e 605 and m/e 607 for cortisol (M+, 31) and 1,2α,-D-cortisol (M+, 31), respectively. The sensitivity at a peak-to-noise ratio of 5:1 was 80 pg.

Calculation of Cortisol Production Rate

Production rates of cortisol (PR[F]) were calculated from the product of the known infusion rate (Rt) and the ratio of tracer infusate enrichment (Et) to tracer dilution in the plasma (Es): (PR[F] = Rt × (Et/Es − 1).[6]

Radioimmunoassay

Concentrations of cortisol in infusates and plasma samples were also determined by radioimmunoassay using a method described previously.[7] The mean difference in concentrations obtained by radioimmu-

noassay versus those obtained by GC/MS was 1.0% ± 0.04% (infusates) and 0.98% ± 0.07% (plasma samples), respectively.

Statistics

Data are given as means ± SD. Student's *t* test (two-tailed) for matched pairs was used for statistical analysis.

RESULTS

Healthy Subjects

As shown in Table 1, mean plasma concentrations of cortisol were similar in healthy men (8.3 ± 2.7 μg/dL) and women (8.9 ± 2.9 μg/dL). However, steady-state concentrations of infused 1α,2α-D-cortisol were higher in women (2.7 ± 0.9 μg/dL) than in men (1.1 ± 0.4 μg/dL). This resulted in calculated cortisol production rates of 0.94 ± 0.15 mg/h (men) and 0.38 ± 0.14 mg/h (women) ($P < .005$, two-tailed). Even after correction for body-surface area, cortisol production rates were higher ($P < .005$, two tailed) in men (0.48 ± 0.09 mg/m^2 · h) than in women (0.22 ± 0.08 mg/m^2 · h). In three healthy men and two healthy women, plasma concentrations of 1α,2α-D-cortisol fell to undectecable values within 100 minutes after the end of the tracer infusion.

Patients With Cushing's Syndrome

The calculated production rates of cortisol in the 12 patients with Cushing's syndrome (Table 2) were outside the sex-specific normal range. However, in four female patients, cortisol production (range, 0.50 to 0.60 mg/m^2 · h) was within a range considered normal for healthy men (0.35 to 0.63 mg/m^2 · h).

DISCUSSION

The mean production rate of cortisol in all 14 healthy subjects was 0.66 ± 0.33 mg/h (0.35 ± 0.16 mg/m^2 · h). These results are similar to those recently reported by Esteban et al,[4] who, in a similar group of healthy volunteers and during the same period of the day, reported cortisol production rates of approximately 0.50 mg/h. These investigators found that, although cortisol production rates were slightly higher in healthy men than in healthy women, this difference was no longer apparent after correction for body-surface area. In contrast, mean cortisol production was twofold higher in our group of healthy men than in the females studied in an analogous fashion. Plasma concentrations of endogenous cortisol were similar in men and women. Assuming that plasma free cortisol concentrations—not determined in our study—and the setpoint of the feedback mechanisms regulating cortisol production are not different in men and women, the higher production rate of cortisol in men has to be due to a higher metabolic clearance rate. This sex-specific difference in calculated cortisol production was also seen after appropriate correction for body-surface area. From this, it appears that daily requirements for glucocortiocid substitution therapy may well be different in male and female patients with adrenocortical insufficiency.

The tracer dose used in our study was higher than that used by others.[4] However, a relevant suppressive effect on endogenous cortisol production is not likely, since the calculated production rates were on average comparable if not higher than those reported by Esteban et al.[4] In any case, this methodologic difference does not account for the observed sex-specific difference in cortisol production, since we administered the same dose to both men and women. The fact that all our female volunteers were studied in the follicular phase of the menstrual cycle is the main difference from the protocol used by Esteban et al,[4] although the potential effect of the menstrual cycle on cortisol production remains to be studied.

It is of note that the sex-specific difference in cortisol production is of diagnostic relevance, since several female patients with Cushing's syndrome presented a cortisol production rate still within the limits of normal of the healthy male control group, albeit above the range determined in healthy women.

The used experimental protocol was meant to be suitable for diagnostic use in an outpatient setting and was therefore limited to 10 hours. Steady-state conditions, a prerequisite for this type of investigation, were certainly achieved, since the preequilibration period (6 hours) was greater then 10-fold the biologic

Table 1. Mean Plasma Concentrations of Native Cortisol and Infused 1α,2α-D-Cortisol as Determined by GC/MS and Calculated Production Rates of Cortisol in Healthy Men and Women

	Men (n = 7)		Women (n = 7)	
Variable	Mean ± SD	Range	Mean ± SD	Range
F (μg/dL)	8.3 ± 2.7	5.6-13.1	8.9 ± 2.9	6.3-13.6
dF (μg/dL)	1.1 ± 0.4	0.6-1.9	2.7 ± 0.9	1.4-4.0
PR[F] (mg/h)	0.94 ± 0.15	0.77-1.2	0.38 ± 0.14	0.12-0.53
PR[F] (mg/m^2 · h)	0.48 ± 0.09	0.35-0.63	0.22 ± 0.08	0.07-0.31

Abbreviations: F, native cortisol; dF, 1α,2α-D-cortisol; PR[F], production rate of cortisol 2 PM to 6 PM.

Table 2. Mean Plasma Concentrations of Native Cortisol and Infused 1α,2α-D-Cortisol as Determined by GC/MS and Calculated Production Rates of Cortisol in 12 Patients with Cushing's Syndrome

Patient No.	Sex/Age	Diagnosis	m^2	F (μg/dL)	dF (μg/dL)	PR[F] (mg/h)	PR[F]/m^2 (mg/m^2 · h)
1	F/64	Adrenal adenoma	1.95	20.7	0.26	0.98	**0.50**
2	F/31	Pituitary	2.20	16.6	1.21	1.25	**0.60**
3	F/37	Pituitary	2.00	13.5	0.67	2.39	1.20
4	F/38	Adrenal carcinoma	1.88	21.3	1.04	2.22	1.18
5	F/38	Pituitary	1.70	16.4	1.19	1.52	0.89
6	F/24	Pituitary	2.35	16.9	0.99	1.81	0.77
7	F/33	Adrenal adenoma	1.96	14.1	1.27	1.14	**0.58**
8	F/22	Adrenal adenoma	2.09	21.5	1.25	1.82	0.87
9	F/53	Pituitary	2.10	12.4	1.01	1.05	**0.50**
10	M/28	Pituitary	2.15	31.3	0.46	7.60	3.67
11	M/50	Pituitary	1.88	14.8	1.06	1.48	0.79
12	M/26	Ectopic ACTH syndrome	2.15	46.6	0.66	4.80	2.23

NOTE. Bold numbers indicate female patients with corticol production within the range considered normal for healthy men.

Abbreviation: F, female; M, male.

half-life of the infused tracer. We had not intended to study the complete diurnal variation of cortisol production and therefore limited the period of blood sampling to 4 hours. Therefore, the data of this study should not be extrapolated for the calculation of daily cortisol production rates. However, as demonstrated by the results obtained in patients with Cushing's syndrome, the evaluation of only one blood sample pooled during a 4-hour period is sufficient to set this group of patients apart from a healthy control group. This procedure therefore is not only of theoretical interest, but also of clinical use in the diagnosis of endogenous hypercortisolism.

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