Serum testosterone levels in women with Cushing's disease

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Abstract  Serum testosterone was measured pre- and post-operatively in 31 women who obtained remission of Cushing's disease following treatment by interstitial irradiation of the pituitary gland. The mean serum testosterone was initially elevated at 3.35 nmol/l, with values ranging from 0.7 to 14.4 nmol/l. Following treatment the mean serum testosterone fell to 1.42 nmol/l (i.e. normal) and all but one of the 15 patients with initially elevated pre-treatment values obtained normal levels. The pre-treatment testosterone concentrations correlated significantly ($r = 0.47; p = 0.019$) with the midnight ACTH concentration. The likely determinant of the raised serum testosterone would appear to be the intensity of the ACTH drive, although an individual's inherent sensitivity to ACTH may also be a factor.

It is commonly assumed that serum testosterone levels are elevated in patients with Cushing's disease. In women with Cushing's syndrome, severe hirsutism and virilisation in association with an elevated serum testosterone concentration is said to be more a feature of adrenal adenomas and carcinomas than pituitary adenomas (1), but hirsutism and acne were included by Cushing in his original series of patients with pituitary adenomas (2) and more recent series have estimated that these features occur in up to 80% of female patients with Cushing's syndrome (3,4). However, there have been no published studies of testosterone levels in a large unselected series of patients with proven pituitary driven Cushing's disease.

We have therefore reviewed the serum testosterone levels in our female patients whose diagnosis of pituitary driven Cushing's disease has been substantiated by their entering into remission following treatment with interstitial irradiation of the pituitary gland (5). The degree of hirsutism of these patients was also assessed, and this was compared with the serum testosterone concentration. Serum testosterone concentrations were also compared with the severity of corticotrope hyperfunction as assessed by the midnight plasma ACTH concentration.

Patients and Methods

The patients included in this study were adult females (age >17 years) in whom a diagnosis of pituitary driven Cushing's disease had been made on the basis of standard biochemical investigations (6,7). None had previously received external beam radiotherapy or pituitary surgery. All underwent interstitial irradiation of the pituitary gland using $^{90}$Y implants (5), and hormone estimations were performed at presentation and again when in biochemical and clinical remission.

Forty-four female patients with biochemical features suggestive of pituitary driven Cushing's disease were treated by interstitial irradiation between January 1975 and March 1988. In 7 of these no follow-up was available and in 8 patients interstitial irradiation did not produce biochemical or clinical remission. These patients were not included in this study and pre-treatment testosterone levels had not been measured in 3 women, leaving 31 women who entered into remission to form the basis of this report. The mean age of these 31 patients was 37.8 years (range 17 to 60). Nine were post-menopausal and the pre-menopausal women were all amenorrheic. Testosterone concentrations had been meas-
duced in 23 women and the data and statistics relating to the effects of treatment are based solely on paired comparisons from patients in whom both pre- and post-treatment values were measured. Pre-treatment midnight plasma ACTH levels had been measured in 24 patients.

Hirsutism
The severity of the patient's hirsutism was graded 0, 1 or 2, without knowledge of the biochemical results on the basis of clinical examination and the need for shaving or plucking of the hairs. Patients with grade 0 hirsutism were not troubled by hirsutism and no excess facial or body hair was found on examination. Patients with grade 1 complained of an increase in facial and/or body hair and this was apparent on physical examination; however, it was less severe than in patients with grade 2 hirsutism who had a heavy growth of facial hair requiring shaving more than once per week and male-pattern chest and pubic hair.

Hormone assays
Total serum testosterone was measured by the Coat-A-Count testosterone kit (Diagnostic Products Ltd, Abingdon, Oxon, UK). Within- and between-batch coefficients of variation (CV) for this assay were 9 and 8% at 2.1 nmol/l, and 4 and 9% at 19.4 nmol/l. This assay has 0.5% cross-reactivity with androstenedione, 0.004% cross-reactivity with androsterone, 0.002% cross-reactivity with dehydroepiandrosterone (DHEA) and 0.006% with DHEA sulphate, 3.3% cross-reactivity with dihydrotestosterone and 0.2% cross-reactivity with androsenediol (Manufacturer's data sheet).

Plasma ACTH was measured by a modification of the radioimmunooassay method of Rees et al. (8) after extraction using Vycor glass. The inter-assay CV was 10.9% at an ACTH concentration of 54 ng/l.

In all cases the values quoted are the means of all available measurements. All samples were taken at 9.00 h unless otherwise stated. Post-treatment samples were taken when the patient was in full biochemical and clinical remission at between 3 and 36 months after implantation, as assessed by normalization of the serum cortisol concentrations, the 24-h urinary excretion of free cortisol, and the resolution of the somatic changes of Cushing's disease (5).

Statistics
Analysis of variance, paired t-tests and linear regression were performed using the Statsgraphics statistical package (Statistical Graphics Corp, Rockville, MD). The values quoted are means together with their 95% confidence intervals.

Results
In the 31 patients in whom it was measured, the mean pre-treatment serum testosterone concentration was 3.35 nmol/l (2.45 to 4.26) with individual values ranging from 0.7 to 14.4 nmol/l. In a group of 98 normal pre-menopausal women the mean serum testosterone was 1.60 nmol/l (1.46 to 1.74), with a range of 0.2 to 3.0 nmol/l. The mean value in the Cushing's group was significantly greater than the mean value in the normal women (1.21 to 2.29 nmol/l; t=6.56, df=131, p=0.0005). In 16 of the patients (52%) the pre-treatment serum testosterone concentration was within the normal range (i.e.<3.0 nmol/l), and in 15 it was elevated.

Eight patients (26%) were classified as having grade 0 hirsutism (i.e. they were not hirsute) and in these the mean testosterone concentration prior to treatment was 2.36 nmol/l (0.62 to 4.10). Thirteen patients (42%) had grade 1 hirsutism and in these patients the mean level was 3.15 nmol/l (1.78 to 4.51), whilst 10 patients (32%) had grade 2 hirsutism and had a mean testosterone concentration of 4.42 nmol/l (2.86 to 5.98) (Fig. 1). There was no significant difference between the mean testosterone levels in these three groups.

There was significant correlation between the pre-treatment serum testosterone and the mean midnight ACTH concentrations (r=0.47, df=23,
p=0.019) in the 24 patients in whom values were available (Fig. 2).

In the 23 patients in whom it was measured the mean serum testosterone concentration after treatment was 1.42 nmol/l (1.09 to 1.75), and treatment produced a mean reduction in the circulating levels of 2.19 nmol/l (1.16 to 3.22, t=4.41, df=22, p=0.0002). The mean testosterone concentration for all 23 patients assessed following treatment was not significantly different from the mean value in normal women (~0.06 to 0.54). Fig. 3 shows that the fall was greatest in those 12 patients whose initial serum testosterone was above the upper limit of the normal range for women (i.e. >3 nmol/l). In these 12 patients the mean reduction was 3.78 nmol/l (2.30 to 5.25, t=5.64, df=11, p=0.0002), whilst in those 11 patients whose initial serum testosterone was in the normal range there was no significant fall after treatment.

Discussion

Previous studies of circulating testosterone levels in patients with Cushing’s disease have either been very selective, reporting levels in a few patients with menstrual irregularities or hirsutism in whom levels were approximately twice normal (9), or limited to a few patients in whom levels were found to be normal (10). Despite this it has commonly been assumed that testosterone levels are elevated in patients with Cushing’s disease. Our study in a large unselected series of patients shows that although the mean value for the group as a whole was elevated, over half the patients had testosterone concentrations within the normal range.

We did not set out to study in detail the hirsutism found in patients with Cushing’s disease; however, over 70% of the women in our series were troubled by clinically significant hirsutism, and although the testosterone levels in the hirsute women tended to be higher than those in women who were not hirsute, the mean values were not significantly different. This may reflect the fact that testosterone is only one of a number of androgens secreted by the adrenal glands. Dihydrotestosterone, androstenedione, androstenediol, dehydroepiandrosterone and dehydroepiandrosterone sulphate are all released from the adrenal; however, apart from dihydrotestosterone, which is approximately 1.25 times as androgenic as testosterone, these are all very weak.

![Fig. 2.](image)

Relationship between serum testosterone concentrations and midnight ACTH concentrations in 24 patients with Cushing’s disease showing the regression line: $y = 1.649 + 0.028x$ ($r=0.47$, df=23, p=0.019).
androgens (11), and with exception of dehydroepiandrosterone are secreted in small quantities (12). Furthermore, in normal women the majority of circulating testosterone is bound to protein (approximately 66% to sex-hormone-binding globulin and 30% to albumin) (12-14) and only the 1-3% of free hormone is biologically active. However, we have shown that, in practice, there is a good correlation between the total testosterone and the serum free testosterone concentrations (15), and it is unlikely, therefore, that differences in the concentration of free testosterone explain the observed variability in the relationship between total testosterone levels and the severity of the hirsutism.

In normal women only 30-40% of the circulating testosterone is secreted by the adrenals and the ovary, the remainder being formed by peripheral conversion from androstenedione (16), and overall, the adrenal accounts for only about 15% of the total circulating testosterone (17). However, in pathological conditions this percentage may be higher, and the correlation between pre-treatment serum testosterone levels and the midnight ACTH levels that we observed, suggests that the stimulation of the adrenals by ACTH may be largely responsible for the elevation in serum testosterone.

Although the exact mechanism by which adrenal androgen secretion is regulated is unknown, a number of studies indicate a major role for ACTH, with exogenously administered ACTH leading to elevation of circulating testosterone (18-22), dehydroepiandrosterone (20,23,24) and androstenedione (18-20,22,24). A direct effect of ACTH on testosterone secretion by isolated human adrenal cells has also been demonstrated (25). In normal women there appears to be considerable variation in both the response of plasma androgen levels to exogenous ACTH (20) and in the threshold at which this response occurs (24). Such variations together with differences in the prevailing ACTH concentrations, may explain the range of testosterone concentrations found before treatment in our patients. They may also explain why the fall in circulating testosterone concentrations following disease remission was most marked in those patients whose levels were elevated initially; ACTH levels may already be subthreshold for stimulation of androgen secretion in those patients with pre-treatment serum testosterone concentrations in the normal range. A separate adrenal androgen-stimulating glycopeptide may also be released from the pituitary gland (26): however, the role of this factor and its relationship to elevated androgen levels in disease states have not been defined.

Fig. 3.
Serum testosterone concentrations in 23 patients before and after treatment with 60Y interstitial irradiation. The upper limit of normal is shown at 3 nmol/l.

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