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Twenty-Four Hour 17-Hydroxyprogesterone Response to Adrenocorticotropine in Adrenal Incidentalomas: Augmented Response after Adrenalectomy in Two Patients

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Abstract. The current study aimed to investigate the midterm (24 hour) response of 17-hydroxyprogesterone (17-OHP) and dehydroepiandrosterone sulphate (DHEA-S) to synthetic high-dose adrenocorticotropin (ACTH) in adrenal incidentalomas (AI). Seventeen patients with AI and 40 age- and sex-matched controls received synthetic ACTH (tetracosactide, 1000 μg, IM). Plasma, 17-OHP and DHEA-S were collected in basal conditions and after 1, 4, 6, 8 and 24 hours. (HPA) axis was also evaluated using circadian serum cortisol, urinary free cortisol and overnight 2 mg dexamethasone suppression. Basal plasma 17-OHP levels did not differ among the groups. However, the increment in plasma 17-OHP in patients both in terms of peak [13.76±2.52, 4.77±0.30 ng/ml, mean±S.E.M, $p<0.001$] and area under the curve [190±46, 96.75±32 ng/ml/h, $p<0.001$] were significantly higher than that of the controls. Stimulated 17OH-P levels never reached 9.1 ng/ml in controls. Sixty-five (11/17) % of the patients were found to have exaggerated response. Three of the patients were found to have subclinical Cushing's syndrome and interestingly, two augmented their 17-OHP response to ACTH after unilateral adrenalectomy and normalisation of their HPA axis. Basal DHEA-S levels of the patients were significantly lower [99.21±45, 230.18±34 μg/dl, $p<0.01$] and stayed persistently lower than that of the controls. Evidence of a heterozygous 21 hydroxylase deficiency, as indicated by the exaggerated 17-OHP response to ACTH, has been widely reported in AI patients. However, to our knowledge to date there is no report on augmented 17-OHP response to ACTH after adrenalectomy. Possible reasons for the augmentation were discussed.

Key words: Adrenal incidentalomas, 17-Hydroxyprogesterone

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AFTER Jaresch *et al.* reported a high incidence of adrenal masses in congenital adrenal hyperplasia (CAH) cases [1], adrenal response to synthetic adrenocorticotropin (ACTH) has been thoroughly investigated and evidence of exaggerated 17-hydroxyprogesterone (17-OHP) and other precursors response, suggestive of heterozygous 21-hydroxylase [2–4], 11-beta-hydroxylase [5] or a combination of adrenal enzyme dysfunction [6], has been widely reported in adrenal incidentalomas (AI). In most studies the frequency of this hormonal abnormality ranged between 17% and 71%, probably reflecting the method used for the definition of normal 17-OHP responsiveness [7]. Whether the enzymatic deficiency is intrinsic to the tumoral tissue or may be attributed to the increased adrenal mass is still controversy, due to

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the small number of patients reinvestigated for this abnormality after surgical removal. Limited data are in favour of normalisation of 17-OHP in the majority of the patients re-evaluated after surgery, thus attributing enzymatic defect to the tumoral tissue itself [2, 3, 8, 9]. On the other hand, the pattern and magnitude of 17-OHP response were unmodified in a few adrenalectomized patients [8] and a normal synthetic steroidogenic system, including P450c21 activity, in non-hyperfunctioning adrenal adenomas, was reported [10]. However, to our knowledge there is to date no report on augmented 17-OHP response to ACTH after adrenalectomy in patients with AI. The testing protocol in the present study is also different in terms of using high-dose (1000 μg), intramuscular synthetic ACTH preparation and investigating the midterm (24 hour) response of 17-OHP in the current study.

Materials and Methods

Subjects and experimental design. Seventeen patients with AI (11 females, 6 males) with a mean age of 52 (34–75) years and 40 age and sex matched controls [28 females, 12 males, 50 (31–76) years] were enrolled for the study. Patients and controls gave their informed consent to enter the study. The study was approved by the institutional review board. Neither of the patients had clinical or overt signs of hormone excess and they were incidentally discovered to have hypodense, round-oval shaped, adrenal lesions (15 unilateral, 2 bilateral) with a mean tumour size of 23 mm (10–40 mm) by computed tomography.

Basal, diurnal (8:00 am, 11:00 p.m.) plasma cortisol, 24 hour urinary free cortisol, were measured and overnight 2 mg dexamethazone suppression test was performed on the patients with AI. After 48 hours both controls and patients received synthetic ACTH (Synacthene®, Sandoz) 1000 μg, IM at 8:00 a.m. Blood samples for plasma cortisol, 17-OHP and dehydroepiandrosterone-sulphate (DHEA-S) were collected through an indwelling catheter and under basal, resting conditions and at 1, 4, 6, 8 and 24 hours after ACTH administration. Serum samples were stored at $-20°C$ until assayed. Premenopausal women were tested in the early follicular phase of the menstrual cycle.

All patients were also subjected to the following endocrine evaluation: 1) measurement of 24 h urinary excretion of vanillylmandelic acid (VMA) and cathecolamines. 2) measurement of PRA and aldosterone levels in supine and standing positions.

Three of the patients underwent surgery for preclinical Cushing's syndrome. Adrenocortical adenoma were histologically confirmed and patients were re-evaluated with the same testing protocol after unilateral adrenalectomy.

Serum cortisol, ACTH, DHEA-S were measured by competitive immunoassay using commercial kits (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA) and 17-alpha hydroxyprogesterone by radioimmunoassay (Diagnostic System Laborotories-5000, Webster, TX).

Area under the curves (AUC) was calculated according to Tai's model [11] and Student's *t*-test and Mann-Whitney U test were used for the statistical analysis. P values equal or less than 0.05 were considered statistically significant.

Results

Fig. 1 reveals that basal and stimulated plasma cortisol levels did not differ between the patients with AI and the control ($p>0.05$). Basal plasma 17-OHP was not significantly different between the two groups, respectively [1.20 ± 0.51 (0.81) ng/ml, mean $\pm$SE (SD)] and [1.25 ± 0.20 (0.83) ng/ml, $p>0.05$] (Fig. 2). However the increment in plasma 17-OHP in patients both in terms of peak [13.76 ± 2.52 (10.39) ng/ml] and AUC [190 ± 46 (156) ng/ml/h] were significantly higher than that of the controls [4.77 ± 0.30 (1.90) ng/ml, $p<0.001$] and [96.75 ± 32 (23) ng/ml/h, $p<0.001$] (Fig. 2). The data indicated that 17OH-P response to ACTH in controls never exceeded 9.1 ng/ml (cut-off for normal response), but that 11 out of 17 (65%) patients with AI exceeded this value. Minimal response value which was considered exaggerated was 6 times larger than that of the basal for the individual patient. There was a borderline weak correlation between basal 17-OHP levels and tumour size at the 8th hour of ACTH stimulation ($p=0.05$) which disappeared during the other hours of testing.

Basal DHEA-S levels were significantly lower [99.21 ± 45 (191) μg/dl vs 230.18 ± 34 (217) μg/dl, $p<0.001$] and stayed persistently and significantly

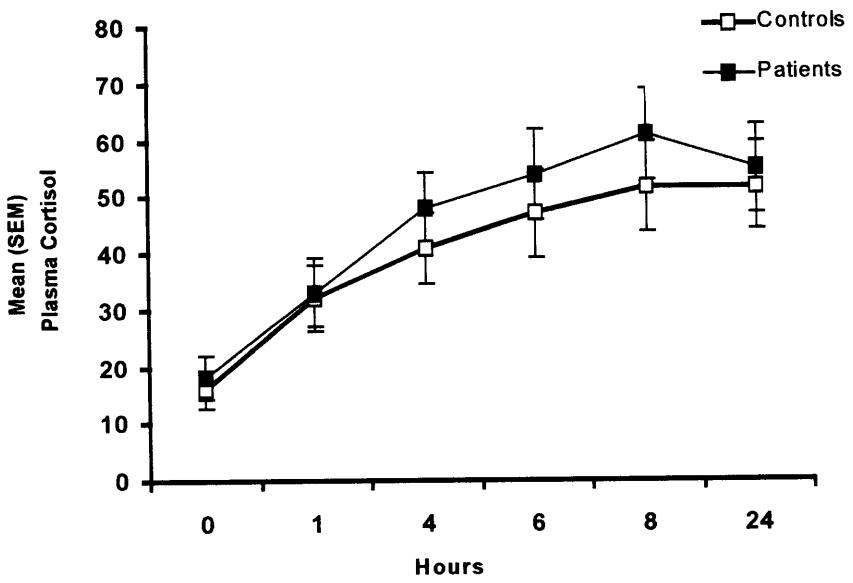


Fig. 1. Basal and ACTH stimulated mean (SEM) plasma cortisol levels of 17 patients with adrenal incidentalomas (AI) and 40 age- and sex-matched controls.

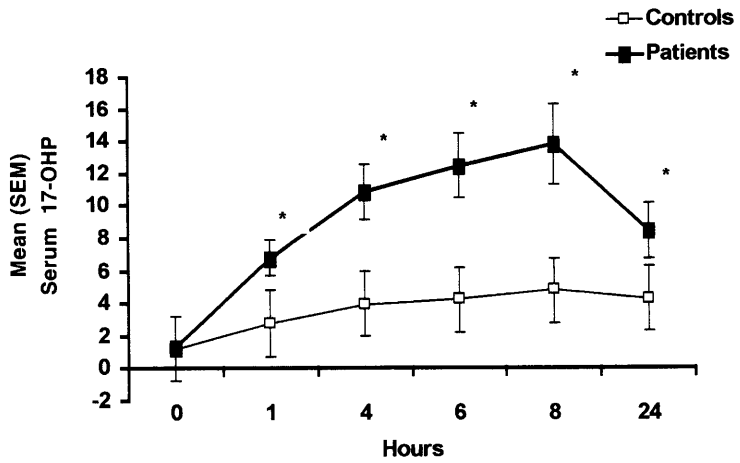


Fig. 2. Basal and ACTH stimulated mean (SEM) serum 17-hydroxyprogesterone (17-OHP) levels of 17 patients with adrenal incidentalomas (AI) and 40 age- and sex-matched controls.
*$p < 0.001$ compared to the controls.

lower than that of the controls during 24 hour ACTH stimulation (Fig. 3).

Three of the 17 (18%) patients were found to have subclinical Cushing's syndrome as assessed by normal to upper normal plasma and/or urinary free cortisol, low normal measurable ACTH, but missing suppression of 8:00 a.m. cortisol (>5 μg/ml) after overnight 2 mg dexamethasone (DXM). Of these three patients, two had noticeably augmented their slightly exaggerated 17-OHP response to ACTH after unilateral adrenalectomy and normalisation of cortisol suppression to 2 mg DXM (<3 μg/ml) (Fig. 4), and one revealed no change in her normal preoperative 17-OHP responsiveness to ACTH although she also normalised her HPA axis. Details about the two patients with augmented 17OH-P response after adrenalectomy are given below.

Patient 1 was a 65 year-old female who had moderate hypertension for 4 years. Physical examination revealed a generalised obesity (BMI: 33 kg/m^2) and

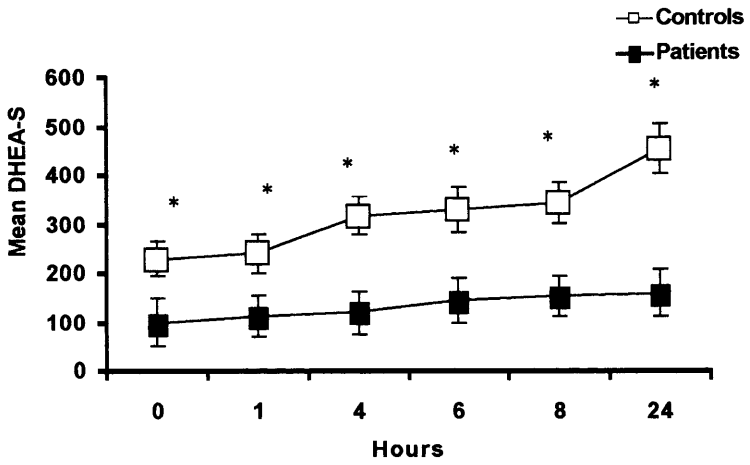


Fig. 3. Basal and ACTH stimulated serum dehydroepiandrosterone sulphate (DHEA-S) levels of 17 patients with adrenal incidentalomas (AI) and 40 age- and sex-matched controls.
*$p < 0.001$ compared to the controls.

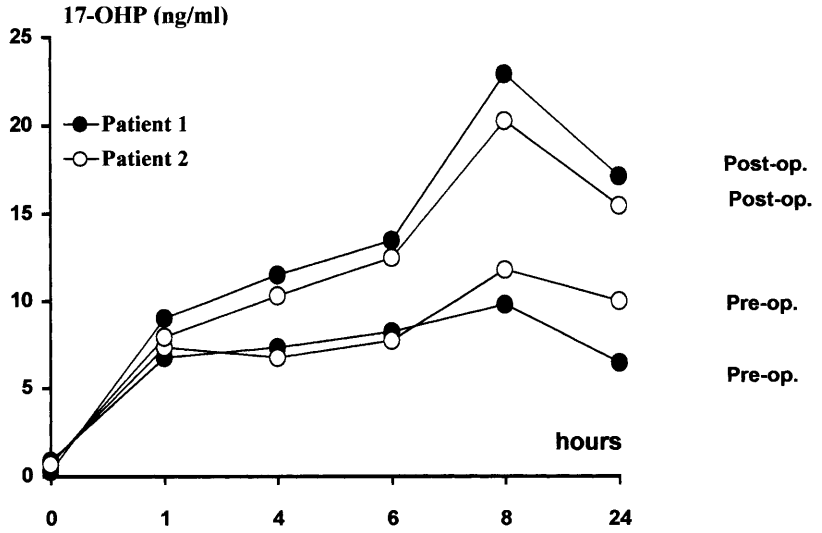


Fig. 4. Postoperative augmentation of 17-hydroxyprogesterone (17-OHP) responsiveness in two patients with bilateral adrenal incidentalomas and subclinical Cushing's syndrome after unilateral adrenalectomy.

no stigmata for Cushing's syndrome. Hormonal evaluation showed mild hypercorticism with high normal basal (0800: 22 μg/dl) normal diurnal (2300: 8 μg/dl) levels of plasma cortisol levels measurable low normal ACTH levels and high normal 24 hour free cortisol excretion [85 μg/gr creatinine (25–95)]. 0800 plasma cortisol stayed at 5.5 μg/dl in response to an overnight 2 mg DXM suppression. Computerized tomography showed bilateral masses of 40 × 25 mm: right, 20 × 25 mm: left adrenal gland origin. Although markedly increased on the right, bilateral uptake was detected on 19-iodo-cholesterol scintigraphy. Right unilateral adrenalectomy was performed and an adrenocortical adenoma was histopathologically diagnosed. Adrenal tissue adjacent to the adenoma was found to be atrophic. Her moderate hypertension disappeared after the surgery. No hydrocortisone compensation was needed. Endocrinological re-evaluation showed a normal basal, diurnal plasma cortisol levels which could be fully suppressed (1 μg/dl) with overnight 2 mg DXM testing. Twenty-four hours plasma cortisol excretion

also normalized to [45 μg/gr creatinine (25–95)] from a high normal level. She augmented her slightly exaggerated 17-OHP response to ACTH after unilateral adrenalectomy (Fig. 4, patient 1).

Patient 2 was a 55 year-old female who also had an incidentally discovered bilateral adrenal mass. She had no complaints regarding the adrenal mass. Physical examination revealed a generalised obesity (BMI: 31 kg/m^2) and no stigmata for Cushing's syndrome. Hormonal evaluation also showed mild hypercorticism with high normal basal (0800: 20 μg/dl) normal diurnal (2300: 6 μg/dl) levels of plasma cortisol levels measurable low normal ACTH levels and high normal 24 hour free cortisol excretion [78 μg/gr creatinine (25–95)]. 0800 plasma cortisol stayed at 6.5 μg/dl in response to an overnight 2 mg DXM suppression. Computerized tomography showed bilateral masses of 30 × 25 mm: right, 10 × 10 mm: left adrenal gland origin. Bilateral uptake, slightly pronounced on the right was detected on 19-iodocholesterol scintigraphy. Right unilateral adrenalectomy was performed and an adrenocortical adenoma was histopathologically diagnosed. Adrenal tissue adjacent to the adenoma was found to be normal. Postoperative course was good and no hydrocortisone compensation was needed. Endocrinological re-evaluation showed a normal basal, diurnal plasma cortisol which could be fully suppressed with overnight 2 mg DXM testing (< 1 μg/dl). Twenty-four hours plasma cortisol excretion also normalized to 44 μg/gr creatinine. She also augmented her slightly exaggerated 17-OHP response to ACTH after unilateral adrenalectomy (Fig 4, patient 2).

Neither of the 17 patients had hypokalemia in salt-replete state; PRA, plasma aldosterone, urinary cathecolamines and VMA were in the normal range in all (data not shown). The remote possibility of adrenal carcinoma has been reliably excluded by either histopathology (3 patients) or 12 months follow-up (14 patients). The size of the mass did not significantly increase in any of the 14 patients followed up and they all remained clinically asymptomatic.

Discussion

The connection between silent adrenal nodules and 21-hydroxylase deficiency and/or a combination of adrenal enzyme dysfunction is well established by using the 17-hydroxyprogesterone (17-OHP) and other precursors response to standard IV 250 μg synthetic adrenocorticotrophin (ACTH) and 60–90 min of testing [2–6]. Although the dose (1000 μg), route (IM), and testing interval (24 hour) used in the current study differs from the previous studies, 65% (11/17) of the AI patients displayed an exaggerated 17-OHP response to ACTH in accordance with the literature [7].

Our data regarding significantly low basal and stimulated DHEA-S levels (Fig. 3) in AI patients are consistent with the previous data [2, 3, 8, 12]. We were also able to stimulate DHEA-S both in controls and in the patients with a high-dose midterm, IM ACTH stimulation test. But serum DHEA-S levels of AI patients stayed persistently and significantly lower than that of the controls during 24 hours of testing (Fig. 3). On the other hand, low dose, short-term ACTH stimulation tests failed to stimulate DHEA-S in normal controls [13]. Low DHEA-S levels in AI patients was previously suggested to be a sensitive and specific marker for the cortical origin, glucocorticoid overproduction and benignity of AI [14, 15]. However, recent data, clearly indicated that DHEA-S measurement does not seem to offer applicable clinical information in the management of AI [12].

An interesting observation from this group was further augmentation of 17-OHP responsiveness in two women with bilateral AI and subclinical Cushing's syndrome after unilateral adrenalectomy. In this regard our findings are in contrast to the findings of Terzolo *et al.* who reported two distinct patterns of steroid secretion in patients with AI: those with disregulated cortisol secretion (established by non-suppression after low dose DXM, blunted ACTH /cortisol response to CRH or a disturbed diurnal secretion) and those who had an exaggerated 17-OHP response to ACTH stimulation [4]. We observed an overlap between these groups with two patients with preclinical Cushing's syndrome having a slightly exaggerated 17-OH response before adrenalectomy and substantially augmented responses after adrenalectomy with the normalisation of ACTH/cortisol axis. Eleven of the twelve published patients who underwent adrenalectomies for preclinical Cushing's syndrome, normalised their cortisol suppression to DXM but also exaggerated 17-OHP response to

ACTH [2, 3, 8, 9]. Our data indicated that exaggerated responses of 17-OHP to ACTH are not restricted to nonfunctional tumors. Cortisol autonomy or disregulation, which is encountered in 9.2% of patients with AI [16], may suppress the 17-OHP response in a subgroup of patients with subclinical Cushing's syndrome which may recover by the normalisation of ACTH/cortisol axis. It is still controversial whether the reduced enzymatic activity among incidentaloma patients is a tumour-associated phenomenon or indicates a heterozygous late-onset CAH. Our two patients, with bilateral masses, showed an augmented response to ACTH after unilateral adrenalectomy, suggesting that the deficit could be generalised. However, recovery of the contralateral tumor, having defective enzymatic pathways, but retaining ACTH receptors, from suppressed responsiveness due to the disregulated cortisol production, may still be the underlying mechanism for the augmentation of the 17-OHP response after surgery.

In conclusion, further investigation of different patterns of steroid secretion, especially on those undergoing an adrenalectomy, may substantially add to our knowledge of this technologically discovered problem.

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