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ORIGINAL ARTICLE

Aberrant cortisol responses to physiological stimuli in patients presenting with bilateral adrenal incidentalomas

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Abstract Aberrant receptors have been implicated in the pathogenesis of several types of adrenal tumours. So far the presence of aberrant receptors has been investigated in patients with massively enlarged adrenals due to ACTH-independent macronodular adrenal hyperplasia (AIMAH) and unilateral adrenal adenomas associated with overt or subclinical Cushing's syndrome. The likelihood of aberrant responses in patients presenting with bilateral adrenal incidentalomas (BI) presenting as discrete solitary bilateral adenomas has not been thoroughly addressed. This is an observational cross-sectional prospective study conducted in a secondary/tertiary care centre. We studied 33 patients; 28 with incidentally discovered bilateral discrete adrenal adenomas and five with massive bilateral adrenal macronodular hyperplasia. We probed responses to physiological stimuli, namely upright posture and meal; in a subgroup of menopausal women the presence of aberrant gonadotropin receptors was assessed by the LHRH test. Abnormal responses obtained in tests performed with no dexamethasone suppression were always repeated and confirmed under dexamethasone suppression. Aberrant cortisol responses were confirmed in 10 patients; 9 to posture, 1 to meal (along with a positive response to posture) and 1 to LHRH tests. Patients who responded to any test compared to those who tested negative had larger adenomas, higher post-LDDST and midnight cortisol and a trend for lower ACTH levels. Patients without subclinical hypercortisolism (SH) did not respond to any test while 50% of patients with SH had an aberrant response ($P = 0.002$). A greater prevalence of aberrant responses was noted in patients with bilateral macronodular hyperplasia compared to those with solitary bilateral adenomas (80 vs. 21.4%, $P = 0.02$). Aberrant cortisol responses, primarily to posture testing, are present in a substantial proportion of patients with bilateral adrenal incidentalomas. Such cortisol responses are observed only in patients with subclinical hypercortisolism and especially in those patients with larger adrenal lesions.

Keywords Bilateral adrenal adenomas · Incidentalomas · Subclinical hypercortisolism · Aberrant receptors · ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) · Posture test · Meal test · LHRH test

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Introduction

Adrenal incidentaloma is a common clinical problem, encountered in 0.4–4.4% of subjects undergoing imaging with CT for reasons unrelated to adrenal pathology [1]. A significant proportion (8–17%) of such patients present with bilateral incidentalomas (BI). The majority of BI appears with an adenoma imaging phenotype in the form of discrete solitary lesions, located in each adrenal [2, 3]. Less commonly, bilateral lesions occur in the form of bilateral diffuse or multinodular adrenal enlargement [4]. Recently, the role of aberrant expression of various ectopic or eutopic membrane-bound G-protein coupled receptors (GPCRs) has emerged as a compelling candidate in the pathogenesis of adrenal tumours. These illicit receptors were originally

implicated in the pathogenesis of ACTH-independent macronodular adrenal hyperplasia (AIMAH) associated with clinically overt Cushing's syndrome. Subsequently, aberrant receptor expression has been implicated in an increasing number of different entities including unilateral cortisol-producing adenomas associated both with overt or subclinical Cushing's syndrome [5–8], unilateral aldosteronomas and even pure androgen-producing adenomas [9]. Aberrant cortisol responses were also found in a substantial proportion of patients with incidentally detected bilateral lesions associated with subclinical hypercortisolism (SH) [10–12]. In these studies, the majority of bilateral adrenal lesions demonstrated an AIMAH radiological phenotype illustrated by massively enlarged adrenals with multiple macronodules [13]. In clinical practice many incidentally presented bilateral adrenal lesions appear with less striking radiological features in the form of distinct bilateral solitary adrenal lesions [14]. It is currently uncertain whether this latter imaging phenotype consists of discrete solitary bilateral adenomas or represents an early or mild form of bilateral nodular adrenal hyperplasia. However, with the exception of a recent study by Libe et al. [12] the likelihood of aberrant hormone receptor expression in patients presenting with such lesions has not been thoroughly addressed.

A diversity of receptors, both ectopic, such as those for glucose-dependent insulinotropic peptide (GIP), catecholamine, vasopressin (V2-V3-vasopressin receptor), serotonin (5-HT7 receptor) and angiotensin II (AT1 receptor), and eutopic, such as those for vasopressin (V1-vasopressin receptor), luteinizing hormone/human/chorionic gonadotropin (LH/hCG-R), serotonin (5-HT4 receptor) and leptin, have been reported to be involved in the control of cortisol secretion [5, 10, 11, 15]. Most in vivo investigation protocols have used both the physiological and pharmacological stimuli to assess for the presence of such aberrant receptors. As a result, an extensive list of pharmacological tests has been introduced rendering investigations of aberrant cortisol responses an extremely cumbersome and expensive process. Such pharmacological stimuli are certainly important for an in depth understanding of aberrant receptor physiology; however, the clinical significance of a cortisol rise after the bolus administration of a pharmacological agent, not necessarily reflecting physiological fluctuations in endogenous levels, remains questionable. In vivo tests with physiological stimuli such as the meal and the supine-to-upright posture tests, evaluate the presence of diverse receptors involved in aberrant cortisol responses, namely GIP, catecholamine, vasopressin and angiotensin II receptors. Moreover, everyday stimulation of the adrenals by the physiological stimuli of food intake or assumption of upright position has unquestionable pathogenic and clinical implications. Therefore, we aimed to investigate the prevalence of abnormal responses to these physiological stimuli in a cohort of patients presenting primarily with incidentally detected bilateral solitary adrenal lesions demonstrating an adenoma imaging phenotype. Since in menopausal women aberrant LH expression may lead to adrenal enlargement and hormonal hypersecretion in a subgroup of menopausal women an LHRH test was performed.

Materials and methods

Subjects

The study population consisted of 33 patients (7 men, 26 women, mean age 60 ± 11 years, age range: 40–78, mean BMI: 29.8 ± 5.7 kg/m^2, range: 19.5–44.0). The adrenal tumours/hyperplasias were discovered on CT scan of the abdomen performed for reasons unrelated to adrenal disease. None of the patients exhibited clinical features of overt Cushing's syndrome. Inclusion criteria were tumors with features highly suggestive of benign adrenocortical adenomas (homogeneous, smoothly marginated, with no change in size on repeat scanning), normal 24-h VMA, normetanephrine and metanephrine excretion, potassium, aldosterone and plasma renin activity levels within the normal range, and a normal aldosterone/renin ratio. Congenital adrenal hyperplasia was excluded by normal levels of 17OH-progesterone. Patients with known malignancies undergoing staging procedures and patients with intervening acute illness were excluded from the study. Most medications were discontinued 10 days prior to admission. Antihypertensive medications, in particular AT-1 inhibitors, angiotensin converting enzyme inhibitors and b-blockers were discontinued 2–4 weeks prior to admission; when hypertension was uncontrolled, we administered nifedipine, a calcium channel blocker, for this period. None of our patients was on medications that increase dexamethasone clearance [16].

Imaging

Slice thickness of the presenting CT scan in the vast majority of cases was 5 mm. On repeat CT scanning thinner slices (2.5 mm) were obtained. A Hounsfield Units cut-off of below 10 was used as indicating a benign imaging phenotype. In those patients with higher values MRI scan was obtained and only patients with signal intensity decrease of 20% or greater on the out-of-phase images were included in the study.

Radiological diagnosis of AIMAH was made in five patients when both adrenals were massively enlarged with

multiple macronodules, distorting and completely obscuring the normal gland [13, 15, 17]. Histological examination confirmed the radiological diagnosis of AIMAH in all five patients. Adenomas were defined as well circumcised homogeneous ovoid masses measuring more than 1 cm with low density and without calcification or necrosis. The range of the bigger adrenal adenomas' largest diameter was 2.0–5.8 cm (mean: 3.8 ± 0.9 cm). The sum of the largest diameters of adenomas on each side (combined size) was used as a surrogate for the bulk of the adrenal masses (range 3.5–9.5 cm). Twenty-eight patients presented with solitary adenomas in both adrenals and constituted the majority of patients investigated in this study. Of this latter group only six patients were operated. Surgery was offered to patients with clear and reproducible evidence of subclinical hypercortisolism. The presence of a >4 cm lesion was also another indication for surgery. The final decision was based on patients' preference. As a rule, unilateral removal of the largest lesion was performed. Histology was compatible with solitary adrenocortical adenoma in four and with nodular hyperplasia in the remaining two patients.

Study protocol

This is an observational cross-sectional prospective study that was conducted between 2005 and 2009. All patients gave informed consent. All investigations were performed in an inpatient basis. BMI was calculated as [body weight (kg)/height (m^2)]. Assessment of cortisol secretion involved: baseline morning serum cortisol, ACTH and dehydroepiandrosterone sulphate (DHEA-S) levels, and midnight cortisol. Subjects also provided at least one complete 24-h urine collection for the measurement of 24-h urinary free cortisol (UFC). In patients where two UFC collections were obtained the highest value is reported in the results of the present study. Trained personnel in an inpatient setting supervised UFC collections. All patients underwent a standard 2-day low-dose dexamethasone suppression test (LDDST-0.5 mg every 6 h for 2 days). Compliance of the patients was established by the supervised administration of each tablet by trained nurses. Post-dexamethasone serum cortisol was measured at 0800, 6 h after the last dose of dexamethasone. The diagnosis of subclinical hypercortisolism (SH) was made on the basis of post-dexamethasone cortisol levels >2.5 μg/dl [18] and at least one of the following criteria: (a) ACTH levels <10 pg/ml [19], (b) high UFC > 120 μg/24 h, corresponding to the upper normal limit of our method and (c) midnight serum cortisol >9 μg/dl; we chose this cut-off as it corresponds to the 97th upper percentile of normal subjects studied in our hospital under similar conditions (unpublished data).

Screening protocol for aberrant receptors

Initial screening included a supine-to-upright posture test to screen for receptors to angiotensin II, vasopressin, catecholamines or atrial natriuretic peptides and a standard mixed meal test to assess the presence of GIP or other gastrointestinal hormone receptors, as has been described previously [11, 20]. All tests were performed following an overnight fast and in a supine position for at least 1 h. Serial measurements of ACTH and cortisol were obtained at 30 min intervals over the course of 2 h following the intervention. ACTH was monitored to ensure that the ligand-mediated cortisol stimulation is ACTH-independent. The cortisol response was calculated using the formula $\Delta F = [(\text{peak cortisol} - \text{basal cortisol})/(\text{basal cortisol})] \times 100\%$. A change of less than 25% of plasma cortisol was defined as no response; a 25–49% change, as a partial response; and a change of 50% or greater, as a positive response [11]. In patients with positive or partial responses the test was repeated on day 3 after the LDDST [8], in order to eliminate the possible confounding effect of ACTH variations on plasma cortisol. In view of a substantial proportion of false positive responses in patients tested without prior dexamethasone suppression an interim modification of the protocol was performed and the most recent patients were tested only following prior dexamethasone administration. Thus, 8 meal and 8 posture tests were performed only under dexamethasone suppression. In these patients partial or positive responses with peak cortisol levels <3 μg/dl were characterised as equivocal.

Since in menopausal women aberrant LH expression may lead to adrenal enlargement and hormonal hypersecretion in a subgroup of menopausal women an LHRH test was performed. Eight postmenopausal women (mean age 57 ± 10; range: 46–76 years) underwent additional testing for the presence of LH receptors (bolus i.v. administration of 100 μg GnRH, with measurements of ACTH and cortisol at 30 min intervals over the course of the following 2 h). Five of the eight tests were performed only under dexamethasone suppression.

Hormone assays

Serum cortisol (F), ACTH, insulin and DHEA-S were assayed using an automated Elecsys/Cobas electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Indianapolis, IN). UFC was measured after dichloromethane extraction by the same method. The reported lower limits of detection of the assays used are 0.018 μg/dl for cortisol, 0.1 μg/dl for DHEA-S, 0.2 μU/ml for insulin and 1 pg/ml for ACTH. The intra-assay coefficient of variation (CV) for cortisol was 1.6% for 7.5 μg/dl, 1.5% for 20.3 μg/dl and 1.6% for 46 μg/dl, respectively. The intra-assay CV for

ACTH was 5.4, 2.4 and 2.6% for 4.9, 74.2 and 1390 pg/ml, respectively. For insulin, the intra-assay CV was 2.6, 2.8 and 2.5% for 6.4, 20.9 and 747 μU/ml, respectively. The intra-assay CV for DHEA-S was 3.6, 4.7 and 2.4% for 117, 395 and 984 μg/dl, respectively.

Statistical analysis

Statistical package SPSS 15.0 was used for data analysis. Data are expressed as mean ± SD, unless otherwise stated. To compare between two groups of independent samples, T-test or Mann–Whitney tests were applied where appropriate. Chi-square or Fischer Exact tests were used to compare the proportions of observations between two groups. Differences were considered statistically significant at $P < 0.05$. Logistic regression multivariate analysis using the backward method was applied to test which of the parameters amongst: combined size, midnight cortisol, ACTH, cortisol post-LDDST and UFC levels, significantly predicted a positive or borderline response. ROC analysis was subsequently applied to identify the best cut-off for the significant parameters.

Results

Cortisol responses to physiological stimuli in patients with bilateral adrenal incidentalomas

The cortisol responses to posture and meal tests for each individual patient are shown in Table 1. ACTH levels remained suppressed (<5 pg/ml) when the tests were performed under dexamethasone suppression (data not shown).

Posture test (n = 26)

Eighteen patients were initially submitted to a posture test without prior dexamethasone suppression; four patients demonstrated a significant (>50%) rise of their cortisol levels, four patients had a partial response and 10 patients did not respond. In the eight patients who had positive or partial responses the test was repeated after the LDDST: in two patients the test was negative; a positive response was confirmed in one patient, who initially had a partial response; four patients had a partial response; in one patient a 50% rise in cortisol levels was noted but this response was taken as equivocal (cortisol increased from 1.4 to 2.1 μg/dl). Amongst the eight additional patients who underwent a posture test only after LDDST, two patients showed positive, and two partial responses. Thus, a total of three patients (11.5%) and six (23.1%) patients were proved to have positive and partial responses, respectively.

Meal test (n = 29)

Twenty-one patients were submitted to a meal test without prior dexamethasone suppression. A positive response was noted in three patients and a partial response in one additional subject. When the test was repeated after the LDDST they all tested negative. Amongst the eight additional patients who underwent a meal test only during LDDST one had a positive, and one an equivocal response (cortisol increased from 0.9 to 1.3 μg/dl). Thus, overall only one patient (3.4%) was proved to have a positive response. Of note this subject also had a partial response to the posture test.

LHRH test (n = 8)

One of the three patients tested positive at initial screening (54% increase in cortisol levels) and this response was confirmed when the test was repeated under dexamethasone suppression (360% increase in cortisol levels and, subsequently, a positive HCG and synthetic LH response was also demonstrated). None of the five additional patients who were tested under dexamethasone suppression showed significant responses.

Comparison of responses in patients with AIMAH versus patients with discrete bilateral adenomas

Amongst the five patients with bilateral macronodular hyperplasia four showed a significant aberrant response; three responded to posture test (one positive and two partial responses) and one additional patient responded to LHRH test. Likewise 6 of the 28 patients with discrete bilateral adenomas displayed significant responses. Thus, a greater prevalence of responses was noted in patients with bilateral macronodular hyperplasia compared to those with discrete bilateral adenomas (80 vs. 21.4%, $P = 0.02$).

Endocrine profile of patients with abnormal responses

Hormonal characteristics of the patients are shown in Table 2. Amongst the 20 patients with SH that were submitted to posture, meal and/or LHRH tests 10 patients had a response (50%); five patients (25%) a positive, and five (25%) a partial response, whereas none of the patients without SH responded to any test ($P = 0.002$). As far as individual HPA axis abnormalities are concerned, patients with abnormal responses compared to those who tested negative had higher cortisol levels post-LDDST, midnight cortisol levels and a trend for lower ACTH levels. No difference in UFC and DHEA-S levels was noted. Patients with positive or partial responses had larger adrenal adenomas (Table 3). Logistic regression

Table 1 Responses to posture and meal tests for each individual patient

Patient	SH	Response to either test	Upright posture test				Mixed meal test			
			ΔF	Post-LDDST		Final response	ΔF	Post-LDDST		Final response
				Baseline/peak F	ΔF			Baseline/peak F	ΔF	
AIMAH										
1	YES	Pa	40	8.8/11.5	31	Pa	13			N
2	YES	N	35	4.6/5.3	15	N	52	5.7/6.8	19	N
3[a]	YES	N[b]	9			N	3			N
4	YES	Pa		9.0/12.0	33	Pa		10.0/9.0	−10	N
5[a]	YES	P		4.2/7.2	71	P	31	5.1/5.9	16	N
Discrete bilateral adenomas										
6	YES	Pa	107	6.4/9.1	42	Pa	11			N
7[a]	YES	Pa	82	7.3/9.2	26	Pa	6			N
8	YES	Pa	49	12.2/15.6	28	Pa				n/d
9	YES	P	26	5.6/9.0	60	P	5	9.8/11.3	15	N
10	YES	N	21			N	6			N
11	YES	N	−2			N	−12			N
12	YES	N	−3			N	95	6.0/7.0	17	N
13	YES	N				n/d	19			N
14	YES	N				n/d	0			N
15	YES	N				n/d	21			N
16	YES	N				n/d	9			N
17	YES	N				n/d	8			N
18	YES	P		3.1/5.8	87	P		4.0/4.8	20	N
19[a]	YES	P		3.5/4.6	28	Pa		4.5/7.8	73	P
20[a]	YES	N		2.6/3.0	15	N		2.8/3.4	19	N
21	NO	N	109	1.5/1.7	13	N	8			N
22	NO	N	12			N	14			N
23	NO	N	8			N				n/d
24	NO	N	6			N				n/d
25	NO	N	4			N	22			N
26	NO	N	−20			N				n/d
27	NO	N	−44			N				n/d
28[a]	NO	EQ	74	1.4/2.1	50	EQ	363	1.4/1.6	14	N
29	NO	N				n/d	−1			N
30[a]	NO	N		0.7/0.8	14	N		0.8/0.9	13	N
31	NO	N		1.3/1.4	8	N		1.4/1.7	21	N
32[a]	NO	EQ		1.1/1.3	18	N		0.9/1.3	44	EQ
33	NO	N				n/d		1.2/1.3	8	N

[a] Patients submitted to LHRH test, [b] Patient tested positive at LHRH test

AIMAH ACTH-independent macronodular adrenal hyperplasia, *SH* subclinical hypercortisolism, *F* cortisol, *P* positive response, *Pa* partial response, *N* negative response, *EQ* equivalent response, *LDDST* low-dose dexamethasone suppression test, *n/d* not done, *peak F* peak cortisol levels during test

multivariate backward analysis showed that amongst the aforementioned parameters (combined size, midnight cortisol, ACTH, cortisol post-LDDST and UFC levels) those that best predict a positive or partial response were the combined size (odds ratio: 5.3, 95% CI 1.5–18.3, $P = 0.008$) and the cortisol levels post-LDDST (odds ratio: 1.5, 95% CI 1.0–2.1, $P = 0.033$). A cortisol post-LDDST level of more than 5 μg/dl had an odds ratio of 25.5 (95% CI 2.7–245.8, $P = 0.005$), whereas a combined size of more than 6.6 cm had an odds ratio of 40.5 (95% CI 4.0–417.4, $P = 0.002$) for a positive or partial response.

Table 2 Hormonal data in each patient

Patient	ACTH	*F* midnight	UFC	*F* post-LDDST
AIMAH				
1	23.0	10.3	199	11.3
2	20.8	3.6	135	2.5
3	8.0	4.8	124	12.6
4	3.0	14.0	128	10.0
5	18.0	16.7	116	6.0
Discrete bilateral adenomas				
6	5.0	8.2	90	5.9
7	1.0	6.0	102	10.0
8	1.0	15.5	165	12.5
9	10.0	4.6	118	5.5
10	7.0	5.0	79	5.9
11	7.0	7.0	42	9.0
12	8.0	7.0	105	5.5
13	3.0	6.5	78	7.3
14	3.0	6.3	54	3.2
15	7.0	4.9	81	5.6
16	22.0	7.8	196	17.4
17	21.0	6.5	243	2.7
18	4.6	11.3	229	3.8
19	4.0	5.2	118	7.3
20	15	7.5	282	2.7
21	27.0	4.0	99	1.8
22	12.0	3.0	17	1.8
23	29.0	4.2	35	1.6
24	15.0	6.1	233	1.3
25	1.0	4.6	130	2.2
26	20.0	3.4	117	0.3
27	24.0	2.3	32	1.3
28	18.0	3.7	108	1.4
29	5.0	4.2	128	2.3
30	11.5	2.0	91	0.5
31	6.0	5.3	186	1.4
32	6.48	2.7	67	0.9
33	25.0	2.7	136	1.3

AIMAH ACTH-independent macronodular adrenal hyperplasia, *F* cortisol, *LDDST* low-dose dexamethasone suppression test, *UFC* 24 h urinary free cortisol

Discussion

The present study aimed to identify the presence of abnormal cortisol responses to physiological stimuli in a cohort of patients with incidentally discovered bilateral discrete solitary adrenal adenomas. So far, detection of aberrant membrane receptors has not been systemically investigated in adrenocortical lesions of this type. The main finding of our study is that a substantial proportion of patients with incidentally discovered bilateral solitary adrenal adenomas exhibit aberrant responses to physiological stimuli, mainly to upright posture test. Interestingly, these aberrant responses were exclusively observed in patients with biochemical evidence of subtle glucocorticoid excess, while in patients who did not meet the diagnostic criteria for SH no aberrant responses were observed.

In our cohort, positive or partial responses to physiological stimuli, indicating the presence of illicit receptors, were seen in 24.1% of the patients with bilateral solitary adenomas. When patients with bilateral adenomas and SH are considered, the percentage rises to 40.0%. In the five patients with AIMAH, we found evidence of illicit receptors in most of them (80%). These results are comparable with data obtained from patients with AIMAH or unilateral incidentalomas reported in previous studies. In 22 patients with incidentally discovered AIMAH and SH, Libe et al.[12] recently reported positive responses to the posture test in 14 patients (66.7%) while none of the patients responded to the meal test. In the study by Bourdeau et al. [10], however, only one out of the four AIMAH patients responded to the posture test and none to the meal test. Reznik et al. [8] investigated 21 patients with unilateral incidentalomas with scintigraphic evidence of SH and reported positive or partial responses to meal and posture tests in eight patients (38.1%). Interestingly, the prevalence of aberrant responses in this group of patients with unilateral adenomas and SH was almost identical to the prevalence of aberrant responses found in our cohort of patients with bilateral lesions.

For patients without overt Cushing's syndrome, in whom ACTH levels are not fully suppressed, it has been proposed that the various tests applied to elicit the presence of aberrant cortisol responses, should be conducted under dexamethasone suppression [8, 11]. This is due to the fact that adrenal nodules retain ACTH responsiveness and, in the setting of mild cortisol excess and incomplete ACTH suppression, it cannot be safely ascertained that an aberrant response is not the result of spontaneously fluctuating ACTH levels. Our study strongly supports this recommendation. In fact, seven tests would have been misclassified as positive or partial if they had not been repeated after dexamethasone suppression. A rational alternative is to monitor variations in ACTH levels throughout the test. However, this is not a well-validated procedure. In fact, due to the infrequent ACTH sampling, fluctuations of ACTH levels could not be confidently detected. Likewise, we observed no significant variation of ACTH levels in one patient with a positive response to a meal test who subsequently tested negative when the test was repeated under dexamethasone suppression.

Our analysis showed that size is an important factor predicting a positive or partial aberrant response. Interestingly, only subjects with SH demonstrated aberrant

Table 3 Demographic and endocrine evaluation in patients with negative compared to patients with partial or positive responses

	Patients with negative responses	Patients with partial or positive responses	*P*
Age (years)	60.2 ± 11.5	58.6 ± 10.8	NS
BMI	31.1 ± 6.3	30.0 ± 4.6	NS
Max size (cm)	3.4 ± 0.8	4.4 ± 0.8	0.005
Combined size (cm)	5.4 ± 1.1	7.4 ± 1.1	<0.001
Baseline cortisol (μg/dl)	15.7 ± 5.5	17.3 ± 8.4	NS
ACTH (pg/ml)	13.6 ± 7.8	8.6 ± 7.4	0.055
Midnight cortisol (μg/dl)	4.8 ± 1.8	9.7 ± 4.6	0.003
UFC (μg/24-h)	116.3 ± 70.7	138.9 ± 44.6	NS
DHEA-S (μg/dl)	53.5 ± 40.0	44.6 ± 33.8	NS
Cortisol post-LDDST (μg/dl)	3.5 ± 3.8	8.5 ± 3.2	<0.001
AIMAH/bilateral adenomas	1/22	4/6	0.021
SH−/SH+	13/10	0/10	0.002

Max size the largest diameter of the largest adenoma, *combined size* the sum of the largest diameters of the largest adenoma on each side, *SH* subclinical hypercortisolism, *F* cortisol, *LDDST* low-dose dexamethasone suppression test, *BMI* body mass index, *AIMAH* ACTH-independent macronodular adrenal hyperplasia, *UFC* 24 h urinary free cortisol, *combined size* the sum of the largest diameters of the adenomas

responses to the applied physiological stimuli. None of the patients without subtle glucocorticoid excess showed an aberrant response to upright posture or meal. One might argue that since aberrant receptors are linked to steroidogenesis, an enhanced secretory activity would be expected only in these patients. However, previous studies in AIMAH patients have shown that the coupling of these receptors to tissue growth and steroidogenesis might not be always concordant [10]. For example, whereas most of the cases of AIMAH present with sizable adrenals they are not all associated with clinically evident cortisol hypersecretion and Cushing's syndrome. Moreover, in the few studies where pharmacological inhibition of the activated receptor was attempted, it ameliorated hypercortisolism with no regression of the adrenal tissue growth whatsoever [21, 22]. Taken together these data do not provide a satisfactory explanation of the predominant appearance of aberrant responses in the cortisol secreting and not the non-secreting patients with bilateral adrenal incidentalomas.

A limitation of our study is the fact that we did not test for the presence of all the reported aberrant GPCRs. However, by using the posture, meal and LHRH tests we screened for the presence of a large number of receptors including catecholamine, vasopressin angiotensin II, GIP and LH receptors. Our protocol did not include testing for the presence of serotonin receptors that, along with vasopressin receptors, are probably the more prevalent functional receptors in the reported AIMAH cases [11]. It should be noted, however, that the clinical relevance of responses to these pharmacological stimuli, in doses not necessarily reflecting the peak attainable physiological levels, remains questionable. These receptors certainly have a pharmacological interest but their endogenous ligands may never reach the concentrations required to induce hypercortisolism [6]. This is not the case for the applied physiological stimuli, where the observed cortisol responses are of undisputed clinical relevance. Moreover, the practical benefit for the patient by documenting an aberrant response is the opportunity to medically control hypercortisolism. So far, the only available medications block the GIP, β-adrenergic and LH receptors and, all these receptors have been investigated in most of the patients of our study by the applied protocol. Another limitation is the lack of a control group or a control study with measurement of cortisol every 30 min over the course of 2 h to exclude random fluctuations. Due to the demanding nature of the protocol such a control collection was not performed. However, this is also the case for all the reported studies dealing with the detection of aberrant responses [5, 11, 12]. To our experience, following dexamethasone suppression, cortisol levels do not fluctuate and, this is clearly seen in those patients that are non-responders to the upright or meal tests.

In conclusion, our data strongly support that screening for the presence of aberrant receptors in patients without overt Cushing's syndrome should be performed under dexamethasone suppression to avoid false positive results. Our findings indicate that aberrant expression of receptors triggered by physiological stimuli, primarily by upright posture, may be implicated in a significant percentage of patients with bilateral adrenal incidentalomas associated with autonomous cortisol production and especially those with fairly large lesions, offering the possibility of future pharmacological manipulation in these patients.

Conflict of interest The authors have nothing to disclose.

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