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Adipokine levels and cardiovascular risk in patients with adrenal incidentaloma

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Abstract

Adrenal incidentalomas (AIs) have been associated with an increased incidence of several cardiovascular risk factors, similar to overt Cushing syndrome. Data about the involvement of the adipokines in the development of insulin resistance and atherosclerosis in AI are completely lacking. The aim of the present study was to evaluate plasma interleukin 6 (IL-6), adiponectin, resistin, tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein 1 (MCP-1) levels in patients with AI. Plasma IL-6, adiponectin, resistin, TNF-α, and MCP-1 levels were measured in 20 healthy subjects (6 males; 14 females; age, 58.5 ± 2.2 years; body mass index, 28.1 ± 0.9 kg/m^2) and in 20 patients (5 males; 15 females; age, 57.9 ± 2.0 years; body mass index, 28.0 ± 0.8 kg/m^2) with AI and typical computed tomographic features of cortical adenoma, who were not affected by diabetes mellitus, hypertension, or other relevant diseases. All patients underwent anthropometric measurements and determination of basal corticotropin, cortisol, and urinary free cortisol excretion. Overnight dexamethasone test and 250-μg corticotropin test were performed in all cases. A subclinical Cushing syndrome was found in 3 patients, whereas the others had apparently nonfunctioning masses. Plasma IL-6, adiponectin, resistin, TNF-α, and MCP-1 levels were higher in patients than in controls (64.4 ± 2.8 vs 5.5 ± 0.6 pg/mL, 13.7 ± 1.3 vs 3.6 ± 0.5 μg/mL, 12.5 ± 1.9 vs 5.1 ± 0.2 ng/mL, 27.0 ± 1.5 vs 22.2 ± 1.5 pg/mL, 172.5 ± 20.0 vs 104.4 ± 19.5 pg/mL, respectively; $P < .05$) and apparently not affected by the presence of visceral obesity. Plasma IL-6 levels were negatively correlated with urinary free cortisol ($r = -0.461$, $P < .05$), and TNF-α levels were positively correlated with cortisol after the administration of 1 mg dexamethasone ($r = 0.636$, $P < .01$). In conclusion, patients with AI may show increased levels of adipokines (apparently not related to the presence of diabetes, hypertension, or obesity), which may be affected by the presence of the adrenal adenoma. For some adipokines, a direct production from the adrenal gland may be hypothesized even if other studies are needed to better investigate the role of adipokines in states of altered cortisol secretion.

1. Introduction

The incidental discovery of an adrenal mass in patients without signs or symptoms of overt hypercortisolism is becoming more and more frequent, and the need for a correct management of these patients is evident. At present, this is a highly controversial matter. In addition to the natural history of incidentally discovered adrenal masses, it would be useful to look into their associated morbidity for planning the optimal follow-up of these patients. The presence of an adrenal incidentaloma (AI) has been associated with an increased incidence of several cardiovascular risk factors. In fact, patients with AI can show a high prevalence of obesity, hypertension, diabetes mellitus, glucose intolerance, and dyslipidemia [1-7]. These abnormalities, typical of overt Cushing syndrome (CS), are more frequent in patients with subclinical Cushing syndrome (SCS); nevertheless, they can be also found in patients with apparently nonfunctioning adrenal masses [1]. This is because patients with AI, even in the absence by definition of signs or symptoms of hypercortisolism, can show a wide and almost continuous spectrum of cortisol hypersecretion, ranging from normality of the hypothalamic-pituitary-adrenal (HPA) axis to the slight cortisol excess conditioning

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SCS. In addition, metabolic and/or clinical abnormalities may be isolated or multiple, to define the metabolic syndrome, possibly reflecting their different degrees of cortisol excess [1].

In the last decades, the endocrine role of adipose tissue has increasingly emerged [8,9]. Indeed, adipose tissue is known to produce several molecules with autocrine, paracrine, and endocrine effects, including also interleukin 6 (IL-6), leptin, adiponectin, resistin, tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein 1 (MCP-1). Abnormalities in adipokine levels have been implicated in the appearance of insulin resistance, atherosclerosis, and left ventricular remodeling and may be a precocious and sensitive index of an increased cardiovascular risk [7,8]; so, we hypothesized that the increased cardiovascular risk described in patients with AI could be at least in part mediated by alterations of these molecules, possibly induced by an even slight glucocorticoid excess.

The relationships between glucocorticoids and adipokines are not fully understood. There are only few studies about adipokine levels in CS [10-17], which are sometimes discordant from in vitro studies [18], and data about adipokine levels in patients with AI are completely lacking.

Therefore, the aim of this study was to evaluate circulating levels of IL-6, adiponectin, resistin, TNF-α, and MCP-1 in 20 patients with incidentally discovered adrenal masses compared with those of 20 healthy subjects similar in age, sex, and body mass index (BMI).

2. Subjects and methods

2.1. Subjects

Twenty consecutive patients (5 males; 15 females; age, 57.9 ± 2.0 years; BMI, 28.0 ± 0.8 kg/m^2; Table 1) with adrenal masses incidentally discovered by computed tomographic or nuclear magnetic resonance scans performed for the evaluation of unrelated diseases were examined. All masses had radiologic characteristics of cortical adenoma. Exclusion criteria were the presence of known extra-adrenal neoplasms and of pheochromocytoma or aldosteronoma (excluded by the determination of 24-hour urinary catecholamines assay and plasma renin activity–aldosterone ratio, respectively).

Table 1
Some characteristics of the 20 patients and controls

	Patients (n = 20)	Controls (n = 20)
Male/female	5/15	6/14
Age (y)	57.9 ± 2.0 (40-71)	58.5 ± 2.2 (39-72)
BMI (kg/m^2)	28.0 ± 0.8 (24.0-34.5)	28.1 ± 0.9 (23.2-36.7)
Fasting glucose (mg/dL)	87.9 ± 2.8 (69-111)	95.3 ± 2.3 (83-113)
Fasting insulin (μU/mL)	8.0 ± 1.2 (2-23.5)	8.3 ± 1.5 (2-23.0)
HOMA index	1.8 ± 0.3 (0.4-6.4)	1.9 ± 0.3 (0.4-4.8)

Data are expressed as mean ± SE (range).

Twenty healthy subjects comparable for sex, age, and BMI (6 males; 14 females; age, 58.5 ± 2.2 years; BMI, 28.1 ± 0.9 kg/m^2; Table 1) were enrolled as controls.

All patients and controls were not affected by arterial hypertension, diabetes mellitus, cardiovascular diseases, chronic inflammatory diseases, chronic hepatitis, or known malignancies; subjects with acute inflammatory states were also excluded from the study.

Informed consent was obtained from all participants, and the study was approved by the local ethical committee.

2.2. Study protocol

All patients with AI underwent physical examination, including anthropometric measurements. In particular, a waist circumference of more than 88 cm in women and more than 102 cm in men was considered the cutoff for visceral obesity in accordance to the Adult Treatment Panel III criteria [19]. Fat mass and fat-free mass were determined by means of bioelectric impedance (BIA 101-S, Akern, Florence, Italy). Systolic and diastolic blood pressures were measured in all patients.

Blood samples from patients and controls were collected after an overnight fast to determine basal plasma levels of IL-6, adiponectin, resistin, TNF-α, and MCP-1; plasma glucose and insulin were also measured. The insulin resistance index HOMA (homeostasis model assessment) was assessed: a HOMA index of greater than 2.5 was considered abnormal [20].

In all patients, serum cortisol, plasma corticotropin (ACTH), and urinary free cortisol (UFC) excretion were determined in basal conditions. All patients underwent an overnight 1-mg dexamethasone test. The suppression was adequate when morning cortisol fell below 1.8 μg/dL [21]. If inadequate, patients underwent other tests, particularly the midnight cortisol measurement and the classic 2-day 2-mg dexamethasone suppression test involving the administration of 0.5 mg oral dexamethasone given every 6 hours for 48 hours. We considered as normal a serum cortisol falling below 1.8 μg/dL in the morning, 6 hours after the last dose of dexamethasone [22]. Moreover, all patients underwent a 250-μg ACTH test, and a 17-OHP peak higher than 5 ng/mL was considered abnormal [23].

Patients qualified for SCS when 2 or more basal or dynamic tests of the HPA axis function were abnormal [23]. In particular, the following parameters were considered for the diagnosis of SCS: plasma ACTH values lower than 5 pg/mL, UFC excretion higher than 110 μg/24 h, serum midnight cortisol higher than 7.5 μg/dL [24], and abnormal cortisol suppression after the 1-mg or the 2-day 2-mg dexamethasone administration.

2.3. Assays

Plasma IL-6, adiponectin, TNF-α, and MCP-1 assays were performed using an enzyme-linked immunosorbent assay method (Quantikine kit, R&D Systems, Minneapolis, MN), with analytical sensitivity of 0.7 pg/mL, 1.6 pg/mL,

Table 2
Some hormonal and metabolic parameters of the 20 patients with AI

	Mean ± SE	Range
Morning cortisol (μg/dL)	10.3 ± 0.9	3.6-18
Midnight cortisol (μg/dL)	5.9 ± 2.2	2.2-14.2
Cortisol after 1 mg dexamethasone (μg/dL)	2.8 ± 0.6	0.5-11.7
ACTH (pg/mL)	11.7 ± 1.1	5.9-21
UFC (μg/24 h)	29.8 ± 4.3	10.0-64.0

5.0 pg/mL, and 0.25 μg/mL, respectively. Resistin assay was performed by an enzyme-linked immunosorbent assay method (Phoenix Pharmaceuticals, Belmont, MA), with analytical sensitivity of 0.26 ng/mL. Assays of the adipocytokines were performed using an EL-800 microplate reader (BioTek Instruments, Los Angeles, CA), and data were analyzed by a KJ software (BioTek Instruments). Intra- and interassay coefficients of variation used for the analysis of plasma IL-6, adiponectin, resistin, TNF-α, and MCP-1 levels were 1.6% to 4.2% and 3.3% to 6.4%, 2.5% to 4.7% and 5.8% to 6.8%, less than 5% and less than 14%, 4.2% to 5.2% and 4.6% to 7.4%, and 4.7% to 7.8% and 4.6% to 6.7%, respectively.

Plasma ACTH, serum cortisol, and UFC were measured by chemiluminescence (Immulite 2000, Diagnostic Products, Los Angeles, CA): the analytical sensitivity was 5 pg/mL for plasma ACTH and 0.2 μg/dL for serum cortisol and UFC. Other biochemical analytes were measured using standard methods.

2.4. Statistical analysis

Data are expressed as mean ± SE. Normal distribution of continuous variables was tested with the Kolmogorov-Smirnov test. The 2-tailed Student *t* test was used for the normally distributed variables, whereas the 2-tailed Mann-Whitney test was used for the others. Correlation analysis was determined by calculating for the Spearman *r* coefficient. Levels of statistical significance were set at $P < .05$. Statistical analysis was performed using the SigmaStat for Windows 3.0 software (SPSS, Chicago, IL).

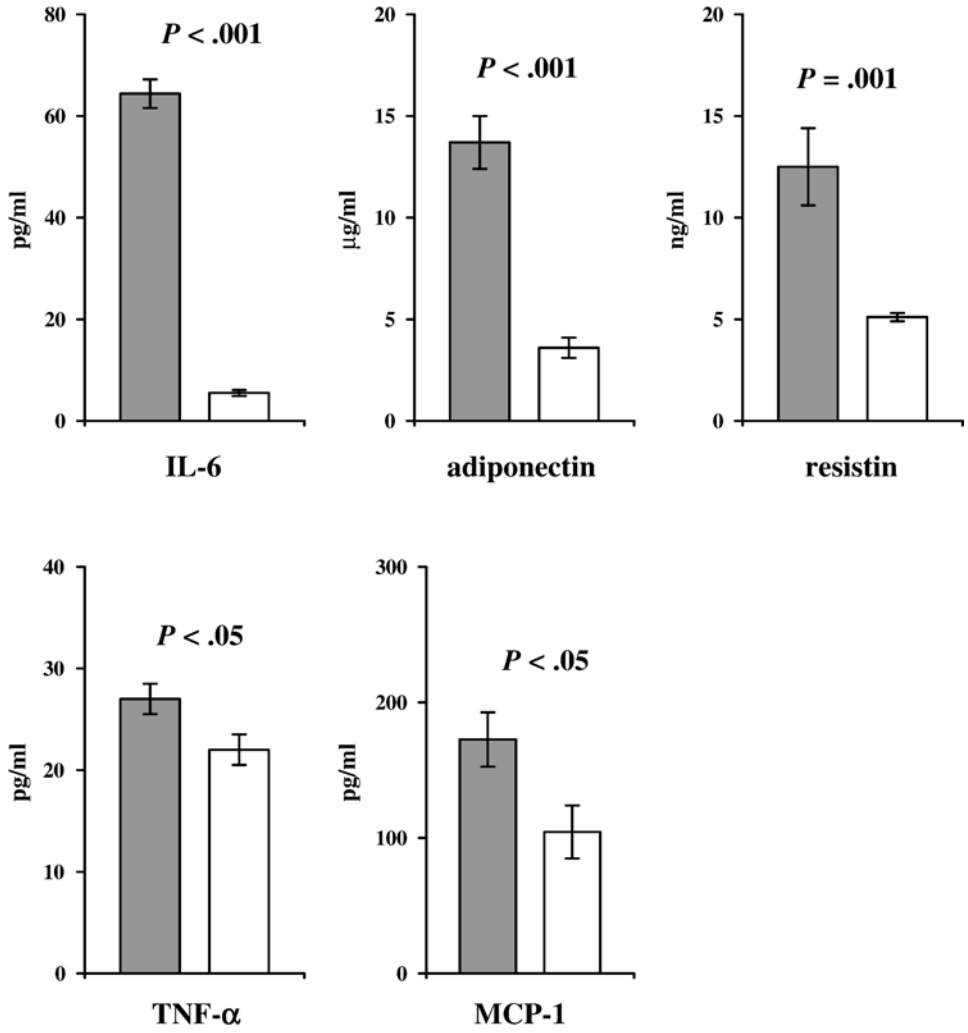

Fig. 1. Mean adipokine levels (±SE) in 20 patients with AI (gray columns) and in controls (white columns).

3. Results

3.1. Hormonal and metabolic evaluation

Based on hormonal evaluation, 3 patients met the criteria of SCS; 2 patients showed only an inadequate cortisol suppression after both the 1-mg and the 2-day 2-mg dexamethasone tests, whereas the other one had also an elevated midnight serum cortisol value. However, even among the 17 patients with an apparently nonfunctioning mass, some isolated alterations of the HPA axis were observed. In fact, 6 patients did not adequately suppress their cortisol levels after 1 mg dexamethasone but only after the 2-day 2-mg dexamethasone administration. Eleven patients showed an increased 17-OHP response after ACTH stimulation (Table 2).

According to waist measurement, 5 patients had visceral obesity, and body fat mass measured by means of bioelectric impedance was greater than the normal range in 14 patients.

Fasting glucose, insulin, and HOMA index did not differ in patients and controls (Table 1). According to HOMA index, 5 patients and 6 controls had insulin resistance.

3.2. Adipokine levels

Patients with AI showed significantly higher circulating levels of IL-6 (64.4 ± 2.8 vs 5.5 ± 0.6 pg/mL, $P < .001$), adiponectin (13.7 ± 1.3 vs 3.6 ± 0.5 μg/mL, $P < .001$), and resistin (12.5 ± 1.9 vs 5.1 ± 0.2 ng/mL, $P = .001$) than healthy subjects (Fig. 1). In particular, all patients had IL-6 levels definitely higher than in controls (Fig. 2). Six patients had particularly high resistin values (Fig. 2); 2 of them had SCS, whereas 3 others showed an inadequate cortisol suppression after 1 mg dexamethasone.

Mean TNF-α and MCP-1 levels were also higher in patients with AI (27.0 ± 1.5 vs 22.2 ± 1.5 pg/mL, $P < .05$; 172.5 ± 20.0 vs 104.4 ± 19.5 pg/mL, $P < .05$; Fig. 1) but with a wide variability in both patients and controls (Fig. 2).

The existence of possible correlations among cytokine levels and the anthropometric, metabolic, and hormonal parameters was investigated: plasma IL-6 levels were negatively correlated with UFC ($r = -0.461$, $P < .05$), whereas TNF-α levels were positively correlated with cortisol after 1 mg dexamethasone ($r = 0.636$, $P < .01$). In our patients, adipokine levels did not correlate with

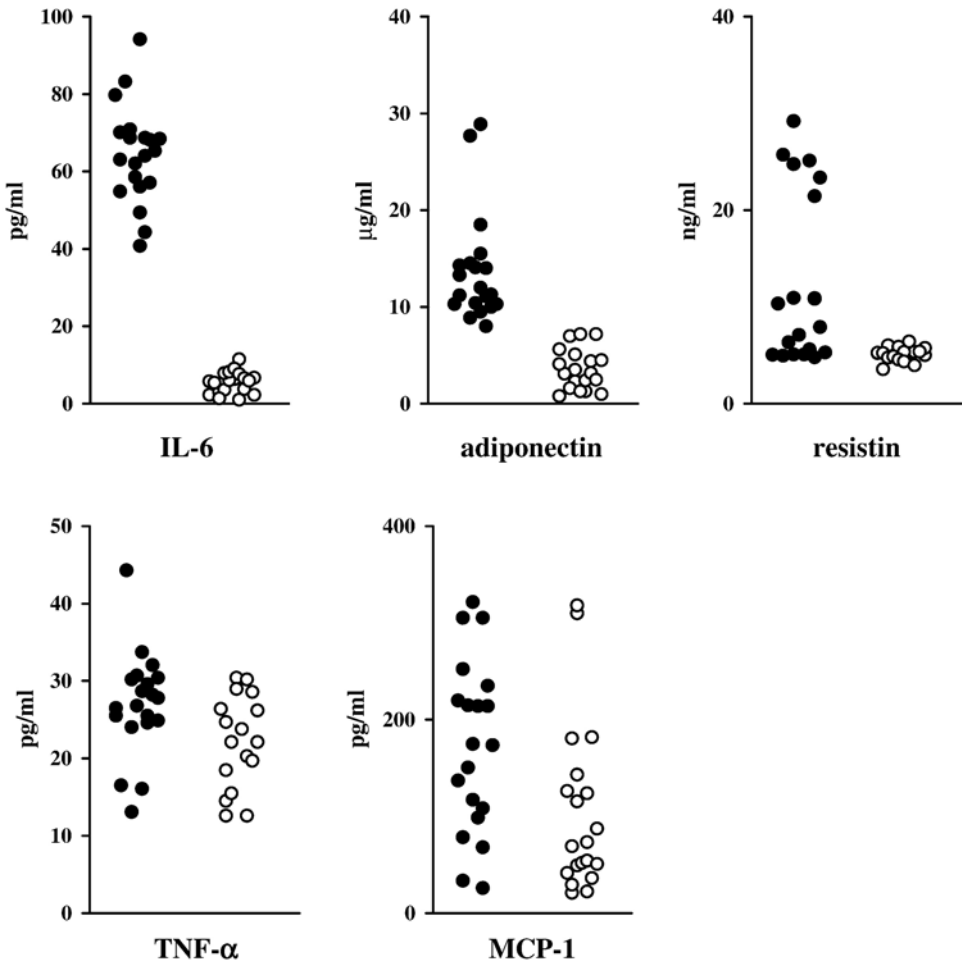

Fig. 2. Adipokine values in 20 patients with AI (●) and in 20 controls (○).

anthropometric, metabolic, or other hormonal parameters; a significant correlation between adenoma size and adipokine levels was neither found. In particular, adipokine levels in the 5 patients with visceral obesity were not different from those in the remaining patients (data not shown).

Patients were divided according to the results of the hormonal evaluation of their adrenal function. A normal HPA axis was shown by 11 patients, whereas 1 or more abnormalities of cortisol secretion were found in 9 patients. Interleukin 6, adiponectin, resistin, TNF-α, or MCP-1 levels did not differ between patients with normal HPA axis and patients with cortisol secretion alterations (data not shown), although TNF-α levels were slightly increased in patients with 1 or more cortisol secretion abnormalities (29.8 ± 2.4 vs 24.6 ± 1.6 pg/mL, $P = .048$).

Similarly, as far as adipokine levels are concerned, the 11 patients with an abnormal 17-OHP rise after ACTH did not differ from those with a normal response (data not shown).

4. Discussion

In this study, we firstly showed that in patients with AI an elevation of adipokines involved in the development of atherosclerosis and cardiovascular disease may exist, and it is apparently independent from the presence of obesity or insulin resistance. In fact, because adipokine levels are strongly influenced by the coexistence of conditions such as obesity, diabetes mellitus, and insulin resistance [8,9], subjects with overt diabetes were excluded from our study, and patients with AI were compared with healthy subjects accurately matched for age and BMI. The presence of visceral obesity did not affect the finding of high adipokine levels in our patients. Moreover, patients and controls were very similar as far as insulin sensitivity, assessed by HOMA index, was concerned.

Chronic glucocorticoid excess has been suggested to induce metabolic alterations that may be correlated with the degree of hypercortisolism [1,25]. Terzolo et al [26] recently reported that an elevated midnight cortisol concentration is a reliable marker of cardiovascular risk in patients with clinically inapparent adrenal adenomas. Although the criteria for the diagnosis of SCS were met only by 3 of 20 patients with AI, other patients showed some alterations of the HPA axis function, confirming the high frequency of these abnormalities and the wide spectrum of hypercortisolism in these patients [3-5,23].

The present data show that patients with AI have a significant elevation of IL-6 not correlated with cortisol levels, although a negative correlation with UFC excretion was found. Because IL-6 is produced also in the adrenal gland (where it has been implicated in the steroidogenesis) and a large expression of IL-6 mRNA has been demonstrated in adrenal adenomas of patients with CS [27,28], an autonomous IL-6 production by adrenal adenomas may be suggested. Nevertheless, a correlation between mass size and IL-6 was not found, and IL-6 levels were not different in patients with an increased 17-OHP response to ACTH. As far as IL-6 concentration in patients with CS is concerned, either elevated [13] or normal levels [12] have been reported. In particular, Kushlinskii et al [13] observed a higher blood content of IL-6 in patients with adrenal tumors compared with healthy subjects, negatively correlated with serum cortisol. This last finding is in line with our results and seems to suggest an inhibitory effect of cortisol on IL-6 secretion, consistent with the anti-inflammatory effects of glucocorticoids. In fact, in vitro studies reported an IL-6 inhibition by glucocorticoids [18], confirmed by the elevation of IL-6 described in patients with severe hypocortisolism [10].

Similar to what was observed for IL-6, an autonomous production of TNF-α by the adrenal tumor may be suggested. In fact, TNF-α seems to be produced at the adrenal level [27]. A high mean TNF-α concentration was observed in our patients, and TNF-α levels positively correlated with cortisol values after 1 mg dexamethasone. However, glucocorticoid inhibition of TNF-α has been described in vitro [18], and in vivo results are conflicting. In patients with CS, normal TNF-α levels have been found [11,12], whereas in patients with adrenal insufficiency, increased TNF-α levels have been reported [10].

The role of MCP-1 in the pathogenesis of several cardiovascular disorders, such as chronic heart failure, atherosclerosis, and ventricular dysfunction, has been recently emphasized [29,30]. To our knowledge, no data about MCP-1 levels in adrenal disorders are available, although an in vitro study showed MCP-1 inhibition by synthetic glucocorticoids [31]. A reciprocal regulation between cytokines exists. In fact, because MCP-1 seems to be stimulated in vitro by IL-6, TNF-α, and resistin [32,33], MCP-1 levels in our patients could be affected also by the elevation of the other cytokines.

It is known that adiponectin has positive effects on insulin sensitivity and anti-inflammatory and antiatherogenic properties, with low levels being considered as a risk factor for several insulin-resistant conditions [34]. Data about the relationship between glucocorticoids and adiponectin are rather conflicting. In fact, whereas in vitro studies suggest an inhibition of adiponectin by glucocorticoids [18], either low or normal adiponectin levels have been found in patients with CS [14-17] and, after administration of glucocorticoids, in healthy subjects [14,15]. On the other hand, in healthy subjects, a positive association between serum cortisol and adiponectin has been reported [35,36]. Gavrila et al [36] showed that circulating adiponectin has diurnal variations following those of cortisol by a few hours, hypothesizing a compensatory mechanism that would tend to keep the degree of insulin resistance stable. No difference in adiponectin levels between patients with or without some abnormality of the HPA axis was found. No evidence of a production of adiponectin at the adrenal level exists at the moment.

Another interesting finding in our study is concerned with resistin concentration observed in patients with AI and particularly with the very high levels found in 6 patients who also showed some alteration in cortisol secretion. These data may be in line with the stimulating effect of glucocorticoid excess on resistin secretion, as previously demonstrated by in vitro and in vivo studies [16,18]. Accordingly, Krsek et al [16] found elevated resistin levels in 8 women with pituitary-dependent CS and in 2 with adrenal adenoma. Notably, a resistin production also at the adrenal level has been reported in rat [37]. Nevertheless, the role of resistin in the pathogenesis of insulin resistance is still unclear. In spite of its involvement in the occurrence of insulin resistance in animal models, its role in human pathophysiology still needs to be clarified [8,9].

In conclusion, an increased concentration of adipokines involved in the development of insulin resistance and atherosclerosis may be demonstrated in patients with AI, independently from obesity, visceral adiposity, and insulin resistance. The finding of an increased resistin production, particularly in patients with an even mildly altered cortisol secretion, suggests a possible role of resistin in affecting insulin sensitivity in these patients. Prospective studies are needed to investigate the role of resistin and of the other cytokines in states of altered cortisol secretion.

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