CLINICAL STUDY

Progressively increased patterns of subclinical cortisol hypersecretion in adrenal incidentalomas differently predict major metabolic and cardiovascular outcomes: a large cross-sectional study

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Abstract

Background: Subclinical Cushing's syndrome (SCS) is defined as alterations in hypothalamic–pituitary–adrenal axis without classic signs/symptoms of glucocorticoid excess. Whether SCS leads to metabolic and cardiovascular diseases is still controversial.

Aim: To evaluate the prevalence of hypertension, type 2 diabetes (T2D), coronary heart disease (CHD), ischemic stroke, osteoporosis, and fractures, and their relationship to increasing patterns of subclinical hypercortisolism, in patients with nonsecreting adrenal adenomas (NSA) and SCS.

Methods: Using the 1 mg dexamethasone suppression test (DST), 348 patients were classified as follows: 203 were defined as NSA and 19 SCS, using the most stringent cutoff values (< 50 and > 138 nmol/l respectively). Patients with cortisol post-DST (50–138 nmol/l) were considered as intermediate phenotypes and classified as minor ($n = 71$) and major ($n = 55$) using plasma ACTH and/or urinary free cortisol as additional diagnostic tools.

Results: SCS patients showed higher prevalence of T2D, CHD, osteoporosis, and fractures with respect to NSA. Intermediate phenotypes also showed higher prevalence of CHD and T2D with respect to NSA. The prevalence of all clinical outcomes was not different between intermediate phenotype patients, which were therefore considered as a single group (IP) for multivariate logistic regression analysis: both IP and SCS-secreting patterns showed a significant association with CHD (odds ratio (OR), 4.09; 95% confidence interval (CI), 1.47–11.38 and OR, 6.10; 95% CI, 1.41–26.49 respectively), independently of other potential risk factors. SCS was also independently associated with osteoporosis (OR, 5.94; 95% CI, 1.79–19.68).

Conclusions: Patterns of increasing subclinical hypercortisolism in adrenal adenomas are associated with increased prevalence of adverse metabolic and cardiovascular outcomes, independently of other potential risk factors.

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Introduction

The clinical consequences of subclinical hypercortisolism in adrenal incidentalomas are still a matter of debate because even defining this condition is controversial due to lack of consensus on diagnostic criteria and the difficulty in defining a specific phenotype (1). Subclinical Cushing's syndrome (SCS) is defined as alterations of the hypothalamic–pituitary–adrenal (HPA) axis without classic signs or symptoms of glucocorticoid excess (2, 3). The widespread use of abdominal imaging techniques has increased the incidental finding of adrenal masses (4, 5) giving primary relevance to the diagnosis of SCS, which

is found in up to 30% of adrenal incidentalomas (6, 7). Previous studies have highlighted the association between subclinical hypercortisolism and increased risk of osteoporosis, vertebral fractures (8), and cardiovascular diseases (CVDs) (9, 10) due to hypertension and metabolic alterations such as dyslipidemia and type 2 diabetes mellitus (T2D) (5). However, to date, no studies have investigated the relationship between adverse clinical outcomes, such as CVDs and stroke, and different degrees of subclinical cortisol hypersecretion.

The aim of the study was to evaluate the prevalence of hypertension, T2D, coronary heart disease (CHD), ischemic stroke, osteoporosis, and osteoporotic

fractures, and the relationship of these outcomes to increasing patterns of subclinical cortisol hypersecretion, in patients with nonsecreting adrenal adenomas (NSA) and SCS.

Materials and methods

Study population

This study included 465 patients (282 females and 183 males, aged 18–87 years) referred to the Endocrinology Unit of the S. Orsola-Malpighi Hospital of Bologna, from 2000 to 2010, for an adrenal incidentaloma, as demonstrated by imaging studies (ultrasound, computed tomography (CT), magnetic resonance imaging (MRI)) performed for unrelated disorders. After referral, each patient underwent a CT scan, if not recently executed or if the adrenal mass was detected by ultrasound. All CT/MRI scans were reviewed by the same radiologists (E Giampalma and C Mosconi). Patients with the suspicion of adrenocortical carcinoma, metastases, myelolipoma, ganglioneuroma, cysts, pheochromocytoma, and infiltrative diseases were excluded, according to the radiological characteristics and/or diagnostic criteria described below. Patients with history of steroid intake within the last 3 months were not included in the study. Oral contraceptives and postmenopausal hormone replacement therapy were withdrawn for at least 3 months. Patients with clinical signs or symptoms specific to overt Cushing's syndrome (e.g. myopathy, plethora, purple striae, and easy bruising) were excluded. Patients with primary hyperaldosteronism were excluded on the basis of the more recent guidelines (11). Twenty-four hour urine for measurement of free metanephrines was carefully collected and conjugated with acid in all patients, who were instructed to abstain from catecholamine-containing products and acetaminophen for 8–10 days before collection. Patients with elevated urinary free metanephrines were also excluded (1).

Overall, 117 (25.2%) subjects were excluded and 348 patients (217 females and 131 males, aged 28–87 years) were enrolled in the study.

Investigational clinical protocol

At study entry, history of cardiac risk factor, including hypertension, T2D, dyslipidemia, and smoking status, was acquired. History of nonfatal acute myocardial infarction, percutaneous transluminal coronary angioplasty/surgical bypass for ischemic heart disease, and ischemic/hemorrhagic stroke was also investigated. All cardiovascular events were reviewed and classified by an experienced cardiologist on the basis of hospital discharge letters. Clinical examination and electrocardiography were performed in all patients. Blood pressure was measured by mercury sphygmomanometer

(WBIC, Wenzhou, China) in each subject in clinostatic position, and 1 and 5 min after assumption of the standing position, according to the European Society of Hypertension guidelines (12). Arterial hypertension was diagnosed with systolic blood pressure ≥ 140 mmHg and/or diastolic ≥ 90 mmHg, as measured on at least two separate occasions. Patients receiving antihypertensive medications were considered to have hypertension. Current smokers were defined as those currently smoking any type of tobacco, former smokers as those who had quit smoking at least 6 months before enrollment in the study, and non-smokers as those who never smoked (13). First-degree family history of T2D, hypertension, CVDs, osteoporosis, and fractures was also investigated. All the patients underwent the same investigational protocol (see below).

Anthropometry

Height, body weight, waist and hip circumferences, and the waist-to-hip ratio were measured according to standardized procedures (14). Body mass index (BMI) was calculated as weight in kilograms per height in square meters.

Hormonal and metabolic evaluation

On the first day, blood samples for hormonal (cortisol and ACTH) and metabolic (glucose, lipids, and HbA1c) routine evaluation were drawn between 0800 and 0900 h after a 12 h overnight fast. Dyslipidemia was defined as total cholesterol levels ≥ 200 mg/dl and/or triglycerides ≥ 150 mg/dl (15). The day before, written instructions for 24 h urine collection for measurement of urinary free cortisol (UFC) (16) were given to each patient. Urinary outputs were normalized to urinary excretion of creatinine. Calcium balance (parathormone, calcium, phosphate, and 25-hydroxyvitamin D₃) was also investigated on the first day to help in the diagnosis of osteopenia/osteoporosis (data not shown). An oral glucose tolerance test (75 g Curvoso; Sclavo, Cinisello Balsamo, Italy) was also performed in patients without known T2D by taking basal blood samples and after 30, 60, 90, and 120 min for glucose determination. The T2D was diagnosed according to the American Diabetes Association position statement (17). Patients who were taking antidiabetic drugs were considered as having T2D. On the same day, 1 mg dexamethasone (Decadron; Visufarma, Rome, Italy) was administered at 2300 h and blood samples were taken the following morning (0800–0900 h), after a 12 h overnight fast, for cortisol measurement. Blood samples for hormone assays were immediately chilled on ice and centrifuged; serum, plasma, and urine aliquots were collected and frozen at -20°C and -80°C until assayed. All blood/urine samples were analyzed in the Central Laboratory of the S. Orsola-Malpighi Hospital of Bologna, Italy.

Grouping criteria of adrenal adenomas according to cortisol secretion

According to National Institutes of Health (2) and American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons (18) guidelines, SCS was diagnosed using the 1 mg overnight dexamethasone suppression test (DST); different criteria were used to define normal cortisol suppression after DST and patterns of progressively increased subclinical cortisol hypersecretion. The most stringent cutoff values were preferred to reduce false positive results: cortisol post-DST >138 nmol/l was used to define SCS, according to the high specificity of this cutoff in defining subclinical hypercortisolism, whereas cortisol post-DST <50 nmol/l was used to define normal suppression. Notably, the cut-point of 50 nmol/l was used to define normal cortisol suppression and not SCS because although this cutoff value has been advocated to increase detection of SCS, it may lead to more false positive results (19, 20) due to its low specificity. Cortisol levels post-DST between 50 and 138 nmol/l were considered as intermediate cortisol suppression states; these patients were classified using at least another HPA axis alteration between high UFC and basal plasma ACTH <10 pg/ml. Although the use of UFC and/or ACTH in addition to the DST is an arbitrary choice, at least two alterations of the HPA axis for definition of subclinical hypercortisolism have been widely found and used in the previous literature (21, 22, 23); moreover, it seems reasonable to consider low morning ACTH and/or high UFC as indices of autonomous cortisol hypersecretion, in addition to the partial cortisol suppression after DST. Finally, patients were classified as follows (shown in Table 1): patients with post-DST cortisol levels <50 nmol/l were classified as having NSA, whereas those with cortisol post-DST >138 nmol/l as having SCS; patients with cortisol post-DST between 50 and 138 nmol/l were divided into two groups: those with at least one alteration between high UFC and plasma ACTH

<10 pg/ml were defined as intermediate major phenotype (IMP), and those remaining as intermediate minor phenotype (ImP).

No further DSTs (e.g. 2-day low dose or 8 mg) were used as diagnostic tools because up to now there is still a lack of a head-to-head comparison of different DSTs to establish a gold standard for diagnosis of SCS (1) in nondiabetic patients (whereas in diabetics, the 2-day low-dose DST has been suggested to be the most specific test) (24). Moreover, similar cortisol suppression has been shown in both 1 and 8 mg DSTs, thus the latter does not change the probability of SCS being defined by the former (25). Finally, as shown in a previous study (26), the standard 1 mg dexamethasone dose did not differ in cortisol suppression from higher doses of dexamethasone (e.g. 0.015 mg/kg body weight), indicating the possibility of achieving the maximum suppressibility of the HPA axis with the 1 mg DST.

Bone metabolism evaluation

Osteoporosis and osteoporotic fractures were recorded on the basis of clinical history, patient's medical records, and laboratory assessments (see above). Moreover, on the same days, Bone mass density (BMD) was measured by dual-energy X-ray absorptiometry (Lunar Corporation, Madison, WI, USA) in the antero-posterior view at femoral neck and lumbar spine. Osteoporosis and osteopenia were defined according to WHO criteria (27). T5–L5 morphometric X-ray absorptiometry was performed to assess vertebral fractures, which were diagnosed with a reduction >25% in anterior, middle, or posterior vertebral height (28). Nonvertebral fractures due to minor trauma (falling from a standing position or while walking) and Colles fractures were also considered.

Biochemical assays

Serum cortisol was determined by electrochemiluminescence immunometric assay (ECLIA; Elecsys E170; Roche); intra-assay coefficient of variation (CV) was <5%. Cortisol was not measured by isotopic dilution-liquid chromatography–mass spectrometry; however, the comparison of this method with ECLIA assay revealed a good agreement for the latter in cortisol determination, as shown in our previous study (29). UFC was measured after extraction (liquid/liquid with dichloromethane) by ECLIA (Modular Elecsys E170; Roche); intra-assay CV was <15%. ACTH was determined by chemiluminescent immunoassay (Immulite2000; Siemens Healthcare Diagnostics Inc, Tarrytown, NY, USA); intra-assay CV was <10%. Urinary metanephrines were measured by solid-phase extraction+HPLC with electrochemical detector. Glucose, lipids, and other hormones were measured as described previously (30).

Table 1 Diagnostic criteria used to classify the different patterns of cortisol secretion.

		Diagnostic criteria		
	<i>n</i>	Cortisol ^a (nmol/l)	Basal ACTH (pg/ml)	UFC/24 h (μg/day)
Secreting pattern				
NSA	203	<50	–	–
ImP ^b	71	50–138	≥10	≤137
IMP ^b	55	50–138	<10	>137
SCS	19	>138	–	–

^aAfter 1 mg overnight DST.

^bIntermediate phenotypes were defined with cortisol levels after DST between 50 and 138 nmol/l as primary criterion; IMP was defined with at least one alteration between UFC >137 μg/day and plasma ACTH <10 pg/ml; the remaining patients were defined as ImP.

Statistical analysis

Statistical analysis was performed by SPSS version 12.0 statistical package (SPSS, Inc., Chicago, IL, USA). Comparison of general characteristics of the studied population among different groups was performed using one-way ANOVA (continuous variables) and the χ^2 tests (categorical variables). Clinical outcomes were analyzed by means of the logistic regression analysis. The four groups were compared by means of the univariate analysis, and the simple contrasts were computed to assess pairwise differences between NSA, ImP, IMP, and SCS. Multivariate logistic regression analysis was used to evaluate the influence of different degrees of cortisol secretion rate (intermediate phenotype and SCS groups respectively) on the prevalence of clinical outcomes, using NSA as the reference group; this analysis was performed by adjusting for age, BMI, and gender for all variables; family history of diabetes was also added in the analysis of T2D, whereas smoking status, hypertension, T2D, and dyslipidemia were considered in the analysis of CHD; smoking status was also added in the analyses of osteoporosis and related fractures. The results are expressed as mean $\pm$ s.d. or frequencies. Two-tailed *P* values <0.05 were considered significant.

Results

According to the diagnostic criteria described above, among 348 patients, 203 (58.3%) were defined as NSA (127 females and 76 males) and 19 (5.5%) were diagnosed as SCS (11 females and eight males). Of the remaining 126 patients (36.2%), 71 (20.4%) were classified as ImP (35 females and 36 males) and 55 (15.8%) as IMP (44 females and 11 males). Mean values of primary and secondary diagnostic tests in each group are shown in Table 2.

Anthropometric characteristics, smoking status, and medical treatments are reported in Table 3. BMI and waist

circumference were comparable, whereas age ($P=0.001$) and female-to-male ratio ($P=0.006$) were significantly different among groups. The prevalence of menopause was similar in female patients. Use of diuretics and calcium-channel blockers was significantly increased in intermediate phenotypes and SCS with respect to NSA. No intergroup difference was detected in first-degree family history of T2D, hypertension, and CVDs (data not shown).

The prevalence of major clinical outcomes in NSA, ImP, IMP, and SCS patients is shown in Table 4. There was no significant difference in the prevalence of hypertension among groups. The prevalence of T2D was significantly higher in SCS (with respect to ImP and NSA) and in IMP patients (compared with NSA). The prevalence of CHD was significantly higher in ImP, IMP, and SCS groups with respect to NSA. IMP patients showed a higher prevalence of ischemic stroke compared with NSA patients. Only SCS patients showed a higher prevalence of osteoporosis (with respect to ImP, IMP, and NSA) and osteoporotic fractures (compared with NSA).

Factors influencing the prevalence of T2D, CHD, osteoporosis, and fractures in intermediate phenotype and SCS patients

Using the univariate logistic regression by applying simple contrast, no significant differences were shown in the prevalence of all clinical outcomes between ImP and IMP patients; therefore, they were considered as a single group (named IP) in the subsequent analyses. Table 5 shows the results of the multivariate logistic regression analyses evaluating the potential risk factors (including the cortisol-secreting pattern itself) for T2D, CHD, osteoporosis, and osteoporotic fractures, by comparing IP and SCS patients with the NSA group. The IP-secreting pattern was not associated with T2D, osteoporosis, and fractures but was significantly associated with CHD (odds ratio (OR), 4.09; 95% confidence interval (CI), 1.47–11.38; $P=0.007$).

Table 2 Values of the diagnostic criteria used to classify the different patterns of cortisol secretion. Criteria for diagnosis of NSA, ImP, IMP, and SCS are defined in Table 1.

Diagnostic criteria	Secreting pattern				<i>P</i> value ^a
	NSA	ImP	IMP	SCS	
1 mg DST					<0.001
Mean (s.d.)	32.0 (10.9)	76.3 ^b (23.6)	84.3 ^b (23.5)	182.2 ^{b,c,d} (37.3)	
Median (min–max)	33.1 (5.5–49.7)	66.2 (52.4–132.4)	80.0 (52.4–137.9)	176.6 (146.2–267.6)	
Basal ACTH					<0.001
Mean (s.d.)	13.9 (7.8)	16.6 ^e (7.7)	6.6 ^{b,c} (2.2)	10.6 ^{c,d,e} (4.8)	
Median (min–max)	12.0 (4.9–49.0)	14.0 (10.0–42.0)	6.0 (4.9–19.0)	11.0 (4.9–22.0)	
24-h UFC					0.567
Mean (s.d.)	56.3 (26.9)	55.0 (23.7)	62.1 (40.8)	59.9 (34.1)	
Median (min–max)	44.0 (36.0–137.0)	48.0 (36.0–133.0)	42.5 (36.0–179.0)	45.0 (36.0–148.0)	

^aOne-way ANOVA.

^bPairwise comparisons between groups (simple contrasts); $P<0.01$ reference category, NSA.

^cPairwise comparisons between groups (simple contrasts); $P<0.01$ reference category, ImP.

^dPairwise comparisons between groups (simple contrasts); $P<0.01$ reference category, IMP.

^ePairwise comparisons between groups (simple contrasts); $P<0.05$ reference category, NSA.

Table 3 General characteristics of patients with different patterns of cortisol secretion. Data are given as mean $\pm$ s.d. or as absolute numbers with percentage in parentheses. Criteria for diagnosis of NSA, ImP, IMP, and SCS are defined in Table 1.

	Secreting pattern				P value
	NSA (n=203)	ImP (n=71)	IMP (n=55)	SCS (n=19)	
General characteristics					
Females/males (n; %)	127/76 (62/38)	35/36 (49/51)	44/11 (80/20)	11/8 (58/42)	0.006 ^a
Age (years)	60.3±10.9	65.6±12.5	61.3±9.9	67.3±8.3	0.001 ^b
BMI (kg/m ²)	28.9±5.7	28.6±6.3	29.2±5.5	28.4±3.7	0.931 ^b
Waist circumference (cm)	97.6±13.8	98.0±15.2	95.9±13.6	98.3±12.3	0.884 ^b
WHR	0.92±0.09	0.92±0.09	0.91±0.09	0.94±0.09	0.748 ^b
Menopause (n; %)	114/127 (89.8)	31/35 (88.6)	40/44 (90.9)	11/11 (100.0)	0.714 ^a
Cigarette smoking status					
Never smoked (n; %)	110 (54.2)	35 (49.3)	24 (43.6)	11 (57.9)	0.182 ^a
Former smoker (n; %)	42 (20.7)	12 (16.9)	8 (14.5)	1 (5.3)	
Current smoker (n; %)	51 (25.1)	24 (33.8)	23 (41.8)	7 (36.8)	
Therapy					
Antihypertensive treatment (n; %)	108 (53.2)	46 (64.8)	36 (65.4)	16 (84.2)	0.021 ^a
Diuretics (n; %)	38 (18.7)	22 (30.9)	25 (45.4)	10 (52.6)	<0.001 ^a
ACEi (n; %)	49 (24.1)	26 (36.6)	19 (34.5)	7 (36.8)	0.126 ^a
ARBs (n; %)	21 (10.3)	9 (12.6)	10 (18.2)	3 (15.8)	0.441 ^a
β Blockers (n; %)	43 (21.2)	15 (21.1)	10 (18.2)	8 (42.1)	0.166 ^a
CCBs (n; %)	28 (13.8)	18 (25.3)	11 (20.0)	7 (36.8)	0.022 ^a
α Blockers (n; %)	9 (4.4)	5 (7.1)	7 (12.7)	0 (0.0)	0.085 ^a
Antidiabetic treatment (n; %)	19 (9.3)	9 (12.7)	10 (18.2)	3 (15.8)	0.300 ^a
Metformin (n; %)	17 (8.4)	1 (1.4)	9 (16.4)	2 (10.5)	0.027 ^a
Sulfonylureas (n; %)	11 (5.4)	7 (9.8)	7 (12.7)	3 (15.8)	0.146 ^a
GLP1a/DPP-IVi (n; %)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0.869 ^a
Insulin (n; %)	2 (0.9)	3 (4.2)	0 (0.0)	1 (5.3)	0.130 ^a
Lipid-lowering treatment (n; %)	26 (12.8)	11 (15.5)	4 (7.3)	3 (15.8)	0.545 ^a
Statins (n; %)	21 (10.3)	7 (9.8)	4 (7.3)	3 (15.8)	0.757 ^a
Omega-3 (n; %)	5 (2.4)	4 (5.6)	1 (1.8)	0 (0.0)	0.417 ^a
Fibrates (n; %)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0.271 ^a
Osteoporosis treatment (n; %)	14 (6.9)	5 (7.0)	4 (7.3)	1 (5.3)	0.993 ^a
Calcium + vitamin D (n; %)	12 (5.9)	1 (1.4)	3 (5.4)	1 (5.3)	0.503 ^a
Bisphosphonates (n; %)	10 (4.9)	4 (5.6)	2 (3.6)	0 (0.0)	0.740 ^a

WHR, waist-to-hip ratio; ACEi, ACE inhibitors; ARBs, angiotensin receptor blockers; CCBs, calcium-channel blockers; GLP1a, GLP1 agonists; DPP-IVi, DPP-IV inhibitors.

^a χ^2 test.

^bOne-way ANOVA.

without the contribution of any other potential risk factor. The SCS-secreting pattern was also associated with CHD (OR, 6.10; 95% CI, 1.41–26.49; $P=0.016$) and osteoporosis (OR, 5.94; 95% CI, 1.79–19.68; $P=0.004$), without any influence of the other potential risk factors; finally, this secreting pattern was significantly associated with T2D (OR, 3.44; 95% CI, 1.18–10.04; $P=0.024$) and osteoporotic fractures (OR, 6.53; 95% CI, 1.29–32.99; $P=0.023$) but with an independent significant contribution of age.

Discussion

This study shows that patients with incidentally discovered adrenal adenomas associated with progressively increased patterns of subclinical hypercortisolism have increasing prevalence of T2D, CHD, osteoporosis, and osteoporotic fractures with respect to those with NSA. Our data also suggest that subclinical hypercortisolism *per se* is a risk factor for specific adverse outcomes, independently of other known risk factors.

It is well known that chronic overt hypercortisolism, as in Cushing's syndrome, is characterized by systemic alterations that may lead to increased risk of metabolic and CVDs (31). However, to date, there are no clear data supporting that subclinical hypercortisolism may lead to severe clinical consequences. This may be due to the lack of large cross-sectional and longitudinal studies and limitations concerning the diagnosis of SCS, which is still a matter of controversial procedures (32); indeed, there is consensus neither on the dosage of dexamethasone for the DST nor on the cutoff values for cortisol post-DST (33). According to our diagnostic criteria, we were able to identify one well-defined group without alterations in cortisol secretion (NSA) and three groups with increased patterns of autonomous cortisol hypersecretion (ImP, IMP, and SCS).

The significant association of SCS and T2D clearly confirms that even subclinical hypercortisolism has a profound impact on glucose metabolism, according to previous findings (9, 34). However, this association was not confirmed in IP patients, suggesting that alterations of glucose metabolism may be related to the degree of

Table 4 Clinical outcomes in groups of patients with different patterns of cortisol secretion. Data are given as absolute numbers with percentage in parentheses. Criteria for diagnosis of NSA, ImP, IMP, and SCS are defined in Table 1.

	Secreting pattern				P value ^a
	NSA (n=203)	ImP (n=71)	IMP (n=55)	SCS (n=19)	
Clinical outcomes					
Hypertension (n, %)	149 (73.4)	58 (81.7)	43 (78.2)	18 (94.7)	0.173
T2D (n, %)	31 (15.2)	13 (18.3)	18 (32.7) ^b	8 (42.1) ^{b,c}	0.004
CHD (n, %)	6 (2.9)	9 (12.6) ^b	6 (10.9) ^d	5 (26.3) ^b	0.002
Stroke (n, %)	1 (0.5)	2 (2.8)	3 (5.4) ^d	1 (5.2)	0.194
Osteoporosis (n, %)	30 (14.8)	7 (9.8)	8 (14.5)	9 (47.3) ^{b,e,f}	0.003
Osteoporotic fractures (n, %)	5 (2.5)	3 (4.2)	1 (1.8)	3 (15.8) ^d	0.056

^aComparisons of outcomes among the four groups (univariate logistic regression).^bPairwise comparisons between groups (simple contrasts applied to the logistic regression): $P < 0.01$ reference category, NSA.^cPairwise comparisons between groups (simple contrasts applied to the logistic regression): $P < 0.05$ reference category, ImP.^dPairwise comparisons between groups (simple contrasts applied to the logistic regression): $P < 0.05$ reference category, NSA.^ePairwise comparisons between groups (simple contrasts applied to the logistic regression): $P < 0.01$ reference category, IMP.^fPairwise comparisons between groups (simple contrasts applied to the logistic regression): $P < 0.01$ reference category, IMP.

cortisol hypersecretion, which may indeed play an independent negative role. In fact, as also demonstrated in patients with Cushing's syndrome (35), cortisol excess has been shown to: inhibit insulin secretion (36, 37), glucose uptake, and glycogen synthesis (38, 39); worsen insulin sensitivity; and increase gluconeogenesis.

The association between different patterns of subclinical hypercortisolism and CHD is novel and deserves more attention. We found that the prevalence of CHD progressively increased in ImP, IMP, and even more in the SCS group when compared with the NSA group. Interestingly, we have shown for the first time that the relationship between CHD and subclinical hypercortisolism was totally independent of other potential contributing factors. It is well known that overt cortisol excess, as in Cushing's syndrome, may lead to systemic complications responsible for increased cardiovascular risk (31) (hypertension, visceral obesity, impaired glucose metabolism, dyslipidemia, and thrombotic diathesis) and cardiovascular complications such as CHD, congestive heart failure, and cardiac stroke, leading to an elevated mortality rate (40, 41, 42). Studies performed on patients with SCS are still unclear, although a prevalence of 17.9% has been reported in one study (43). Our data also suggest that subclinical hypercortisolism may represent *per se* an independent risk factor for CHD and that this risk tends to increase according to the cortisol-secreting pattern. Potential mechanisms involved in the pathogenesis of CHD in subclinical hypercortisolism may involve morphological and functional changes in vascular smooth muscle (44), endothelial cells (45), myocardium (46), and left ventricular dysfunction (47), although this should be adequately confirmed.

Finally, we found that the prevalence of osteoporosis (independently of other potential risk factors) and osteoporotic fractures (with an independent significant contribution of age) increased in patients with SCS. The worsening of BMD and the deleterious effects of cortisol on bone turnover are well described in patients with Cushing's syndrome (48) who have increased bone

resorption rate, reduced osteoblastic activity, and increased osteoblast and osteocyte apoptosis (49, 50). Whether the same mechanisms are involved in patients with SCS is still unclear as previous studies reported worsening in BMD and spinal deformity index and increased risk of vertebral fractures (51, 52), while others did not confirm these findings (53). A recent longitudinal study reported an independent association between SCS and vertebral fractures, with an incidence of new fractures of 48% after 2 years of follow-up (54). Our data confirm that even moderate cortisol excess may worsen osteoporosis and increase osteoporotic fractures. However, caution must be exercised in interpreting the data because none of the SCS patients with fractures were on specific therapy at the time of diagnosis, which was first performed during the work-up of the adrenal mass.

Additional limits of the study are the lack of data about follow-up, due to its cross-sectional design, and the inability to define the length of exposure to the hypercortisolism. Finally, we have to acknowledge that some of the criteria used to stratify the groups were relatively arbitrary; however, we tried to use the available guidelines. Conversely, the strengths of the study are the homogeneity of the groups, given to the strict inclusion and exclusion criteria, and the strict clinical and biochemical criteria used in the diagnosis of different outcomes.

In conclusion, this is the first large cross-sectional study showing that adverse metabolic and cardiovascular outcomes are significantly associated with increasing patterns of subclinical hypercortisolism due to adrenal adenomas, independently of other potential risk factors for each specific outcome. Although these findings need to be confirmed by prospective studies, they raise questions about the need for more reliable and sensitive tests to diagnose subclinical hypercortisolism. On the other hand, our findings could indeed help in therapeutic decision making (conservative vs surgery approach), particularly in patients with adenomas associated with SCS.

Table 5 Odds ratios for T2D, CHD, osteoporosis, and osteoporotic fractures for potential risk factors in groups of patients with different patterns of cortisol secretion.

	Clinical outcomes											
	T2D				CHD				Osteoporosis			
	OR	95% CI	P		OR	95% CI	P		OR	95% CI	P	
Risk factors												
IP-secreting pattern (IP vs NSA)	1.702	0.939–3.082	0.079		4.094	1.473–11.378	0.007		0.637	0.312–1.300	0.215	
Age (1-year increase)	1.026	1.004–1.049	0.021		1.023	0.999–1.047	0.055		1.031	1.009–1.055	0.006	
BMI (1 unit increase)	0.989	0.948–1.032	0.617		0.993	0.950–1.038	0.752		0.992	0.951–1.034	0.710	
Gender (female vs male)	0.875	0.533–1.435	0.597		0.794	0.475–1.325	0.377		0.795	0.474–1.334	0.385	
Family history of T2D	0.988	0.592–1.651	0.965		–	–	–		–	–	–	
(presence vs absence)												
Hypertension (presence vs absence)	–	–	–		0.948	0.512–1.757	0.866		–	–	–	
T2D (presence vs absence)	–	–	–		1.643	0.891–3.029	0.112		–	–	–	
Dyslipidemia (presence vs absence)	–	–	–		0.875	0.531–1.442	0.600		–	–	–	
Smoking status (smokers vs nonsmokers)	–	–	–		1.050	0.768–1.436	0.760		1.080	0.796–1.466	0.621	
SCS-secreting pattern (SCS vs NSA)	3.443	1.181–10.038	0.024		6.104	1.407–26.490	0.016		5.940	1.793–19.677	0.004	
Age (1-year increase)	1.059	1.006–1.116	0.029		1.045	0.989–1.103	0.116		1.043	0.987–1.103	0.132	
BMI (1 unit increase)	0.962	0.872–1.060	0.434		0.965	0.872–1.068	0.489		1.015	0.917–1.123	0.774	
Gender (female vs male)	0.807	0.285–2.286	0.686		0.757	0.258–2.220	0.613		1.872	0.557–6.286	0.310	
Family history of T2D	0.869	0.284–2.654	0.805		–	–	–		–	–	–	
(presence vs absence)												
Hypertension (presence vs absence)	–	–	–		2.932	0.341–25.177	0.327		–	–	–	
T2D (presence vs absence)	–	–	–		2.736	0.926–8.085	0.069		–	–	–	
Dyslipidemia (presence vs absence)	–	–	–		0.935	0.330–2.654	0.900		–	–	–	
Smoking status (smokers vs nonsmokers)	–	–	–		0.764	0.379–1.538	0.450		0.664	0.317–1.395	0.280	
									0.742	0.366–1.501	0.406	

IP, intermediate phenotype; SCS, subclinical Cushing's syndrome, using multivariate logistic regression analysis. The reference category is NSA.

Declaration of interest

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki, and all patients provided written informed consent for all the investigations performed according to usual clinical practice as well as for anonymous data publication. No IRB protocol approval was needed because the data were collected in the course of usual clinical practice. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and no other relationships or activities that could appear to have influenced the submitted work.

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