Cardiovascular features of possible autonomous cortisol secretion in patients with adrenal incidentalomas

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Abstract

Background: Low-grade incomplete post-dexamethasone cortisol suppression in patients with adrenal incidentalomas – recently defined as possible autonomous cortisol secretion (pACS) – has been associated with increased cardiovascular events and mortality. However, prospective studies documenting cardiac abnormalities in these patients are lacking.

Subjects and methods: Between July 2016 and September 2017, 71 consecutive patients with adrenal lesions were prospectively screened for hypercortisolism by dexamethasone suppression test (NCT 02611258). Complete anthropometric, metabolic and hormonal parameters were recorded along with full cardiac ultrasound assessment and noninvasive measurement of arterial stiffness. All patients underwent chemical-shift magnetic resonance imaging to characterize the lesions. Cardiovascular outcomes were recorded in blind.

Results: According to post-dexamethasone suppression cortisol values (post-DST), 34 patients had pACS and 37 non-functioning adenomas (NFA). The two groups were similar in sex, BMI, age distribution, cardiovascular risk factors and comorbidities. Left ventricular mass index ($LVMI^{BSA}$) was increased in pACS compared to NFA ($P=0.006$) and mildly correlated to the post-DST cortisol level (rho = 0.347; $P=0.004$). The post-DST cortisol levels explained up to 13.7% of $LVMI^{BSA}$ variance ($P=0.002$). Compared to NFA, patients with pACS had a higher prevalence of diastolic dysfunction (35.1% vs 82.6%; $P=0.001$) and worse arterial stiffness assessed by pulse wave velocity ($P=0.033$).

Conclusions: In apparently asymptomatic patients, mild autonomous cortisol secretion can sustain early cardiac and vascular remodeling, independently of other risk factors. The morphological and functional cardiovascular changes observed in pACS underline the need for further studies to correctly define the long-term management of this relatively common condition.

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Introduction

Adrenal incidentalomas are clinically silent masses discovered inadvertently during diagnostic imaging procedures performed for unrelated reasons. Depending on the criteria applied, up to 50% of patients with adrenal incidentalomas may have biochemical evidence of cortisol excess (1, 2). Possible autonomous cortisol secretion (pACS), as recently defined in the European Society of Endocrinology Guidelines, is characterized by

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a partial, incomplete suppression of the hypothalamic–pituitary–adrenal (HPA) axis without the typical signs of overt cortisol hypersecretion (i.e. moon face, truncal obesity, easy bruising, thin extremities, proximal myopathy, cutaneous purple striae) (2). It is widely recognized that overt endogenous hypercortisolism (Cushing's syndrome CS) is associated with excessive mortality, mainly due to cardiovascular (CV) disease (3). However, the mechanism leading to cardiac damaging remains unclear (3, 4). For this reason, the ERGO trial (Endocrine Cardiomyopathy in Cushing Syndrome: Response to Cyclic GMP PDE5 inhibitOrs) was designed. Retrospective analysis unexpectedly revealed that also asymptomatic patients with adrenal adenomas and low-grade autonomous cortisol secretion suffer from a higher rate of CV events and mortality than patients with normal HPA axis suppression (5, 6, 7). Despite these epidemiological associations, only two small studies investigated morphological and functional changes to the CV system in patients with mild hypercortisolism (8, 9). In particular, they did not investigate whether CV changes correlated to cortisol levels. The fact that pACS is, by definition, asymptomatic prompted us to investigate the cardiac features of these patients, taking advantage of patients screened during enrollment for the ERGO trial. All patients with pACS underwent a complete ultrasound assessment of structural and functional left ventricular (LV) abnormalities accompanied, for the first time, by arterial stiffness assessment. Patients were then stratified according to response to the dexamethasone suppression test. The main hypothesis of this sub-analysis of the ERGO trial was to test whether left ventricular mass index (LVMI) is higher in pACS than in nonfunctioning adenoma (NFA) patients.

Subjects and methods

Protocol

109 consecutive outpatients aged between 18 and 75 years with an incidentally detected adrenal mass were screened for inclusion in the ERGO trial (NCT02611258). The main inclusion criterion to enter the ERGO trial is a diagnosis of CS. The study population of the current sub-analysis consists of all screened patients with an adrenal lesion not matching a diagnosis of CS (Fig. 1). All patients were evaluated at the Department of Experimental Medicine, Sapienza, University of Rome between July 2016 and September 2017.

All patients were clinically examined and evaluated for signs and symptoms typical of adrenal hormones excess. Anthropometric measurements were assessed by the same person. BMI was calculated by dividing weight (in kg) by height (in m^2). Arterial hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on repeated evaluations and/or reported use of antihypertensive medication (10). Type 2 diabetes mellitus (T2DM) was diagnosed according to ESC guidelines or reported use of antidiabetic drugs (11). Dyslipidemia was diagnosed according to ESC/EAS Guidelines or reported use of hypolipidemic drugs (12). Current smokers were patients who smoked any tobacco during the observation period or patients who had smoked in the last twenty years. The study was performed following Declaration of Helsinki principles and the protocol approved by the Ethics Committee of 'Sapienza' University of Rome on the 19 November 2015 with reference number 3875, and subsequently registered on NIH (NCT02611258). An informed consent has been obtained from each patient.

Biochemical and radiological evaluation

Fasting baseline sampling was performed for measurement of blood glucose, insulin, glycated hemoglobin, sodium, potassium, creatinine, total cholesterol, HDL cholesterol and triglycerides. Insulin resistance was assessed using HOMA-IR.

The functional status of incidentally discovered adrenal masses was determined according to the ESE 2016 guidelines (2). Blood tests included an endocrine workup taken after an overnight fast and performed between 08:00 h and 09:00 h. Baseline cortisol (radioimmunoassay), ACTH (immunoradiometric assay), dehydroepiandrosterone sulfate (DHEAS) (chemiluminescence), androstenedione (radioimmunoassay), 17OH-progesterone (radioimmunoassay) and aldosterone/renin ratio (radioimmunoassay) were measured in all patients. Urinary free cortisol (UFC) (radioimmunoassay) and 24-h urine metanephrine (radioimmunoassay) tests were also performed.

Adrenal lesions were discovered by abdominal ultrasonography, CT scan or MRI and confirmed in all cases with chemical shift MRI. All adrenal masses were larger than 10 mm and showed a MRI pattern consistent with benign adenoma (i.e. regular margins, loss of signal intensity on out-of phase images compared with in-phase images, size <6 cm).

Of the 109 patients with adrenal incidentalomas , those with overt Cushing's syndrome, pheocromocytoma,

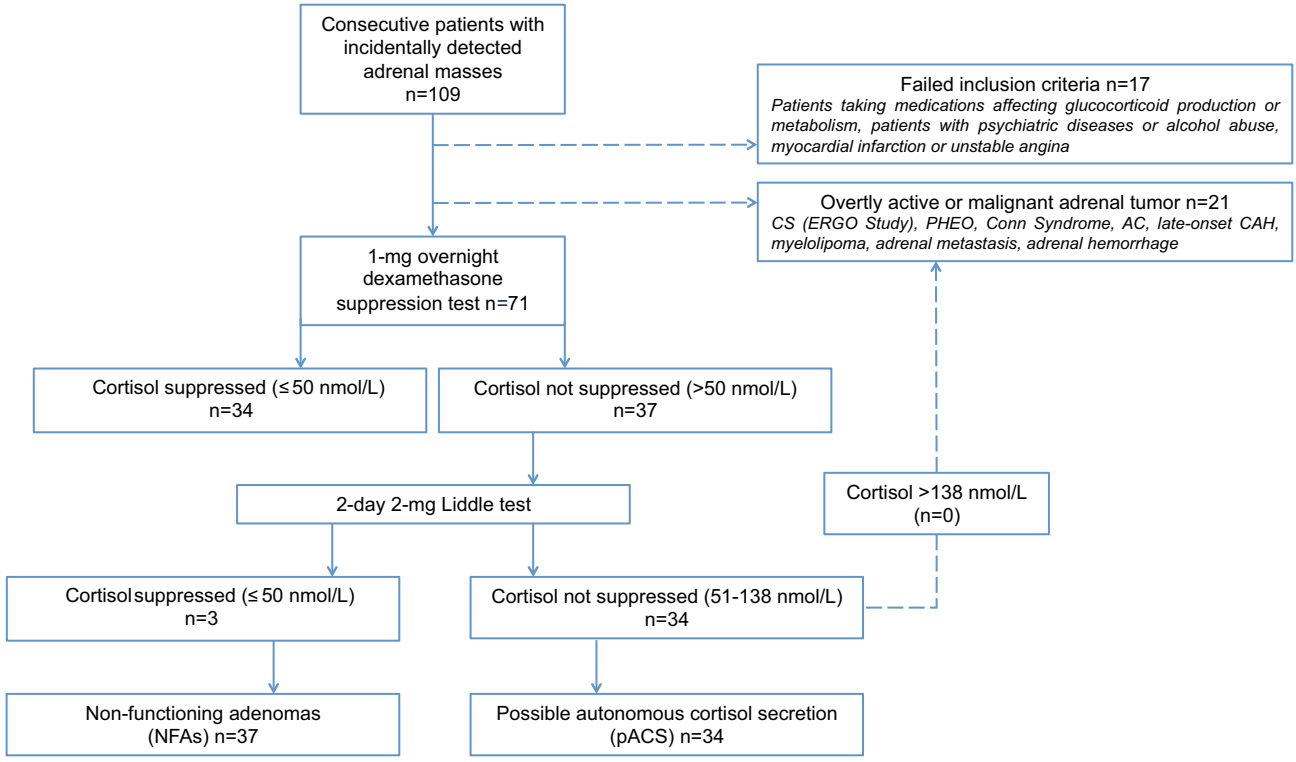

Figure 1

Study flow diagram. AC, adrenal carcinoma; CAH, congenital adrenal hyperplasia; CS, Cushing syndrome; NFA, non-functioning adenoma; PHEO, pheochromocytoma; pACS, possible autonomous cortisol secretion.

Conn syndrome, adrenocortical carcinoma, late-onset congenital adrenal hyperplasia, myelolipoma, adrenal metastasis and adrenal hemorrhage were not included in the current analysis. Patients taking medications influencing glucocorticoid production or metabolism, patients with psychiatric diseases or alcohol abuse, myocardial infarction or unstable angina, history of malignancy, infections and infiltrative diseases potentially affecting the adrenal glands were also excluded. This left a group of 71 patients (Fig. 1).

Interpretation of the results of the 1 mg overnight dexamethasone test (1 mg-DST) was based on the 2016 European Society of Endocrinology guidelines (2). 1 mg-DST cortisol levels between 51 and 138 nmol/L were considered as possible autonomous cortisol secretion (pACS) and greater than 138 nmol/L were considered as Cushing's syndrome sustained by autonomous cortisol secretion. 1 mg-DST cortisol levels below 50 nmol/L were considered to identify patients with nonfunctioning adenoma (NFA). Since the 1 mg-DST has the same sensitivity (98%) but lower specificity than the classic 2-day 2-mg Liddle test (2 mg-DST) (87.5 vs 97–100%) (13), in all patients with 1 mg-DST cortisol above 50 nmol/L, the test was confirmed by 2 mg-DST. In the final data set analysis, the cortisol values for patients with normal suppression on 1 mg-DST and the 2 mg-DST values for all remaining patients were combined (post-DST).

Cardiovascular evaluation

Transthoracic echocardiography was performed by a blinded outcome assessor using an Aplio CV Toshiba with a 3 MHz transducer in 2D, Doppler, color-Doppler and Tissue Doppler Imaging (TDI). Patients were examined in the left lateral decubitus position according to the American Society of Echocardiography guidelines (14). Left ventricle end-diastolic diameter (LVED), interventricular septal thickness (IVS) and posterior wall thickness (PWT) were measured at end diastole. Left ventricular mass (LVM) was calculated by the corrected American Society of Echocardiography method: LVM = 0.8 (1.04 ((LVED + IVS + PWT)^3–(LVED)^3) + 0.6). LVM index (LVMI^{BSA}) was calculated as LVM divided by body surface area (BSA). LV hypertrophy was defined according to gender-specific criteria as 2D measurements greater than 88 g/m^2 in women and 102 g/m^2 in men. The relative wall

thickness (RWT) was calculated as (IVS+PWT)/LVED. RWT>0.42 was considered pathologic. Patients with increased $LVMI^{BSA}$ and increased RWT were considered to have concentric LV hypertrophy, while those with increased $LVMI^{BSA}$ and normal RWT were considered to have eccentric LV hypertrophy. $LVMI^{BSA}$ is widely used in routine practice, and it has been documented to detect hypertension-related cardiac hypertrophy. We also calculated LVM normalized to $height^{2.7}$ to obtain $LVMI^{h2.7}$, which is considered to be more sensitive in detecting obesity-related LVH (15).

Left atrial (LA) anteroposterior size was assessed in the parasternal long axis. Left ventricular ejection fraction (LVEF) was determined by manual tracing using Simpson's biplane method.

Left ventricular diastolic dysfunction (LVDD) was assessed by pulse-wave Doppler evaluating the following parameters: early-E-wave (E) and late A-wave (A) diastolic filling velocities and E/A ratio. TDI measurements were performed in the apical 4-chamber view and the sample volume was placed in the septal mitral annulus and lateral mitral annulus. Early diastolic tissue velocities (E′) from both the septal (E′s) and lateral (E′l) wall were assessed and mean E′ (E′m) was calculated; E/E′ ratio was derived, reflecting the LV filling pressures. LVDD was diagnosed on the basis of E′s<8 cm/s or E′l<10 cm/s. Diastolic dysfunction (grade I, mild; grade II, moderate or grade III, severe) was quantified using EAE recommendations (16). Right ventricular function was assessed as the tricuspid annular plane systolic excursion (TAPSE). TAPSE<17 mm is highly suggestive of RV (right ventricular) systolic dysfunction.

Blood pressure and arterial stiffness

Arterial stiffness and blood pressure (BP) were assessed by BPLab (Vasotens Technology), using brachial oscillometric blood pressure waves for a noninvasive estimation. The measurements were performed in the supine position after the patients had been resting for a minimum of 5 min. The distance from the jugulum to the symphysis was measured in every patient. The optimal adult cuff was wrapped around the non-dominant arm. In a single session measurements of brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken three times, at 5-min interval. Their average was used for analyses. During the BP measurement the pressure waveforms in the cuff were registered and digitalized. Thereafter, an aortic pulse wave was generated to derive the aortic pressure wave. The difference in time between the beginning of the first wave and the beginning of the second (reflected wave) was related to the distance from the jugulum to the symphysis, resulting in the pulse-wave velocity (PWV) in m/s. Therefore, PWV and reflected wave transit time (RWTT), which have been used to evaluate arterial stiffness, were obtained after the end of measurement.

Statistical analysis

Based on previously published data (17) describing 25 g/m^2 standard deviation (S.D.) for $LVMI^{BSA}$ in the population with hypercortisolism, and considering cautiously 20 g/m^2 as the minimal clinically relevant difference between groups, a calculated sample size of 54 would have provided 90% power to detect the specified minimum detectable difference in $LVMI^{BSA}$ at a 2-sided significance level of 0.05.

Distribution of continuous variables was tested with the Shapiro–Wilk test; linearity was established by visual inspection of a scatterplot. Categorical variables were expressed as percentage and frequency; continuous variables were reported as mean and 95% confidence interval (95% CI) or median and interquartile range (25th–75th percentile). For group comparisons unpaired Student's *T*-test, Mann–Whitney, χ^2 or Fisher's exact test were used as appropriate. A general linear model including the presence or absence of autonomous cortisol secretion as a fixed effect and factors known to affect cardiovascular features as covariates was developed. In the analysis of covariance (ANCOVA) model, log transformation or reciprocal transformation were used for skewed data. Regression analysis was performed to estimate the contributors to $LVMI^{BSA}$ variability. Standardized residuals were tested for normality. Homoscedasticity and homogeneity of variances were assessed by visual inspection. Multicollinearity was assessed by calculation of the variance inflation factor (VIF). Sensitivity analysis was performed excluding both patients with a diagnosis of diabetes mellitus and those with discrepancies between the two suppression tests. Correlations between parameters were analyzed using Pearson and Spearman's correlation coefficients. A $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software (Version 19.0; IBM, Normal).

Results

Clinical and biochemical evaluation

A total of 109 patients with incidentally discovered adrenal mass were screened in the ERGO trial, of whom 38 did

Table 1 Clinical and biochemical characteristics of all enrolled subjects.

	Adrenal incidentalomas ($n=71$)	**NFA** ($n=37$)	**pACS** ($n=34$)	$P^{§,*,\#}$
Age (years)*	67 (59–72)	67 (60–72)	68 (57–73)	0.161
Male/Female gender	24/47	11/26	13/21	0.449
Body mass index (kg/m^2)*	26.3 (23.5–29.4)	25.9 (23.7–31.2)	26.7 (22.6–29.3)	0.275
Overweight patients$^+$	30/71 (42.3%)	15/37 (40.5%)	15/34 (44.1%)	0.761
Obese patients$^+$	17/71 (23.9%)	7/37 (18.9%)	10/34 (29.4%)	0.301
Diameter of adenoma (mm)§	23.8 (21.2–26.4)	20.2 (17.2–23.1)	27.4 (23.4–31.5)	0.004
Blood glucose (mg/dL)*	92 (85–105)	90 (85–96)	94 (85–110)	0.475
Glycated hemoglobin (%)*	5.5 (5.2–5.7)	5.5 (5.3–5.7)	5.5 (5.2–5.7)	0.294
Insulin (μU/mL)*	8.7 (6.9–10)	8.6 (6.6–10.4)	8.7 (7.3–10)	0.850
HOMA-IR Index*	2 (1.5–2.4)	1.9 (1.4–2.4)	2 (1.5–2.5)	0.917
Total cholesterol (mg/dL)§	205 (197–212)	199 (187–210)	212 (201–220)	0.114
LDL cholesterol (mg/dL)§	121 (113–130)	114 (102–126)	130 (116–141)	0.089
HDL cholesterol (mg/dL)*	62 (54–71)	59 (53–67)	63 (54–72)	0.510
Triglycerides (mg/dL)*	90 (75–119)	104 (83–131)	90 (73–122)	0.550
Creatinine (mg/dL)*	0.7 (0.7–0.8)	0.7 (0.7–0.8)	0.7 (0.7–0.8)	0.217
Sodium (mmol/L)§	142 (141–143)	142 (141–143)	142 (140–143)	0.765
Potassium (mmol/L)§	4.3 (4.2–4.4)	4.2 (4–4.3)	4.3 (4.2–4.5)	0.146
Hypertension$^+$	45/71 (63.4%)	23/37 (62.2%)	22/34 (64.7%)	0.824
Number of antihypertensive drugs$^+$				0.463
1–2	30/45 (66.7%)	17/23(73.9%)	13/22 (59.1%)	
3–4	15/45 (33.3%)	6/23 (26.1%)	9/22 (40.9%)	
Duration of hypertension$^\#$				0.488
<10 years	21/45 (46.7%)	10/23 (43.5%)	11/22 (50%)	
10–20 years	16/45 (35.5%)	10/23 (43.5%)	6/22 (27.3%)	
>20 years	8/45 (17.8%)	3/23 (13%)	5/22 (22.7%)	
Type 2 diabetes mellitus$^\#$	9/71 (12.7%)	2/37 (5.4%)	7/34 (20.6%)	0.077
Taking antidiabetic drugs$^+$	8/9 (88.9%)	1/2 (50%)	7/7(100%)	0.222
Dyslipidemia$^+$	45/71 (63.4%)	21/37 (56.8%)	24/34 (70.6%)	0.227
Taking hypolipidemic drugs$^+$	14/45 (31.1%)	6/21 (28.6%)	8/24 (33.3%)	0.371
Smoker$^+$	24/71 (33.8%)	13/37 (35.1%)	11/34 (32.4%)	0.804

Categorical variables are expressed as frequency and percentages. Continuous variables are expressed as §mean (95% CI) or *median (25th–75th percentile).

§Unpaired sample *T*-test or *Mann–Whitney test and $^\#\chi^2$ or Fisher's exact test, as appropriate.

not meet the inclusion criteria for the current analysis. The remaining 71 patients with AI fulfilling the inclusion criteria were enrolled (Fig. 1). Of these, 37 had 1 mg-DST serum cortisol levels greater than 50 nmol/L and underwent confirmatory 2 mg-DST, giving rise to a diagnosis of pACS in 34. The remaining three cases (1 mg-DST serum cortisol levels respectively 180, 92.8, 69.1 nmol/L) were found to be false positives, with complete suppression on 2 mg-DST (respectively 27.6, 46.9, 33.5 nmol/L). Of the four patients with 1 mg-DST above 138 nmol/L (respectively 181, 180, 161, 147 nmol/L), none had a 2 mg-DST higher than 138 nmol/L (respectively 135, 27.6, 131, 134 nmol/L). In all, therefore, 37 patients had a post-DST cortisol level below 50 nmol/L and were defined as NFA (Fig. 1).

Table 1 describes the clinical and biochemical characteristics of all patients with AI, divided into pACS and NFA. The two groups were similar for sex, BMI and age distribution. There was no statistically significant difference in blood glucose, insulin, insulin resistance, glycated hemoglobin, sodium, potassium, creatinine, total cholesterol, HDL-cholesterol or triglycerides between the groups. Cardiovascular risk factors and comorbidities (arterial hypertension, dyslipidemia, smoking) were equally distributed among NFA and pACS, as were duration of hypertension and the number of antihypertensive, antidiabetic and hypolipidemic drugs (Table 1). The relative prevalence of T2DM was apparently higher in the pACS compared to NFA, although this was not statistically significant due to the small number of patients in each group.

The diameter of the lesions was higher in pACS than NFA patients (27.4 (95% CI: 23–32) vs 20.2 mm (95% CI: 17–23); $P=0.004$) and positively correlated with post-DST cortisol levels (rho=0.441; $P<0.001$). Hormone parameters are shown in Table 2. pACS had lower ACTH (18 (95% CI: 15–20) vs 25 (95% CI: 19–31) pg/mL; $P=0.028$) and DHEAS (37.4 (18–54) vs 78 (45–189) μg/dL; $P=0.012$) levels compared to NFA patients. No correlation was found between age and post-DST cortisol levels (rho=0.123; $P=0.32$).

Table 2 Hormonal parameters.

	Adrenal incidentalomas (*n*=71)	**NFA** (*n*=37)	**pACS** (*n*=34)	*P*§,*
Baseline cortisol (nmol/L)§	493 (434–537)	470 (372–512)	517 (445–600)	0.244
ACTH (pg/mL)§	22 (18–25)	25 (19–31)	18 (15–20)	0.028
Aldosterone/renin ratio*	30 (20–39)	29 (21–37)	31 (19–64)	0.084
17OH progesterone (ng/mL)*	0.5 (0.3–0.9)	0.6 (0.3–0.9)	0.6 (0.4–0.8)	0.773
DHEAS (µg/dL)*	48 (24–88)	77.8 (45–189)	37.5 (18–54)	0.012
Urinary free cortisol (nmol/24 h)*	121 (97–184)	110 (81–167)	145 (106–202)	0.708
Androstenedione (ng/dL)*	97 (84–172)	109 (87–173)	89 (63–178)	0.613
Urine metanephrine (µg/24 h)*	58 (41–87)	57 (40–96)	75 (41–87)	0.685
1 mg-DST cortisol (nmol/L)*	58 (42–77)	41.4 (34.2–49.0)	76.3 (62–93)	0.001
Post-DST cortisol (nmoL/L)*	54 (42–77)	41.4 (34.2–48.6)	73.4 (57–90)	0.001

Continuous variable are expressed as §mean (95% CI) or *median (25th–75th percentile).
§Unpaired sample *T*-test or *Mann–Whitney test as appropriate.

Echocardiographic findings

Morphological analysis showed that both $LVMI^{BSA}$ and $LVMI^{h2.7}$ were significantly higher in the pACS than NFA group ($LVMI^{BSA}$: 90.25 (71.7–110.3) vs 76.6 (64–85) g/m^2; *P*=0.006; $LVMI^{h2.7}$: 46.3 (33.6–54.6) vs 34.7 (30–41) g/m$^{2.7}$; *P*=0.003). Two linear models were developed adjusting for known contributors of $LVMI^{BSA}$. The first model included age and BMI and the second age, BMI, HbA1c, LDL cholesterol and SBP (Table 3). Estimated marginal means of the main CV outcomes are given in Table 3. After adjusting for all covariates a statistically significant difference in $LVMI^{BSA}$ and $LVMI^{h2.7}$ was still found between the two groups (*P*=0.001). There was also a significantly higher prevalence of LV hypertrophy in the pACS than the NFA group (53% vs 13.5%; *P*=0.001), including both concentric LV hypertrophy (26.5% vs 5.4%) (*P*=0.014) and eccentric LV hypertrophy (26.5% vs 8.1%) (*P*=0.039). $LVMI^{BSA}$ and $LVMI^{h2.7}$ were both positively correlated to post-DST cortisol (rho=0.347, *P*=0.004 and rho=0.336, *P*=0.005, respectively Fig. 2).

A regression model was used to estimate the contributors to $LVMI^{BSA}$ in patients with adrenal incidentalomas. The model included age, BMI, SBP, Hba1C, LDL cholesterol and post-DST cortisol. Step-wise post-DST cortisol, SBP and Hba1C were the only independent determinants of $LVMI^{BSA}$ explaining up to 31.7% of $LVMI^{BSA}$. Specifically, the post-DST cortisol level was the main independent contributor to $LVMI^{BSA}$ (*P*=0.002), accounting for 13.7% of $LVMI^{BSA}$ variability, followed by SBP (9.9%) and Hba1C (8.1%). No collinearity between SBP and post-DST cortisol was found (VIF=1), suggesting an independent effect of cortisol on cardiac function.

Interventricular septal thickness (IST) was greater in the pACS than in the NFA group (10.8 (95% CI: 9.9–11.8) vs 9.9 (95% CI: 9.3–10.3); *P*=0.015). LVEF was similar between the two groups (63.2 (95% CI: 61.2–65.9) vs 62.8 (95% CI: 59.9–64.5); *P*=0.786). Mean TAPSE values were significantly lower in the pACS than in the NFA group (19.9 (95% CI: 18.5–21.3) vs 22.1 (95% CI: 20.9–23.4); *P*=0.046).

Functional analysis showed a higher prevalence of LVDD in patients with pACS (82.3% vs 35.1%; *P*=0.001). pACS patients, compared with NFA group, had lower septal E' (7.3 (95% CI: 6.5–8.0) vs 8.61 (95% CI: 7.7–9.5) cm/s; *P*=0.027), lower E'm (8.24 (95% CI: 7.6–8.9) vs 9.57 (95% CI: 8.6–10.5); *P*=0.024) and higher E/e'm (8.99 (95% CI: 8–10) vs 7.38 (95% CI: 6.6–8.1) cm/s; *P*=0.010). The severity of diastolic dysfunction in pACS patients appeared worse than that in the NFA group (Grade I: 38.2% vs 27%; Grade II: 35.3% vs 8.1%; Grade III: 8.8% vs 0%).

To exclude that an odd distribution of T2DM prevalence among the two groups might explain some of the observed differences in the CV assessment, a sensitivity analysis was performed excluding both patients with T2DM and those with discrepant findings on 1-mg DST vs 2-mg DST. This produced similar findings for $LVMI^{BSA}$ and diastolic dysfunction (Table 4), confirming the validity of the dataset. To rule out an influence of glycemic control, HbA_{1c} was also included in the regression models.

Arterial stiffness

RWTT was significantly lower (123.5 (113–129) vs 129.5 (121–135) m/s; *P*=0.048), whereas PWV was significantly higher (11.05 (95% CI: 10.4–11.7) vs 10.3 (95% CI: 9.9–10.6) m/s; *P*=0.033), in the pACS compared to the NFA group. After adjusting for age and BMI, a significant difference in PWV was found between the two groups (*P*=0.040, Table 3).

Table 3 Cardiac and arterial stiffness features comparisons between groups.

	NFA ($n=37$)	pACS ($n=34$)	*P* value
ANCOVA model[a]			
Echocardiographic measurements			
LV end-diastolic diameter (mm)	45.6 (43.9–47.2)	47.1 (45.5–48.8)	0.194
IST (mm)	10.0 (9.5–10.5)	10.7 (10.2–11.3)	0.042
Posterior wall thickness (mm)	8.4 (7.9–8.9)	9.1 (8.6–9.6)	0.071
Relative wall thickness	0.41 (0.39–0.43)	0.42 (0.4–0.45)	0.279
LV mass (g)	141 (127–166)	168 (153–182)	0.014
LV mass^{BSA} (g/m^2)	77.0 (70–85)	92.8 (85.5–100.2)	0.009
LV mass$^{h2.7}$ (g/m$^{2.7}$)	37.3 (33.5–40.9)	44.9 (41.3–48.7)	0.004
Left atrial size (mm)	36.2 (34.6–37.8)	36.3 (34.6–37.9)	0.955
LV ejection fraction (%)	63.1 (60.9–65.3)	60.7 (60.7–65.0)	0.850
E/A	0.9 (0.8–1.1)	1.0 (0.8–1.2)	0.530
E'l (cm/s)	10.3 (9.3–11.3)	9.5 (8.5–10.3)	0.211
E's (cm/s)	8.5 (7.7–9.2)	7.5 (6.7–8.2)	0.093
E'm (cm/s)	9.4 (8.6–10.2)	8.4 (7.7–9.2)	0.065
E/e' l	7.0 (6.2–7.9)	7.9 (7.1–8.8)	0.155
E/e's	8.6 (7.5–9.6)	10.2 (9.1–11.3)	0.044
E/e' m	7.6 (6.8–8.3)	8.7 (7.9–9.6)	0.061
TAPSE (mm)	21.9 (20.8–23.1)	20.3 (18.9–21.5)	0.064
Arterial stiffness measurements			
Systolic blood pressure (mmHg)	137.2 (131.1–143.4)	136.8 (131.1–142.9)	0.917
Diastolic blood pressure (mmHg)	83.3 (79.3–87.2)	83.1 (79.2–86.9)	0.946
Mean blood pressure (mmHg)	110.3 (106–115)	109.9 (106–114)	0.918
Heart rate (bpm)	69.9 (65.1–74.7)	76.6 (71.1–82.1)	0.143
Reflected wave transit time (m/s)	127.5 (123–132)	120.6 (115.9–125.3)	0.031
Pulse-wave velocity (m/s)	10.3 (9.9–10.8)	11.0 (10.5–11.5)	0.040
ANCOVA model[b]			
Echocardiographic measurements			
LV end-diastolic diameter (mm)	45.6 (44.0–47.3)	47.3 (45.5–49.0)	0.198
IST (mm)	9.9 (9.4–10.4)	10.7 (10.2–11.2)	0.015
Posterior wall thickness (mm)	8.4 (7.9–9)	9.1 (8.5–9.6)	0.047
Relative wall thickness	0.40 (0.39–0.4)	0.42 (0.39–0.43)	0.193
LV mass (g)	140 (126–155)	169 (154–184)	0.013
LV mass^{BSA} (g/m^2)	76.6 (69.6–83.6)	95.3 (88.0–102.5)	0.001
LV mass$^{h2.7}$ (g/m$^{2.7}$)	36.2 (32.8–39.6)	46.1 (42.5–49.6)	0.001
Left atrial size (mm)	35.5 (34.1–36.9)	35.9 (34.3–37.5)	0.685
LV ejection fraction (%)	63.4 (61.09–65.7)	62.7 (60.4–65.1)	0.692
E/A	0.9 (0.79–1.0)	0.9 (0.8–1.1)	0.524
E'l (cm/s)	10.4 (9.3–11.5)	9.3 (8.1–10.5)	0.199
E's (cm/s)	8.5 (7.7–9.3)	7.4 (6.4–8.2)	0.059
E'm (cm/s)	9.4 (8.6–10.2)	8.3 (7.4–9.2)	0.067
E/e' l	6.8 (5.9–7.8)	8.0 (6.9–9.1)	0.125
E/e's	8.3 (7.2–9.5)	10.3 (9.0–11.6)	0.028
E/e' m	7.4 (6.6–8.3)	8.85 (7.8–9.8)	0.044
TAPSE (mm)	22.2 (21.1–23.3)	20.2 (18.9–21.6)	0.036

[a,b]ANCOVA models for group comparisons with possible autonomous cortisol secretion (pACS) as fixed factor and the following as covariates: model[a], age and BMI; model[b], age, BMI, SBP, LDL cholesterol and Hba1C. Values represent the estimated marginal means (lower-upper limit of 95% CI).

Discussion

Our findings suggest that exposure to mild levels of autonomous cortisol secretion sustains structural and functional changes of the LV and medium-sized vessel arterial wall that may account for the observed increase in CV events and related mortality described in other studies.

In our cohort of patients with AI, both mean LVMI and prevalence of LV hypertrophy was higher in pACS than in NFA patients. LVMI is a sensitive indicator of cardiac end-organ damage and an independent risk factor for the development of heart failure. LV hypertrophy is the hallmark of several detrimental cardiovascular diseases and is associated with a worse prognosis after cardiac events (18). We also find a mild positive

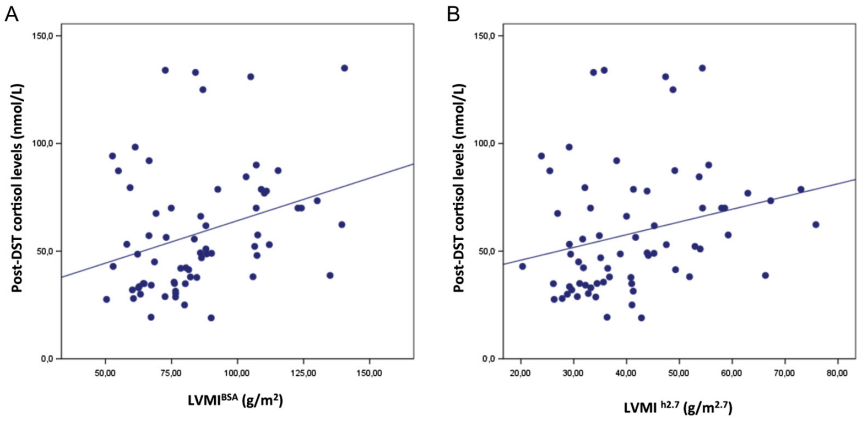

Figure 2
Correlation between post-DST cortisol levels and left ventricular mass index normalized to BSA ($LVMI^{BSA}$) (A) and normalized to height$^{2.7}$ ($LVMI^{h2.7}$) (B).

correlation between both $LVMI^{BSA}$ and $LVMI^{h2.7}$ and post-DST cortisol. For the first time, we documented a diastolic dysfunction associated with pACS. The presence of LVDD is considered an independent risk factor for heart failure and death from cardiac causes. LVDD is also associated with a reduced quality of life (19, 20). TAPSE has been proposed as a simple and reproducible parameter for qualitative RV systolic function. We found a mild reduction of TAPSE in pACS compared to NFA patients. Although TAPSE may slightly overestimate or underestimate RV function because of cardiac translation, it still correlates well with the parameters estimating RV global systolic function (14).

Arterial stiffness analysis showed that patients with pACS had significantly higher PWV and lower RWTT. PWV is the most validated method for the noninvasive quantification of arterial stiffness and is considered the gold standard index (21). Increased PWV, and thus arterial stiffness, has been associated with increased morbidity and cardiovascular mortality and is considered an independent early predictor of coronary heart disease and stroke in the general population (22). In our study, compared to NFA patients, pACS patients presented a worse metabolic profile, although this difference was not statistically significant. There was no significant difference between the two groups in age, gender distribution, BMI, arterial hypertension, T2DM, dyslipidemia and smoking habit, all important parameters associated with increased cardiovascular risk; therefore, these were reasonably excluded as possible confounders. The two groups also showed similar duration of arterial hypertension and number of antihypertensive drugs.

Cortisol is thought to have direct relevant effects on cardiac function: it potentiates cardiac angiotensin II and noradrenaline responsiveness and also stimulates the local renin-angiotensin system (4). Complex mechanisms including endothelial damage, activation of inflammation, increased oxidative stress and fibroproliferation are correlated with high cortisol levels, leading to functional and structural change in the heart and blood vessels. An increased prevalence of LV hypertrophy and LV diastolic dysfunction (albeit with preserved LV ejection fraction)

Table 4 Sensitivity analysis of cardiac and arterial stiffness excluding patients with of diabetes mellitus or discrepancies between the 1 mg DST and 2-mg DST.

	Adrenal incidentalomas ($n=59$)	**NFA** ($n=32$)	**pACS** ($n=27$)	$P^{§,*,+}$
Pulse-wave velocity (m/s)§	10.6 (10.2–11)	10.3 (9.9–10.7)	11.0 (10.3–11.7)	0.039
LV $mass^{BSA}$ (g/m^2)*	76.1 (64.3–86)	75.9 (63.4–80.1)	83.8 (64.7–105.5)	0.032
LV $mass^{h2.7}$ ($g/m^{2.7}$)*	34.1 (30.7–41)	33.2 (30.1–36.6)	37.4 (31.1–48.8)	0.022
LV hypertrophy+	19/59 (32.2%)	5/32 (15.6%)	14/27 (51.8%)	0.003
E's (cm/s)§	8.0 (7.3–8.7)	8.6 (7.6–9.5)	7.3 (6.4–8.2)	0.039
E'm (cm/s)§	9.1 (8.4–9.8)	9.7 (8.7–10.8)	8.3 (7.6–9.1)	0.022
E/e'l§	7.0 (6.3–7.7)	6.3 (5.6–7.1)	7.8 (6.7–8.9)	0.029
E/e's§	9.0 (8.1–9.9)	8.0 (5.6–7.1)	10.2 (8.6–11.7)	0.021
E/e' m§	7.7 (7.1–8.4)	7.0 (6.2–7.7)	8.6 (7.6–9.6)	0.011
Diastolic dysfunction+	33/59 (55.9%)	10/32 (31.25%)	23/27 (85.2%)	0.001

Continuous variable are expressed as §mean (lower-upper limit of 95% CI) or *median (25th–75th percentile). Categorical variables are expressed as frequency and percentages.
§Unpaired sample *T*-test or *Mann–Whitney test and +X-square as appropriate.

are consistently found in Cushing's syndrome (CS). In patients with CS, systolic and diastolic dysfunction can develop independently of the presence of hypertension (17, 23, 24, 25). We found a higher prevalence of LV hypertrophy in pACS than that reported in overt CS by previous echocardiography-based studies, where LV hypertrophy was found in 24–42% of affected patients (23, 25, 26, 27). However, cardiac hypertrophy increases with age, and in those studies on CS (23, 25, 26, 27) the mean age was significantly lower than in our cohort of pACS patients (39–41 vs 65 years). Finally, a recent report on arterial stiffness in CS reported structural changes in the arterial wall occurring independently of blood pressure levels (28).

Recent studies suggest that even patients lacking the full clinical picture of CS having an adrenal incidentaloma and a non-suppressed cortisol after DST suffer from increased cardiovascular events and mortality when compared to NFA (5, 7, 29, 30). To our knowledge, this is the first study evaluating the relationship between pACS, LV function and structure and arterial stiffness and the first to describe a dose–response relationship between post-DST cortisol and LVMI. Only two previous studies presented data about echocardiographic findings of subclinical hypercortisolism. However, both were completed before the publication of the 2016 ESE Guidelines defining pACS (2). In 2013, Iacobellis and coworkers reported echocardiographic data on 6 patients with mild CS compared with 40 patients with NFA. In this study, mild CS was defined by two positive tests among UFC >70 µg per 24 h, post-DST serum cortisol levels >138 nmol/L (5 µg/dL) or ACTH levels <10 pg/mL. Patients matching these criteria had a higher LVM than those without (8). In 2015, Evran and coworkwers described the US findings of 5 patients with 'subclinical Cushing's syndrome' defined as a post-DST cortisol values >50 nmol/L (1.8 µg/dL) and ACTH < 10 pg/mL. No echocardiographic differences were found between the 5 patients with 'subclinical Cushing's syndrome' and a group of 76 patients without CS (9).

The strengths of the present study are the presence of a confirmatory test for the diagnosis of pACS, the balanced distribution between the two groups and its prospective design: all echocardiography and arterial stiffness measurements were performed by the same clinician blinded to the diagnosis of pACS or NFA (blinded outcome assessor).

However, the study also has a number of limitations. The size of the two groups, although significantly higher than in other published studies, is still low to provide definitive data. In addition, there was no control group (patients without adrenal adenoma). Moreover, 2D-echocardiogram and BPlab are not the gold standard methods to assess cardiac hypertrophy and arterial stiffness. Cardiac MRI or aortic catheterization would have provided more definitive findings. However, they are more affordable, have good reproducibility and are often used in the clinical setting for population studies (14, 31, 32), expanding the applicability of our findings. Finally, some recent reports suggested that patients with NFA may also carry an increased CV risk compared with the general population, thus our study may underestimate the magnitude of cardiac damage associated with pACS. Among the hypotheses proposed, there is the possibility that NFAs produce excess cortisol in a cyclic manner, or during stress, or release excess steroid precursors, such as deoxycorticosterone and corticosterone (6, 33, 34).

In conclusion, our study shows that possible autonomous cortisol secretion (pACS) can sustain early cardiac and vascular dysfunction even in asymptomatic patients. Our results suggest that the finding of post-DST cortisol levels above 50 nmol/L requires careful monitoring of cardiovascular risk and long-term evaluation in non-operated patients. These results underline the need for further studies, including second line cardiovascular assessment, to correctly characterize the molecular mechanism or pathway through which mild autonomous secretion can induce cardiac damage.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement
E S and M M researched the data, wrote the manuscript, contributed to the discussion and edited the manuscript; D D performed cardiac ultrasound and measurement of arterial stiffness; L R, M D G and R P enrolled and evaluated patients and controls; F V critically revised cardiovascular data; E G and M A V collected and analyzed data; A V provided statistical support; S M, A L and A M I established the study protocol, coordinated the study and critically revised the manuscript.

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