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Cardiometabolic disease burden and steroid excretion in benign adrenal tumors: a cross-sectional multi-center study

Running title: Cardiometabolic disease burden in benign adrenal tumors

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ABSTRACT

BACKGROUND: Benign adrenal tumors are commonly discovered on cross-sectional imaging. Mild autonomous cortisol secretion (MACS) is regularly diagnosed but its impact on cardiometabolic disease in affected individuals is ill-defined.

OBJECTIVE: To determine cardiometabolic disease burden and steroid excretion in persons with benign adrenal tumors with and without MACS.

DESIGN: Cross-sectional study.

SETTING: 14 endocrine secondary/tertiary care centers (recruitment 2011-2016).

PARTICIPANTS: 1305 prospectively recruited persons with benign adrenal tumors.

MEASUREMENTS: Cortisol excess was defined by clinical assessment and the 1mg-overnight dexamethasone suppression test (serum cortisol <50 nmol/L: non-functioning adrenal tumor [NFAT]; 50-138 nmol/L: possible MACS [MACS-1]; >138 nmol/L and absence of typical clinical Cushing's syndrome [CS] features: definitive MACS [MACS-2]). Net steroid production was assessed by multi-steroid profiling of 24-hour urine by tandem mass spectrometry.

RESULTS: Of the 1305 participants, 49.7% had NFAT (n=649; 64.1% women), 34.6% MACS-1 (n=451; 67.2% women), 10.7% MACS-2 (n=140; 73.6% women), and 5.0% CS (n=65; 86.2% women). Prevalence and severity of hypertension were higher in MACS-2 and CS than NFAT (adjusted prevalence ratios (aPRs) for hypertension: MACS-2 1.15 [95%CI 1.04-1.27], CS 1.37 [95%CI 1.16-1.62]; aPR for use of ≥3 anti-hypertensives: MACS-2 1.31 [95%CI 1.02-1.68], CS 2.22 [95%CI 1.62-3.05]). Type 2 diabetes was more prevalent in CS than NFAT (aPR 1.62 [95%CI 1.08-2.42]), and more likely to require insulin therapy in MACS-2 (aPR 1.89 [95%CI 1.01-3.52]) and CS (aPR 3.06 [95%CI 1.60-5.85]). Urinary multi-steroid profiling revealed an increase in

glucocorticoid excretion from NFAT over MACS-1 and MACS-2 to CS whilst androgen excretion decreased.

LIMITATIONS: Cross-sectional design, selection bias possible.

CONCLUSION: MACS is a cardiometabolic risk condition that predominantly affects women and warrants regular assessment for hypertension and type 2 diabetes.

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INTRODUCTION

Adrenal masses are discovered in approximately 5% of cross-sectional imaging studies (1, 2). Benign adrenal tumors are the most common underlying entity; in the largest prospective study to date, ENSAT EURINE-ACT (3), they represented 1513 (89.7%) of 1686 incidentally discovered adrenal masses. Benign adrenal masses can be non-functioning adrenal tumors (NFAT) or autonomously overproduce steroids, most frequently cortisol. Clinically overt cortisol excess, Cushing's syndrome (CS), usually presents with typical clinical signs including proximal myopathy and purple striae (4). CS is rare but potentially life-threatening due to the metabolically adverse consequences of cortisol excess, including type 2 diabetes, hypertension, and dyslipidemia, the main drivers of increased cardiovascular mortality in the affected persons (4, 5). Mild autonomous cortisol secretion (MACS), previously also termed subclinical CS, is regularly diagnosed in persons with benign adrenal tumors. MACS is defined by failure to suppress serum cortisol sufficiently after overnight administration of 1mg dexamethasone (6), but in the absence of the typical clinical signs of cortisol excess. Previous case series identified MACS in up to 35% of persons with benign adrenal tumors, making it the most common hormonal abnormality observed in this population (6, 7). However, while CS is a well-established cause of increased cardiometabolic morbidity and mortality, the evidence regarding the impact of MACS on cardiometabolic disease risk is scarce and heterogeneous. In a recent systematic review and meta-analysis of studies reporting on the prevalence of cardiometabolic comorbid conditions in persons with NFAT and MACS (8), hypertension was the most common occurrence (64.0% in MACS vs. 58.2% in NFAT). Persons with MACS were more likely to present with prediabetes (50.0% vs. 14.4%) and type 2 diabetes (28.1% vs. 14.4%), while the prevalence of dyslipidemia was at a similar level in MACS and NFAT (approximately 34%). However, evidence regarding the

cardiometabolic risk of persons with MACS is almost exclusively derived from observational studies of small sample size, thereby limiting the interpretation of the results.

Here we report a cross-sectional study investigating the clinical characteristics, cardiometabolic burden, and urinary steroid excretion in 1305 prospectively recruited persons with benign adrenal tumors and different degrees of cortisol excess.

METHODS

Subject selection

Persons with benign adrenal tumors were drawn from the ENSAT EURINE-ACT study (3), which had prospectively recruited adults (≥18 years) with newly diagnosed adrenal tumors >1cm from 2011 to 2016 through 14 secondary and tertiary care centers with expertise in the management of adrenal tumors in 11 countries, participating in the European Network for the Study of Adrenal Tumors (ENSAT; www.ensat.org). We included all EURINE-ACT participants who were diagnosed with benign adrenocortical adenomas and had undergone standardized endocrine assessment for exclusion of cortisol excess (9, 10), with measurement of endocrine parameters carried out in the recruitment center. We excluded participants with confirmed primary aldosteronism diagnosed according to current guidelines (11) and participants with cortisol excess due to bilateral macronodular adrenal hyperplasia. We included 1305 (82%) of 1588 otherwise eligible persons with benign adrenal tumors, as 283 had no available results for the 1-mg overnight dexamethasone suppression test (1mg-DST) that is required for the diagnosis of MACS (**Fig. 1**).

In accordance with recent guidelines (6), we defined the presence of MACS as failure to suppress morning serum cortisol concentration to <50 nmol/L after administration of 1mg dexamethasone orally at 11 pm the preceding night (1mg-DST) in the absence of clinical features indicative of CS

(e.g. proximal myopathy, moon face, dorsocervical and supraclavicular fat pads, purple striae). Persons with MACS were further subdivided into MACS-1 (possible autonomous cortisol secretion; serum cortisol in the 1mg-DST 50-138 nmol/L) and MACS-2 (definitive autonomous cortisol secretion; serum cortisol in the 1mg-DST >138 nmol/L) (6). Persons with current or recent (<6 months) intake of drugs known to alter steroid synthesis or metabolism were excluded. All centers had ethical approval for pseudonymized phenotype recording in the online ENSAT database and all participants of the EURINE-ACT study provided written informed consent.

We used the information available at the time of adrenal tumor diagnosis (baseline assessment). Variables obtained through the online ENSAT database included demographic data (sex, age, body mass index, BMI), tumor characteristics (maximum diameter and location), information about cardiometabolic morbidity (hypertension, dysglycemia, dyslipidemia), and endocrine test results (adrenocorticotropic hormone, ACTH; serum dehydroepiandrosterone sulfate, DHEAS; 24-hour urinary free cortisol, UFC). We then asked each site to review the available information against their local databases to obtain any variables that were missing in the online ENSAT database (for details see **Appendix Table 1**).

Definitions of cardiometabolic outcomes

We calculated the prevalence of hypertension, prediabetes, type 2 diabetes, and dyslipidemia considering the clinical information available at the time of adrenal tumor diagnosis. We also identified subjects with a more severe clinical phenotype, specifically those with hypertension treated with ≥3 anti-hypertensives and those requiring insulin to manage their type 2 diabetes (for details see **Appendix Table 1**).

Hypertension: Participants were considered as having hypertension if they had a doctor diagnosis or if they were prescribed medications for hypertension.

Treatment with ≥3 anti-hypertensives: Participants with hypertension were chosen for a subgroup analysis to study prescription of ≥3 antihypertensives as an outcome, in line with established American Heart Association criteria (12).

Glucose metabolism status: Participants were considered as having type 2 diabetes if they had a doctor diagnosis or if they were prescribed antidiabetic medications. Prediabetes and type 2 diabetes were also diagnosed based on glycated hemoglobin results according to American Diabetes Association criteria (13).

Type 2 diabetes requiring insulin: Participants with type 2 diabetes were chosen for a subgroup analysis to study insulin therapy as an outcome.

Dyslipidemia: The prescription of lipid-lowering agents was considered as a proxy for dyslipidemia. We only considered subjects taking lipid-lowering agents other than for secondary cardiovascular prevention, after excluding those with a history of stroke, cerebral hemorrhage, cerebral thrombosis, ischemic heart disease, or angina, in line with American College of Cardiology/American Heart Association criteria (14).

Urine multi-steroid profiling

Each study participant provided a 24-hour urine sample that was sent for centralized measurement at the Steroid Metabolome Analysis Core, Institute of Metabolism and Systems Research, Birmingham, UK. Multi-steroid profiling was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with quantification of the 24-hour urinary excretion of 16 distinct steroid metabolites (**Appendix Table 2** and **Appendix Fig.1**), as previously described (3). Multi-steroid profiling results in persons with MACS-1, MACS-2, and CS were compared to those with NFAT.

Statistical analysis

Poisson regression with robust variance (15) was fitted to obtain crude and adjusted prevalence ratios (PR) of hypertension, prediabetes, type 2 diabetes, and dyslipidemia in persons with MACS-1, MACS-2, and CS using NFAT as the reference group. The models were adjusted for age, sex, and BMI. In order to provide prevalence ratios using Poisson models, the categorical glucose metabolism outcome variable was replaced with two separate binary outcomes: (a) dysglycemia (combination of pre-diabetes and type 2 diabetes – subjects with NFAT and normal glucose metabolism were used as the reference) and (b) type 2 diabetes (the combined group of subjects with NFAT and with either pre-diabetes or normal glucose metabolism was used as the reference). In sub-groups of subjects with hypertension and type 2 diabetes, Poisson regression models were fitted to estimate the crude and adjusted PRs of treatment with ≥3 anti-hypertensives and insulin use, respectively. Missing data for the clinical outcomes were replaced using multiple imputation using chained equations through logistic models with the following covariates: age, sex, and BMI category. Resistant hypertension, type 2 diabetes and insulin treatment were imputed within a conditional sample of subjects with hypertension, dyslipidemia, and type 2 diabetes, respectively. Outside these conditional samples, missing values for these variables were replaced with the conditional constant (0/absent).

Associations between continuous outcomes, including 24-hour urine steroid excretion, were determined by linear regression after log-transformation of all outcomes to reduce skewness in the dataset. Associations between the log-transformed outcome and the variable of interest were reported as sympercents (16) and all models were adjusted for age, sex, and BMI. Statistical analyses were carried out using Stata Statistical Software: Release 16 (College Station, TX: StataCorp LLC) and GraphPad Prism 9 (San Diego, CA: GraphPad Software Inc.).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had access to all the data and had final responsibility for the decision to submit for publication.

RESULTS

Clinical and endocrine characteristics

Between 2011 and 2016, 1305 persons with newly diagnosed non-aldosterone producing adenomas underwent a 1mg-DST and were prospectively assessed for clinical signs of cortisol excess (**Fig. 1**, **Appendix Table 3**). Less than half of them achieved normal suppression of serum cortisol after the 1mg-DST (NFAT n=649, 49.7%). The vast majority of those with abnormal results lacked the distinctive clinical features of overt cortisol excess (MACS-1, n=451 [34.6%]; MACS-2, n=140 [10.7%]), while 65 (5.0%) were diagnosed with clinically overt CS including 37 incidentally discovered cases. Women represented 67.3% of the subjects included in the study and the female predominance was most pronounced in MACS-2 (73.6%) and CS (86.2%) (**Table 1**). The median age at the time of adrenal tumor diagnosis was 60 years (interquartile range 52-67 years). Subjects with MACS were older than those with NFAT (**Fig. 2A**). By contrast, CS was diagnosed at a younger age (median 48 years, interquartile range 38-60 years) (**Table 1**). Subjects with abnormal 1mg-DST results had larger adrenal tumors, with over half of those with tumors >2 cm failing to suppress serum cortisol during the 1mg-DST (**Fig. 2B**).

Plasma ACTH was negatively associated with 1mg-DST results (**Appendix Table 4**), which was reflected in a progressive decrease in ACTH from MACS-1 over MACS-2 to CS (**Table 1, Fig. 2C**). Serum DHEAS had a similar trend, but the differences among groups were less pronounced (**Appendix Table 4, Fig. 2D**).

Persons with MACS were almost twice as likely to present with bilateral tumors than persons with NFAT (30.1% vs. 16.5%) (**Table 1**). Persons with bilateral tumors had abnormal 1mg-DST results in 62.3% and presented with larger adrenal masses (the maximum diameter of the larger adrenal mass was considered), lower plasma ACTH, and higher 24-hour UFC (**Appendix Table 5**).

Cardiometabolic disease burden

In comparison to NFAT, subjects with MACS-2 and CS showed higher prevalence of hypertension (age-, sex-, and BMI-adjusted prevalence ratios [aPRs] 1.15 [95%CI 1.04-1.27] and 1.37 [95%CI 1.16-1.62], respectively) (**Table 2, Fig. 3A**) and more often required ≥3 anti-hypertensives, increasing with the degree of cortisol excess (MACS-2 aPR 1.31 [95%CI 1.02-1.68] and CS aPR 2.22 [95%CI 1.62-3.05]) (**Table 2, Fig. 3B**).

The prevalence of type 2 diabetes was increased in subjects with CS (aPR 1.62 [95%CI 1.08-2.42]). In a subgroup analysis of persons with type 2 diabetes, both MACS-2 and CS more often required insulin treatment (aPR 1.89 [95%CI 1.01-3.52] and 3.06 [95%CI 1.60-5.85], respectively) (**Table 2, Fig. 3B**).

The prevalence of dyslipidemia did not differ from NFAT in MACS and CS.

None of the available clinical or biochemical characteristics (such as tumor diameter, 1mg-DST results considered as a continuous variable, plasma ACTH, serum DHEAS, and 24-hour UFC) correlated in a clinically meaningful way with the presence of cardiometabolic disease in the EURINE-ACT study participants (**Appendix Table 6**).

Patients with bilateral adrenal tumors more often required ≥3 anti-hypertensives (43.4% vs. 35.2% in unilateral tumors; aPR 1.28 [95%CI 1.06-1.55]) and were more frequently diagnosed with dysglycemia (58.3% vs. 49.7%; aPR 1.15 [95%CI 1.02-1.31]) (**Appendix Table 5**). When we

238 further stratified these observations according to the 1mg-DST results, only patients with bilateral
239 tumors and MACS had an increased cardiometabolic burden (**Appendix Table 5**).

240 **Urinary steroid excretion**

241 When compared to NFAT, persons with MACS-1, MACS-2, and CS showed a gradual decrease
242 in the 24-hour urinary excretion of androgen metabolites (androsterone, etiocholanolone,
243 dehydroepiandrosterone [DHEA]) and of pregnenetriol (5-PT), the metabolite of the immediate
244 DHEA precursor 17-hydroxypregnenolone, (**Table 3**). Conversely, we observed a progressive
245 increase in the 24-hour urinary excretion of cortisol and tetrahydro-11-deoxycortisol (THS), the
246 metabolite of the immediate cortisol precursor 11-deoxycortisol. In MACS-2 and CS, the excretion
247 of cortisone was also increased (**Table 3**).

248 **DISCUSSION**

249 In this cross-sectional study, we showed that persons with benign adrenal tumors diagnosed with
250 MACS-2 and adrenal CS had an increased prevalence and severity of hypertension as compared
251 to NFAT. Persons with adrenal CS were also more likely to have a diagnosis of type 2 diabetes
252 and persons with MACS-2 and CS who had type 2 diabetes more often required insulin therapy to
253 achieve adequate glycemic control. Our data demonstrate that persons with MACS-2 carry an
254 increased cardiometabolic burden similar to that observed in CS, even if they do not display typical
255 features of clinically overt cortisol excess. We also show progressive changes in steroid excretion
256 in all four adrenal tumor subgroups, with decreased androgen and increased glucocorticoid
257 precursor excretion already present in persons with NFAT and increased glucocorticoid excretion
258 in MACS-1.

These findings were generated utilizing the largest ever prospectively recruited group of persons with benign adrenal tumors, participants of the ENSAT EURINE-ACT study (3). We classified subjects into four subgroups, NFAT, MACS-1, MACS-2, and CS, based on 1mg-DST results and clinical presentation, according to the criteria defined in the 2016 European Society of Endocrinology/ENSAT guidelines on adrenal incidentalomas (6).

Increased cardiometabolic risk is a well-established feature of clinically overt CS, while the evidence regarding a metabolically adverse impact of MACS has been limited by small study sizes and heterogeneous definitions of diagnosis and clinical outcomes (8). However, a picture of increased cardiometabolic disease burden and frailty in persons with MACS has emerged from previous studies (8, 17-21). Our data demonstrate in a large prospective group that failure to suppress serum cortisol in the 1mg-DST increased the prevalence of cardiometabolic disease in persons with MACS-2 and CS. Though cardiometabolic disease burden was not increased in MACS-1, urinary multi-steroid profiling by mass spectrometry demonstrated decreased androgen excretion and increased excretion of cortisol. Our steroid data suggest that NFAT, MACS-1 and MACS-2 represent a gradually progressive continuum, which is also supported by the fact that approximately 9% of subjects with NFAT develop MACS over time (8). To explore this further, we stratified the EURINE-ACT NFAT group at a more granular level according to their 1mg-DST result, demonstrating an increased cardiometabolic burden with each 10 nmol/L increment in serum cortisol in the 1-mg DST (**Appendix Fig. 2**). We speculate that a subgroup of subjects with NFAT may have underlying autonomous cortisol secretion that is not detected when applying the current diagnostic criteria for cortisol excess, namely the 1mg-DST.

In our study of 1305 persons with benign adrenal tumors, 45.3% fulfilled the diagnostic criteria for MACS according to 1mg-DST results. The prevalence of MACS in our study is higher than

previously reported, though direct comparison is hampered because of the heterogeneous approaches to the definition of MACS prior to the 2016 consensus (6), including different DST protocols and cut-offs and combination of DST results with other parameters such as ACTH, 24-hour urinary free cortisol excretion, and salivary cortisol (8). However, a retrospective study in 198 persons with adrenal incidentalomas diagnosed MACS in 34.8% of cases according to the same diagnostic criteria we used in this study (7). A very recent study (26) reported increased mortality in patients with adrenal incidentaloma who had a serum cortisol of 83 nmol/L or higher in the 1-mg DST, which increased in persons with a post-dexamethasone cortisol of 138 nmol/L or higher, i.e. MACS-2, adding further evidence to a continuum of gradually increasing cardiometabolic burden.

Persons included in the study were predominantly women and more than half of those were over the age of 60 at the time of adrenal tumor diagnosis; the demographics of our prospectively recruited study participants resemble those of large retrospective studies on adrenal incidentalomas (27-29). We also found that the proportion of women increased with the degree of cortisol excess, corroborating previous observations that cortisol excess predominantly affects women (7, 30).

Previous smaller studies found that subjects with bilateral and larger tumors are more likely to be diagnosed with MACS (31, 32). We found in our much larger study that individuals with MACS and bilateral tumors were more frequently diagnosed with dysglycemia and prescribed ≥3 anti-hypertensives. We did not include subjects with cortisol excess due to primary bilateral macronodular adrenal hyperplasia in whom this diagnosis had been ascertained by typical imaging findings, positive family history and/or documentation of gene mutations in germline DNA. Primary bilateral macronodular adrenal hyperplasia is a very rare cause of hypercortisolism that

304 regularly presents with MACS. Thus, some further cases of undiagnosed primary bilateral
305 macronodular adrenal hyperplasia in our study cannot be ruled out (33).
306 Strengths of our study include the prospective recruitment, the large sample size, the standardized
307 classification of different degrees of cortisol excess, and the 24-hour urine multi-steroid profiling
308 carried out by a centralized tandem mass spectrometry assay. To our knowledge, this is the largest
309 prospective study to establish the extent of the cardiometabolic disease burden in persons with
310 benign adrenal tumors with and without cortisol excess.
311 Weaknesses of our study include its cross-sectional design, precluding the collection of
312 longitudinal data about cardiometabolic outcomes, and the absence of a comparator group of
313 persons who also underwent imaging under similar circumstance but without being diagnosed with
314 an adrenal tumor. Routine biochemical assessments were not standardized across participating
315 centers and not measured in a centralized fashion. However, while we acknowledge that results
316 for 24h UFC, plasma ACTH, and serum DHEAS should be interpreted with caution, inter-assay
317 variability of serum cortisol measurements is unlikely to affect the cut-off of 50 nmol/L used to
318 diagnose MACS (34). We could not include 283 (18%) of the overall 1588 eligible ENSAT
319 EURINE-ACT participants with benign adrenal tumors in this study as they had no recorded 1mg-
320 DST results at the time of adrenal tumor diagnosis. Therefore, a degree of selection bias is possible
321 and should be taken into account when interpreting the high prevalence of MACS in our study.
322 However, 213 of the 283 persons excluded due to missing 1-mg DST results were recruited by the
323 four German centers who initially did not test their participants with the 1-mg DST, which makes
324 a relevant impact of selection bias unlikely.
325 In conclusion, our study demonstrates that MACS-2 and CS are clinically highly relevant
326 metabolic risk conditions, which predominantly affect women and come with increased prevalence

of hypertension and type 2 diabetes, and present with a more severe clinical phenotype than persons with NFAT. Affected individuals should receive a comprehensive cardiovascular risk assessment at the time of adrenal tumor diagnosis, with particular attention to blood pressure and glucose metabolism. Future studies are required to further dissect cardiometabolic risk in MACS-1 and NFAT and to identify biomarkers suitable for prediction of metabolic risk and assessment of risk-mitigating interventions.

Contributors

A.P. and W.A. designed the study, with contributions from I.B., V.C., A.S. and K.N. A.P. contributed to data collection, data analysis, data interpretation, and co-wrote the manuscript. A.S. and K.N. performed statistical analyses and co-wrote the manuscript. A.J.S. reviewed the statistical analyses and edited the manuscript. A.E.T., L.C.G, and C.J. carried out the steroid excretion analysis by mass spectrometry and edited the manuscript. I.B., V.C. S.T., K.L., M.M., D.A.D, I.D.P., G.R, L.V.M., T.D., M.B., M.W.O.R., T.B., C.Z., T.D., A.D., M.A., A.K., D.L., J.R.M., M.Q., G.A.U., M.C.D., F.B., A.T., M.F., M.I., M.T., D.K., W.F.Y. Jr, K.N.M., U.A., and D.A.V. contributed to data collection and edited the manuscript. W.A. contributed to data analysis and data interpretation, co-wrote the manuscript, and supervised all steps of the conduct of the study.

Declaration of interests

The authors do not declare a conflict of interest in relation to this work.

Reproducible Research Statement

Protocol: not available.

Computer Code: available upon request to be sent to the corresponding author.

Data: we have provided a detailed description of the statistical analysis undertaken. We may share de-identified, individual participant-level data that underlie the results reported in this article on receipt of a request detailing the study hypothesis and statistical analysis plan; all requests should be sent to the corresponding author.

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Figure legends

Figure 1: Flow-chart of patient inclusion.

Figure 2: Endocrine assessment results.

Distribution of serum cortisol (median, range) after the 1mg-overnight dexamethasone suppression test (1mg-DST) according to age (A) and maximum tumor diameter (B) in subjects without clinical signs of Cushing's syndrome. Plasma ACTH (C) and serum DHEAS (D) measured in these subjects are shown as boxplots, with boxes representing median and interquartile range, and whiskers representing 5^{th} to 95^{th} centile. The dotted lines in panels A and B represent the cortisol cut-offs that separate non-functioning adrenal tumors (NFAT) from possible mild autonomous cortisol secretion (MACS-1) and definitive mild autonomous cortisol secretion (MACS-2).

Figure 3: Impact of different degrees of cortisol excess on the cardiometabolic risk.

Poisson regression models with robust variance exploring the cardiometabolic risk of patients with mild autonomous cortisol secretion (MACS) and adrenal Cushing's syndrome (CS) in comparison to patients with non-functioning adrenal tumors (NFAT). Age-, sex-, and BMI-adjusted prevalence ratios and 95% confidence intervals are reported. Panel A: adjusted prevalence ratios for hypertension, dysglycemia, type 2 diabetes, and dyslipidemia. Panel B: adjusted prevalence ratios for treatment with ≥3 anti-hypertensives (in subjects with hypertension) and insulin (in subjects with type 2 diabetes).

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Table 1: Demographics, radiological, and biochemical parameters of EURINE-ACT participants with benign adrenocortical adenomas who underwent assessment for cortisol excess.

Values are reported as median (interquartile range), unless otherwise stated. Abbreviations: 1mg-DST, 1mg-overnight dexamethasone suppression test; ACTH, adrenocorticotropic hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; UFC, urinary free cortisol. NFAT, non-functioning adrenal tumors; MACS, mild autonomous cortisol secretion; CS, Cushing's syndrome.

	Overall cohort (n=1305)	**NFAT (n=649)**	**MACS-1 (n=451)**	**MACS-2 (n=140)**	**Adrenal CS (n=65)**
Women, n (%)	878 (67.3)	416 (64.1)	303 (67.2)	103 (73.6)	56 (86.2)
Age (years)	60 (52-67)	58 (51-65)	64 (56-71)	63 (54-69)	48 (38-60)
BMI (kg/m^2)	29.0 (25.4-33.4)	29.4 (25.8-33.9)	28.8 (25.1-33.1)	28.6 (24.0-32.9)	28.7 (25.2-31.7)
- Lean (BMI <25), n (%)	292 (22.9)	129 (20.6)	106 (23.8)	42 (30.0)	15 (23.4)
- Overweight (BMI 25-30), n (%)	429 (33.6)	202 (32.2)	160 (35.9)	41 (29.3)	26 (40.6)
- Obesity (BMI ≥30), n (%)	556 (43.5)	296 (47.2)	180 (40.4)	57 (40.7)	23 (35.9)
Maximum tumor diameter (mm)*	26 (19-36)	22 (16-30)	30 (23-38)	32 (24-44)	30 (26-38)
Tumor location:					
- Left adrenal, n (%)	616 (47.2)	323 (49.8)	196 (43.5)	63 (45.0)	34 (52.3)
- Right adrenal, n (%)	391 (30)	219 (33.7)	119 (26.4)	35 (25.0)	18 (27.7)
- Bilateral, n (%)	298 (22.8)	107 (16.5)	136 (30.2)	42 (30.0)	13 (20.0)
Serum cortisol in the 1mg-DST (nmol/L)	51 (33-92)	33 (27-41)	72 (60-93)	200 (165-283)	435 (271-574)
Plasma ACTH (pmol/L)	2.38 (1.34-3.96)	3.00 (1.89-4.89)	2.20 (1.30-3.43)	1.43 (0.55-2.60)	0.66 (0.55-1.43)
Serum DHEAS (µmol/L)	1.40 (0.70-2.70)	1.90 (1.00-3.40)	1.14 (0.65-2.19)	0.83 (0.40-1.85)	0.54 (0.23-1.58)
24-hour UFC (nmol/24h)	132 (66-226)	127 (66-207)	141 (69-229)	130 (47-207)	472 (149-1319)

* For bilateral tumors, the maximum diameter of the larger adrenal mass was considered.

Table 2: Cardiometabolic disease burden in benign adrenocortical tumors with different degrees of cortisol excess.
Series of Poisson regression model with robust variance was employed to investigate the cardiometabolic burden of 1305 persons from the EURINE-ACT study. Unadjusted and adjusted prevalence ratios are reported; adjusted models included age, sex and BMI as covariates. Missing outcome data were replaced using multiple imputation using chained equations with age, sex, and BMI as covariates. Imputations for treatment with ≥3 anti-hypertensives, type 2 diabetes and insulin treatment were conditional to patients with hypertension, dysglycemia and type 2 diabetes, respectively. Abbreviations: NFAT, non-functioning adrenal tumors; MACS, mild autonomous cortisol secretion; CS, Cushing's syndrome.

	NFAT (n=649)	MACS-1 (n=451)	MACS-2 (n=140)	Adrenal CS (n=65)
Hypertension, n (%)	416 (64.1)	339 (75.2)	107 (76.4)	47 (72.3)
Prevalence ratios (95% CI)		1.17 (1.08-1.27)	1.19 (1.07-1.33)	1.13 (0.96-1.33)
Adjusted prevalence ratios (95% CI)		1.07 (0.99-1.16)	1.15 (1.04-1.27)	1.37 (1.16-1.62)
Treatment with ≥3 anti-hypertensives, n (%)[*]	142 (34.3)	132 (39.1)	46 (43.0)	27 (57.4)
Prevalence ratios (95% CI)		1.14 (0.94-1.38)	1.25 (0.97-1.63)	1.68 (1.26-2.23)
Adjusted prevalence ratios (95% CI)		1.12 (0.92-1.37)	1.31 (1.02-1.68)	2.22 (1.62-3.05)
Dysglycemia, n (%)[†]	321 (49.5)	243 (53.9)	77 (55.0)	32.4 (49.8)
Prevalence ratios (95% CI)		1.09 (0.97-1.23)	1.11 (0.91-1.35)	1.01 (0.75-1.34)
Adjusted prevalence ratios (95% CI)		1.00 (0.89-1.13)	1.07 (0.89-1.29)	1.23 (0.92-1.65)
Type 2 diabetes, n (%)	171 (26.4)	145 (32.2)	47 (33.7)	20 (31.5)
Prevalence ratios (95% CI)		1.22 (1.00-1.49)	1.27 (0.95-1.72)	1.19 (0.80-1.78)
Adjusted prevalence ratios (95% CI)		1.10 (0.91-1.33)	1.23 (0.92-1.64)	1.62 (1.08-2.42)
Insulin treatment, n (%)[‡]	29 (16.9)	37 (25.8)	15 (32.6)	8 (41.0)
Prevalence ratios (95% CI)		1.53 (0.92-2.56)	1.94 (1.05-3.59)	2.44 (1.25-4.76)
Adjusted prevalence ratios (95% CI)		1.45 (0.83-2.52)	1.89 (1.01-3.52)	3.06 (1.60-5.85)
Dyslipidemia, n (%)	187 (28.8)	161 (35.7)	50 (35.9)	10 (15.7)
Prevalence ratios (95% CI)		1.24 (1.04-1.47)	1.24 (0.96-1.60)	0.54 (0.30-0.97)
Adjusted prevalence ratios (95% CI)		1.08 (0.91-1.29)	1.18 (0.91-1.52)	0.76 (0.43-1.32)

[*] Considering only subjects with a diagnosis of hypertension (n=909).
[†] Dysglycemia includes subjects with pre-diabetes and type 2 diabetes.
[‡] Considering only subjects with a diagnosis of type 2 diabetes (n=383).

Table 3: 24-hour steroid metabolite excretion in persons with benign adrenocortical tumors and different degrees of cortisol excess. Steroid metabolites measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) of 24-h urine collected by persons with non-functioning adrenal tumors (NFAT), mild autonomous cortisol secretion (MACS-1 and MACS-2 listed separately), and adrenal Cushing's syndrome (CS). Values are reported as median (interquartile range) (μg/24h). The urinary excretion of each steroid metabolite in persons with MACS-1, MACS-2, and adrenal CS was compared to those with NFAT using a linear regression model with the log-transformed steroid metabolite as the outcome (adjusted for age, sex and BMI). Associations between the log-transformed outcome and the variable of interest are reported as sympercents.

		NFAT (n=649)	MACS-1 (n=451)	MACS-2 (n=140)	Adrenal CS (n=65)
An	median (IQR) 24-h excretion (μg/24h)	577 (258-1034)	290 (127-642)	191 (97-474)	167 (61-314)
	% change compared to NFAT (95% CI)		-38 (-51, -25)	-69 (-88, -50)	-115 (-142, -88)
Etio	median (IQR) 24-h excretion (μg/24h)	540 (264-1073)	364 (167-747)	329 (144-689)	331 (221-725)
	% change compared to NFAT (95% CI)		-26 (-38, -13)	-45 (-63, -26)	-39 (-65, -13)
DHEA	median (IQR) 24-h excretion (μg/24h)	26 (22-54)	22 (22-30)	22 (22-24)	22 (22-22)
	% change compared to NFAT (95% CI)		-17 (-27, -8)	-34 (-49, -20)	-56 (-76, -36)
5-PT	median (IQR) 24-h excretion (μg/24h)	92 (49-177)	63 (43-126)	56 (43-101)	71 (43-115)
	% change compared to NFAT (95% CI)		-16 (-25, -7)	-30 (-43, -16)	-28 (-47, -10)
5-PD	median (IQR) 24-h excretion (μg/24h)	81 (55-144)	64 (55-106)	56 (55-105)	89 (55-158)
	% change compared to NFAT (95% CI)		-9 (-17, -1)	-18 (-30, -7)	7 (-9, 24)
PD	median (IQR) 24-h excretion (μg/24h)	328 (190-597)	281 (157-479)	254 (149-503)	536 (206-813)
	% change compared to NFAT (95% CI)		-8 (-19, 3)	-17 (-33, -0.3)	16 (-7, 39)
PT	median (IQR) 24-h excretion (μg/24h)	333 (179-567)	257 (143-465)	210 (118-452)	222 (145-368)
	% change compared to NFAT (95% CI)		-8 (-17, 1)	-19 (-32, -6)	-26 (-45, -7)
17HP	median (IQR) 24-h excretion (μg/24h)	69 (39-135)	63 (37-127)	51 (32-108)	74 (45-116)
	% change compared to NFAT (95% CI)		6 (-4, 17)	-8 (-24, 7)	-1 (-23, 21)
THS	median (IQR) 24-h excretion (μg/24h)	141 (87-222)	142 (91-239)	177 (90-271)	317 (181-500)
	% change compared to NFAT (95% CI)		9 (0.4, 17)	20 (8, 32)	84 (67, 102)
Cortisol	median (IQR) 24-h excretion (μg/24h)	45 (28-65)	54 (32-82)	57 (33-92)	151 (76-344)
	% change compared to NFAT (95% CI)		23 (15, 32)	33 (21, 46)	131 (113, 148)

THF	median (IQR) 24-h excretion (µg/24h)	1362 (914-2011)	1460 (888-2165)	1563 (998-2293)	3163 (1466-6425)
	% change compared to NFAT (95% CI)		10 (-1, 22)	14 (-3, 30)	92 (69, 116)
5α-THF	median (IQR) 24-h excretion (µg/24h)	568 (287-986)	543 (267-947)	506 (206-823)	642 (315-1088)
	% change compared to NFAT (95% CI)		5 (-6, 15)	-5 (-21, 11)	30 (7, 52)
11β-OH-Et	median (IQR) 24-h excretion (µg/24h)	305 (120-541)	335 (135-613)	413 (156-769)	602 (182-1310)
	% change compared to NFAT (95% CI)		8 (-3, 19)	23 (7, 39)	60 (37, 83)
Cortisone	median (IQR) 24-h excretion (µg/24h)	73 (47-105)	76 (47-108)	82 (49-115)	141 (95-317)
	% change compared to NFAT (95% CI)		6 (-2, 14)	16 (4, 27)	84 (67, 100)
THE	median (IQR) 24-h excretion (µg/24h)	2223 (1457-3409)	2181 (1334-3329)	2323 (1296-3170)	3812 (1939-5865)
	% change compared to NFAT (95% CI)		3 (-6, 12)	0 (-13, 13)	47 (28, 66)
β-cortolone	median (IQR) 24-h excretion (µg/24h)	634 (401-964)	624 (389-989)	658 (341-937)	998 (622-1632)
	% change compared to NFAT (95% CI)		5%(-4, 13)	0 (-13, 13)	54 (35, 73)