Cardiometabolic disease burden and steroid excretion in benign adrenal tumors

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Appendix

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

List of ENSAT EURINE-ACT Investigators who were involved in the data collection and analysis of the original EURINE-ACT study (1)

(listed in alphabetical order by country and institution)

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APPENDIX TABLES

Appendix Table 1: Clinical assessments, methods for data collection and analysis, and definition of clinical outcomes in the ENSAT EURINE-ACT study.

Type of clinical assessments carried out to assess cardiometabolic risk	Nurse- or doctor-led clinical assessment which included:
	• Collection of medical data including:
	  o Demographics.
	  o General health.
	  o Longstanding illness.
	  o Doctor-diagnosed hypertension.
	  o Doctor-diagnosed diabetes.
	  o Prescribed medicines.
	• Anthropometric measurements including height and weight.
	• Blood sample including glycated hemoglobin (carried out at the discretion of the medical professional).
	• Collection of radiological information concerning the newly diagnosed adrenal tumor(s) including:
	  o Maximum tumor diameter.
	  o Tumor location.
	• Laboratory work-up to exclude adrenal steroid excess including:
	  o 1mg-overnight dexamethasone suppression test.*
	  o Adrenocorticotropic hormone.
	  o Serum dehydroepiandrosterone sulfate.
	  o 24-hour urinary free cortisol.
	• Physical exam to exclude signs of adrenal steroid excess.

Clinical and laboratory data analysis and collection	• Anthropometric measurements were carried out at the recruiting centers. Height was measured to the nearest cm. Weight was measured in kg (one decimal place) using clinically validated scales. • Laboratory workup was carried out at the recruiting center according to standardized local protocols which were in agreement with the 2016 European Society of Endocrinology/ENSAT guidelines on adrenal incidentalomas, the Endocrine Society guidelines on Cushing's syndrome, and the Endocrine Society guidelines on primary aldosteronism (2-4). Laboratory measurements were carried out at the clinical laboratory of each recruiting center. • Clinical information was collected through the online ENSAT database. Access to the ENSAT database occurs through a unified, security-driven portal that allows targeted upload of pseudonymized patient data. The principal investigator at each recruiting center was responsible for data entry in the ENSAT database. All data collectors with access to the ENSAT database had to undergo training on how to use the online platform and the same person was responsible at each site for data entry throughout the study. • After recruitment was completed, we asked each site to review the available information against their local databases to obtain any variables that were missing in the online ENSAT database. Any variable that was inconsistent with the rest of the available records was queried and resolved with each site.
Centralized 24-hour urine multi-steroid profiling	• Each study participant provided a 24-hour urine sample, and the volume of the 24-h collection was recorded. • The samples were aliquoted on the day of collection and stored locally at -20°C.

	• The samples were transported on dry ice to the University of Birmingham, UK, for mass spectrometry analysis in the Steroid Metabolome Analysis Core of the Institute of Metabolism and Systems Research, as previously described (1).	
Definitions of clinical outcomes – hypertension	Hypertension was defined as: • Doctor-diagnosed hypertension at the time of the clinical assessment (i.e. the patient presented with a previous diagnosis of hypertension from a medical doctor), or • Treatment with anti-hypertensives at the time of the clinical assessment.	
Definitions of clinical outcomes – treatment with 3 or more anti-hypertensives	The outcome was defined as the combination of: • Diagnosis of hypertension (see criteria above), and • Treatment with three or more anti-hypertensive medications at the time of the clinical assessment.	
Definitions of clinical outcomes – pre-diabetes	The outcome was defined as glycated hemoglobin 5.7-6.4% (39–47 mmol/mol).	
Definitions of clinical outcomes – type 2 diabetes	Diabetes was defined as: • Doctor-diagnosed diabetes at the time of the clinical assessment (i.e. the patient presented with a previous diagnosis of diabetes from a medical doctor), or • Treatment with anti-diabetes medications at the time of the clinical assessment, or • Glycated hemoglobin ≥6.5% (48 mmol/mol) at the time of the clinical assessment. Type 1 diabetes was excluded based on clinical assessment and medical history.	
Definitions of clinical outcomes – type 2 diabetes treated with insulin	The outcome was defined as the combination of: • Diagnosis of type 2 diabetes (see criteria above), and • Treatment with any type of injectable insulin at the time of the clinical assessment.	
Definitions of clinical outcomes – dyslipidemia	The outcome was defined as treatment with lipid-lowering agents other than for secondary cardiovascular prevention at the time of the clinical assessment. Persons on treatment following a major cardiovascular event such as ischemic heart disease and stroke (secondary prevention) were excluded. Blood samples for lipids taken at the time of the clinical assessment were not considered for the diagnosis of dyslipidemia.	
Percentage of subjects for whom the information on clinical outcomes and anthropometric measurements was missing at the time of the clinical assessment†	Hypertension	0.1%
	Treatment with 3 or more anti-hypertensives	0
	Glucose metabolism status	25.6%
	Type 2 diabetes treated with insulin	0
	Dyslipidemia	1.1%
	Body mass index	2.1%
Percentage of subjects for whom the information on biochemical results was missing at the time of the clinical assessment†	1mg-overnight dexamethasone suppression test	0
	Adrenocorticotropic hormone	19.2
	Serum dehydroepiandrosterone sulfate	25.4
	24-hour urinary free cortisol	31.7

* Four German centers did not perform the 1mg-DST in patients lacking clinical signs of overt cortisol excess (Cushing's syndrome) during the initial stages of recruitment (see also Appendix Table 3).

† This information refers to the 1305 patients included in this study.

Appendix Table 2: Nomenclature and origin of the 16 urinary steroid metabolites quantified by liquid chromatography-tandem mass spectrometry.

Abbreviation	Name	Metabolite of
An	Androsterone	Androstenedione, testosterone, 5α-dihydrotestosterone
Etio	Etiocholanolone	Androstenedione, testosterone
DHEA	Dehydroepiandrosterone	DHEA, DHEAS
5-PT	Pregnenetriol	17-hydroxypregnenolone
5-PD	Pregnenediol	Pregnenolone
PD	Pregnanediol	Progesterone
PT	Pregnanetriol	17-hydroxyprogesterone
17HP	17-hydroxypregnanolone	17-hydroxyprogesterone
THS	Tetrahydro-11-deoxycortisol	11-deoxycortisol
F	Cortisol	Cortisol
THF	Tetrahydrocortisol	Cortisol
5α-THF	5α-tetrahydrocortisol	Cortisol
11β-OH-Et	11β-hydroxyetiocholanolone	Cortisol
E	Cortisone	Cortisone
THE	Tetrahydrocortisone	Cortisone
β-cortolone	β-cortolone	Cortisone

Appendix Table 3: Centre-specific distribution of ENSAT EURINE-ACT study participants with benign adrenocortical adenomas categorized according to cortisol excess.

Abbreviations: 1mg-DST, 1mg-overnight dexamethasone suppression test; CS, Cushing's syndrome; MACS, mild autonomous cortisol secretion; NFAT, non-functioning adrenal tumor.

Centre	All EURINE-ACT participants with benign adrenocortical adenomas*, n (%)	EURINE-ACT participants with benign adrenocortical adenomas and available 1mg-DST result†, n (%)	NFAT, n (%)	MACS-1, n (%)	MACS-2, n (%)	CS, n (%)
Athens, Greece	215 (13.5)	206 (15.8)	106 (51.5)	70 (34.0)	17 (8.3)	13 (6.3)
Birmingham, UK	209 (13.2)	196 (15.0)	105 (53.6)	61 (31.1)	23 (11.7)	7 (3.6)
Warsaw, Poland	199 (12.5)	196 (15.0)	100 (51.0)	71 (36.2)	19 (9.7)	6 (3.1)
Rochester, MN, USA	203 (12.8)	175 (13.4)	94 (53.7)	52 (29.7)	20 (11.4)	9 (5.1)
Zagreb, Croatia	116 (7.3)	116 (8.9)	57 (49.1)	42 (36.2)	12 (10.3)	5 (4.3)
Turin, Italy	89 (5.6)	85 (6.5)	38 (44.7)	40 (47.1)	5 (5.9)	2 (2.4)
Belgrade, Serbia	68 (4.3)	68 (5.2)	39 (57.4)	27 (39.7)	2 (2.9)	0 (0)
Würzburg, Germany	103 (6.5)	56 (4.3)	19 (33.9)	18 (32.1)	12 (21.4)	7 (12.5)
Bordeaux, France	57 (3.6)	47 (3.6)	24 (51.1)	16 (34.0)	4 (8.5)	3 (6.4)
Munich, Germany	109 (6.9)	43 (3.3)	17 (39.5)	13 (30.2)	6 (14.0)	7 (16.3)
Galway, Ireland	45 (2.8)	42 (3.2)	27 (64.3)	11 (26.2)	3 (7.1)	1 (2.4)
Bergen, Norway	34 (2.1)	34 (2.6)	6 (17.6)	17 (50.0)	9 (26.5)	2 (5.9)
Berlin, Germany	108 (6.8)	23 (1.8)	7 (30.4)	9 (39.1)	5 (21.7)	2 (8.7)
Dresden, Germany	33 (2.1)	18 (1.4)	10 (55.6)	4 (22.2)	3 (16.7)	1 (5.6)
TOTAL	**1588**	**1305 (100.0)**	**649 (49.7)**	**451 (34.6)**	**140 (10.7)**	**65 (5.0)**

* After exclusion of participants with primary aldosteronism and participants with cortisol excess due to primary bilateral macronodular adrenal hyperplasia.

† These are the EURINE-ACT participants included in this study.

Appendix Table 4: Associations between clinical, radiological, and biochemical parameters of persons with benign adrenocortical tumors and different degrees of cortisol excess.

Percentage changes are reported, derived from the linear regression with log-transformed outcomes. All models were adjusted by age, sex, and BMI. Abbreviations: 1mg-DST, 1mg-overnight dexamethasone suppression test; ACTH, adrenocorticotropic hormone; CS, Cushing's syndrome; DHEAS, dehydroepiandrosterone sulfate; MACS, mild autonomous cortisol secretion; NFAT, non-functioning adrenal tumor; UFC, urinary free cortisol.

OUTCOMES	VARIABLES			
	1mg-DST	Maximum tumor diameter	Plasma ACTH	Serum DHEAS
	Percentage change (95% CI)	Percentage change (95% CI)	Percentage change (95% CI)	Percentage change (95% CI)
All subjects (n=1305)				
Maximum tumor diameter	0.08 (0.06, 0.11)			
Plasma ACTH	-0.28 (-0.33, -0.24)	-0.86 (-1.22, -0.49)		
Serum DHEAS	-0.24 (-0.29, -0.19)	-0.92 (-1.32, -0.53)	3.88 (2.04, 5.72)	
24-hour UFC	0.19 (0.14, 0.24)	0.82 (0.38, 1.26)	-2.01 (-4.20, 0.18)	1.93 (-1.60, 5.46)
After excluding subjects with CS (n=1240)				
Maximum tumor diameter	0.13 (0.10, 0.17)			
Plasma ACTH	-0.30 (-0.36, -0.24)	-0.76 (-1.10, -0.41)		
Serum DHEAS	-0.24 (-0.31, -0.17)	-0.93 (-1.32, -0.55)	3.32 (1.52, 5.13)	
24-hour UFC	0.07 (-0.01, 0.14)	0.71 (0.29, 1.14)	-1.25 (-3.37, 0.88)	4.17 (0.67, 7.68)

Appendix Table 5: Clinical characteristics of ENSAT EURINE-ACT study participants with benign adrenocortical adenomas comparing participants with unilateral vs. those with bilateral adrenocortical tumors.

Persons with Cushing's syndrome were excluded from the analysis because of their low number (n=65). Values are reported as median (interquartile range), unless otherwise stated. The analysis of cardiometabolic outcomes is based on a Poisson regression model (unilateral tumors used as the reference). Results are reported as prevalence ratios and 95% confidence intervals (CI); both unadjusted and age-, sex-, and BMI-adjusted prevalence ratios are reported. Missing cardiometabolic 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: 1mg-DST, 1mg-overnight dexamethasone suppression test; ACTH, adrenocorticotropic hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; MACS, mild autonomous cortisol secretion; NFAT, non-functioning adrenal tumor; UFC, urinary free cortisol.

	NFAT + MACS (n=1240)		**NFAT only (n=649)**		**MACS only (n=591)**	
	Unilateral tumors (n=955)	**Bilateral tumors (n=285)**	**Unilateral tumors (n=542)**	**Bilateral tumors (n=107)**	**Unilateral tumors (n=413)**	**Bilateral tumors (n=178)**
Women, n (%)	631 (66.1)	190 (66.7)	350 (64.6)	66 (61.7)	281 (68.0)	124 (69.7)
Age (years)	61 (52-67)	60 (54-68)	59 (50-65)	58 (53-65)	64 (56-71)	62 (55-70)
BMI (kg/m^2)	29.2 (25.3-33.7)	28.7 (25.4-33.0)	29.6 (25.8-34.1)	28.7 (25.9-33.4)	28.9 (24.7-33.1)	28.7 (25.4-32.4)
- Lean (BMI <25), n (%)	216 (22.6)	61 (21.4)	108 (19.9)	21 (19.6)	108 (26.2)	40 (22.5)
- Overweight (BMI 25-30), n (%)	294 (30.8)	109 (38.2)	163 (30.1)	39 (36.4)	131 (31.7)	70 (39.3)
- Obesity (BMI ≥30), n (%)	420 (44.0)	113 (39.6)	249 (45.9)	47 (43.9)	171 (41.4)	66 (37.1)
Maximum tumor diameter (mm)[*]	25 (18-35)	29 (20-37)	21 (15-30)	23 (18-30)	30 (23-40)	30 (24-40)
1mg-DST (nmol/L)	45 (31-75)	62 (41-100)	33 (27-40)	34 (29-43)	83 (63-135)	85 (65-134)
Plasma ACTH (pmol/L)	2.64 (1.54-4.18)	2.02 (1.29-3.51)	3.07 (2.02-4.89)	2.42 (1.56-4.73)	2.01 (1.15-3.26)	1.83 (1.20-3.00)
Serum DHEAS (µmol/L)	1.44 (0.73-2.81)	1.39 (0.79-2.61)	1.90 (0.97-3.42)	1.90 (1.11-2.84)	1.02 (0.51-2.08)	1.17 (0.68-2.05)
24-hour UFC (nmol/24h)	127 (58-204)	174 (86-254)	124 (63-196)	177 (83-257)	127 (55-213)	168 (94-246)
Hypertension, n (%)	649 (67.9)	213 (74.7)	3413(63.3)	73 (68.2)	306 (74.1)	140 (78.7)
Unadjusted prevalence ratios (95% CI)		1.10 (1.01-1.19)		1.08 (0.93-1.25)		1.06 (0.96-1.17)
Adjusted prevalence ratios (95% CI)		1.08 (1.00-1.17)		1.06 (0.92-1.22)		1.07 (0.98-1.17)
Treatment with ≥3 anti-hypertensives, n (%)[†]	228 (35.2)	92 (43.4)	115 (33.5)	27 (37.7)	113 (37.1)	65 (46.4)
Unadjusted prevalence ratios (95% CI)		1.23 (1.02-1.49)		1.12 (0.80-1.57)		1.25 (0.98-1.59)
Adjusted prevalence ratios (95% CI)		1.28 (1.06-1.55)		1.21 (0.87-1.69)		1.28 (1.01-1.62)
Dysglycemia, n (%)	475 (49.7)	166 (58.3)	262 (48.4)	58 (54.7)	212 (51.4)	108 (60.4)
Unadjusted prevalence ratios (95% CI)		1.17 (1.03-1.34)		1.28 (0.91-1.39)		1.17 (0.99-1.39)
Adjusted prevalence ratios (95% CI)		1.15 (1.02-1.31)		1.09 (0.89-1.34)		1.20 (1.01-1.41)
Type 2 diabetes, n (%)	283 (29.6)	81 (28.3)	146 (26.9)	26 (24.0)	137 (33.3)	55 (30.8)

Unadjusted prevalence ratios (95% CI)		0.95 (0.76-1.19)		0.89 (0.59-1.35)		0.92 (0.70-1.22)
Adjusted prevalence ratios (95% CI)		0.94 (0.76-1.16)		0.86 (0.58-1.28)		0.95 (0.73-1.24)
Insulin treatment, n (%)‡	68 (23.9)	14 (17.7)	27 (18.9)	1 (5.8)	40 (29.1)	13 (23.3)
Unadjusted prevalence ratios (95% CI)		0.74 (0.42-1.30)		0.29 (0.05-1.79)		0.80 (0.45-1.44)
Adjusted prevalence ratios (95% CI)		0.75 (0.43-1.30)		0.32 (0.05-1.91)		0.82 (0.46-1.47)
Dyslipidemia, n (%)	303 (31.7)	95 (33.6)	155 (28.5)	32 (30.4)	148 (35.9)	63 (35.4)
Unadjusted prevalence ratios (95% CI)		1.06 (0.87-1.28)		1.06 (0.77-1.46)		0.99 (0.78-1.25)
Adjusted prevalence ratios (95% CI)		1.04 (0.86-1.25)		1.02 (0.75-1.39)		1.01 (0.80-1.28)

* For bilateral tumors, the maximum diameter of the larger adrenal mass was considered.
† Considering only subjects with a diagnosis of hypertension.
‡ Considering only subjects with a diagnosis of type 2 diabetes.

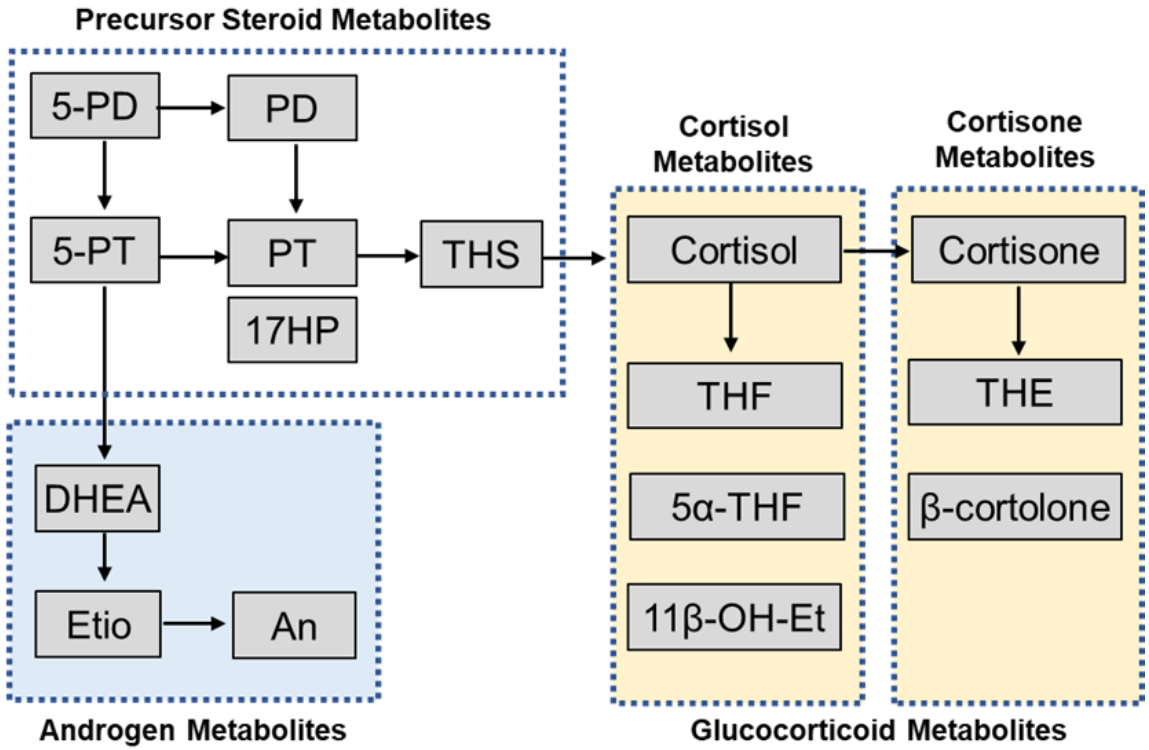
Appendix Table 6: Poisson regression models to investigate the relationship between cardiometabolic disease and clinical, radiological, and biochemical parameters.

Relationships reported as prevalence ratios and 95% confidence intervals (CI). Unadjusted and adjusted prevalence ratios are reported; adjusted models included age, sex and BMI as covariates. All continuous exposure variables (1mg-DST, tumor diameter, plasma ACTH, serum DHEAS, and 24-hour UFC) were scaled to clinically meaningful incremental units, as indicated in the "Variables" column. 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: 1mg-DST, 1mg-overnight dexamethasone suppression test; ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone sulfate; UFC: urinary free cortisol.

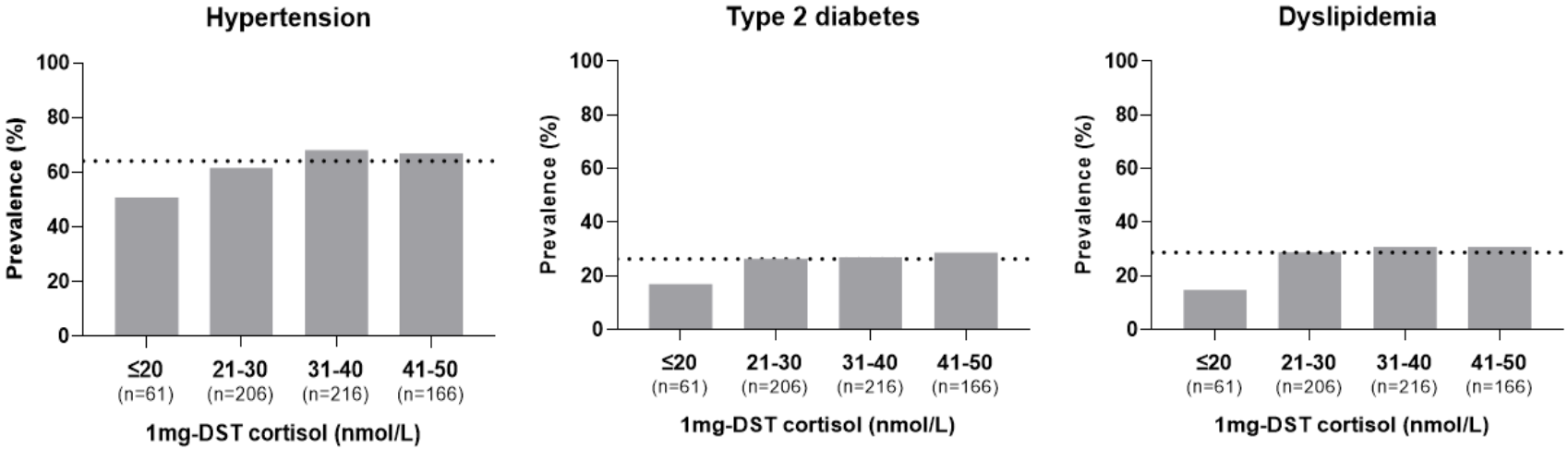
VARIABLES		Hypertension	Treatment with ≥3 anti-hypertensives	Dysglycemia	Type 2 diabetes	Insulin treatment	Dyslipidemia
		Prevalence ratio (95% CI)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)
All subjects (n=1305)							
1mg-DST (unit: 10 nmol/L)	Unadj.	1.00 (1.00-1.01)	1.01 (1.00-1.01)	1.00 (0.99-1.00)	1.00 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (0.99-1.00)
	Adj.	1.01 (1.00-1.01)	1.01 (1.01-1.02)	1.00 (1.00-1.01)	1.01 (1.00-1.01)	1.02 (1.00-1.03)	1.00 (0.99-1.01)
Maximum tumor diameter (unit: 5mm)	Unadj.	1.00 (0.99-1.02)	1.01 (0.98-1.04)	1.00 (0.98-1.02)	1.00 (0.97-1.04)	1.02 (0.96-1.08)	0.97 (0.95-1.00)
	Adj.	1.00 (0.99-1.02)	1.01 (0.98-1.04)	0.99 (0.97-1.02)	1.00 (0.97-1.04)	1.02 (0.96-1.08)	0.97 (0.95-1.00)
Bilateral tumors	Unadj.	1.10 (1.02-1.19)	1.17 (0.97-1.41)	1.17 (1.03-1.33)	0.97 (0.78-1.20)	0.77 (0.46-1.30)	1.06 (0.88-1.28)
	Adj.	1.08 (1.01-1.17)	1.21 (1.00-1.45)	1.15 (1.02-1.30)	0.95 (0.77-1.17)	0.78 (0.47-1.29)	1.04 (0.86-1.24)
Plasma ACTH (unit: 1.1 pmol/L)	Unadj.	0.99 (0.98-1.01)	0.97 (0.93-1.01)	1.00 (0.98-1.02)	1.01 (0.99-1.04)	1.00 (0.95-1.05)	1.00 (0.98-1.03)
	Adj.	0.99 (0.97-1.01)	0.95 (0.91-1.00)	1.00 (0.98-1.02)	1.01 (0.98-1.05)	0.99 (0.94-1.05)	0.99 (0.96-1.02)
Serum DHEAS (unit: 0.5 µmol/L)	Unadj.	0.99 (0.98-1.00)	0.99 (0.97-1.01)	0.98 (0.97-1.00)	0.99 (0.96-1.01)	0.94 (0.87-1.01)	0.95 (0.92-0.98)
	Adj.	1.00 (0.99-1.01)	0.99 (0.96-1.01)	0.99 (0.98-1.01)	1.00 (0.98-1.02)	0.96 (0.89-1.03)	0.96 (0.93-0.99)
24-hour UFC (unit: 25 nmol/24h)	Unadj.	1.00 (1.00-1.00)	1.01 (1.00-1.01)	1.00 (0.99-1.01)	1.00 (0.99-1.01)	0.99 (0.96-1.02)	0.99 (0.98-1.00)
	Adj.	1.00 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (0.97-1.03)	1.00 (0.99-1.01)
After excluding subjects with CS (n=1240)							
1mg-DST (unit: 10 nmol/L)	Unadj.	1.00 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (0.99-1.01)	1.01 (1.00-1.02)	1.01 (1.00-1.03)	1.01 (1.00-1.02)
	Adj.	1.00 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (0.99-1.01)	1.00 (1.00-1.01)	1.01 (0.99-1.03)	1.01 (1.00-1.02)

Maximum tumor diameter (unit: 5mm)	Unadj.	1.00 (0.99-1.02)	1.00 (0.97-1.03)	0.99 (0.97-1.01)	1.00 (0.97-1.03)	1.02 (0.95-1.09)	0.98 (0.95-1.01)
	Adj.	1.00 (0.99-1.01)	1.00 (0.97-1.03)	0.99 (0.97-1.01)	1.00 (0.97-1.03)	1.01 (0.95-1.08)	0.98 (0.95-1.00)
Bilateral tumors	Unadj.	1.10 (1.01-1.19)	1.23 (1.02-1.49)	1.17 (1.03-1.34)	0.95 (0.76-1.19)	0.74 (0.43-1.30)	1.06 (0.87-1.28)
	Adj.	1.08 (1.00-1.17)	1.28 (1.06-1.55)	1.15 (1.02-1.31)	0.94 (0.76-1.16)	0.75 (0.43-1.30)	1.04 (0.86-1.25)
Plasma ACTH (unit: 1.1 pmol/L)	Unadj.	0.99 (0.98-1.01)	0.98 (0.94-1.02)	1.00 (0.98-1.02)	1.01 (0.99-1.04)	1.01 (0.97-1.05)	1.00 (0.97-1.02)
	Adj.	0.99 (0.98-1.01)	0.97 (0.92-1.01)	1.00 (0.98-1.02)	1.02 (0.99-1.05)	1.01 (0.97-1.06)	0.99 (0.95-1.02)
Serum DHEAS (unit: 0.5 μmol/L)	Unadj.	0.99 (0.98-1.00)	0.99 (0.97-1.01)	0.98 (0.97-1.00)	0.99 (0.96-1.01)	0.95 (0.89-1.03)	0.94 (0.91-0.97)
	Adj.	1.00 (0.99-1.01)	0.99 (0.97-1.01)	0.99 (0.97-1.01)	1.00 (0.98-1.02)	0.97 (0.91-1.05)	0.95 (0.92-0.98)
24-hour UFC (unit: 25 nmol/24h)	Unadj.	1.00 (0.99-1.00)	1.00 (0.99-1.01)	1.00 (0.99-1.01)	1.00 (0.99-1.02)	0.96 (0.90-1.02)	1.00 (0.99-1.01)
	Adj.	1.00 (1.00-1.01)	1.01 (0.99-1.02)	1.01 (1.00-1.01)	1.01 (1.00-1.02)	0.97 (0.91-1.03)	1.01 (0.99-1.02)

Appendix Figure 1: Schematic overview of urine steroid metabolites analyzed in the study participants. Multi-steroid profiling of 24-hour urine samples was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with quantification of 16 distinct steroid metabolites. Steroid metabolites are schematically mapped onto the steroidogenic pathways leading to glucocorticoid and androgen biosynthesis.



Appendix Figure 2: Prevalence of hypertension, type 2 diabetes, and dyslipidemia in subjects with non-functioning adrenal tumors (NFAT; post-dexamethasone serum cortisol <50 nmol/L) stratified according to serum cortisol concentrations after 1mg dexamethasone overnight in 10 nmol/L increments. The dotted lines represent the prevalence of cardiometabolic disease in the entire NFAT group.



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