Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors : A Cross-Sectional Multicenter Study

Cardiometabolic disease risk in patients with adrenocortical adenomas: a case-control study

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Word count: 3308

ABSTRACT

BACKGROUND: Mild autonomous cortisol excess (MACE) is frequently diagnosed in patients with benign adrenocortical adenomas (ACA) and defined by failure to appropriately suppress cortisol in the 1mg dexamethasone suppression test (1mg-DST), in the absence of clinical features of Cushing's syndrome (CS). Accumulating evidence suggests that MACE is associated with an increased prevalence of cardiometabolic disease. However, this is mostly derived from small-scale, heterogenous studies lacking comparison to a reference cohort.

METHODS: We performed a large-scale case-control study in 1305 prospectively recruited ACA patients; clinical and endocrine assessment included a 1mg-DST. We compared their cardiometabolic disease burden to 5268 population-based controls.

FINDINGS: According to 1-mg DST results and clinical assessment, the 1305 ACA patients (67% women, median age 60 years) were classified into 649 patients with non-functioning adrenal tumours (NFAT, 49.7%), 591 patients with MACE (45.3%; 451 (34.6%) with MACE-1, 140 (10.7%) with more severe MACE-2), and 65 patients with clinically overt CS (5%). Cardiometabolic disease burden in all four subgroups was significantly higher than in controls and increased in line with cortisol excess (adjusted odds ratios (aORs) for hypertension: NFAT 3·66 (95%CI 3·02-4·43), MACE-1 5·03 (3·96-6·40), MACE-2 6·72 (4·37-10·32), CS 11·86 (6·50-21·65), all $p<0·001$; aORs for type 2 diabetes: NFAT 2·62 (2·00-3·44), MACE-1 2·75 (2·05-3·69), MACE-2 4·06 (2·45-6·71), CS 11·00 (5·31-22·77), all $p<0·001$).

INTERPRETATION: Both MACE and NFAT are clinically highly relevant cardiometabolic risk conditions, predominantly affecting women, and warrant careful assessment for hypertension and type 2 diabetes. Novel biomarkers for accurate prediction of metabolic risk are urgently needed.

FUNDING: Diabetes UK, European Commission, UK Medical Research Council, the UK Academy of Medical Sciences, Wellcome Trust, and UK National Institute for Health Research, US National Institutes of Health, the Claire Khan Trust Fund at University Hospitals Birmingham Charities, and the Mayo Clinic Foundation for Medical Education and Research.

RESEARCH IN CONTEXT

Evidence before this study

Adrenal masses are discovered upon cross-sectional imaging in approximately 5% of adult patients. Benign adrenocortical adenomas (ACA) are the most common underlying entity; ACA can be non-functioning (NFAT) or autonomously overproduce steroids, most frequently cortisol. Florid, clinically overt cortisol excess, Cushing's syndrome (CS), is rare while mild autonomous cortisol excess (MACE) is a much more frequent occurrence in ACA patients. However, while CS is a well-established cause of increased cardiometabolic morbidity and mortality, the evidence regarding the impact of MACE on cardiometabolic disease risk is scarce and heterogeneous. In a recent systematic review and meta-analysis, we undertook a comprehensive search of MEDLINE, Embase, Cochrane, and Scopus (January 1990 to February 2019) and identified studies that reported on the prevalence of cardiometabolic comorbid conditions in patients with NFAT and MACE. Cardiometabolic disease was highly prevalent, with hypertension as the most common occurrence (64·0% in MACE vs. 58·2% in NFAT). Patients with MACE 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 dyslipidaemia was at a similar level in MACE and NFAT (34%). The main limitations of these data were the small sample sizes of the included studies, the heterogeneity in the definitions of MACE and clinical outcomes, and that none of the studies provided a comparison of the morbidity risk in MACE to a control group, except for one small-scale retrospective study.

Added value of this study

This study analysed the prevalence and severity of cardiometabolic disease in a large cohort of patients with ACA prospectively recruited to an international multi-centre study, EURINE-ACT, in comparison to controls extracted from a population surveillance study, the Health Survey for England (HSE). We found that ACA patients had a significantly increased

risk of hypertension, type 2 diabetes, and dyslipidaemia. After stratifying patients according to different degrees of cortisol excess in accordance with the recent joint guideline by the European Society of Endocrinology and European Network for the Study of Adrenal Tumours, we found a high prevalence of MACE amongst ACA patients (45%) while the prevalence of CS was expectedly low (5%). Our analysis showed that individuals in all three ACA subgroups, NFAT, MACE and CS, had an increased prevalence of type 2 diabetes, hypertension and dyslipidaemia, with a stepwise increase in risk with increasing degrees of cortisol excess. Two thirds of ACA patients were women and the proportion of women, age and tumour diameter was higher in MACE than in NFAT.

Implications of all the available evidence

Our study demonstrates an increased cardiometabolic risk in ACA patients, with substantially increased prevalence of type 2 diabetes, hypertension and dyslipidaemia in MACE and also in NFAT. These findings identify both MACE and NFAT as cardiometabolic risk conditions, which predominantly affect women and will require regular monitoring and management.

INTRODUCTION

Clinically overt endogenous Cushing's syndrome (CS) is a life-threatening albeit rare disorder caused by excessive adrenal cortisol production. CS usually presents with typical clinical signs including proximal myopathy, purple striae, and moon face,[1] as well as much less distinct but metabolically highly adverse consequences, such as type 2 diabetes, hypertension and dyslipidaemia, drivers of increased cardiovascular mortality in the affected patients.[1,2]

Mild autonomous cortisol excess (MACE), previously also termed subclinical CS, is regularly diagnosed in patients with incidentally discovered adrenal masses, detected in approximately 5% of cross-sectional imaging studies.[3] MACE is defined by failure to suppress serum cortisol sufficiently after overnight administration of 1mg dexamethasone,[4] but in the absence of the typical clinical signs of cortisol excess. Previous case series identified MACE in up to 35% of patients with benign adrenocortical adenomas (ACA), making it the most common hormonal abnormality observed in this population.[4,5] In the largest prospective study to date, EURINE-ACT, 1513 (89·7%) of 1686 incidentally discovered adrenal masses were ACA.[6]

Previous evidence suggests that a substantial proportion of patients with MACE – similarly to CS – present with a high cardiometabolic burden including hypertension, type 2 diabetes, dyslipidaemia, cardiovascular disease, fragility fractures, and mortality.[4,5,7] Of note, one previous study reported that patients with NFAT may also be at increased risk of hypertension, type 2 diabetes, and dyslipidaemia.[8] However, the evidence regarding the cardiometabolic risk of patients with NFAT and MACE is mostly derived from observational studies of small sample size without control cohort for reference, thereby limiting the interpretation of the results.

Here we report a case-control study investigating the cardiometabolic risk of a large prospectively recruited cohort of ACA patients with different degrees of cortisol excess in comparison to population controls drawn from the Health Survey for England (HSE).

METHODS

Case cohort

Patients with benign adrenocortical tumours were part of the part of the EURINE-ACT study,[6] which prospectively recruited adult patients (≥18 years) with newly diagnosed adrenal tumours >1cm from 2011 to 2016 through 14 clinical centres in 11 countries participating in the European Network for the Study of Adrenal Tumours (ENSAT; www.ensat.org). We included all EURINE-ACT participants who (1) were diagnosed with ACA and (2) had undergone standardized endocrine assessment for exclusion of adrenocorticotropic hormone (ACTH)-independent cortisol excess (MACE or CS),[9,10] with measurement of endocrine parameters carried out in the recruitment centre. After exclusion of aldosterone-producing ACAs and patients with cortisol excess due to bilateral macronodular hyperplasia, we included 1305 (74%) of the overall 1767 ACA patients in the EURINE-ACT cohort (**Fig. 1**).

In accordance with recent guidelines,[4] we defined the presence of MACE as failure to suppress morning serum cortisol to less than 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). Patients with MACE were further subdivided into MACE-1 (possible MACE; serum cortisol after the 1mg-DST 50-138 nmol/L) and MACE-2 (definitive MACE; cortisol after the 1mg-DST >138 nmol/L), as defined by recent guidelines.[4] Patients with current or recent (<6 months) intake of drugs known to alter steroid synthesis or metabolism were excluded. All centres 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 tumour diagnosis (baseline assessment). Variables obtained through the online ENSAT database included demographic data (sex, age, body mass index, BMI), tumour characteristics (maximum diameter, location, tumour attenuation measured as Hounsfield units by non-contrast CT scan), information about cardiometabolic morbidity, and endocrine test results (plasma 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.

Control cohort

The Health Survey for England (HSE) is an annual survey of randomly selected nationally representative private households in England. HSE obtains data throughout the year in survey respondents' own homes in various stages including health interview, nurse visits, and blood sample collection. For this study, HSE data from the year 2014 was chosen as the community control pool, as this was the mid-point of EURINE-ACT recruitment period (2011-2016). HSE benefits from a combinatorial data acquisition derived from self-reported diagnosis and an objective medical examination by a trained nurse. This aids identification of those with conditions who may have been undiagnosed in a primary care or hospital setting. This survey also collects data on prescribed medications. Participants from the HSE 2014 were eligible if they were aged ≥18 years and completed their nurse appointment. Participants were excluded if they were prescribed oral glucocorticoids or if they self-reported having type 1 diabetes (**Fig. 1**, **Suppl. Table 1**). Data collection by HSE is approved by the relevant National Health Service (NHS) National Research Ethics Service (NRES) and the data is available for access from the UK data service.[11]

Definitions of cardiometabolic disease

Hypertension: Participants were considered as having hypertension if they had a doctor diagnosis or if they were prescribed medications for hypertension (**Suppl. Table 2**).

Treatment with ≥3 anti-hypertensives: Participants with hypertension were chosen for a subgroup analysis to study prescription of ≥3 antihypertensive as an outcome, in line with established American Heart Association criteria (**Suppl. Table 2**).[12]

Abnormal glucose metabolism: 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 oral glucose tolerance test, random/fasting plasma glucose, or glycated haemoglobin results according to American Diabetes Association criteria (**Suppl. Table 3**).[13] The Health Survey for England (HSE) does not distinguish between type 1 and type 2 diabetes; participants who self-reported diabetes diagnosed after the age of 35 and/or not treated with insulin were considered as having type 2 diabetes.[14]

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

Dyslipidaemia: The prescription of lipid-lowering agents was considered as a proxy for dyslipidaemia. We only considered subjects taking lipid-lowering agents for primary prevention of cardiovascular disease, after excluding those with a history of stroke, cerebral haemorrhage, cerebral thrombosis, ischaemic heart disease, or angina, in line with American College of Cardiology/American Heart Association criteria (**Suppl. Table 4**).[15]

Calculation of the prevalence of cardiometabolic disease

We calculated the prevalence of hypertension, prediabetes, type 2 diabetes, and dyslipidaemia in the EURINE-ACT and HSE 2014 cohorts. For the EURINE-ACT patients, we considered the clinical information available at the time of tumour diagnosis. In both

cohorts, 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 (**Suppl. Tables 2-4**). The prevalence of cardiometabolic disease in the EURINE-ACT and HSE 2014 cohorts was compared using a two-tiered approach, i.e. (1) an unmatched analysis and (2) a 1:1 matched analysis.

Unmatched analysis

Logistic and multinomial logistic regression was run to obtain crude and adjusted odds ratio (OR) of hypertension, prediabetes, type 2 diabetes, and dyslipidaemia among EURINE-ACT ACA patients (n=1305) compared to the control population from HSE 2014 (n=5268). The models were adjusted for age, sex, and body mass index (BMI). In sub-cohorts of subjects with hypertension and type 2 diabetes, a logistic regression model was run to obtain the crude and adjusted ORs of treatment with ≥3 anti-hypertensives and insulin use, respectively. In an ancillary analysis, patients with NFAT, MACE-1, MACE-2, and adrenal CS were considered as the independent variable for each model.

Matched Analysis

For each patient in the EURINE-ACT ACA cohort, one patient from the HSE 2014 data was randomly selected after matching for age (±4 years), sex, and BMI (±2·5 kg/m^2). Matching was performed thrice in patients with complete data on hypertension, glucose metabolism status, and dyslipidaemia. Logistic regression was run in the matched subset of patients to obtain crude and adjusted ORs. Subgroup analyses were performed among patients with NFAT, MACE-1, MACE-2, and adrenal CS and their corresponding matched controls.

Statistical analysis

The normal distribution of the continuous variables was verified using the Shapiro–Wilk and the Kolmogorov–Smirnov tests for normality. The Mann–Whitney U test and the Kruskal–

Wallis test with Dunn's post hoc test were used for the analysis of nonparametric data. The $\chi 2$ test was applied for categorical variables. Spearman rank-order correlation was used to measure the strength and direction of association between continuous variables in the EURINE-ACT ACA cohort. Multivariate logistic regression was run to assess the relationship between cardiometabolic disease and clinical, radiological, and biochemical parameters in the EURINE-ACT ACA cohort; the model was adjusted for age, sex, and BMI. Statistical significance was considered when the p-value was <0·05. 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 of the case cohort

Between 2011 and 2016, 1305 patients with newly diagnosed non-aldosterone producing adenomas underwent a 1mg-DST and were prospectively assessed for clinical signs of cortisol excess (**Fig. 1**, **Suppl. Table 5**). Less than half of these patients achieved normal suppression of serum cortisol after the 1mg-DST (NFAT n=649, 49·7%), with the vast majority of those with abnormal results lacking the distinctive clinical features of overt cortisol excess (MACE-1, n=451 [34.6%]; MACE-2, n=140 [10·7%]; CS, n=65 [5·0%]). Women represented 67·3% of the entire cohort and the female predominance was more pronounced in MACE-2 (73·6%) and CS (86·2%) (**Table 1**). The median age at the time of tumour diagnosis was 60 years (interquartile range [IQR] 52-67 years). Patients with MACE

were older than those with NFAT (**Fig. 2A**). By contrast, CS was diagnosed at a significantly younger age than the other groups (median 48 years, IQR 38-60 years) (**Table 1**). Patients with abnormal 1mg-DST results had significantly larger adrenal tumours, with over half of patients with tumours larger than 2 cm failing to suppress serum cortisol during the 1mg-DST (**Fig. 2B**, **Suppl. Table 6**).

Plasma ACTH negatively correlated with 1mg-DST results (ρ -0·41, p<0·001; **Suppl. Table 6**), which was reflected in progressively lower levels in MACE-1, MACE-2, and CS (**Table 1, Fig. 2C**). Serum dehydroepiandrosterone sulfate (DHEAS) had a similar trend, but the differences among groups were less pronounced possibly because of the observed negative relationship between age and DHEAS (**Suppl. Table 6, Fig. 2D**).

Patients with MACE were almost twice more likely to present with bilateral tumours than patients with NFAT (30·1% vs. 16·5%, p<0·001) (**Table 1**). Accordingly, 62·3% of patients with bilateral tumours had abnormal 1mg-DST results and presented with larger adrenal masses. Bilateral tumours were associated with lower plasma ACTH in NFAT and higher 24-hour UFC in both NFAT and MACE (**Suppl. Table 7**).

Cardiometabolic disease prevalence

In comparison to the population-based HSE 2014 cohort, the EURINE-ACT ACA patients carried an increased risk of hypertension, which gradually increased with the degree of cortisol excess. In the unmatched analysis age-, sex, and BMI-adjusted ORs (aORs) for hypertension were 3·66 for NFAT (95%CI 3·02-4·43), 5·03 for MACE-1 (95%CI 3·96-6·40), 6·72 for MACE-2 (95%CI 4·37-10·32), and 11·86 for CS (95%CI 6·50-21·65) (all p<0·001; **Fig. 3A**, **Table 2**). Results in the matched analysis were similar (aORs: NFAT 4·05 (95%CI 3·06-5·36), MACE-1 5·67 (95%CI 3·99-8·07), MACE-2 8·40 (95%CI 4·19-16·82), CS 8·98 (95%CI 3·43-23·55), all p<0·001; **Suppl. Table 8**). In a subgroup analysis in the

hypertensive patients, we also observed that those with higher degrees of cortisol excess more frequently required ≥3 anti-hypertensives (**Table 2**, **Fig. 3B**).

EURINE-ACT ACA patients also had an increased risk of type 2 diabetes that positively correlated with the degree of cortisol excess, with aORs ranging from 2·62 in NFAT (95%CI 2·00-3·44, p<0·001) to 11·00 in CS (95%CI 5·31-22·77, p<0·001) in the unmatched analysis (**Table 2** and **Figure 3**). The matched analysis reinforced these observations and found an even higher risk of type 2 diabetes in patients with CS (aOR 22·05 [95%CI 3·86-125·80], p<0·001), which is possibly explained by their younger age (**Suppl. Table 8**).

The use of lipid-lowering agents for primary prevention of cardiovascular disease was used as a proxy for dyslipidaemia. Both the unmatched and matched analysis yielded similar results, showing an increased risk of dyslipidaemia in patients with NFAT and MACE, but not CS (**Table 2**, **Figure 3**, and **Suppl. Table 8**).

None of the available clinical or biochemical information strongly correlated with the presence of cardiometabolic disease in the EURINE-ACT cohort (**Suppl. Table 9**). However, patients with bilateral adrenal tumours had an increased risk of requiring ≥3 anti-hypertensives (44·6% vs. 35·1% in unilateral tumours; aOR 1·66 [95%CI 1·18-2·35], p=0·004) and being diagnosed with prediabetes (31·7% vs. 20·0%; aOR 2·01 [95%CI 1·38-2·93], p<0·001) (**Suppl. Table 7**). When we further stratified these observations according to the 1mg-DST results, only patients with bilateral tumours and MACE had a significantly increased risk (aOR for ≥3 anti-hypertensives 1·75 [95%CI 1·12-2·74], p=0·014; aOR for prediabetes 2·52 [95%CI 1·49-4·29], p<0·001) (**Suppl. Table 7**).

DISCUSSION

In this case-control study, we showed that patients with ACA have a significantly increased cardiometabolic disease burden as compared to a population-based reference cohort and that this risk increases with the degree of cortisol excess. We also observed a significantly increased cardiometabolic burden in patients with ACA classified as "non-functioning" based on current criteria for the biochemical assessment of cortisol excess. These findings were made utilising the largest ever study prospectively recruiting patients with adrenal tumours, EURINE-ACT,[6] in comparison to a population-based reference cohort, drawn from the Health Survey for England.

Patients with ACA are classified into four subgroups, NFAT, possible MACE (MACE-1), definitive MACE (MACE-2), and CS, based on 1mg-DST results and clinical presentation, according to the recent European Society of Endocrinology/ENSAT guidelines on adrenal incidentalomas.[4] Increased cardiometabolic risk is a well-established feature of CS, while the evidence around MACE has been inconsistent, limited mainly by small study sizes and heterogenous definitions of diagnosis and clinical outcomes.[7] However, a picture of increased cardiometabolic disease burden and frailty in this group of patients has emerged from previous studies.[7,16,17] In our cohort of 1305 patients with ACA, cortisol excess was highly prevalent (50·3%), with most cases falling within the indeterminate category of MACE-1 (34·6%). The prevalence of MACE in our study is higher than previously reported though direct comparison is hampered because of the heterogeneous approaches to the definition of MACE prior to the 2016 consensus agreement,[4] including different DST protocols and cut-offs and combination of DST results with other parameters such as ACTH, 24h urinary free cortisol excretion, and salivary cortisol.[7] However, a retrospective study on 198 patients with adrenal incidentalomas diagnosed MACE in 34·8% of cases according to the same diagnostic criteria we used in this study.[5]

Our case cohort was predominantly female and 52·4% of women were over the age of 60 at the time of tumour diagnosis; the demographics of our study are similar to those of large retrospective studies on adrenal incidentalomas.[18-20] We also found that women were more likely to be diagnosed with MACE-2 and CS, confirming previous observations that cortisol excess predominantly affects women.[5,21]

The comparison with the HSE 2014 cohort demonstrated that ACA patients had an increased prevalence of cardiometabolic disease, most commonly hypertension (69·7%), followed by dyslipidaemia (31·4%) and type 2 diabetes (30·9%). The risk of hypertension and type 2 diabetes increased with the degree of cortisol excess and was highest in patients with MACE-2 and CS. Moreover, ACA patients with hypertension more often required multiple anti-hypertensives and ACA patients with type 2 diabetes more frequently required insulin therapy than hypertensive and diabetic patients in the population-based control cohort. These results were identically obtained when employing two different approaches – an unmatched logistic regression considering all the eligible HSE 2014 controls and a logistic regression after 1:1 matching of cases and controls for sex, age, and BMI. Our data show that patients with MACE carry an increased cardiometabolic burden similar to the one observed in CS, even if they do not display typical features of clinically overt, florid cortisol excess.

Of note, in our study, patients with NFAT were also found to have a higher prevalence of hypertension (63·7% vs. 33·3%), dyslipidaemia (29·3% vs. 19·7%), and type 2 diabetes (24·1% vs. 14·8%), indicating that NFAT represent a metabolic risk condition even if these patients suppress their circulating cortisol below 50 nmol/L in the 1mg-DST. Our findings add significant weight to the evidence previously provided by a cohort study in 166 retrospectively collected patients describing an increased incident metabolic disease risk.[8] To explore this further, we stratified patients in the NFAT group at a more granular level according to their 1mg-DST result (**Suppl. Figure 1**). This revealed that only those with fully

suppressed serum cortisol (<20 nmol/L) had rates of dyslipidaemia and type 2 diabetes similar to controls; the rate of hypertension, on the other hand, remained consistently above that of the HSE cohort in all NFAT subgroups (**Suppl. Figure 1**). We propose that the increased cardiometabolic disease in patients with NFAT may be due to underlying autonomous cortisol secretion that is not picked up by currently employed routine biochemical testing. This is supported by a previous study describing increased 24-h urinary total glucocorticoid metabolite excretion in 69 patients with NFAT,[22] and by the fact that approximately 9% of patients with NFAT develop MACE over time.[7]

Previous small-scale studies found that patients with bilateral and larger tumours are more likely to be diagnosed with MACE.[23,24] We provided corroborating evidence for this in our much larger cohort and also found that patients with MACE and bilateral tumours were more likely to require ≥3 anti-hypertensive medications and be diagnosed with prediabetes. In our cohort, we did not include patients with cortisol excess and typical imaging findings of primary bilateral macronodular adrenal hyperplasia, which is a very rare cause of hypercortisolism. However, these patients often present with MACE and, thus, some cases of undiagnosed primary bilateral macronodular adrenal hyperplasia in our study cannot be ruled out.[25]

Strengths of our study include the prospective recruitment, the large sample size, a standardised classification of different degrees of cortisol excess, and the use of a large, population-based control cohort. To our knowledge, this is the first large-scale study to establish the extent of the cardiometabolic burden of ACA patients by comparing them to population-based control subjects and adjusting for multiple confounding variables.

A weakness of our study is its cross-sectional nature, hence, we lack longitudinal data about the cardiometabolic outcomes. Baseline biochemical and clinical assessments were not standardised across centres and were not measured in a centralised fashion. However, while

we acknowledge that results for 24h urinary free cortisol, plasma ACTH, and serum DHEAS should be interpreted with caution, inter-assay variability of serum cortisol measurements is unlikely to affect the cut-off of 50 nmol/L used to diagnose MACE.[26] We had to exclude 287 (18%) of the overall 1592 eligible ACA patients as they had no recorded 1-mg DST results at the time of adrenal tumour diagnosis, thus, the high MACE prevalence in our study may be interpreted with some caution.

However, having taken into account these limitations, we believe that our study conclusively demonstrates that both NFAT and MACE are clinically highly relevant metabolic risk conditions, which predominantly affect women and come with increased prevalence of hypertension, dyslipidaemia and type 2 diabetes. We recommend that these patients, regardless of 1mg-DST results and clinical signs of cortisol excess, should receive a comprehensive cardiovascular assessment at the time of ACA diagnosis, with particular attention to blood pressure and glucose and lipid metabolism. Future studies are required to identify biomarkers that can be utilised to reliably predict metabolic risk in patients with ACA.

Contributors

A.P. and W.A. designed the study, with contributions from I.B., V.C., A.S. and K.N. In addition, A.P. contributed to data collection, data analysis, data interpretation, and co-wrote the manuscript. A.S. and K.N. extracted data from the Health Survey for England, performed statistical analyses, and co-wrote the manuscript. A.J.S. reviewed the statistical analyses and edited the manuscript. I.B., V.C., S.T., K.L., M.M., D.A.D, CRZA-2, ITTU-2, L.M., GYWU-2, GRAT-3, M.W.O.R., PLWW2-3, C.Z., CRZA-3, GRAT-4, M.A., PLWW2-4, 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. all authors to confirm

Data sharing

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.

Acknowledgments

This work was supported by Diabetes UK (Sir George Alberti Research Training Fellowship 18/0005782, to A.P.), the European Commission under the 7th Framework Programme (FP7/2007-2013, grant agreement 259735, ENSAT-CANCER, to W.A., M.F., and F.B.) and under the European Union's Horizon 2020 research and innovation programme under grant agreement no. 633983 (ENSAT-HT, to W.A. and F.B.), the Medical Research Council UK (Strategic Biomarker Grant G0801473, to W.A.), the Claire Khan Trust Fund at University Hospitals Birmingham Charities (Project grant, to W.A.), the Mayo Clinic Foundation for Medical Education and Research (Mayo Scholarship, to I.B.), the Wellcome Trust (Clinical Research Training Fellowship WT101671, to V.C.), and the Academy of Medical Sciences (Starter Grant for Clinical Lecturers, to V.C.). This research was also partly supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) USA under award K23DK121888 (to I.B). W.A. and A.J.S. receive support from the National Institute for Health Research (NIHR) Birmingham Biomedical Research Centre at the University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham (Grant Reference Number BRC-1215-20009). The views

expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care UK, or the National Institutes of Health USA.

Table 1: Demographics, radiological, and biochemical parameters of participants of the Health Survey for England (HSE) 2014 and of the EURINE-ACT participants with benign adrenocortical adenomas (ACA) 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.

	HSE (n=5268)	EURINE-ACT ACA Cohort				
		Overall cohort (n=1305)	NFAT (n=649)	MACE-1 (n=451)	MACE-2 (n=140)	Adrenal CS (n=65)
Women, n (%)	2934 (55·7)	878 (67·3)	416 (64·1)	303 (67·2) p=0·290*	103 (73·6) p=0·032† p=0·155‡	56 (86·2) p<0·001§ p=0·002‖ p=0·045¶
Age (years)	51 (38-66)	60 (52-67)	58 (51-65)	64 (56-71) p<0·001*	63 (54-69) p=0·005† p=0·450‡	48 (38-60) p<0·001§ p<0·001‖ p<0·001¶
BMI (kg/m^2)	26·0 (23·0-30·0)	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)
- BMI <25, n (%)	1678 (34·3)	292 (22·9)	129 (20·6)	106 (23·8)	42 (30·0)	15 (23·4)
- overweight (BMI 25-30), n (%)	1846 (37·7)	429 (33·6)	202 (32·2)	160 (35·9)	41 (29·3)	26 (40·6)
- obesity (BMI ≥30), n (%)	1368 (28·0)	556 (43·5)	296 (47·2)	180 (40·4) p=0·372*	57 (40·7) p=0·223† p>0·999‡	23 (35·9) p>0·999§ p>0·999‖ p>0·999¶
Missing information, n (%)	376 (7·1%)	28 (2·1)	22 (3·3)	5 (1·1)	0	1 (1·5)
Maximum tumour diameter (mm)#	NA	26 (19-36)	22 (16-30)	30 (23-38) p<0·001*	32 (24-44) p<0·001† p=0·661‡	30 (26-38) p<0·001§ p>0·999‖ p>0·999¶
Tumour location:	NA					
- Left, n (%)		616 (47·2)	323 (49·8)	196 (43·5)	63 (45·0)	34 (52·3)
- Right, 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) p<0·001*	42 (30·0) p<0·001† p=0·972‡	13 (20·0) p=0·470§ p=0·091‖ p=0·133¶
Hounsfield units (HU):	NA					
- HU <10, n (%)		773 (69·5)	392 (70·6)	283 (70·0)	72 (65·5)	26 (59·1)
- HU 10-20, n (%)		185 (16·6)	85 (15·3)	72 (17·8)	22 (20)	6 (13·6)
- HU >20, n (%)		155 (13·9)	78 (14·1)	49 (12·1) p=0·556*	16 (14·5) p=0·845†	12 (27·3) p=0·136§

					p=0·852‡	p=0·076‖ p=0·172¶
Missing information, n (%)		192 (14·7)	94 (14·5)	47 (10·4)	30 (21·4)	21 (32·3)
Serum cortisol in the 1mg-DST (nmol/L)	NA	51 (33-92)	33 (27-41)	72 (60-93)	200 (165-283)	435 (271-574) p<0·001‖ p>0·999¶
Plasma ACTH (pmol/L)	NA	2·38 (1·34-3·96)	3·00 (1·89-4·89)	2·20 (1·30-3·43) p<0·001*	1·43 (0·55-2·60) p<0·001† p<0·001‡	0·66 (0·55-1·43) p<0·001§ p<0·001‖ p0·033¶
Missing information, n (%)		250 (19·2)	150 (23·1)	83 (18·4)	13 (9·3)	4 (6·2)
Serum DHEAS (µmol/L)	NA	1·40 (0·70-2·70)	1·90 (1·00-3·40)	1·14 (0·65-2·19) p<0·001*	0·83 (0·40-1·85) p<0·001† p=0·157‡	0·54 (0·23-1·58) p<0·001§ p=0·016‖ p>0·999¶
Missing information, n (%)		331 (25·4)	180 (27·7)	111 (24·6)	28 (20·0)	12 (18·5)
24-hour urinary free cortisol excretion (nmol/24h)	NA	132 (66-226)	127 (66-207)	141 (69-229) p>0·999*	130 (47-207) p>0·999† p>0·999‡	472 (149-1319) p<0·001§ p<0·001‖ p<0·001¶
Missing information, n (%)		414 (31·7)	224 (34·5)	132 (29·3)	37 (26·4)	21 (32·3)

* MACE-1 vs. NFAT. † MACE-2 vs. NFAT. ‡ MACE-2 vs. MACE-1. § Adrenal CS vs. NFAT. ‖ Adrenal CS vs. MACE-1. ¶ Adrenal CS vs. MACE-2.
# For bilateral tumours, the maximum diameter of the larger adrenal mass was considered.

Table 2: Cardiometabolic disease burden of ACA patients with different degrees of cortisol excess. A multinomial logistic regression model was employed to investigate the cardiometabolic burden of 1305 ACA patients from the EURINE-ACT study in comparison to the control cohort of Health Survey for England (HSE). Unadjusted odds ratios and age-, sex-, and body mass index (BMI)-adjusted ORs are reported.

	HSE (n=5268)	EURINE-ACT ACA Cohort				
		Overall cohort (n=1305)	NFAT (n=649)	MACE-1 (n=451)	MACE-2 (n=140)	Adrenal CS (n=65)
Hypertension, n (%)	1521 (28·9)	907 (69·7)	414 (64·0)	339 (75·2)	107 (76·4)	47 (72·3)
Odds ratios (95% CI)		5·65 (4·95-6·45) p<0·001	4·39 (3·70-5·21) p<0·001	7·45 (5·97-9·30) p<0·001	7·98 (5·38-11·84) p<0·001	6·42 (3·72-11·10) p<0·001
Adjusted odds ratios (95% CI)		4·58 (3·95-5·31) p<0·001	3·66 (3·02-4·43) p<0·001	5·03 (3·96-6·40) p<0·001	6·72 (4·37-10·32) p<0·001	11·86 (6·50-21·65) p<0·001
Missing information, n (%)	5 (0·1)	3 (0·2)	3 (0·5)	0	0	0
Treatment with ≥3 anti-hypertensives, n (%)*	128 (8·42)	320 (38·6)	131 (31·6)	118 (34·8)	44 (41·1)	27 (57·4)
Odds ratios (95% CI)		6·86 (5·45-8·62) p<0·001	5·73 (4·33-7·56) p<0·001	7·06 (5·26-9·47) p<0·001	8·26 (5·36-12·71) p<0·001	15·47 (8·37-28·58) p<0·001
Adjusted odds ratios (95% CI)		7·60 (5·92-9·75) p<0·001	6·40 (4·72-8·68) p<0·001	7·43 (5·43-10·16) p<0·001	10·04 (6·36-15·86) p<0·001	31·37 (16·00-61·50) p<0·001
Missing information, n (%)	0	79 (8·7)	34 (8·2)	39 (11·5)	5 (4·7)	1 (2·1)
Prediabetes, n (%)	912 (23·3)	224 (22·4)	119 (24·1)	77 (21·8)	20 (19·4)	8 (16·7)
Odds ratios (95% CI)		1·37 (1·15-1·63) p=0·001	1·39 (1·10-1·75) p=0·006	1·41 (1·07-1·88) p=0·017	1·29 (0·76-2·21) p=0·344	0·99 (0·44-2·22) p=0·981
Adjusted odds ratios (95% CI)		1·02 (0·84-1·23) p=0·850	1·12 (0·87-1·43) p=0·374	0·85 (0·63-1·15) p=0·298	0·93 (0·53-1·62) p=0·796	1·57 (0·68-3·61) p=0·293
Type 2 diabetes, n (%)	414 (10·6)	308 (30·9)	130 (26·4)	122 (34·5)	39 (37·9)	17 (35·4)
Odds ratios (95% CI)		4·14 (3·47-4·95) p<0·001	3·34 (2·64-4·23) p<0·001	4·94 (3·81-6·39) p<0·001	5·56 (3·57-8·66) p<0·001	4·63 (2·46-8·75) p<0·001
Adjusted odds ratios (95% CI)		2·99 (2·43-3·67) p<0·001	2·62 (2·00-3·44) p<0·001	2·75 (2·05-3·69) p<0·001	4·06 (2·45-6·71) p<0·001	11·00 (5·31-22·77) p<0·001
Missing information, n (%)	1346 (25·6%)	307 (23·5)	156 (24·0)	97 (21·5)	37 (26·4)	17 (26·2)
Insulin treatment, n (%)†	56 (13·5)	69 (24·5)	19 (15·8)	29 (26·9)	13 (34·2)	8 (50)
Odds ratios (95% CI)		2·07 (1·40-3·06)	1·20 (0·68-2·12)	2·35 (1·41-3·91)	3·32 (1·61-6·88)	6·39 (2·31-17·72)

		p<0·001	p=0·522	p=0·001	p=0·001	p<0·001
Adjusted odds ratios (95% CI)		2·44 (1·58-3·76)	1·41 (0·76-2·61)	2·70 (1·58-4·62)	4·24 (1·98-9·09)	11·08 (3·62-33·90)
		p<0·001	p=0·270	p<0·001	p<0·001	p<0·001
Missing information, n (%)	0	26 (8·4)	10 (7·7)	14 (11·5)	1 (2·6)	1 (5·9)
Dyslipidaemia, n (%)	770 (14·6%)	405 (31·4)	185 (28·9)	160 (35·7)	50 (36·0)	10 (15·6)
Odds ratios (95% CI)		2·67 (2·32-3·07)	2·37 (1·97-2·86)	3·24 (2·63-3·99)	3·28 (2·30-4·67)	1·08 (0·55-2·13)
		p<0·001	p<0·001	p<0·001	p<0·001	p=0·823
Adjusted odds ratios (95% CI)		2·19 (1·87-2·56)	2·11 (1·71-2·59)	2·20 (1·75-2·76)	2·65 (1·80-3·89)	1·80 (0·87-3·73)
		p<0·001	p<0·001	p<0·001	p<0·001	p=0·111
Missing information, n (%)	4 (0·08%)	14 (1·1)	9 (1·4)	3 (0·7)	0	1 (1·5)

* Considering only patients with a diagnosis of hypertension (case cohort n=907; control HSE cohort n=1521).
† Considering only patients with a diagnosis of type 2 diabetes (case cohort n=308; control HSE cohort n=414).

Figure legends

Figure 1: Flow-chart of case and control inclusion.

Figure 2: Endocrine assessment results in the case cohort.

Distribution of serum cortisol (median, range) after the 1mg-overnight dexamethasone suppression test (1mg-DST) according to age (A) and maximum tumour diameter (B) in patients without clinical signs of Cushing's syndrome. Plasma ACTH (C) and serum DHEAS (D) measured in the EURINE-ACT ACA cohort are shown as boxplots, with boxes representing median and IQR 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 tumours (NFAT) from possible mild autonomous cortisol excess (MACE-1) and definitive mild autonomous cortisol excess (MACE-2). Symbols: n.s., $p>0.05$; *, $p\leq0.05$; **, $p\leq0.01$; ***, $p\leq0.001$.

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

Multinomial logistic regression model exploring the cardiometabolic risk of the EURINE-ACT ACA cohort in comparison to the HSE 2014 population-based reference cohort. Age-, sex-, and body mass index (BMI)-adjusted odds ratios, 95% confidence intervals and the level of significance are reported. Panel A: adjusted odds ratios for hypertension, type 2 diabetes, and dyslipidaemia. Panel B: adjusted odds ratios for treatment with ≥3 anti-hypertensives (in patients with hypertension) and insulin (in patients with type 2 diabetes).

Figure 1

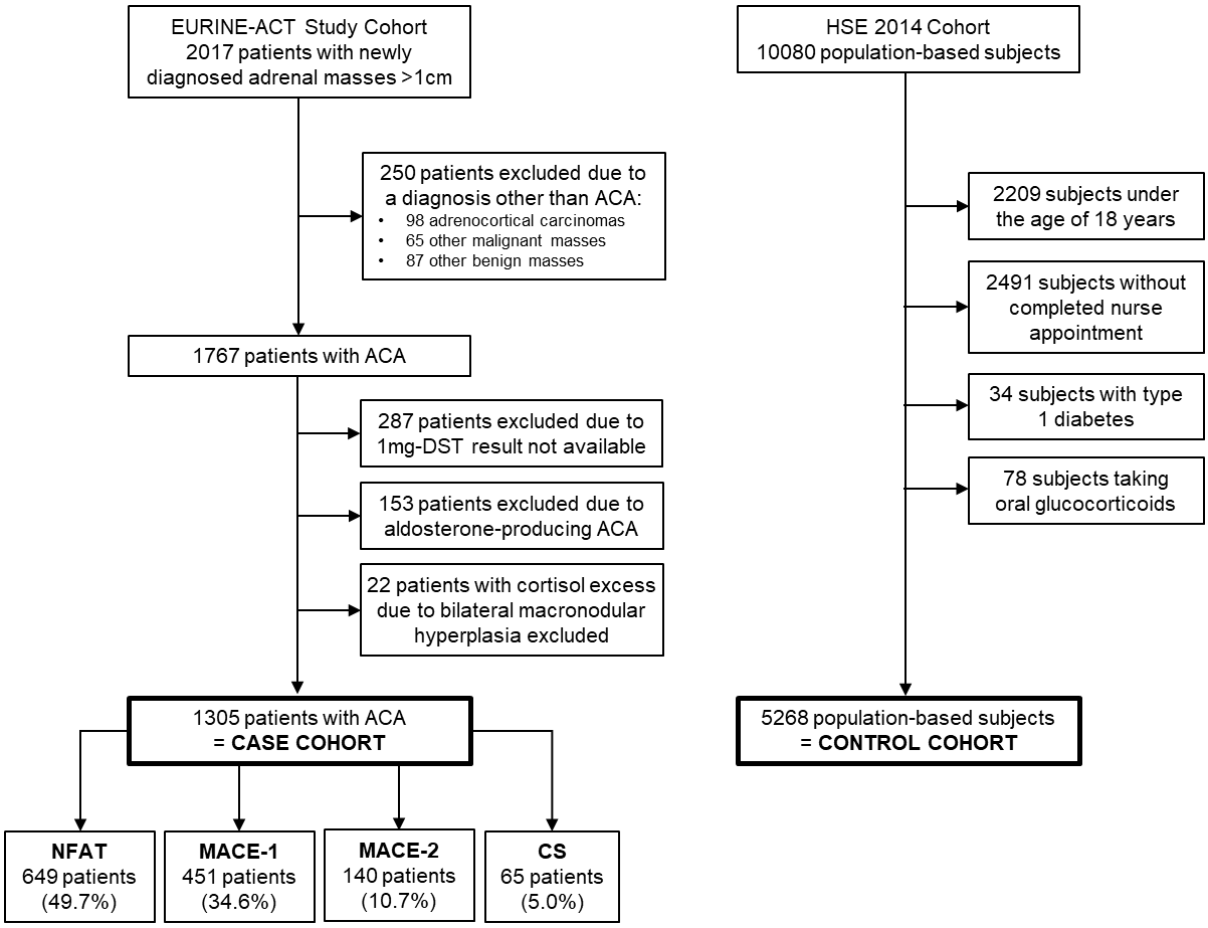

EURINE-ACT Study Cohort
2017 patients with newly diagnosed adrenal masses >1cm
250 patients excluded due to a diagnosis other than ACA:
• 98 adrenocortical carcinomas
• 65 other malignant masses
• 87 other benign masses
1767 patients with ACA
287 patients excluded due to 1mg-DST result not available
153 patients excluded due to aldosterone-producing ACA
22 patients with cortisol excess due to bilateral macronodular hyperplasia excluded
1305 patients with ACA
= CASE COHORT
NFAT
649 patients
(49.7%)
MACE-1
451 patients
(34.6%)
MACE-2
140 patients
(10.7%)
CS
65 patients
(5.0%)
HSE 2014 Cohort
10080 population-based subjects
2209 subjects under the age of 18 years
2491 subjects without completed nurse appointment
34 subjects with type 1 diabetes
78 subjects taking oral glucocorticoids
5268 population-based subjects
= CONTROL COHORT

Figure 2

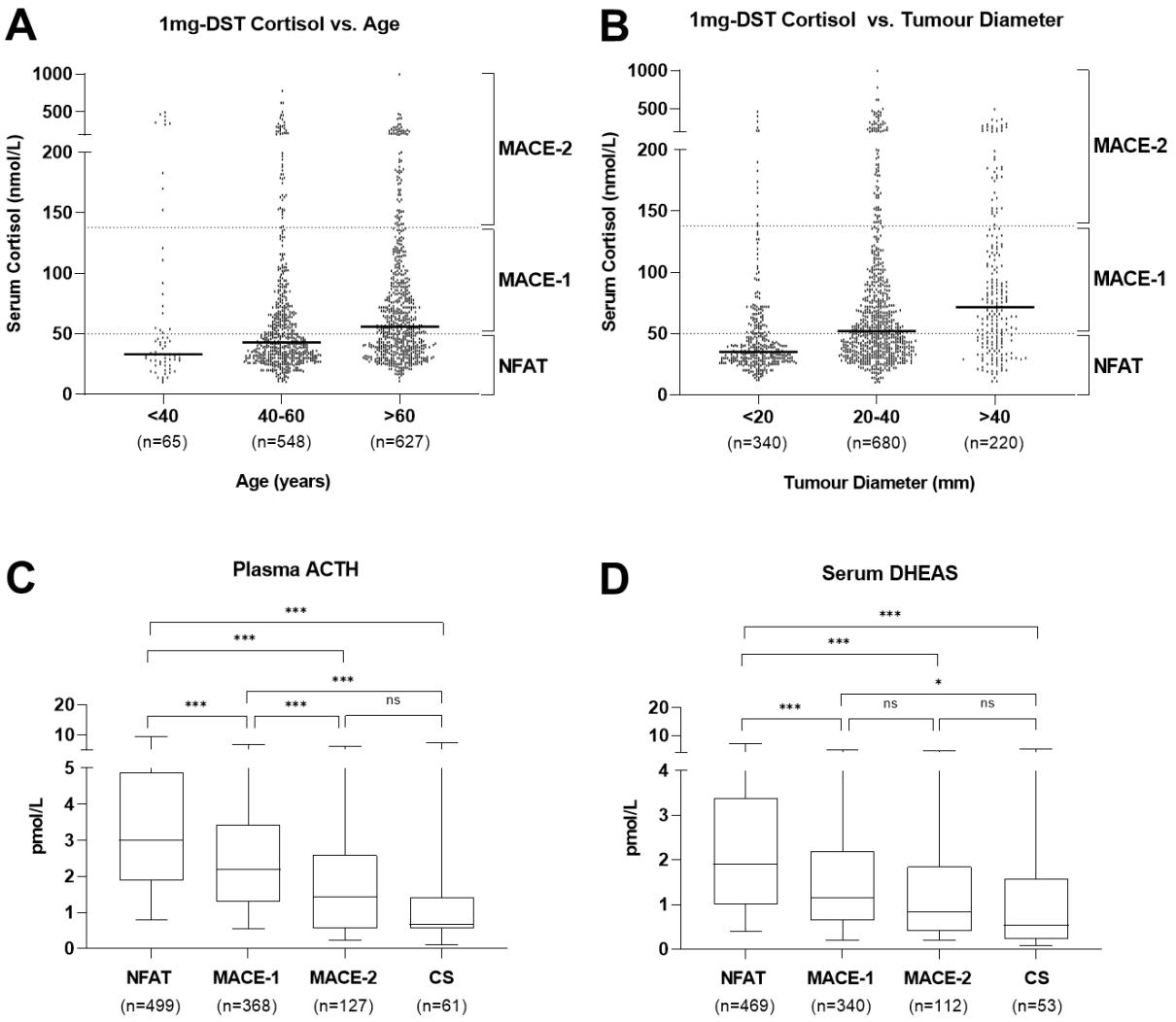
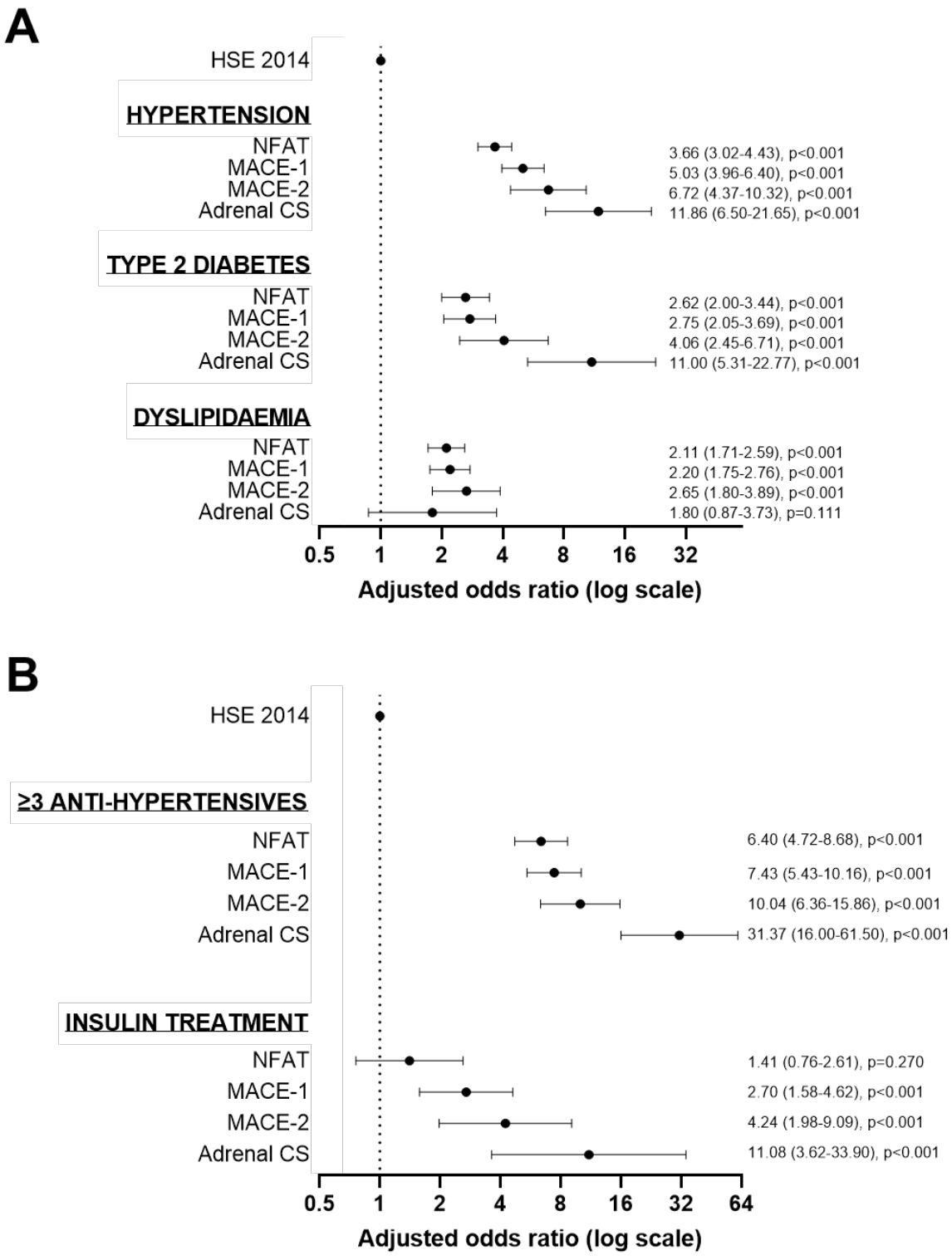

A
1mg-DST Cortisol vs. Age
Serum Cortisol (nmol/L)
1000
500
200
150
100
50
0
MACE-2
MACE-1
NFAT
<40
(n=65)
40-60
(n=548)
>60
(n=627)
Age (years)
B
1mg-DST Cortisol vs. Tumour Diameter
Serum Cortisol (nmol/L)
MACE-2
MACE-1
NFAT
<20
(n=340)
20-40
(n=680)
>40
(n=220)
Tumour Diameter (mm)
C
Plasma ACTH
pmol/L
***
***
***
***
***
ns
NFAT
(n=499)
MACE-1
(n=368)
MACE-2
(n=127)
CS
(n=61)
D
Serum DHEAS
pmol/L
***
***
*
***
ns
ns
NFAT
(n=469)
MACE-1
(n=340)
MACE-2
(n=112)
CS
(n=53)

Figure 3


A
HSE 2014
HYPERTENSION
NFAT
MACE-1
MACE-2
Adrenal CS
3.66 (3.02-4.43), p<0.001
5.03 (3.96-6.40), p<0.001
6.72 (4.37-10.32), p<0.001
11.86 (6.50-21.65), p<0.001
TYPE 2 DIABETES
NFAT
MACE-1
MACE-2
Adrenal CS
2.62 (2.00-3.44), p<0.001
2.75 (2.05-3.69), p<0.001
4.06 (2.45-6.71), p<0.001
11.00 (5.31-22.77), p<0.001
DYSLIPIDAEMIA
NFAT
MACE-1
MACE-2
Adrenal CS
2.11 (1.71-2.59), p<0.001
2.20 (1.75-2.76), p<0.001
2.65 (1.80-3.89), p<0.001
1.80 (0.87-3.73), p=0.111
0.5 1 2 4 8 16 32
Adjusted odds ratio (log scale)
B
HSE 2014
≥3 ANTI-HYPERTENSIVES
NFAT
MACE-1
MACE-2
Adrenal CS
6.40 (4.72-8.68), p<0.001
7.43 (5.43-10.16), p<0.001
10.04 (6.36-15.86), p<0.001
31.37 (16.00-61.50), p<0.001
INSULIN TREATMENT
NFAT
MACE-1
MACE-2
Adrenal CS
1.41 (0.76-2.61), p=0.270
2.70 (1.58-4.62), p<0.001
4.24 (1.98-9.09), p<0.001
11.08 (3.62-33.90), p<0.001
0.5 1 2 4 8 16 32 64
Adjusted odds ratio (log scale)

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