Non-Functional Adrenal Tumors and Incident Cardiometabolic Outcomes

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Scholarly Report Title: Non-Functional Adrenal Tumors and Incident Cardiometabolic Outcomes

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Title: Non-Functional Adrenal Tumors and Incident Cardiometabolic Outcomes

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Background: Adrenal tumors are incidentally discovered on abdominal imaging with increasing frequency. Small cross-sectional studies have shown that non-functional adrenal tumors have a higher prevalence of cardiovascular risk factors. However, longitudinal studies are lacking. We hypothesized that non-functional adrenal tumors (NFAT) may increase the risk for incident cardiovascular and metabolic outcomes when compared to controls without adrenal tumors.

Methods: We conducted a retrospective longitudinal cohort study. We identified patients who underwent abdominal imaging between 1991-2014 (n=234,267). After a rigorous exclusion process, cases of NFAT were selected using medical chart review to confirm adrenal tumor on imaging and the absence of adrenal hormone dysfunction or malignancy (n=242). Controls were computer-matched by age, gender, and race after confirming no adrenal hormone dysfunction or neoplasia on imaging (n=1237). Multivariable Cox proportional hazards regression was used to evaluate the association between NFAT and the risk of developing incident clinical outcomes in participants with >3 years of follow-up.

Results: NFAT were associated with a significantly higher hazard for incident composite diabetes when compared to no adrenal tumor (adjusted HR=2.36, 95% CI: 1.45, 3.84). Larger NFAT size strongly associated with higher "normal" cortisol levels following 1 mg of dexamethasone (<1.8 mcg/dL) (P<0.0001) and higher "normal" 24h urinary cortisol levels (<50 mcg/24h) (P<0.0001). Higher "normal" cortisol levels following dexamethasone predicted a higher prevalence of type 2 diabetes among participants with NFAT, P-trend=0.04.

Conclusions: NFAT were associated with a greater than two-fold increased risk of developing diabetes when compared to no adrenal tumor. Since "non-functional" adrenal tumors are

common and considered to pose no health risk, these results prompt a re-assessment of how incidentally discovered adrenal tumors are biochemically assessed and subsequently treated.

Student contribution to the SMO Project

Design

To decide on the variables I would need to study to conduct a retrospective study, I reviewed the current literature on adrenal incidentalomas. I reviewed existing evidence, if any, connecting subclinical hypercortisolism and nonfunctional adenomas with cardiometabolic disease. I incorporated that review into the final version of my database, the inclusion criteria, and the exclusion criteria for the cases and controls. I met with my PI at various point in the design and he approved the final design.

Data Collection

I reviewed the medical records of all ~2500 cases and controls. An undergrads helped review ~50 cases during the months I was on the wards (Amy Steele). I manually entered the data for all the patients and reviewed the data of the undergrads for quality control.

Data Analyses

I cleaned and formatted the raw data for SAS analyses. I performed the preliminary Chi-squared, T tests, and regression studies examining the prevalence of each outcome in the controls versus the groups. Dr. Vaidya performed the more advanced SAS analyses. As we progressed, additional questions came up requiring I perform additional data mining and data cleaning.

Manuscript

I wrote the complete initial draft of the manuscript. This version underwent multiple revisions by my PI. The final draft was shared with our collaborators for feedback. The final version was submitted for publication (see Appendix A).

Other collaborators:

Drs. Turchin, Adler, Luque Fernandez served as consultants. We ran design and statistical questions by them throughout this project. Their feedback was very helpful and much appreciated.

----------APPENDIX A: MANUSCRIPT SUBMITTED FOR PUBLICATION----------

INTRODUCTION

The frequent use of cross-sectional abdominal imaging has increased the incidental detection of adrenal tumors(1, 2). Imaging and autopsy series estimate that the prevalence of adrenal tumors is 1-10% (3-5), with higher occurrences with older age(4, 5). Although nearly all of these adrenal findings will represent benign adrenocortical tumors that are "non-functional" in that they do not apparently secrete hormones (1, 2, 6), subset may be "functional" in that they do secrete detectable levels of adrenocortical hormone(s). It is estimated that 5-10% of adrenocortical tumors may secrete excess cortisol without the classical signs or symptoms of Cushing syndrome, known as subclinical hypercortisolism (1, 6-9). Subclinical hypercortisolism has been associated with hypertension, insulin resistance, type 2 diabetes, hyperlipidemia, osteoporosis, and obesity(10, 11), and recent studies suggest that subclinical hypercortisolism may increase the risk of developing incident cardiovascular events and mortality when compared to "non-functional" adrenal tumors (NFAT)(12-14). Therefore, it is recommended that all adrenal tumors be screened for subclinical or overt hypercortisolism (1, 2, 15, 16).

However, emerging evidence suggests that even apparent NFAT have a higher cross-sectional association with cardiometabolic derangements, such as insulin resistance, dyslipidemia, hypertension, and endothelial dysfunction when compared to matched controls without adrenal tumors (17-19), possibly because NFAT secrete low-levels of adrenal hormones that evade detection or because NFAT secrete atypical glucocorticoid metabolites that are not routinely measured (17, 20). Although the size of these aforementioned studies was small and lacked longitudinal follow-up, collectively they suggest that NFAT, defined by our current consensus practice guidelines (1, 2, 15, 16, 21), may not be "non-functional" after all; they may impart cardiometabolic risk by secreting small but inappropriate amounts of glucocorticoids and/or mineralocorticoids that evade our traditional clinical criteria and detection capabilities.

Given the rapid rise of incidentally discovered adrenal tumors on cross-sectional imaging, and the fact that the vast majority will not be considered to pose a health risk because they are deemed to be "non-functional," a better understanding of whether NFAT represent independent cardiometabolic risk factors is of public health importance. We hypothesized that NFAT are associated with an increased risk for developing incident cardiovascular and/or metabolic diseases, when compared to similar patients without adrenal tumors.

METHODS

Study Population

We conducted a retrospective cohort study. We evaluated patients at Brigham and Women's Hospital and Massachusetts General Hospital and their affiliated partner hospitals who had undergone either abdominal computed tomography (CT) or abdominal magnetic resonance imaging (MRI) (**Figure 1**). Detailed methodology for the selection of the study participants is presented in the **Supplementary Appendix**. After excluding participants who had a documented diagnosis of any adrenal hormone disorder or malignancy, we identified participants with adrenal tumors with the potential to be non-functional (n=941), and age-, sex-, and race-matched participants without any adrenal tumors (n=1,559).

Assessment of the Main Exposure: Non-functional Adrenal Tumor vs. No Adrenal Tumor

Individual medical chart review was used to confirm exposures status (**Figure 1**). Detailed methodology for confirming exposure status is presented in the **Supplementary Appendix**. We excluded participants with an adrenal tumor that did not appear benign on imaging and who lacked biochemical evaluation to assess for subclinical hypercortisolism. From the remaining participants with adrenal tumors who had assessments for hypercortisolism, we excluded those with evidence for potential subclinical or overt hypercortisolism defined as: a serum cortisol of >1.8 μg/dl (50 nmol/liter) following a 1 mg dexamethasone suppression testing (DST) (21) and/or a urinary free cortisol (UFC) ≥50 mcg/24h (21). We also excluded participants with potential subclinical or overt primary aldosteronism (22). Following these exclusions, there were 242 participants with the NFAT exposure, of which subclinical hypercortisolism was excluded by DST in 164/242, by 24h UFC in 104/242, and by both DST and UFC in 28/242. Similarly, we used medical chart review to confirm 1,237 unexposed participants who had no adrenal tumors on imaging and no adrenal hormone dysfunction diagnoses (**Figure 1**).

Assessment of Other Relevant Exposures

Medical chart review was used to collect details of pertinent demographic information (age, sex, race, and body mass index [BMI]), prevalent medical diagnoses (hypertension, hyperlipidemia, pre-diabetes, type 2 diabetes, heart failure, chronic kidney disease, myocardial infarction, ischemic stroke, atrial fibrillation, interventional coronary procedure [such as coronary catheterization or coronary artery bypass graft surgery]), smoking history, and use of relevant medication classes (anti-hypertensive medications, anti-diabetes medications [oral antihyperglycemics or insulin], medications for coronary artery disease and/or hyperlipidemia [aspirin, statins, fibrates, niacin]).

Assessment of Main Outcome Measures: Incident Cardiovascular and Metabolic Diseases

The main clinical outcomes of interest included: hypertension, composite diabetes, hyperlipidemia, cardiovascular events (CVE), and chronic kidney disease (CKD). Hypertension was defined by documented diagnosis. Pre-diabetes was defined by documented diagnosis and/or having a hemoglobin A1c value between 5.7-6.5% on $\geq$ 2 occasions among patients who were not on any hypoglycemic agent other than metformin. Type 2 diabetes defined by documented diagnosis and/or having hemoglobin A1c values $\geq$ 6.5% on $\geq$ 2 occasions. Since pre-diabetes and type 2 diabetes are on a pathophysiologic continuum of insulin resistance, we defined "composite diabetes" as either pre-diabetes or type 2 diabetes. Hyperlipidemia was defined by documented diagnosis and/or LDL $\geq$ 150 mg/dL. CVE was a composite of any documented diagnosis of history of myocardial infarction, ischemic stroke, heart failure, atrial fibrillation, or a coronary intervention procedure. CKD was defined by documented diagnosis.

These outcomes were assessed at baseline (the time of first abdominal imaging to evaluate exposure status) and again in follow-up. Follow-up assessment of outcomes was conducted only in individuals who had at least $\geq$ 3 years of available longitudinal data to ensure sufficient time of exposure and because the detection and documentation of clinical diagnoses by healthcare providers may not always coincide ($\geq$ 3 years of longitudinal data: n=166/242 with NFAT, n=740/1237 without any adrenal tumor) (**Figure 1**)..

When assessing prospective outcomes, participants who had only one follow-up visit following ≥ 3 years had clinical outcomes assessed at this time; participants who had more than one follow-up visit had clinical outcomes assessed at the most current annual or complete clinical evaluation.

Statistical analyses

We described demographic and clinical characteristics using the number of counts and proportions for categorical variables and mean with standard deviations (SD) for continuous variables. Categorical and continuous variables were compared using Fisher's exact test or Chi-squared test, and Student's t-test, respectively.

Multivariable logistic regression models were used to assess the cross-sectional association between the exposure (NFAT vs. no adrenal mass) and cardiometabolic diagnoses. Model 1 adjusted for age, BMI, sex, race, and smoking status. Model 2 adjusted for variables in model 1 as well as other clinically relevant cardiovascular and metabolic diagnoses (all adjustments listed in table footnotes).

Adjusted Cox proportional hazards regression was used to evaluate the independent relation between exposure status and the development of incident cardiometabolic outcomes over time in individuals who did not have each outcome in question at baseline. We used three multivariable models. Models 1 and 2 are as described above. Model 3 included all variables in models 1 and 2 as well as adjustments for the use of individual medication classes. In secondary analyses, we evaluated associations between NFAT size, hormone levels, and the incidence of cardiometabolic outcomes.

Results of logistic regression are presented as odds ratios with 95% confidence intervals, and results of Cox proportional hazards regression are presented as the hazard ratio (HR) with 95% confidence intervals. A two-tailed *P* value of ≤0.05 was deemed as statistically significant. All statistical analyses were performed using SAS v9.3 (Cary, North Carolina, USA).

Role of the Funding Source

The study investigators were funded by the National Institutes of Health and the Doris Duke Charitable Foundation. Neither funding source played a role in the study's design, conduct, or reporting.

RESULTS

Study Population and Baseline Cardiometabolic Outcomes

The baseline characteristics of the study population are described in **Table 1**. Participants with NFAT were slightly younger, had higher BMI and a higher prevalence of pre-diabetes and diabetes when compared to those without adrenal tumors. In adjusted logistic regression models, there was a significant association between NFAT exposure and the prevalence of baseline composite diabetes (OR = 1.79 [1.22, 2.62]) (**Appendix Table 1**). This independent association between NFAT and having baseline composite diabetes was stable whether NFAT participants had subclinical hypercortisolism excluded using DST (adjusted OR=1.88 [1.21, 2.92]; n=164 with NFAT, n=1,237 without adrenal tumors) or using 24h UFC (adjusted OR=2.11 [1.24, 3.60]; n=104 with NFAT, n=1,237 without adrenal tumors).

Non-Functional Adrenal Tumors and Incident Cardiometabolic Outcomes

The risk of developing incident cardiometabolic outcomes was assessed in participants with ≥ 3 years of follow-up. The mean duration of follow-up was 7.2 years (SD=3.5, range 3.0-23.1) for participants with NFAT and 7.8 years (SD=3.6, range 3.0-22.1) for participants without adrenal tumors. During the course of follow-up, incident composite diabetes was detected in 27.3% of participants with NFAT and 11.7% of participants without adrenal tumors. NFAT exposure was associated with a significantly higher adjusted risk of developing incident composite diabetes (HR=2.36 [1.45, 3.84]) (**Table 2**). There were no other significant associations between exposure status and incident outcomes. The relationship between NFAT and incident composite diabetes remained similarly robust and significant regardless of whether subclinical hypercortisolism was excluded using the 1mg DST (adjusted HR=2.12 [1.20, 3.75]) or the 24h UFC (adjusted HR=3.11 [1.61, 5.98]) (**Figure 2A,B,C**). Since 24h UFC is regarded to be less sensitive at excluding subclinical hypercortisolism when compared to a 1mg DST (21), we also used a stricter criteria of <35 mcg/24h to define non-functionality with a 24h UFC, and observed no material difference in the result (HR=3.13 [1.59, 6.14]). Importantly, among eligible participants in these analyses for incident composite diabetes, there were no major differences in demographic profiles, comorbidities, or

medication use (**Table 3**). Similarly, both exposure groups had comparable duration of follow-up (7.3 [3.3] vs. 8.0 [9.7] years; *P*=0.06), and similar rates of laboratory assessments for glucose and creatinine (100% vs. 100%), lipids (75.5% vs. 67.6%; *P*=0.27), hemoglobin A1c (43.8% vs. 35.7%; *P*=0.17), and assessment of blood pressure (100% vs. 100%) (**Table 3**).

Sensitivity Analyses for Incident Composite Diabetes

Higher adiposity (higher BMI) may be a confounder of the association between NFAT and incident composite diabetes; however, higher adiposity may also represent a consequence of NFAT that secrete low-grade glucocorticoids and therefore be in the causal pathway between NFAT and incident diabetes. Considering the potential for confounding by higher BMI, we repeated our analysis for incident composite diabetes after adjusting for the change in BMI over time (ΔBMI = BMI at the end of longitudinal follow-up – baseline BMI) since weight gain may be an important risk factor for developing diabetes and observed a stable adjusted HR of 2.49 (1.52, 4.07). Further, we repeated our analysis for incident composite diabetes after restricting the eligible participants to only those with a BMI<30 kg/m^2 such that the BMI for exposed participants with NFAT was 25.0 (3.4) and the BMI for unexposed participants with adrenal tumors was 24.5 (3.1), *P*=0.21. There were no other notable differences in demographic of comorbid factors between exposure groups. The adjusted HR for incident composite diabetes after restricting BMI to <30 kg/m^2 remained stable at 3.04 (1.39, 6.65).

Exploratory Analyses Including Potential Subclinical Hypercortisolism

We explored whether there was a continuum of risk for incident diabetes when including participants with potential subclinical hypercortisolism. Of the 89 participants with adrenal tumors we excluded for adrenal hormone excess, 35 were excluded for subclinical hypercortisolism, and of these only 25 were eligible for analyses for incident composite diabetes (≥3 years of follow-up and no diabetes at baseline). Subclinical hypercortisolism was defined by 1mg DST in 21/25 with a mean post-dexamethasone cortisol of 2.8 (0.8) mcg/dL and by 24h UFC in 4/25 with a mean 24h UFC of 72.8 (19.3) mcg. During a mean follow-up

duration of 7.9 (3.2) years, 32.0% of participants with subclinical hypercortisolism developed incident composite diabetes (**Figure 2D**). In an exploratory analysis where participants with no adrenal tumors were the reference, the unadjusted hazard for incident diabetes was 3.10 (2.01, 4.79) for participants with NFAT and 3.30 (1.58, 6.85) for participants with adrenal tumors with subclinical hypercortisolism.

Size of NFAT, Adrenal Steroid Levels, and Cardiometabolic Outcomes

In secondary analyses, we observed that larger NFAT size was associated with higher BMI (r=0.20, =0.25, P<0.01) but was not associated with age (r=0.07, =0.05, P=0.29). Further, larger NFAT size was strongly associated with higher "normal" cortisol levels following dexamethasone (r=0.32, =0.015, *P*<0.0001) and higher 24h urinary free cortisol levels within the "normal" range (r=0.36, =0.48, *P*<0.0001) (**Figure 3A & 3B**). Both relationships remained significant following adjustments for age, sex, race, BMI, smoking status, and prevalent hypertension, diabetes, hyperlipidemia, cardiovascular events, and chronic kidney disease (=0.015, P<0.001 and =0.43, P=0.001, respectively). We observed that the prevalence of type 2 diabetes was higher with higher quartiles of cortisol following 1 mg DST (*P*-trend=0.04) (**Figure 3C**). The mean serum cortisol following DST in NFAT with type 2 diabetes at baseline was 1.22 (0.4) mcg/dL in comparison to 1.05 (0.4) mcg/dL in NFAT without baseline type 2 diabetes (*P*=0.02).

There was no significant association between the size of NFAT, or the degree of cortisol suppression following dexamethasone, with the prevalence or incidence of composite diabetes or other outcomes; however, the number of incident cases in these analyses was small enough to limit a robust analysis.

DISCUSSION

Adrenal tumors are incidentally discovered in 1-10% of all abdominal imaging studies (1-5). Despite this high prevalence, adrenal tumors are often lost to clinical follow-up or not given dedicated recognition (23) either because they were detected incidentally when imaging was performed for another priority non-adrenal indication, and/or because many health-care providers may not understand the importance of screening for adrenal hormone excess. This is relevant because adrenal tumors may be "functional" in that they autonomously secrete hormones that cause overt or subclinical adrenal hormone excess and increase the risk for adverse cardiovascular and/or metabolic clinical outcomes. However, most adrenal tumors are ultimately determined to be "non-functional," and therefore considered to pose no health risk. The findings from our current investigation substantially extend and potentially redefine the current paradigm of a "non-functional" adrenal tumor: we observed that individuals with apparent NFAT, as defined by current consensus practice guidelines, had a >2-fold higher risk of developing incident diabetes when compared to participants without adrenal tumors. Our findings were independent of other risk factors for developing glucose intolerance or insulin resistance, and suggest that a potential mechanism underlying this increased risk for diabetes may be glucocorticoid excess that is currently considered "normal" by our accepted practice standards.

Our findings could have important public health implications even by conservative estimates. More than 85 million CT scans and 32 million MRI scans were performed in 2011 in the U.S. alone (24). Conservatively estimating that only 60% of these scans were in unique individuals and that only 40% of these were of the abdomen and that only 1% of these abdominal scans included an incidentally discovered adrenal tumor, at least 290,000 individuals per year in the U.S. may have imaging evidence of an adrenal tumor. This is striking since approximately 90% (12, 14) of incidentally discovered adrenal tumors will either be determined to be "non-functional" and therefore presumed to pose no health risk (1, 2), or will not receive appropriate follow-up to evaluate functionality. Therefore, conservatively >290,000 patients per year in the U.S. alone may have an unrecognized and significantly increased risk of developing

impaired glucose tolerance and incident diabetes which are now considered to be among the leading causes of death in the U.S. (25).

Prior longitudinal studies by Di Dalmazi et al.(13), Debono et al.(12), and Morelli et al.(14) (each with a total of 198, 206, and 206 participants respectively) have described associations between adrenal tumors with subclinical hypercortisolism and incident cardiovascular events (14) as well as cardiovascular and all-cause mortality(12, 13). These three studies used the NFAT group as the referent control, and did not include comparisons with individuals without adrenal tumors. Whether the higher risk of cardiovascular events and mortality associated with hypercortisolism in these studies was mediated by development of incident insulin resistance or diabetes, a known cardiovascular risk factor, was not directly evaluated. In contrast to these important studies, our study was designed to focus only on NFAT in comparison to no adrenal tumors; however, we were able to conduct an exploratory analysis that included a small population of participants with potential subclinical hypercortisolism that suggested a continuum of risk. Collectively, our studies (12-14) suggest a "*continuum of metabolic and cardiovascular risk*:" NFAT may impart an increased risk of developing insulin resistance and diabetes when compared to individuals without adrenal tumors, and the addition of subclinical hypercortisolism may further increase the risk of developing cardiovascular events and death, which are dramatically increased in states of overt Cushing syndrome (**Figure 4**).

Some small cross-sectional studies have suggested a potential link between NFAT and metabolic and cardiovascular diseases (10, 17-19). Androulakis et al. showed that individuals with NFAT had greater insulin resistance indices and carotid intima-media thickness, and less flow-mediated dilation, when compared to healthy controls without adrenal tumors (17). Further, they observed associations between higher carotid intima-media thickness and the level of cortisol within the "non-functional" range, suggesting that even the spectrum of "normal" cortisol concentrations may confer cardiometabolic risk. The findings of our secondary analyses demonstrate that larger NFAT size associated with more cortisol

secretion within the presumed "normal" range, and that higher "normal" cortisol levels following DST associated with a higher prevalence of type 2 diabetes. Although we did not observe any relation between NFAT size and incident outcomes, we presume that this effect may have been negated by the fact that larger NFAT (>4 cm) were preferentially surgically resected in accordance with current guidelines (1, 2, 4, 15, 16).

Cortisol is a potent glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) agonist, but it is certainly *not* the only adrenal steroid capable of activating these receptors. Morphologically normal and neoplastic adrenal glands may secrete many other potential GR and MR agonists. As elegantly demonstrated by Arlt et al. in their studies of adrenal steroid profiling using mass spectroscopy (20), when compared to healthy controls without adrenal tumors, NFAT secrete higher levels of non-cortisol glucocorticoids that are not typically measured or captured by our standard clinical assays of cortisol. Thus, our reliance on assessing cortisol as a surrogate for the functionality of adrenal tumors may be inadequate in that the spectrum of GR and MR agonists that are secreted may be much greater. Together, our findings provide some general support for the speculation that adrenal tumors may continue to secrete low concentrations of GR and/or MR agonists that can contribute to cardiometabolic disease risk, and therefore, future studies of NFAT should incorporate methods such as broad adrenal steroid profiling to evaluate a larger spectrum of potential GR and MR agonists that might account for our findings of incident diabetes (20).

Our findings must be interpreted in the context of the limitations of our study design. First, observational studies can suffer from potential confounding and selection bias. There may have been potential confounders that we did not account for because they were either unknown, unmeasured, or in the case of family history, not recorded consistently in the medical charts to include. Second, since the root cause of adrenal neoplasia remains unresolved, an alternative interpretation of our findings could be that an unknown factor that induces adrenal neoplasia may also increase the risk of diabetes. Regardless of the

interpretation, the ultimate clinical applicability of our findings remains that patients with adrenal tumors have a higher risk for developing diabetes and therefore more frequent screening should be considered. Third, we recognize that selection bias may have played a role in how we classified NFAT and bias in the ascertainment of outcomes could have influence the result. We used documentation of diagnoses to screen for adrenal tumor and observed that only 1,346 participants from the >220,000 eligible participants without an adrenal hormonal diagnosis who underwent abdominal imaging. This <1% prevalence of adrenal tumors on imaging is much lower than previous reports (3-5), and likely represents the fact that most incidentally discovered adrenal tumors were not officially included as diagnoses in the patient record, or were lost to follow-up during the clinical care of the primary indication for imaging. Therefore, it is possible that our selection of NFAT may have represented patients who had more visits to their healthcare providers; and/or had more conscientious healthcare providers; and/or had more abdominal imaging due to a greater burden of medical problems. However, we observed no major demographic or comorbidity differences in the eligible participants included in our incident composite diabetes analyses, and our evidence suggests that both exposure groups were followed for comparable durations of time with comparable screening and assessments of outcomes. Fourth, our findings may not be generalizable to men since the majority of our study population was female. The female predominance of our study population was unexpected, and may be due to the fact that women are much more likely than men to visit with and maintain longitudinal follow-up with their physicians (26, 27) and more likely to undergo abdominal imaging than men (of the >234,000 of abdominal scans available to us, 65% were in women). Fifth, we cannot exclude meaningful associations between NFAT and other cardiometabolic outcomes, where confidence intervals suggest the potential for a relationship. It should be noted that our classification of diabetes outcomes was the most refined since it incorporated documented diagnosis and/or supportive HbA1c levels. In contrast, the classification of some other clinical outcomes (such as hypertension) relied on documentation of diagnoses alone. Sixth, we did not have repeated and longitudinal assessments of adrenal hormones or NFAT size to assess whether new or worsening adrenal hormone excess could account for our findings (12-14). It is possible that NFAT are related to risk for diabetes due to the

development of progressive subclinical glucocorticoid secretion that we did not assess; however, the underlying message of our study, that NFAT should be recognized and monitored as potential risk factors for diabetes, is unlikely to change even if we had repeated measures of hormones or NFAT size. Seventh, we did not have corticotropin levels since they were rarely measured in clinical practice. Our data suggest that corticotropin levels should be low or suppressed with NFAT, while other studies suggest that adrenal tumors may even secrete local corticotropin, and future studies should investigate this hypothesis and consider using corticotropin-releasing hormone stimulated dexamethasone-suppression testing as well(28, 29). Lastly, our study was not designed to evaluate whether the causal mechanism behind our finding was mild excess secretion of cortisol or other GR or MR agonists not captured by cortisol assays; however, our secondary analyses do suggest that this hypothesis is worth pursuing in future studies.

In summary, our findings demonstrate a greater than 2-fold higher risk of developing incident diabetes in individuals with NFAT when compared to those without adrenal tumors. Given the high prevalence of incidentally discovered adrenal tumors that predominantly represent benign NFAT, our findings have important implications for general clinical practice and future research investigations: 1) the findings underscore the importance of recognizing incidentally discovered adrenal tumors as potential risk factors for developing diabetes that may warrant more frequent surveillance for glucose intolerance; 2) the currently accepted criteria by which we classify adrenal tumors as “non-functional” may need re-evaluation since our findings suggest that NFAT may increase risk for diabetes by secreting clinically relevant excesses of adrenocortical hormone(s); and 3) future studies that include broad adrenal steroid metabolite profiling are needed to confirm whether NFAT secrete inappropriate amounts of GR and/or MR agonists that evade our current clinical practice or detection capabilities and contribute to adverse cardiometabolic outcomes.

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FIGURES

Figure 1: Selection of the study population and identification of exposure status (NFAT vs. no adrenal tumors).

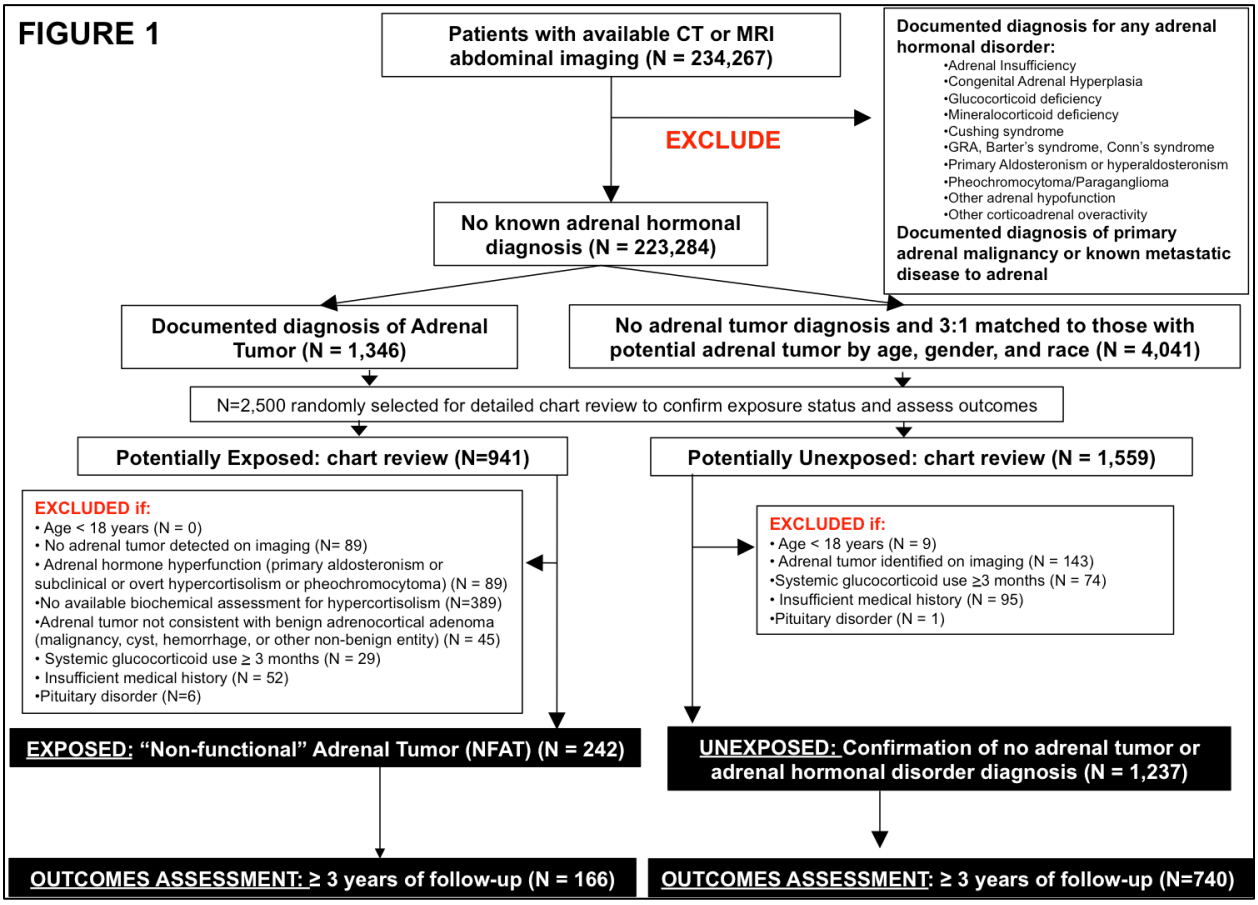
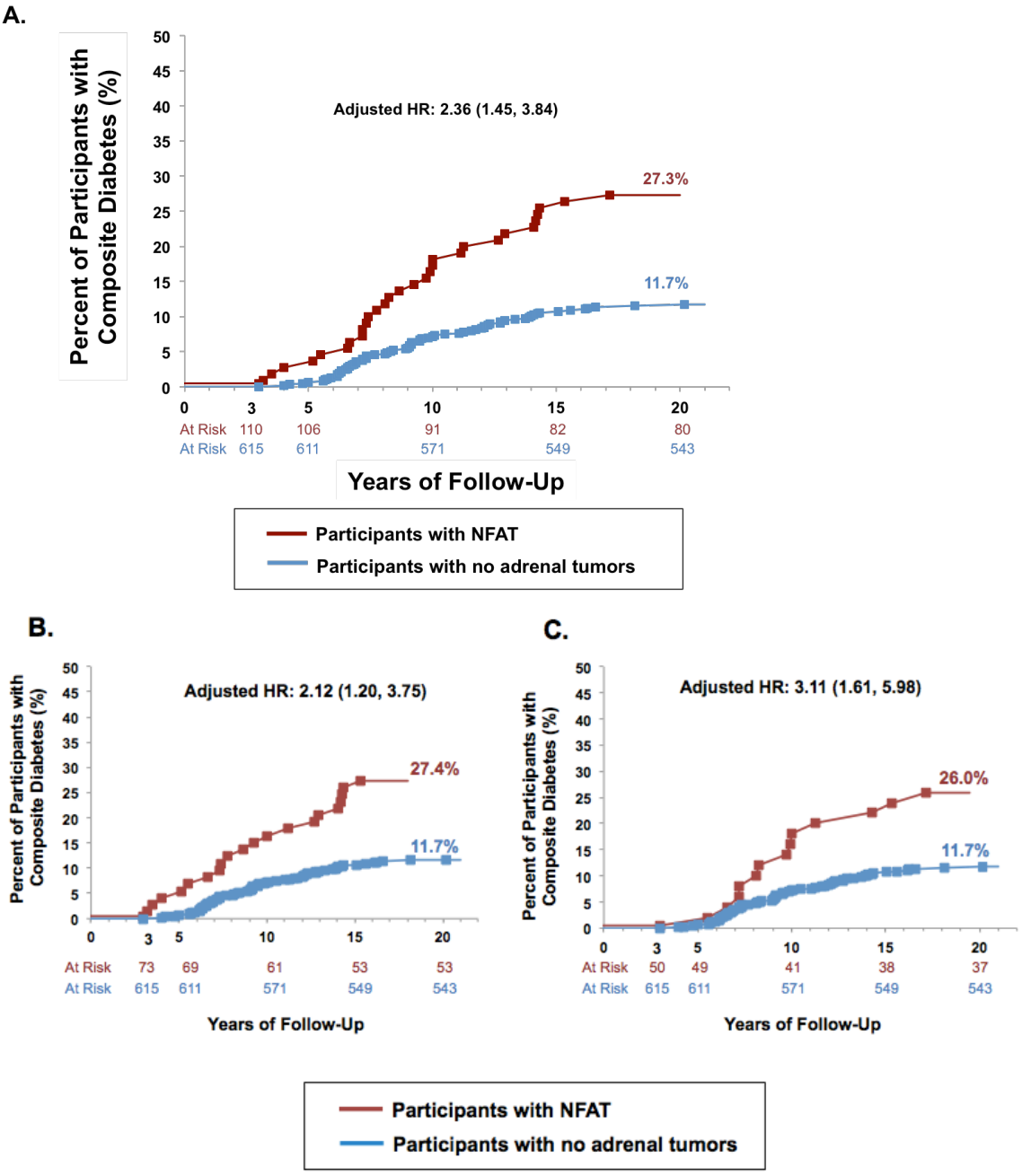


Figure 2: Cases of incident composite diabetes during longitudinal follow-up. Shown is the proportion of participants who developed incident composite diabetes during follow-up. Panel (**A**) shows all 725 eligible participants (110 with NFAT and 625 without adrenal tumors), where participants with NFAT had subclinical hypercortisolism excluded using either a 1mg DST or a 24h UFC. Panel (**B**) shows only those participants with NFAT who had subclinical hypercortisolism excluded using a 1mg DST≥1.8 mcg/dL (73 eligible), and panel (**C**) shows only those participants with NFAT who had subclinical hypercortisolism excluded using a 24h UFC<50 mcg (50 eligible). Panel (**D**) depicts an exploratory analysis, whereby participants who were excluded from the main analysis due to potential subclinical hypercortisolism were included, comparing participants without adrenal tumors, to those with NFAT, and those with adrenal tumors with potential subclinical hypercortisolism.



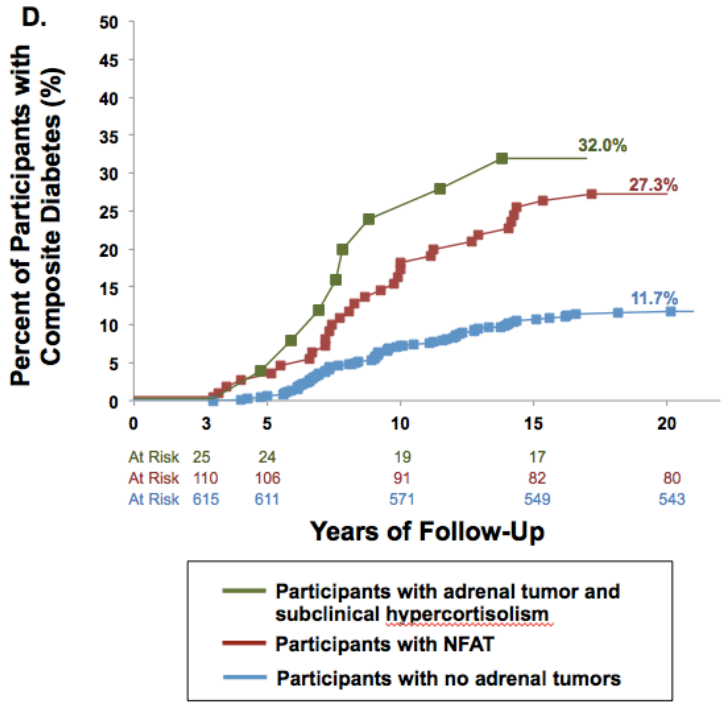

D.
Percent of Participants with Composite Diabetes (%)
50
45
40
35
30
25
20
15
10
5
0
32.0%
27.3%
11.7%
0
3
5
10
15
20
At Risk 25 24 19 17
At Risk 110 106 91 82 80
At Risk 615 611 571 549 543
Years of Follow-Up
Participants with adrenal tumor and subclinical hypercortisolism
Participants with NFAT
Participants with no adrenal tumors

Figure 3: The relationship between the size of NFAT and (**A**) the degree of serum cortisol suppression following a 1 mg DST where all values are <1.8 mcg/dL, and (**B**) the 24 hour urinary free cortisol where all values are <50 mcg/24h. Shown are the mean regression line (bold), the 95% C.I. for the mean regression (shaded), and the 95% C.I. for the observed values (dashed). (**C**) The prevalence of type 2 diabetes in participants with NFAT by quartile of "normal" serum cortisol levels following 1 mg DST, where all cortisol levels are < 1.8 mcg/dL.

Figure 3

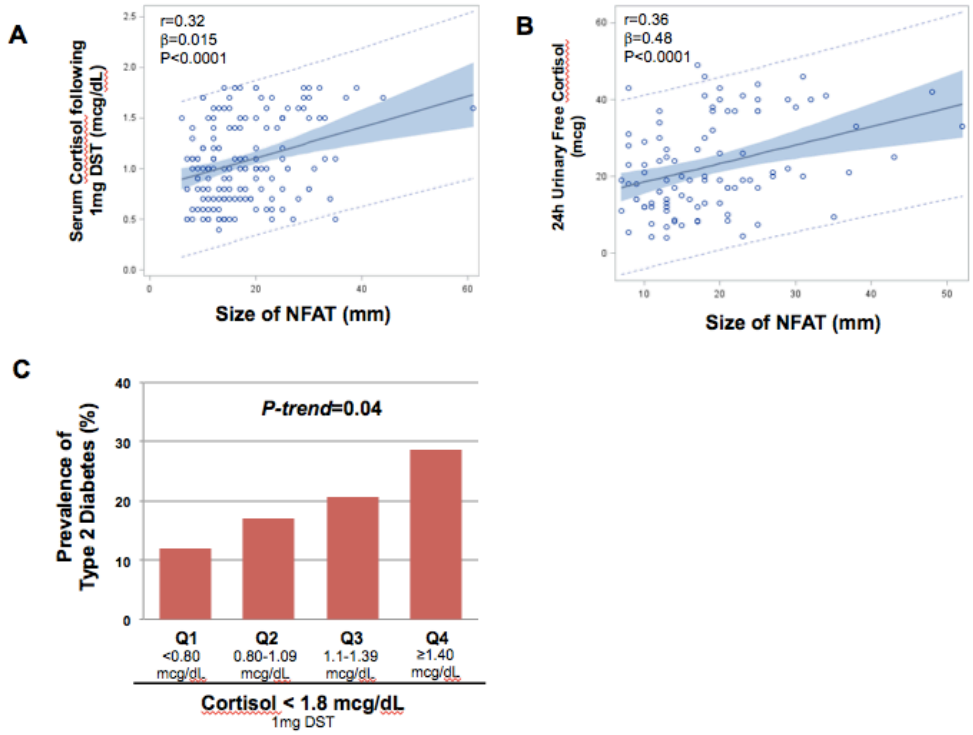
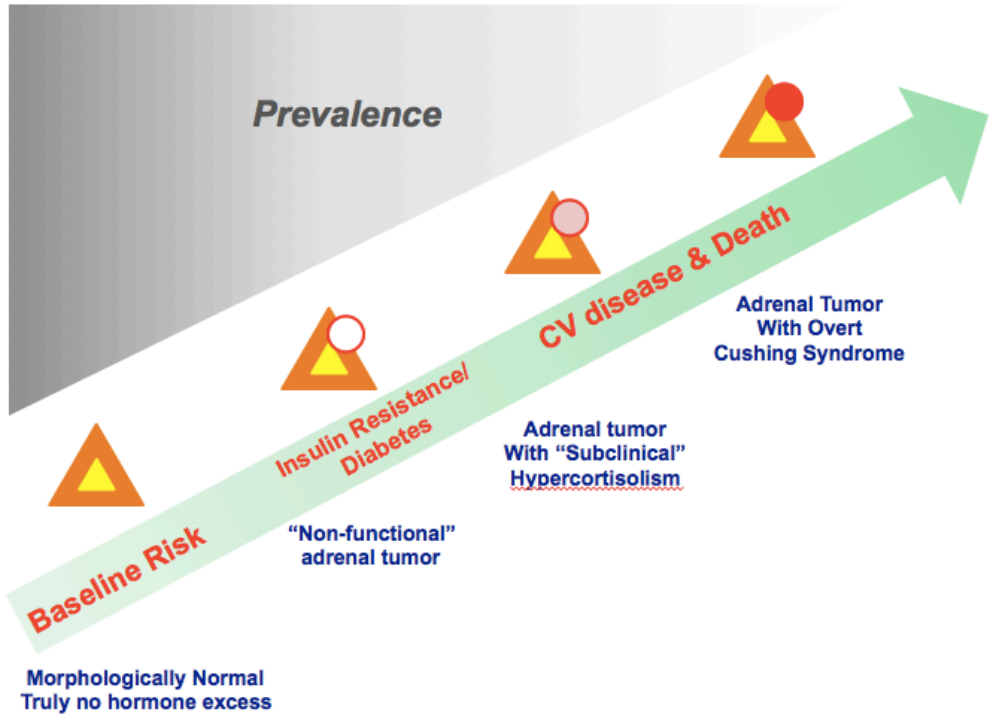


Figure 4: Schematic representation of the "continuum of metabolic and cardiovascular risk" associated with adrenal tumors. When compared to morphologically normal adrenal glands that secrete no adrenocortical hormones in excess, NFAT defined by current criteria are associated with a higher risk of developing insulin resistance states such as diabetes. This risk of diabetes associated with NFAT may be attributed to low-grade secretion of adrenal glucocorticoids (and/or mineralocorticoids) within ranges we currently consider to be "normal." The development of subclinical hypercortisolism, when compared to NFAT, increases the risk of developing cardiovascular events and death, whereas overt hypercortisolism (Cushing syndrome) has a very high risk of developing severe metabolic and cardiovascular complications.



TABLES

Table 1. Demographic and clinical characteristics of the study population at baseline.

	Non-Functional Adrenal Tumor	No Adrenal Tumor	*P*
N	242	1237	
Age (y)	56.8 (11.4)	59.6 (14.6)	<0.01
BMI (kg/m^2)	30.9 (7.3)	28.1 (6.8)	<0.0001
Female (%)	77.7	70.7	0.03
Race			
White (%)	60.7	62.3	
Black (%)	15.7	8.9	0.01
Hispanic (%)	8.3	6.7	
Other (%)	15.3	22.1	
Smoking			
Non-smoker (%)+	68.6	72.4	0.24
Current smoker (%)	31.4	27.6	
Hypertension (%)	54.6	50.4	0.26
Pre-Diabetes* (%)	7.9	3.1	<0.001
Type 2 Diabetes**(%)	20.7	14.2	0.01
Composite diabetes [†] (%)	28.5	17.3	0.0001
Hyperlipidemia (%)	46.3	39.9	0.07
Coronary Artery Disease (%)	11.2	10.2	0.64
Stroke (%)	2.1	3.8	0.25
Atrial Fibrillation (%)	3.7	6.4	0.14
Chronic Kidney Disease (%)	6.2	5.7	0.76
History of Cardiovascular Event (%)‡	16.9	18.1	0.71
Medications for hypertension (%)	48.8	48.3	0.94
Medications for diabetes[#] (%)	15.3	10.3	0.03
Medications for hyperlipidemia or coronary heart disease[##] (%)	35.6	27.9	0.01

Data are mean (SD) or number of patients (%) where applicable. P values were calculated with t tests (continuous variables) or Chi $\chi 2$ or Fisher's exact test (categorical variables).
+Presumed never smokers or former smokers who quit > 6 months ago
*Pre-Diabetes: Physician diagnosis and/or two or more separate HbA1c values between 5.7-6.5% without treatment with oral hypoglycemic agents (other than metformin) or injectable diabetes medications.
**Diabetes: Physician diagnosis of "type 2 diabetes mellitus" or two or more separate HbA1c values ≥ 6.5%.
[†]Composite diabetes: Combination of Pre-diabetes and Diabetes.
‡History of myocardial infarction, ischemic stroke, heart failure, atrial fibrillation, or interventional coronary procedure (such as coronary catheterization or coronary artery bypass graft surgery).
oral antihyperglycemics or insulin
aspirin, statins, fibrates, niacin

Table 2: NFAT and the risk of developing incident cardiometabolic outcomes. The table shows the results of adjusted Cox proportional hazards regression models to evaluate the risk for developing incident outcomes in participants with non-functional adrenal tumors when compared to participants without any adrenal tumors.

	Hypertension	Hyper-lipidemia	Composite Diabetes	Pre-Diabetes	Type 2 Diabetes	CKD	CVE
Eligible Participants+	474	548	725	859	772	867	767
Number of Incident Events†	144	81	102‡	59	58	77	68
Unadjusted HR	1.33 [0.76, 2.01]	1.59 [0.91, 2.80]	3.00 [1.95, 4.61]	3.35 [1.98, 5.67]	2.11 [1.16, 3.87]	1.24 [0.69, 2.22]	1.18 [0.63, 2.20]
Multivariable Model 1 HR*	1.11 [0.68, 1.83]	1.35 [0.74, 2.47]	2.65 [1.69, 4.16]	3.41 [1.93, 6.03]	2.04 [1.08, 3.89]	1.29 [0.68, 2.43]	0.97 [0.50, 1.87]
Multivariable Model 2 HR**	0.98 [0.58, 1.64]	1.31 [0.69, 2.48]	2.52 [1.57, 4.04]	3.47 [1.94, 6.23]	1.84 [0.93, 3.62]	1.13 [0.58, 2.20]	0.66 [0.33, 1.33]
Multivariable Model 3 HR***	1.24 [0.73, 2.11]	1.19 [0.63, 2.25]	2.36 [1.45, 3.84]	4.09 [2.27, 7.39]	1.36 [0.67, 2.78]	1.12 [0.57, 2.19]	0.62 [0.30, 1.27]

+Eligible participants include those who did not have the outcome of interest at baseline and who had ≥ 3 years of follow-up data to assess the incident development of this outcome over time.
†Incident event is defined as not having the specific outcome at baseline assessment and developing the outcome during the follow-up time.
‡Incident events of composite diabetes do not add up to the sum of incident type 2 diabetes events and pre-diabetes events because 15 individuals who developed incident type 2 diabetes had a diagnosis of pre-diabetes at baseline.
*Multivariable Model 1 includes adjustment for: age, BMI, sex, race, and smoking status
**Multivariable Model 2 includes adjustment for: Model 1 + other clinically relevant cardiovascular and metabolic diagnoses (hypertension, composite diabetes, hyperlipidemia, CVE, CKD).
***Multivariable Model 3 includes adjustment for: Model 2 + use of anti-hypertensive medications, use of anti-diabetes medications (oral antihyperglycemics and insulin), and use of medications to treat coronary artery disease or hyperlipidemia (anti-platelets, nitrates, statins, fibrates, niacin).

Table 3: Demographic and clinical characteristics of the eligible participants for analyses on incident composite diabetes. This group of eligible participants had no baseline diabetes and ≥3 years of follow-up.

	Non-Functional Adrenal Tumor	**No Adrenal Tumor**	***P***
N	110	615	
Age (y)	56.1 (12.1)	56.3 (14.5)	0.92
Female (%)	84.6	80.5	0.37
Race			
White (%)	65.5	67.3	0.97
Black (%)	9.1	9.3	
Hispanic (%)	7.3	6.2	
Other (%)	18.2	17.3	
BMI (kg/m^2)	29.5 (6.9)	27.7 (6.4)	0.01
Years of Follow-up (y)	7.34 (3.3)	8.0 (3.7)	0.06
Smoking			
Non-smoker (%)+	66.4	75.0	0.08
Current smoker (%)	33.6	25.0	
Hypertension (%)	45.5	38.9	0.20
Pre-Diabetes* (%)	0.0	0.0	-
Type 2 Diabetes**(%)	0.0	0.0	-
Composite Diabetes [†] (%)	0.0	0.0	-
Hyperlipidemia (%)	40.0	31.7	0.10
Coronary Artery Disease (%)	8.2	5.5	0.27
Stroke (%)	0.9	3.4	0.23
Atrial Fibrillation (%)	1.8	4.2	0.29
Chronic Kidney Disease (%)	7.3	2.4	0.02
History of Cardiovascular Event (%)‡	10.9	11.5	1.0
Medications for hypertension (%)	40.5	37.4	0.60
Medications for diabetes[#] (%)	0.0	0.0	-
Medications for hyperlipidemia or coronary heart disease[##] (%)	22.7	17.6	0.23
Assessment of basic metabolic panel within 1 year of final follow-up (%)^	100.0	100.0	1.0
Assessment of lipid profile within 1 year of final follow-up (%)^^	75.5	67.6	0.27
Assessment of hemoglobin A1c within 1 year of final follow-up (%)^^^	43.8	35.7	0.17
Assessment of blood pressure within 1 year of final follow-up (%)^^^^	100.0	100.0	1.0

Data are mean (SD) or number of patients (%) where applicable. P values were calculated with t tests (continuous variables) or Chi $\chi 2$ and Fischer's exact tests (categorical variables).

+Presumed never smokers or former smokers who quit > 6 months ago

*Pre-Diabetes: Physician diagnosis and/or two or more separate HbA1c values between 5.7-6.5% without treatment with oral hypoglycemic agents (other than metformin) or injectable diabetes medications.

**Type 2 Diabetes: Physician diagnosis of "type 2 diabetes mellitus" or two or more separate HbA1c values ≥ 6.5%.

†Total Diabetes: Combination of Pre-diabetes and Diabetes.
‡History of myocardial infarction, ischemic stroke, heart failure, atrial fibrillation, or interventional coronary procedure (such as coronary catheterization or coronary artery bypass graft surgery).
^Laboratory assessment of a basic metabolic panel (which includes electrolytes, creatinine, and glucose) within one year of the final assessment of outcomes.
^^ Laboratory assessment of a lipid profile (which includes total cholesterol, triglycerides, and low and high density lipoprotein cholesterol) within one year of the final assessment of outcomes among participants not on lipid lowering medications.
^^^ Laboratory assessment of hemoglobin A1c within one year of the final assessment of outcomes.
^^^^ Measurement of in-office blood pressure within one year of the final assessment of outcomes.

SUPPLEMENTARY DATA

Extended Details of Methods: Study Population

The study population was selected from the Research Patient Data Registry at Partners Inc., a centralized clinical data registry including patients from the Brigham and Women's Hospital and Massachusetts General Hospital and their partner affiliate hospitals. All study procedures where approved by the Partners Institutional research and ethics review board. Our study protocol permitted use of the data registry to build a cohort of patients with adrenal disorders, and controls without adrenal disorders, to evaluate exposures and outcomes. The cohort was de-identified once relevant data were extracted from medical charts; personal identifiers were not saved in the database. We followed the STROBE statement of reporting.

We performed queries within the Research Patient Data Registry to identify all patients who had undergone either abdominal computed tomography (CT) or abdominal magnetic resonance imaging (MRI) for any indication between 1991-2014 (n=234,267) (**Figure 1**). Adrenal tumors can also occasionally be detected on some chest CT or MRI that extended into the abdominal cavity; however, chest imaging was not included in the current selection process. From this population, we excluded participants who had a documented diagnosis for any adrenal hormonal disorder or malignancy ("documented diagnosis" refers to healthcare provider registration of the diagnosis in the medical record problem list or use of an ICD-9 code indicating the diagnosis) (**Figure 1**).

Potentially "exposed" individuals were identified by a documented diagnosis of an "adrenal tumor," "adrenal mass," "adrenal nodule," or "adrenal incidentaloma" (n=1,346). Although it is likely that more than 1,346 participants may have had evidence for adrenal tumor on imaging, this methodology to screen for potentially exposed participants only captured those whose healthcare providers officially documented the diagnosis. We identified potentially "unexposed" individuals by performing a blinded computer-based match to identify participants who did not have a documented diagnosis for any adrenal tumor in a 3:1 manner matched by age, se, and race (n=4,041). From these 5,387 potentially exposed and unexposed study participants, we randomly selected 2,500 to undergo individual and detailed medical chart review to confirm exposure status and to assess cardiometabolic outcomes and other potential confounders (**Figure 1**).

Extended Details of Methods: Assessment of Main Exposure

To confirm exposure status, we analyzed individual medical charts. To confirm benign NFAT, we excluded participants who: did not have an adrenal mass detected on their official abdominal imaging report, had imaging characteristics to suggest a malignant or non-adenomatous adrenal mass, did not have biochemical assessments for subclinical hypercortisolism, had any biochemical evidence of adrenal hormone hyperfunction (see below), had a documented diagnosis of any adrenal hormone disorder, used any systemic glucocorticoid use for ≥ 3 months, were not adults (<18 years of age), and who had insufficient medical history to confirm the presence of an adrenal mass (**Figure 1**). Radiographic reports were reviewed to confirm the size and location of adrenal masses on their first detection. In instances where multiple or bilateral adrenal masses occurred, we recorded the size of the largest mass/nodule. We used biochemical test results to exclude participants with adrenal tumors who might potentially have subclinical or overt adrenal hormone excess. Participants with potential subclinical hypercortisolism were excluded if they had a serum cortisol of >1.8 μg/dl (50 nmol/liter) following a 1 mg dexamethasone suppression testing (DST) and/or a urinary free cortisol (UFC) ≥ 50 mcg/24h. Of the remaining 244 participants, 70% had serum aldosterone and plasma renin activity measurements to assess for primary aldosteronism and we therefore further excluded participants with potential subclinical or overt primary aldosteronism if they had an aldosterone-to-renin ratio >30 ng/dL per ng/mL/h or a combination of a serum aldosterone > 15 ng/dL with a plasma renin activity < 1.0 ng/mL/h[1]. Following these exclusions, we confirmed 242 participants with NFAT (**Figure 1**), of which subclinical hypercortisolism was excluded using the 1mg DST (n=164), 24h UFC (n=104), or both (n=28). We conducted similar detailed chart reviews on potentially unexposed participants to confirm that they had no adrenal mass or nodules on abdominal imaging reports and had no evidence or documentation of adrenal hormonal disorders or diagnoses, resulting in a population of 1,237 matched participants with confirmation of no adrenal tumors or adrenal hormonal disorders.

1. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2008;93(9):3266-3281.

Appendix Table 1: Odds ratios and 95% confidence intervals for the association between having a non-functional adrenal tumor and individual clinical diagnoses at baseline, when compared to participants without any adrenal tumor.

	Hypertension	Hyper-lipidemia	Composite diabetes	Pre-diabetes	Type 2 Diabetes	CKD	CVE
Unadjusted	1.12 [0.89, 1.55]	1.30 [0.98, 1.71]	1.91 [1.39, 2.61]	2.69 [1.52, 4.75]	1.57 [1.11, 2.23]	1.10 [0.62, 1.96]	0.92 [0.64, 1.33]
Multivariable Model 1*	1.35 [0.98, 1.87]	1.35 [0.99, 1.84]	1.97 [1.37, 2.83]	2.52 [1.38, 4.62]	1.59 [1.07, 2.38]	1.48 [0.79, 2.78]	1.37 [0.88, 2.14]
Multivariable Model 2**	1.18 [0.84, 1.65]	1.20 [0.87, 1.66]	1.79 [1.22, 2.62]	2.33 [1.26, 4.29]	1.38 [0.91, 2.10]	1.28 [0.67, 2.45]	1.19 [0.75, 1.88]

*Multivariable Model 1 includes adjustment for: age, BMI, sex, race, and smoking status.
**Multivariable Model 2 includes adjustment for: Model 1 and other clinically relevant cardiovascular and metabolic diagnoses (hypertension, composite diabetes, hyperlipidemia, CKD, CVE).