1 **LOW DHEAS: A SENSITIVE AND SPECIFIC TEST FOR THE DETECTION OF**

2 **SUBCLINICAL HYPERCORTISOLISM IN ADRENAL INCIDENTALOMAS.**

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12 **Abbreviated title:** DHEAS and subclinical hypercortisolism in AI.

13 **Key words:** DHEAS, subclinical hypercortisolism, adrenal incidentaloma

14 **Word Count:** Abstract 249; Text 3580; Figures 3; Table 1

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21 **Disclosures:** The authors have nothing to disclose.

22   **Abstract**

23   **Context:** Subclinical hypercortisolism (SH) occurs in 5-30% of adrenal incidentalomas (AIs). Common

24   screening tests for adrenocorticotropin (ACTH)-independent hypercortisolism have significant false

25   positive rates, mandating further time and resource intensive investigations.

26   **Objective:** To determine whether low basal dehydroepiandrosterone sulphate (DHEAS) is a sensitive

27   and specific screening test for SH in AI.

28   **Setting and Patients:** 185 patients with AI were screened for adrenal medullary (plasma

29   metanephrines) and cortical [1 mg overnight dexamethasone suppression test (ONDST), 24h urinary

30   free cortisol (UFC), serum DHEAS, plasma renin and aldosterone] hyperfunction. Positive ONDST

31   [≥1.8 mcg/dL (≥50 nmol/L)] and/or UFC (>upper limit of reference range) results were further

32   investigated. We diagnosed SH when at least two of the following were met: raised UFC, raised

33   midnight serum cortisol, 48h DST cortisol ≥1.8 mcg/dL (≥50 nmol/L).

34   **Results:** Twenty-nine patients (16%) were diagnosed with SH. ACTH was <10 pg/mL (<2.2 pmol/L)

35   in all patients with SH. We calculated age- and gender-specific DHEAS ratios (derived by dividing the

36   DHEAS by the lower limit of the respective reference range) for all patients. ROC analyses,

37   demonstrated that a ratio of 1.12 was sensitive (>99%) and specific (91.9%) for the diagnosis of SH.

38   Cortisol following 1mg ONDST of 1.9 mcg/dL (53 nmol/L) was a sensitive (>99%) screening test for

39   SH, but had lower specificity (82.9%). 24 h UFC lacked sensitivity (69%) and specificity (72%).

40   **Conclusion:** A single basal measurement of DHEAS offers comparable sensitivity and greater

41   specificity to the existing gold-standard 1 mg DST for the detection of SH in patients with AIs.

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Introduction

Incidentally discovered adrenal lesions [so-called adrenal incidentalomas (AIs)] are a common cause of endocrine referral (1). Although most are benign and not associated with overt endocrine dysfunction, subtler forms of adrenal hypersecretion (hypercortisolism, hyperaldosteronism, sex-steroid excess or phaeochromocytoma) have been variably reported in up to 20−40 % of AIs (2,3). The terms subacute autonomous glucocorticoid hypersecretion (SAGH), subclinical Cushing's syndrome (SCS), (possible) autonomous cortisol secretion, and subclinical hypercortisolism (SH) are often interchangeably used to denote adrenocorticotropin (ACTH)-independent cortisol secretion from a benign adrenal adenoma or nodular adrenal hyperplasia that is not associated with clinically-overt hypercortisolism (2,4,5). Several groups have reported adverse clinical sequelae in individuals with SH, with recent studies highlighting an increase in cardiovascular morbidity and mortality compared to the general population (6-12). While independent medical treatment of cardiovascular risk factors may represent a reasonable long-term management strategy for individuals with SH, recent advances in laparoscopic and retroperitonoscopic adrenalectomy raise the possibility of a potentially curative intervention for a subgroup of patients (13,14). A related, but frequently under-appreciated risk in these patients is chronic hypothalamic-pituitary-adrenal axis suppression, resulting in an impaired or absent stress response to intercurrent illness (7). Accurate exclusion or confirmation of a diagnosis of SH is therefore a key step in the investigation and management of patients with AIs.

The 1 mg (overnight) dexamethasone suppression test (ONDST) is often employed as a sensitive screening test to exclude SH (1,4,15), but its relatively poor specificity (70–80%) results in a significant number of false positive tests, requiring further evaluation that is both time and resource intensive (4). This has led some workers to propose a higher threshold [≥5mcg/dL (≥138 nmol/L)] for triggering additional screening/confirmatory investigations in the context of AI (15), but with the inevitable consequence of failing to diagnose milder cases of SH. Measurement of 24 h urinary free cortisol excretion rates (UFCs) is also potentially problematic with causes of both false positive (e.g. obesity) and false negative (e.g. mild hypercortisolism, renal impairment) results recognised (4,16). Demonstration of the loss of the normal circadian rhythm of cortisol secretion is considered a sensitive

69   test for the detection of hypercortisolism, but opinions vary as to the thresholds that should be employed

70   to optimize detection while minimizing false positive results (for example, although a sleeping midnight

71   serum cortisol of <1.8 mcg/dL (<50 nmol/L) effectively excludes Cushing's syndrome in the absence

72   of cyclical hypercortisolism, false positive results are common, especially in patients hospitalized for

73   short periods (<48h) (4,17-19). Similarly, late night salivary cortisol is sensitive for the detection of

74   hypercortisolism, but may be falsely elevated in shift workers, smokers or if there is blood

75   contamination of the sample (4,20,21). Early morning (9 AM) plasma ACTH levels are typically low

76   or suppressed in SH, but there is overlap with ACTH levels seen in normal individuals, reflecting its

77   pulsatile secretion and short half-life (4,22).

78   Synthesis and secretion of the adrenal androgen dehydroepiandrosterone (DHEA), and its sulphated

79   form DHEAS, are regulated by pituitary ACTH. Sustained suppression of central ACTH drive therefore

80   leads to a reduction in DHEA and DHEAS levels (23-25). However, DHEA has a short half-life (25

81   mins) and is secreted in a circadian manner similar to ACTH; hence, the interpretation of a single DHEA

82   measurement is subject to the same caveats as with ACTH. In contrast, DHEAS has a prolonged half-

83   life in serum (10−16h), with relatively stable levels throughout the day, making it a more attractive

84   marker for the detection of chronically suppressed ACTH. (26,27). Indeed, the potential utility of

85   DHEAS to reflect ambient ACTH levels over a longer time interval has led to DHEAS being proposed

86   as an indicator of persisting autonomous ACTH secretion in Cushing's disease following pituitary

87   surgery (28), and as a possible marker of SH in patients with AI (29, 30, 31).

88   We therefore performed a systematic comparison of a single DHEAS measurement at presentation with

89   the current gold-standard 1 mg dexamethasone suppression test, for the detection of SH in 185

90   consecutive individuals with AI referred to the endocrine department of a university hospital. We

91   describe the utility of DHEAS as a sensitive and specific marker of SH within this cohort.

Patients and Methodology

Patients

The records of 185 consecutive patients with adrenal incidentalomas referred to the Endocrine Department of Cambridge University Hospitals Foundation Trust between January 2006 and April 2013 were reviewed. All patients had undergone standardized clinical, biochemical and radiological assessments according to an Institutional protocol, as part of routine clinical care. We performed a retrospective analysis of this systematically collected dataset. Appropriate Institutional approval was obtained.

Seventeen patients were excluded on the basis of: (i) concomitant use of drugs influencing glucocorticoid metabolism or secretion; (ii) major psychiatric illness or history of excess alcohol intake; or (iii) overt clinical features of hypercortisolism. One patient had previously undergone pituitary surgery and was also excluded. All AIs were discovered by computed tomography (CT) scanning or magnetic resonance imaging (MRI) performed for unrelated reasons, and all lesions were >1cm in diameter. Adrenal adenomas displayed the following radiological features: either ≤10 HU on unenhanced CT (in which case no further characterization was performed); or >50% absolute washout at 10 minutes (or >60% at 15 minutes) on triple phase adrenal CT; or signal dropout on chemical shift MRI (32,33).

All patients were assessed by one of three clinicians experienced in the diagnosis and management of Cushing's syndrome, and each underwent standardized clinical and biochemical screening at first presentation according to an institutional clinical protocol. This included measurement of plasma metanephrines, 24 h urine collection for urinary free cortisol estimation (UFC), plasma renin and aldosterone, serum DHEAS, electrolytes, liver blood tests, fasting plasma glucose, fasting lipids and complete blood count. All patients also underwent a 1 mg overnight dexamethasone suppression test, with values <1.8 mcg/dL (<50nmol/L) signifying adequate suppression (34). Measurement of UFC was performed separately on two 24 h urine collections, with the higher of the two values used for subsequent analysis. Patients returning a post-dexamethasone cortisol ≥1.8 mcg/dL (≥50 nmol/L)

118   and/or raised UFC [>6.5 mcg/dL (>180 nmol)/24 h prior to 2011 and >5.3 mcg/dL (>146 nmol)/24 h

119   after 2011] underwent more detailed evaluation during an inpatient admission as follows: repeat 24 h

120   UFC, midnight serum cortisol, 48 h low dose (0.5 mg qds) dexamethasone suppression test (LDDST),

121   plasma ACTH (measured on 2 occasions between 08:00 and 09:00). We diagnosed SH in the presence

122   of at least two of the following: failure to suppress serum cortisol to <1.8 mcg/dL (<50 nmol/L)

123   following dexamethasone; sleeping midnight serum cortisol >1.8 mcg/dL (>50 nmol/L) or awake

124   midnight serum cortisol >7.5 mcg/dL (>207 nmol/L); raised UFC (32). Although not used as a

125   diagnostic criterion, all patients subsequently confirmed with SH had a 09:00 ACTH level <10 pg/mL

126   (<2.2 pmol/L). All investigations were reviewed by two endocrinologists (MCD, MG), who were

127   blinded to the DHEAS result for each patient. For the purpose of analyses, a DHEAS ratio was

128   calculated by dividing the measured DHEAS by the lower limit of the respective reference range (age-

129   and sex-matched). DHEAS values reported as falling below the lower limit of detection of the assay

130   [<15.0 mcg/dL (<0.4 micromol/L)] were interpreted as equivalent to the lowest measurable value [i.e.

131   15.0 mcg/dL (0.4 micromol/L)].

132   *Assays*

133   Biochemical assays were run in a UKAS (http://www.ukas.com/default.asp) accredited clinical

134   laboratory. External Quality Assurance (EQA) for all assays was provided by UKNEQAS

135   (http://www.ukneqas.org.uk).

136   DHEAS and ACTH were assayed using a Siemens (Surrey, UK) Immulite 2000 platform with reagents

137   provided by the same manufacturer. DHEAS was analyzed by solid phase competitive immunoassay.

138   Age and sex adjusted reference ranges for DHEAS are provided in Table 1. ACTH was analysed by

139   sequential two site solid phase chemiluminescent immunoassay.

140   Aldosterone was measured using solid phase radioimmunoassay (Coat-A-Count, Siemens, Los

141   Angeles, USA). The Laboratory working range was 70–3330 pmol/L. Plasma Renin was measured

142   using a Diasorin XL (Kent, UK) chemillumunescent immunometric assay. The Laboratory working

143   range was 2–3000 μIU/mL.

144 Plasma metanephrines were analyzed using a derivitization ELISA assay provided by Labor

145 Diagnosticka Nord GmbH (Nordhorn, Germany). The Laboratory working range was 41-2100 pg/mL

146 for metanephrine and 58-58000 pg/mL for normetanephrine.

147 Urine cortisol was measured by competitive radioimmunoassay using reagents provided by Siemens

148 (Coat-a-Count) or using an in-house liquid chromatography – tandem mass spectrometry method. The

149 Laboratory working range was 25-1380 nmol/L and the laboratory reference range for the RIA was

150 <180 nmol/24 h. The mass spectrometric assay was performed using an API 5500 triple quadrupole

151 mass spectrometer (ABPsiex, UK) using atmospheric pressure chemical ionization. Deuterated (d4)

152 cortisol was used as an internal standard. Urine samples were mixed with internal standard in 50%

153 methanol/water. Samples were pre-cleaned using a C8 reverse phase column before HPLC separation

154 using a pheny-hex column with a shimadzu HPLC system. The Laboratory working range was 12–2330

155 nmol/L and the laboratory reference interval for the liquid chromatography–tandem mass spectrometry

156 method was <146 nmol/24 h.

157 Between batch imprecision was <10% across the working ranges for all assays.

158
159 *Statistical Analysis*

160 With the exception of age and body mass index, which are reported as mean ± standard deviation (SD),

161 all data are expressed as medians with interquartile ranges. For parametric data, hypothesis testing was

162 performed using an unpaired students t-test. For non-parametric data, statistical analysis was performed

163 using the *Mann Whitney U Test*.

164 Receiver operated characteristic (ROC) curves were generated to assess the utility of DHEAS in the

165 diagnosis of SH, using the first, randomly sampled DHEAS ratio for each patient included in the study.

166 ROC curves were also generated for the 1 mg overnight dexamethasone suppression test and the original

167 24 h UFC measurement.

168 ROC analysis evaluated the performance of each parameter for the diagnosis of SH against non-

169 functioning adrenal adenomas (NFAs). Performance of each screening / diagnostic test is expressed as

170   area under the curve (AUC) with 95% confidence intervals. Data are also presented for sensitivity and

171   specificity for each screening / diagnostic test with 95% confidence intervals. All analyses were

172   performed using SPSS V 21.0 or GraphPad Prism V 6.0.

173   Due to the systematic nature of investigation for AI in our Institution, and robust data collection, there

174   were no missing data for ONDST, LDDST, UFC or demographic details in the study cohort.

175 **Results**

176 *Patient Characteristics*

177 As described above, 18 subjects were excluded from further analyses due to confounding clinical

178 disorders or medication usage. Final diagnoses in the remaining 167 patients were: non-functioning

179 adrenal adenoma (n=97), subclinical hypercortisolism (n=29), phaeochromocytoma (n=19), adrenal

180 metastasis (n=8), primary aldosteronism (n=4), adrenal hemorrhage (n=2), adrenocortical carcinoma

181 (n=2), myelolipoma (n=2), oncocytoma (n=1) and three subjects had Cushing's disease with an adrenal

182 incidentaloma (Supplemental Fig. 1A). There was no difference in age [NFA: 63.4 ± 12.8 yr (mean $\pm$

183 SD); SH: 65.8 ± 11.3 yr (p=0.36)], sex [NFA: Male 44/97; SH: Male 14/29 (p=0.41)] or body mass

184 index [NFA: 31.8 ± 9.2 kg/m^2 (mean $\pm$ SD); SH: 29.4 ± 7.5 kg/m^2 (p=0.91)] between the NFA and SH

185 subgroups. The age distribution of the cohort as a whole was consistent with other published series

186 (Supplemental Fig. 1B) (6-11). Twenty-three patients had bilateral adrenal adenomas (16 NFA; 7 SH).

187 *DHEAS ratio for the screening and diagnosis of SH*

188 There was a significant difference in the DHEAS ratio between the two study groups [median 2.62 (1.8,

189 5.5) for NFAs versus 1.0 (0.77, 1.0) for SH; p < 0.0001]. DHEAS ratio was a sensitive and specific test

190 both with respect to screening and diagnosis of SH versus NFA [AUC 0.95 (0.91, 0.99); p<0.0001].

191 Evaluation for SH versus NFA showed that for a DHEAS ratio of 1.12, sensitivity was 100% (86.2,

192 100) and specificity was 91.9% (83.9, 96.7) (Fig. 1). This finding was consistent in a subgroup analysis

193 of patients with bilateral adrenal adenomas (Supplemental Fig. 2A). In fact, DHEAS ratio also

194 performed well as a sensitive and a specific test for the diagnosis of SH against all other causes of

195 adrenal lesions within this cohort, as all other non-SH causes of adrenal nodules returned a DHEAS

196 ratio >1.12. Included in this, a low DHEAS ratio also differentiated between ACTH-independent and

197 ACTH-dependent hypercortisolemia in the presence of an adrenal lesion (Supplemental Fig. 3).

198 *1 mg dexamethasone suppression test for the screening and diagnosis of SH*

199 There was a significant difference between the two study groups with respect to post-1mg

200 dexamethasone cortisol levels: median 1.3 mcg/dL (0.91, 1.71) [36 nmol/L (25.0, 47.3)] for NFAs,

201 versus 4.2 mcg/dL (1.96, 8.73) [116 nmol/L (92.0, 241.0)] for SH (p<0.0001). As expected, the 1 mg

202 overnight dexamethasone suppression test performed well as a screening test for SH when compared

203 with NFAs [AUC 0.97 (0.95, 0.99); p<0.0001] (Fig. 2). A post-dexamethasone value of 1.9 mcg/dL (53

204 nmol/L) yielded a sensitivity >99% (87.2, 100) and a specificity of 82.9% (73.4, 90.1). As a diagnostic

205 test, a value of 4.0 mcg/dL (109 nmol/L) had a sensitivity of 55.5% (35.3, 74.5) with a specificity of

206 >99% (95.9, 100). A post-dexamethasone cortisol of 2.1 mcg/dL (58.5 nmol/L) gave the best overall

207 test performance: sensitivity 92.5% (75.7, 99.0); specificity 88.6% (80.1, 94.4). Findings in those with

208 bilateral adrenal adenomas were largely consistent with this (Supplemental Fig. 2B). The 1 mg

209 overnight dexamethasone suppression test did not differentiate between SH and other causes of

210 hypercortisolism such as ACTH-mediated or adrenocortical carcinoma.

211 *24 hour UFC for the screening and diagnosis of SH*

212 For the purpose of analyses, a UFC ratio was calculated by dividing the measured 24 hour UFC by the

213 upper limit of the reference range. There was a significant difference in the UFC ratio between the two

214 study groups: median 0.74 (0.46, 1.16) for NFAs versus 1.3 (0.95, 1.84) for SH (p < 0.0001) (Fig. 3).

215 However, UFC performed relatively poorly both as a screening test and as a diagnostic test for SH

216 versus NFA [AUC 0.77 (0.67, 0.87); p<0.01], and this pattern held true for bilateral adenomas

217 (Supplemental Fig 2C). A ratio of 1.01 gave a sensitivity of 69 % (48.2, 85.7) and a specificity of 67%

218 (55.5, 78.2). A ratio of 0.47 gave a sensitivity of 100% (86.8, 100) but a specificity of 25.3% (15.8,

219 37.1), while a ratio of 2.1 gave a specificity of 100% (94.9, 100), but a sensitivity of just 19.2% (6.6,

220 39.4). Therefore, while UFC did not perform well as a screening test, a value greater than twice the

221 upper limit of the reference range performed well as a confirmatory test (Fig. 3).

Discussion

In this study we have robustly evaluated the diagnostic utility of a single DHEAS measurement as a sensitive and specific marker of SH in adrenal incidentalomas, using a complete and systematically collected dataset. Consecutive patients referred to our service were investigated using widely accepted screening and confirmatory tests (4). For every patient we also calculated a ratio of measured DHEAS to the age- and sex-specific lower reference interval. Using this approach, DHEAS ratio performed well as a screening test for SH, with a threshold of ≤1.12 demonstrating a sensitivity >99% while showing higher specificity than the ONDST (91% versus 86% respectively) (Figs. 1 & 2). Moreover, DHEAS ratio reliably differentiated between SH and non-SH etiologies of adrenal nodules within our cohort (Supplemental Fig. 3).

Our findings lend important support to previous proposals and guidelines, which have observed that a low or suppressed DHEAS level should be considered a potential indicator of SH in the context of AI (29-31). Yener and colleagues have also explored the association between low DHEAS levels and SH, noting that an age-unadjusted DHEAS threshold of 40.0 mcg/dL provided a sensitivity of 68% and specificity of 75% for the diagnosis of SH (29). In the study reported here, we have demonstrated a higher sensitivity and specificity for low DHEAS in the diagnosis of SH. This may be explained by some important differences in study design. Firstly, rather than adopting a higher ONDST threshold [3.0 mcg/dL (83 nmol/L)] as a confirmatory test for SH, we have used the diagnostic criteria recommended in the Endocrine Society Clinical Practice Guidelines (4), which include the LDDST as a 'gold standard' confirmatory test. In our hands, when compared with the LDDST, the higher ONDST threshold of 3.0 mcg/dL (83 nmol/L) performed less well as a confirmatory test for SH, returning a sensitivity of 85% and specificity of 95%. Secondly, we have accounted for the expected age-related decline and sex-related differences in DHEAS by calculating a sex- and age-adjusted DHEAS ratio based on locally validated reference ranges, rather than using absolute DHEAS values (Table 1). Although a previous report by Bencsik and colleagues suggested that DHEAS may not be a useful indicator of hormonal activity in AI (34), the main focus of this study was to explore the potential for DHEAS to discriminate benign from malignant AI. Moreover, the analysis was restricted to only a

11

249 small number of histologically-confirmed cases (thus introducing potential selection bias). Importantly

250 the authors noted that DHEAS measurement may have a role once the cortical origin and benign feature

251 of the tumor has been confirmed.

252 In this study we employed robust criteria for the diagnosis of SH across multiple testing modalities,

253 which allowed us to also examine the performance of investigations that are commonly used to

254 detect/exclude hypercortisolism. The ONDST performed well as a screening test for SH. The traditional

255 cortisol threshold of ≥1.8 mcg/dL (≥50 nmol/L) yielded a sensitivity >99% and a specificity of 81%,

256 consistent with previous findings for this test (Fig. 2) (4) . The higher suggested cut-off value for cortisol

257 of ≥5 mcg/dL (>138 nmol/L) post-dexamethasone provided a specific test (>99%), but at the cost of a

258 low sensitivity of 41% which would have missed the majority of subsequently diagnosed SH within

259 this cohort. In our hands the lowest cut-off cortisol value which produced a specificity >99% was 109

260 nmol/L. However, this was associated with lower sensitivity (56%) and hence performed poorly as a

261 screening test. As anticipated, the ONDST did not differentiate between ACTH-dependent and

262 independent forms of hypercortisolaemia. Accordingly, we support the traditional cortisol threshold of

263 ≥1.8 mcg/dL (≥50 nmol/L) in the ONDST as the trigger for further confirmatory testing as

264 recommended in the Endocrine Society and European Society guidelines (4).

265 The utility of urinary free cortisol in the diagnosis of SH and in the broader context of Cushing's

266 syndrome has long been debated. There are numerous pitfalls associated with this test. Urine collection

267 over a 24 h period is not always convenient for the patient and it is often difficult to obtain a complete

268 sample in the clinic setting. Several studies have shown that 24 h UFC measurement performs poorly

269 overall both as a screening and a diagnostic test, particularly if reliance is placed on a single

270 measurement (20). Within our cohort 24 h UFC (measured on two occasions with the highest value

271 used for subsequent analyses) demonstrated a low sensitivity (67%) and specificity (69%), although

272 values ≥ 2 times the upper limit of the reference range carried a specificity >99% but a sensitivity of

273 only 25% (Fig. 3). It is important to note however, that we used a univariate UFC measurement, rather

274 than the multi-analyte, steroid metabolomic profiling that is increasingly available, with more recent

275 data suggesting superior sensitivity and specificity of urinary steroid metabolomics for the differential

276 diagnosis of steroid excess in the context of adrenal disease (35).

277 In the setting of SH, ACTH levels are typically suppressed and we also observed this, with all affected

278 patients returning levels <10 ng/L (<2.2 pmol/L). However, measurement of ACTH in the clinical

279 setting carries multiple sampling challenges and can produce false positive results when sampled under

280 non-ideal circumstances, reflecting the short half-life, pulsatile secretion and diurnal variability of

281 ACTH release (4).

282 Measurement of DHEAS also offers some other potential advantages when screening in AI: firstly, it

283 allows detection of tumors that are co-secretory (and therefore suspicious for malignancy); secondly, it

284 has recently been recognized that some cases of congenital adrenal hyperplasia may present with AI,

285 although we did not identify any such cases in our cohort (36).

286 It is important to note however that the reliability of low or suppressed DHEAS as a marker for SH test

287 may be limited under certain circumstances, for example when there is pre-existing ACTH-suppression

288 in the context of chronic glucocorticoid or opioid use, pituitary disease or hypothalamic dysfunction.

289 Under these circumstances a false positive result may be obtained (37). However, each of the existing

290 screening tests have well recognized limitations and pitfalls, and also require specific sampling

291 conditions.

292 Our study has other potential limitations. The reported data are representative of a patient population

293 attending a single center, using a single platform for DHEAS measurement. Multi-center studies and/or

294 meta-analyses, specifically investigating the use of the DHEAS ratio reported here as a screening test

295 for SH in the context of AI will be required to support and validate our findings, across patient

296 populations, using different DHEAS assays. It will also be important to validate the utility of DHEAS

297 ratio alone or in combination with the ONDST (at differing threshold values) for confirming or

298 excluding SH without the need to progress to more expensive, time-consuming investigations.

299   Finally, low DHEAS has also been directly linked with increased cardiovascular risk (38), and it is

300   interesting to speculate that undiagnosed SH may underlie this association given the recent

301   demonstration that SH is associated with excess cardiovascular mortality (7,10,39).

302   In conclusion, our data support the use of an age-adjusted DHEAS ratio as a sensitive and specific

303   screening test for SH in patients with incidentally detected adrenal incidentalomas. Pending further

304   studies, we suggest that the identification of a low or suppressed DHEAS level in a patient with AI

305   should trigger more detailed assessment for SH.

306   **Acknowledgements**

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