

1 **ASSOCIATIONS OF ‘RELATIVE CORTICOSTERONE DEFICIENCY’ WITH GENETIC**
2 **VARIATION IN *CYP17A1* AND METABOLIC SYNDROME FEATURES**

3 Scott D Mackenzie^{1*}, Andrew A Crawford^{1,2,3*}, Daniel Ackermann⁴, Katharina E Schraut¹, Caroline
4 Hayward⁵, Jennifer L Bolton¹, Christopher Saunders¹, Emad Al-Dujaili¹, Bernhard Dick⁴, Geneviève
5 Escher⁴, Bruno Vogt⁴, Menno Pruijm⁶, Belen Ponte⁷, James F Wilson^{5,8}, Mark W J Strachan¹, Jackie F
6 Price⁸, David I W Phillips⁹, Scott M MacKenzie¹⁰, Eleanor Davies¹⁰, Rebecca M Reynolds¹, and Brian
7 R Walker^{1,11}

8 *These authors contributed equally

9 (1) BHF Centre for Cardiovascular Science, Queen’s Medical Research Institute, University of
10 Edinburgh, United Kingdom, (2) MRC Integrative Epidemiology Unit at the University of Bristol,
11 Bristol, UK (3) Population Health Sciences, Bristol Medical School, University of Bristol, Bristol,
12 UK (4) University Clinic for Nephrology, Hypertension and Clinical Pharmacology, Inselspital, Bern
13 University Hospital, University of Bern, Switzerland, (5) MRC Human Genetics Unit, Institute of
14 Genetics and Molecular Medicine, University of Edinburgh, United Kingdom, (6) Service of
15 Nephrology, Lausanne University Hospital (CHUV), Switzerland, (7) Service of Nephrology,
16 University Hospitals of Geneva (HUG), Switzerland, (8) Centre for Global Health Research, Usher
17 Institute for Population Health Sciences and Informatics, University of Edinburgh, United Kingdom,
18 (9) MRC Lifecourse Epidemiology Unit, Southampton General Hospital, United Kingdom, (10)
19 Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom. (11)
20 Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK

21

22 ABBREVIATED TITLE: Corticosterone, *CYP17A1* and metabolic syndrome

23 KEYWORDS: Glucocorticoids; Metabolic Syndrome; 17 α -Hydroxylase; *CYP17A1*

24 CORRESPONDENCE (including for reprint requests):

25 Professor Brian R Walker

26 Newcastle University

27 Executive Office

28 King's Gate

29 Newcastle upon Tyne

30 NE1 7RU

31 UK

32

33 Tel +44 (0)191 208 7701

34 Email: Brian.Walker@ncl.ac.uk

35

36 FINANCIAL DISCLOSURE:

37 The Wellcome Trust Scottish Translational Medicine and Therapeutics Initiative (STMTI), Medical

38 Research Council, British Heart Foundation, Wellcome Trust and the Swiss National Science

39 Foundation (FN 33CM30-124087, Schweizerischer Nationalfonds (SNF), Wildhainweg 3, Postfach

40 8232, 3001 Bern, Switzerland). The funders had no role in study design, data collection and analysis,

41 decision to publish, or preparation of the manuscript.

42 COMPETING INTERESTS:

43 The authors have declared that no competing interests exist.

45 **ABSTRACT**

46 **Context and objective.** Common genetic variants in *CYP17A1* associate with higher blood pressure,
47 putatively from impaired 17 α -hydroxylase activity and mineralocorticoid excess. However, the same
48 variants protect against obesity and insulin resistance. We tested whether *CYP17A1* variants that
49 enhance 17 α -hydroxylase activity cause ‘relative corticosterone deficiency’. Since corticosterone is
50 thought to contribute disproportionately to negative feedback in the hypothalamic-pituitary-adrenal
51 axis, we also tested whether lower corticosterone associates with higher cortisol and hence with
52 metabolic syndrome.

53

54 **Design:** Cross-sectional studies within the population-based Orkney Complex Disease Study
55 (ORCADES; n=2018), VIKING Health Study Shetland (VIKING; n=2098), East Hertfordshire study
56 (EHERTS; n=279), Edinburgh Type 2 Diabetes Study (ET2DS; n=903), and the Swiss Kidney Project
57 on Genes in Hypertension (SKIPOGH; n=888).

58

59 **Outcome measures.** Cortisol and corticosterone in morning plasma samples in ORCADES, VIKING
60 and ET2DS, and in EHERTS in plasma following overnight dexamethasone suppression (0.25mg)
61 and 30 mins after ACTH₁₋₂₄ (1 μ g); cortisol and corticosterone metabolites in day and night urine
62 samples in SKIPOGH. Features of the metabolic syndrome including body mass index, systolic blood
63 pressure, lipid profile, fasting glucose, fasting insulin and HOMA-IR.

64

65 **Results.** In ORCADES, ET2DS and SKIPOGH, *CYP17A1* variants were associated with
66 corticosterone:cortisol ratio. In ORCADES, VIKING and ET2DS there were consistent associations
67 of morning plasma cortisol and corticosterone with BMI, blood pressure, lipid profile, fasting glucose
68 and HOMA-IR. In EHERTS, however, after dexamethasone suppression and ACTH₁₋₂₄ stimulation,
69 impaired glucose tolerance and insulin sensitivity were associated with higher cortisol but lower
70 corticosterone. Similarly, in SKIPOGH, low corticosterone:cortisol metabolite ratios were associated
71 with high BMI and dyslipidemia.

72

73 **Conclusions.** ‘Relative corticosterone deficiency’, due to a primary alteration in adrenal
74 steroidogenesis favouring cortisol over corticosterone, may mediate the associations of genetic
75 variation in *CYP17A1* with metabolic syndrome. However, additional determinants of variation in
76 plasma corticosterone are likely to explain its generally positive associations with features of
77 metabolic syndrome.

78 INTRODUCTION

79 In genome-wide association studies (GWAS) common variants in the *CYP17A1* locus are consistently
80 associated with hypertension (1-4) and cardiovascular disease (5, 6). *CYP17A1* encodes the
81 steroidogenic enzyme CYP17A1, which is expressed in human but not rat or mouse adrenal cortex and
82 catalyses 17 α -hydroxylase activity, converting precursors for synthesis of 11-deoxycorticosterone and
83 corticosterone into 17-hydroxylated precursors for synthesis of 11-deoxycortisol and cortisol. Rare
84 mutations causing near-absent CYP17A1 activity result in low-renin hypertension (7), attributed to
85 accumulation of the mineralocorticoid 11-deoxycorticosterone. Ratios of steroid metabolites reflecting
86 CYP17A1 activity are highly heritable (8). Associations of more common variation in *CYP17A1* with
87 hypertension might therefore be explained by reduced 17 α -hydroxylase activity.

88 Intriguingly, *CYP17A1* risk alleles for hypertension are also associated with lower body mass index
89 (BMI) (9-11) and enhanced insulin sensitivity (12). Conversely, alleles which are protective for
90 hypertension, and therefore predicted to increase 17 α -hydroxylase activity, confer increased risk of
91 obesity and insulin resistance. This paradox is unexplained, but may relate to variations in production
92 of the major glucocorticoid, cortisol. Elevated cortisol in Cushing's syndrome causes obesity and
93 insulin resistance, while plasma cortisol is more subtly increased in metabolic syndrome (13). However,
94 if elevated 17 α -hydroxylase activity contributes to obesity and insulin resistance by increasing cortisol
95 production, why would plasma cortisol concentration not be 'corrected' by enhanced negative feedback
96 suppression of ACTH secretion and a compensatory fall in cortisol production? An explanation may be
97 found by considering the role of the second glucocorticoid in humans, corticosterone.

98 Widely neglected in humans, corticosterone circulates at concentrations of 5-10 % those of cortisol.
99 However, concentrations of corticosterone in human cerebrospinal fluid and brain are relatively high,
100 at ~30% of cortisol (14, 15). This discrepancy has been attributed to differential trans-membrane
101 trafficking of steroids by the ATP-binding cassette (ABC) transporter ABCB1, which is expressed in
102 the human CNS and selectively exports cortisol rather than corticosterone (15). Corticosterone may
103 therefore make a disproportionate contribution to negative feedback control of the hypothalamic-

104 pituitary-adrenal (HPA) axis within the CNS. Notably, the endogenous negative feedback signal is
105 known to be impaired in people with metabolic syndrome (16).

106 We hypothesised that individuals with a genetically determined increase in 17 α -hydroxylase activity
107 have relatively low corticosterone which causes impaired negative feedback to the HPA axis, and hence
108 sustains higher plasma cortisol. We predict this pattern of ‘relative corticosterone deficiency’ is
109 associated with obesity and insulin resistance but lower blood pressure. We tested this hypothesis in
110 population-based cohorts, by studying the associations of corticosterone and cortisol in plasma and of
111 their metabolites in urine with: (i) genetic variation in *CYP17A1*; and/or (ii) features of metabolic
112 syndrome.

113

114 MATERIALS AND METHODS

115 Participants

116 All studies conformed with the Declaration of Helsinki and ethical approval and written informed
117 consent were obtained.

118 *Orkney Complex Disease Study (ORCADES)*

119 The ORCADES study is a genetic epidemiological study based in the Scottish archipelago of Orkney,
120 comprising 2039 subjects aged 18-100 years, with at least two Orcadian grandparents, recruited
121 between 2005 and 2011 (17). Subjects attended a local or mobile venepuncture clinic between 0730h
122 and 1100h (mean 0923 h \pm SD 46 min), after fasting from 2200h the previous night. On another occasion
123 subjects attended for measurement of weight, height and blood pressure. Genotyping was undertaken
124 as described (17).

125 *Viking Health Study – Shetland (VIKING)*

126 The VIKING study is a population cohort study based in an isolated population in the north of Scotland
127 that recruited 2105 volunteers between 2013 and 2015. Subjects were required to have at least two
128 grandparents from the Shetlands in order to participate. Each participant attended a measurement clinic
129 and a venepuncture clinic to give a fasting blood sample. Participants' DNA was genotyped using the
130 Illumina HumanOmniExpressExome8v1-2_A.

131 *Edinburgh Type 2 Diabetes Study (ET2DS)*

132 The Edinburgh Type 2 Diabetes Study (ET2DS) is a prospective cohort study comprising 1066 men
133 and women with type 2 diabetes, living in Lothian, Scotland. Recruitment and study design has been
134 reported (18). Briefly, participants aged 60-75 years with a diagnosis of type 2 diabetes according to
135 WHO criteria (19) were recruited from a clinical database. Following overnight fast, subjects attended
136 a research clinic at 0800-0830h where they underwent venepuncture and physical examination.
137 Genotyping was performed by KBioscience (Herts, UK) using a competitive allele-specific PCR system
138 (KASPar).

139 *East Hertfordshire Study (EHERTS)*

140 All births in EHERTS from 1911 onwards were notified by the attending midwife (20). As described,
141 these records were used to recruit 309 women and 370 men, with no history of diabetes, for a standard
142 75-g oral glucose tolerance test (OGTT), performed at a clinic at 0800-1020 h following overnight fast
143 from 2100 h (21). In follow up studies (21), 312 of these individuals, aged 67-78 years, ingested 0.25
144 mg dexamethasone at 2200 h, fasted overnight and attended a local clinic at 0830h next morning. A
145 baseline blood sample was obtained before 1.0 μ g ACTH₁₋₂₄ (tetracosactrin, Synacthen, Alliance,
146 Chippenham, UK) was administered intravenously, and repeat samples obtained after 30 minutes.

147 *Swiss Kidney Project on Genes in Hypertension (SKIPOGH)*

148 The SKIPOGH study is a family-based cross-sectional study comprising 1093 subjects aged 18-82 years
149 from 2 regions (Bern and Geneva) and 1 city (Lausanne) of Switzerland (8). A random sample of the
150 inhabitants were invited to participate if they were of European ancestry and had at least 1 first degree
151 family member also willing to participate. They attended hospital in the morning after an overnight fast
152 for venepuncture and physical examination. Five consecutive blood pressure (BP) measurements were
153 taken from the arm with the higher BP, and the average of the last four was used. Urine samples were
154 collected separately for day- and night-time periods, as described (8). DNA samples were genotyped
155 on the Illumina MetaboChip array.

156 **Laboratory analyses**

157 In ORCADES , VIKING, EHERTS, and SKIPOGH, the homeostasis model assessment was used to
158 quantify insulin resistance (HOMA-IR) (22). Serum cortisol was measured by radioimmunoassay with
159 Guildhay antisera (23). After exclusion of individuals prescribed glucocorticoid therapy within the
160 previous 3 months (ORCADES n = 21; ETD2S n=163; EHERTS n = 33), there was sufficient sample
161 for analysis of corticosterone in 2018 individuals from ORCADES, 2098 in VIKING, 903 in ETD2S,
162 and 279 in EHERTS. An in-house radioimmunoassay, modified for microtiter plate scintillation
163 proximity assay (SPA), was used to measure plasma corticosterone with assay characteristics as
164 previously reported (24); cross-reactivity for dexamethasone and cortisol was less than 1%. Results
165 were accepted if the coefficient of variation between duplicates was <15%. In SKIPOGH, urinary

166 steroid metabolites were extracted and analysed by gas chromatography-mass spectrometry (GC-MS)
167 as previously described (8); analyses comprised 888 individuals after exclusion of 205 subjects with
168 missing steroid metabolites. To estimate the apparent CYP17A1 activity, the ratio (tetrahydro-11-
169 dehydrocorticosterone (THA) + tetrahydrocorticosterone (THB) + 5 α -tetrahydrocorticosterone
170 (5 α THB)) / (tetrahydrocortisone (THE) + tetrahydrocortisol (THF) + 5 α -tetrahydrocortisol (5 α THF))
171 was calculated.

172 **Statistical analysis**

173 Associations with genotype were analysed using Stata v14.1 (Stata Statistical Software: Release 14.
174 College Station, TX: StataCorp LP). Given limited statistical power, we restricted analysis to candidate
175 single nucleotide polymorphisms (SNPs) in *CYP17A1* which have functional effects; these included
176 rs2486758, located in the *CYP17A1* promoter region and associated with ovarian steroidogenesis (25,
177 26), and the other SNPs in Table 2 which affect CYP17A1 transcription *in vitro* (27). All analyses were
178 adjusted for age and sex. Because clearance of glucocorticoids is increased and plasma cortisol
179 decreased in obesity (28), relevant analyses were adjusted for BMI. Because timing of sampling varied
180 in ORCADES, venesection time (as minutes after the first sample in the cohort) was included as a
181 predictor variable in all models (29). In ORCADES many of the participants are related; analysis of
182 associations of SNPs with plasma glucocorticoid concentrations and metabolic risk factors was
183 therefore undertaken following adjustment after fitting both the first 3 principal components of ancestry
184 and the kinship matrix using a mixed model (mmscore function of GenABEL for the association test in
185 ProbABEL) (30) under an additive model. The kinship matrix used the identity-by-state function of
186 GenABEL (using weight = "freq" option).

187 Other analyses for ORCADES, VIKING, ET2DS and EHERTS were undertaken using Minitab (version
188 16; State College PA). Continuous response variables were normalised by log transformation. Student's
189 t-tests were used for group comparisons of corticosterone:cortisol ratios and responses to ACTH.
190 Simple linear regression analysis of corticosterone or cortisol with a given 'response' (or dependent)
191 variable was used to determine unadjusted p values for the regression co-efficient. Analyses were
192 repeated with potentially confounding co-variables in multiple regression analyses, adjusting for age,
193 sex, BMI and time of sampling. Histograms and normal plots of residuals were examined to confirm

194 the validity of the linear regression model. To maintain this validity, and to minimise confounding due
195 to prescribed medication, those prescribed glucose-lowering medications (see Table 1 for numbers)
196 were excluded from regression analyses involving glucose or insulin in the ORCADES, VIKING and
197 EHERTS cohorts. Because the majority of participants in ET2DS were prescribed these agents, their
198 prescription was encoded as a binary variable and entered into the regression equation. This binary
199 variable approach was also used in all cohorts to adjust for the effect of relevant medications on lipids
200 and blood pressure.

201 The magnitude of the association of plasma cortisol and corticosterone with response variables is
202 presented using standardised Z scores of log transformed variables in the adjusted regression models.
203 The coefficients from these regression models are interpreted as the SD change in log transformed
204 outcome for a 1SD higher log transformed cortisol or corticosterone. This allows direct comparison of
205 all associations investigated.

206 In SKIPOGH statistical analyses were conducted using STATA 14.0 (StataCorp, College Station,
207 Texas, USA). Simple mixed linear or multiple regression analyses were used to explore associations of
208 total urinary corticosterone (THA+THB+5 α -THB) or cortisol (THE+THF+5 α -THF) metabolites, while
209 taking familial correlations into account using a random family-effect. In the multiple regression model,
210 analyses were adjusted for age, sex, study center, BMI (unless BMI was the outcome variable) and
211 relevant medication. (THA+THB+5 α THB)/(THE+THF+5 α THF) ratio (i.e. CYP17A1 ratio) was
212 analysed after log transformation in a mixed linear model to investigate associations with variables of
213 interest. Covariates included age, sex, study center, and antihypertensive, lipid- and glucose-lowering
214 treatment, BMI (unless BMI was the outcome variable), estimated GFR, urine flow rate, urinary
215 sodium, potassium and creatinine excretion (24h per kg body weight).

217 RESULTS

218 Participant characteristics are summarised in Table 1. Plasma cortisol and corticosterone were
219 positively correlated in ORCADES and ET2DS cohorts (Figure 1A), in VIKING (Coefficient
220 correlation = 0.08, $p < 0.001$) and in both dexamethasone-suppressed and ACTH-stimulated samples in
221 EHERTS (Figure 1B), while urinary cortisol and corticosterone metabolites were positively correlated
222 in SKIPOGH (Figure 1C and 1D). After ACTH stimulation in EHERTS, the increase from baseline was
223 greater for corticosterone than cortisol (6.2 ± 0.3 vs 3.9 ± 0.4 fold, respectively; $p < 0.001$).

224 Associations of plasma corticosterone and cortisol with *CYP17A1* genotype

225 In ORCADES, associations of functional SNPs in *CYP17A1* with morning plasma cortisol and
226 corticosterone are shown in Table 2. The minor allele (C; frequency 0.16) of rs2486758 was associated
227 with higher plasma corticosterone and corticosterone:cortisol ratio but not with plasma cortisol. These
228 associations were replicated in ET2DS, in which the minor allele (C; frequency 0.21) of rs2486758
229 similarly tended to be associated with higher plasma corticosterone ($\beta = 0.074 \pm 0.043$, $p = 0.09$) and
230 corticosterone:cortisol ratio ($\beta = 0.078 \pm 0.040$, $p = 0.05$) but not with cortisol ($\beta = -0.004 \pm 0.017$, $p = 0.83$).
231 Similar associations were observed in SKIPOGH with the minor allele (C; frequency 0.23) of
232 rs2486758 associated with higher overnight urinary corticosterone ($\beta = 11.0 \pm 5.7$, $p = 0.05$) and with day
233 and night urinary corticosterone:cortisol ratio ($\beta = 0.004 \pm 0.002$, $p = 0.03$; $\beta = 0.004 \pm 0.002$, $p = 0.05$), but
234 not with daytime or overnight urinary cortisol ($\beta = -133.5 \pm 96.6$, $p = 0.17$; $\beta = 11.1 \pm 44.6$, $p = 0.80$). There
235 was no evidence of an association between *CYP17A1* variants with morning plasma cortisol or
236 corticosterone in the VIKING cohort.

237 Associations of morning plasma cortisol and corticosterone with features of metabolic syndrome

238 In ORCADES (Table 3 and Figure 2A), both higher plasma cortisol and higher plasma corticosterone
239 were associated with lower BMI. After adjustment for BMI, age and sex, both higher plasma cortisol
240 and corticosterone were associated with higher fasting plasma glucose and higher triglycerides,
241 although the magnitude of this association was greater for cortisol than corticosterone. Moreover, higher
242 cortisol, but not corticosterone, was associated with raised systolic blood pressure. Neither cortisol nor
243 corticosterone was significantly associated with total cholesterol or LDL cholesterol. In contrast with

244 these predominant associations with cortisol, lower corticosterone but not cortisol was associated with
245 lower HDL-cholesterol, while higher corticosterone but not cortisol was associated with higher fasting
246 insulin and HOMA-IR. The corticosterone:cortisol ratio was positively associated with fasting glucose,
247 insulin and HOMA-IR but not with BMI, blood pressure or lipid profile.

248 In VIKING (Table 3 and Figure 2B), similar associations were observed as in ORCADES. Both cortisol
249 and corticosterone were negatively associated with BMI. Cortisol was more strongly positively
250 associated with systolic blood pressure and triglycerides than corticosterone. The corticosterone:cortisol
251 ratio was negatively associated with BMI and positively associated with fasting glucose.

252 In ET2DS (Table 3 and Figure 2C), fewer biochemical phenotypes were available, but both higher
253 plasma cortisol and plasma corticosterone were associated with lower BMI and higher fasting plasma
254 glucose, as in ORCADES. However, cortisol and corticosterone were unrelated to systolic blood
255 pressure in ET2DS, and the corticosterone:cortisol ratio was not significantly associated with BMI,
256 blood pressure or glucose.

257 **Associations of ACTH-stimulated cortisol and corticosterone with features of metabolic** 258 **syndrome**

259 Given the limited associations of the corticosterone:cortisol ratio in morning plasma samples with
260 features of metabolic syndrome, despite its robust association with *CYP17A1* genotype, we sought to
261 test the association of 17 α -hydroxylase activity with features of metabolic syndrome using potentially
262 more sensitive dynamic testing in the EHERTS cohort. See Table 3 and Figures 2D and 2E. After low
263 dose overnight dexamethasone suppression followed by low dose ACTH₁₋₂₄ administration, plasma
264 cortisol and corticosterone levels changed as expected (Table 1). Plasma cortisol was not associated
265 with any features of metabolic syndrome, except that ACTH-stimulated values were positively
266 correlated with plasma glucose 30 min after an oral glucose load. However, plasma corticosterone
267 showed different associations with metabolic syndrome variables. After dexamethasone suppression,
268 lower plasma corticosterone was associated with higher BMI and, independently, with higher fasting
269 insulin and HOMA-IR. After ACTH stimulation, lower plasma corticosterone (and
270 corticosterone:cortisol ratio) was associated with higher plasma glucose before and after an oral glucose

271 load, and with higher HOMA-IR. There were no significant associations between cortisol,
272 corticosterone and blood pressure in the EHERTS cohort.

273 **Associations of urinary cortisol and corticosterone metabolites with features of metabolic**
274 **syndrome**

275 In SKIPOGH (Table 4 and Figure 3), after adjustment for confounders (age, sex, study center, relevant
276 medication and BMI), higher cortisol metabolite excretion during daytime and overnight was associated
277 with higher BMI, during the day with higher LDL cholesterol and lower HDL cholesterol, and during
278 the night with higher fasting glucose. Plasma triglycerides were, surprisingly, inversely associated with
279 day and night urinary cortisol metabolite excretion. By contrast, corticosterone metabolite excretion
280 during the day was inversely associated with insulin levels and plasma triglycerides, but during the
281 night was positively associated with fasting glucose and inversely with plasma triglycerides. A lower
282 ratio of corticosterone:cortisol metabolites during both day and night was strongly associated with
283 higher BMI and LDL cholesterol, and during the night was associated with higher systolic BP, higher
284 fasting triglycerides and insulin as well as lower HDL cholesterol levels.

285

286 **DISCUSSION**

287 This is the largest study to date investigating associations between endogenous glucocorticoids and
288 features of metabolic syndrome. Using 3 cohorts comprising 3200 participants, we confirm previous
289 observations (13, 31-34) that elevated morning plasma cortisol is associated with higher blood pressure
290 and blood glucose in subjects with and without type 2 diabetes. Moreover, we confirm previous
291 observations (35) in by far the largest sample to date that urinary cortisol metabolite excretion is
292 increased in association with obesity and dyslipidemia. With a large number of participants, the chance
293 of statistical errors is much reduced.

294 This is the first study to investigate whether similar associations exist for corticosterone. We measured
295 plasma corticosterone and analysed variability in *CYP17A1* to determine whether ‘relative
296 corticosterone deficiency’, favouring production of cortisol over corticosterone, underlies associations
297 between variation in *CYP17A1* and features of metabolic syndrome identified in GWAS consortia. The
298 sample size was too small to detect associations at genome-wide significance, but using a candidate
299 gene approach we demonstrated that variation in *CYP17A1* associates with plasma
300 corticosterone:cortisol ratio in a general adult population, and confirmed our findings in two additional
301 cohorts (one in a general adult population and one in a cohort of patients with type 2 diabetes).
302 Moreover, these observations were supported by an association of *CYP17A1* genotype with urinary
303 corticosterone/cortisol metabolite ratios. The association was restricted to just one SNP of 3 that are
304 known to have functional effects on transcription *in vitro* (27), rs2486758; the replication in a second
305 independent cohort, however, makes it highly unlikely to be due to chance. rs2486758 is located in the
306 5’ regulatory region of *CYP17A1* and in LD with rs2150927 but less so with rs138009835 or the variants
307 previously associated with hypertension by GWAS (27). Paradoxically, the major T allele of rs2486758,
308 which we find associated with ‘relative corticosterone deficiency’, induced lower, rather than higher,
309 *CYP17A1* expression *in vitro* (27) but this may be an artefact of the *in vitro* system or rs2486758 may
310 be linked with other functional variants.

311 Previous large GWAS consortia have shown opposite directions of association between variants in
312 *CYP17A1* with hypertension versus other features of metabolic syndrome. We therefore anticipated that
313 ‘relative corticosterone excess’ is associated with higher blood pressure while ‘relative corticosterone

314 deficiency' is associated with cortisol-mediated insulin resistance and obesity. However, despite
315 elevated corticosterone excretion rate being reported previously in hypertension (36), we did not find
316 an association between morning plasma corticosterone and blood pressure. Moreover, our data show
317 that morning plasma cortisol and corticosterone were positively correlated. Further, plasma cortisol and
318 corticosterone showed similar associations with BMI; it is likely that reduced plasma corticosterone in
319 obesity is explained by increased activity of A-ring reductases which also underlie increased clearance
320 of cortisol in obesity (37). When we did find discrepancies in associations with plasma cortisol or
321 corticosterone, it was generally higher, rather than lower, morning plasma corticosterone that was the
322 stronger predictor than morning plasma cortisol, e.g. of fasting plasma glucose, plasma insulin and
323 HOMA-IR. This suggests that *CYP17A1* genotype is not the major determinant of morning plasma
324 corticosterone:cortisol ratio. It is possible that insulin resistance or deficiency causes a shift in
325 steroidogenesis in favour of corticosterone rather than cortisol, since *CYP17A1* expression is up-
326 regulated by insulin (38). Alternatively, the combined elevation of cortisol and corticosterone with
327 insulin resistance could reflect central activation of the HPA axis, consistent with altered responses to
328 habituation and stress (34).

329 Associations between morning plasma glucocorticoid levels and lipid profile provided limited support
330 for our hypothesis of 'relative corticosterone deficiency'. Thus, elevated triglycerides were consistently
331 associated with plasma cortisol and less strongly with plasma corticosterone, and low HDL-cholesterol
332 was associated only with low corticosterone, although the latter association has been reported
333 previously for cortisol (39). However, effects of glucocorticoids on lipid metabolism are complex (40),
334 and previous studies investigating associations of glucocorticoid excess with dyslipidaemia have been
335 inconsistent (13).

336 A different pattern emerged when we studied associations of corticosterone and cortisol in EHERTS
337 subjects who had received dexamethasone to suppress any compensatory increase or primary drive to
338 the HPA axis, and a fixed dose of ACTH₁₋₂₄ to stimulate cortisol and corticosterone. The results are
339 striking, with opposite associations of cortisol and corticosterone with metabolic syndrome features,
340 consistent with a primary shift in adrenocortical production in favour of cortisol rather than
341 corticosterone in metabolic syndrome. Since, as a result of the activity of ABCB1 excluding cortisol

342 from the brain (15), corticosterone is thought to make a disproportionate contribution to negative
343 feedback suppression of the HPA axis in humans, these observations could provide a key insight into
344 the activation of the HPA axis and elevated plasma cortisol in metabolic syndrome. This insight is
345 obscured by measurement of morning plasma corticosterone and cortisol alone, perhaps because of
346 greater variability as a result of unmeasured confounding.

347 Fasting plasma corticosterone was low in the ET2DS cohort, whereas cortisol was similar to the values
348 in the ORCADES cohort (mean corticosterone:cortisol was 3.0% in ET2DS vs. 7.5% in ORCADES,
349 $p < 0.0001$). This is unlikely to be due to older age of the ET2DS cohort, since corticosterone:cortisol
350 ratio was not associated with age ($r^2 = 0.01$, $p = 0.27$). Again, insulin deficiency might favour cortisol
351 rather than corticosterone secretion, consistent with loss of up-regulation of *CYP17A1* by insulin (38).
352 If confirmed, this mechanism may exacerbate the ‘relative corticosterone deficiency’ apparent with
353 metabolic syndrome in EHERTS, and thereby exacerbate HPA axis activation in type 2 diabetes.

354 Given the limitations of morning plasma cortisol and corticosterone, we investigated the hypothesis of
355 relative corticosterone deficiency in another cohort, the SKIPOGH study, in which integrated
356 measurements of cortisol and corticosterone metabolite excretion in urine were available in a large
357 number of participants. These results confirmed that the pattern observed for plasma glucocorticoids
358 after ACTH₁₋₂₄ stimulation in EHERTS, with relatively low corticosterone and high cortisol, is similarly
359 associated in urine with glucose and lipid metabolism and, strikingly, with obesity. Surprisingly,
360 ‘relative corticosterone deficiency’ was also associated with higher systolic clinic blood pressure,
361 although previous studies in this cohort have suggested that ambulatory blood pressure is inversely
362 associated with *CYP17A1* activity only in the presence of high sodium intake (8).

363 An important limitation of these studies is the cross-sectional design which precludes inferences of
364 causation. Moreover, genotype data was not available for EHERTS subjects because samples of DNA
365 from this cohort have been exhausted. Indeed, no sample of sufficient size exists in which ACTH
366 stimulation tests have been conducted to be adequately powered to detect associations of steroidogenic
367 response with *CYP17A1* genotype. Also, effects of intensive lipid-lowering and antihypertensive
368 treatments in the ET2DS may have obscured associations with corticosterone and cortisol. A further
369 limitation is that the dynamic HPA axis tests and urinary steroid analyses were conducted in different

370 cohorts from the measurement of fasting plasma glucocorticoids. The corollary is, however, that we
371 replicated associations of candidate *CYP17A1* genotype with phenotype in three independent studies
372 and we have been drawn upon comprehensive datasets to adjust for a number of potentially confounding
373 variables and assess the corticosterone:cortisol relationship in urine as well as plasma. We present our
374 findings unadjusted for multiple testing because, as discussed above, there are distinct associations of
375 glucocorticoid levels with individual components of metabolic syndrome such that each analysis tests
376 a distinct hypothesis.

377 In conclusion, elevated morning plasma corticosterone accompanies insulin resistance and elevated
378 cortisol in metabolic syndrome, consistent with activation of the HPA axis at the time of blood
379 sampling. However, in addition, there are marked discrepancies between associations with ACTH-
380 stimulated cortisol and corticosterone with metabolic syndrome which likely reflect genetically
381 determined differences in adrenal steroidogenesis, particularly by 17α -hydroxylase, and may also be
382 influenced by dysregulated insulin signaling. When sustained HPA axis activation is assessed by urinary
383 metabolites, the variation in adrenal steroidogenesis predominates in the associations with metabolic
384 syndrome and, in particular, obesity. These findings throw the spotlight on corticosterone and suggest
385 that further dissection of its biology in humans may be fruitful in understanding the basis for altered
386 glucocorticoid signaling in metabolic syndrome.

387 **ACKNOWLEDGEMENTS**

388 We are grateful to: Lynne Ramage and Jill Harrison for technical support; Stela McLachlan for data
389 processing in ET2DS; the study nurses Marie-Odile Levy, Guler Gök-Sogüt, Ulla Schüpbach,
390 Dominique Siminski and Sandrine Estoppey for the data collection in SKIPOGH; and all of the
391 participants and researchers involved. We also acknowledge the investigators in the Swiss Kidney
392 Project on Genes in Hypertension (SKIPOGH) team: (in alphabetical order) Daniel Ackermann¹,
393 Murielle Bochud², Michel Burnier³, Georg Ehret⁴, Idris Guessous⁵, Pierre-Yves Martin⁶, Markus
394 Mohaupt¹, Fred Paccaud², Antoinette Pechère-Bertschi⁷, Belen Ponte⁶, Menno Pruijm³, and Bruno
395 Vogt¹.

396 ¹University Clinic for Nephrology, Hypertension and Clinical Pharmacology, Inselspital, Bern
397 University Hospital, University of Bern, Switzerland

398 ²Institute of Social and Preventive Medicine (IUMSP), Lausanne University Hospital and University of
399 Lausanne, Switzerland

400 ³Department of Nephrology, Lausanne University Hospital, Lausanne, Switzerland.

401 ⁴Cardiology, Department of Specialties of Internal Medicine, Geneva University Hospitals, Geneva,
402 Switzerland.

403 ⁵Unit of Population Epidemiology, Department of Community Medicine and Primary Care and
404 Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland.

405 ⁶Department of Nephrology, Geneva University Hospitals, Geneva, Switzerland.

406 ⁷Unit of Hypertension, Departments of Community Medicine and Primary Care and Emergency
407 Medicine, Geneva University Hospitals, Switzerland.

408

409

REFERENCES

- 410 1. Ehret GB, Munroe PB, Rice KM. Genetic variants in novel pathways influence blood
411 pressure and cardiovascular disease risk. *Nature*. 2011;478(7367 SRC - BaiduScholar):103-9.
- 412 2. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, et al. Meta-analysis of genome-
413 wide association studies identifies common variants associated with blood pressure variation in east
414 Asians. *Nature genetics*. 2011;43(6):531-8.
- 415 3. Fedorowski A, Franceschini N, Brody J, Liu C, Verwoert GC, Boerwinkle E, et al.
416 Orthostatic hypotension and novel blood pressure-associated gene variants: Genetics of Postural
417 Hemodynamics (GPH) Consortium. *Eur Heart J*. 2012;33(18):2331-41.
- 418 4. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide
419 association study identifies eight loci associated with blood pressure. *Nature genetics*.
420 2009;41(6):666-76.
- 421 5. Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale
422 association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nature genetics*.
423 2011;43(4):333-8.
- 424 6. Dai C-F, Xie X, Yang Y-N, Li X-M, Zheng Y-Y, Fu Z-Y, et al. Relationship between
425 CYP17A1 genetic polymorphism and coronary artery disease in a Chinese Han population.
426 2015;14(1):16.
- 427 7. Yanase T, Simpson ER, Waterman MR. 17 alpha-hydroxylase/17,20-lyase deficiency: from
428 clinical investigation to molecular definition. *Endocr Rev*. 1991;12(1):91-108.
- 429 8. Ackermann D, Pruijm M, Ponte B, Guessous I, Ehret G, Escher G, et al. CYP17A1 Enzyme
430 Activity Is Linked to Ambulatory Blood Pressure in a Family-Based Population Study. *Am J*
431 *Hypertens*. 2016;29(4):484-93.
- 432 9. Yan H, Guo Y, Yang T-L, Zhao L-J, Deng H-WJG, GMR mr. A family-based association
433 study identified CYP17 as a candidate gene for obesity susceptibility in Caucasians. 2012;11(3):1967.
- 434 10. Hotta K, Kitamoto A, Kitamoto T, Mizusawa S, Teranishi H, Matsuo T, et al. Genetic
435 variations in the CYP17A1 and NT5C2 genes are associated with a reduction in visceral and
436 subcutaneous fat areas in Japanese women. *J Hum Genet*. 2012;57(1):46-51.
- 437 11. Wen W, Zheng W, Okada Y, Takeuchi F, Tabara Y, Hwang J-Y, et al. Meta-analysis of
438 genome-wide association studies in East Asian-ancestry populations identifies four new loci for body
439 mass index. *Hum Mol Genet*. 2014;23(20):5492-504.

- 440 12. Echiburú B, Pérez-Bravo F, Maliqueo M, Sánchez F, Crisosto N, Sir-Petermann TJM.
441 Polymorphism T→C (− 34 base pairs) of gene CYP17 promoter in women with polycystic ovary
442 syndrome is associated with increased body weight and insulin resistance: a preliminary study.
443 2008;57(12):1765-71.
- 444 13. Walker BR. Glucocorticoids and cardiovascular disease. *European journal of endocrinology*.
445 2007;157(5):545-59.
- 446 14. Raubenheimer PJ, Young EA, Andrew R, Seckl JR. The role of corticosterone in human
447 hypothalamic-pituitary-adrenal axis feedback. *Clin Endocrinol (Oxf)*. 2006;65(1):22-6.
- 448 15. Karssen AM, Meijer OC, van der Sandt IC, Lucassen PJ, de Lange EC, de Boer AG, et al.
449 Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse
450 and human brain. *Endocrinology*. 2001;142(6):2686-94.
- 451 16. Mattsson C, Reynolds RM, Simonyte K, Olsson T, Walker BR. Combined receptor antagonist
452 stimulation of the hypothalamic-pituitary-adrenal axis test identifies impaired negative feedback
453 sensitivity to cortisol in obese men. *The Journal of clinical endocrinology and metabolism*.
454 2009;94(4):1347-52.
- 455 17. McQuillan R, Leutenegger A-L, Abdel-Rahman R, Franklin CS, Pericic M, Barac-Lauc L, et
456 al. Runs of homozygosity in European populations. *Am J Hum Genet*. 2008;83(3):359-72.
- 457 18. Price JF, Reynolds RM, Mitchell RJ, Williamson RM, Fowkes FGR, Deary IJ, et al. The
458 Edinburgh Type 2 Diabetes Study: study protocol. *BMC endocrine disorders*. 2008;8:18.
- 459 19. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its
460 complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO
461 consultation. *Diabetic medicine : a journal of the British Diabetic Association*. 1998;15(7):539-53.
- 462 20. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death
463 from ischaemic heart disease. *Lancet (London, England)*. 1989;2(8663):577-80.
- 464 21. Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Phillips DI. Is there a gender
465 difference in the associations of birthweight and adult hypothalamic-pituitary-adrenal axis activity?
466 *European Journal of Endocrinology*. 2005;152(2):249-53.
- 467 22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis
468 model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin
469 concentrations in man. *Diabetologia*. 1985;28(7):412-9.

- 470 23. Moore A, Aitken R, Burke C, Gaskell S, Groom G, Holder G, et al. Cortisol assays:
471 guidelines for the provision of a clinical biochemistry service. *Ann Clin Biochem.* 1985;22 (Pt
472 5):435-54.
- 473 24. Al-Dujaili EAS, Mullins LJ, Bailey MA, Andrew R, Kenyon CJ. Physiological and
474 pathophysiological applications of sensitive ELISA methods for urinary deoxycorticosterone and
475 corticosterone in rodents. *Steroids.* 2009;74(12):938-44.
- 476 25. Flote VG, Furberg A-S, McTiernan A, Frydenberg H, Ursin G, Iversen A, et al. Gene
477 variations in oestrogen pathways, CYP19A1, daily 17 β -estradiol and mammographic density
478 phenotypes in premenopausal women. *Breast cancer research : BCR.* 2014;16(6):499.
- 479 26. Iversen A, Thune I, McTiernan A, Makar KW, Wilsgaard T, Ellison PT, et al. Genetic
480 polymorphism CYP17 rs2486758 and metabolic risk factors predict daily salivary 17 β -estradiol
481 concentration in healthy premenopausal Norwegian women. The EBBA-I study. *The Journal of
482 clinical endocrinology and metabolism.* 2012;97(5):E852-7.
- 483 27. Diver LA, MacKenzie SM, Fraser R, McManus F, Freel EM, Alvarez-Madrado S, et al.
484 Common Polymorphisms at the CYP17A1 Locus Associate With Steroid Phenotype: Support for
485 Blood Pressure Genome-Wide Association Study Signals at This Locus. *Hypertension.*
486 2016;67(4):724-32.
- 487 28. Lottenberg SA, Giannella-Neto D, Derendorf H, Rocha M, Bosco A, Carvalho SV, et al.
488 Effect of fat distribution on the pharmacokinetics of cortisol in obesity. *Int J Clin Pharmacol Ther.*
489 1998;36(9):501-5.
- 490 29. Reynolds RM, Fischbacher C, Bhopal R, Byrne CD, White M, Unwin N, et al. Differences in
491 cortisol concentrations in South Asian and European men living in the United Kingdom. *Clin
492 Endocrinol (Oxf).* 2006;64(5):530-4.
- 493 30. Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. GenABEL: an R library for genome-wide
494 association analysis. *Bioinformatics.* 2007;23(10):1294-6.
- 495 31. Phillips D, Barker D, Fall C, Whorwood C, Seckl J, Wood P, et al. Elevated plasma cortisol
496 concentrations: an explanation for the relationship between low birthweight and adult cardiovascular
497 risk factors. *J Clin Endocrinol Metab.* 1998;83(757):60.
- 498 32. Reynolds RM, Labad J, Strachan MW, Braun A, Fowkes FGR, Lee AJ, et al. Elevated fasting
499 plasma cortisol is associated with ischemic heart disease and its risk factors in people with type 2
500 diabetes: the Edinburgh type 2 diabetes study. 2010;95(4):1602-8.
- 501 33. Ward AMV, Fall CHD, Stein CE, Kumaran K, Veena SR, Wood PJ, et al. Cortisol and the
502 metabolic syndrome in South Asians. *Clin Endocrinol (Oxf).* 2003;58(4):500-5.

- 503 34. Reynolds RM, Walker BR, Syddall HE, Whorwood CB, Wood PJ, Phillips DI. Elevated
504 plasma cortisol in glucose-intolerant men: differences in responses to glucose and habituation to
505 venepuncture. *The Journal of clinical endocrinology and metabolism*. 2001;86(3):1149-53.
- 506 35. Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, et al. Altered
507 Control of Cortisol Secretion in Adult Men with Low Birth Weight and Cardiovascular Risk Factors.
508 *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(1):245-50.
- 509 36. Soro A, Ingram MC, Tonolo G, Glorioso N, Fraser R. Mildly raised corticosterone excretion
510 rates in patients with essential hypertension. *J Hum Hypertens*. 1995;9(6):391-3.
- 511 37. Andrew R, Phillips DI, Walker BR. Obesity and gender influence cortisol secretion and
512 metabolism in man. *The Journal of clinical endocrinology and metabolism*. 1998;83(5):1806-9.
- 513 38. Baptiste CG, Battista M-C, Trottier A, Baillargeon J-P. Insulin and hyperandrogenism in
514 women with polycystic ovary syndrome. *The Journal of steroid biochemistry and molecular biology*.
515 2010;122(1-3):42-52.
- 516 39. Varma VK, Rushing JT, Ettinger WH. High density lipoprotein cholesterol is associated with
517 serum cortisol in older people. *J Am Geriatr Soc*. 1995;43(12):1345-9.
- 518 40. Macfarlane DP, Forbes S, Walker BR. Glucocorticoids and fatty acid metabolism in humans:
519 fuelling fat redistribution in the metabolic syndrome. *The Journal of endocrinology*. 2008;197(2):189-
520 204.

521

522

523

524 **Figure Legends**

525

526 **Figure 1. Relationships between plasma cortisol and corticosterone**

527 A) Plasma cortisol and corticosterone in the Orkney complex diseases study group (ORCADES, open symbols)
528 and the Edinburgh Type 2 Diabetes Study (ET2DS, closed symbols). Lines indicate simple linear regression with
529 95% confidence bands. Coefficient of determination (r^2): ORCADES = 0.062, $p < 0.001$; ET2DS = 0.070, $p < 0.001$.

530 B) Cortisol and corticosterone in the East Hertfordshire Cohort. Sampling was undertaken following overnight
531 dexamethasone (0.25mg) suppression testing (pre synacthen, open symbols); and 30 minutes following
532 intravenous injection of ACTH₁₋₂₄ (synacthen 1 μ g, closed symbols). Lines indicate simple linear regression with
533 95% confidence bands. Coefficient of determination (r^2): pre-synacthen = 0.488, $p < 0.001$; post synacthen = 0.168,
534 $p < 0.001$.

535 C and D) Total urinary cortisol and corticosterone metabolites in the Swiss Kidney Project on Genes in
536 Hypertension. Sampling was undertaken for day (C, open symbols) and night (D, closed symbols) separately.
537 Lines indicate simple linear regression with 95% confidence bands. Coefficient of determination (r^2): day = 0.641,
538 $p < 0.001$; night = 0.69, $p < 0.001$.

539

540 **Figure 2. Associations of plasma cortisol and corticosterone with features of metabolic syndrome**

541 Data in A-E are for plasma cortisol (solid bars) and corticosterone (striped bars) from A) Orkney complex diseases
542 study (ORCADES), B) Viking Health Study Shetland (VIKING), C) Edinburgh Type 2 Diabetes Study (ET2DS),
543 D) East Hertfordshire study (EHERTS) after overnight dexamethasone (250 μ g) suppression, and E) EHERTS 30
544 mins after ACTH₁₋₂₄ (1 μ g). The plots show the SD change (and standard error) in log transformed outcome
545 for a 1SD higher log transformed cortisol or corticosterone.

546

547

548 **Figure 3. Associations of urine cortisol and corticosterone metabolites with features of metabolic syndrome**

549 Data are from 888 participants in the Swiss Kidney Project on Genes in Hypertension (SKIPOGH). Results are
550 from multiple regression analysis of daytime log-transformed ratio (THA+THB+5 α THB)/(THE+THF+5 α THF)
551 with BMI (left panel) and plasma LDL cholesterol (right panel). The model was adjusted for age, sex, center,
552 lipid- and glucose-lowering and antihypertensive treatment, BMI (for LDL cholesterol only), estimated GFR,
553 urine flow rate, urinary sodium, potassium and creatinine excretion. Results were similar for night-time
554 corticosteroid excretion.

555

Table 1. Characteristics of participants

	Cohort				
	ORCADES	EHERTS	ET2DS	SKIPOGH	VIKING
Sex (M/F)	794/1224 (39/61%)	189/90 (68/32%)	476/427 (53/47%)	380/508 (43/57%)	838/1260 (40/60%)
Age (years)	53.3 ± 0.3 (range 16-91)	71.3 ± 0.2 (range 67-78)	67.9 ± 0.1 (range 60-68)	47.1 ± 0.6 (range 18-82)	49.8 ± 0.3 (range 18-92)
BMI (kg/m ²)	27.7 ± 0.1	27.1 ± 0.2	31.2 ± 0.2	24.7 ± 0.1	27.4 ± 0.1
Taking anti-hypertensive (n (%))	412 (20%)	105 (38%)	704 (79%)	124 (14%)	344 (16%)
Systolic BP (mmHg)	129.9 ± 0.4	159.3 ± 1.3	133.3 ± 0.6	116.8 ± 0.6	127.0 ± 0.4
Diastolic BP (mmHg)	75.5 ± 0.2	86.9 ± 0.7	69.2 ± 0.3	75.1 ± 0.3	74.7 ± 0.2
Plasma corticosterone at baseline (nM)	50.7 ± 1.2	20.2 ± 1.0*	22.3 ± 0.5	n/a	20.3 ± 1.4
Plasma corticosterone 30 min after ACTH ₁₋₂₄ (nM)	n/a	84.0 ± 2.5	n/a	n/a	n/a
Plasma cortisol at baseline (nM)	724 ± 7	188 ± 6*	732 ± 6	n/a	293 ± 4
Plasma cortisol 30 min after ACTH ₁₋₂₄ (nM)	n/a	460 ± 8	n/a	n/a	n/a
Lipid lowering therapy (n (%))	255 (13%)	71 (25%)	755 (84%)	34 (3.8%)	n/a
Total cholesterol (mM)	5.39 ± 0.03	6.82 ± 0.07	4.28 ± 0.03	5.1 ± 0.03	5.30 ± 0.02
LDL cholesterol (mM)	3.37 ± 0.02	4.89 ± 0.08	n/a	3.11 ± 0.03	3.33 ± 0.02
HDL cholesterol (mM)	1.50 ± 0.01	1.33 ± 0.02	1.28 ± 0.01	1.55 ± 0.01	1.52 ± 0.01
Triglycerides (mM)	1.15 ± 0.02	1.54 ± 0.05	n/a	1.02 ± 0.02	1.00 ± 0.01
Medication for diabetes (n)	47 (2.3%)	10 (3.6%)	734 (81%)	8 (0.8%)	44 (2.1%)
Oral agents			660 (74%)	7 (0.7%)	
Insulin			156 (18%)	1 (0.1%)	
Fasting plasma glucose (mM)	5.33 ± 0.02	5.99 ± 0.08	7.57 ± 0.07	5.14 ± 0.02	4.88 ± 0.02
Plasma glucose 30 min after oral glucose (mM)	n/a	9.38 ± 0.14	n/a	n/a	n/a
Plasma glucose 120 min after oral glucose (mM)	n/a	7.13 ± 0.18	n/a	n/a	n/a
Fasting plasma insulin mU/L	6.44 ± 0.10	7.18 ± 0.35	n/a	6.19 ± 0.21	8.31 ± 0.80
HOMA-IR	1.59 ± 0.34	1.96 ± 0.11	n/a	1.48 ± 0.06	1.86 ± 0.17
Daytime urinary steroids					
Tetrahydrodehydrocorticosterone (µg/day)	n/a	n/a	n/a	67.3 ± 1.3	n/a
Tetrahydrocorticosterone (µg/day)	n/a	n/a	n/a	91.7 ± 1.7	n/a

5 α -Tetrahydrocorticosterone ($\mu\text{g}/\text{day}$)	n/a	n/a	n/a	188.5 \pm 4.2	n/a
Tetrahydrocortisol ($\mu\text{g}/\text{day}$)	n/a	n/a	n/a	1080.5 \pm 18.2	n/a
5 α -Tetrahydrocortisol ($\mu\text{g}/\text{day}$)	n/a	n/a	n/a	779.9 \pm 20.8	n/a
Tetrahydrocortisone ($\mu\text{g}/\text{day}$)	n/a	n/a	n/a	1861.2 \pm 33.8	n/a
Nighttime urinary steroids					
Tetrahydrodehydrocorticosterone ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	27.3 \pm 0.7	n/a
Tetrahydrocorticosterone ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	41.8 \pm 1	n/a
5 α -Tetrahydrocorticosterone ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	60.7 \pm 1.7	n/a
Tetrahydrocortisol ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	362.9 \pm 8.2	n/a
5 α -Tetrahydrocortisol ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	247.1 \pm 6.8	n/a
Tetrahydrocortisone ($\mu\text{g}/\text{night}$)	n/a	n/a	n/a	642.6 \pm 14.2	n/a

Data presented as mean \pm SEM. n/a = not available.

* Dexamethasone suppressed in EHERTS cohort

Table 2. Associations of functionally significant SNPs in *CYP17A1* with morning plasma cortisol and corticosterone

Study	SNP ID	Minor allele frequency	Cortisol		Corticosterone		Corticosterone:cortisol ratio	
			β	P	β	p	β	p
ORCADES	rs2486758	0.16	0.000	0.98	0.074	0.01	0.070	0.01
	rs138009835	0.09	0.003	0.89	0.004	0.91	0.003	0.93
	rs2150927	0.36	-0.008	0.52	0.023	0.29	0.032	0.14
ET2D	rs2486758	0.21	-0.004	0.83	0.074	0.09	0.077	0.05
	rs138009835	0.08	0.006	0.82	0.004	0.95	-0.002	0.97
	rs2150927	0.36	-0.005	0.70	-0.025	0.49	-0.019	0.56
VIKING	rs2486758	0.21	-0.003	0.85	-0.009	0.77	-0.004	0.88
	rs138009835	0.09	0.006	0.78	-0.024	0.60	0.040	0.26
	rs2150927	0.35	0.012	0.35	0.019	0.51	-0.012	0.58
SKIPOGH (day)	rs2486758	0.23	-133.5	0.17	6.86	0.60	0.004	0.03
	rs138009835	0.10	-119.7	0.37	-27.58	0.14	-0.004	0.22
	rs2150927	0.42	20.71	0.80	4.66	0.68	0.000	0.90
SKIPOGH (night)	rs2486758	0.23	11.09	0.80	11.03	0.05	0.004	0.05
	rs138009835	0.10	-29.08	0.64	-9.60	0.24	-0.006	0.06
	rs2150927	0.42	-0.04	0.99	-1.71	0.73	0.001	0.61

β = regression coefficient. Analyses were adjusted for age, sex, BMI, time of sampling, and relatedness.

Table 3. Associations of plasma cortisol and corticosterone with features of the metabolic syndrome

		Cortisol				Corticosterone				Corticosterone:cortisol ratio			
		Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
		β	P	β	p	β	p	B	p	β	p	β	p
ORCADES (n=2018)	BMI	-0.069	<0.001	-0.064	<0.001	-0.026	<0.001	-0.028	<0.001	-0.006	0.33	-0.009	0.11
	Systolic BP	-0.021	0.01	0.022	0.001	-0.004	0.41	0.005	0.25	0.003	0.48	-0.002	0.57
	Total cholesterol	-0.006	0.61	0.021	0.09	-0.002	0.73	-0.002	0.73	-0.010	0.17	-0.008	0.27
	LDL cholesterol	-0.042	0.02	0.006	0.74	-0.030	0.004	-0.018	0.08	-0.021	0.06	-0.018	0.09
	HDL cholesterol	0.063	<0.001	0.022	0.14	0.026	0.004	0.021	0.02	0.007	0.45	0.014	0.12
	Triglycerides	0.047	0.12	0.176	<0.001	0.016	0.35	0.057	0.001	0.012	0.52	0.010	0.56
	Fasting glucose	-0.007	0.31	0.014	0.02	0.011	0.003	0.014	<0.001	0.015	<0.001	0.011	0.004
	Insulin	-0.066	0.03	0.030	0.27	0.009	0.58	0.042	0.007	0.038	0.03	0.038	0.02
	HOMA-IR	-0.073	0.03	0.045	0.13	0.020	0.27	0.057	0.001	0.053	0.01	0.048	0.01
VIKING (n=2093)	BMI	-0.047	<0.001	-0.035	<0.001	-0.018	<0.001	-0.014	<0.001	-0.012	0.001	-0.010	0.004
	Systolic BP	-0.009	0.11	0.020	<0.001	-0.003	0.30	0.004	0.04	-0.002	0.49	0.001	0.80
	Total cholesterol	-0.019	0.03	-0.001	0.89	-0.006	0.11	-0.002	0.64	-0.005	0.27	-0.003	0.46
	LDL cholesterol	-0.048	<0.001	-0.021	0.09	-0.013	0.02	-0.005	0.35	-0.006	0.32	-0.002	0.69
	HDL cholesterol	0.035	0.002	0.013	0.21	0.014	0.004	0.003	0.41	0.007	0.18	-0.001	0.91
	Triglycerides	0.017	0.48	0.112	<0.001	-0.015	0.13	0.014	0.13	-0.022	0.04	-0.006	0.59
	Fasting glucose	-0.006	0.22	0.012	0.008	0.001	0.72	0.006	0.002	0.002	0.32	0.005	0.02
	Insulin	-0.041	0.10	0.037	0.09	-0.020	0.06	0.008	0.37	-0.017	0.15	0.001	0.96
	HOMA-IR	-0.047	0.08	0.048	0.04	-0.020	0.09	0.014	0.15	-0.015	0.25	0.005	0.62
ET2DS (n=903)	BMI	-0.064	0.003	-0.055	0.01	-0.018	0.05	-0.013	0.12	-0.010	0.30	-0.006	0.50
	Systolic BP	0.015	0.34	0.014	0.37	-0.003	0.66	-0.003	0.66	-0.005	0.40	-0.005	0.42
	Fasting glucose	0.179	<0.001	0.190	<0.001	0.027	0.03	0.030	0.02	0.001	0.95	0.003	0.84
EHERTS dex suppressed (n=279)	BMI	-0.018	0.13	-0.017	0.18	-0.036	0.003	-0.036	0.004	-0.023	0.14	-0.024	0.12
	Systolic BP	0.008	0.49	0.005	0.67	0.008	0.49	0.008	0.49	-0.002	0.89	0.000	1.00
	Fasting glucose	0.018	0.06	0.010	0.28	0.003	0.74	-0.001	0.89	-0.019	0.12	-0.012	0.30
	30 min glucose	0.033	0.05	0.027	0.12	0.015	0.39	0.015	0.38	-0.027	0.23	-0.014	0.51
	120 min glucose	-0.049	0.04	-0.034	0.17	-0.050	0.04	-0.032	0.20	0.016	0.61	0.012	0.69
	Insulin	-0.138	0.01	-0.096	0.06	-0.192	<0.001	-0.131	0.01	-0.046	0.80	-0.040	0.52
	HOMA-IR	-0.119	0.03	-0.085	0.11	-0.189	0.001	-0.133	0.01	-0.105	0.59	-0.053	0.42
EHERTS after ACTH ₁₋₂₄ (n=279)	BMI	0.012	0.71	-0.020	0.61	-0.015	0.19	-0.018	0.12	-0.019	0.11	-0.017	0.15
	Systolic BP	0.014	0.65	0.048	0.19	-0.007	0.58	-0.004	0.77	-0.014	0.32	-0.011	0.41
	Fasting glucose	-0.031	0.19	0.037	0.20	-0.033	0.003	-0.027	0.01	-0.021	0.06	-0.026	0.02
	30 min glucose	0.019	0.66	0.146	0.01	-0.023	0.25	-0.010	0.63	-0.021	0.32	-0.019	0.35
	120 min glucose	0.092	0.15	0.010	0.90	-0.054	0.06	-0.056	0.05	-0.072	0.02	-0.059	0.05
	Insulin	0.136	0.32	0.036	0.82	-0.120	0.04	-0.099	0.07	-0.046	0.02	-0.096	0.09
	HOMA-IR	0.103	0.48	0.072	0.66	-0.153	0.01	-0.126	0.03	-0.485	0.02	-0.123	0.04

β = regression coefficient. P values refer to regression co-efficient, unadjusted or adjusted in multiple regression analyses for co-variables (age, sex, time of sample, relevant medication, and BMI (unless BMI is the outcome variable)). All continuous variables were normalised by log transformation.

Table 4. Associations of day- and night-time excretion of urinary cortisol and corticosterone metabolites with features of the metabolic syndrome

		Cortisol metabolites (THE+THF+5 α THF) day				Corticosterone metabolites (THA+THB+5 α THB) day				Corticosterone:cortisol metabolites (THA+THB+5 α THB)/(THE+THF+5 α THF) day				
		Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted		
		β	p	B	p	β	p	B	p	β	p	β	p	
				(THE+THF+5 α -THF) night		(THA+THB+5 α -THB) night		(THA+THB+5 α THB)/(THE+THF+5 α THF) night						
SKIPOGH (n=888)	BMI	2.351	<0.001	1.824	<0.001	0.563	0.02	0.143	0.564	-4.618	<0.001	-3.388	<0.001	
	Systolic BP	5.996	<0.001	0.385	0.688	0.428	0.65	-0.631	0.449	-13.97	<0.001	-2.524	0.067	
	Total cholesterol	-0.045	0.47	0.096	0.173	-0.239	<0.001	-0.032	0.6	-0.542	<0.001	-0.290	0.004	
	LDL cholesterol	0.166	0.002	0.174	0.005	-0.052	0.32	0.025	0.64	-0.569	<0.001	-0.295	0.001	
	HDL cholesterol	-0.240	<0.001	-0.020	0.026	-0.157	<0.001	0.003	0.908	0.190	<0.001	0.049	0.188	
	Triglycerides	0.093	0.006	-0.118	0.002	-0.045	0.17	-0.120	<0.001	-0.368	<0.001	-0.085	0.123	
	Fasting glucose	0.244	<0.001	0.055	0.16	0.093	0.01	0.028	0.401	-0.380	<0.001	-0.036	0.523	
	Insulin	1.173	0.001	-0.562	0.163	-0.278	0.43	-0.805	0.021	-3.821	<0.001	-1.050	0.071	
	HOMA-IR	0.360	0.001	-0.157	0.184	-0.036	0.73	-0.196	0.058	-1.033	<0.001	-0.212	0.217	
			(THE+THF+5 α -THF) night				(THA+THB+5 α -THB) night				(THA+THB+5 α THB)/(THE+THF+5 α THF) night			
	BMI	2.094	<0.001	1.274	<0.001	0.676	0.002	-0.025	0.910	-4.252	<0.001	-3.123	<0.001	
	Systolic BP	8.146	<0.001	1.444	0.111	2.765	0.002	-0.091	0.903	-13.58	<0.001	-3.152	0.023	
	Total cholesterol	0.011	0.85	0.005	0.938	-0.096	0.088	-0.036	0.508	-0.331	0.001	-0.153	0.136	
	LDL cholesterol	0.179	0.001	0.090	0.129	0.026	0.596	0.001	0.982	-0.423	<0.001	-0.207	0.023	
	HDL cholesterol	-0.195	<0.001	-0.031	0.212	-0.095	<0.001	0.021	0.294	0.281	<0.001	0.137	<0.001	
	Triglycerides	0.078	0.018	-0.114	0.002	-0.047	0.121	-0.128	<0.001	-0.420	<0.001	-0.176	0.001	
	Fasting glucose	0.299	<0.001	0.093	0.011	0.170	<0.001	0.060	0.047	-0.294	<0.001	0.013	0.824	
	Insulin	1.213	0.001	-0.135	0.726	0.008	0.979	-0.499	0.110	-3.850	<0.001	-1.355	0.022	
	HOMA-IR	0.394	<0.001	-0.033	0.772	0.060	0.533	-0.110	0.230	-1.041	<0.001	-0.306	0.079	

β = regression coefficient. P values refer to regression co-efficient, unadjusted or adjusted in multiple regression analyses for co-variables (age, sex, center, relevant medication, and BMI (unless BMI is the outcome variable)). The ratio (THA+THB+5 α THB)/(THE+THF+5 α THF) was normalised by log transformation before regression analyses.

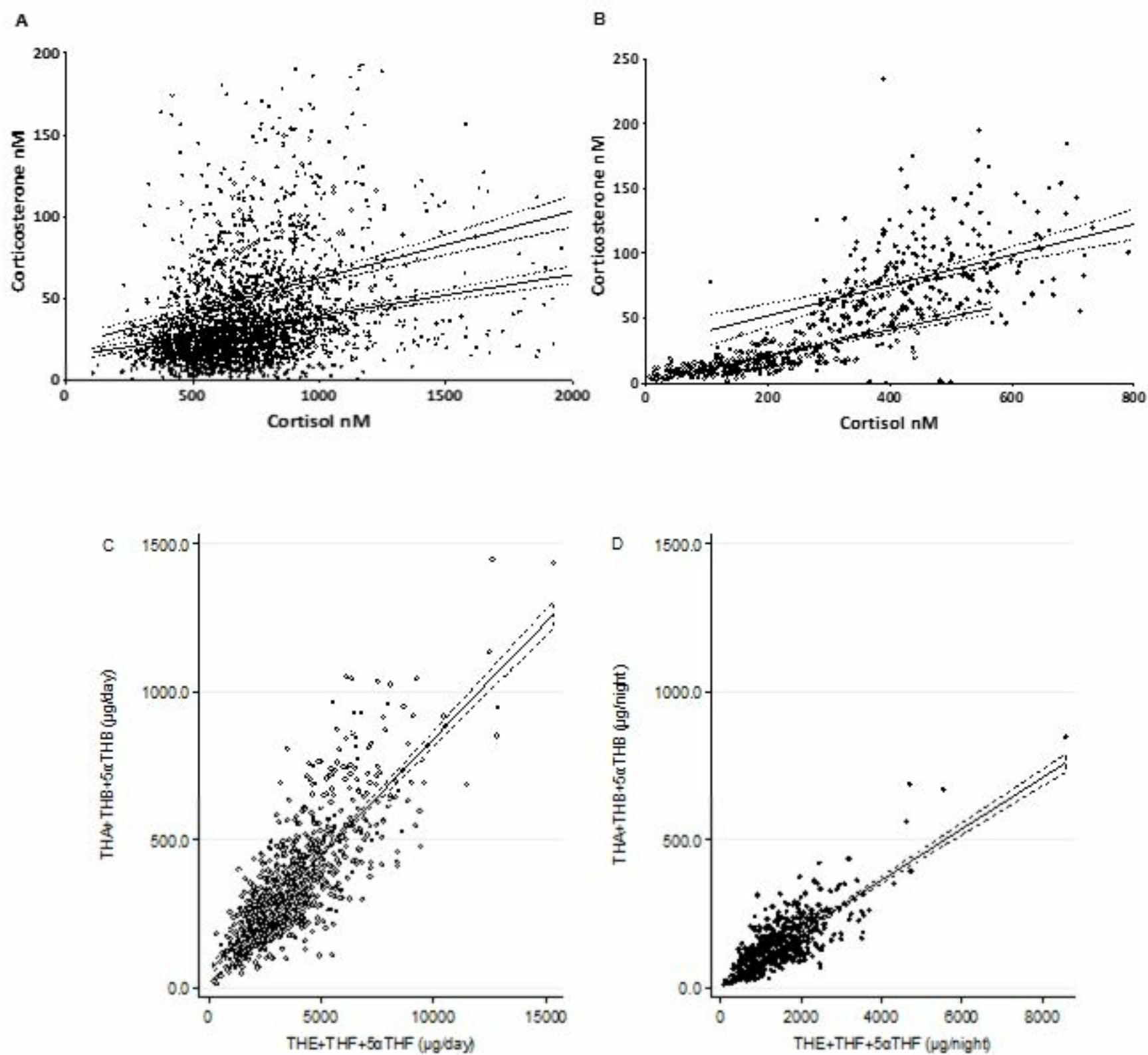


Figure 1

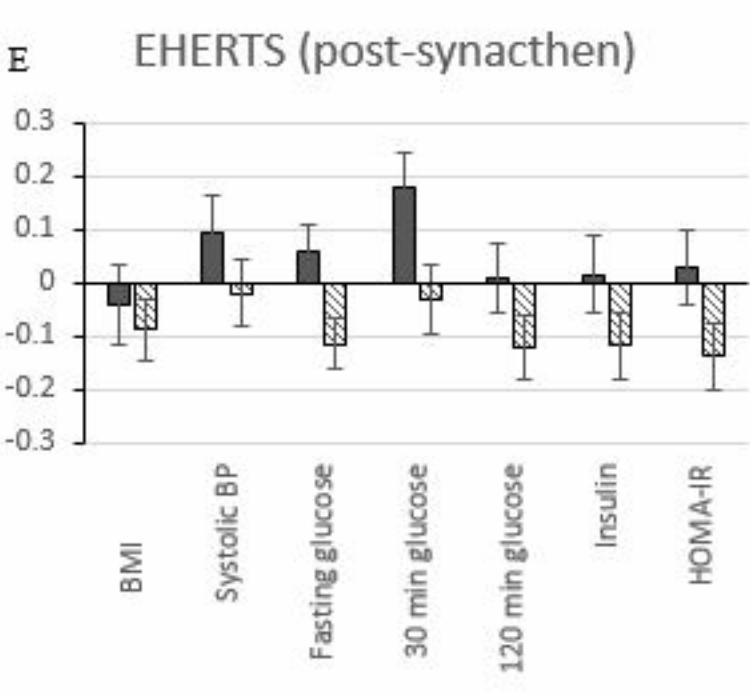
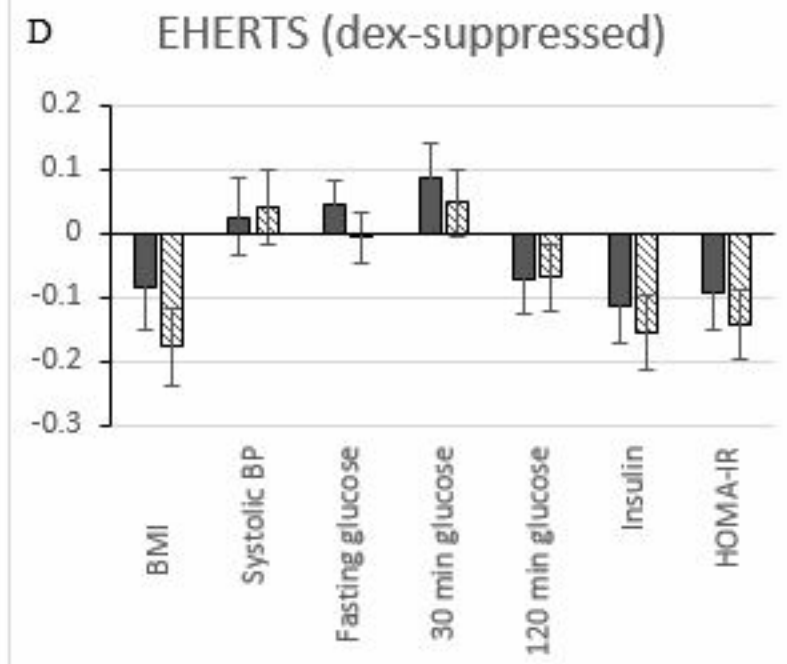
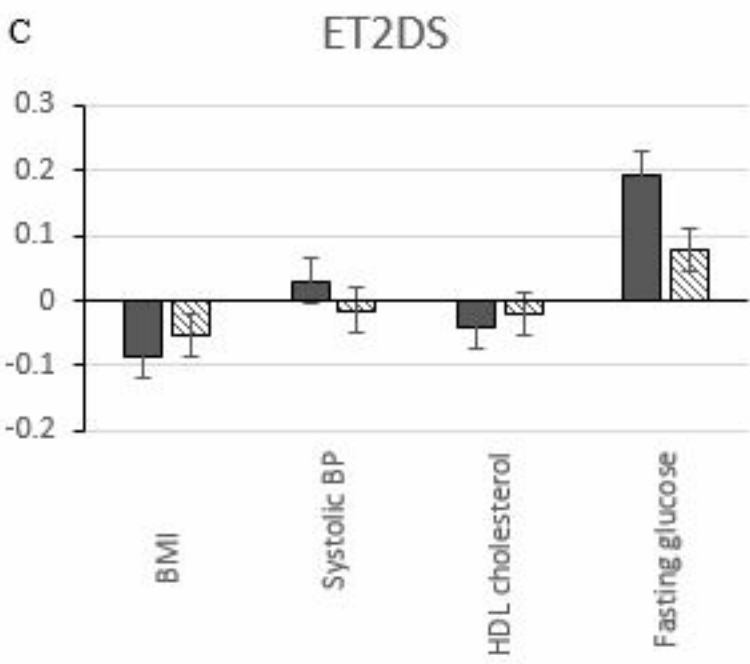
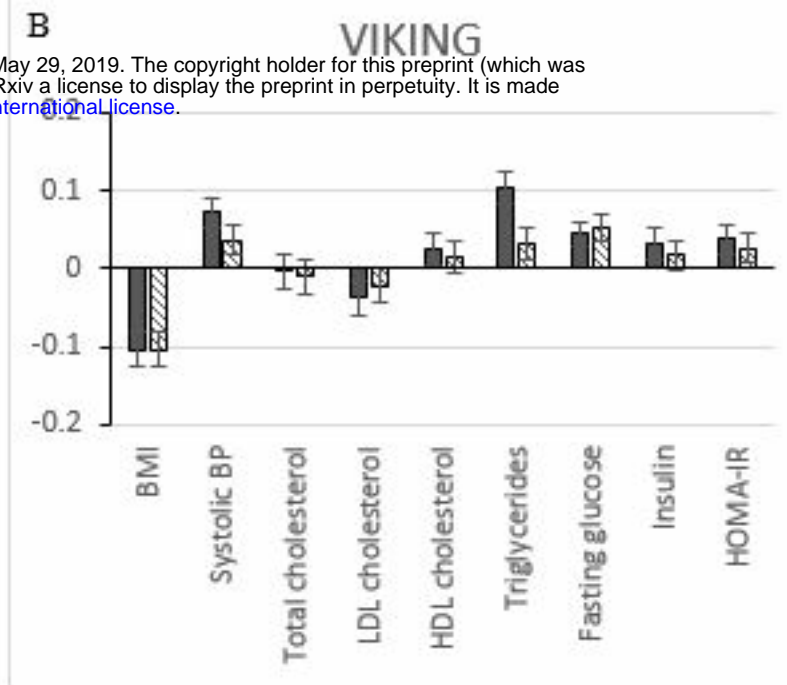
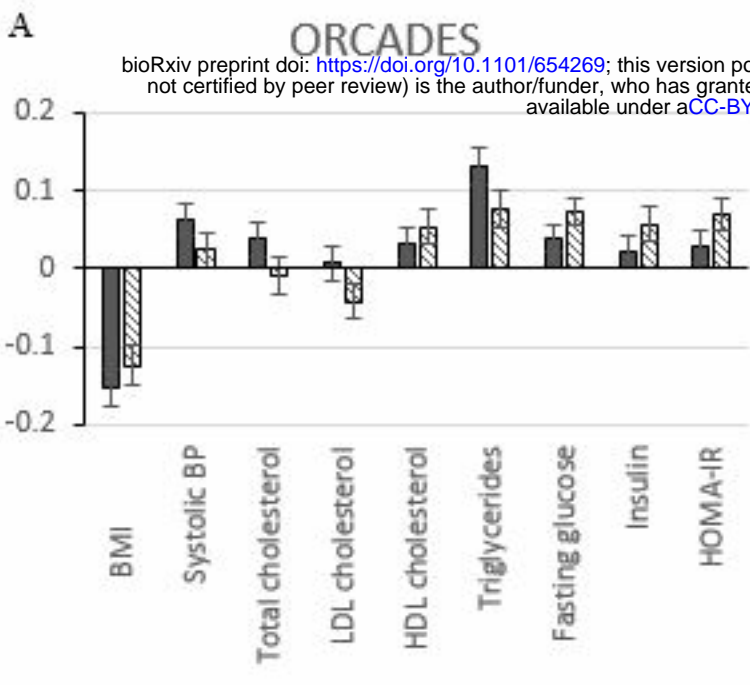


Figure 2

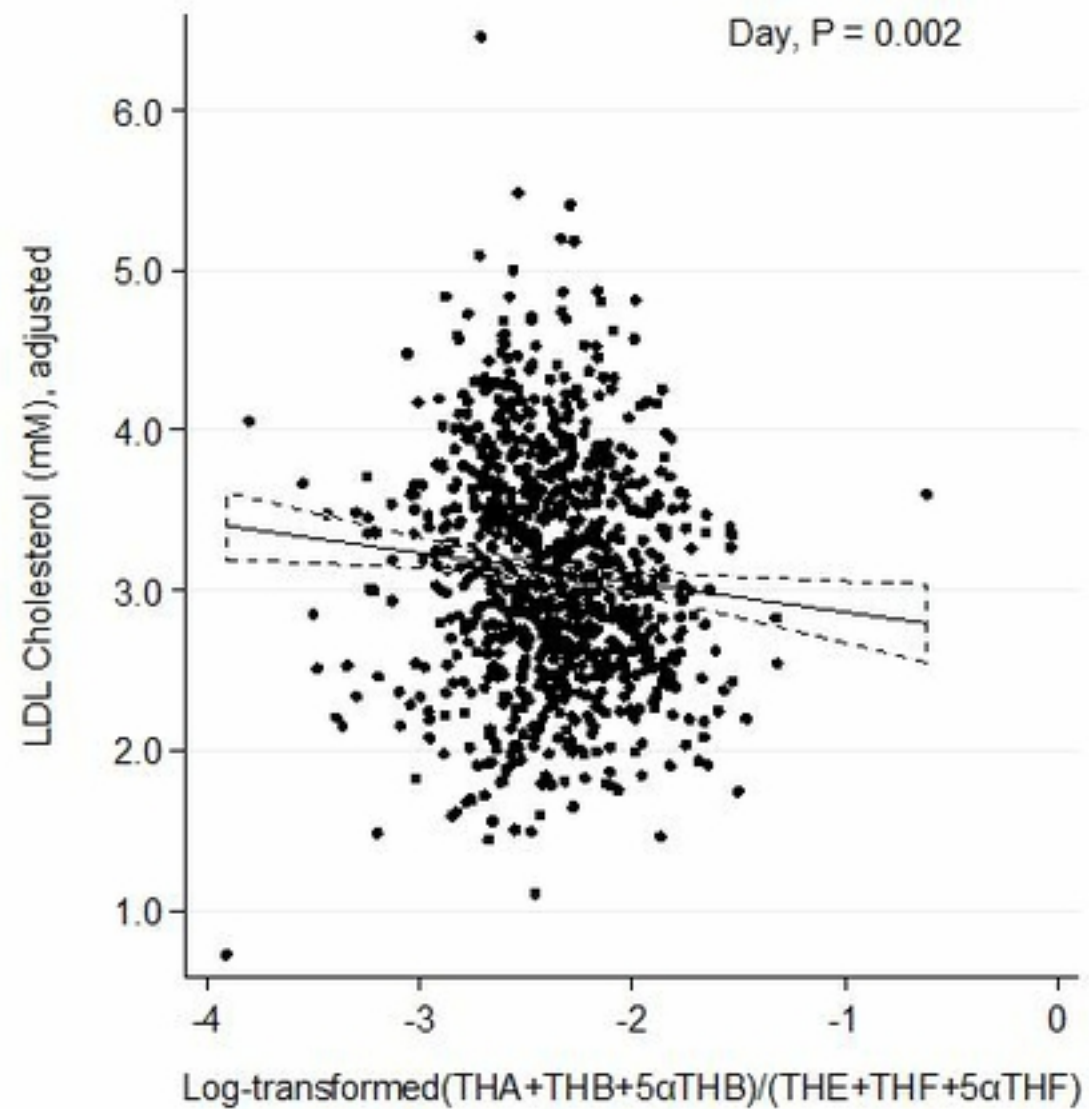
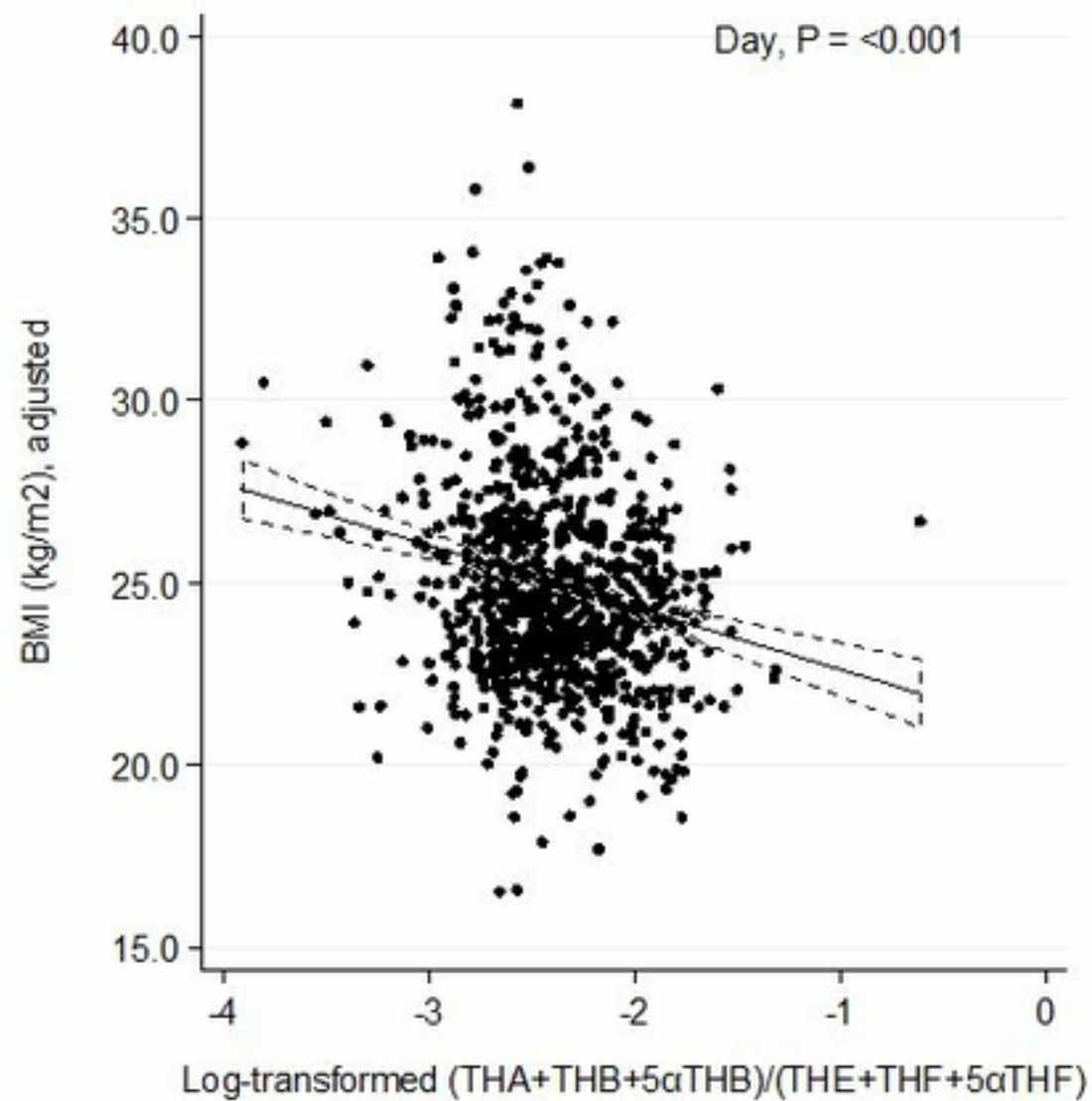


Figure 3