



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Maternal glucocorticoid metabolism across pregnancy: a potential mechanism underlying fetal glucocorticoid exposure

Citation for published version:

Stoye, DQ, Andrew, R, Grobman, WA, Adam, EK, Wadhwa, PD, Buss, C, Entringer, S, Miller, GE, Boardman, J, Seckl, J, Keenan-Devlin, LS, Borders, AEB & Reynolds, R 2020, 'Maternal glucocorticoid metabolism across pregnancy: a potential mechanism underlying fetal glucocorticoid exposure', *Journal of Clinical Endocrinology & Metabolism*, vol. 105, no. 3, dgz313. <https://doi.org/10.1210/clinem/dgz313>

Digital Object Identifier (DOI):

[10.1210/clinem/dgz313](https://doi.org/10.1210/clinem/dgz313)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Journal of Clinical Endocrinology & Metabolism

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1 **Maternal glucocorticoid metabolism across pregnancy: a potential mechanism underlying fetal**
2 **glucocorticoid exposure**

3

4 David Q. Stoye¹, Ruth Andrew², William A. Grobman^{3,4}, Emma K. Adam⁵, Pathik D. Wadhwa⁶,
5 Claudia Buss^{6,7}, Sonja Entringer^{6,7}, Gregory E. Miller⁸, James P. Boardman¹, Jonathan R. Seckl²,
6 Lauren S. Keenan-Devlin⁹, Ann E.B. Borders^{4,9}, Rebecca M. Reynolds^{1,2}

7

8 **Affiliations:** ¹MRC Centre of Reproductive Health, University of Edinburgh, Edinburgh, UK; ²Centre
9 for Cardiovascular Sciences, University of Edinburgh, Edinburgh, UK; ³Department of Obstetrics and
10 Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; ⁴Center for
11 Healthcare Studies, Institute for Public Health and Medicine, Northwestern University, Chicago, IL,
12 USA; ⁵School of Education and Social Policy, Institute for Policy Research, Northwestern University,
13 Evanston, IL, USA; ⁶Development, Health and Disease Research Program, University of California,
14 Irvine, CA, USA; ⁷Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin,
15 Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Institute of Medical Psychology,
16 Berlin, Germany; ⁸Department of Psychology, Institute for Policy Research, Northwestern University,
17 Evanston, IL, USA; ⁹Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine,
18 NorthShore University Health System, University of Chicago Pritzker School of Medicine, Chicago,
19 IL, USA

20

21 **Short title:** Glucocorticoid metabolism across pregnancy

22

23 **Key words:** cortisol, glucocorticoid, metabolism, HPA, pregnancy, birthweight

24

25 **Corresponding Author:** Professor Rebecca M. Reynolds. Centre for Cardiovascular Science, Queen's
26 Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ. Telephone: + 44 (0) 131
27 242 6762. Email: r.reynolds@ed.ac.uk. Reprint requests should be made to Professor Rebecca M.
28 Reynolds.

29

30 **Funding sources:** This project was supported by The National Children’s Study, Vanguard Study, Task
31 Order 5 (HHSN275201200007I-HHSN27500005), and Auxiliary Research Scholar and Research
32 Career Development Awards from NorthShore University Health System to Ann E.B. Borders. We
33 acknowledge the support of the British Heart Foundation. David Q. Stoye is supported by a fellowship
34 from Theirworld. This work was undertaken in the MRC Centre for Reproductive Health at the
35 University of Edinburgh, which is funded by MRC Centre Grant (MRC G1002033). We acknowledge
36 the support of the Wellcome Trust (202794/Z/16/Z).

37

38 **Disclosure summary:** The authors have no conflicts of interest relevant to this article to disclose.

39

40 **Word count (excluding references, tables and figures):** 3692

41

Abstract

43

44 *Context:* Across pregnancy maternal serum cortisol levels rise up to threefold. It is not known whether
45 maternal peripheral cortisol metabolism and clearance change across pregnancy, or influence fetal
46 cortisol exposure and development.

47

48 *Objectives:* The primary study objective was to compare maternal urinary glucocorticoid metabolites,
49 as markers of cortisol metabolism and clearance, between the 2nd and 3rd trimester of pregnancy.
50 Secondary objectives were to test associations of total maternal urinary glucocorticoid excretion, with
51 maternal serum cortisol levels and offspring birthweight z-score.

52

53 *Design, participants and setting:* 151 women with singleton pregnancies, recruited from prenatal clinic
54 at the Pittsburgh site of the Measurement of Maternal Stress (MOMS) study, had 24-hour urine
55 collections during both the 2nd and 3rd trimester.

56

57 *Results:* Between the 2nd and 3rd trimester total urinary glucocorticoid excretion increased (ratio of
58 geometric means (RGM) 1.37, 95% CI 1.22-1.52, $p < 0.001$), and there was an increase in calculated 5 β -
59 reductase compared to 5 α -reductase activity (RGM 3.41, 95% CI 3.04-3.83, $p < 0.001$). During the 3rd
60 trimester total urinary glucocorticoid excretion and serum cortisol were negatively correlated ($r = -$
61 0.179, $p = 0.029$). Mean total urinary glucocorticoid excretion across both trimesters and offspring
62 birthweight z-score were positively associated ($\beta = 0.314$, $p = 0.001$).

63

64 *Conclusions:* The estimated activity of maternal enzymes responsible for cortisol metabolism change
65 between the 2nd and 3rd trimester of pregnancy. Additionally, maternal peripheral metabolism and
66 clearance of cortisol may serve as a novel mechanism impacting fetal cortisol exposure and growth.

67

68 **Précis:** Maternal urine was sampled as part of a pregnancy cohort. Estimated cortisol metabolism
69 changes across pregnancy, and total urinary glucocorticoid excretion is positively associated with fetal
70 growth.

71 **Introduction**

72

73 Glucocorticoids play a critical role in fetal maturation. While a surge in glucocorticoid exposure
74 towards the end of pregnancy helps prime a fetus for life outside the womb¹, excess or inappropriately
75 timed exposure can adversely programme offspring development^{2,3}. There is growing evidence that
76 circulating levels of maternal cortisol influence both fetal cortisol exposure and development. Maternal
77 blood cortisol levels correlate with cortisol levels measured in fetal blood⁴ and amniotic fluid⁵. Elevated
78 cortisol levels measured in maternal blood or saliva are associated with offspring growth restriction and
79 adverse neurodevelopment and metabolic health⁶⁻⁸.

80

81 Maternal regulation of glucocorticoids changes profoundly across pregnancy, with circulating cortisol
82 levels rising approximately threefold by delivery⁹. Multiple factors contribute to maternal
83 hypercortisolism including rising cortisol binding globulin (CBG)¹⁰, placental secretion of corticotropin
84 releasing hormone (CRH)¹¹, and reduced sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis
85 to glucocorticoid mediated central negative feedback¹². Altered breakdown, clearance and regeneration
86 of cortisol within maternal peripheral tissues could also influence maternal serum levels and fetal
87 glucocorticoid exposure.

88

89 Relatively little intact cortisol is excreted from the body passively, with the majority instead being
90 metabolised to compounds considered more inert before urinary excretion¹³. Metabolism of cortisol to
91 5 β -tetrahydrocortisol (THF), and its derivatives α -cortol and β -cortol, and 5 α -tetrahydrocortisol (α -
92 THF), are reliant on the activity of A-ring reductases, 5 β -reductase, predominantly expressed in the
93 liver, and 5 α -reductase, expressed in both liver and fat. 11 β -hydroxysteroid dehydrogenase type 2 (11 β -
94 HSD2) acts in the kidney and placenta, converting cortisol to cortisone. In contrast, 11 β -hydroxysteroid
95 dehydrogenase type 1 (11 β -HSD1) is most highly expressed in the liver, where it regenerates active
96 cortisol from inert cortisone. These processes are outlined in figure 1. Peripheral glucocorticoid
97 metabolism varies as a function of age, gender and obesity and in many disease states¹⁴⁻¹⁶.

98

99 The sum of glucocorticoid metabolites measured in a 24-hour sample of urine represents total urinary
100 glucocorticoid excretion. As the majority of glucocorticoids are excreted in urine this measurement has
101 also been used as an estimate of glucocorticoid production by the adrenal gland¹⁷. Additionally,
102 comparison of the relative levels of metabolites offers insight into the activity of enzymes converting
103 cortisol in peripheral tissues.

104

105 To date there has been limited investigation of maternal peripheral glucocorticoid metabolism and
106 clearance in pregnancy. Longitudinal studies of maternal peripheral glucocorticoid metabolism in
107 pregnancy have been limited by small sample size¹⁸, or have relied on metabolites collected in spot
108 urine or blood samples that are subject to diurnal variation^{19,20}. There is growing evidence that maternal
109 peripheral glucocorticoid metabolism and clearance are altered in preeclampsia²⁰⁻²². There is also
110 preliminary data supporting a role for peripheral glucocorticoid metabolism influencing fetal
111 development, with a higher plasma cortisone to cortisol ratio (representing more inert compared to
112 active glucocorticoid) measured in mothers with psychiatric morbidity during the 3rd trimester, being
113 associated with higher offspring birthweight²³.

114

115 The aims of this study were to assess how maternal urinary glucocorticoid excretion, measured in 24-
116 hour urine, changes between the 2nd and 3rd trimester of pregnancy, and to test the associations of total
117 urinary glucocorticoid excretion with maternal serum cortisol levels and offspring birth weight z-score.
118 We tested the hypothesis that total urinary glucocorticoid excretion, as a marker of maternal adrenal
119 cortisol production, increases across pregnancy, and is negatively associated with offspring birthweight
120 z-score.

121

122 **Materials and Methods**

123

124 **Participants and clinical protocol**

125 The Measurement of Maternal Stress (MOMS) study was a multisite prospective cohort that recruited
126 women with singleton pregnancies from antenatal clinics in Pittsburgh, PA, Chicago, IL, Schuylkill

127 County, PA and San Antonio, TX between June 2013 and May 2014. Exclusion criteria were fetal
128 congenital abnormality, chromosomal abnormalities, progesterone use before 14 weeks' gestation, or
129 regular maternal corticosteroid use. All participating women gave written informed consent, and the
130 study protocol was approved by the Institutional Review Board of each site. A description of the cohort
131 has been presented previously²⁴.

132

133 This study reports data from a subset (151 of 200) of mother-baby dyads, recruited from the Pittsburgh
134 site, who had 24-hour urine collected for measurement of total glucocorticoids and metabolites on two
135 occasions during pregnancy, between 12.7 and 22.1 weeks' gestation (2nd trimester), and between 31.9
136 and 36.4 weeks' gestation (3rd trimester).

137

138 Participants also had blood collected for measurement of serum cortisol at study visits during the 2nd
139 and 3rd trimester. Maternal demographic and medical information including body mass index (BMI),
140 age, ethnicity, diabetes mellitus, preeclampsia, gestational hypertension and offspring outcomes
141 including birthweight and birth gestation, were recorded either during study visits, or on review of
142 participants' medical records. Offspring birthweight z-scores were calculated according to International
143 Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) standards²⁵.

144

145 **Laboratory methods**

146

147 **Serum**

148 Serum was obtained by centrifuging whole blood at 1000 g at 4 °C for 15 minutes, then aliquoting
149 serum into 2mL cryovials. Cortisol was assessed by radioimmunoassay at the Development, Health and
150 Disease Research Program's laboratory at the University of California, Irvine. 10% of samples were
151 measured in duplicate, and inter-assay and intra-assay CVs were <10%.

152

153 **Urinary glucocorticoids**

154 Urinary glucocorticoid metabolites were analysed by gas chromatography triple quadrupole mass
 155 spectrometry (GC-MS/MS), at the Edinburgh Clinical Research Facility Mass Spectrometry Core as
 156 previously described²⁶. The inter- and intra-assay CVs were <13%. Analytes included cortisol (F),
 157 cortisone (E), α -THF, THF, α -cortol, β -cortol, THE, α -cortolone and β -cortolone. The sum of these
 158 measured analytes is referred to as total urinary glucocorticoid excretion.

159

160 The following ratios of urinary metabolites were used as parameters to estimate peripheral
 161 glucocorticoid metabolism:

162 i) 11β -HSD2 activity = F / E

163 ii) 11β -HSD total activity = (THF + α -THF) / THE.

164 iii) Relative 5β -reductase and 5α -reductase activity = THF / α -THF

165 iv) 5α -reductase activity = F / α -THF

166 v) 5β -reductase metabolism of F = F / (THF + α -cortol + β -cortol)

167 vi) 5β -reductase metabolism of E = E / (THE + α -cortolone + β -cortolone)

168

169 **Statistical Analysis**

170 All analyses were performed using IBM SPSS Statistics Version 24. Data distributions were assessed
 171 for normality visually using histograms. Serum cortisol levels were normally distributed amongst the
 172 study population. Levels of all excreted urinary glucocorticoid metabolites were positively skewed, and
 173 log base 10 transformed prior to statistical analysis.

174

175 Demographic data is presented as mean \pm SD. Change of urinary metabolite excretion between the 2nd
 176 and 3rd trimester was tested using paired *t* tests, and the degree of change is represented through the
 177 ratio of the geometric means (RGM), with 95% confidence intervals. To assess if peripheral metabolism
 178 has a maintained trait component across pregnancy, the rank stability, i.e. the similarity of where
 179 participants' estimated enzymatic function fell within the study population's distribution, at the 2nd
 180 compared to the 3rd trimester, was tested by a linear regression model adjusting for the gestation of urine
 181 sampling. The relationship between maternal total urinary glucocorticoid excretion and serum cortisol

182 levels was tested using Pearson's Coefficient within both the whole study population and in a subgroup
183 of patients with blood sampled before 10 am. Finally, the association of maternal total urinary
184 glucocorticoid excretion and offspring birthweight z-score was tested by linear regression adjusting for
185 confounding factors. These included the gestation at urine sampling and maternal ethnicity, smoking
186 status, age, preeclampsia, gestational hypertension, diabetes mellitus (pre-gestational and gestational),
187 BMI and gravidity. Associations with birthweight z-score were tested for both 2nd and 3rd trimester
188 glucocorticoid excretion, and for mean glucocorticoid excretion across pregnancy. A p-value < 0.05
189 was considered statistically significant.

190

191 **Results**

192

193 **Demographics**

194 Table 1 shows the characteristics of study participants. Mothers were aged 30.5 ± 5.0 years, with BMI
195 27.6 ± 7.1 kg/m², and were predominantly white non-smokers. Mean gestational age at birth was 39.4
196 ± 1.4 weeks, and mean birthweight was 3487 ± 489 grams.

197

198 **Changing glucocorticoid levels across pregnancy**

199 Figure 2 and table 2 depict urinary glucocorticoid metabolite excretion for collections during the 2nd
200 and 3rd trimester. Across pregnancy total urinary glucocorticoid excretion increased (RGM 1.37,
201 $p < 0.001$). Excretion of all individual metabolites increased except for α -THF which decreased between
202 the 2nd and 3rd trimester (RGM 0.55, $p < 0.001$). Assessing individual metabolic pathways, the ratio of F
203 / E (RGM 0.90, $p < 0.001$) decreased likely representing increased estimated 11 β -HSD2 (inactivation of
204 cortisol to cortisone) activity across pregnancy. Total body 11 β -HSD activity represented by (THF + α -
205 THF) / THE (RGM 1.27, $p < 0.001$) shifted in favour of excretion of cortisol metabolites relative to
206 cortisone metabolites. The activity of A-ring reductases shifted towards 5 β -reductase metabolism
207 compared to 5 α -reductase metabolism with increased THF / α -THF ratio (RGM 3.41, $p < 0.001$).
208 Between the 2nd and 3rd trimester serum cortisol also increased (ratio of means 1.63, 95% CI 1.40-
209 1.85, $p < 0.001$).

210

211 Individual stability in peripheral glucocorticoid metabolism

212 Table 3 and figure 3 represent rank-order stability of total urinary glucocorticoid excretion and estimates
213 of peripheral metabolism of glucocorticoids for participants across the 2nd and 3rd trimester. Despite the
214 whole group changes in peripheral glucocorticoid metabolism across pregnancy the relative enzymatic
215 activity of individual participants compared to the whole group was well maintained across both time
216 points, with women with higher estimated activity for peripheral glucocorticoid metabolism during the
217 2nd trimester tending to have higher estimated enzyme activity measured in the third trimester.

218

219 Associations between total urinary glucocorticoid excretion and serum cortisol levels

220 During the 2nd trimester serum cortisol was not associated with total urinary glucocorticoid excretion
221 ($r=0.076$, $p=0.358$). During the 3rd trimester, total urinary glucocorticoid excretion was negatively
222 associated with serum cortisol within the whole group ($r=-0.179$, $p=0.029$). This association between
223 3rd trimester serum cortisol and total urinary glucocorticoid excretion was largely driven by the
224 subgroup of participants with 3rd trimester blood samples taken before 10am ($n=66$, $r=-0.354$, $p=0.004$).
225 In contrast, for participants with 3rd trimester blood taken after 10am ($n=83$, $r=-0.096$, $p=0.390$).

226

227 Associations between total urinary glucocorticoid excretion and infant birthweight z-score

228 In the adjusted models, there were positive associations between total urinary glucocorticoid excretion
229 during the 2nd trimester and offspring birth weight z-score ($\beta=0.198$, r-square change 0.028, $p=0.033$),
230 total urinary glucocorticoid excretion during the 3rd trimester and offspring birth weight z-score
231 ($\beta=0.202$, r-square change 0.032, $p=0.023$), and mean total glucocorticoid excretion across both
232 trimesters with offspring birth weight z-score ($\beta=0.314$, r-square change 0.066, $p=0.001$). In contrast,
233 there was no association between mean serum cortisol levels and offspring birthweight z-score. A
234 visual representation of maternal glucocorticoid excretion across trimesters according to infant
235 birthweight quantile is shown in figure 4.

236

237 **Associations between glucocorticoid metabolite ratios, with serum cortisol and infant birthweight**
238 **z-score**

239 Having demonstrated that total urinary glucocorticoid excretion was negatively associated with serum
240 cortisol during the 3rd trimester and positively associated with birthweight z-score, further exploratory
241 analysis was undertaken to investigate whether these effects were being driven by the action of
242 individual metabolic pathways. In this exploratory analysis, higher 3rd trimester serum cortisol was
243 associated with estimates of reduced 5 α -reductase activity (F / α -THF; whole group $r=0.168$, $p=0.041$;
244 venepuncture <10am subgroup $r=0.318$, $p=0.009$), and reduced 5 β -reductase activity (F / (THF + α -
245 cortol + β -cortol); whole group $r=0.206$, $p=0.012$; venepuncture <10am subgroup $r=0.281$, $p=0.022$)
246 and (E / (THE + α -cortolone + β -cortolone); whole group $r=0.252$, $p=0.002$; venepuncture <10am
247 subgroup $r=0.251$, $p=0.042$). No associations were seen between 3rd trimester serum cortisol and
248 estimated 11 β -HSD1 or 11 β -HSD2 activity. Additionally, no association were seen between infant
249 birthweight z-score and urine metabolite ratios.

250

251

252 **Discussion**

253

254 In this study of pregnant women with detailed measurements of glucocorticoid metabolism we have
255 demonstrated that glucocorticoid metabolism changes across pregnancy, and that total urinary
256 glucocorticoid excretion is positively associated with offspring birthweight z-score.

257

258 Within the cohort total maternal glucocorticoid excretion increased between the 2nd and 3rd trimester.
259 This builds on previous observations of increased urinary free cortisol excretion across pregnancy⁹, and
260 likely represents an increase in adrenal cortisol release across pregnancy. There were also differences
261 in the ratios of urinary metabolites between the 2nd and 3rd trimester. This provides evidence that the
262 global actions of enzymes working to metabolise cortisol in peripheral tissues changes across
263 pregnancy. A reduced F/E ratio represents increased 11 β -HSD2 activity. An increase in (THF + α -THF)
264 / THE ratio, in the context of estimated increased 11 β -HSD2 likely represents an increase in 11 β -HSD1

265 activity across pregnancy. The ratio of A-ring reductase metabolism shifted profoundly towards 5 β -
266 reductase meta²⁷bolism compared to 5 α -reductase metabolism with increased THF / α -THF ratio. A
267 reduction of 5 α -reductase cortisol metabolism is in keeping with results from a study where α -THF
268 excretion measured in maternal urine rose across the first year postpartum²⁸. The action of 5 α -reductase
269 in pregnancy has received attention due to its important role in converting testosterone to
270 dihydrotestosterone, with 5 α -reductase genetic mutation or pharmacological inhibition causing *in utero*
271 under-virilization of male offspring²⁹. 5 α -reductase metabolism of progesterone has also been
272 investigated in the context of parturition, with 5 α -reductase type 1 deficient mice failing to undergo
273 cervical ripening at term³⁰. However, to our knowledge the physiological importance of 5 α -reductase
274 metabolism of cortisol in pregnancy has not previously been considered.

275

276 Changes in glucocorticoid metabolism may offer specific advantages to the mother and fetus. In
277 addition to controlling systemic cortisol inactivation and clearance, peripherally located enzymes play
278 an important role in regulating glucocorticoid exposure to specific tissues. This is most commonly
279 discussed in relation to the kidney, where local 11 β -HSD2 acts to prevent excessive activation of
280 mineralocorticoid receptors by cortisol¹³. 5 α -reductase influences cortisol clearance and action within
281 the liver, and its activity has been shown to be modifiable either by early life stress³¹, or by variation in
282 nutritional demands^{32,33}. Within pregnancy, marked reduction in 5 α -reductase activity during the 3rd
283 trimester may act to enhance cortisol activity in the liver, allowing mobilisation of fuels at a time of
284 increased metabolic requirements.

285

286 Alternatively, changing glucocorticoid metabolism across pregnancy may be a bystander influenced by
287 other physiological changes in the mother across pregnancy. Maternal glucocorticoid metabolism could
288 be influenced by a changing inflammatory milieu. For example it has both been demonstrated that
289 tumor necrosis factor alpha (TNF- α) rises across pregnancy²⁷, and that inhibiting TNF α in patients with
290 inflammatory arthritis increases 5 α -reductase activity³⁴. Changing biliary physiology may also
291 influence maternal glucocorticoid metabolism, with bile acids holding the potential to inhibit A-ring
292 reductases and 11 β -HSDs³⁵. Increases in insulin resistance across pregnancy may also influence

293 glucocorticoid metabolism. However, insulin sensitizing therapies and weight loss have both previously
294 been associated with decreases in 5α -reductase activity^{36,37}, making it unlikely that changes in insulin
295 sensitivity are driving the reductions in 5α -reductase activity seen within the 3rd trimester. There is also
296 likely to be a placental contribution to maternal whole-body glucocorticoid metabolism estimated
297 through urinary glucocorticoids. In an ex vivo placental perfusion model the majority of cortisone
298 converted from cortisol at term gestation was transferred back into the maternal circulation rather than
299 fetal circulation³⁸.

300

301 During the 2nd trimester there was no association between maternal urinary glucocorticoid excretion
302 and serum cortisol, whilst during the 3rd trimester higher serum cortisol correlated with lower total
303 urinary glucocorticoid excretion. Additionally, in exploratory analysis, higher serum cortisol in the third
304 trimester was associated with lower estimated activity of 5β -reductase and 5α -reductase. Individual
305 differences in peripheral glucocorticoid metabolism and clearance may influence serum cortisol levels
306 in the later stages of pregnancy. In healthy non-pregnant populations differences in peripheral
307 glucocorticoid metabolism are generally not associated with serum cortisol levels, likely due to
308 compensatory glucocorticoid release by the HPA axis in response to changing negative feedback^{39,40}.
309 However in critically ill patients reduced peripheral metabolism and clearance of cortisol contributes to
310 raised serum cortisol levels¹⁶. Throughout pregnancy regulation of the maternal HPA axis changes,
311 becoming progressively less sensitive to negative feedback by glucocorticoids¹². It therefore seems
312 physiologically plausible that by the 3rd trimester individual differences in glucocorticoid metabolism
313 and clearance influence serum cortisol levels.

314

315 An unexpected finding was the modest positive association between total urinary glucocorticoid
316 excretion and offspring birthweight z-score, with maternal total urinary glucocorticoid excretion
317 measured in the 2nd and 3rd trimesters of pregnancy explaining 6.6% of variance in offspring birthweight
318 z-score. Previous studies have typically reported a negative association between synthetic
319 glucocorticoid exposure², or maternal cortisol levels measured in saliva⁷ or blood⁴¹, with infant
320 birthweight. A negative association has also previously been reported between urinary free cortisol

321 measured in the morning between 18-20 weeks' gestation and fetal growth⁴². The relationship between
322 total urinary glucocorticoid excretion and infant birthweight z-score has not previously been tested.
323 Increased maternal peripheral metabolism and clearance of glucocorticoids may serve as a mechanism
324 reducing cortisol exposure to the fetus. This theory is strengthened by the negative association found
325 between serum cortisol and total urinary glucocorticoids observed in the third trimester. In the
326 exploratory analyse no associations were found between birthweight z-score and any of the urinary
327 metabolite ratios used to estimate peripheral enzymatic function, and so it cannot be concluded that this
328 relationship is driven through the effects of a single enzyme's function. Alternatively, the relationship
329 between maternal total urinary glucocorticoid excretion and infant birthweight z-score could be
330 mediated by other maternal factors. For example, increased urinary glucocorticoid excretion has
331 previously been associated with insulin resistance³⁶, and increased maternal insulin resistance during
332 pregnancy may also act to increase offspring birthweight⁴³.

333

334 Despite whole group changes in peripheral metabolism across pregnancy, individuals' rank within the
335 cohort remained relatively stable with those who had higher calculated enzymatic activity during the
336 2nd trimester also tending to have higher activity during the 3rd trimester. This implies that individual's
337 peripheral metabolism shows a consistent trait across pregnancy, increasing the likelihood that
338 peripheral glucocorticoid metabolism could influence fetal exposure to cortisol, and play a role in fetal
339 development.

340

341 Strengths of this study include the use of a modern technique for accurate quantification of urinary
342 glucocorticoid metabolites²⁶, the large sample size, and longitudinal study design allowing comparison
343 of urinary metabolites across pregnancy. Limitations include the fact that there was variation in the time
344 of day blood samples were collected, that participants did not fast before venepuncture, and the lack of
345 measurement of other serum glucocorticoid metabolites in addition to cortisol.

346

347 **Conclusions**

348

349 Between the 2nd and 3rd trimester the ratios of urinary glucocorticoids, acting as markers of peripheral
350 metabolism, changed suggesting a relative decrease in 5 α -reductase metabolism and relative increase
351 in 5 β -reductase metabolism of cortisol. However inter-individual differences among study participants
352 were relatively well preserved between the two testing periods. The negative association between total
353 urinary glucocorticoids and 3rd trimester serum cortisol, along with the positive association between
354 total urinary glucocorticoids and birthweight z-score, provides preliminary data that peripheral
355 glucocorticoid metabolism may influence fetal glucocorticoid exposure and fetal growth.

356

357 **Acknowledgements**

358

359 We are grateful for the support of the MOMS Study Collaboration including research staff and
360 participants. We also appreciate the important contribution of the MOM-led pilot study collaboration.
361 We acknowledge the support of the University of Edinburgh Mass Spectrometry Core.

362

363 **Data Availability**

364

365 The dataset generated during the current study is not publicly available but is available from the
366 corresponding author on reasonable request.

367

368 **References**

369

- 370 1. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are
371 there long-term consequences of the life insurance? *The Proceedings of the Nutrition Society*.
372 1998;57(1):113-122.
- 373 2. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades
374 of testing the hypothesis—2012 Curt Richter Award Winner. *Psychoneuroendocrinology*.
375 2013;38(1):1-11.
- 376 3. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 1: Outcomes. *Nat*
377 *Rev Endocrinol*. 2014;10(7):391-402.
- 378 4. Gitau R, Cameron A, Fisk NM, Glover V. Fetal exposure to maternal cortisol. *Lancet*
379 *(London, England)*. 1998;352(9129):707-708.
- 380 5. Glover V, Bergman K, Sarkar P, O'Connor TG. Association between maternal and amniotic
381 fluid cortisol is moderated by maternal anxiety. *Psychoneuroendocrinology*. 2009;34(3):430-
382 435.

- 383 6. Zijlmans MA, Riksen-Walraven JM, de Weerth C. Associations between maternal prenatal
384 cortisol concentrations and child outcomes: A systematic review. *Neuroscience and*
385 *biobehavioral reviews*. 2015;53:1-24.
- 386 7. Cherak SJ, Giesbrecht GF, Metcalfe A, Ronksley PE, Malebranche ME. The effect of
387 gestational period on the association between maternal prenatal salivary cortisol and birth
388 weight: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2018;94:49-62.
- 389 8. Stinson LJ, Stroud LR, Buka SL, et al. Prospective evaluation of associations between
390 prenatal cortisol and adulthood coronary heart disease risk: the New England family study.
391 *Psychosom Med*. 2015;77(3):237-245.
- 392 9. Jung C, Ho JT, Torpy DJ, et al. A longitudinal study of plasma and urinary cortisol in
393 pregnancy and postpartum. *J Clin Endocrinol Metab*. 2011;96(5):1533-1540.
- 394 10. Qureshi AC, Bahri A, Breen LA, et al. The influence of the route of oestrogen administration
395 on serum levels of cortisol-binding globulin and total cortisol. *Clin Endocrinol (Oxf)*.
396 2007;66(5):632-635.
- 397 11. Sasaki A, Shinkawa O, Yoshinaga K. Placental corticotropin-releasing hormone may be a
398 stimulator of maternal pituitary adrenocorticotrophic hormone secretion in humans. *Journal of*
399 *Clinical Investigation*. 1989;84(6):1997-2001.
- 400 12. Odagiri EMI, Ishiwatari N, Abe Y, et al. Hypercortisolism and the Resistance to
401 Dexamethasone Suppression during Gestation. *Endocrinologia Japonica*. 1988;35(5):685-
402 690.
- 403 13. Walker BR, Seckl JR. Cortisol metabolism. *International Textbook of Obesity*. 2001:241-268.
- 404 14. Nixon M, Upreti R, Andrew R. 5 α -Reduced glucocorticoids: a story of natural selection. *J*
405 *Endocrinol*. 2012;212(2):111-127.
- 406 15. Andrew R, Phillips DI, Walker BR. Obesity and gender influence cortisol secretion and
407 metabolism in man. *J Clin Endocrinol Metab*. 1998;83(5):1806-1809.
- 408 16. Boonen E, Vervenne H, Meersseman P, et al. Reduced Cortisol Metabolism during Critical
409 Illness. *New England Journal of Medicine*. 2013;368(16):1477-1488.
- 410 17. Remer T, Maser-Gluth C, Wudy SA. Glucocorticoid measurements in health and disease--
411 metabolic implications and the potential of 24-h urine analyses. *Mini reviews in medicinal*
412 *chemistry*. 2008;8(2):153-170.
- 413 18. Stirrat LI, O'Reilly JR, Riley SC, et al. Altered maternal hypothalamic-pituitary-adrenal axis
414 activity in obese pregnancy is associated with macrosomia and prolonged pregnancy.
415 *Pregnancy Hypertens*. 2014;4(3):238.
- 416 19. Mistry HD, Eisele N, Escher G, et al. Gestation-specific reference intervals for
417 comprehensive spot urinary steroid hormone metabolite analysis in normal singleton
418 pregnancy and 6 weeks postpartum. *Reprod Biol Endocrinol*. 2015;13:101.
- 419 20. Vasku M, Kleine-Eggebrecht N, Rath W, Mohaupt MG, Escher G, Pecks U. Apparent
420 systemic 11 β -dehydroxysteroid dehydrogenase 2 activity is increased in preeclampsia but not
421 in intrauterine growth restriction. *Pregnancy Hypertension*. 2018;11:7-11.
- 422 21. Jayasuriya NA, Hughes AE, Sovio U, Cook E, Charnock-Jones DS, Smith GCS. A Lower
423 Maternal Cortisol-to-Cortisone Ratio Precedes Clinical Diagnosis of Preterm and Term
424 Preeclampsia by Many Weeks. *J Clin Endocrinol Metab*. 2019;104(6):2355-2366.
- 425 22. Kosicka K, Siemiątkowska A, Szpera-Goździewicz A, Krzyścin M, Bręborowicz GH,
426 Główska FK. Increased cortisol metabolism in women with pregnancy-related hypertension.
427 *Endocrine*. 2018;61(1):125-133.
- 428 23. Hellgren C, Edvinsson Å, Olivier JD, et al. Tandem mass spectrometry determined maternal
429 cortisone to cortisol ratio and psychiatric morbidity during pregnancy—interaction with birth
430 weight. *Psychoneuroendocrinology*. 2016;69:142-149.
- 431 24. Miller GE, Culhane J, Grobman W, et al. Mothers' childhood hardship forecasts adverse
432 pregnancy outcomes: Role of inflammatory, lifestyle, and psychosocial pathways. *Brain*
433 *Behav Immun*. 2017;65:11-19.
- 434 25. Villar J, Ismail LC, Victora CG, et al. International standards for newborn weight, length, and
435 head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the
436 INTERGROWTH-21st Project. *The Lancet*. 2014;384(9946):857-868.

- 437 26. Homer N, Kothiya S, Rutter A, Walker BR, Andrew R. Gas chromatography tandem mass
438 spectrometry offers advantages for urinary steroids analysis. *Analytical Biochemistry*.
439 2017;538:34-37.
- 440 27. Beckmann I, Visser W, Struijk PC, van Dooren M, Glavimans J, Wallenburg HCS.
441 Circulating bioactive tumor necrosis factor- α , tumor necrosis factor- α receptors, fibronectin,
442 and tumor necrosis factor- α inducible cell adhesion molecule VCAM-1 in uncomplicated
443 pregnancy. *American Journal of Obstetrics and Gynecology*. 1997;177(5):1247-1252.
- 444 28. Rogers SL, Hughes BA, Jones CA, et al. Diminished 11beta-hydroxysteroid dehydrogenase
445 type 2 activity is associated with decreased weight and weight gain across the first year of
446 life. *J Clin Endocrinol Metab*. 2014;99(5):E821-831.
- 447 29. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5alpha-reductase
448 deficiency in man: an inherited form of male pseudohermaphroditism. *Science*.
449 1974;186(4170):1213-1215.
- 450 30. Mahendroo MS, Porter A, Russell DW, Word RA. The parturition defect in steroid 5alpha-
451 reductase type 1 knockout mice is due to impaired cervical ripening. *Molecular*
452 *endocrinology (Baltimore, Md)*. 1999;13(6):981-992.
- 453 31. Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe
454 developmental adversity, including nutritional deprivation, on cortisol metabolism in aging
455 Holocaust survivors. *Journal of psychiatric research*. 2009;43(9):877-883.
- 456 32. Tomlinson JW, Finney J, Gay C, Hughes BA, Hughes SV, Stewart PM. Impaired Glucose
457 Tolerance and Insulin Resistance Are Associated With Increased Adipose 11 β -
458 Hydroxysteroid Dehydrogenase Type 1 Expression and Elevated Hepatic 5 α -Reductase
459 Activity. *Diabetes*. 2008;57(10):2652-2660.
- 460 33. Stimson RH, Johnstone AM, Homer NZM, et al. Dietary Macronutrient Content Alters
461 Cortisol Metabolism Independently of Body Weight Changes in Obese Men. *The Journal of*
462 *Clinical Endocrinology & Metabolism*. 2007;92(11):4480-4484.
- 463 34. Nanus DE, Filer AD, Hughes B, et al. TNFalpha regulates cortisol metabolism in vivo in
464 patients with inflammatory arthritis. *Annals of the rheumatic diseases*. 2015;74(2):464-469.
- 465 35. McNeilly AD, Macfarlane DP, O'Flaherty E, et al. Bile acids modulate glucocorticoid
466 metabolism and the hypothalamic-pituitary-adrenal axis in obstructive jaundice. *Journal of*
467 *hepatology*. 2010;52(5):705-711.
- 468 36. Tomlinson JW, Finney J, Hughes BA, Hughes SV, Stewart PM. Reduced glucocorticoid
469 production rate, decreased 5alpha-reductase activity, and adipose tissue insulin sensitization
470 after weight loss. *Diabetes*. 2008;57(6):1536-1543.
- 471 37. Glintborg D, Hermann AP, Hagen C, et al. A randomized placebo-controlled study on the
472 effects of pioglitazone on cortisol metabolism in polycystic ovary syndrome. *Fertility and*
473 *sterility*. 2009;91(3):842-850.
- 474 38. Stirrat LI, Sengers BG, Norman JE, et al. Transfer and Metabolism of Cortisol by the Isolated
475 Perfused Human Placenta. *J Clin Endocrinol Metab*. 2018;103(2):640-648.
- 476 39. Purnell JQ, Brandon DD, Isabelle LM, Loriaux DL, Samuels MH. Association of 24-hour
477 cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body
478 composition, leptin levels, and aging in adult men and women. *J Clin Endocrinol Metab*.
479 2004;89(1):281-287.
- 480 40. White PC, Mune T, Agarwal AK. 11 β -Hydroxysteroid Dehydrogenase and the Syndrome of
481 Apparent Mineralocorticoid Excess*. *Endocrine Reviews*. 1997;18(1):135-156.
- 482 41. Goedhart G, Vrijkotte TG, Roseboom TJ, van der Wal MF, Cuijpers P, Bonsel GJ. Maternal
483 cortisol and offspring birthweight: results from a large prospective cohort study.
484 *Psychoneuroendocrinology*. 2010;35(5):644-652.
- 485 42. Diego MA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, Gonzalez-Quintero VH.
486 Prenatal depression restricts fetal growth. *Early Human Development*. 2009;85(1):65-70.
- 487 43. Yamashita H, Yasuhi I, Fukuda M, et al. The association between maternal insulin resistance
488 in mid-pregnancy and neonatal birthweight in uncomplicated pregnancies. *Endocr J*.
489 2014;61(10):1019-1024.
- 490

491 **Figure legends**

492

493 Figure 1. Peripheral cortisol metabolism enzymes and metabolites

494

495 Figure 2. Geometric mean and 95% confidence intervals of glucocorticoid metabolites from 24-hour
496 urine collections during the 2nd and 3rd trimester. * p<0.01, ** p<0.001

497

498 Figure 3. Rank correlation across the 2nd and 3rd trimesters of participant total urinary glucocorticoid
499 excretion or estimated enzymatic function, ** p<001

500

501 Figure 4. Geometric means and 95% confidence intervals of mothers' mean total urinary
502 glucocorticoid excretion across trimesters according to offspring birthweight z-score quintile

503

504 **Table Legends**

505 Table 1. Maternal, infant and sampling demographics

506 Table 2. Changes in urinary metabolites excretion and ratios across pregnancy

507 Table 3. Rank Correlation across the 2nd and 3rd trimesters of participant total urinary glucocorticoid

508 excretion or estimated enzymatic function

509

510 **Table 1. Maternal, infant and sampling demographics**

Maternal demographics	Number (%), Mean ± SD
Maternal Age (years)	30.5 ± 5.0
Maternal BMI (kg/m ²)	27.6 ± 7.1
Gravidity	
-1	50 (33.1%)
-2	41 (27.2%)
-≥3	60 (39.7%)
Ethnicity	
-Hispanic White	1 (0.7%)
-White	118 (78.1%)
-Black	27 (17.9%)
-Other	5 (3.3%)
Current Smoker	
-Yes	10 (6.6%)
-No	141 (93.4%)
Preeclampsia	
-Yes	4 (2.8%)
-No	139 (97.2%)
Hypertension	
-Yes	15 (10.5%)
-No	128 (89.5%)
Diabetes	
-Yes	9 (6.3%)
-No	134 (93.7%)
Infant Demographics	
Infant sex	
-Female	61 (42.7%)
-Male	82 (57.3%)
Birthweight (grams)	3487 ± 489
Birth gestation (weeks)	39.4 ± 1.4
Birthweight Z-Score	0.56 ± 0.99
Sampling Demographics	
2 nd trimester urine sample gestation (weeks)	17.3 ± 2.4
3 rd trimester urine sample gestation (weeks)	33.9 ± 1.2
2 nd trimester blood sample gestation (weeks)	16.7 ± 2.4
3 rd trimester blood sample gestation (weeks)	33.3 ± 1.1
2 nd trimester blood sample time (hours after midnight)	11.0 ± 2.2
3 rd trimester blood sample time (hours after midnight)	10.6 ± 2.5

511

512 Of the 151 participants included in the study the following data was missing: maternal BMI n = 2,

513 infant demographics and maternal health during pregnancy n = 8, 2nd trimester serum cortisol n = 1,514 3rd trimester serum cortisol n = 2.

515

516 **Table 2. Changes in urinary metabolites excretion and ratios across pregnancy**

	2 nd Trimester: Median (lower quartile-upper quartile)	3 rd Trimester: Median (lower quartile-upper quartile)	Change across gestations: RGM (95% CI)
Urinary metabolites (Mg / 24 hours)			
THF	1043 (691-1397)	1768 (1066-3269)	1.88 (1.65 to 2.15) ²
α -THF	494 (331-781)	291 (177-436)	0.55 (0.50 to 0.61) ²
THE	2500 (1588-3579)	2799 (1805-4222)	1.13 (1.04 to 1.23) ¹
α -cortol	586 (368-917)	641 (455-1140)	1.19 (1.05 to 1.34) ¹
β -cortol	545 (259-947)	849 (540-1410)	1.65 (1.45 to 1.88) ²
α -cortolone	2420 (1589-4473)	3685 (2371-6241)	1.46 (1.25 to 1.71) ²
β -cortolone	632 (424-979)	796 (574-1189)	1.29 (1.13 to 1.47) ²
F	231 (160-315)	272 (215-361)	1.23 (1.13 to 1.35) ²
E	228 (171-292)	316 (227-410)	1.36 (1.26 to 1.48) ²
Total urinary glucocorticoids	9691 (6157-12805)	13523 (8955-18269)	1.37 (1.22 to 1.52) ²
Ratios of metabolites			
11 β -HSD2 activity = F / E	0.99 (0.78-1.28)	0.88 (0.73-1.16)	0.90 (0.86 to 0.95) ²
11 β -HSD total activity = (THF + α -THF) / THE	0.61 (0.52-0.85)	0.76 (0.48-1.23)	1.27 (1.14 to 1.42) ²
Relative 5 β -reductase and 5 α -reductase activity = THF / α -THF	1.78 (1.33-2.83)	7.19 (3.64-11.74)	3.41 (3.04 to 3.83) ²
5 α -reductase activity = F / α -THF	0.45 (0.27-0.60)	0.98 (0.61-1.51)	2.24 (2.00 to 2.50) ²
5 β -reductase metabolism of F = F / (THF + α -cortol + β - cortol)	0.10 (0.07-0.14)	0.07 (0.05-0.11)	0.72 (0.65 to 0.81) ²
5 β -reductase metabolism of E = E / (THE + α -cortolone+ β -cortolone)	0.04 (0.02-0.06)	0.04 (0.03-0.06)	1.05 (0.96 to 1.15)

517

518 Paired T-Test (2-tailed) of log transformed urine values. ¹p< 0.01, ²p<0.001

519

520

521

522

523 **Table 3. Rank Correlation across the 2nd and 3rd trimesters of participant total urinary**
 524 **glucocorticoid excretion or estimated enzymatic function**

	Standardised Coefficient, β
Total urinary glucocorticoids	.387 ²
11 β -HSD2 activity = F / E	.652 ²
11 β -HSD total activity = (THF + α -THF) / THE	.352 ²
Relative 5 β -reductase and 5 α -reductase activity = THF / α -THF	.581 ²
5 α -reductase activity = F / α -THF	.438 ²
5 β -reductase metabolism of F = F / (THF + α -cortol + β -cortol)	.328 ²
5 β -reductase metabolism of E = E / (THE + α -cortolone + β -cortolone)	.608 ²

525

526 Adjusted according to the gestation of urine collection. ²p<0.001







