CLINICAL STUDY

Is there a gender difference in the associations of birthweight and adult hypothalamic–pituitary–adrenal axis activity?

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Abstract

Objective: Increased hypothalamic–pituitary–adrenal (HPA) axis activity in men of low birthweight may be an important link between early life and the adult metabolic syndrome. In animal models females are more sensitive than males to HPA axis programming, but whether gender influences susceptibility in humans is unknown.

Design: Birth cohort study.

Methods: We studied 106 women aged 67–78 years, from Hertfordshire, UK, in whom birthweight was recorded. Negative feedback sensitivity was assessed by an overnight low-dose (0.25 mg) dexamethasone suppression test, and adrenal sensitivity by a low-dose (1 μg) ACTH1-24 stimulation test. Cortisol and its metabolites were analysed in a 24 h urine collection. Data were compared with previously published identical measurements in 205 men aged 66–77 years from the same cohort.

Results: In women, plasma cortisol levels after dexamethasone were lower (P, 0.0001) and peak cortisol following ACTH1-24 were higher (P, 0.0001) than in men, suggesting a more responsive HPA axis. As in men, women with lower birthweight had enhanced plasma cortisol responses to ACTH1-24 (P = 0.05 for trend) but no difference in plasma cortisol after dexamethasone or in urinary cortisol metabolite excretion. The strength of the association in women was not different from that in men: a 1 lb decrease in birthweight was associated with an incremental rise in cortisol of 12.6 nmol/l (95% confidence interval (CI) 1.4, 23.8) in men, P = 0.03, and 14.8 nmol/l (95% CI –0.4, 29.9) in women, P = 0.05 (P = 0.82 for birthweight £ gender interaction). In a combined analysis of men and women adjusted for gender (n = 302), a 1 lb decrease in birthweight was associated with a 13.4 nmol/l (95% CI 4.5, 22.4) greater incremental rise in plasma cortisol, P = 0.003.

Conclusions: Associations between lower birthweight and increased HPA axis activity are similar in men and women, supporting the hypothesis that HPA axis activation is an important mechanism underlying programming of adult disease.

Introduction

There is now substantial evidence that small size at birth is associated with a higher prevalence of cardiovascular and metabolic disease in adult life, but the underlying mechanisms remain unknown (1). Animal experiments and supportive evidence in humans have suggested that in utero resetting of the hypothalamic–pituitary–adrenal (HPA) axis may be an important change initiating the metabolic syndrome. In animal studies, interventions both during pregnancy, including administration of synthetic glucocorticoids to the mother or maternal undernutrition, and also postnatally, such as offspring ‘handling’ or maternal deprivation, are associated with long-term changes in offspring HPA axis activity and metabolic responses (2–5). In human population studies, low birthweight is associated with elevated fasting plasma cortisol (6–10). In addition, in a group of men aged 70 years born in Hertfordshire, UK, we showed that those with low birthweight and the metabolic syndrome had increased adrenal responsiveness to synthetic adrenocorticotropic hormone (ACTH) and increased urinary glucocorticoid metabolite excretion consistent with activation of the HPA axis (11).

Data from animal studies suggest that the associations described in men between cortisol, birthweight and the metabolic syndrome may not be the same in women. In animal models there is evidence for sexually dimorphic responses to programming of the HPA axis. For example, following fetal alcohol exposure (12) or prenatal stress (13, 14), female rats are more sensitive than males to activation of the HPA axis. Moreover, fetal growth restriction in guinea pig appears to impair glucose tolerance and insulin sensitivity through activation of the HPA axis in females but not
males (15). In humans, most of the published cross-sectional studies relating cortisol levels to cardiovascular risk factors have been performed in men, although an association between the simple measurement of fasting plasma cortisol with birthweight and the metabolic syndrome has been found in both men and women (7, 8). It is known that urinary cortisol metabolite excretion differs between men and women (16), and this may reflect different metabolic clearance rates of cortisol. In one small study, women who were born prematurely exhibited differences in cortisol metabolite excretion rate which were not apparent in men (17). Moreover, characteristics of HPA axis control differ, with women exhibiting lower cortisol responses to stress (18–20), and in a recent study there was no correlation between birthsize and the adrenal response to synthetic ACTH (21). Thus, it is possible that central feedback sensitivity and the responses to stimulation of the axis may differ between sexes.

We aimed to test whether there were differences in the associations between birthweight and dynamic regulation of the HPA axis in women and men.

**Subjects and methods**

**Subject selection**

We have previously studied a cohort of 297 women born between 1920 and 1930 in east Hertfordshire, UK, for whom birthweights were recorded by midwives. In 1994, blood pressure was measured, and 75 g oral glucose tolerance tests were performed (22). In 1998 we approached the surviving 154 women, of whom 106 met the inclusion criteria and agreed to take part in the present study. Baseline characteristics of the participants including birthweight did not differ from those unable to take part. Subjects with clinical evidence of pituitary or adrenal disease and those who had received oral, topical or inhaled glucocorticoids in the previous 3 months were excluded, and none of the subjects were receiving hormone-replacement therapy. Ethical approval and written informed consent were obtained.

**Clinical protocol**

The clinical protocol was identical to that used in a previous study of 205 men from the same cohort (11). Briefly, at a preliminary interview, information about medical and social history, family history of diabetes and hypertension, smoking habits, alcohol consumption and current medication was recorded, and subjects completed a ten-item ‘Geriatric Depression Screening Scale’ questionnaire to assess mood (23). On another occasion, subjects ingested 0.25 mg dexamethasone at 2200 h and fasted overnight. The following morning they attended a local clinic at 0830 h, a 21 gauge butterfly cannula was inserted in an antecubital fossa vein, and, after 30 min rest, a baseline blood sample was obtained before 1.0 μg of freshly diluted ACTH1–24 (tetracosactrin, Synacthen: Alliance, Chippenham, Wilts, UK) was injected as a bolus with a 10 ml saline flush. Venous blood was sampled through the cannula at 20, 30, 40 and 60 min after ACTH1–24 administration. Samples were centrifuged immediately and plasma was stored at −80°C. Height and weight were recorded, and waist and hip circumferences were measured with a steel tape measure at the level of the umbilicus and greater trochanter respectively. Finally subjects collected a 24 h urine sample at least 1 week before or 1 week after the dexamethasone/ACTH1–24 test.

**Laboratory methods**

Measurements of glucose, triglycerides and insulin concentrations have been reported previously (22). Plasma cortisol was measured by RIA using Guildhay antisera as previously described (11). The intra-assay coefficient of variation was 5.6–8.2% and inter-assay coefficient of variation 7.4–10.3%. Cortisol, cortisone and their metabolites were measured in urine by gas chromatography/electron impact mass spectrometry (24). Total cortisol metabolite excretion was calculated as tetrahydrocortisols + tetrahydrocortisone.

**Statistical analysis**

To obtain normally distributed variables, measurements of glucose, triglycerides, post-dexamethasone plasma cortisol, urinary cortisol metabolites, peak cortisol following ACTH1–24 and cortisol area under the curve (AUC) calculated by the trapezoidal rule, were loge-transformed. Geometric means and standard deviations are therefore presented for these variables. Associations between continuously distributed variables were assessed by the Pearson correlation coefficient, and associations between continuous and categorical variables by the Mann–Whitney U-test or the two-sample t-test as appropriate. Multiple linear regression was then used to explore the relationship between continuously distributed variables with adjustment for confounding factors. All analyses used birthweight as a continuous variable but categories of birthweight are used in presentation (see Table 3). All statistical analysis was carried out using STATA, Release 7 (Statacorp 2001, Stata Statistical Software Release 7; Stata Corporation, College Station, TX, USA).

**Results**

**Subject characteristics and potential confounders**

Table 1 shows the characteristics of the women compared with our published findings in the comparator cohort of men (11). The two cohorts were of similar age. Women had lower waist/hip ratio but similar body mass index (BMI) to men. Blood pressure and fasting plasma glucose were lower in women. As in men,
none of the measurements of cortisol in plasma or urine correlated with age or differed in subjects receiving topical or inhaled corticosteroid therapy \((n = 17)\). In women, central obesity, as measured by waist circumference, was associated with lower peak plasma cortisol levels \((r = -0.20, P = 0.05)\) and cortisol AUC after ACTH_{1–24} \((r = -0.22, P = 0.02)\) but not with post-dexamethasone plasma cortisol or total urinary glucocorticoid metabolites. Associations with BMI were similar. There were also no associations of cortisol measurements with depression or with social class.

**Responses to dynamic testing of the HPA axis**

In women, plasma cortisol levels after dexamethasone were lower, and peak cortisol and cortisol AUC following ACTH_{1–24} were higher than in men (Table 2). These gender differences remained after adjustment for differences in men and women described above including waist/hip ratio, diastolic blood pressure and glucose tolerance status. As in men, women with lower birthweight had a greater incremental rise in plasma cortisol concentrations after ACTH_{1–24}. The inverse relationship between birthweight and adrenal ACTH_{1–24} responsiveness was not confounded by obesity. In women there were no associations between birthweight and plasma cortisol after dexamethasone or urinary cortisol metabolite excretion (Table 3).

In a combined analysis of men and women adjusted for gender \((n = 302)\), a 1 lb decrease in birthweight was associated with a 13.4 nmol/l (95% confidence interval (CI) 4.5, 22.4) greater incremental rise in plasma cortisol, \(P = 0.003\). The strength of the association in women was not different from in men: a 1 lb decrease in birthweight was associated with a greater incremental rise in cortisol of 12.6 nmol/l (95% CI 1.4, 23.8) in men, \(P = 0.03\), and 14.8 nmol/l (95% CI –0.4, 29.9) in women, \(P = 0.05\) \((P = 0.82\) for birthweight \(\times\) gender interaction). These results were not significantly altered after adjustment for potential confounding factors including waist/hip ratio, blood pressure or glucose tolerance status.

**Cortisol and the metabolic syndrome**

In the male cohort we have previously described greater peak cortisol concentrations after ACTH_{1–24} in those with the most adverse metabolic profiles (11). In women there were no associations of blood pressure, or fasting glucose, insulin, triglycerides or high density lipoprotein cholesterol concentrations with any cortisol measurement in plasma or urine (data not shown).

### Table 1
Characteristics of cohorts of women and men studied. Data are means (s.d.) or number (%).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Females ((n = 106))</th>
<th>Males(^a) ((n = 205))</th>
<th>(P) value for gender difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (lb)</td>
<td>7.6 (1.3)</td>
<td>8.0 (1.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.0 (2.6)</td>
<td>70.9 (3.1)</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.5 (3.9)</td>
<td>26.9 (3.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.80 (0.04)</td>
<td>0.93 (0.05)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>157.2 (21.9)</td>
<td>161.5 (22.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.2 (11.1)</td>
<td>89.3 (11.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td>45 (42%)</td>
<td>73 (36%)</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)(^a)</td>
<td>5.7 (1.1)</td>
<td>6.0 (1.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Impaired glucose tolerance(^c)</td>
<td>35 (33%)</td>
<td>33 (17%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Type 2 diabetes(^d)</td>
<td>2 (2%)</td>
<td>15 (8%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Depression(^e)</td>
<td>12 (11%)</td>
<td>13 (6%)</td>
<td>ns</td>
</tr>
<tr>
<td>Manual social class IIIM–V</td>
<td>58 (56%)</td>
<td>134 (66%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Non-manual social class I–IIIN</td>
<td>46 (44%)</td>
<td>69 (34%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\(^a\) Geometric mean and s.d.

\(^b\) Data as previously presented (11).

\(^c\) 2 h glucose 7.8–11.0 mmol/l.

\(^d\) 2 h glucose \(\geq\) 11.1 mmol/l.

\(^e\) Current or previous history of depression.

### Table 2
Plasma cortisol levels in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males(^a)</th>
<th>(P) value for gender difference(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-dexamethasone cortisol (nmol/l)(^c)</td>
<td>106</td>
<td>117.2 (2.4)</td>
<td>203 178.6 (1.7) (&lt;0.0001)</td>
</tr>
<tr>
<td>Peak cortisol post ACTH (nmol/l)(^c)</td>
<td>103</td>
<td>612.0 (1.2)</td>
<td>199 431.3 (1.2) (&lt;0.0001)</td>
</tr>
<tr>
<td>Cortisol increment (nmol/l)</td>
<td>103</td>
<td>460.9 (99.4)</td>
<td>199 238.5 (93.7) (&lt;0.0001)</td>
</tr>
<tr>
<td>Cortisol area under curve post-ACTH (nmol/l per min)</td>
<td>101</td>
<td>29 305.0 (5965.6)</td>
<td>197 21 290.9 (4226.9) (&lt;0.0001)</td>
</tr>
</tbody>
</table>

\(^a\) Data as previously presented (11).

\(^b\) Tests for gender difference based on two-sample \(t\)-tests.

\(^c\) Geometric mean (s.d.).
Women with impaired glucose tolerance or type 2 diabetes mellitus (n = 37) tended to have higher post-dexamethasone plasma cortisol levels (142.0, S.D. 2.3, vs 105.7, S.D. 2.4 nmol/l, P = 0.07, after adjustment for BMI).

Discussion

We have shown that dynamic changes in the HPA axis do relate to birthweight in women as well as in men, supporting the hypothesis that activation of the HPA axis may be an important mechanism underlying the fetal programming of adult disease. In this respect, the associations observed in humans differ from the results of early life interventions in a number of animal models in which the female HPA axis is more sensitive to programming (12–15). We cannot tell whether this represents a species difference or indicates that gender-specific mechanisms which operate in animals are not the same in humans, and therefore cannot be invoked in explaining epidemiological associations of low birthweight.

As in men, we found no associations of cortisol following dexamethasone suppression and birthweight in women, even after adjustment for body size. In our previous study, there were still no associations after adjustment for dexamethasone levels or corticosteroid-binding globulin (11). We have previously suggested this may be due to poor access of low doses of dexamethasone to the brain (25). Another reason could be the confounding effect of gestational age, which is not known in this cohort, as alterations in central negative feedback using low-dose dexamethasone have been reported depending on gestational age (21). In women we also did not find associations of birthweight with total cortisol metabolite excretion. However, in men this was a complex and rather weak parabolic relationship, confounded by obesity. It may be that the less striking effect of obesity on total cortisol metabolite excretion in women leaves it more obvious that basal cortisol secretion rate is not increased in those of low birthweight. In studies of the HPA axis aimed to minimise stress, for example 24 h plasma sampling (26) or salivary cortisol measurements (27), no relationship is seen between birthweight and cortisol measurements. This emphasises that it is reactivity rather than basal activity of the HPA axis which is related to birthweight.

There are several studies reporting associations between elevated cortisol levels and glucose intolerance, hypertension and hyperlipidaemia (6–11, 21, 28–33). The lack of association between dynamic measurements of cortisol and metabolic variables in women in this study is most likely because of limited power. The previously reported associations between fasting cortisol and metabolic variables in women from a larger cohort (7) were weakened in the smaller number of women in the present study (data not shown).

In summary, these data support the concept of programming of the control of cortisol secretion by events in early life in humans, but do not suggest that gender-specific mechanisms which operate in other species are so important in humans.

Acknowledgements

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