Epidemiological studies have shown that low birthweight, a marker of an adverse intrauterine environment, is associated with development of hypertension in adulthood.1 One hypothesis to explain this phenomenon is excessive activity of glucocorticoids.2 Treatment of pregnant rats with dexamethasone reduces birthweight and leads to permanent hypertension and increased hypothalamic-pituitary-adrenal (HPA) axis activity in the offspring.2–4 Similarly, in men and women of low birthweight and with cardiovascular risk factors, including hypertension, we observed increased fasting cortisol, increased excretion of urinary glucocorticoid metabolites, and increased cortisol responses to ACTH stimulation.5–8 Thus, it has been proposed that elevated glucocorticoid activity resulting from activation of the HPA axis may explain the link between low birthweight and high blood pressure.

Chronic activation of the HPA axis may also increase mineralocorticoid activity. This mechanism has been invoked, for example, to explain higher aldosterone levels in subjects with polymorphisms in \( CYP11B1 \) and \( CYP11B2 \) that associate with lower 11\( \beta \)-hydroxylase activity, reduced cortisol secretion, and compensatory HPA axis activation.9 There is substantial evidence implicating mineralocorticoids in the pathogenesis of hypertension. For example, data from the Framingham Offspring Study showed that higher aldosterone levels within the normal range predict development of hypertension.10 In addition, recent studies showing that up to 15% of unselected patients with hypertension have a raised aldosterone/renin ratio.11–14 Although these reports have led to wide debate about the true meaning of relative aldosterone excess in hypertensive patients,15 overall, they suggest that altered regulation of aldosterone synthesis or release may be relatively frequent in essential hypertension.

In animal models of intrauterine growth retardation, there are also alterations in activity of the renin-angiotensin-aldosterone system (RAS) that may underlie hypertension. For example, prenatal exposure of rats to glucocorticoids results in elevated hepatic angiotensinogen mRNA expression and increased plasma angiotensinogen and renin activity in association with hypertension in adulthood.16 Further, in a rodent model of placental insufficiency, the subsequent hypertension

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was associated with increases in renal angiotensin-converting enzyme activity and renal renin and angiotensinogen mRNA in adult offspring.17 Whether aldosterone is altered in humans of low birthweight has not been explored in detail.

Against this background, we aimed to investigate whether aldosterone levels and their responsiveness to ACTH are associated with HPA axis activity, hypertension, and low birthweight in a cohort of elderly men and women, and whether any such associations are explained by CYP11B2 genotype.

Methods

Participants and Protocol

We previously reported studies on a cohort of 370 men and 297 women, born between 1920 and 1930, born and still residing in East Hertfordshire, for whom birthweights were recorded by midwives. Seventy-five-gram oral glucose tolerance tests were performed, fasting cortisol was measured, and measurements of blood pressure recorded.5,18,19 In 1997 and 1998, we approached the surviving 245 men and 154 women and invited them to take part in a cross-sectional study examining the relationship between the early environment and the HPA axis.7,8 Subjects with clinical evidence of pituitary or adrenal disease and those who had received oral glucocorticoids in the previous 3 months were excluded. A total of 205 men and 106 women met the inclusion criteria and were willing to take part. Baseline characteristics of participants, including birthweight, did not differ from those unable to take part. Ethical approval was obtained from the East and North Hertfordshire Health Authority local research ethics committee, and written informed consent was obtained.

The study protocol has been described in detail previously.7,8 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. On another occasion, subjects took 0.25 mg dexamethasone at 2230 hours and attended a local clinic, a 21-gauge butterfly cannula was inserted in an antecubital fossa vein, and, after 30 minutes of rest, a baseline blood sample was obtained before 0.1 μg of freshly diluted ACTH1–24 (tetracosactrin, Synacthen; Alliance, Chippenham, 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 minutes 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-hour urine sample at least a week before or a week after the dexamethasone/ACTH1–24 test. Dexamethasone (0.25 mg) and ACTH1–24 (1 μg) doses were selected to approximate the ED50 (ie, to provide ∼50% of maximal suppression or stimulation, respectively). More conventional doses (eg, 1 mg dexamethasone or 250 μg ACTH1–24)20,21 used in clinical practice are designed to induce maximal effects in all of these otherwise healthy participants and would not detect subtle alterations in the control of corticosteroid secretion.

Laboratory Assays

We measured aldosterone in stored samples by radioimmunoassay using the Coat-A-Count system (Euro/DPC Ltd; Caernarfon, Wales) in the samples collected after dexamethasone, and 30 and 60 minutes after ACTH stimulation, for which there was sufficient plasma (185 men and 98 women). The intra-assay and interassay coefficients of variation were <5% and 10%, respectively.

For the 232-bp fragment containing the -344 polymorphism in CYP11B2, a 25-ng template DNA was amplified in a 25-μL reaction volume using 1.25 U Thermos-Start DNA Polymerase (ABGene; Surrey, UK) according to manufacturer protocol. Oligonucleotide primers SF1F and SF1R were added at a final concentration of 10 μmol/L (sequences: SF1F 5′-GTGTCAAGGGCAGGGTGTA-3′ and SF1R 5′-AGGCCTGGGTCTGGACT-3′). Reactions were cycled at 15 minutes at 94°C, 30 s at 94°C, 30 s at 68°C, 1 minute at 72°C for 30 cycles, and then incubated at 72°C for 7 minutes. Polymerase chain reaction products were cleaned up using AMPure (Agencourt) according to manufacturer protocol. A total of 10 μL of polymerase chain reaction product was transferred to a new polymerase chain reaction plate. Sequencing reactions were performed using the BigDye Terminator v3.1 (Applied Biosystems), also according to manufacturer protocol and the corresponding sequencing primers. The -344 products were sequenced with SF1F at a concentration of 3.2 μmol/L and cycled at 45 s at 96°C, 25 s at 50°C, and 4 minutes at 60°C for 25 cycles. Sequencing products were cleaned using CleanSEQ (Agencourt) according to manufacturer protocol. DNA sequences were analyzed using a 3730 DNA Analyzer (Applied Biosystems) and visualized using SeqScape version 2.1.1.

Statistical Analysis

Aldosterone measurements were skewed and were normalized by log transformation for analysis. Aldosterone levels were divided into tertiles for presentation but analyzed as a continuous variable. Associations between continuously distributed variables were assessed by the Pearson correlation coefficient, and associations between continuous and categorical variables were assessed by the 2-sample t test or the Mann–Whitney U test as appropriate. Multiple linear regression was then used to explore the relationship between continuously distributed response variables and possible explanatory variables, with adjustment for confounding factors. Multiple logistic regression was used to analyze binary response variables. To account for multiple measurements of aldosterone, an ANOVA for repeated measurements was also performed to test the relationship of aldosterone with birthweight. All statistical analysis was performed using Statistica Release 6.

Results

Associations of Aldosterone With Cortisol

The Table shows the characteristics of the participants. In women, aldosterone levels were lower after dexamethasone, and higher after ACTH stimulation than in men (P<0.0001), findings that were similar to the cortisol responses we reported previously. Aldosterone and cortisol levels after dexamethasone were correlated (men r = −0.33, P<0.001; women r = −0.29, P = 0.003), with similar associations for ACTH-stimulated values. Aldosterone levels after both dexamethasone and ACTH stimulation were higher in subjects taking antihypertensive therapy (P<0.0001) but were not associated with age, obesity, or any other potential confounding factor. There were no associations of aldosterone levels with urinary glucocorticoid metabolites.

Associations of Aldosterone With Birthweight

Aldosterone levels were associated inversely with birthweight after both dexamethasone (r = −0.16; P = 0.008) and ACTH stimulation (r = −0.21; P = 0.001 at T = 30 minutes and r = −0.21, P = 0.001 at T = 60 minutes; Figure 1). The findings were significant in both men and women and remained after adjustment for age and obesity. The effect of birthweight remained significant using repeated-measures ANOVA (P = 0.01). There were similar inverse associations between aldosterone and weight at 1 year.

Associations of Aldosterone With Blood Pressure

Figure 2 shows that there were significant correlations between systolic blood pressure and aldosterone after dexam-
Table. Characteristics of Participants and Plasma Steroid Concentrations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men</th>
<th>Women</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>70.9 (3.1)</td>
<td>71.0 (2.5)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8 (3.5)</td>
<td>27.4 (3.9)</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93 (0.05)</td>
<td>0.80 (0.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birthweight, oz</td>
<td>127.6 (15.6)</td>
<td>122.5 (20.1)</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Weight at 1 year, oz</td>
<td>364.1 (39.7)</td>
<td>334.7 (40.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP mm Hg</td>
<td>161 (21.2)</td>
<td>157 (21.7)</td>
<td>P=0.10</td>
</tr>
<tr>
<td>Diastolic BP mm Hg</td>
<td>89 (10.9)</td>
<td>83 (11.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No. on antihypertensive therapy, %</td>
<td>64 (35%)</td>
<td>44 (45%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol after dex, nmol/L</td>
<td>207 (94)</td>
<td>164 (114)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol 30 min after ACTH, nmol/L</td>
<td>411 (89)</td>
<td>572 (117)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol 60 min after ACTH, nmol/L</td>
<td>366 (80)</td>
<td>540 (133)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aldo after dex ng/dL</td>
<td>8.4 (6.0)</td>
<td>6.2 (5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aldo 30 min after ACTH</td>
<td>20.2 (9.6)</td>
<td>22.9 (13.4)</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Aldo 60 min after ACTH</td>
<td>11.3 (7.6)</td>
<td>14.0 (9.0)</td>
<td>P=0.003</td>
</tr>
</tbody>
</table>

Values are mean (SD).
BP indicates blood pressure; BMI, body mass index; WHR, waist/hip ratio; dex, dexamethasone; Aldo, aldosterone.

methasone (r=0.20; P=0.001) and after ACTH stimulation (r=0.33; P<0.0001). The associations were stronger in men than in women and remained after adjustment for potential confounding factors and after excluding subjects taking antihypertensive therapy. There were similar significant correlations between aldosterone levels after dexamethasone with higher diastolic blood pressure (r=0.21; P<0.0001).

**Aldosterone levels were inversely correlated with birthweight after both dexamethasone (r=−0.16; P=0.008) and ACTH stimulation (r=−0.21, P=0.001 at T=30 minutes; r=−0.21, P=0.001 at T=60 minutes) and in repeated-measures ANOVA (P=0.01).**

![Figure 1](http://hyper.ahajournals.org/)

Figure 1. Aldosterone levels after dexamethasone and ACTH stimulation according to birthweight. T=0 minutes: aldosterone levels after dexamethasone (0.25 mg); T=30 minutes and T=60 minutes: aldosterone levels after stimulation with 1 µg ACTH. **Aldosterone levels were inversely correlated with birthweight after both dexamethasone (r=−0.16; P=0.008) and ACTH stimulation (r=−0.21, P=0.001 at T=30 minutes; r=−0.21, P=0.001 at T=60 minutes) and in repeated-measures ANOVA (P=0.01).**

![Figure 2](http://hyper.ahajournals.org/)

Figure 2. Systolic blood pressure (BP) according to tertiles of aldosterone levels. Data were analyzed using aldosterone and systolic blood pressure as continuous variables but grouped according to tertiles for presentation. Correlations between systolic blood pressure and aldosterone levels in all subjects after dexamethasone (black solid bars; r=0.20; P=0.001), those taking no antihypertensive therapy (striped bars; r=0.20; P=0.005), and all subjects after ACTH (dotted bars; r=0.33; P<0.0001).

**CYP11B2 Polymorphisms**

A total of 278 subjects had sufficient and suitable samples for genotyping at the -344 locus of CYP11B2. The relative allele frequencies were T 0.53 and C 0.47. There were 31 CC, 96 TT, and 151 TC subjects. Genotypes were in Hardy–Weinberg equilibrium. There was no difference in birthweight according to genotype. Systolic blood pressure was higher in those with the T allele (CC mean 154±SE 20.1, TC 163±23.1, TT 158±19.8 mm Hg; P=0.04), but diastolic blood pressure and aldosterone levels did not vary according to genotype. In multiple regression analysis, adjustment for genotype removed the association between birthweight and postdexamethasone aldosterone levels (β=−0.10; P=0.11), but the relationships between birthweight and aldosterone levels after ACTH stimulation remained (aldosterone at T=30 minutes, β=−0.17; P=0.006; aldosterone at T=60 minutes, β=−0.18, P=0.004).

**Discussion**

Over the past decade, an expanded role for aldosterone in the pathogenesis of hypertension and cardiovascular disease has emerged. Increasing use of the aldosterone/renin ratio has led to the suggestion that a greater proportion of patients with hypertension than described previously have altered regulation of aldosterone, although in the largest study examining aldosterone levels within the normal range, aldosterone levels at baseline did not predict baseline blood pressure. The current report describes aldosterone measurements in a cross-sectional study of men and women in whom the prevalence of hypertension and its antecedents, including obesity and birthweight, have been carefully characterized. The principal findings are that men and women with low birthweight and higher blood pressure have increased aldosterone levels measured after both dexamethasone and ACTH stimulation. Moreover, aldosterone levels after suppression or stimulation were associated closely with cortisol levels, suggesting shared regulation by the HPA axis. These findings are consistent with existing evidence that aldosterone is an
important determinant of blood pressure and a potential target for early life programming.

The explanation for higher aldosterone levels in men and women with low birthweight and high blood pressure is not known but is likely to relate to variations in the RAS or HPA axis. There are limited studies in animals suggesting the RAS is activated in association with low birthweight. However, in humans, the data are limited to a few studies examining the RAS in preterm infants or in early infant life, reporting increased angiotensin II levels in small-for-gestational-age children in the neonatal period and increased angiotensin-converting enzyme activity at 3 months of age. Whether this is related to subsequent hypertension has not been explored, although a recent small study suggested RAS activation in association with low birthweight and hypertension in boys born small for gestational age. There is also some evidence of associations between low birthweight and polymorphisms in components of the RAS, but these have not yet been linked with current or later blood pressure measurements.

In previous studies of the current cohort, we proposed that activation of the HPA axis explained the link between low birthweight and hypertension. This could be attributable to increased central drive to the axis from higher centers or to increased cortisol clearance, with subsequent activation of the axis, as is observed in obesity. It has also been hypothesized that in patients with hypertension, impaired 11β-hydroxylation as a consequence of single-nucleotide polymorphisms in CYP11B1 and CYP11B2 leads to a subtle activation of the HPA axis. These single-nucleotide polymorphisms are in close linkage disequilibrium, and a representative single-nucleotide polymorphism (CYP11B2 -344 C/T) was assessed in our subjects. We found there was no relationship between CYP11B2 genotype and birthweight, and that adjustment for genotype in regression analysis did not remove the association between low birthweight and aldosterone measurements, but the numbers were small. The hypothesis that there is a genetically determined lifelong increase in activation of the HPA axis is supported by a study showing higher ACTH levels after dexamethasone administration in subjects with the genotype associated with high blood pressure and relative aldosterone excess. The present data, which show a close correlation between aldosterone and cortisol, further support the notion of activation of the HPA axis in association with hypertension and suggest that this also translates into an increase in aldosterone levels.

The idea that ACTH is an important chronic regulator of aldosterone is somewhat controversial. Administration of pharmacological doses of ACTH causes only a short-term increase in aldosterone secretion, followed by suppression of its release. However, this is not necessarily relevant to chronic endocrine physiology, and several observations suggest that more modest changes of ACTH within the physiological range are capable of influencing chronic variation in aldosterone levels. First, patients with Cushing’s disease who have moderately raised ACTH values do not show suppression of aldosterone, indicating that, in this circumstance, there is no inhibition of responsiveness. Second, in normal subjects, there is a recognized diurnal variation in aldosterone that mirrors that of cortisol, consistent with an effect of ACTH on synthesis of the mineralocorticoid. We have also shown positive correlations between aldosterone and glucocorticoids (and androgens) in a large population-based study, which we suggest is a consequence of shared regulation, and have shown that this might be related to genetic variation at the CYP11B1/B2 locus. Finally, aldosterone regulation is typically maintained as a result of input from multiple tropins, and we proposed previously that the effect of ACTH is to modulate the aldosterone responsiveness to other factors such as angiotensin II and potassium.

Regardless of possible mechanism, it is of considerable interest that there was a significant relationship between aldosterone and systolic and diastolic blood pressure. This adds to the previous data from the Framingham Offspring Study that showed that aldosterone levels were not correlated with prevalent blood pressure, but predicted the rise in blood pressure over a 5-year period and development of hypertension. Our findings suggest that circulating aldosterone may have an important role in determining the level of blood pressure in older subjects; there was an approximate 10 mm Hg difference in systolic pressure when subjects in the lowest and highest tertile of aldosterone were compared. This finding complements data that show that between 10% and 15% of hypertensive patients have a raised aldosterone/renin ratio, consistent with relative and inappropriate aldosterone secretion, and supports the concept that aldosterone is an important therapeutic target in treating high blood pressure.

Several limitations of this study should be acknowledged. Serum potassium levels were not available, and appropriate samples were not obtained to measure plasma renin activity or urinary sodium. Likewise, we did not have any measurements of aldosterone without previous administration of dexamethasone or ACTH. Although it is possible that some participants had undiagnosed mild primary hyperaldosteronism, we did not observe very high aldosterone levels, and the relationship between aldosterone and blood pressure was continuous across the range of aldosterone measurements.

Perspectives

Our findings suggest a common component in the regulation of aldosterone and cortisol. The results support existing evidence that aldosterone is an important determinant of blood pressure and suggest that control of aldosterone secretion may be determined with that of cortisol in early life. This highlights the importance of aldosterone as a therapeutic target in treating high blood pressure.

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Programming of Hypertension: Associations of Plasma Aldosterone in Adult Men and Women With Birthweight, Cortisol, and Blood Pressure
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