Increased Glucocorticoid Activity in Men With Cardiovascular Risk Factors


Abstract—The association between hypertension and insulin resistance might be explained by increased activity of the principal glucocorticoid, cortisol. Recent data show that the intensity of dermal vasoconstriction after topical application of glucocorticoids is increased in patients with essential hypertension. In this report, we examine whether increased glucocorticoid sensitivity or secretion is associated with insulin resistance and is a cause or consequence of hypertension. We studied 32 men (aged 47 to 56 years) from a cross-sectional study and 105 men (aged 23 to 33 years) in whom predisposition to high blood pressure has been defined by their own blood pressure and the blood pressures of their parents. In both populations, increased dermal glucocorticoid sensitivity was associated with relative hypertension, insulin resistance, and hyperglycemia. In young men with higher blood pressure whose parents also had high blood pressure, enhanced glucocorticoid sensitivity was accompanied by enhanced secretion of cortisol, enhanced ligand-binding affinities for dexamethasone in leukocytes, and impaired conversion of cortisol to inactive metabolites (cortisone and 5β-dihydrocortisol). Increased tissue sensitivity to cortisol, amplified by enhanced secretion of cortisol, is a feature of the familial predisposition to high blood pressure rather than a secondary effect of high blood pressure. It may be mediated by an abnormal glucocorticoid receptor, and it may contribute to the association between hypertension and insulin resistance. (Hypertension. 1998;31:891-895.)

Key Words: receptors, corticosteroid ■ adrenal cortex hormones ■ blood pressure ■ insulin ■ blood vessels

Many patients with atheromatous disease have identifiable risk factors, including hypertension, insulin resistance, glucose intolerance, and central obesity. These risk factors often occur together, but the reason for their association is not clear. The same list of abnormalities can be reproduced in Cushing’s syndrome, caused by excessive activity of glucocorticoid hormones such as cortisol. In health, the activity of cortisol is determined by a combination of factors, including secretion rate from the adrenal, metabolic clearance rate, and tissue sensitivity. Recent studies suggest that these factors may be abnormal in patients with essential hypertension, in whom impaired metabolism of cortisol is accompanied by increased sensitivity to glucocorticoids in the skin. Similarly, in inbred rats with hypertension, glucocorticoid metabolism is impaired and sensitivity of mesenteric vessels to dexamethasone is enhanced. In addition, in rats and humans, polymorphisms of the glucocorticoid receptor gene have been linked with high blood pressure and insulin resistance.

The aims of the present studies were to establish whether abnormalities of glucocorticoid secretion, metabolism, or tissue sensitivity are (1) associated with hypertension, insulin resistance, glucose intolerance, and obesity and (2) likely to be a cause or consequence of hypertension. These aims were addressed in two distinct populations of men: one study was of conventional cross-sectional design; the second study was in a cohort of young men in whom predisposition to high blood pressure has been characterized using the novel “four-corners” approach.

Methods

These studies were approved by local Ethics of Medical Research Committees, and written informed consent was obtained from all participants.

Study 1: Cross-sectional Study Sample

We approached 53 men in Preston, Lancashire, who have participated in previous investigations of the relationship between measurements at birth and carbohydrate metabolism in adulthood and who were selected in equal numbers from each of the quartiles of glucose tolerance by World Health Organization criteria. None was receiving relevant medication.
Variable Parental BP: they inherited this trait from their parents (ie, OH/PH pressure may be greater in offspring with high blood pressure only if disposition to high blood pressure, ie, OH/PH (evident when comparing subjects with maximum contrast in pre-

abnormalities that precede the development of clinical hypertension

Study 2: “Four-Corners” Study Sample

The sampling method has been described elsewhere. Blood pressure was measured in 603 married couples in 1979 and in 864 of their offspring (then aged 16 to 24 years) in 1986. Age-adjusted Z scores were used to define tertiles for both offspring and mean parental blood pressure. Offspring for whom both their own blood pressure and the mean blood pressure of their parents were in the highest or lowest tertiles were identified as belonging to one of the “four corners”: OL/PL (offspring blood pressure “low,” parental blood pressure “high”), OH/PL (offspring blood pressure “high,” parental blood pressure “low”), OL/PH (offspring blood pressure “low,” parental blood pressure “high”), or OH/PH (offspring blood pressure “high,” parental blood pressure “high”). Subgroups of offspring randomly selected from these corners have participated in previous investigations.

Comparison of subjects from the four corners may elucidate abnormalities that precede the development of clinical hypertension (evident when comparing subjects with maximum contrast in pre-disposition to high blood pressure, ie, OH/PH>OL/PL) and may establish whether these abnormalities are likely to be a cause or consequence of higher blood pressure. A variable that is elevated as “high,” parental blood pressure “high”). Subgroups of offspring (then aged 16 to 24 years) in 1986. Age-adjusted Z scores was measured in 603 married couples in 1979 and in 864 of their children. The mean blood pressure of the age-adjusted distribution

Selected Abbreviations and Acronyms

HOMA = homeostasis model of assessment
OH = offspring with blood pressure in the highest tertile of the age-adjusted distribution
OL = offspring with blood pressure in the lowest tertile of the age-adjusted distribution
PH = offspring of parents with mean blood pressure in the highest tertile of the age-adjusted distribution
PL = offspring of parents with mean blood pressure in the lowest tertile of the age-adjusted distribution
THE = tetrahydrocortisone
THF = tetrahydrocortisol

We studied 105 male offspring drawn at random from the four corners (Table). No subject was taking relevant medication. Each provided a 24-hour urine sample and a 30-mL blood sample obtained at 9:30 AM after an overnight fast and 30 minutes in a supine position. Insulin sensitivity was assessed by the relationship between fasting plasma insulin and glucose using the HOMA. Blood pressure was recorded using a Hawksley random-zero sphygmomanometer (Hawksley & Sons). Dermal glucocorticoid sensitivity was measured as described below. Sixty-eight subjects agreed to return for an additional blood sample for studies of glucocorticoid receptor binding in leukocytes. This sample was obtained at any convenient time of day (from 8:30 AM to 6 PM) and without fasting. There was no systematic diurnal variation in results obtained, and studies of subjects from each of the four corners were distributed at random throughout the day. The characteristics of subjects who provided leukocytes were not different from those of the other subjects (Table).

Dermal Vasoconstrictor Sensitivity to Glucocorticoids

Dermal glucocorticoid sensitivity was measured using the classic skin vasoconstrictor bioassay, as previously described. In brief, 50 μL of beclomethasone dipropionate (Sigma Chemical Co; at 0, 1, 5, 10, 100, or 1000 μg/mL in 95% ethanol) was applied in random order to circles of 14 mm diameter on the volar surface of the nondominant forearm. After the surface had been covered with polyethylene for 16 to 18 hours, the intensity of blanching was assessed on a visual scale from 0 to 3 by an observer who was blind to the order of application and to all other data. The response in each subject is expressed as the area under the dose-response curve (ie, the sum of scores for all six circles; maximum, 18 U). This technique has been validated against objective measurement of skin blanching by reflectance spectrophotometry. The intersubject and intrasubject coefficients of variation are 31% and 18%, respectively.

Laboratory Measurements

We measured plasma insulin using a microparticle enzyme immunoassay on an Abbott Laboratories IMX analyzer. Cortisol and its metabolites were measured by electron impact gas chromatography/mass spectrometry, using epi-tetrahydrocortisol as the internal standard. Glucocorticoid receptor binding was performed in freshly isolated peripheral blood leukocytes at 24°C as previously described.

Statistics

Results are mean±SEM. In both studies, correlations were identified by linear regression analysis, for which concentrations of glucose

Characteristics of Subjects From Study 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Offspring BP:</th>
<th>Parental BP:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n=30)</td>
<td>High (n=25)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>29±0.5 (29±0.6)</td>
<td>29±0.5 (29±0.6)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.1±0.6 (25.7±0.6)</td>
<td>24.4±0.5 (24.4±0.6)</td>
</tr>
<tr>
<td>Alcohol intake, U/wk</td>
<td>13±2</td>
<td>13±2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg*</td>
<td>111±1 (112±1)</td>
<td>118±1 (118±2)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg*</td>
<td>72±1 (72±1)</td>
<td>72±1 (72±1)</td>
</tr>
<tr>
<td>Urine sodium excretion, mmol/d</td>
<td>215±20 (203±25)</td>
<td>207±14 (211±16)</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.6±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>HOMA insulin resistance index, arbitrary units</td>
<td>1.1±0.1</td>
<td>1.5±0.3</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Numbers in parentheses are for the subgroup in whom dexamethasone binding was studied in leukocytes. *P<0.001 by ANOVA.
were normalized by logarithmic transformation; cortisol production rate was estimated by the sum of urinary cortols + cortolones + 5β-THF + 5α-THF + THE:\(^{-24}\). 11β-dehydrogenase activity was estimated by \((5β-THF + 5α-THF)/THE\) ratio; and relative 5α/5β-reductase activities were estimated by \(5β-THF/5α-THF\) ratio.\(^{-3}\)

Comparisons among the four corners in study 2 were made by factorial ANOVA, followed by comparison of pairs of corners by Fisher’s probability of least-squares difference test when values for ANOVA were \(P<.05\). To avoid multiple testing of interdependent variables, ANOVA was restricted to blood pressures, sodium excretion, the five variables in Fig 2, and \(B_{max}\) for dexamethasone in leukocytes (Table).

### Results

#### Tissue Sensitivity to Glucocorticoids

In both groups of men, increased dermal sensitivity to beclomethasone dipropionate was associated with (1) insulin resistance, measured in study 1 by the rate of decline in arterialized plasma glucose during a short insulin tolerance test (\(r = -.43, P < .02\)) (Fig 1) and in study 2 by HOMA insulin resistance index (\(r = .20, P < .05\)), and (2) relative hyperglycemia, measured in study 1 by plasma glucose 120 minutes after 75 g oral glucose (\(r = .54, P < .001\)) and in study 2 by fasting plasma glucose (\(r = .19, P < .05\)). In study 1, increased dermal glucocorticoid sensitivity was associated with higher systolic blood pressure (\(r = .34, P < .05\)). In study 2, increased dermal glucocorticoid sensitivity was associated with high blood pressure in offspring whose parents had high blood pressure (ie, OH/PH > OL/PH) but not in offspring whose parents had low blood pressure (ie, OH/PL < OL/PL) (Fig 2a). Dermal glucocorticoid sensitivity did not correlate with body mass index, waist/hip ratio, or alcohol intake.

In study 2, glucocorticoid receptor affinity for dexamethasone was increased (indicated by decreased \(B_{max}\)) in offspring with maximum predisposition to high blood pressure compared with offspring with minimum predisposition to high blood pressure (ie, OH/PH > OL/PH) (Fig 2b). The number of binding sites per leukocyte was not different (Table). However, indices of dexamethasone binding did not correlate with other cardiovascular risk factors.

#### Cortisol Secretion and Metabolism

In study 1, cortisol production rate (9.2 ± 1.0 mg/d) correlated positively with alcohol intake (\(r = .40, P < .02\)) but not with insulin sensitivity, glucose tolerance, or blood pressure. In study 2, cortisol production rate (8.3 ± 0.5 mg/d) correlated positively with body mass index (\(r = .37, P < .001\)), the HOMA index of insulin resistance (\(r = .37, P < .001\)), and fasting plasma glucose (\(r = .20, P < .05\)) but not with alcohol intake (\(r = .11\)). Cortisol metabolite ratios reflecting 11β-dehydrogenase and 5β-reductase activities did not correlate with cardiovascular risk factors in either study.

In study 2, an increased cortisol production rate in subjects with higher blood pressure was attributable in large part to an increase in 5α-THF excretion. However, elevated 5α-THF was associated with high blood pressure only in offspring whose parents had high blood pressure (ie, OH/PH > OL/PH) and not in offspring whose parents had low blood pressure (ie, OH/PL < OL/PL) (Fig 2c). 5β-THF and THE excretions were not different among the four corners. Differences in cortisol metabolite excretion were not associated with differences in sodium excretion (Table).

In multiple regression analyses to examine the relationships of age, body mass index, alcohol intake, dexamethasone sensitivity, and cortisol production rate to 120 minute plasma glucose during the oral glucose tolerance test (study 1) or fasting glucose 2 hours after a 75-g oral glucose load (\(r = .54, P < .001\)); b, rate of decline of arterialized plasma glucose (taken from a heated hand vein) in the 15 minutes following an intravenous bolus of soluble insulin (0.05 U/kg body wt) (\(r = -.43, P < .02\)).
plasma glucose (study 2), only the effect of dermal glucocorticoid sensitivity ($P<.01$, study 1; $P<.05$, study 2) was significant. With insulin sensitivity as the dependent variable, only the effects of dermal glucocorticoid sensitivity ($P<.01$; $P<.05$), cortisol production rate in study 2 ($P>.30$; $P<.02$), and body mass index ($P<.0001$; $P<.0001$) were significant. With mean arterial blood pressure as the dependent variable in study 1, only the effect of dermal glucocorticoid sensitivity approached statistical significance ($P<.10$).

**Discussion**

These data show that increased dermal vasoconstrictor response to glucocorticoids is associated with higher blood pressure, glucose intolerance, and insulin resistance in two distinct populations of healthy men from different age groups. In the face of the imprecision of measurement of glucocorticoid sensitivity and cardiovascular risk factors, these associations were remarkably strong: in middle-aged men dermal glucocorticoid sensitivity alone accounts for 30% of the variance in glucose tolerance and 18% of the variance in insulin sensitivity; in combination with the independent influence of obesity, dermal glucocorticoid sensitivity accounts for 46% of the variance in insulin sensitivity. In addition, in the younger men, enhanced cortisol production rate was associated with higher blood pressure, glucose intolerance, insulin resistance, and obesity. The combination of enhanced tissue sensitivity and enhanced secretion may allow cortisol to make an important contribution to the association between insulin resistance and hypertension.

Associations such as these cannot distinguish causes from consequences of cardiovascular risk factors. The data from the “four-corners” study show that dermal vasoconstrictor sensitivity to glucocorticoids and cortisol production rate are only increased together in young men with high blood pressure if their parents also had high blood pressure. This pattern would not be expected if these phenomena were secondary to high blood pressure per se, and it suggests that enhanced cortisol activity may be an element of the familial predisposition to high blood pressure.3 Importantly, dermal glucocorticoid sensitivity was similar in the groups with maximum (OL/PL) predisposition to high blood pressure, but the amount of endogenous cortisol available to stimulate the response was markedly different, being higher in OH/PH subjects. This emphasizes that a combination of abnormalities in cortisol secretion and sensitivity may be required to induce changes in blood pressure and insulin sensitivity.

This hypothesis requires that sensitivity to cortisol is enhanced in other tissues as well as the skin. The dermal blanching response to glucocorticoids is mediated by glucocorticoid receptors25,26 and reflects, at least in part, sensitivity to glucocorticoids in nonvascular peripheral tissues because it correlates with the bronchodilator response to prednisolone in asthmatic patients.19 Moreover, it is not influenced by other local variables including basal skin blood flow, microvascular structure and function, sympathetic tone, or skin thickness (B.R. Walker, J.P. Noon, and D.J. Webb, unpublished observations, 1997). Thus, the skin test might reasonably be expected to reflect sensitivity to glucocorticoids in other blood vessels and in liver, skeletal muscle, and/or adipose tissue where glucocorticoids raise blood pressure and antagonize the actions of insulin.27

However, if enhanced sensitivity to cortisol in peripheral tissues contributes to cardiovascular risk, then it should not be associated with enhanced negative feedback of the hypothalamic-pituitary-adrenal axis, which would result in compensatory suppression of adrenocorticotropic hormone (ACTH) and cortisol secretion. Discrepancies between peripheral and central sensitivity to glucocorticoids have been described in essential hypertension1 and steroid-resistant asthma,28 and they may be attributable to tissue-specific regulation of glucocorticoid receptor expression.29 In the present study, the lack of compensatory suppression of cortisol secretion in the face of enhanced peripheral sensitivity to cortisol could be explained by factors that increase central drive to corticotropin-releasing hormone and ACTH secretion and thereby overcome any tendency for increased negative feedback. These include psychological stress,30 depression, alcohol excess,31 and obesity,32,33 all of which increase cortisol secretion and have been proposed as independent risk factors for cardiovascular disease.34,35 In our data, indices of obesity and alcohol intake did not correlate with peripheral glucocorticoid sensitivity, but they were associated with enhanced cortisol secretion and had an additive effect with dermal glucocorticoid sensitivity on insulin resistance. In other studies, it is centripetal obesity that is associated with the greatest increase in cortisol secretion and the highest risk of cardiovascular disease.32,33,36 The contribution of central obesity and alcohol to cardiovascular risk may therefore be mediated by enhanced cortisol secretion, which induces hypertension and insulin resistance in subjects who are predisposed by an intrinsic increase in tissue sensitivity to cortisol.

Potential mechanisms for increased cortisol sensitivity include abnormalities of the glucocorticoid receptor or of the isozymes of 11$\beta$-hydroxysteroid dehydrogenase, which modulate access of cortisol to its receptors. In patients with essential hypertension, 11$\beta$-dehydrogenase and 5$\beta$-reductase enzyme activities are impaired.23 In the present studies, urinary metabolite ratios reflecting these enzyme activities were not related to other cardiovascular risk factors, although 5a-THF was disproportionately elevated in offspring with high blood pressure whose parents had high blood pressure. Also, beclomethasone dipropionate, the synthetic glucocorticoid that we used in the skin test, is not metabolized by 11$\beta$-dehydrogenase,18,20 so defective enzymatic inactivation of glucocorticoids is unlikely to account for the increased dermal vasoconstrictor response. We therefore suggest that although there may be a subtle defect in cortisol metabolism in subjects with a familial predisposition to high blood pressure, this does not contribute to the relationship between enhanced dermal glucocorticoid sensitivity and insulin resistance or hypertension. By contrast, an intrinsic polymorphism of the glucocorticoid receptor gene is associated with both insulin resistance2 and a familial predisposition to high blood pressure.7 The present data show that young men with a familial predisposition to high blood pressure have increased glucocorticoid receptor affinity in leukocytes (Table). However, relatively few of the subjects provided leukocyte samples, and we could not demonstrate a relationship between altered glucocorticoid receptor affinity and insulin resistance or hyperglycemia.
Others have shown that affinity for dexamethasone is increased in skin fibroblasts of patients with hypercholesterolemia. Further studies of the genetic and functional heterogeneity of the glucocorticoid receptor may provide important clues to the pathogenesis of hypertension and insulin resistance.

In summary, these data provide a novel description of abnormalities of glucocorticoid secretion and sensitivity in men with hypertension and insulin resistance. This provides a model for the etiology of cardiovascular risk factors that could explain their polygenic inheritance, reversible amplification by obesity, and clinical similarities to Cushing’s syndrome.

Acknowledgments

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References


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