Increased Plasma Noncortisol Glucocorticoid Activity in Open-Angle Glaucoma

George R. McCary* and Bernard Schwartz

Total biologic plasma glucocorticoid activity of normal, ocular hypertensive, and open-angle glaucoma patients was compared using a glucocorticoid receptor-based competitive binding assay. Multiple linear-regression analysis was used to adjust for the effects of significant ocular and nonocular variables, including therapy for glaucoma. The glaucoma patients had significantly greater plasma glucocorticoid activities than did normal subjects. A comparison of receptor-based assay values to values obtained with a cortisol radioimmunoassay showed that significant amounts of biologic glucocorticoid activity in the plasma of the glaucoma patients could not be explained by cortisol alone. In the normal and ocular hypertensive groups, however, virtually all of the plasma glucocorticoid activity could be accounted for by cortisol. These results suggest that in open-angle glaucoma patients, noncortisol glucocorticoids are responsible for elevating biologic plasma glucocorticoid activity. Thus, open-angle glaucoma may be associated with a disturbance of the hypothalamic-pituitary-adrenal axis that produces increased plasma levels of both cortisol and other noncortisol glucocorticoids. Invest Ophthalmol Vis Sci 32:1600-1608, 1991

The glucocorticoid cortisol has been implicated in the pathogenesis of ocular hypertension (OH) and primary open-angle glaucoma (OAG).12 Several clinical studies show that pharmacologic levels of glucocorticoids, given either topically to the eye or systemically, can raise intraocular pressure.3-9 This elevation in intraocular pressure is often accompanied by visual field loss and optic disc changes characteristic of OAG.10 Similarly, patients with Cushing's syndrome, also associated with an elevation of glucocorticoids, especially plasma cortisol, often have OAG.11-13 Compared with normal individuals (NOR), patients with OH or OAG have significantly elevated levels of total plasma cortisol1,2,14-17 and greater amounts and higher percentages of free plasma cortisol.18

Both OH and OAG may be associated with glucocorticoids other than cortisol. These glucocorticoids may elevate the biologic plasma glucocorticoid activity beyond that expected on the basis of the total plasma cortisol level. This elevated glucocorticoid activity could be present at normal or only slightly elevated total plasma cortisol levels and would go undetected with a standard cortisol radioimmunoassay (RIA). We wanted to determine whether, in comparison to NOR, patients with OH and OAG have plasma glucocorticoid activity in excess of that attributable to cortisol.

Materials and Methods

Subjects

We studied NOR, OH, and OAG populations. All subjects underwent a complete ocular examination, including measurement of intraocular pressures with the Goldmann applanation tonometer on at least two separate occasions, slit-lamp gonioscopy, tonography, determination of visual fields with the Goldman perimeter by kinetic and static means, and stereophotography of the optic disc.

The NOR consisted primarily of volunteers from senior-citizen groups who had ocular pressures less than 21 mm Hg with normal visual fields and optic discs on at least two occasions. The OH patients had normal visual fields but had ocular pressures less than 21 mm Hg with normal visual fields and optic discs on at least two occasions. The OH patients had normal visual fields but had ocular pressures determined on two or more independent occasions to be 21 mm Hg or more. The OAG patients may or may not have had changes characteristic of glaucoma. The OAG patients had ocular pressures of 21 mm Hg or
more, and visual field and optic disc changes characteristic of glaucoma.

All OAG subjects were examined with slit-lamp gonioscopy and categorized into: (1) those with increased pigmentation graded as 2+ or more in all angles, (2) those with the exfoliation syndrome, and (3) those with neither pigmentary changes in the angle nor exfoliation syndrome. Subjects in the first two categories were excluded from this study. Fifty-five of the 85 subjects had been evaluated for plasma free-cortisol levels in a previous study.18

On the day of the study, ocular pressure was measured after a blood sample was drawn. The values for maximum applanation pressure were obtained from the patient's record. Areas of cupping and pallor of the optic discs, expressed as the percent area relative to the area of the optic disc, were measured using a semi-quantitative technique from disc photographs made with a Zeiss fundus camera (Carl Zeiss, Inc., Oberkochen, Germany) and from stereophotographs taken with a Donaldson stereocamera.20,21 Low-speed color slide film (Kodachrome 25; Kodak, Rochester, NY) was used for all photographs. For each subject, blood pressure was measured in one arm on the morning of blood sampling, with the subject in a seated position, with the standard mercury sphygmomanometer technique.

Subjects receiving phenytoin sodium, barbiturates, cyproheptadine, or any form of steroid medication, including estrogens, progesterones, and antimineralocorticoids (such as spironolactone) were excluded from the study. Seventeen subjects were receiving medication for systemic vascular hypertension, of whom two were taking systemic beta-adrenergic blockers (propranolol hydrochloride). Only two subjects had a diagnosis of diabetes mellitus. All but 1 of 25 OAG patients and only 12 of 31 OH patients were receiving medication for elevated ocular pressure. Twenty subjects were receiving the beta-adrenergic blocker, timolol, as drops, 16 were receiving epinephrine drops, 11 were receiving a carbonic anhydrase inhibitor, 9 were receiving demecarium bromide drops, and 27 were receiving pilocarpine drops, either alone or in combination.

All subjects gave informed consent before participating in the study. Approval for the investigation was obtained from the Institutional Human Investigation Review Committee of the New England Medical Center, Boston.

Blood Samples

All samples of blood for analysis for both total plasma cortisol and free cortisol were taken between 9:00 AM and 11:00 AM on an outpatient visit. The samples were drawn only during or after a subject's second visit. They were centrifuged immediately at room temperature for 15 min. An aliquot of the plasma was collected to determine the total cortisol. Free cortisol was separated as described later, and then the sample and the plasma sample for total cortisol were frozen at −20°C.

Chemical Analysis

For the receptor-based competitive assay, 8-week-old male New Zealand white rabbits weighing about 1.6 kg were used as a source of ocular tissue. This source was chosen both because the rabbit eye is sensitive to the ocular hypertensive effects of glucocorticoids and because specific glucocorticoid receptors are present in this tissue.26,27 Preparations of rabbit iris–ciliary body glucocorticoid receptors were made using the method previously described by McCarty and Schwartz.27

The Glucocorticoid Receptor Competitive Binding Assay (RRA) was used for the determination of total plasma glucocorticoid activity. The method used was an adaptation of the method originally published by Ballard and co-workers, who used rat hepatoma glucocorticoid receptors to measure plasma glucocorticoid activity in both rabbits and humans.

Steroid-free plasma prepared from pooled plasma from NOR was stirred at room temperature with 50 mg/ml of Norit A charcoal (Amend Drug and Chemical Co., New York, NY) for 1 hr. The charcoal was removed by centrifugation at 25,000 × g for 15 min. The supernatant was treated with the same procedure twice more. This procedure has been used successfully by previous investigators to deplete plasma of steroids.28 A cortisol RIA on the steroid-free plasma did not detect the presence of cortisol.

Corticosteroid extracts were prepared both from standards and from plasma obtained from subjects. For standards, concentrations of cortisol of 2.5–40 ng in ethanol were dispensed in triplicate into clean tubes. The ethanol vehicle was evaporated with N2. One hundred microliters of steroid-free plasma was placed into each tube, mixed, and incubated at room temperature for 15 min. After a second mixing, 1.0 ml of methylene chloride was added to all tubes, and each sample was extracted by vortexing for 20 sec at room temperature. The aqueous phase was aspirated, and 0.5 ml of the methylene chloride extract was transferred to a clean, labeled tube. This produced standards of 1.25–20 ng. Using 3H-cortisol as a tracer showed that this method had a consistent recovery of >95%.

The analyses of samples from subjects were done without prior knowledge of patient diagnosis. Three 100-μl aliquots of the samples were extracted at the same time and in the same way as the standards. The
portion of the extract analyzed contained corticosteroids equivalent to 50 μl of plasma.

For the analysis of plasma from subjects, the extracts from standards and from the subjects were dried under N₂. Radioactive dexamethasone was then added to all tubes so that, when it was redissolved in 200 μl, the final concentration was 10 nM. The ethanol vehicle was dried with N₂, and the tubes were placed on ice. Two hundred microliters of rabbit iridociliary body glucocorticoid receptor preparation (cytosol) was then added to all samples. All samples were vortexed at 15-min intervals three times, then incubated at 0°C for 16-18 hr. After incubation, the receptor-bound and unbound ³H-dexamethasone were separated with hydroxyapatite as previously described. 27

A standard curve was developed by making a ratio of the amount of ³H-dexamethasone bound by the cytosol in the absence of a competing steroid to the amount of ³H-dexamethasone bound in the presence of increasing quantities of unlabeled cortisol and plotting it against the cortisol concentration in the standards. The glucocorticoid activity of the subjects' samples was determined by using the quantity of ³H-dexamethasone bound in the samples as the denominator in this ratio. The total receptor-determined glucocorticoid activity (RRA) in the “cortisol equivalents” of the unknown was then read by extrapolation from the standard curve.

Total plasma cortisol was measured by RIA using a method outlined by Niswender et al 29 and described previously. 18 Briefly, tenfold diluted plasma was incubated at 60°C for 30 min to inactivate all binding proteins. Triplicate aliquots of the heat-treated samples was determined by using the quantity of ³H-dexamethasone bound in the samples as the denominator in this ratio. The total receptor-determined glucocorticoid activity (RRA) in the “cortisol equivalents” of the unknown was then read by extrapolation from the standard curve.

Statistical Methods

Univariate: Univariate analysis of the data was done using two-tailed nonparametric statistical procedures 30,31 with a 0.05 level of significance. Nonparametric procedures were chosen for the univariate analysis because they require few assumptions about the underlying distribution from which the data were obtained. Chi-square tests were done to ascertain whether the diagnostic groups differed in composition with respect to sex, race, season of the year of patient visits, and frequency of occurrence of various systemic diseases, such as vascular hypertension and diabetes mellitus. A diagnosis of vascular hypertension was presumed if the subject reported use of prescription medication for vascular hypertension. Diagnostic groups were analyzed for differences in distribution in age and systemic blood pressure using the Kruskal-Wallis test.

The latter test was used also to compare the distributions of RRA, RIA, and the RRA/RIA ratio of the diagnostic groups. When only two groups were being compared, such as patients who were and were not being treated with a beta-adrenergic blocker (both ocular and systemic), the Mann-Whitney U test was used to compare RRA with RIA and the RRA/RIA ratios.

Spearman correlation coefficients were computed and used to test for statistical independence between RIA and RRA. Two-tailed Wilcoxon signed-rank tests were done in each of the three diagnostic groups to determine whether the frequency distributions of RRA differed significantly from RIA. For comparison of correlation coefficients, the Fisher’s Z transformation was used. 32

Multivariate: Multiple linear regression 33,34 was used to examine the relationship between the experimental findings and ocular and systemic therapy in the presence of other, potentially influential nondiagnostic variables. The RRA, RIA, and RRA/RIA ratios were the dependent variables of interest. We transformed the data to logarithmic values to normalize them.

Indicator variables such as meds (1) and meds (2) were created for the purpose of identifying the different categories of medication use, ie, no ocular medication or beta-blocker use [meds (1) = 0, meds (2) = 0], use of ocular medication other than timolol or demecarium bromide [meds (1) = 0, meds (2) = 1], and beta-blocker and/or demecarium bromide use [meds (1) = 1, meds (2) = 0].

The univariate tests established that there were no significant differences between OH and OAG patients. Consequently, for the multiple linear-regression analysis, they were combined into one group, and only one indicator variable was needed for the purpose of distinguishing ocular diagnosis (ie, the diagnosis was coded as: normal, 0 and OH or OAG, 1). The other variables included in the models because of their potentially influential relationships with the dependent variables included the logarithm of total plasma cortisol (which was mean centered), age (in yr), sex (female as 1 and male as 2), systolic (or diastolic) blood pressure (in mm Hg), and time of blood sampling (measured in hours from the previous
Table 1. Characteristics of study population: systemic and ocular variables

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Normal</th>
<th>Ocular hypertension</th>
<th>One-angle glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Median</td>
<td>65.0</td>
<td>65.0 (60.0, 70.5)</td>
<td>72.0 (66.5, 75.5)</td>
</tr>
<tr>
<td>Percentile (30th, 70th)</td>
<td>60.5, 72.5</td>
<td>60.0, 70.5</td>
<td>66.5, 75.5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Sex/male</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Race/white</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg) at time of blood sample</td>
<td>15.0 (13.3, 15.5)</td>
<td>21.0 (18.0, 22.3)</td>
<td>17.8 (15.0, 18.5)</td>
</tr>
<tr>
<td>Maximum intraocular pressure (mm Hg)</td>
<td>16.0 (14.0, 17.0)</td>
<td>26.0 (24.0, 34.8)</td>
<td>27.5 (22.3, 28.5)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>130</td>
<td>140 (120, 140)</td>
<td>140 (130, 140)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>80</td>
<td>80 (70, 80)</td>
<td>80 (78, 81)</td>
</tr>
<tr>
<td>Optic disc percent cupping†</td>
<td>42.5 (40.0, 50.0)</td>
<td>55.0 (47.5, 61.8)</td>
<td>70.0 (56.8, 73.8)</td>
</tr>
<tr>
<td>Percent pallor‡†</td>
<td>6.0 (3.8, 8.0)</td>
<td>19.0 (8.5, 25.0)</td>
<td>47.5 (31.8, 55.0)</td>
</tr>
</tbody>
</table>

*Ocular variables are expressed as average of both eyes.
† Percent cupping = area of cup/area of optic disc × 100.
‡‡ Percent pallor = area of pallor/area of optic disc × 100.

midnight). A term representing the interaction of total plasma cortisol and diagnosis was also included in the models because of differences in the relationship between total cortisol and each of the other dependent variables (especially percent free cortisol) for the different diagnosis groups.

Results

Univariate Analyses

Comparisons for systemic and ocular characteristics of subjects: Plasma samples were obtained from 29 NOR and 31 OH and 25 OAG patients. The characteristics of the three groups with respect to systemic and ocular parameters and plasma cortisol parameters are shown in Table 1. Nonparametric multiple comparison testing revealed no significant differences in the frequency distributions of age between the NOR and OH patients, or between the OH and the OAG patients, or between the NOR and OAG patients. There were no significant differences between the groups with respect to sex or systemic blood pressure. There was a significant difference with respect to race between the three groups (chi-square = 8.211, P = 0.02) with the OAG having more blacks than the OH.

Comparison of optic disc cupping and pallor measurements by the Kruskal-Wallis test showed a significant difference in the distribution of these variables among the NOR, OH, and OAG groups. Multiple comparison testing indicated that the NOR were significantly different from the OH group for cupping (cupping, P = 0.001; pallor, P = 0.000). Similarly the NOR were significantly different from the OAG group (cupping, P = 0.000; pallor, P = 0.000), and also the OH group was significantly different from the OAG group (cupping, P = 0.002; pallor, P = 0.000).

Comparisons for diagnosis: Comparisons of cortisol parameters are shown in Table 2 and Figure 1.

Table 2. Characteristics of study population experimental variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Ocular hypertension</th>
<th>Open-Angle Glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Median</td>
<td>9.5 (8.8, 11.8)</td>
<td>11.3 (10.3, 13.6)</td>
<td>11.9 (10.0, 13.6)</td>
</tr>
<tr>
<td>Percentile (30th, 70th)</td>
<td>8.8, 11.8</td>
<td>10.3, 13.6</td>
<td>10.0, 13.6</td>
</tr>
<tr>
<td>Radioimmunoassay-determined cortisol (RIA) (µg/100 ml)</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Radioreceptor-determined glucocorticoids (RRA) (µg/100 ml)</td>
<td>29</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Ratio = RRA/RIA</td>
<td>0.97 (0.71, 1.27)</td>
<td>1.02 (0.77, 1.33)</td>
<td>1.09 (0.95, 1.55)</td>
</tr>
</tbody>
</table>

* Percent change from normal group.
Fig. 1. Scatter plot of RRA value radioreceptor-determined glucocorticoid activity (in cortisol equivalents μg/100 ml) for normals, ocular hypertensives (OH), and open-angle glaucomas (OAG).

Although the median values of RIA cortisol were the least for the NOR group (19.0% greater for OH and 25.3% greater for OAG), the Kruskal-Wallis test showed no significant difference in the distributions of this variable. For both the RRA and the RRA/RIA ratios, the medians were smallest for the NOR group and greatest for the OAG group (For RRA: OH was 6.9% greater and OAG was 37.1% greater than NOR; for RRA/RIA: OH was 5.2% greater and OAG was 13.4% greater than NOR). The Kruskal-Wallis test showed a significant difference (H = 8.94, P = 0.0115) in the distribution of RRA among the three groups, but this was not significant for the distribution of the ratio RRA/RIA.

A comparison of the frequency distributions of RRA with RIA using the two-tailed Wilcoxon signed-rank test showed no significant difference for NOR or OH but a significant difference for OAG patients (P = 0.0264). This indicated that cortisol could account for virtually all of the receptor-determined glucocorticoid activity among NOR and OH patients (Table 2) but not in the OAG group; there is a significant amount of noncortisol glucocorticoid activity in the plasma of OAG patients.

Comparisons for beta-blocker and other therapy for glaucoma: The OAG and OH patients were categorized according to their use of certain medications that could be readily absorbed systemically and thus possibly affect the hypothalamic-pituitary-adrenal axis. The medications of interest were beta-blocker medications (timolol and/or propranolol hydrochloride), epinephrine, carbonic anhydrase inhibitors, and demecarium bromide. The Mann-Whitney U test showed a significant difference in the distribution of RRA (P = 0.040) between those OAG and OH patients who were receiving topical beta-blocker medication (mean = 18.5 mg/100 ml, n = 20) and those who were not (mean = 13.5 mg/100 ml, n = 36), but no significant differences were observed in the distribution of RIA or the ratio RRA/RIA. A borderline significant difference was observed using the Mann-Whitney U test in the distribution of RRA (P = 0.054) between those OAG and OH patients who were receiving demecarium bromide (mean = 20.3 mg/100 ml, n = 9) versus those who were not (mean = 14.3 mg/100 ml, n = 47). No significant differences between demecarium bromide users versus nonusers were found for RIA and the ratio RRA/RIA.

No significant differences were observed using the Mann-Whitney U test for RIA, RRA, or RRA/RIA between those OAG and OH patients who were (n = 16) versus those who were not (n = 40) receiving epinephrine drops, between those OAG and OH subjects who were (n = 11) versus those who were not (n = 45) receiving carbonic anhydrase inhibitors, and between those who were (n = 22) versus those who were not (n = 34) receiving a beta-blocker drug (topical and systemic). Similarly no significant differences were seen between those who were (n = 27) versus those who were not (n = 29) receiving pilocarpine drops.

Correlations between RIA and RRA: The relationship between RIA and RRA values are shown in Table 3 and Figure 2. Highly significant positive correlation coefficients for the total population and the OAG group were found, ie, as the amount of RIA increased, the amount of RRA also increased. The correlation coefficient was also significant for the OH group but was of borderline significance in the NOR group.
Fig. 2. Correlation between RRA (radioceptor-determined glucocorticoid activity in cortisol equivalents µg/100 ml) and RIA (radioimmunoassay cortisol levels µg/100 ml) for (A) normals, (B) ocular hypertensives, and (C) open-angle glaucomas.

These correlations were not significantly different between the NOR and OAG groups or between the OH and OAG groups (Fisher's Z-transformation test).

Multivariate Analysis

The multiple-regression analyses were divided into two parts. The first considered the association of systemic parameters with RIA, RRA, and RRA/RIA values such as age, sex, and diastolic blood pressure with the diagnostic groups and used the whole population. The second evaluated the effect of therapy on the systemic variables and types of medications in the OH and OAG groups, since these groups were the only ones receiving medications to lower ocular pressure.
Table 3. Spearman correlation coefficients of glucocorticoids determined by radioreceptor competitive binding assay (RRA) vs. cortisol determined by radioimmunoassay (RIA)

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population (n = 85)</td>
<td>0.4568</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Normal subjects (n = 29)</td>
<td>0.3458</td>
<td>0.0661</td>
</tr>
<tr>
<td>Ocular hypertensive patients (n = 31)</td>
<td>0.3862</td>
<td>0.0319</td>
</tr>
<tr>
<td>Open-angle glaucoma patients (n = 25)</td>
<td>0.5593</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

Table 4 shows the significant model obtained for the first analysis. The diagnosis variable (NOR versus OH plus OAG) was significantly associated with the RRA value and diastolic pressure. For the model using RIA as the dependent variable, age and sex were significantly associated with the RIA level. For the model with the ratio RRA/RIA as the dependent variable, only diastolic pressure was significantly associated.

Since timolol and demecarium bromide therapy were found to be associated with a significant increase in RRA values, an analysis was done to determine whether other medications are associated with changes in RRA, RIA, or RRA/RIA. A multiple-regression model was constructed for the OH and OAG groups with the following independent variables: diagnosis (OH versus OAG), age, sex, diastolic blood pressure, meds (1) (ie, timolol drops and/or demecarium bromide drops versus no medication), meds (2) (ie, epinephrine drops, pilocarpine drops, and a carbonic anhydrase inhibitor versus no medication), and meds (1–2) (ie, timolol drops and/or demecarium bromide drops versus epinephrine drops, pilocarpine drops, and a carbonic anhydrase inhibitor). No significant models could be found for RRA, RIA, and RRA/RIA, nor were any of the independent variables significant.

Analysis of Other Factors

Finally, additional characteristics of the subjects were analyzed to determine other factors which could influence RRA and RRA/RIA and contribute to the differences found between the diagnostic groups. There was a difference of borderline significance (Pearson’s chi square = 0.064) in the prevalence of systemic hypertension in the OH and OAG groups compared with the NOR group, with the OH and OAG groups having a greater prevalence of systemic hypertension. There were no significant differences between the groups for distribution of subjects’ visits categorized by season of the year.

Discussion

We used the approach employed by Ballard and colleagues28 to measure the combined activities of cortisol and other glucocorticoids because glucocorticoid receptors, unlike the specific cortisol antibodies used in the RIA, will accept both cortisol and other biologically active glucocorticoids. To compare the findings from the RRA with those from the cortisol RIA, the former were determined in cortisol equivalents, ie, the amount of glucocorticoid activity if cortisol were the only glucocorticoid present in plasma. By comparing the RRA value in cortisol equivalents with the amount of cortisol present in plasma (measured

Table 4. Multiple regression models for total population

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>F</th>
<th>P</th>
<th>n</th>
<th>R²</th>
<th>Independent variables</th>
<th>Standardized coefficient</th>
<th>Coefficient</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN (RRA) (intercept = 1.1532)</td>
<td>2.715</td>
<td>0.0361</td>
<td>80</td>
<td>0.1265</td>
<td>Diagnosis* (normal vs OH plus OAG)</td>
<td>0.26</td>
<td>0.2854</td>
<td>2.37</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>0.05</td>
<td>0.0024</td>
<td>0.43</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sex†</td>
<td>0.03</td>
<td>0.0356</td>
<td>0.29</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diastolic pressure</td>
<td>0.25</td>
<td>0.0119</td>
<td>2.26</td>
<td>0.03</td>
</tr>
<tr>
<td>LN (RIA) (intercept = 2.5034)</td>
<td>3.762</td>
<td>0.0076</td>
<td>80</td>
<td>0.1671</td>
<td>Diagnosis* (normal vs OH plus OAG)</td>
<td>0.18</td>
<td>0.1342</td>
<td>1.72</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>-0.22</td>
<td>-0.0075</td>
<td>-2.07</td>
<td>0.04</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Sex†</td>
<td>0.28</td>
<td>0.1952</td>
<td>2.65</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Diastolic pressure</td>
<td>0.03</td>
<td>0.0009</td>
<td>0.25</td>
<td>0.80</td>
</tr>
<tr>
<td>LN (ratio RRA/RIA) (intercept = -1.3448)</td>
<td>2.888</td>
<td>0.0279</td>
<td>80</td>
<td>0.1335</td>
<td>Diagnosis* (normal vs OH plus OAG)</td>
<td>0.14</td>
<td>0.1526</td>
<td>1.32</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Age</td>
<td>0.20</td>
<td>0.0098</td>
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<td>0.07</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Sex†</td>
<td>-0.16</td>
<td>-0.1621</td>
<td>-1.49</td>
<td>0.14</td>
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<tr>
<td></td>
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<td>Diastolic pressure</td>
<td>0.24</td>
<td>0.0111</td>
<td>2.20</td>
<td>0.03</td>
</tr>
</tbody>
</table>

LN = natural logarithm; RRA = radioimmunoassay-determined cortisol levels (ng/100 ml); RIA = radioreceptor-determined glucocorticoid activity (in cortisol equivalent, ng/100 ml).

* Diagnosis: 0 = normal; 1 = OH plus OAG.
† Sex: 1 = female; 2 = male.
by cortisol RIA), it was possible to determine whether appreciable amounts of glucocorticoid activity not attributable to cortisol were present. Nevertheless, the method measures only noncortisol glucocorticoid activity, not the actual quantities of these hormones. Since cortisol is by far the most biologically active glucocorticoid in humans, other active glucocorticoids, such as corticosterone or 11-deoxycortisol, would have to be present in amounts normally high for a human to produce a significant discrepancy between the quantity of cortisol equivalent glucocorticoid activity determined by the RRA and the quantity of cortisol determined by RIA. Although the identity of possible active glucocorticoids responsible for such an observation cannot be determined by the methods in this report, the data suggest that such a phenomenon may exist in primary OAG compared with NOR and OH.

We confined our study to subjects who had no evidence of the pigmentary dispersion syndrome and/or exfoliation. This decision was based on our previous study which showed statistically significant greater differences with NOR when such subjects with pigmentary dispersion and/or exfoliation were excluded from the analysis.

Our results indicate that when compared with NOR or OH, there were significant differences in the RRA and the RRA/RIA ratios of the OAG group. These differences were not significant for OH versus NOR and were borderline in significance between OH and OAG. Furthermore the mean RRA/RIA ratio was found to be significantly greater than unity for the OAG (with RRA significantly greater than RIA for OAG). With multiple-regression analysis, a significant difference of RRA was also observed between the NOR versus the OH plus OAG groups.

The subjects in this study consisted of 29 whites and no blacks in the NOR group, 28 whites and 3 blacks in the OH group, and 25 whites and 6 blacks in the OAG group. When the data for RIA, RRA, and RRA/RIA values were categorized by race, there was no trend for the OH for the blacks being different from the whites (median values: RIA whites, 12.3; RIA blacks, 10.7; RRA whites, 13.5; RRA blacks, 15.4; RRA/RIA whites, 1.18; RRA/RIA blacks 1.09). However, for the OAG, the blacks appeared to have lower values than the whites (median values [30th and 70th percentiles]: RIA whites, 13.7 [9.4, 16.3]; RIA blacks, 11.0 [10.6, 11.3]; RRA whites, 18.6 [14.0, 19.0]; RRA blacks, 13.0 [10.4, 15.6]; RRA/RIA whites, 1.45 [0.95, 1.79]; RRA/RIA blacks, 1.09 [0.95, 1.55]). The lower values for blacks tend to minimize the differences between whites among the NOR, OH, and OAG groups. However, the number of blacks in the OAG was only six, and any interpretation of differences between blacks and whites should not be considered of significance until a further study is done with a larger sample size of blacks in comparison to whites.

It appears that OAG, but not OH patients, have increased amounts of nonplasma cortisol glucocorticoid in their plasma compared with NOR (37% greater in OAG than in NOR groups). Furthermore, the multiple-regression models indicate that the increase of the plasma noncortisol glucocorticoid is associated with diagnosis of glaucoma and tends to increase with increased diastolic blood pressure, but it is not associated with ocular medication for glaucoma including topical and systemic beta-adrenergic blockers. Our observations of increased RRA values for OAG patients compared with NOR support previous studies in which OAG and OH was found to be associated with elevated plasma cortisol.

The increase in RRA is also associated with a significant increase in RIA in OH and OAG groups but a borderline significant increase in NOR (Table 3). Therefore, there may be hypothalamic-pituitary-adrenal abnormalities in OH and OAG patients which cause them to produce significant amounts of other glucocorticoids in addition to cortisol. In diseases resulting in severe adrenal abnormalities, such as Cush- ing's syndrome and hyperaldosteronism, glucocorticoids other than cortisol and aldosterone are produced. The observation that OH patients are not as significantly associated with increased RRA values as are OAG patients may result from the greater severity of disease, or that OH may be a mixed group of patients, some of whom are essentially normal and others of whom are in the early stages of glaucoma. The identification of the other plasma noncortisol glucocorticoids would be important in determining the nature of any hypothalamic-pituitary-adrenal abnormality in glaucoma.

Key words: glucocorticoid, receptor, glaucoma, ocular hypertension, plasma glucocorticoids

Acknowledgments

The authors thank Alice Wysocki, BS, for performing the chemical analytic determinations, Mark Manning, BS, for statistical analysis, and Sarah Boardman, BA, for editorial assistance.

References