Variable Effects of Different Corticosteroids on Plasma Lipids, Apolipoproteins, and Hepatic Apolipoprotein mRNA Levels in Rats

Bart Staels, Arie van Tol, Lawrence Chan, Guido Verhoeven, and Johan Auwerx

Treatment of male rats with hydrocortisone provoked a dose- and time-dependent decrease in plasma cholesterol concentration without a change in plasma triglyceride levels. In contrast, administration of triamcinolone and dexamethasone at equipotent glucocorticoid doses increased plasma cholesterol and triglyceride levels, respectively. Small effects on apolipoprotein E (apo E) and apo B mRNA levels were observed, but all corticosteroids increased apo A-I and apo A-IV mRNA and decreased apo A-II mRNA levels in the liver. Triamcinolone and dexamethasone, however, were three times more potent in stimulating hepatic apo A-IV gene expression than was hydrocortisone, whereas liver apo A-I and apo A-II mRNA levels were altered to a similar extent by all corticosteroids. Plasma apo A-I and apo B concentrations always varied in a similar fashion with their respective liver mRNA levels after administration of the distinct corticoids. For apo A-IV and apo E, discrepancies between plasma and liver mRNA levels after administration of the different steroids, however, point to additional regulatory effects on plasma apolipoprotein levels. We conclude that 1) in contrast to plasma apo A-I and apo B, alterations in plasma lipid, apo A-IV, and apo E levels depend on the type of corticosteroid used; and 2) glucocorticoids have a differential effect on hepatic mRNA levels of apo A-I and apo A-IV on the one hand and apo A-II on the other hand, an effect that may be of consequence in the process of reverse cholesterol transport. (Arteriosclerosis and Thrombosis 1991;11:760-769)

Corticosteroids are widely used as anti-inflammatory agents. These hormones are ligands for receptors belonging to the erb A/steroid receptor superfamily. After binding of these ligands to specific receptors, these complexes interact with specific elements (hormone-responsive elements) in the upstream region of genes, such as those involved in lipoprotein metabolism. Treatment with these hormones causes profound changes in plasma lipid levels that may lead to an increased risk for developing atherosclerosis. In humans treated with different doses of prednisone for variable periods of time, any or all of the following changes in plasma lipid levels have been reported: elevated very low density lipoprotein (VLDL) cholesterol and triglycerides, increased or decreased high density lipoprotein (HDL) cholesterol, and increased low density lipoprotein (LDL) cholesterol. Administration of glucocorticoids to laboratory animals such as the rat has been reported to result in a selective or combined increase in plasma cholesterol and triglyceride levels.

However, the mechanisms through which corticosteroids alter plasma lipid concentrations are poorly understood. These hormones appear to act both on the synthesis and clearance of plasma lipoproteins. Indeed, it has been suggested that hepatic VLDL production is increased in patients treated with adrenocortical steroids. Similarly, corticosteroids increase VLDL secretion in livers of intact rats as well as in rat hepatocyte cultures. On the other hand, lipoprotein catabolism appears to be decreased after corticosteroid treatment. Indeed, binding and degradation of human LDL by dexamethasone-treated hepatocytes is diminished due to a decrease in liver LDL receptor activity.

The most likely mechanism through which corticosteroids may influence plasma lipid levels is by...
changing the expression of genes involved in lipoprotein metabolism. Therefore, the aim of this study was to determine the effects of hydrocortisone on plasma lipid and apolipoprotein levels and to investigate whether these changes might be explained by alterations in the expression of the corresponding genes. In addition, since synthetic corticosteroids were used in the majority of the reported studies, the effects of treatment with the natural corticosteroid hydrocortisone were compared with the effects of the synthetic corticosteroids triamcinolone and dexamethasone given at an equipotent glucocorticoid dose.

**Methods**

**Animals and Treatments**

Eighty-day-old male rats of the Wistar or R/A strain received once-daily subcutaneous injections with the indicated corticosteroids at a dose and for the period of time indicated. At the end of the experiment, animals were fasted overnight and killed by exsanguination while under ether anesthesia. Blood was collected in EDTA-containing tubes, and plasma was used within 1 day for flotation of plasma lipoproteins. The remaining plasma was stored at −20°C for determination of cholesterol, triglyceride, and apolipoprotein levels. The liver was removed immediately, rinsed with 0.9% NaCl, and frozen in liquid N2. The intestine was removed and rinsed with ice-cold 0.9% NaCl, and the epithelium was scraped off and frozen in liquid N2.

**Measurement of Plasma Lipid and Apolipoprotein Concentrations**

Plasma total cholesterol and triglyceride values were measured as described previously. Plasma apolipoprotein A-I, apo A-IV, and apo E concentrations were measured by electroimmunoassay exactly as described previously. Plasma apo B was measured by radial immunodiffusion as described.

Total plasma lipoproteins were isolated by flotation through a KBr gradient. The floated lipoproteins were lyophilized and delipidated for 10 minutes at 95°C in 4% sodium dodecyl sulfate (SDS), and the apolipoproteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (15% resolving gel, 4% stacking gel). Equal volumes of each sample were loaded to estimate relative changes in apolipoproteins. The different apolipoproteins were identified by comparing their mobility with those of purified apolipoproteins and by their apparent molecular weights.

**RNA Analysis**

RNA was prepared by the guanidine isothiocyanate–CsCl procedure from the liver and intestinal epithelium of individual animals. Northern and dot blot hybridizations of total cellular RNA were performed as described previously. The following apolipoprotein probes were used: a rat apo A-I cDNA probe comprising an 800-bp fragment of the rat apo A-I mRNA starting from the 3’ end; a rat apo A-II cDNA clone containing the whole 3’ untranslated region, mature peptide, propeptide coding regions, and part of the signal peptide region; a 42-base oligonucleotide probe constructed complementary to bases 73–114 of rat apo A-IV; a rat apo E cDNA clone containing all the coding region of the rat apo E sequence; and a rat 1.2-kb apo B cDNA probe.

**Results**

Analysis of variance (ANOVA) was used to evaluate the results of the dose-response and time-course experiments. Values observed between different groups were compared by contrast statements. A two-tailed unpaired Student’s t test was used to evaluate differences between means in all other experiments.

**Influence of 20 Days of Treatment With Hydrocortisone on Plasma Lipid, Apolipoprotein, and Liver mRNA Levels**

Administration of hydrocortisone (100 μg/g body wt) for 20 days to male Wistar rats decreased the body weight from 350 ± 24 to 306 ± 14 g (control versus treated rats), whereas the liver weight remained unchanged (9.3 ± 1.0 versus 9.3 ± 0.5 g in control versus treated rats). The average daily food intake of the animals was not influenced by the treatment with hydrocortisone (data not shown).

Plasma total cholesterol levels dropped by approximately 30%, whereas plasma triglyceride levels were not significantly influenced after treatment with hydrocortisone (Table 1). In contrast, plasma apo A-I
TABLE 1. Influence of Hydrocortisone on Plasma Lipid, Apolipoprotein, and Hepatic mRNA Levels

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Control</th>
<th>HC</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>62±8</td>
<td>42±6</td>
</tr>
<tr>
<td></td>
<td>Triglyc</td>
<td>48±4</td>
<td>65±27</td>
</tr>
<tr>
<td></td>
<td>Apo A-I</td>
<td>27±4</td>
<td>40±3</td>
</tr>
<tr>
<td></td>
<td>Apo B (AU)</td>
<td>70±19</td>
<td>67±27</td>
</tr>
<tr>
<td></td>
<td>Apo E (mg/dl)</td>
<td>9±2</td>
<td>4±1</td>
</tr>
<tr>
<td>Liver</td>
<td>Apo A-I (RAU)</td>
<td>100±14</td>
<td>188±44</td>
</tr>
<tr>
<td></td>
<td>Apo A-II (RAU)</td>
<td>100±10</td>
<td>48±8</td>
</tr>
<tr>
<td></td>
<td>Apo B (RAU)</td>
<td>100±41</td>
<td>92±20</td>
</tr>
<tr>
<td></td>
<td>Apo E (RAU)</td>
<td>100±17</td>
<td>95±10</td>
</tr>
<tr>
<td></td>
<td>LDL-R (RAU)</td>
<td>100±24</td>
<td>64±14</td>
</tr>
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Male Wistar rats (n=4) were treated with hydrocortisone (HC, 100 μg/g body wt) for 20 days. Plasma cholesterol, triglyceride (Triglyc), and apolipoprotein (Apo) concentrations and liver mRNA levels were measured and expressed as described in “Methods.” A two-tailed unpaired Student’s t test was used to determine statistically significant differences between groups. NS, not significant.

LDL-R, low density lipoprotein receptor; AU, arbitrary units; RAU, relative absorbance units.

concentration rose to nearly 150%, whereas plasma apo E fell to less than 45% of controls (Table 1). Plasma apo B levels were similar in treated and untreated animals (Table 1). The changes in plasma apo A-I and apo E, as determined by immunoassay, were also observed after SDS-PAGE of the floated d<1.21 g/ml lipoproteins (Figure 1). In addition, it can be seen that plasma apo A-IV remained constant after hydrocortisone treatment (Figure 1), an observation that confirms previous plasma apo A-IV measurements.4

The change in plasma apo A-I concentration was accompanied by a twofold increase in liver apo A-I mRNA levels (Table 1). Apo A-II mRNA levels, however, dropped to less than 50% of controls, whereas no significant changes in hepatic apo B and apo E mRNA concentrations were observed after hydrocortisone treatment (Table 1). Northern blot analysis confirmed the observed effects of hydrocortisone on liver apo A-I, apo A-II, and apo E mRNA levels by dot blot analysis (Figure 2).

In contrast, intestinal apo A-I and apo B mRNA levels were only slightly influenced after hydrocortisone treatment. Apo A-I and apo B mRNA levels, respectively, increased from 100±11% and 100±16% in control to 130±19% and 134±13% in hydrocortisone-treated rats. Northern blot analysis of intestinal apo A-I mRNA confirmed that the increase after hydrocortisone treatment is only very small (Figure 2).

![Figure 1. Photograph showing influence of hydrocortisone (HC) on plasma apolipoprotein levels. Adult male Wistar rats received hydrocortisone (HC, 100 μg/g body wt for 20 days) or vehicle (CON). Plasma lipoproteins (d<1.21 g/ml) were isolated and delipidated, and apolipoproteins (Apo B, A-IV, E, A-I, C, and A-II) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described in “Methods.” Coomassie Brilliant Blue R250 stain.](http://atvb.ahajournals.org/fig/1a.jpg)
FIGURE 2. Autoradiographs of Northern blot analysis showing influence of hydrocortisone (HC) on mRNA levels for apolipoproteins (Apo) A-I, A-II, and E. RNA was prepared from livers and intestines of HC- or sham-injected (CON) adult male Wistar rats. Twelve micrograms of each RNA preparation was subjected to electrophoresis in a 1% agarose gel. RNA was transferred to a nylon membrane (Hybond-N, Amersham, Buckinghamshire, U.K.) and hybridized with a mixture of nick-translated cDNA probes for apo A-I, A-II, and E as described. Localization of the different apolipoprotein mRNAs and their corresponding sizes (in bases) are indicated on the autoradiograph. 18S, migration of 18S human ribosomal RNA.

Finally, hepatic LDL receptor mRNA levels showed a borderline significant decrease (Table 1), whereas intestinal LDL receptor mRNA levels remained constant after treatment with hydrocortisone (117±38% versus 100±18% in treated and control rats, respectively).

Influence of Hydrocortisone on Plasma Cholesterol in Different Rat Strains

Since previous studies all showed either an increase or no change in plasma cholesterol levels after treatment with corticosteroids,12-14 we wondered whether the hydrocortisone-induced decrease in plasma cholesterol observed in rats from the Wistar strain could also be induced in rats from a different strain. Therefore, rats from the R/A strain were treated for 4 days with hydrocortisone (100 μg/g body wt/day), and changes in plasma cholesterol were compared with the changes observed in identically treated Wistar rats. In male R/A rats, treatment with hydrocortisone decreased plasma cholesterol concentrations from 82±9 mg/100 ml in controls to 56±2 mg/100 ml in treated rats. In Wistar rats, a similar decrease in plasma cholesterol was observed after treatment with hydrocortisone (from 80±15 mg/100 ml to 54±6 mg/100 ml in hydrocortisone-treated rats).

Time-Dependent Influence of Hydrocortisone on Plasma Lipids and Apolipoproteins and Hepatic Apolipoprotein and LDL Receptor mRNA Levels

Next we investigated if the discrepancies between our findings and previous reports could be explained by differences in duration of treatment. Therefore, a more detailed time-course study was initiated. Plasma cholesterol levels were already lower in hydrocortisone-treated R/A rats than in control R/A rats after 2 days and remained low after an additional 2 days (Figure 3A). This decrease was comparable to the decrease observed in Wistar rats treated for similar periods of time (Figure 3A). In contrast, plasma triglycerides were not significantly influenced by these treatments in both strains (Figure 3C). In male R/A rats, treatment with hydrocortisone decreased plasma triglyceride concentrations from 95±3 mg/100 ml in controls to 79±2 mg/100 ml in treated rats. In Wistar rats, a similar decrease in plasma triglyceride was observed after treatment with hydrocortisone (from 90±15 mg/100 ml to 75±6 mg/100 ml in hydrocortisone-treated rats).

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mRNA content (Figure 4B). Liver apo E mRNA levels remained constant (Figure 4B). Hepatic LDL receptor mRNA levels were only slightly influenced: they decreased insignificantly from 100 ± 26% to 78 ± 13% after 4 days of hydrocortisone.

**Dose-Dependent Influence of Hydrocortisone on Plasma Lipids and Apolipoproteins As Well As on Hepatic Apolipoprotein and Low Density Lipoprotein Receptor mRNA Levels**

Since differences in rat strain as well as in duration of treatment could not explain the discrepancy in plasma cholesterol changes after treatment with corticosteroids between this study and previous reports, we analyzed whether the decrease in plasma cholesterol was dose dependent. Therefore, rats from the R/A strain were treated for 4 days with the indicated doses of hydrocortisone (100 μg/g/day) or with equipotent doses of the synthetic glucocorticoids triamcinolone (20 μg/g/day) and dexamethasone (3.7 μg/g/day). Marked differences in plasma lipid levels were observed after 4 days of treatment (Figure 6): whereas hydrocortisone lowered plasma cholesterol without changing triglyceride values, triamcinolone caused an increase of more than 50% in cholesterol without changing triglyceride levels, and dexamethasone increased triglyceride levels more than twofold without affecting cholesterol levels in plasma.

Liver apo A-I mRNA increased to a similar extent in dexamethasone- and triamcinolone-treated animals compared with hydrocortisone-treated rats (Figure 7A). Although plasma apo A-I increased in all treatment groups, both triamcinolone and dexamethasone were significantly more potent than hydrocortisone (Figure 7C). Liver apo A-II mRNA decreased in all treatment groups to a similar extent (Figure 7A). In contrast, apo A-IV mRNA levels increased nearly sixfold in livers from dexamethasone- and triamcinolone-treated rats but only twofold in hydrocortisone-treated animals (Figure 7A). Plasma apo A-IV showed large variation between all groups: a decrease in hydrocortisone-, no change in triamcinolone-, and an increase in dexamethasone-treated rats (Figure 7C). Although liver apo B mRNA levels tended to decrease in all groups, the differences were not statistically significant (Figure 7B). In plasma, apo B levels decreased to a similar extent in all groups (Figure 7D). Finally, liver apo E mRNA showed very small but significant decreases in dexamethasone- and triamcinolone-treated animals (Figure 7D). In plasma, however, apo E decreased after hydrocortisone, remained constant after dexamethasone, and increased after triamcinolone treatment (Figure 6D). Finally, hepatic LDL receptor mRNA levels were not significantly influenced by any of the corticosteroids used (data not shown).

**Discussion**

Treatment with corticosteroids causes profound changes in plasma lipid and apolipoprotein concen-
FIGURE 4. Bar graphs showing influence of duration of treatment with hydrocortisone on liver apoprotein mRNA levels (relative absorbance units) (panel A and B) and on plasma apoprotein concentrations (apos A-I, A-IV, E: mg/100 ml; apo B, arbitrary units) (panels C and D). Adult male RJA rats were treated during the indicated number of days with hydrocortisone (100 μg/g body wt). Plasma apoprotein concentrations and liver mRNA levels were measured and expressed as described in "Methods." Statistically significant differences (analysis of variance, p<0.05) observed between values are followed by different letters. Each value represents mean±SD of three animals.

Hydrocortisone provokes a decrease in plasma cholesterol concentrations without changing plasma triglyceride values. These results conflict with those from previous studies, all of which showed either no effect or an increase in plasma cholesterol and/or triglycerides after treatment with corticosteroids.12-14 All these studies, however, used synthetic corticosteroids such as dexamethasone and triamcinolone. The results from this study show that this apparent discrepancy is related to the type of corticosteroid used since triamcinolone and dexamethasone, given at equipotent glucocorticoid doses,28 cause increases in plasma cholesterol and triglycerides, respectively. In addition to these differences, differences in the response of plasma apolipoproteins are also observed after administration of these corticosteroids.

A first interesting observation in this respect is that both plasma apo A-IV and apo E change in opposite directions after treatment with these distinct hormone preparations. Their respective hepatic mRNA levels, however, are similarly influenced by all corticosteroids. Since intestinal apo A-IV mRNA levels do not decrease in hydrocortisone-treated rats,4 it is unlikely that intestinal apolipoprotein production accounts for the observed decrease in plasma apo A-IV levels. The decrease in plasma apo E levels after hydrocortisone also cannot be explained by a decrease in liver apo E mRNA message. This decrease may be a consequence of either a decreased secretion by the liver or an increased catabolism of apo E-containing lipoproteins. Alternatively, the decrease in plasma apo E could be a consequence of a lowered production of apo E by extrahepatic tissues. Indeed, it has been shown that extrahepatic apo E production may contribute significantly to the plasma pool of apo E.29,30 Furthermore, it has been demonstrated in rabbits that extrahepatic apo E gene expression can be modulated selectively by probucol, whereas liver apo E mRNA levels remain constant.31

Plasma apo A-I concentrations increase after all these corticosteroids, but the effect is much more pronounced when triamcinolone and dexametha-
Figure 5. Bar graphs showing influence of treatment with different doses of hydrocortisone on liver apolipoprotein mRNA levels (panels A and B) and plasma apolipoprotein concentrations (panels C and D). Adult male RIA rats were treated for 4 days with the indicated doses (µg/g body wt [BW]/day) of hydrocortisone. Plasma apolipoprotein concentrations (apo A-I, A-IV, E; mg/100 ml; apo B, arbitrary units) and liver mRNA levels (relative absorbance units) were measured and expressed as described in “Methods.” Statistically significant differences (analysis of variance, p<0.05) observed between values are followed by different letters. Each value represents mean±SD of three animals.

Figure 6. Bar graph showing influence of treatment with hydrocortisone (HC), triamcinolone (T), or dexamethasone (D) on plasma cholesterol (■) and triglyceride (□) values. Adult male RIA rats were treated with HC (100 µg/g body wt), T (20 µg/g body wt), or D (3.7 µg/g body wt) for 4 days. Plasma cholesterol and triglyceride levels were measured as described in “Methods.” Values represent mean±SD. Significant differences from controls are indicated by an asterisk (analysis of variance, p<0.05).
expression of these genes directly via interaction of the glucocorticoid receptor with responsive elements in the regulatory regions of these genes. Hepatic apolipoprotein mRNA levels only change significantly after the highest dose of steroid used. Since none of the animals were adrenalectomized, this could indicate that the endogenous corticosteroid production already may have a strong impact on the expression of these genes.

The distinct response of plasma lipids and apolipoproteins after the different corticosteroids used is highly interesting. These distinct effects could be related to differences in half-life, to differences of gluco-/mineralocorticoid action, or to different affinities and interactions of these glucocorticoid-glucocorticoid receptor complexes with the responsive elements in the regulatory regions of the respective genes. This may consequently lead to distinct regulation of the activity of proteins involved in lipoprotein metabolism (such as lecithin: cholesterol acyltransferase [LCAT], hepatic lipase, and lipoprotein lipase), cholesterol synthesis (such as hydroxymethyl glutaryl coenzyme A reductase) or catabolism (such
as the LDL receptor) by the respective corticosteroid preparations. Experiments are currently underway to investigate these possibilities. Alterations in hepatic LDL receptor activity could be involved in the observed variation in plasma cholesterol levels. Our results, however, show that hepatic LDL receptor mRNA levels are not significantly influenced by any of the corticosteroids used. Changes in LDL receptor mRNA levels therefore cannot be invoked as an explanation for the observed discrepancies. Further studies, however, are required to determine whether LDL receptor activity also remains unchanged after the different corticosteroids.

Most interesting is the differential regulation of hepatic apo A-I and apo A-II mRNA levels. A similar opposite regulation of liver apo A-I and apo A-II gene expression has been observed during early development and after ethinylenestradiol treatment. In addition, administration of L-thyroxine increases apo A-I mRNA and decreases apo A-II mRNA levels in the liver of adult rats, whereas n-propylthiouracil, a thyroid hormone antagonist, changes liver apo A-I and apo A-II mRNA in the opposite direction. The opposite regulation of apo A-I and apo A-II by different hormones, such as estrogens, corticosteroids, and thyroid hormones, may be of importance since these apolipoproteins are the major constituents of HDL and therefore may be involved in the process of reverse cholesterol transport in humans. Both apolipoproteins may influence the removal of cholesterol from peripheral cells in humans as well as in rats and mice. On the one hand, apo A-I has been shown to promote cholesterol efflux from human, mouse, and rat cells. In addition, apo A-I is a potent activator of LCAT, which mediates the formation of HDL3 from HDL2. On the other hand, apo A-II appears to antagonize the removal of cholesterol from peripheral cells since it inhibits binding of HDL to peripheral cells and cholesterol efflux from human and mouse adipocytes.

In conclusion, the present study confirms that corticosteroids have a major impact on plasma lipid and apolipoprotein levels and shows that their effects may vary with the type of corticosteroid used. Since all corticosteroids tested have qualitatively similar effects on hepatic apolipoprotein mRNA levels, these differences cannot be explained solely by the changes in apolipoprotein gene expression. Finally, the distinct effect of corticosteroids on apo A-I and apo A-II gene expression in vivo is of interest. Since both apo A-I and apo A-II are involved in the regulation of reverse cholesterol transport in humans, these results warrant further investigation on the regulation of these apolipoproteins in humans.

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