# Glucocorticoids Upregulate High-Affinity, High-Density Lipoprotein Binding Sites in Rat Hepatocytes

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**Glucocorticoid hormones (GL) regulate high-density lipoprotein (HDL) plasma concentrations by increasing synthesis and secretion of HDL by the liver. However, little is known about the effect of GL on the uptake and processing of HDL by hepatocytes (HEP). To investigate this question, we studied the effects of dexamethasone (DEX) on the expression of high-affinity HDL-binding sites via the specific binding and internalization of iodine-labeled apolipoprotein E (apo E)-free $HDL_3$ in a culture of rat HEP. Specific binding and internalization of $HDL_3$ decreased by 60% in cells cultured in the absence of DEX for 48 hours. At concentrations of $10^{-7}$ and $10^{-5}$ mol/L, DEX prevented the decrease, maintaining specific binding and internalization versus the control level (at 24 hours). HDL-binding sites with a $K_d$ of 20 μg/mL were revealed on the surface of cultured HEP. HEP demonstrated a greater binding capacity in the presence of DEX at concentrations of $10^{-7}$ and $10^{-5}$ mol/L (125 *v* 45 ng/mg cell protein). The effect of the hormone has demonstrated to be dose-dependent at concentrations between $10^{-9}$ and $10^{-7}$ mol/L, leveling off at $10^{-7}$. Higher concentrations did not induce a further increase in specific binding and internalization. Withdrawal of the hormone from culture medium was associated with a decrease in specific binding of the ligand by 60% in the following 24 hours. To investigate the effect of glucocorticoid deficiency on liver uptake of HDL in vivo, specific binding and internalization were studied in a culture of HEP isolated from adrenalectomized rats (AER) at 2 hours after seeding. The HEP demonstrated a 2.5-fold decrease in specific binding versus those from normal rats (NR). Correction of the glucocorticoid deficiency in AER by intramuscular injection of DEX at a dose of 5 μg per rat restored the specific binding of $HDL_3$ at 2 hours after seeding. Replacement of the hormone by incubation of HEP from AER with DEX increased specific binding and internalization of the ligand in a dose-dependent fashion until NR levels were attained. In both cases, the upregulating effect of DEX was stopped by 0.5 mmol/L cycloheximide. Lovastatin (100 nmol/L) blocked the DEX-dependent increase in cholesterol synthesis, but failed to prevent the DEX-induced increase in specific binding and internalization of $^{125}I$-$HDL_3$. It is concluded that the specific binding and internalization of $^{125}I$-$HDL_3$ by liver parenchymal cells are reversibly regulated by physiologic and pharmacologic concentrations of DEX. Its effect does not depend on availability of the pool of newly synthesized cholesterol, but it seems to be a result of the increase in the synthesis of HDL-binding protein.**


HIGH-DENSITY LIPOPROTEINS (HDLs) are widely accepted to play a protective role in the development of arteriosclerosis.[1] This hypothesis is based on data that demonstrate a strong inverse correlation between plasma HDL cholesterol concentration and the prevalence of premature atherosclerosis.[2,3] The antiatherogenic properties of HDL are probably based on the ability of HDL to promote efflux of cholesterol from peripheral tissues and to deliver it to the liver for further oxidation into bile acids.[4,5]

Special attention has been paid to the effects of glucocorticoids on lipoprotein metabolism. This interest results from data demonstrating a relationship between glucocorticoid hormones (GL) therapy and a marked increase of both plasma cholesterol and HDL concentrations in response to GL treatment. It has been shown that short-term and low-dose administration of prednisone increases cholesterol in HDL and plasma apolipoprotein (apo) A-I levels in patients with inflammatory diseases and healthy volunteers.[6,7] A chronic excess of GL has been associated with elevated concentrations of both HDL and low-density lipoprotein cholesterol, followed by the accelerated development of atherosclerosis.[8-11] The precise mechanism by which GL can alter lipoprotein metabolism remains unknown. However, it has been demonstrated that GL affect apolipoprotein synthesis by promoting expression of mRNA for apo A-I and apo A-IV genes in the liver. There were no observed changes in the synthesis of apo E mRNA.[12] The increase in expression of lecithin:cholesterol acyltransferase and 7α-hydroxylase mRNA in the liver after GL treatment has been suggested to be a cause, among others, of the accumulation of HDL and HDL cholesterol in the blood.[13,14]

Other mechanisms by which GL may alter HDL metabolism include a change in the specific binding and internalization of the ligand in the liver. Although the hypothesis about the significant role of high-affinity HDL-binding sites in the trafficking of cholesterol into the liver failed to find general acceptance, they are considered to play a marked role in the uptake of HDL and their catabolism of the liver, regulating the concentration of HDL in the blood plasma. High-affinity HDL-binding sites have been demonstrated on the surface of a number of cell types, including liver plasma membranes, parenchymal liver cells, liver endothelial and Kupffer cells, and hepatoma cell lines.[15-21] Moreover, ligand blot analysis has been successfully used to identify candidate receptor proteins. This approach has allowed description of a group of HDL-binding proteins that were revealed in the liver and peripheral tissues.[22-24] However, there are no reports about the effects of GL on

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the expression of HDL-binding sites, receptor-mediated processing of HDL, and their catabolism in the liver.

In this communication, we investigate the effect of dexamethasone (DEX), a synthetic glucocorticoid, on the specific binding and internalization of iodine-labeled $HDL_3$ by cultured hepatocytes (HEP), demonstrating a relationship between DEX treatment and expression of HDL-binding sites.

## MATERIALS AND METHODS

### *Materials*

Collagenase, protease, and dispase were purchased from Boehringer, (Mannheim, Germany). DEX, albumin (fraction V), and cycloheximide were purchased from Sigma (St Louis, MO). $^{125}I$ (carrier-free) in NaOH was obtained from Amersham International. Culture medium and HEPES were obtained from Flow Laboratories.

### *$HDL_3$ Isolation and Iodination*

Human $HDL_3$ ($1.125 < d < 1.216$) were isolated from plasma of healthy donors by two consecutive centrifugations.[25] $HDL_3$ were passed through a Sepharose-heparin column, and the apo E–free $HDL_3$ fraction was checked for the presence of apo E and albumin by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by Coomassie Blue R-250 staining and densitometric scanning.[26] $HDL_3$ were radiolabeled with $^{125}I$ by the iodine monochloride (ICl) method reported by McFarlane,[27] as modified by Bilheimer et al.[28] $HDL_3$ contained less than 1% of trichloroacetic acid–soluble radioactivity; the most radioactivity was present in apolipoproteins, as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and further autoradiography.

### *Animals and Surgery*

In all experiments, male Wistar rats weighing 250 to 350 g were used. The rats were provided water and standard chow.

Adrenalectomy was performed as described elsewhere.[29] After surgery, rats were kept in a temperature (25°C)-, humidity-, and light (12 hours on)-controlled room and allowed standard chow. Instead of water, rats were provided saline solution. Adrenalectomized rats (AER) were used in experiments 2 weeks after surgery. To replace GL, DEX was injected intramuscularly at a dose of 5 μg/rat/d as a solution in ricini oil for 3 days. At day 4, animals were used in the experiment. In the control group, ricini oil was injected by the same schedule.

### *Isolation and Cultivation of HEP*

HEP isolation was performed as reported elsewhere,[19,20,30] using a regular two-step collagenase-perfusion technique.[31,32] The cells (98% viability according to trypan blue exclusion test) were plated on collagen-coated 12-well culture clusters at a density of $10^5$ cells per $cm^2$ in Williams' E medium containing 10 μg/mL insulin, 100 μg/mL kanamycine, and 20 mmol/L HEPES. HEP were allowed to attach for 2 to 3 hours, and thereafter the medium was renewed to remove unattached cells for the fresh medium additionally supplemented with DEX at concentrations of $10^{-9}$ to $10^{-5}$ mol/L. Afterward, the medium was renewed every 24 hours.

After isolation, HEP from normal rats (NR) and AER demonstrated similar viability (98% according to trypan blue exclusion test), with a yield of approximately $10^8$ cells per gram liver tissue. The cells maintained 98% viability and a negligible level of release of cellular lactate dehydrogenase (LDH) for 5 days in the absence of DEX and for 8 to 9 days in the presence of $10^{-7}$ or $10^{-5}$ mol/L of the hormone, respectively. The activity of LDH in culture medium was determined on the Spectrum High-Performance Diagnostic System (Abbot, North Chicago, IL) using a Boehringer test combination kit. Data on LDH release in cells cultured under hormone-free conditions and in the presence of $10^{-7}$ mol/L DEX are shown in Fig 1A. In both cases, there was a temporary increase of LDH release for the first 24 hours. Afterward, membrane permeability was decreased to a low level and further maintained in culture until day 5 under hormone-free conditions and day 8 in the presence of the hormone, respectively. There were no observed differences in the pattern of LDH release in the culture of HEP in the presence of $10^{-7}$ mol/L DEX and in hormone-free conditions until day 4 of culture. Afterward, there was a gradual increase in LDH release at day 5 of culture in hormone-free conditions and at days 8 to 9 of culture in the presence of $10^{-7}$ mol/L DEX. To further characterize cell integrity in response to treatment by the hormone, we have also investigated the time course of DNA synthesis in HEP cultured in the presence and absence of $10^{-7}$ mol/L DEX (Fig 1B). DNA synthesis was estimated via $^3H$-thymidine incorporation. For this, cells were incubated with $^3H$-thymidine (5 μQu/mL) in culture medium containing $10^{-7}$

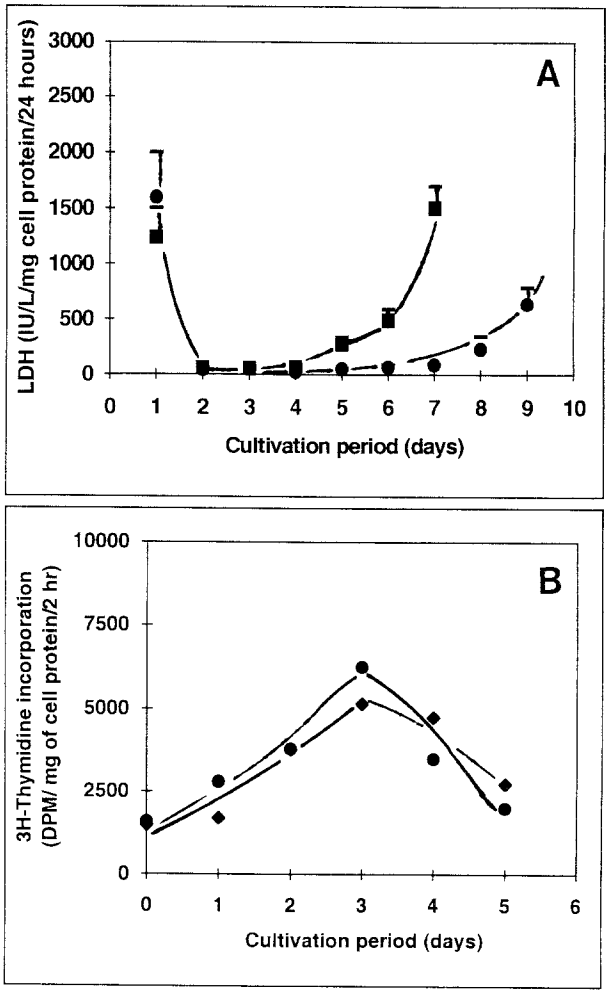


**Fig 1. Effect of DEX on LDH release (A) and $^3H$-thymidine incorporation (B) in cultured HEP. HEP isolated from NR were cultured for 9 days in the presence of $10^{-7}$ mol/L DEX (■, ◆) and in the absence of the hormone (●). At indicated times, LDH release and $^3H$-thymidine incorporation were determined.**

mol/L DEX or in hormone-free conditions for 2 hours in a $CO_2$ incubator. Afterward, cells were washed with cold Hanks balanced salt solution (HBSS) three times and 5% trichloroacetic acid two times. HEP was solubilized with 1N NaOH with further neutralization with 1N HCl, and radioactivity was counted in an LKB-Wallac Ultragamma counter (Sweden) using Toluene-Triton scintillator. The cells demonstrated a similar pattern of DNA synthesis at 72 hours and a further slow decrease for the next 2 days. There were no statistically significant differences in DNA synthesis in HEP cultured under hormone-free conditions and in the presence of the hormone. The cumulative data consider the 4-day cultivation period as a period of maximal maintenance of cell integrity irrespective of the presence of the hormone.

### *Binding Assays*

Measurements of the uptake of labeled $^{125}$I-HDL$_3$ were performed at 37°C at a concentration of 10 μg/mL to determine both the specific binding and specific internalization of lipoproteins for the same portion of cultured cells.[19,20] Instead of the routinely used short-term, high-temperature method of release of the surface-bound ligand by trypsin,[16] treatment of the cells with the dispase/protease solution at 4°C was introduced to avoid the detachment and damage of HEP associated with the use of trypsin. Before every experiment, HEP were rinsed two times with HBSS at 37°C, and the medium was replaced by a fresh one containing 2% bovine serum albumin and 10 μg/mL $^{125}$I-HDL$_3$ in the absence or presence of a 20-fold excess of unlabeled ligand. After a 2-hour incubation at 37°C, the culture clusters were placed on ice and the cells were rinsed three times with ice-cold HBSS. The washing protocol took less than 3 seconds for every culture well. Cultured cells were further incubated with the protease/dispase solution in HBSS (100 μg/mL of each) for 30 minutes at 4°C to release surface-bound radioactivity (the specific binding). Intracellular radioactivity after incubation of HEP with protease solution was considered as the specifically internalized ligand. For this, cells were scraped with a rubber policeman and counted for radioactivity. Both the specific binding and specific internalization were determined as a difference between radioactivity measured in cells incubated in the absence and presence of unlabeled ligand.

Scatchard plot analysis was performed at 4°C to avoid an internalization of surface-bound HDL. Saturation curves were built at concentrations of $^{125}$I-HDL$_3$ between 2.5 and 40 μg/mL. Nonspecific binding was determined in the presence of a 20-fold excess of unlabeled ligand. To determine the specific bound radioactivity, cells were scraped by a rubber policeman and radioactivity was counted in an LKB-Wallac Ultragamma counter. The protein content of samples was determined after hydrolysis in 0.1 mol/L NaOH followed by neutralization with HCl using the method of Bradford with albumin as a standard.[33]

### *Cholesterol Synthesis*

To determine the rate of cholesterol synthesis, HEP ($5 \times 10^5$ cells in monolayer culture) were rinsed with HBSS at 37°C two times and incubated essentially as described earlier, but $^{14}$C-acetate (final concentration, 10 μCi/mL) was added to the culture medium. After incubation with the label, cells were rinsed twice with cold HBSS and dissolved in 1N NaOH. Samples were saponified by incubation for 2 hours at 100°C in 5N KOH in 50% ethanol, and cholesterol was precipitated with digitonin (Sigma) according to the method reported by Sperry and Webb.[34]

## RESULTS

### *Effect of DEX on Specific Binding and Internalization of $^{125}$I-HDL$_3$ in Cultured HEP Isolated From NR*

In the absence of DEX, specific binding and internalization of $^{125}$I-HDL$_3$ by HEP from NR were decreased with cultivation time (Fig 2A and B). During the first 48 hours, specific binding and internalization decreased by 60% and 65%, respectively. Further culture of the cells was associated with a gradual decrease of both parameters to 40%. DEX at concentrations of $10^{-7}$ and $10^{-5}$ mol/L restored the specific binding and internalization of $^{125}$I-HDL$_3$ to the level observed at the first 24 hours. Both pharmacologic ($10^{-5}$ mol/L) and physiologic concentrations ($10^{-7}$) of the hormone demonstrated similar potency to retain normal processing of HDL, but failed to increase the specific

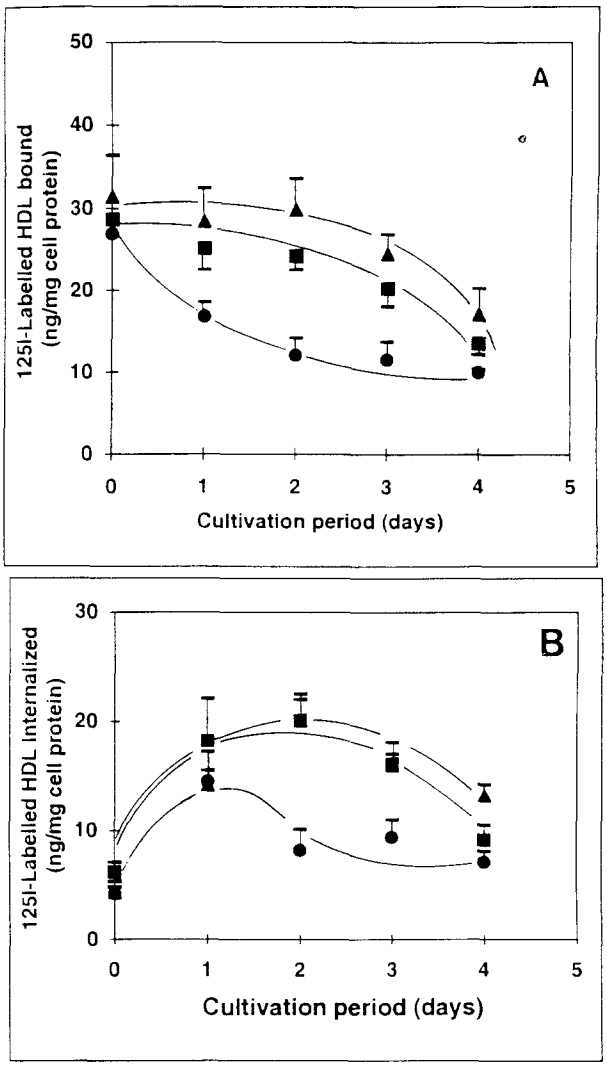


**Fig 2. Effect of DEX on specific binding (A) and internalization (B) of $^{125}$I-HDL$_3$ by cultured rat HEP from intact rats. HEP isolated from NR were cultured for 4 days in the presence of $10^{-5}$ mol/L DEX (▲) and $10^{-7}$ mol/L DEX (■), and in the absence of DEX (●). At indicated times, specific binding and internalization of $^{125}$I-HDL$_3$ were determined.**

binding and internalization of HDL over the level demonstrated for cells cultured during the first 24 hours. Despite the presence of DEX in culture medium, both the specific binding and internalization had a tendency to decrease at 96 hours after initiation of culture.

Scatchard plot analysis of specific binding of $^{125}$I-HDL$_3$ showed the existence of a single type of HDL-binding sites with a $K_d$ of 20 μg/mL on the surface of cultured HEP (Fig 3). The maximal number of binding sites was increased from 45 ng/mg (in the absence of DEX) to 125 ng/mg of cell protein (in the presence of $10^{-7}$ and $10^{-5}$ mol/L DEX in culture medium). The concentration of DEX ($10^{-7}$) corresponding to the physiologic concentration of cortisone in the blood was demonstrated to be nearly as effective as $10^{-5}$ mol/L.

DEX upregulated in a dose-dependent fashion both specific binding and internalization of $^{125}$I-HDL$_3$. Under incubation of HEP with the hormone, there was a dose-dependent 2.5-fold increase of specific binding and internalization of $^{125}$I-HDL$_3$ at a concentration of DEX between $10^{-10}$ and $10^{-5}$ mol/L at 48 hours of culture (Fig 4). The effect of the hormone was nearly leveled off at the concentration of $10^{-7}$ mol/L; however, a further slow increase of the specific binding and internalization was observed at concentrations higher than $10^{-7}$ mol/L.

*Effect of DEX on Specific Binding and Internalization of $^{125}$I-HDL in Cultured HEP Isolated From AER: Reversibility of Effects of DEX In Vivo and In Vitro*

Adrenalectomy was used to investigate the effect of GL deficiency on the specific binding and internalization of apo

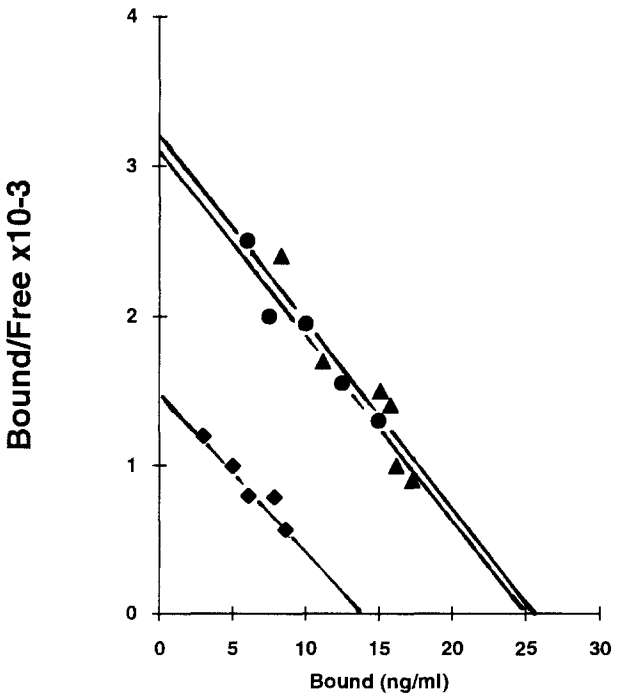


**Fig 3. Scatchard plot analysis of the specific binding of $^{125}$I-HDL$_3$ with cultured HEP. HEP isolated from NR were cultured for 2 days in the presence of $10^{-5}$ mol/L DEX (●), $10^{-7}$ mol/L DEX (△), or in the absence of the hormone (◆).**

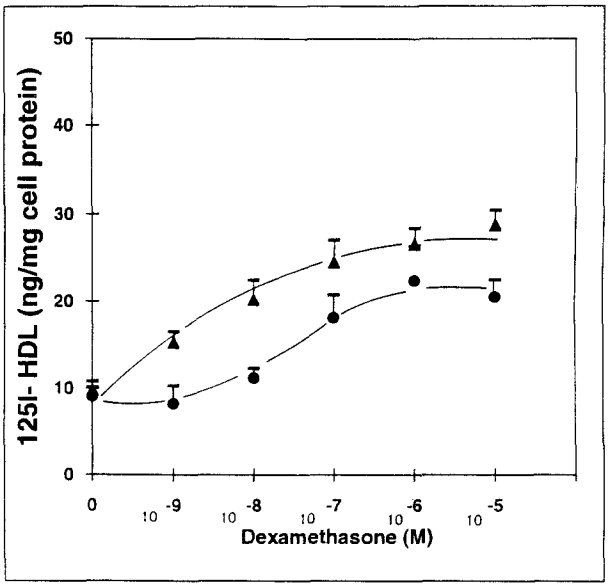


**Fig 4. Dose-dependent effect of DEX on the specific binding (▲) and internalization (●) of $^{125}$I-HDL$_3$ by HEP. HEP from NR were cultured for 2 days in the presence of different concentrations of DEX. At 48 hours, cells were used in experiments.**

E-free $^{125}$I-HDL$_3$ by cultured rat HEP. The yield, viability, and release of LDH from cells isolated from AER are demonstrated to be similar to those in cells isolated from NR. We failed to show a substantial loss of the cells during 96 hours of culture; the cells maintained 98% viability and negligible release of intracellular enzymes. To test the effect of adrenalectomy on specific binding and internalization of $^{125}$I-HDL$_3$, freshly isolated HEP from NR and AER were allowed to attach and spread for 2 hours, and thereafter were further used for 2-hour incubation with $^{125}$I-HDL$_3$ for examination of specific binding and internalization. Table 1 lists the data on specific binding and internalization of $^{125}$I-HDL$_3$ in short-term–cultured HEP isolated from NR, from AER 2 weeks after surgery, and from AER 2 weeks after surgery and treated with DEX for 3 days at a dose of 5 μg/rat/d. There was a threefold reduction of the specific binding of HDL$_3$ in short-term–cultured HEP isolated from AER versus NR (9.0 ± 0.4 *v* 29.0 ± 1.8 ng/mg cell protein). A similar tendency was observed for the measurement of specific internalization, demonstrating a decrease from 9.2 ± 1.2 to 5.4 ± 1.2 ng/mg of cell protein. Prolongation of the postsurgery period for more than 2 weeks was not associated with a further decrease of the processing of $^{125}$I-HDL$_3$ by cells (data not

**Table 1. Effect of Adrenalectomy on Specific Binding and Internalization of $^{125}$I-HDL$_3$ in Cultured Rat HEP**

| Parameter | NR | AER | AER Treated With DEX |
|---|---|---|---|
| Specific binding (ng/mg cell protein) | 29.0 ± 1.8 | 9.0 ± 0.4 | 29 ± 3.1 |
| Specific internalization (ng/mg cell protein) | 9.2 ± 1.2 | 5.4 ± 1.2 | 10 ± 2.1 |

NOTE. Results are the mean ± SEM.

shown). Under incubation of HEP from AER in culture for 48 hours in the presence of different concentrations of DEX, the hormone upregulated the specific binding and internalization of HDL in a dose-dependent fashion at concentrations between $10^{-9}$ and $10^{-7}$ mol/L (Fig 5). As with the culture of HEP isolated from NR, the effect of the hormone was nearly leveled off at the concentration of $10^{-7}$ mol/L.

To estimate the effect of the hormone in vivo, DEX was intramuscularly injected in AER in a 3-day schedule at a dose of 5 μg/rat/d (which corresponds to the concentration of the hormone, $10^{-6}$ mol/L, in the blood). The reversibility of the effect of DEX in vivo was illustrated by the fact that specific binding and internalization of $^{125}$I-HDL$_3$ in short-term–cultured HEP from DEX-treated AER had been demonstrated to be nearly as effective as that measured for NR (29 ± 3.1 and 10 ± 2.1 ng/mg cell protein *v* 29.0 ± 1.8 and 9.2 ± 1.2, respectively). To investigate the reversibility of GL insufficiency in vitro, DEX at the concentration of $10^{-7}$ mol/L was added in culture medium at different periods after plating the cells isolated from AER. When incubation with the hormone was started at 2 hours after seeding HEP, replacement of the hormone was associated with an increase of specific binding of the ligand for the following 24 hours. When the hormone was added at 24 hours after seeding of HEP, it was followed by an increase in the specific binding of $^{125}$I-HDL$_3$ from 9 ± 2 to 23.6 ± 5.4 ng/mg cell protein for the next 24 hours. The hormone had no effect on the specific binding of HDL when cells were cultured with the hormone for less than 3 hours, demonstrating a lag period of GL action (Fig 6A).

Withdrawal of DEX from the culture of HEP earlier stimulated by the hormone for 24 hours was associated with a decrease of specific binding of HDL by 65% for the next 24 hours (Fig 6B).

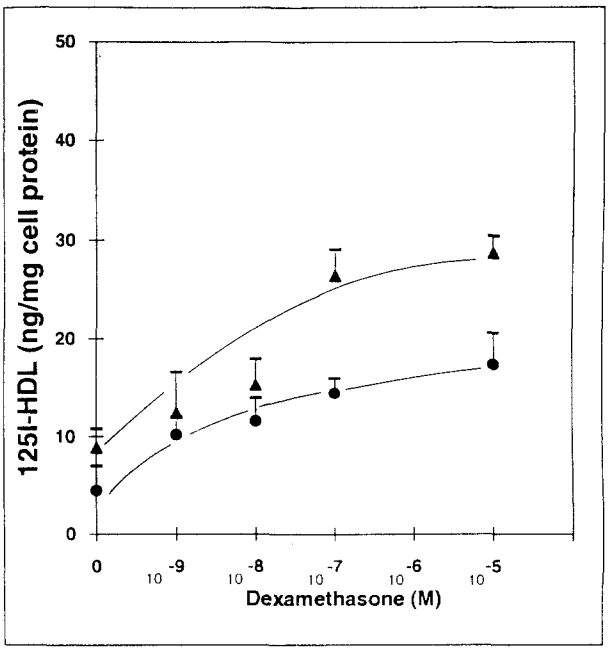


**Fig 5. Dose-dependent effect of DEX on the specific binding (▲) and internalization (●) of $^{125}$I-HDL$_3$ by HEP from AER. HEP isolated from AER were cultured for 2 days in the presence of different concentrations of DEX. At 48 hours, cells were used in experiments.**

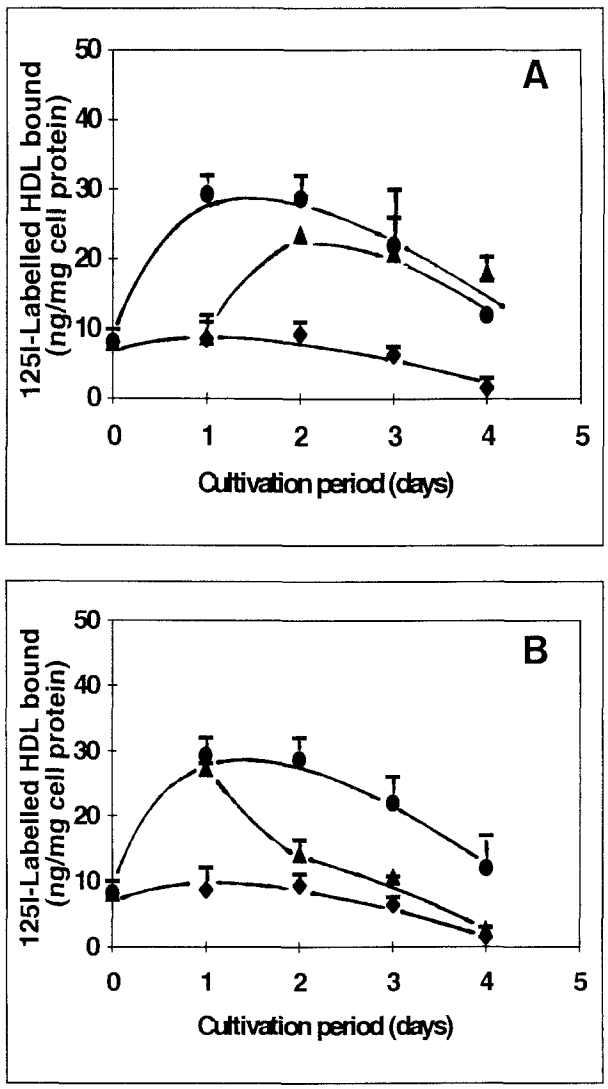


**Fig 6. Reversibility of the effect of DEX on the specific binding of $^{125}$I-HDL$_3$ by cultured rat HEP from AER. (A) HEP isolated from AER were cultured for 4 days in the presence of $10^{-7}$ mol/L DEX (●) 24 hours in the absence of DEX and further in the presence of $10^{-7}$ mol/L DEX (▲), or 4 days in the absence of the hormone (◆). At indicated times, the specific binding of $^{125}$I-HDL$_3$ was determined. (B) HEP isolated from AER were cultured for 4 days in the presence of $10^{-7}$ mol/L DEX (●), 24 hours in the presence of $10^{-7}$ mol/L DEX and further in the absence of DEX (▲), or 4 days in the absence of the hormone (◆). At indicated times, the specific binding of $^{125}$I-HDL$_3$ was determined. The line represents binding measured for NR at 2 hours after isolation.**

*Effect of Cycloheximide on the Processing of $^{125}$I-HDL$_3$ in Culture of HEP*

To answer the question of whether the increase in specific binding and internalization of $^{125}$I-HDL$_3$ observed in the presence of DEX is a result of the elevated synthesis of HDL-binding proteins, the effect of cycloheximide (CH)

was tested in HEP cultured in the presence of $10^{-5}$ mol/L DEX. CH at a concentration of 0.5 mmol/L did not affect the viability of cells tested via the trypan blue exclusion test and the release of LDH (data not shown). Table 2 demonstrates that the specific binding and internalization of $^{125}$I-HDL were inhibited by 82% in the presence of CH. The effect of the inhibitor was shown for HEP isolated from NR, as well as from AER. Although the inhibition was observed for cells cultured both in the presence and absence of the hormone, a more pronounced effect was found for cells stimulated by the hormone.

*Relationship Between Cholesterol Synthesis and the Processing of $^{125}$I-HDL in Response to Lovastatin*

To exclude the possibility that the effect of DEX on the expression of HDL-binding sites was mediated through an elevation of intracellular cholesterol metabolism, we have studied the effect of DEX on both cholesterol synthesis and the processing of HDL in the presence and absence of lovastatin. Figure 7A demonstrates that DEX induced an increase of cholesterol synthesis, as shown by incorporation of $^{14}$C-acetate into digitonin-insoluble lipids in a dose-dependent fashion. There was a fivefold increase in the synthesis of cholesterol at a concentration of $10^{-6}$ mol/L of the hormone. The effect leveled off at concentrations between $10^{-6}$ and $10^{-7}$ mol/L. At concentration of 100 nmol/L, lovastatin inhibited the synthesis of cholesterol by 95% in a cells cultured both in the presence and absence of the hormone at a concentration of $10^{-5}$ mol/L (Fig 7B). Despite the effective inhibition of cholesterol synthesis in both NR and AER by lovastatin, it failed to alter the specific binding and internalization of $^{125}$I-HDL$_3$ by HEP cultured with or without DEX (Table 3).

## DISCUSSION

It has been shown earlier that both short-term administration of GL and chronic GL excess are associated with an elevated level of HDL and HDL cholesterol in the blood.[6-9,11] Long-term administration of GL resulted in an increase in plasma concentrations of both HDL and LDL.[11,35] It is known that GL alter hepatic synthesis and secretion of apo A-I, increasing the HDL concentration; however, little information is available about the effects of GL on cholesterol synthesis and receptor-mediated processing of HDL in the liver, which regulates both cholesterol and HDL concentrations in blood.[6-14,35]

The purpose of this study was to examine the effects of DEX, a synthetic GL, on the expression of HDL-binding

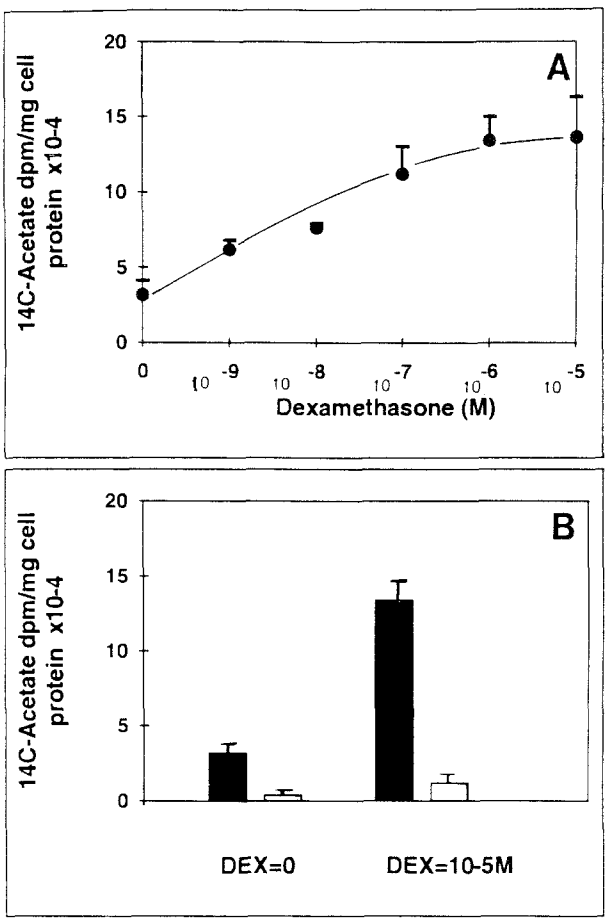


**Fig 7. Dose-dependent effect of DEX on cholesterol synthesis by cultured rat HEP. (A) HEP were cultured for 2 days in the presence of different concentrations of DEX. At 48 hours, cells were used in experiments $^{14}$C-acetate incorporation into digitonin-precipitated lipids (●) was measured. (B) HEP were cultured for 2 days in the absence or presence of $10^{-5}$ mol/L DEX (■). The medium was renewed daily. At 24 hours after seeding in some dishes, lovastatin at 100 mmol/L was added to culture medium (□). At 48 hours, $^{14}$C-acetate incorporation into digitonin-precipitible lipids was measured.**

**Table 2. Effect of CH on Specific Binding and Internalization of $^{125}$I-HDL$_3$ by Cultured Rat HEP**

| Rats | CH (0.5 mmol/L) | DEX ($10^{-6}$ mol/L) | Specific Binding of HDL (ng/mg cell protein) | Specific Internalization of HDL (ng/mg cell protein) |
|---|---|---|---|---|
| Intact | No | No | 9.5 ± 1.1 | 6.8 ± 0.5 |
| | Yes | No | 2.6 ± 0.15 | 2.4 ± 0.12 |
| | No | Yes | 28.9 ± 2.7 | 25.3 ± 2.1 |
| | Yes | Yes | 4.7 ± 0.5 | 6.4 ± 1.31 |
| Adrenalectomized | No | No | 9.2 ± 0.83 | 5.1 ± 0.42 |
| | Yes | No | 2.5 ± 0.3 | 1.7 ± 0.22 |
| | No | Yes | 28.3 ± 0.41 | 20.8 ± 1.67 |
| | Yes | Yes | 5.1 ± 0.34 | 3.8 ± 0.41 |

NOTE. Results are the mean ± SEM and represent the average of at least 4 independent experiments.

**Table 3. Effect of Lovastatin on Specific Binding and Internalization of $^{125}$I-HDL$_3$ by Cultured Rat HEP**

| Specific Binding of HDL (ng/mg cell protein) | Specific Internalization of HDL (ng/mg cell protein) | Lovastatin (100 nmol/L) | DEX ($10^{-5}$ mol/L) |
|---|---|---|---|
| 9.5 ± 1.1 | 6.8 ± 0.5 | No | No |
| 8.3 ± 0.95 | 5.9 ± 1.1 | Yes | No |
| 28.9 ± 2.7 | 25.3 ± 2.1 | No | Yes |
| 22.5 ± 2.5 | 23.3 ± 3.1 | Yes | Yes |

NOTE. Results are the mean ± SEM and represent the average of at least 4 independent experiments.

sites and the processing of iodine-labeled apo E–free $HDL_3$ by cultured parenchymal liver cells. The relationship between intracellular cholesterol metabolism and the receptor-mediated processing of $^{125}I$-$HDL_3$ in response to DEX was studied on cultured HEP.

Since GL affect a number of liver-specific functions, including lipoprotein metabolism,[12-14] we hypothesized that GL would alter cholesterol synthesis and the expression of HDL-binding sites in HEP cultured with DEX. To test this hypothesis, we used cultured rat HEP as a model to examine the effect of GL on cholesterol synthesis and $HDL_3$ uptake in the liver. It is known that cultured HEP retain the responsiveness to GL treatment, increasing transcription of albumin and other protein genes.[36-40] Moreover, it has been found that GL receptors were detected in freshly isolated HEP.[41]

To exclude the influence of uncontrolled plasma-derived factors, the effect of the hormone was studied on cells cultured in well-defined serum-free culture medium.[19,20,30] It is of great significance for our experiments, since the individual concentrations of plasma steroids were found to vary strongly from batch to batch. Moreover, natural GL are metabolized in HEP culture, resulting in uncontrolled changes of hormone concentrations in culture medium. Our choice of DEX instead of natural GL (cortisol) was a result of the observation that DEX, in contrast to natural GL, is not catabolized by cultured HEP and a steady-state concentration of the hormone is maintained in culture medium though the 24-hour period of culture. At these conditions, we have observed a single type of HDL-binding sites on the surface of HEP, with a $K_d$ similar to that reported previously.[15-21,23]

The results of our study demonstrate that both the expression of HDL-binding sites and the specific receptor-mediated internalization of the ligand are upregulated by incubation of HEP with DEX or by injection of the hormone into GL-deficient male rats. The hormone has shown a dose-dependent effect on the specific binding within the wide range of concentrations, including physiologic ones. DEX failed to increase the specific binding and internalization of $^{125}I$-$HDL_3$ over the level demonstrated for freshly isolated cells. Its effect was to maintain a certain level of HDL binding, since DEX prevented the decrease in HDL binding that occurs with an increased time of cell culture. The effect of the hormone was hardly associated with the general anabolic effects of DEX, ie, increasing the metabolic index of cultured cells and preventing the decrease of HDL binding due to an increased proportion of metabolically active viable cells. First, HEP isolated from AER demonstrated the same viability, LDH release, and morphology in 4-day culture as those isolated from NR. Second, during cultivation of HEP isolated from NR, we did not observe substantial release of LDH for the first 4 days of culture in hormone-free conditions and for more than 6 days in the presence of DEX at concentrations of $10^{-7}$ and $10^{-5}$ mol/L (excluding the first 24 hours, where there was a high LDH release). Third, the cells demonstrated a similar pattern of DNA synthesis in the presence of DEX and in hormone-free conditions. Moreover, the cells maintained responsiveness to the action of DEX, demonstrating reversibility of hormone action at 48 hours after isolation. Our cumulative data suggest that DEX may maintain the physiologic level of hepatic receptor-mediated uptake of HDL. As a result of incubation of the cells with DEX, we have observed an increase in the capacity of HDL-binding sites on the surface of the cells, suggesting that the increase of specific binding was due to the expression of a greater number of receptors. This is supported by the observation that CH, an inhibitor of protein synthesis, was found to prevent the upregulating effect of DEX on both the specific binding and internalization of $^{125}I$-$HDL_3$. Moreover, incubation of the cells with CH also decreased the specific binding of $^{125}I$-$HDL_3$ by HEP cultured without DEX. Although DEX did not increase the specific uptake over the physiologic level at concentrations higher than $10^{-7}$ mol/L (corresponding to the physiologic concentration of cortisone), the upregulating effect of DEX on cholesterol synthesis occurred at concentrations higher than $10^{-7}$ mol/L. These data are consistent with the effects of short- and long-term administration of GL to humans and other animals in whom there is an increase in plasma cholesterol.[6-11] The physiologic relevance of these observations remains to be determined. However, the increase in the number of HDL-binding sites would be a result of the increase in cellular cholesterol content, an effect that has been reported previously.[42,43] We have investigated the relationship between the rate of cholesterol synthesis and the expression of HDL-binding sites in response to an incubation of HEP with DEX at a concentration that maximally stimulated cholesterol synthesis in the presence or absence of lovastatin. Our data clearly demonstrate that despite the 95% decrease in cholesterol synthesis, lovastatin was without an effect on the specific binding and internalization of $^{125}I$-$HDL_3$. Therefore, it is reasonable to suggest the existence of a direct cholesterol-independent effect of GL on physiologic levels of HDL-binding sites on the surface of HEP.

So far, GL under physiologic concentrations take part in the maintenance of a certain level of uptake of lipoproteins containing apo A in the liver. Its effect is dose-dependent, reversible, and independent of the availability of de novo synthesized cholesterol in HEP. Both the reversibility of the effect of DEX on the capacity of HDL-binding sites and downregulating effects of CH suggest that DEX probably acts through an interaction with corresponding GL receptors. This is supported by the observation that the increase of GL over physiologic concentrations does not induce the following increase of the processing of HDL in terms of specific binding and internalization. The upregulating effect of GL is limited by GL deficiency, as we show in experiments with AER. At the same time, GL at a pharmacologic dose induced an increase in the synthesis of cholesterol, probably resulting in the elevated plasma cholesterol concentrations, which has been reported previously.[6-11] Taking into consideration that GL decreased LDL-receptor activity in the liver, it is possible to suggest that the

increase of cholesterol synthesis, among other factors, may be the cause of the increase of plasma cholesterol concentration during long-term administration of GL.[44]

Under physiologic circumstances, there are not available data concerning the consequences of GL on reverse cholesterol transport in the literature. However, it is possible to speculate that GL maintenance of a certain level of HDL uptake in the liver upregulates a turnover of HDL cholesterol across the liver. Although the role of HDL-binding sites in cholesterol delivery to the liver has failed to find general acceptance, several data have demonstrated that a substantial portion of cholesterol can be delivered to the liver as a result of the receptor-mediated retroendocytosis of HDL in hepatic cells.[45] Moreover, our data (manuscript in preparation) demonstrate that the incubation of rat HEP with $HDL_2$ in the presence of DEX induces pronounced activation of bile acid synthesis, whereas there are not statistically significant effects under hormone-free conditions. This allows us to speculate that GL upregulation of HDL binding in the liver can stimulate or maintain a certain level of cholesterol delivery to hepatic parenchymal cells. This body of questions is under investigation.

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