Effect of ursodeoxycholic acid on hepatic LDL binding and uptake in dietary hypercholesterolemic hamsters

Susan Ceryak a, Bernard Bouscarel a,b,*, Mauro Malavolti c, Sander J. Robins d, Kathleen L. Caslow a, Hans Fromm a

a Department of Medicine, Division of Gastroenterology and Nutrition, The George Washington University Medical Center, 2300 I St, N.W. 523 Ross Hall, Washington, DC 20037, USA
b Department of Biochemistry and Molecular Biology, The George Washington University Medical Center, 2300 I St, N.W. 523 Ross Hall, Washington, DC 20037, USA
c Istituto di Clinica Medica e Gastroenterologica, University of Bologna, Bologna, Italy
d Lipid Metabolism Laboratory and Department of Medicine, Veterans Administration Medical Center, Boston, MA, USA

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Abstract

Administration of ursodeoxycholic acid (UDCA) has been shown to decrease serum total and low density lipoprotein (LDL) cholesterol in hypercholesterolemic patients with primary biliary cirrhosis. Results of previous studies prompted us to postulate that the cholesterol-lowering effect of UDCA may be due, at least in part, to a direct increment in hepatic LDL receptor binding [Bouscarel et al., Biochem J, 1991;280:589; Bouscarel et al., Lipids 1995;30:607]. The aim of the present investigation was to determine the ability of UDCA to enhance hepatocellular LDL receptor recruitment, as determined by its effect in vivo on LDL uptake, and its effect in vitro on LDL binding, under conditions of moderately elevated serum cholesterol. Study groups consisted of male golden Syrian hamsters fed either a standard chow diet (control), a 0.15% cholesterol-containing diet, or a 0.15% cholesterol-containing diet supplemented with either 0.1% UDCA, or 0.1% chenodeoxycholic acid (CDCA). Cholesterol feeding increased ($P<0.01$) total serum cholesterol by 44%, and was associated with a 10-fold accumulation of cholesteryl esters in the liver ($P<0.01$). In vivo, hepatic uptake of [U-14C]sucrose-labeled hamster LDL was increased ($P<0.05$) to a level of 454 ± 101 ml in animals fed a cholesterol-containing diet supplemented with UDCA, compared to that either without UDCA (337 ± 56 ml), or with CDCA (240 ± 49 ml). The hepatic uptake of [U-14C]sucrose-labeled methylated human LDL, a marker of LDL receptor-independent LDL uptake, was unaffected by bile acid feeding. In vitro, specific binding of [125I]hamster LDL to isolated hepatocytes was determined at 4°C, in presence and absence of 700 μmol/l UDCA. The $K_D$ ranged from 25 to 31 μg/ml, and was not affected by either cholesterol feeding or UDCA. In the presence of UDCA, the $B_{max}$ was increased by 19% ($P<0.05$) in cells isolated from control animals and by 29% ($P<0.01$) in cells isolated from hamsters fed a cholesterol-supplemented diet. In conclusion, in dietary hypercholesterolemic hamsters, both chronic in-vivo and acute in-vitro treatments with UDCA resulted in restoration of hepatic LDL binding and uptake to levels observed in control hamsters. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cholesterol metabolism; Isolated hepatocytes; Bile acids

1. Introduction

The low density lipoprotein (LDL) receptor plays a central role in the regulation of cholesterol homeostasis, while the liver is a key organ in the maintenance of whole body cholesterol balance [1,2]. In a number of species, including the hamster, most of receptor-mediated clearance of LDL occurs in the liver [3–5] by rapid internalization of the complex formed by the binding of the apolipoprotein-B100-containing particle to the LDL receptor [6]. Once in the cell, the LDL particle dissociates, and the receptor generally is cycled back to the cell surface membrane [7].
The activity of the LDL receptor is tightly regulated by changes in cholesterol supply and demand. Hepatic LDL receptor synthesis and cycling can be markedly suppressed by dietary cholesterol [8,9]. Conversely, administration of both bile acid binding resins, which augment hepatocellular bile acid synthesis, and cholesterol synthesis inhibitors, increase the number of LDL receptors, by increasing the cellular cholesterol demand [10,11]. The consequent changes in cellular cholesterol pools modulate the production of LDL receptor mRNA and the synthesis of the LDL receptor.

It has previously been shown in vivo, in the golden Syrian hamster, that chronic feeding of ursodeoxycholic acid (UDCA), in contrast to that of its 7α-hydroxyl epimer, chenodeoxycholic acid (CDCA), evoked a significant increment in hepatic LDL uptake. However, this occurred in spite of both a 55–71% enrichment of the bile acid pool with CDCA and a marked suppression of bile acid synthesis [12]. This increased LDL receptor-dependent LDL uptake and degradation in vitro have also been documented, upon acute exposure to UDCA, in hepatocytes isolated from standard rodent chow-fed hamsters [13,14]. Furthermore, recent studies from our laboratory have demonstrated that UDCA interacts directly with the LDL receptor, in the absence of any effects on either the LDL particle or on the membrane lipid composition. It is independently of any effects on LDL receptor synthesis or cycling [13,15]. Thus, the ability of UDCA to directly augment LDL receptor-dependent LDL uptake is independent of changes in cellular cholesterol pools.

Administration of UDCA has been shown to decrease serum total and LDL cholesterol in hypercholesterolemic patients with primary biliary cirrhosis [16]. In light of results of previous studies, it was postulated that the cholesterol-lowering effect of UDCA may be due, in part, to a direct increment in LDL receptor binding [13,15]. The present investigation was undertaken in order to determine the ability of UDCA to enhance hepatocellular LDL receptor recruitment, as determined by its effect both in vivo on LDL uptake, and in vitro on LDL binding, under conditions of moderately elevated serum cholesterol concentrations [17]. This was achieved by feeding male golden Syrian hamsters an excess of cholesterol in order to induce mild hypercholesterolemia. Under these conditions, the effects of UDCA on parameters of hepatic LDL metabolism were studied both in vivo and in vitro.

2. Materials and methods

2.1. Materials

Sodium $^{125}$I (specific activity 16–20 mCi/μg) and [U-$^{14}$C]sucrose (specific activity 380 μCi/μmol) were purchased from Amersham Corporation (Arlington Heights, IL). Gelatin and bovine serum albumin (fraction V) were purchased from Sigma (St. Louis, MO). UDCA was supplied by Tokyo Tanabe (Tokyo, Japan), and CDCA by Dr Falk GmbH (Freiburg, Germany). Both UDCA and CDCA were 98–99% pure, as judged by gas–liquid chromatography. All other chemicals used were of analytical grade available from commercial sources.

2.2. Animals

Male golden Syrian hamsters (115–140 g body weight; Harlan Sprague Dawley, Indianapolis, IN), were divided into four groups, each of which consisted of 10–13 age-matched animals. The first group was fed a standard rodent chow diet (Ralston Purina, St. Louis, MO), containing 0.027% cholesterol (control, CONT). The second group received the standard diet in which the cholesterol content had been increased to 0.15% (cholesterol, CHOL). In the other two groups, the 0.15% cholesterol-containing diet was supplemented with 0.1% UDCA (CHOL-UDCA) and 0.1% CDCA (CHOL-CDCA), respectively. The relative percentage of saturated, monounsaturated, and polyunsaturated fatty acids in the diet was 1.41, 1.44, and 1.65%, respectively, with the predominant species of 16:0 (66%), 18:1 (92%), and 18:2 (84%) (Purina Mills). Each group received the specific diet for 3 weeks. There were no differences among the treatment groups as far as food intake and weight gain were concerned. Mean food intake was 6.5 g/day, while mean body weight ranged from 120–140 g following treatment. The percentage of cholesterol in the cholesterol-supplemented diets corresponded to a daily intake of 0.075 g/day per kg body weight. Both bile acids were well tolerated by the animals, and livers appeared macroscopically normal after 3 weeks of the respective cholesterol and bile acid feeding. All animals received humane care in compliance with the George Washington University guidelines.

2.3. Serum lipid determinations

The total serum cholesterol concentration was determined enzymatically using the cholesterol esterase–cholesterol oxidase/phenol-4-amine phenazone reaction (Boehringer Mannheim Diagnostics, Indianapolis, IN). Serum high density lipoprotein (HDL) cholesterol was measured as described for total serum cholesterol after precipitation of apo B-containing lipoproteins with dextran sulfate-MgCl₂, using the method of Warnick et al. [18].
2.4. Hepatocellular lipid determinations

Livers were removed from Nembutal-anesthetized hamsters 3 weeks after feeding a control diet, or a cholesterol-supplemented diet. Liver homogenates and enriched plasma membrane fractions were prepared by the method of Prip' et al. [19], as previously described [20]. Cellular and membrane protein content was assessed with a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

Total lipids were extracted from aliquots of liver homogenate and enriched plasma membrane fractions, respectively, according to the method of Bligh and Dyer [21]. Lipid extracts were kept under nitrogen in glass tubes to prevent oxidative degradation. Phospholipid and cholesterol amounts were determined in liver homogenate and plasma membrane lipid extracts using HPLC procedures previously described [22]. Quantitation was performed by integration of peak areas in conjunction with internal standards. Total phospholipid was quantitated by phosphorus measurement [23]. Lipid concentrations were expressed per mg of protein.

2.5. Biliary bile acid composition

In order to confirm the enrichment of the respective bile acid pool by either UDCA or CDCA in the hamsters receiving the bile acid-supplemented diets, the relative bile acid composition was determined, by gas–liquid chromatography, in gallbladder bile as previously described [12,15].

2.6. Biliary lipid determinations

Total bile acid concentration was measured, using 3α-hydroxysteroid dehydrogenase (Worthington Biochemicals, Malvern, PA) [24]. Biliary phosphatidylcholine concentration was determined by the enzymatic measurement of choline content using a choline oxidase/peroxidase reaction following treatment with phospholipase D (Nippon Shoji Kaisha, Higashi-Ko, Osaka, Japan) [25]. The biliary cholesterol content was analyzed enzymatically, using a cholesterol oxidase/catalase reaction (Boehringer Mannheim Biochemicals, Indianapolis, IN) [26].

2.7. Separation of plasma LDL

Blood was collected from normal human subjects, as well as from normocholesterolemic hamsters, into EDTA-containing vacuum collection tubes. The plasma lipoproteins were separated by density gradient ultracentrifugation, as previously reported [12–14,27]. The total protein concentration of the LDL fraction was determined by the method ofBradford [28], using the Bio-Rad® protein assay (Bio-Rad Laboratories, Richmond, CA). The purity of the LDL fraction was confirmed by SDS-page as described [12–14]. In some studies, human LDL was reductively methylated, as previously described [12–14].

2.8. In-vivo hepatic uptake of [14C]sucrose-labeled LDL

Animals were maintained under diethyl ether anesthesia for the duration of the surgical procedure as well as the subsequent infusion experiments. For in-vivo studies, both hamster LDL and methylated human LDL were radiolabeled with [U-14C]sucrose [12,27]. The final [14C]sucrose-labeled LDL had a specific activity of 1188 ± 719 dpm/μg protein. Either [14C]sucrose-labeled hamster LDL or methylated human LDL was administered via a jugular vein catheter (Silastic®, I.D., 0.012 in.; O.D., 0.025 in., Dow Corning, Midland, MI) in a bolus, containing 20 μg of LDL protein, followed by a constant infusion for 1 h at a rate of 1 μg/70 μl normal saline/min [12,27]. All LDL infusion experiments were carried out between 09:00 and 12:00 h. Each study on a given day was carried out in three animals, one of each group (CHOL, UDCA-CHOL, CDCA-CHOL), using the same LDL preparation. After completing the LDL infusion at 60 min, the abdomen was opened with a midline incision, and a 50 μl sample of blood was withdrawn from the inferior vena cava, the gallbladder bile was aspirated, the liver was removed, and the animals were exsanguinated. Livers were combusted in toto in a Packard Oxidizer (Packard Instrument, Downers Grove, IL) [29]. The radioactivity in the combusted liver and blood was determined by scintillation counting.

The LDL tissue space in the liver was obtained by subtracting the [14C]albumin tissue space, which was previously determined by the infusion of [14C]sucrose-labeled hamster albumin (Research Plus, Bayonne, NJ) [12,27], from the [14C]LDL tissue space. The uptake in the liver was normalized for an animal weighing 100 g.

2.9. Hepatocyte isolation

Hamsters were anesthetized with Nembutal (70 mg/kg body wt), and hepatocytes were isolated by collagenase perfusion, as previously described [13–15]. Cells were suspended in a Krebs–Henseleit bicarbonate buffer, pH 7.4, containing 1.5% gelatin, at a concentration of approximately 40–50 mg cell wet wt/ml. Prior to each experiment, hepatocytes were incubated for 20–30 min at 37°C under constant agitation and gassing, in order for the cells to reach a steady-state [13–15].
2.10. In-vitro hepatocellular $^{125}$I/LDL binding

Native hamster LDL isolated from normocholesterolemic animals was labeled with $^{125}$I sodium iodide to a specific radioactivity of 80–200 cpm/ng of protein, as previously described [13–15], using the iodine monochloride technique [30,31].

With hepatocytes isolated in tandem from 1 hamster of each group fed the 0.027 and the 0.15% cholesterol-containing diet, respectively, LDL binding was studied [13,14]. The cells (150–250 mg protein/ml) were incubated with increasing concentrations of $^{125}$I-labeled hamster LDL (1–120 μg protein/ml) in the presence and absence of 700 μmol/l UDCA. Previous studies have shown that, at this concentration, UDCA is not toxic to the cell [32], and induces a maximum increase in hepatocellular LDL binding [13,14]. After 1 h incubation at 4°C, the cell-associated radioactivity was measured in a Beckman model 4000 gamma-radiation counter. Nonspecific binding was determined by incubating the cells under the same conditions, but with an excess of native human LDL (2–2.5 mg/ml) [13,15]. Specific binding was determined by subtracting nonspecific binding from total binding. Saturation binding curves were derived, and the maximum number of binding sites ($B_{\text{max}}$, ng/mg protein) and the dissociation constant ($K_d$, μg LDL protein/ml) were calculated [13,15].

Table 1
Serum lipid concentrations in cholesterol-fed hamsters*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>HDL</th>
<th>Non-HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>%</td>
</tr>
<tr>
<td>CONT</td>
<td>127 ± 6</td>
<td>53 ± 4</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>CHOL</td>
<td>183 ± 15*</td>
<td>74 ± 17</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>CHOL-UDCA</td>
<td>200 ± 14*</td>
<td>84 ± 6*</td>
<td>43 ± 3***</td>
</tr>
<tr>
<td>CHOL-CDC</td>
<td>210 ± 10*</td>
<td>66 ± 5</td>
<td>32 ± 3**</td>
</tr>
</tbody>
</table>

* Respective groups of hamsters were fed either a standard chow diet, containing 0.027% cholesterol (CONT, control); a 0.15% cholesterol-containing diet (CHOL); the 0.15% cholesterol-containing diet supplemented with 0.1% ursodeoxycholic acid (CHOL-UDCA); or the same diet supplemented with 0.1% chenodeoxycholic acid (CHOL-CDCA) for 3 weeks. At the end of this period, serum cholesterol was determined photometrically, by standard enzymatic methods. High density lipoprotein (HDL) cholesterol was determined photometrically in serum following precipitation of the apoB-containing lipoproteins with 2% dextran sulfate.1 M MgCl$_2$, Non-HDL cholesterol was determined by subtraction. Results are the means ± S.E.M. of determinations in 8–13 animals of each group.

** Significantly different from CONT group, $P<0.01$.

*** Significantly different from CHOL group, $P<0.01$.

2.11. Statistical analysis

The paired $t$-test was used to compare the acute effect of the respective UDCA treatment and dietary cholesterol supplementation with that of the respective controls. An analysis of variance, and Student–Neuman–Keuls test were used to compare the differences in the measured parameters as a result of bile acid feeding.

3. Results

3.1. Serum lipid concentrations

After 3 weeks of feeding a 0.15% cholesterol-supplemented diet, total serum cholesterol concentrations increased by 44%, (Table 1). The percentage of serum cholesterol associated to HDL particles was significantly decreased from 48 to 44% of the total serum cholesterol in the hypercholesterolemic animals.

In those hamsters fed a 0.15% cholesterol-supplemented diet, CDCA supplementation induced a significant decrease in the percentage of serum HDL cholesterol, and a consequent increase in the proportion of non-HDL cholesterol, when compared to both the UDCA-fed animals, and the bile acid-free diet group (CHOL).

3.2. Hepatocellular lipid concentrations

Both the unesterified cholesterol and total phospholipid concentrations were markedly higher, per mg of protein in the isolated liver membrane fraction, when compared to that in the total homogenate (data not shown). As a result of cholesterol feeding, there was a 10-fold accumulation of cholesteryl esters observed in the liver homogenate (Table 2), and the absolute concentrations of unesterified and esterified cholesterol were calculated to be 3.8 ± 0.08 and 38.7 ± 5.7 μmol/g tissue. However, there were no changes in the unesterified cholesterol content, in either the homogenate or membrane fractions. Furthermore, there was no change in the phospholipid content or composition of either the liver homogenate or of the plasma membrane, as a result of cholesterol feeding (data not shown).

3.3. Biliary bile acid and lipid composition

The biliary bile acid composition in the bile acid-treated groups is shown in Table 3. In the animals fed the 0.15% cholesterol-containing diet, CDCA content in bile increased significantly during chronic treatment with CDCA, but was not increased by UDCA treatment, in contrast to what has previously been observed in normocholesterolemic hamsters [12]. Corresponding decreases in the relative percentages of biliary deoxy-
Table 2
Lipid content of liver homogenates of cholesterol-fed hamsters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unesterified cholesterol (nmol/mg protein)</th>
<th>Cholesteryl esters (nmol/mg protein)</th>
<th>Phospholipids (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONT</td>
<td>26 ± 3</td>
<td>3 ± 0.3</td>
<td>22 ± 16</td>
</tr>
<tr>
<td>CHOL</td>
<td>30 ± 0.3</td>
<td>300 ± 31*</td>
<td>17 ± 2</td>
</tr>
</tbody>
</table>

* Respective groups of hamsters were fed either a standard, 0.027% cholesterol-containing chow diet (CONT, control), or a 0.15% cholesterol-containing diet (CHOL) for 3 weeks. At the end of this period, livers were removed, homogenized, and the lipids associated with the homogenate were extracted into chloroform/methanol, 1:2, v/v and measured as indicated in Section 2. Results are the means ± S.E.M. of determinations in three animals of each dietary treatment group.

* Significantly different from respective control, P<0.01.

Table 3
Biliary bile acid composition in dietary hypercholesterolemic hamsters following chronic bile acid feeding

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LCA</th>
<th>DCA</th>
<th>CDCA</th>
<th>UDCA</th>
<th>CA</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOL</td>
<td>2 ± 0.2</td>
<td>15 ± 2</td>
<td>45 ± 3</td>
<td>n.d.</td>
<td>41 ± 4</td>
<td>1 ± 0.7</td>
</tr>
<tr>
<td>CHOL-UDCA</td>
<td>3 ± 0.1</td>
<td>8 ± 4</td>
<td>48 ± 5</td>
<td>30 ± 3*</td>
<td>12 ± 3*</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>CHOL-CDCA</td>
<td>5 ± 0.5*</td>
<td>6 ± 1*</td>
<td>77 ± 2*</td>
<td>n.d.</td>
<td>10 ± 1*</td>
<td>1 ± 0.8</td>
</tr>
</tbody>
</table>

* Respective groups of hamsters were fed either a 0.15% cholesterol-containing diet (CHOL), the 0.15% cholesterol-containing diet supplemented with 0.1% ursodeoxycholic acid (CHOL-UDCA), or the same diet supplemented with 0.1% chenodeoxycholic acid (CHOL-CDCA) for 3 weeks. At the end of this period, relative bile acid composition was determined by GLC in gallbladder bile. Results are the means ± S.E.M. of determinations in four animals of each treatment group. ‘Other’ represents unidentified gas chromatographic peaks. Other abbreviations used: LCA, lithocholic acid; DCA, deoxycholic acid; CA, cholic acid.

* Significantly different from control, P<0.02.

Cholic and cholic acids were observed following chronic treatment with both bile acids, due to the relative enrichment in the respective fed bile acid, and its metabolites.

No significant differences were observed in the respective biliary cholesterol, phospholipid and bile acid concentrations in the gallbladder bile obtained from animals fed a 0.15% cholesterol-supplemented diet when compared to those of the control animals, and the respective values were around 1.6, 3.2, and 62 mmol/l (data not shown). In a small number (2–3) of animals fed the bile acid-supplemented diets, there were also no significant differences in the biliary lipid composition as a result of bile acid supplementation (data not shown).

3.4. In-vivo hepatic LDL uptake

As reported in Table 4, in the animals fed a 0.15% cholesterol-containing diet supplemented with UDCA, the total hepatic uptake of [U-14C]sucrose-labeled hamster LDL was significantly increased by 35 and 89% compared to the respective bile acid-free (CHOL) and CDCA-supplemented diets. In contrast, the receptor-independent LDL uptake showed no significant differences among the different study groups. Furthermore, as shown in Fig. 1, in hypercholesterolemic hamsters, stimulation of hepatic LDL uptake was positively correlated (r = 0.6729, P < 0.02) with an increase in the percentage of serum HDL cholesterol.

Table 4
In-vivo hepatic low density lipoprotein (LDL) uptake and serum cholesterol concentrations in hypercholesterolemic hamsters following chronic bile acid feeding

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hepatic LDL uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster LDL (µl/organ)</td>
<td>Meth Human LDL (µl/organ)</td>
</tr>
<tr>
<td>CONT(a)</td>
<td>412 ± 135</td>
</tr>
<tr>
<td>CHOL</td>
<td>337 ± 56</td>
</tr>
<tr>
<td>CHOL-UDCA</td>
<td>454 ± 101**</td>
</tr>
<tr>
<td>CHOL-CDCA</td>
<td>240 ± 49</td>
</tr>
</tbody>
</table>

* For experimental design and abbreviations, see legends of Table 1. Hepatic LDL uptake was determined in hamsters receiving the respective indicated diets, after 1 h intravenous infusion of either [U-14C]sucrose-labeled hamster LDL or methylated (meth) human LDL. The tissue space of LDL (µl/organ) was calculated, and liver uptake was normalized for an animal weighing 100 g. Results are the means ± S.E.M. of determinations of LDL uptake in five animals of each group. (a) Values obtained from a previously published study conducted with the same group of hamsters [12], in which animals were fed a standard chow diet, containing 0.027% cholesterol (CONT, control), results are the means ± S.E.M. of determinations in five animals, and are not used in statistical analysis.

* Significantly different from CHOL group, P < 0.05
** Significant difference between bile acid-fed animals, P < 0.05.

3.5. Effect of UDCA on the specific binding of [125I]hamster LDL to hepatocytes isolated from hypercholesterolemic hamsters

With hepatocytes isolated in tandem from one animal of each group of hamsters receiving either a control diet, or a 0.15% cholesterol-supplemented diet, LDL binding was studied at 4°C, using increasing concentrations (1–120 μg/ml protein) of [125I]hamster LDL (Fig. 2). An excess of native human LDL (2.5 mg/ml) was used to measure nonspecific LDL binding. The nonspecific binding was not affected by cholesterol feeding in either the presence or absence of UDCA and represented around 25% of the total binding. The $B_{\text{max}}$ was 103 ± 43 and 94 ± 38 ng/mg of cells, in the control and cholesterol-fed groups, respectively, and was not significantly different. In the presence of UDCA, the $B_{\text{max}}$ was significantly increased by 19% in the hepatocytes isolated from the control-fed animals, and by 29% in the hepatocytes isolated from hamsters fed a 0.15% cholesterol-supplemented diet. Analysis of the saturation curves showed that the $K_D$ was not significantly different from control following cholesterol feeding and furthermore, the $K_D$ was not affected by UDCA (Table 5, Fig. 2).

4. Discussion

Results of the present study in dietary hypercholesterolemic hamsters indicate that both chronic, in-vivo and acute, in-vitro treatments with UDCA were able to restore the respective hepatic LDL binding and uptake to the levels observed in normocholesterolemic hamsters. Furthermore, the administration of UDCA in conjunction with a high cholesterol diet suppressed the biotransformation of UDCA to CDCA. This possibly removed some of the repressive effects of CDCA on hepatic LDL uptake, namely, an inhibition of bile acid synthesis [33], and the consequent decrease in LDL receptor activity [34], thus augmenting the stimulatory effects of UDCA.

Hamster serum cholesterol was significantly increased after 3 weeks of cholesterol supplementation, reflecting an increase in the total amount of cholesterol associ-

![Fig. 1. Correlation between in-vivo hepatic uptake of [U-14C]sucrose-labeled hamster low density lipoprotein (LDL) and percent serum high density lipoprotein (HDL) cholesterol. Respective groups of hamsters were fed either a 0.15% cholesterol-containing diet (CHOL), the 0.15% cholesterol-containing diet supplemented with 0.1% ursodeoxycholic acid (CHOL-UDCA), or the same diet supplemented with 0.1% chenodeoxycholic acid (CHOL-CDCA). After 3 weeks of feeding the respective indicated diets, total liver uptake of LDL was determined, following 1 h intravenous infusion of [U-14C]sucrose-labeled hamster LDL. The tissue space of LDL ($\mu$L:g) was calculated, and the hepatic uptake of LDL was normalized for an animal weighing 100 g. Both total and HDL cholesterol concentrations in serum were determined photometrically. The correlation coefficient ($r$), $P$-value, and equation of the regression line are also reported.](image1)

![Fig. 2. Effect of ursodeoxycholic acid (UDCA) on the specific binding of [125I]hamster low density lipoprotein (LDL) to hepatocytes isolated from cholesterol-fed hamsters. Hepatocytes were isolated in tandem from one animal of each group of hamsters receiving either the 0.027% cholesterol-containing control diet (A) or the diet containing 0.15% cholesterol (B). The binding of increasing concentrations (1–120 μg/ml) of [125I]hamster LDL to isolated hamster hepatocytes (40–50 mg of cells) was measured after incubation for 60 min, at 4°C, in the absence (+UDCA, ▲) and in the presence of 700 μmol/l UDCA (+UDCA, ▲). Nonspecific binding was determined by incubating the cells under the same conditions but in the presence of an excess of native human LDL (2.5 mg/ml). The saturation curves are representative of four experiments performed with hepatocytes isolated in tandem from animals of each treatment group.](image2)
Table 5
Effect of ursodeoxycholic acid (UDCA) in vitro on apparent low density lipoprotein (LDL) binding constants determined in hepatocytes isolated from cholesterol-fed hamsters

<table>
<thead>
<tr>
<th></th>
<th>$K_D$ (µg/ml)</th>
<th>$B_{\text{max}}$ (ng/mg of cells)</th>
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<tbody>
<tr>
<td></td>
<td>−UDCA</td>
<td>+UDCA</td>
</tr>
<tr>
<td></td>
<td>−UDCA</td>
<td>+UDCA</td>
</tr>
<tr>
<td>CONT</td>
<td>28 ± 6</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>CHOL</td>
<td>39 ± 12</td>
<td>41 ± 11</td>
</tr>
</tbody>
</table>

* For experimental design and abbreviations, see legend of Table 1. Hepatocytes were isolated in tandem from one animal of each group of hamsters receiving either a CONT or CHOL diet. The binding of increasing concentrations (1–120 µg/ml) of [125I]hamster LDL to isolated hamster hepatocytes (40–50 mg of cells) was measured after incubation for 60 min, at 4°C in the absence (− UDCA, control) and in the presence of 700 µmol/l UDCA (+ UDCA). Nonspecific binding was determined by incubating the cells under the same conditions but in the presence of an excess of native human LDL (2.5 mg/ml). Results are the means ± S.E.M. of four different experiments performed with hepatocytes isolated from one animal of each treatment group.

* $P<0.05$: significantly different in the presence of UDCA.

** $P<0.01$.

Furthermore, in spite of the absence of an absolute serum LDL cholesterol-lowering effect of UDCA, the enhanced LDL uptake should result in a decreased residence time in the bloodstream. Walzem et al. [39] have demonstrated that the susceptibility to in-vitro oxidation of LDL particles was highly correlated with lipoprotein age in-vivo. Furthermore, there is considerable evidence that the level of oxidation of the LDL particle may directly influence its atherogenicity [40]. Therefore, if the residence time of the LDL particle in the bloodstream is shorter, due to UDCA-induced enhanced hepatic uptake, the potential for oxidative modification of the LDL particle may be decreased. This could support a therapeutic role for UDCA in the treatment of mild hypercholesterolemia, however, this remains to be confirmed.

Increased cholesterol/phospholipid ratios in lipid bilayers have been shown to increase the stability, and consequently, the activity of integral membrane spanning proteins, such as the nicotinic acetylcholine receptor [41]. In addition, van de Heijning et al. [42] have shown that UDCA protects the cholesterol integrity of vesicles. This stabilizing effect of UDCA is enhanced with increasing vesicular cholesterol/phospholipid ratio [43,44]. In this study it has been shown that cholesterol feeding does not alter the hepatic membrane cholesterol/phospholipid ratio. In light of the present results, it was postulated that under the condition of hypercholesterolemia, domains of the plasma membrane may be selectively enriched in cholesterol, which, in turn, may affect the interaction of the LDL receptor protein with UDCA, as well as further facilitate its binding to the LDL particle.

The in-vivo 35% increment of LDL uptake associated with UDCA feeding was paralleled by the in-vitro 29% increase in LDL binding effected by UDCA in hepatocytes isolated from hypercholesterolemic hamsters. Administration of UDCA has been shown to decrease serum total and LDL cholesterol in hypercholesterolemic patients with primary biliary cirrhosis [16]. The putative cholesterol-lowering effect of UDCA was thought to occur due to its ability to decrease cholesterol absorption in the intestine [45]. A strong association between intestinal cholesterol absorption and total plasma cholesterol level through regulation of hepatic cholesterol pools has been well-documented [46]. Indeed, the addition of UDCA to a cholesterol-supplemented diet significantly lowered cholesterol absorption and hepatic cholesterol concentration in hamsters [47]. While it can not be ruled out that UDCA may have additional regulatory effects on hepatocellular cholesterol pools, in light of the present results, it is clear that a direct, UDCA-induced augmentation of LDL receptor binding may be responsible, at least in part, for this effect in hypercholesterolemic patients.
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