Low-density lipoproteins are degraded in HepG2 cells with low efficiency

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In previous studies we have shown that in HepG2 cells, as compared with fibroblasts, the low-density lipoprotein (LDL) receptor is only weakly down-regulated upon incubation of the cells with LDL. To explain this difference in down-regulation of the LDL-receptor activity, we studied simultaneously the intracellular processing of ¹²⁵I-labelled LDL in both cell lines. Upon incubation of HepG2 cells with ¹²⁵I-LDL, the appearance of degradation products started at 90 min, whereas in fibroblasts this lag time was only 30 min. The degradation efficiency (representing the ratio degradation/cell association of LDL) in HepG2 was less than 50% of that in fibroblasts up to 5 h of incubation at 37 °C. The longer lag time and low efficiency of the degradation of LDL in HepG2 cells were independent of the cell density. Pulse-chase experiments indicated that the internalization rate of surface-bound LDL in HepG2 cells is similar to that of fibroblasts. Endosomal loading of ¹²⁵I-LDL by incubation

at 18 °C for 4.5 h, followed by a shift to 37 °C, resulted in degradation of LDL within 30 min in fibroblasts, whereas in HepG2 cells the lag time of the degradation was 90 min. In parallel experiments using subcellular fractionation by Percoll-gradient centrifugation of homogenized cells and ¹²⁵I-tyramine-cellobiose-labelled LDL, we observed that in both cell types LDL is equally rapidly shifted from a low- to a high-density compartment (within 15 min), representing the endosomal and the late-endosomal plus lysosomal compartment respectively. We conclude that in HepG2 cells the cell-bound LDL, upon internalization, goes through the intracellular itinerary at the same rate as in fibroblasts, but that either the fusion between late endosomes and lysosomes or the lysosomal degradation itself is proceeding at a lower efficiency. A low degradation rate of LDL may contribute to explain the relatively weak down-regulation of the LDL-receptor activity in HepG2 cells by LDL.

INTRODUCTION

It has been widely demonstrated that the liver is the major site of removal of low-density lipoproteins (LDL) from the circulation, accounting for about 80% of the total LDL catabolism [1]. Up to 70% of LDL is cleared from the plasma via high-affinity binding to receptors gathered in coated pits on the plasma membrane. After binding, the coated pits invaginate, after which endosomes are formed and, upon acidification, the LDL dissociates from the receptor. The receptor returns to the surface, binds another lipoprotein particle and initiates another cycle of endocytosis. After dissociation from the receptor, LDL is delivered to the lysosomes, where the protein component of LDL is hydrolysed to amino acids. The cholesterol released from the degraded LDL leads to a decrease in the cholesterol biosynthesis *de novo*, activates the esterification of cholesterol to cholesteryl esters and suppresses the synthesis of LDL receptors *de novo* by blocking gene expression. These co-ordinated actions allow the cells to be provided with sufficient cholesterol for metabolic needs without causing over-accumulation of free cholesterol (for review, see [2]).

To study the regulation of LDL-receptor activity and cellular cholesterol homeostasis, HepG2 cells are often used as a model for human hepatocytes [3–5]. HepG2 cells have been shown to possess functional LDL receptors with properties similar to those of human fibroblasts [3]. Previous studies performed in our laboratory have shown that the LDL receptors in both HepG2 cells and freshly isolated human hepatocytes are much less responsive to down-regulation by extracellular LDL than are LDL receptors in fibroblasts [6,7]. In addition, the LDL-receptor activity in HepG2 cells and human hepatocytes was found to be

stimulated 2–3-fold by the presence of cholesterol acceptors such as heavy high-density lipoproteins (HDL), whereas in fibroblasts the LDL-receptor activity was almost insensitive to the presence of heavy HDL.

Studies of the cellular cholesterol homeostasis in relation to the LDL-receptor activity in HepG2 cells have suggested that both the LDL cholesterol and the endogenously synthesized cholesterol are primarily directed to a cholesteryl ester pool or, if present, to extracellular cholesterol acceptors, like heavy HDL, rather than to the cholesterol pool involved in the regulation of the LDL-receptor activity [8]. We reasoned that more information about the intracellular pathway of LDL was needed to explain the marked difference between HepG2 cells and fibroblasts in their ability to modulate LDL-receptor activity in response to extracellular LDL cholesterol. Therefore, we studied the kinetics of binding, internalization, distribution into the endosomal-lysosomal compartment and degradation of LDL in both HepG2 cells and fibroblasts. We found that in HepG2 cells the LDL particles are internalized and delivered to the late-endosomal or lysosomal compartment at the same rate as in fibroblasts, whereas in HepG2 cells the degradation of LDL occurs rather inefficiently. This observation may provide an additional explanation for our previous findings that in HepG2 cells the LDL-receptor activity is poorly down-regulated upon incubation of the cells with exogenous (LDL) cholesterol.

MATERIALS AND METHODS

Materials

Fetal-calf serum (FCS) and Dulbecco's modified Eagle's medium (DMEM; cell culture medium) were obtained from Flow Labora-

Abbreviations used: LDL, low-density lipoproteins; DMEM, Dulbecco's modified Eagle's medium; HSA, human serum albumin; PBS, phosphate-buffered saline; FCS, fetal-calf serum; TC, tyramine-cellobiose; LPDS, lipoprotein-depleted serum; HDL, high-density lipoproteins.

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tories (Irvine, Scotland, U.K.). Human serum albumin (HSA) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Na¹²⁵I (sp. radioactivity 13.3 µCi/µg) was purchased from Amersham International (Amersham, Bucks, U.K.). Multiwell cell-culture dishes were from Costar (Cambridge, MA, U.S.A.). Percoll (density 1.13 g/ml) was obtained from Pharmacia (Uppsala, Sweden). Trypsin (from pig pancreas) was from ICN Biomedicals (Cleveland, OH, U.S.A.).

Lipoproteins

LDL was isolated from serum of normolipidaemic donors by density-gradient ultracentrifugation as described by Redgrave et al. [9]. LDL was immediately labelled with ¹²⁵I as described by Bilheimer et al. [10]. The specific radioactivity ranged from 100 to 150 c.p.m./ng of LDL protein. After iodination, the LDL sample was dialysed against phosphate-buffered saline (PBS, pH 7.4) and stabilized with 1% (w/v) HSA [3]. The stabilized ¹²⁵I-labelled LDL was stored at 4 °C and used within 2 weeks. Less than 1% of the radioactivity was soluble in 10% (w/v) trichloroacetic acid. For ¹²⁵I-tyramine-cellobiose (TC) labelling, LDL was first dialysed exhaustively against PBS/0.01% (w/v) EDTA. The labelling was then performed exactly as described by Pittman and Taylor [11]. ¹²⁵I-TC-LDL (specific activity in the range 60–80 c.p.m./ng) was stabilized as above and used within 2 weeks. Whenever unlabelled LDL was used, it was immediately stabilized after isolation with 1% HSA, followed by extensive dialysis against PBS and subsequently DMEM supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml).

Lipoprotein-depleted serum (LPDS) was obtained by ultracentrifugation of serum at a density of 1.21 g/ml, followed by extensive dialysis of the infranatant against PBS and subsequently DMEM supplemented with penicillin and streptomycin.

Cell culture

HepG2 cells and fibroblasts were cultured in flasks in DMEM supplemented with 10% (v/v) heat-inactivated FCS, 20 mM Hepes, 10 mM NaHCO₃, 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in equilibration with air/CO₂ (19:1). Then 6–7 days before each experiment, cells were seeded in 2 cm² multiwell dishes.

At 24 h before the assays, the cells were washed with Puck's saline [0.136 M NaCl, 5.3 mM KCl, 4 mM NaHCO₃, 0.1% (w/v) glucose, 5 mg/l Phenol Red, pH 7.4] and further incubated with DMEM containing 10% (v/v) LPDS instead of FCS.

Binding assays

Each experiment was started by washing the cells three times in DMEM/1% HSA, followed by incubating the cells in the same medium containing 10 µg of ¹²⁵I-LDL/ml, in the presence or absence of a 50-fold excess of unlabelled lipoprotein. Temperature and duration of the respective incubations are described in the text and in Figure legends. After the incubation with labelled LDL, cells were cooled to 0 °C. Degradation of LDL was measured exactly as described in [12]. After removal of the incubation medium, the cells were washed extensively as described in [13]. To measure total cell association, the washed cells were dissolved in 0.2 M NaOH, and a portion of the cell lysate was counted for radioactivity. Another portion of the cell lysate was used for protein determination as described by Lowry et al. [14].

When the internalization of ¹²⁵I-LDL was measured, cells were washed and further incubated with 0.05% (w/v) trypsin and

0.02% (w/v) EDTA in PBS for 10 min at room temperature [15]. The radioactivity released into the buffer represents the amount of ¹²⁵I-LDL bound to the cell membrane. The radioactivity which remains cell-associated represents the amount of ¹²⁵I-LDL internalized.

Values for the specific (receptor-mediated) binding, internalization and degradation were calculated by subtracting the amount of labelled LDL that was bound, internalized or degraded in the presence of a 50-fold excess of unlabelled LDL (non-specific binding) from the amount of labelled LDL that was bound, internalized or degraded in the absence of excess unlabelled LDL (total binding).

Subcellular fractionation using Percoll

HepG2 cells and fibroblasts were subcellularly fractionated on Percoll density gradients as previously described [16]. Cells were seeded in 100 mm dishes (fibroblasts) or 60 mm dishes (HepG2 cells). After incubation for 4.5 h at 18 °C in the presence of 10 µg/ml ¹²⁵I-TC-LDL, cells were washed twice with DMEM/1% HSA to remove the unbound ligand and further incubated at 37 °C for the indicated periods of time in medium without ¹²⁵I-TC-LDL. The cells were then washed extensively with 0.28 M sucrose/2 mM CaCl₂/0.01 M Tris/HCl, pH 7.6 (homogenization buffer), and scraped from the dishes with a rubber policeman in the homogenization buffer (1 ml/dish). The cells from three 100 mm dishes (for fibroblasts) or from two 60 mm dishes (for HepG2 cells) were combined for homogenization in a Dounce homogenizer by 20 complete strokes with a tight-fitting pestle. The homogenates were centrifuged at 280 g for 10 min in order to remove remaining intact cells. The supernatants, containing 65–80% of the cell-associated radioactivity, were made 20% in Percoll by addition of 3 ml of 80% (v/v) Percoll in homogenization buffer and adjustment of the total volume of 12 ml with homogenization buffer. After thorough mixing, the samples in Percoll were placed in cellulose nitrate tubes fitting a 50Ti rotor (Beckman) and centrifuged at 10000 g for 45 min. Fractions (~0.3 ml) were collected by aspiration from top to bottom, and the radioactivity in each sample was counted. The density of each fraction was measured in a PAAR-DMA-45 density meter equipped with a DMA-602M small sample cell (~170 µl). The distribution of the lysosomal marker (acid phosphatase) was measured by the method of Torriani [17].

RESULTS

Time course of receptor-mediated association and degradation of ¹²⁵I-LDL in fibroblasts and HepG2 cells

The receptor-mediated association and degradation (defined as difference between the binding in the absence and in the presence of a 50-fold excess of unlabelled LDL) of ¹²⁵I-LDL by HepG2 cells and fibroblasts as a function of incubation time at 37 °C are shown in Figure 1(a). In both cell lines, LDL-association increased progressively over the first 2–3 h, before reaching an apparent steady-state level. In fibroblasts, after a lag period of 30 min, the degradation of ¹²⁵I-LDL proceeds rapidly. However, in HepG2 cells a lag period of 90 min was observed before the degradation of ¹²⁵I-LDL started at a slower rate than in fibroblasts. When the degradation efficiency is calculated as the amount of LDL degraded relative to the amount of LDL which becomes cell-associated (Figure 1b), it is clear that in HepG2 cells the degradation efficiency is less than 50% of that in fibroblasts (at 5 h of incubation). In Figure 1 10 µg/ml ¹²⁵I-LDL was used. Similar results were observed in the presence of 20 µg/ml ¹²⁵I-LDL.

Figure 1 Time course at 37 °C of the LDL-receptor-mediated association ($\blacktriangle$) and degradation ($\bullet$) in fibroblasts (—) and HepG2 cells (---)

Cells (mean cell density of 61 and 273 $\mu\text{g}/\text{well}$ for fibroblasts and HepG2 cells respectively) were incubated with 10 μg of ^{125}I -LDL/ml, with or without a 50-fold excess of unlabelled LDL. Values are means $\pm$ S.D. of triplicate incubations. Panel (b) represents the degradation efficiency of LDL calculated from panel (a) as the ratio high-affinity degradation/association in fibroblasts (—) and HepG2 cells (---). This Figure shows the results of one representative experiment out of three.

Influence of the cell density on cell association and degradation of ^{125}I -LDL in HepG2 cells

In fibroblast cultures it has been shown that the LDL-receptor activity decreases with an increase in cell density [18]. We wondered whether the same correlation holds true for the expression of the LDL receptor in HepG2 cells and whether this could explain the observed delay and relatively low efficiency of the degradation of LDL in HepG2 cells. As for fibroblasts, with HepG2 cells both the cell association and degradation were higher at lower cell densities, on the basis of cell protein (Figure 2a). The degradation started after a lag period of about 90 min, irrespective of the cell density. The calculated degradation efficiency appeared thus to be independent of the cell density (Figure 2b). As a next step, we investigated whether the decreased degradation efficiency of LDL in HepG2 cells could be due to (i) a decreased internalization rate of surface-bound LDL, (ii) a delayed and less efficient transport of LDL from the early-endosomal to the late-endosomal or lysosomal compartment, or (iii) a less efficient degradation of LDL in the lysosomes itself.

Rate of internalization of surface-bound ^{125}I -LDL in fibroblasts and HepG2 cells

The rate of internalization of ^{125}I -LDL was examined by first incubating HepG2 cells and fibroblasts at 4 °C with 10 $\mu\text{g}/\text{ml}$ ^{125}I -LDL for 2 h, to allow binding of LDL to its cell surface

Figure 2 Influence of the cell density on the high-affinity cell association and degradation of ^{125}I -LDL in HepG2 cells

(a) Time course at 37 °C of LDL-receptor-mediated cell association (black symbols) and degradation (white symbols) in HepG2 cells seeded at three different cell densities, corresponding to a mean protein content of 360 $\mu\text{g}/\text{well}$ ($\bullet$, $\circ$), 175 $\mu\text{g}/\text{well}$ ($\blacksquare$, $\square$) and 60 $\mu\text{g}/\text{well}$ ($\blacktriangle$, $\triangle$). Cells were incubated with 10 μg of ^{125}I -LDL/ml with or without a 50-fold excess of unlabelled LDL. Values are means $\pm$ S.D. of triplicate determinations. (b) Degradation efficiency calculated as the ratio degradation/association at each cell density. The symbols are as above. The results are obtained from one representative experiment out of two.

receptors. Thereafter, the cells were washed and further incubated at 37 °C. As shown in Figure 3, most of the cell-bound LDL is already internalized within 5 min, whereas the maximal internalization is reached within 15–20 min with both fibroblasts and HepG2 cells. These data indicate that in HepG2 cells the internalization of surface-bound LDL is similarly rapid as compared with fibroblasts. As a remaining explanation, it is possible that in HepG2 cells either the internalized LDL is not rapidly released from the receptor within the early-endosomal compartment and thus cannot be further processed, or the internalized LDL is normally targeted to the late-endosomal and lysosomal compartment but cannot readily be degraded, due either to an impairment in the late-endosome–lysosome fusion or to a defect in the lysosomal degradation itself.

Rate of transport of ^{125}I -LDL from the early endosomes to the late endosomes or lysosomes in fibroblasts and HepG2 cells

To evaluate whether the delayed and less efficient degradation of LDL in HepG2 cells is due more specifically to an impaired transport of LDL from the early-endosomal compartment to the late-endosomal or lysosomal compartment, cells were incubated with ^{125}I -LDL at 18 °C. At this temperature the degradation of LDL is inhibited, owing to an impairment in the dissociation of the internalized LDL from the receptor in the early-endosomal compartment [19] and to a block in endosome–lysosome fusion

Figure 3 Internalization rate of LDL in fibroblasts (●) and HepG2 cells (○)

After 2 h of incubation at 4 °C in the presence of 10 µg of ^{125}I -LDL/ml with or without a 50-fold excess of unlabelled LDL, cells were chased for the indicated periods of time at 37 °C and the amount of LDL internalized was measured as described in the Materials and methods section. Values are means $\pm$ S.D. of triplicate incubations. The values for the zero-time binding to fibroblasts and HepG2 cells are 12.26 ± 0.7 and 7.9 ± 0.6 ng of LDL/mg of cell protein respectively, and are indicated on the ordinate by black and white arrows. This Figure shows the results of one representative experiment out of two.

[20]. As a result, the cell-associated lipoproteins will accumulate in the early-endosomal compartment at this temperature.

In a time-course experiment at 18 °C, we verified that in both fibroblasts and HepG2 cells indeed no detectable degradation of LDL occurred during the 4.5 h of incubation at 18 °C (Figure 4a). After that time the cells were washed in order to remove the unbound ligand and further incubated at 37 °C (Figure 4b). After the temperature shift to 37 °C, in fibroblasts we observed a lag period of 30 min followed by a rapid appearance of degradation products into the medium. In HepG2 cells this lag period was about 90 min and the degradation of LDL appeared to occur at a slower rate than in fibroblasts.

In a parallel experiment, the incubation for 4.5 h at 18 °C was carried out in the presence of ^{125}I -TC-LDL, after which the temperature was shifted to 37 °C and, at the indicated time

Figure 5 Subcellular distribution of ^{125}I -LDL

HepG2 cells were incubated for 4.5 h at 18 °C with 10 µg/ml ^{125}I -TC-LDL in DMEM/1 % HSA. After washing to remove unbound ligand, the cells were incubated at 37 °C for various periods of time, after which they were homogenized and subjected to subcellular fractionation as described in the Materials and methods section. The subcellular distribution of ^{125}I -TC-LDL after 0 (Δ) and 30 min ($\circ$) of incubation at 37 °C is shown. The broken line (---) represents the density profile, and the horizontal bar indicates the fractions in which an activity above 0.4 unit of the lysosomal marker acid phosphatase was measured.

points, cells were homogenized. The subcellular fractionation of cell homogenates shows (Figure 5, time 0 at 37 °C) that, owing to their lower buoyant density, the early endosomes were separated from the lysosomes by Percoll-gradient centrifugation [16]. The late endosomes, having a density similar to that of the lysosomes [21], are recovered with the high-density fractions.

As shown in Figure 5, at zero time all the label was found in the early-endosomal (light) fractions, whereas after 30 min of incubation at 37 °C most of the label was found in the bottom fractions of the gradient. That these fractions contain lysosomal activity has been tested by measuring acid phosphatase activity as lysosomal marker [22].

As shown in Figure 6, for both cell lines at zero time only

Figure 4 Intracellular processing of ^{125}I -LDL in fibroblasts (—) and HepG2 cells (---)

Cells were preincubated at 18 °C for 4.5 h in the presence of 10 µg of ^{125}I -LDL/ml with or without a 50-fold excess of unlabelled LDL and then chased for the indicated periods of time at 37 °C. Receptor-mediated association (●) and degradation (▲) were measured. After 4.5 h at 18 °C, less than 10% of the cell association appeared to be surface-bound, measured as that trypsin-releasable. Association and degradation are expressed as the percentage of the amount of ^{125}I -LDL that became cell-associated after 4.5 h at 18 °C. The 100% values for association are 216 ± 21 and 114 ± 3 ng of LDL/mg of cell protein for fibroblasts and HepG2 cells respectively. This Figure shows the results of one representative experiment out of two.

Figure 6 Accumulation of ¹²⁵I-TC-LDL in the late-endosomal and lysosomal fractions of fibroblasts and HepG2 cells as a function of time

Cell homogenates were fractionated on Percoll gradients as described in Figure 5. For each time point the fractions representing the high-density fractions (30–38; see Figure 5) were pooled and expressed as the percentage of the total amount of radioactivity present in the homogenate. The Figure shows the results of one representative experiment out of two.

about 5% of the total amount of radioactivity could be found in the high-density fractions, whereas the accumulation of ¹²⁵I-TC-LDL in the high-density fractions reached its maximum within almost 15 min.

DISCUSSION

Several studies have shown that HepG2 cells possess functional LDL receptors with properties similar to those of human fibroblasts [3]. However, some aspects of the mechanism regulating the LDL-receptor activity in both HepG2 cells and primary cultures of human hepatocytes are likely to differ from those that have been characterized in fibroblasts and in cells derived from other peripheral tissues [2]. In this respect, we have demonstrated that both HepG2 cells [6] and freshly isolated human hepatocytes [7] are much less responsive to feedback regulation by LDL than are fibroblasts. In these studies it also appeared that the LDL-receptor activity in HepG2 cells and human hepatocytes was stimulated 2–3-fold by the presence of cholesterol acceptors such as heavy HDL, whereas in fibroblasts the LDL-receptor activity was almost insensitive to the presence of heavy HDL. Studies on the cellular cholesterol homeostasis in relation to the LDL-receptor activity in HepG2 cells suggested that both the exogenously delivered (LDL) cholesterol and the endogenously synthesized cholesterol are primarily directed to a cholesteryl ester pool or, if present, to extracellular cholesterol acceptors, like heavy HDL, rather than to the regulatory free cholesterol pool involved in the regulation of the LDL-receptor activity [8].

In the present work we compared the processing of LDL in HepG2 cells and fibroblasts in more detail, reasoning that information about this pathway might help to explain the marked difference between the two cell lines in their ability to modulate the LDL-receptor activity in response to the presence of exogenous cholesterol.

Data reported in the literature [23,24] have revealed that in hepatocytes the LDL receptors are distributed diffusely on the basal surfaces, and only a small number are localized in endocytic vesicles. This is in sharp contrast with the distribution of LDL receptors in human fibroblasts, where most of the receptors are located in coated pits and in the membranes of the

endocytic recycling pathway. Nevertheless, it has also been found that in liver cells the ligand–receptor complexes are only internalized after moving into coated pits [24]. It seems likely therefore that this difference in the cellular distribution of the LDL receptors could result in a slower internalization rate of LDL in hepatocytes. The present results show, however, that in HepG2 cells LDL is internalized at the same rate as in fibroblasts.

Our results show that the degradation of LDL in HepG2 cells appears to be severely impaired, being not only delayed but also inefficient, as expressed by the low ratio of degradation/cell association (Figures 1 and 2). A number of other studies concur with our findings of a relatively inefficient degradation of LDL in HepG2 cells. Edge et al. [25] also found that the binding and uptake of LDL in cultured human hepatocytes was similar to that seen in fibroblasts, whereas the degradation efficiency of LDL was lower than in fibroblasts. Kamps et al. [26] showed that in human parenchymal liver cells the LDL uptake is also not efficiently coupled to catabolism.

In our attempt to delineate the events responsible for the observed defect in the degradation of LDL in HepG2 cells, we measured the rate of distribution of LDL between the endosomes and the lysosomes in both cell lines. In these experiments cells were incubated at 18 °C, since at this temperature no ligand–receptor dissociation and no fusion between endocytic vesicles and lysosomes occurs, and the ligand thus accumulates in the early-endosomal compartment [20]. When this approach was used, the difference in LDL degradation between fibroblasts and HepG2 cells was still apparent (Figure 4). Subsequently, the distribution of LDL between the endosomes and the lysosomes was measured with ¹²⁵I-TC-LDL. Upon degradation of proteins, ¹²⁵I-TC remains attached to short peptide fragments, and in this form no release of label from the lysosomes occurs [27]. Using this experimental design, we did not observe any difference between both cell lines in the processing of LDL from the early-endosomal compartment to the late-endosomal or lysosomal compartment. This indicates that the dissociation of LDL from the receptor does not represent a rate-limiting step in the degradation of LDL. The method for subcellular fractionation used does not enable us to discriminate further between a pre-lysosomal and the lysosomal compartment. Therefore from our data we can conclude that in HepG2 cells either the fusion process between the late endosomes and the lysosomes is altered, or the degradation of LDL itself in the lysosomes is less efficient than in fibroblasts.

Several studies in fibroblasts [18], smooth-muscle cells [28], endothelial cells [29], A431 cells [30] and rat hepatocytes [31] have shown that the LDL-receptor activity decreases with increasing cell densities. Our results with HepG2 cells confirm this inverse correlation between cell density and LDL-receptor activity (Figure 2). However, the lag period and the efficiency of the degradation, expressed as the ratio degradation/cell association, was not influenced by the cell density (Figure 2).

The absence of a relationship between LDL-receptor activity and degradation efficiency is sustained by our previous observation that the stimulation of the LDL receptor in HepG2 cells by incubation with heavy HDL does not result in an increased efficiency of LDL degradation [6]. Similarly, ursodeoxycholic acid was found to enhance receptor-dependent LDL uptake to a similar extent to the degradation in isolated hamster hepatocytes [32].

A primarily direction of exogenously delivered LDL cholesterol and endogenously synthesized cholesterol to an intracellular cholesteryl ester pool or to extracellular cholesterol acceptors rather than to the regulatory free cholesterol pool involved in LDL-receptor activity has been reported [8]. With the

present results, we argue that also a relatively low degradation efficiency of LDL in HepG2 cells may contribute to provide an explanation for the relatively weak down-regulation of the LDL-receptor activity in HepG2 cells on incubation of the cells with LDL.

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