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Uptake of LDL in parenchymal and non-parenchymal rabbit liver cells *in vivo*

LDL uptake is increased in endothelial cells in cholesterol-fed rabbits

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1. Hepatic uptake of low-density lipoprotein (LDL) in parenchymal cells and non-parenchymal cells was studied in control-fed and cholesterol-fed rabbits after intravenous injection of radioiodinated native LDL (^{125}I-TC-LDL) and methylated LDL (^{131}I-TC-MetLDL). 2. LDL was taken up by rabbit liver parenchymal cells, as well as by endothelial and Kupffer cells. Parenchymal cells, however, were responsible for 92% of the hepatic LDL uptake. 3. Of LDL in the hepatocytes, 89% was taken up via the B,E receptor, whereas 16% and 32% of the uptake of LDL in liver endothelial cells and Kupffer cells, respectively, was B,E receptor-dependent. 4. Cholesterol feeding markedly reduced B,E receptor-mediated uptake of LDL in parenchymal liver cells and in Kupffer cells, to 19% and 29% of controls, respectively. Total uptake of LDL in liver endothelial cells was increased about 2-fold. This increased uptake is probably mediated via the scavenger receptor. The B,E receptor-independent association of LDL with parenchymal cells was not affected by the cholesterol feeding. 5. It is concluded that the B,E receptor is located in parenchymal as well as in the non-parenchymal rabbit liver cells, and that this receptor is down-regulated by cholesterol feeding. Parenchymal cells are the main site of hepatic uptake of LDL, both under normal conditions and when the number of B,E receptors is down-regulated by cholesterol feeding. In addition, LDL is taken up by B,E receptor-independent mechanism(s) in rabbit liver parenchymal, endothelial and Kupffer cells. The non-parenchymal liver cells may play a quantitatively important role when the concentration of circulating LDL is maintained at a high level in plasma, being responsible for 26% of hepatic uptake of LDL in cholesterol-fed rabbits as compared with 8% in control-fed rabbits. The proportion of hepatic LDL uptake in endothelial cells was > 5-fold higher in the diet-induced hypercholesterolaemic rabbits than in controls.

INTRODUCTION

The liver plays a central role in the control of blood cholesterol levels by removing lipoproteins from the circulation, via receptor mediated endocytosis [1,2]. In rabbits about 70% of the low-density lipoprotein (LDL) receptors in the body are associated with the liver cells [3]. By feeding rabbits a cholesterol-enriched diet, the hepatic uptake of LDL is markedly reduced, due to a decrease in the number of LDL receptors [4]. By chemical modification of lysine and arginine residues of LDL, the lipoprotein particles do not bind to the LDL receptor; the modified LDL is instead taken up via a B,E receptor-independent mechanism [5–7]. This pathway is also available for native LDL, and by determining total and B,E receptor-independent uptake, the B,E receptor-dependent removal can be estimated. Previous studies have shown that plasma decay curves for native and cyclohexanedione-modified LDL were superimposable in cholesterol fed rabbits [7]. All LDL was removed from the blood via a B,E receptor-independent pathway in these animals. LDL-turnover studies in homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits indicated that essentially all LDL was catabolized via B,E receptor-independent processes [8]. Studies of LDL uptake in various tissues have shown that the liver is by far the largest contributor to the overall body catabolism of LDL [9,10]. However, the role of various hepatic cell types in removing LDL from plasma under different metabolic conditions has not been thoroughly studied. Some studies of LDL uptake in rat liver cells have suggested that non-parenchymal cells (particularly the Kupffer cells) may play an important role in the uptake of this lipoprotein [11,12]. However, lipoprotein metabolism in rats is clearly very different from that of humans. In the present studies we have used rabbits as a model system, as rabbits have much higher plasma LDL levels than rats, and LDL metabolism in rabbits resembles that in humans. Non-parenchymal liver cells might participate in hepatic uptake of LDL in several ways: by endocytosis mediated via the B,E receptor; by an alternative lipoprotein receptor; by fluid phase endocytosis; and by uptake of modified LDL through the scavenger receptor [13–15]. The latter mechanism would be of particular interest in hypercholesterolaemic animals as lasting high plasma levels of cholesterol may lead to modification of LDL which then is taken up via the scavenger receptor [1].

Abbreviations used: ^{125}I-TC-LDL, ^{125}I-tyramine cellobiose-labelled low-density lipoprotein; ^{131}I-TC-MetLDL, ^{131}I-tyramine cellobiose-labelled methylated low-density lipoprotein; WHHL, Watanabe heritable hyperlipidaemic.

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The present studies were undertaken to determine: (*a*) the role of various liver cells in LDL uptake *in vivo*; (*b*) the role of the B,E receptor-dependent and B,E receptor-independent uptake of LDL in parenchymal and non-parenchymal liver cells; and (*c*) the effect of cholesterol feeding on the hepatic uptake of LDL. The results indicate that cholesterol feeding markedly reduces the B,E receptor-dependent uptake of LDL in parenchymal and Kupffer cells, whereas the total uptake in the endothelial cells was increased. The B,E receptor-independent uptake of LDL in parenchymal cells was not affected by cholesterol feeding.

EXPERIMENTAL PROCEDURES

Animals and diets

Male Chinchilla rabbits, 2–3 kg, were purchased from GMBH Versuchstierkunde und -zucht, West Germany. Some rabbits were fed Ewos Maintenance Feed containing 2% (w/w) cholesterol for 1 week. Control animals received an unsupplemented diet. All animals were given food and water *ad libitum*.

Lipoproteins

LDL was isolated from fresh, pooled rabbit plasma by sequential ultracentrifugation in a Centrikon T-2060 ultracentrifuge in the density range 1.019–1.063 g/ml, in a TFT 70.38 rotor for 20 h at 43000 rev./min and 10 °C [16]. The LDL fraction was washed and concentrated by a second ultracentrifugation in a TFT 45.6 rotor, under the same conditions. One aliquot of LDL was labelled with ^{125}I-tyramine cellobiose, another with ^{131}I-tyramine cellobiose [17,18]. The final preparations were dialysed against 0.15 M-NaCl, 20 mM-sodium phosphate and 1 mM-EDTA, pH 7.4. The advantage of labelling a protein with radioiodinated tyramine cellobiose is that the degradation products are trapped in the organelles where degradation takes place. LDL preparations analysed by SDS/polyacrylamide gradient gel electrophoresis (5–20%) [19], contained a single band corresponding to apoprotein B-100. More than 99% of the radioactivity in the labelled lipoproteins was precipitated by 10% (w/v) trichloroacetic acid. The final specific activity of ^{125}I-TC-LDL ranged from 536 to 1774 c.p.m./ng of protein. The ^{131}I-TC-LDL preparations in 0.15 M-NaCl/0.01% EDTA (pH 7.4) were reductively methylated with formaldehyde and sodium borohydride by using a 30 min reaction sequence as described by Weisgraber *et al.* [20]. The specific activity of ^{131}I-labelled methylated LDL (^{131}I-TC-MetLDL) ranged from 72 to 217 c.p.m./ng.

Studies *in vivo*

^{125}I-TC-LDL and ^{131}I-TC-MetLDL were simultaneously injected into the marginal ear vein. Blood samples were drawn 3 min later from the vein of the opposite ear, and then 5, 10, 20, 40 and 60 min and 2, 4, 6 and 24 h after injection. Plasma was obtained and radioactivity quantified using Kontron Automatic Gamma Counter MB252 (2 channel run). A plasma sample was obtained 24 h after injection for lipoprotein fractionation (density 1.019, 1.063 and 1.21 g/ml). Fractional catabolic rate was calculated from plasma tracer data by using the SAAM 29 (Simulation, Analysis and Modeling [21]) and CONSAM programs (Conversational SAAM [22]) run on a VAX computer. The absolute catabolic rate was calculated by multiplying the fractional catabolic rate (pools per day) by the apolipoprotein LDL pool size. Plasma volume was assumed to be 3.28% of body weight [23]. After 24 h the rabbits were anaesthesized by intraperitoneal injection of Dormicum (Roche), 0.5 ml/kg and 5 min later by intramuscular injection of Hypnorm (Janssen), 0.4ml/kg. All rabbits were sacrificed at the same time of the day (9.30 a.m.–10.00 a.m.). Isolated liver cells were prepared by collagenase perfusion of the liver, essentially as described for rat liver [24]. The liver *in situ* was first perfused with Ca^{2+}-free buffer for 10 min and then with a solution containing 0.1% (w/v) collagenase for 5–10 min. The perfusion rate was 100 ml/min. A lobe was tied off and removed for determination of total hepatic uptake at the end of preperfusion. After collagenase perfusion, parenchymal and non-parenchymal cells were separated by centrifugal elutriation using a Beckman JE-6 rotor [25]. Hepatocytes were eluted at 1200 rev./min at a flow rate of 25–55 ml/min. Non-parenchymal cells were eluted at 2500 rev./min at a flow rate of 20 ml/min. Endothelial and Kupffer cells were separated at 2500 rev./min at flow rates of 20 ml/min and 20–55 ml/min, respectively. Non-parenchymal cells were prepared free of contaminating parenchymal cells, as judged by microscopy. Pure endothelial and Kupffer cells were also prepared by a selective attachment culturing method, according to Laakso & Smedsrød [26]. Briefly, the culture dishes were pre-treated with bovine serum albumin and glutaraldehyde, and then thoroughly washed with phosphate buffer solution. Elutriated non-parenchymal cells, free of contaminating hepatocytes, were seeded on the coated dishes in RPMI medium. After 20 min of incubation the Kupffer cells had attached, and non-attached cells in the

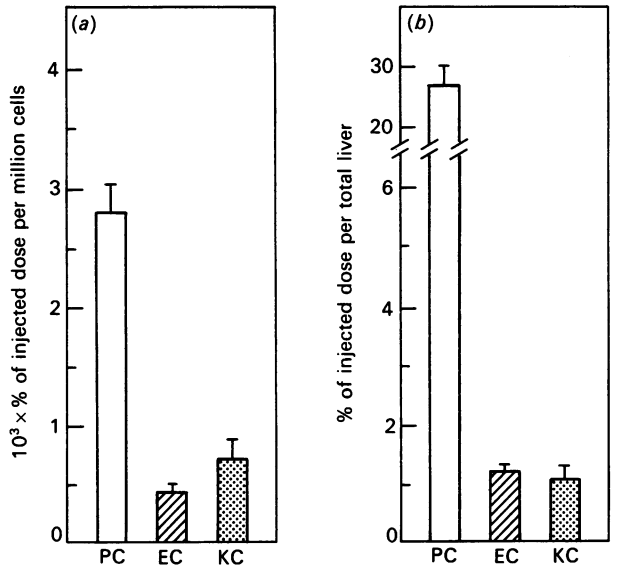

Fig. 1. Total (receptor-dependent and -independent) uptake of intravenously injected ^{125}I-TC-LDL in rabbit liver parenchymal cells (PC), endothelial cells (EC) and Kupffer cells (KC)

The various types of liver cells were isolated 24 h after injection. The results are expressed as means ± S.D. (%) of injected dose per million cells (*a*), and percentage of injected dose distributed in total liver (*b*) in four rabbits. Recovery of radioiodinated LDL in cells was 116%.

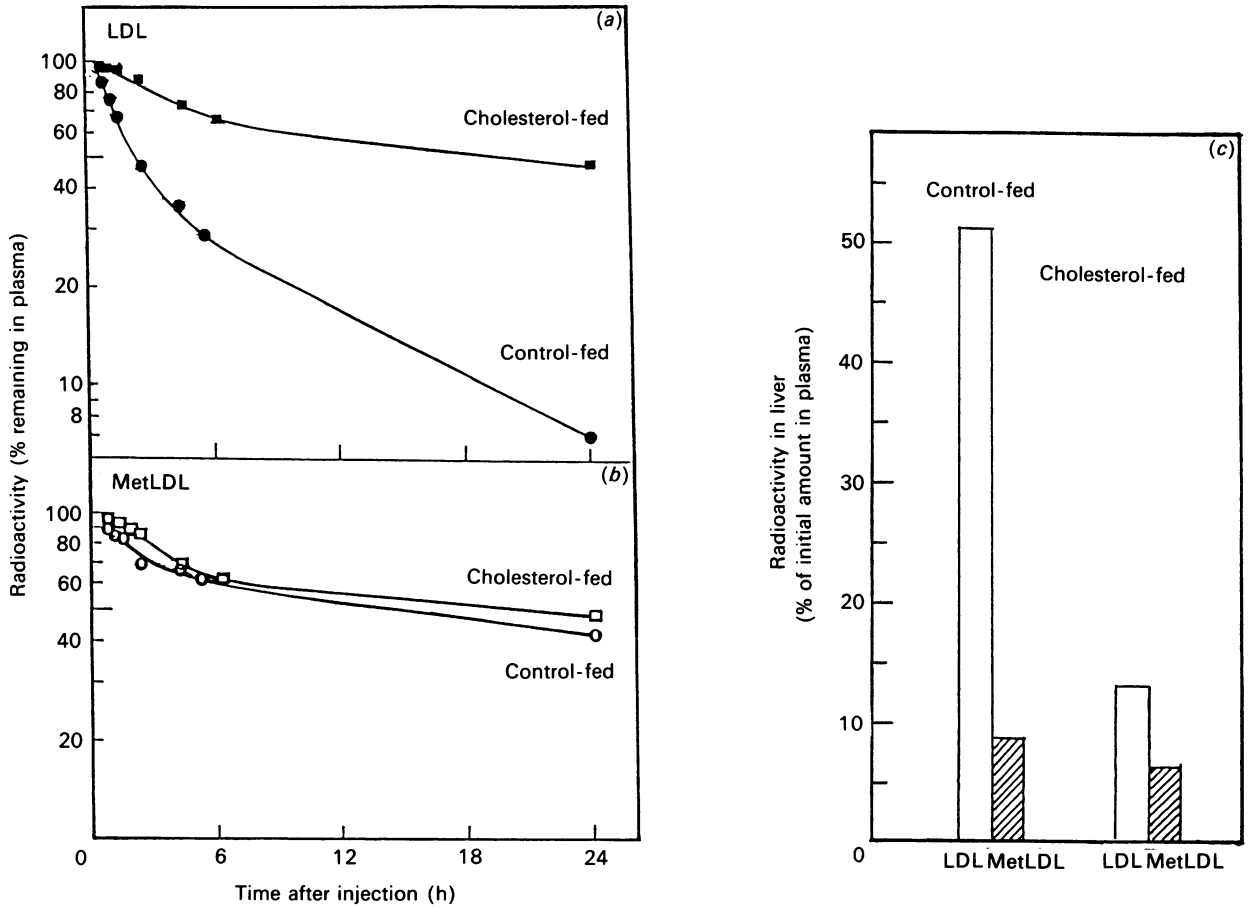

Fig. 2. Plasma clearance curves (*a* and *b*) and total hepatic uptake (*c*) of ^{125}I-TC-LDL and ^{131}I-TC-MetLDL in control- and cholesterol-fed rabbits

Plasma clearance curves in control-fed (●,○) and cholesterol-fed (■,□) rabbits after intravenous injection of ^{125}I-TC-LDL (*a*) and ^{131}I-TC-MetLDL (*b*). A liver lobe was tied off and excised 24 h after intravenous injection (*c*). Open bars show the uptake of ^{125}I-TC-LDL (receptor-dependent and -independent) and hatched bars the uptake of ^{131}I-TC-MetLDL (B,E receptor-independent) in control-fed and cholesterol-fed rabbits. The liver weight was found to be 3.59 ± 0.38% of total body weight ($n = 3$). Data are expressed as % of the amount of radioactivity present in plasma 5 min after injection, and represent one typical experiment.

medium and washes were seeded on dishes coated with fibronectin. After 2 h of incubation at 37 °C, the cells were washed and dissolved in a solution of 0.1% SDS in 0.3 M-NaOH, and the radioactivity was counted. The number of cells in the cultures was determined microscopically. Cellular distribution of ^{125}I-TC-LDL and ^{131}I-TC-MetLDL in parenchymal, endothelial and Kupffer cells was expressed as a percentage of injected dose per million cells. To estimate the contribution of each cell type to the total hepatic uptake of LDL, the above-mentioned values were multiplied by the number of each cell type (in millions) in whole liver. It was assumed that 1 g of liver contained 125×10^6 parenchymal, 36×10^6 endothelial and 19×10^6 Kupffer cells [27,28]. These values refer to rat, because corresponding values for rabbit have not been published, to our knowledge. However, when the cell number from rat liver was used for rabbit liver, it was found that the recovery of radioactive LDL in the cells was about 92% of that in the liver from which the cells were derived. The liver weight was found to be 3.59 ± 0.38% of body weight ($n = 3$).

Chemical assays

Cholesterol and triacylglycerol were determined enzymatically by Nycotest kits (Nycomed A/S, Oslo, Norway). Protein concentration was determined by the method of Lowry *et al.* [29], using bovine serum albumin (Bio-Rad Laboratories, CA, U.S.A.) as standard. SDS/polyacrylamide-gradient-gel electrophoresis (5–20%) of lipoprotein preparations was performed according to Laemmli [19]. The gels were stained and apolipoprotein bands identified as described previously [30].

RESULTS

What types of liver cells take up LDL?

Hepatocytes, endothelial cells, and Kupffer cells were isolated 24 h after intravenous injection of ^{125}I-TC-LDL into rabbits, to determine the cellular distribution of LDL taken up by the liver. The results (Fig. 1*a*) show that LDL was associated with all the cell types isolated. Parenchymal cells were by far the most active cells, being

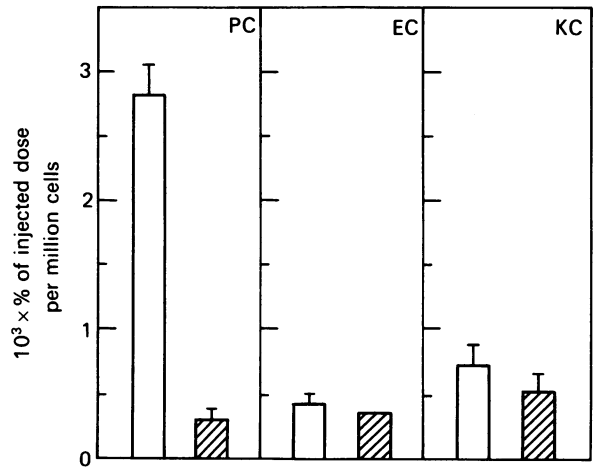

Fig. 3. Total uptake of intravenously injected ^{125}I-TC-LDL (open bars) and B,E receptor-independent uptake ^{131}I-TC-MetLDL (hatched bars) in parenchymal cells (PC), endothelial cells (EC), and Kupffer cells (KC) in control-fed rabbits

The various cell types were isolated 24 h after injection. The results are expressed as means ± S.D. (%) of injected dose per million cells in four rabbits. Recovery of ^{131}I-TC-MetLDL in the cells was 78%.

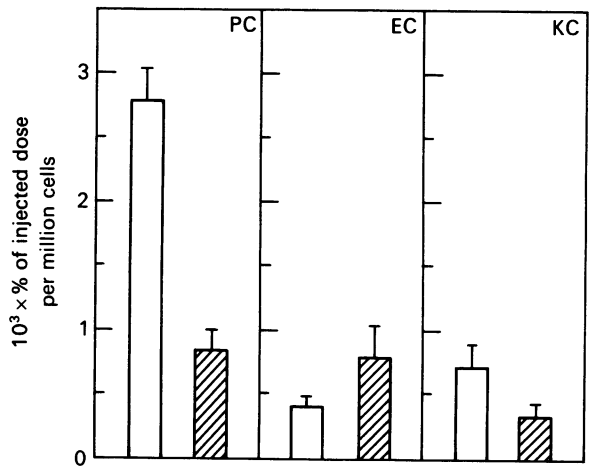

Fig. 4. Total (receptor-dependent and -independent) uptake of intravenously injected ^{125}I-TC-LDL in liver parenchymal cells (PC), endothelial cells (EC) and Kupffer cells (KC) in control-fed (open bars) and cholesterol-fed rabbits (hatched bars)

The various cell types were isolated 24 h after injection. The results are expressed as means ± S.D. (%) of injected dose per million cells in four control-fed and four cholesterol-fed rabbits. Recovery of radioiodinated LDL in the cells was 86%.

responsible for about 7-fold and 4-fold higher LDL uptake per cell than endothelial and Kupffer cells, respectively.

Estimation of the contribution of hepatocytes, liver endothelial cells and Kupffer cells to the total hepatic uptake of LDL, suggested that 92% of the liver uptake of LDL took place in the parenchymal cells, whereas 4% and 3.6% of the hepatic uptake of LDL was attributable to endothelial and Kupffer cells, respectively (Fig. 1*b*).

How much LDL is taken up via the hepatic B,E receptor?

To estimate the hepatic B,E receptor-dependent uptake of LDL, ^{125}I-TC-LDL and ^{131}I-TC-MetLDL were simultaneously injected into rabbits. Plasma clearance was followed, and the results show that the decay of methylated LDL was considerably slower than that of native LDL (Fig. 2*a* and *b*). In accordance with this, the total hepatic uptake of methylated LDL after 24 h was 8.5% of the initial amount of radioactivity in plasma, as compared with 51% of native LDL (Fig. 2*c*). The various types of liver cells were isolated 24 h after injection. If B,E receptor-mediated uptake is represented by the difference between the uptake of native and methylated LDL, our results suggest that 89%, 16%, and 32% of the hepatic uptake of LDL in the parenchymal, endothelial and Kupffer cells, respectively, is taken up via the B,E receptor (Fig. 3). However, the differences between the uptake of native and methylated LDL in endothelial and Kupffer cells are not statistically significant.

How is the LDL uptake in parenchymal and non-parenchymal liver cells regulated by cholesterol feeding?

To determine the effects of cholesterol feeding on the hepatic uptake of LDL in various liver cell types *in vivo*, radioiodinated LDL was injected intravenously into four control-fed and four cholesterol-fed rabbits. In the cholesterol-fed rabbits the concentration of plasma cholesterol increased to a value [13 ± 3 mmol/l (mean ± S.D., $n = 4$)] that was > 20-fold higher than that in the control group [0.6 ± 0.1 mmol/l (mean ± S.D., $n = 4$)]. Most of this increase could be accounted for by an increase in the combined very- and intermediate-low-density lipoprotein fraction and in the LDL fraction. Only a slight increase was observed in the high-density lipoprotein fraction. Plasma triacylglycerol levels decreased from 0.9 ± 0.2 mmol/l (mean ± S.D., $n = 4$) to 0.5 ± 0.1 mmol/l (mean ± S.D., $n = 4$) after cholesterol feeding. In accordance with previous data [5], the plasma clearance of LDL in the cholesterol-fed rabbits was slower than in controls (Fig. 2*a*). The fractional catabolic rate of LDL was markedly reduced after cholesterol feeding, to about 40% of normal. However, taking into account the much larger plasma pool size in the diet-induced hypercholesterolaemic group, the absolute catabolic rate was about 6-fold higher than in control animals. In agreement with the plasma clearance data, the hepatic LDL uptake 24 h after injection appeared to be 13% of the initial amount of plasma radioactivity in cholesterol-fed rabbits, as compared with 51% in the control-fed rabbits (Fig. 2*c*). The total uptake of LDL was markedly reduced in hepatocytes and Kupffer cells after cholesterol feeding, to about 29% and 50% of the controls, respectively (Fig. 4). In the endothelial cells, however, the total uptake of LDL was about 2-fold higher than in the control rabbits [significantly different ($P < 0.03$) by two-tailed Mann–Whitney test]. Furthermore, in the cholesterol-fed rabbits the uptake of injected LDL per parenchymal cell was about the same as the uptake per endothelial cell. Based on the assumption that the cell number per g of liver was 125×10^6 parenchymal, 36×10^6 endothelial and 19×10^6 Kupffer cells, it followed

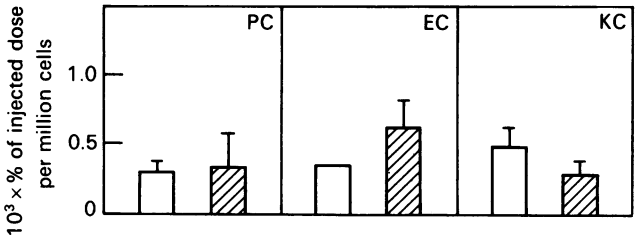

Fig. 5. B,E receptor-independent uptake of intravenously injected ^{131}I-TC-Methylated LDL in liver parenchymal cells (PC), endothelial cells (EC) and Kupffer cells (KC) in control-fed (open bars) and cholesterol-fed rabbits (hatched bars)

The various cell types were isolated 24 h after injection. The results are expressed as means ± S.D. (%) of injected dose per million cells in four control-fed and four cholesterol-fed rabbits. Recovery of radioiodinated methylated LDL in the cells was 89%.

that 74% of the total hepatic uptake of LDL was attributable to parenchymal cells, as compared with 92% in the control-fed rabbits. Twenty-one % and 5% of the uptake of native LDL in cholesterol-fed animals were attributable to endothelial and Kupffer cells, respectively, as compared with 4% and 3.6% in the control animals. The results indicate that cholesterol feeding down-regulated the hepatic uptake of LDL in hepatocytes and Kupffer cells, and enhanced the uptake in endothelial cells.

Does cholesterol feeding regulate hepatic uptake of methylated LDL?

^{131}I-TC-MetLDL was intravenously injected into four control-fed and four cholesterol-fed rabbits and plasma clearance was followed. Despite a > 20-fold higher plasma cholesterol pool size in the cholesterol-fed rabbits, the B,E receptor-independent clearance of LDL was similar in both groups of animals (Fig. 2*b*). The decay curves of native (*a*) and methylated LDL (*b*) in cholesterol-fed rabbits suggest that LDL is cleared mainly via a B,E receptor-independent mechanism. The present findings are in agreement with the plasma clearance of native and methylated LDL reported in fed and fasted rabbits [31], and data obtained in WHHL rabbits in which a functional B,E receptor is lacking [6]. In accordance with these findings, our data show that total hepatic uptake of methylated LDL after 24 h was of the same order of magnitude in both groups of rabbits (Fig. 2*c*). Uptake of methylated LDL in hepatocytes, isolated 24 h after injection, was not affected by cholesterol feeding, whereas cholesterol feeding slightly increased the uptake of methylated LDL in endothelial cells and slightly decreased the uptake in Kupffer cells (Fig. 5).

DISCUSSION

The current studies demonstrate that LDL is taken up by rabbit hepatocytes as well as liver endothelial cells and Kupffer cells. However, parenchymal cell are the major site of hepatic uptake of LDL under normal conditions (Fig. 1*b*) and even when the number of B,E receptors is down-regulated by cholesterol feeding (calculated on the basis of data in Fig. 4). The parenchymal cells are also the main site for hepatic

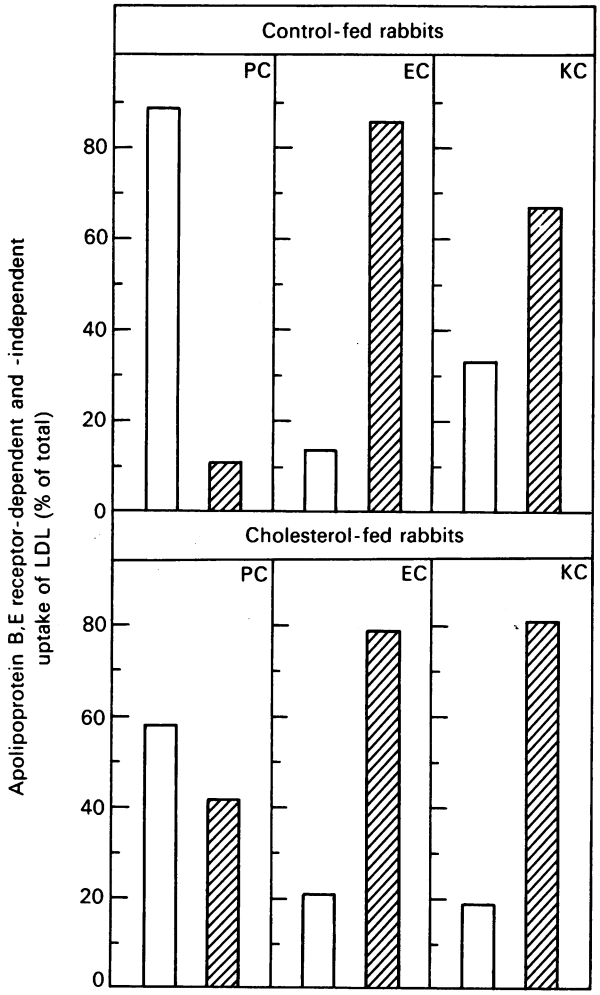

Fig. 6. B,E receptor-dependent (open bars) and -independent uptake (hatched bars) of LDL in control-fed and cholesterol-fed rabbits

Results are derived from experiments presented in Figs. 3, 4 and 5, and are expressed as % of total uptake of LDL in parenchymal cells (PC), endothelial cells (EC) and Kupffer cells (KC), respectively.

uptake of methylated LDL. These data are in accordance with previous findings, indicating that hepatocytes are responsible for most of the hepatic LDL uptake both in control and WHHL rabbits [32]. Our data show, in addition, that the non-parenchymal cells were responsible for a higher proportion of the hepatic uptake of LDL and methylated LDL in cholesterol-fed rabbits as compared with controls. The present data also indicate that most of the LDL in hepatocytes is taken up via the B,E receptor in the control animals (Fig. 6). Cholesterol feeding down-regulated the B,E receptor-dependent component of the total uptake in parenchymal and Kupffer cells, to about 19% and 29% of controls, respectively.

The present studies therefore suggest that Kupffer cells, in addition to hepatocytes, contain B,E-specific binding sites for LDL, and that these receptors could be down-regulated by cholesterol feeding. This is further supported in preliminary experiments where we have identified mRNA for the B,E receptor in non-parenchymal rabbit liver cells (M. Nenseter, unpublished

results). The presence of an LDL receptor in Kupffer cells has previously been reported by Van Berkel *et al.* [11,12]. In contrast to our data, however, they found that Kupffer cells in rat were the main cell type in which the liver accumulated LDL.

Our data indicated an increased association of LDL to endothelial cells when the B,E receptor route was blocked either by cholesterol feeding or by reductive methylation of LDL. The proportion of hepatic LDL uptake that took place in endothelial cells in diet-induced hypercholesterolaemic rabbits was more than 5-fold higher than in controls. This result may conceivably be explained by the presence of the scavenger receptor on the liver endothelial cells in addition to a B,E receptor [33,34]. The scavenger receptor may be involved in uptake of modified LDL when the concentration of LDL is maintained at a high level in plasma [1]. Experiments *in vivo* as well as *in vitro* have shown that modified LDL is taken up by hepatic endothelial cells via a high-affinity, saturable pathway. If cholesterol feeding results in down-regulation of the classical LDL receptor, and if LDL at the same time is biologically modified in cholesterol-fed rabbits, then the net result may be an increased uptake of LDL in the endothelial cells. The increased uptake via the scavenger receptor may mask the down-regulation of a possible B,E receptor in this cell type. In a previous study [34], we have shown that a significant amount of cholesterol taken up by liver endothelial cells via the scavenger receptor, shortly after appeared in the bile. The scavenger receptor in liver endothelial cells may therefore function as an important protection system by removing atherogenic lipoproteins. Taken together, the present studies indicate that the parenchymal, as well as the non-parenchymal, cells of the liver possess a specific apolipoprotein B,E-binding site for LDL that is down-regulated by cholesterol feeding. Under normal conditions the parenchymal cells are by far the most important cell type in the liver for uptake of LDL. In addition, the liver cells do also take up LDL by a B,E receptor-independent pathway. Further experiments are needed to elucidate the mechanism(s) of this apolipoprotein B,E receptor-independent uptake. Our results may also suggest that another receptor on the endothelial cells (the scavenger receptor) may be quantitatively important when the concentration of circulating LDL is maintained at a high level in plasma, by forming a protection system against atherogenic particles in the blood. Experiments are underway to elucidate the mechanism for the increased uptake of LDL in endothelial cells in cholesterol-fed rabbits.

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