Uptake of apolipoprotein E-rich and apolipoprotein E-poor subfractions of high-density lipoprotein by liver membranes and HepG2 cells

Yara D. FRAGOSO and E. Roy SKINNER*

Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

Apolipoprotein (apo) E plays an important role in mediating high-density lipoprotein (HDL) cholesterol transport and uptake by the liver. Evidence for and against the existence of conventional liver receptors for HDL containing apoE have been reported, although the selective uptake of the cholesterol moiety of HDL has been demonstrated. The present study investigated the hepatic uptake of subfractions of HDL separated on the basis of their apoE content. Rabbit HDL and its apoE-rich and apoE-poor subfractions, separated by heparin–Sepharose affinity chromatography, were labelled in their apoprotein moieties with [¹⁴C]sucrose and in their cholesteryl ester moiety with ³H. No binding of either subfraction to rabbit liver membranes could be

detected. With cultured HepG2 cells, however, there was a high uptake of ³H but a very low uptake of ¹⁴C from both HDL subfractions, demonstrating that selective uptake was operating. Addition of unlabelled apoE-poor HDL inhibited the uptake of both labels from the two subfractions to the same extent. These studies, which differed from previously reported investigations by employing native homologous HDL subfractions of known apolipoprotein composition, demonstrated that apoE is not directly involved in the selective uptake of HDL cholesterol by the liver. In the absence of specific binding sites on liver membranes, it is suggested that an alternative mechanism might exist for the clearance of HDL cholesterol from the plasma.

INTRODUCTION

During reverse cholesterol transport, cholesterol effluxed from peripheral cells becomes associated with high-density lipoprotein (HDL) and is ultimately taken up by the liver for excretion as bile [1–3]. The mechanism by which HDL cholesterol is delivered to hepatic cells is, however, unclear, although several processes have been proposed. A substantial proportion of the cholesterol is taken up indirectly by cholesteryl ester/triacylglycerol exchange, via cholesteryl-ester-transfer-protein (CETP) activity, with low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) which are taken up by hepatic LDL receptors [3]. HDL cholesterol may also be taken up directly. It has been proposed that under certain conditions, such as high cholesterol loading, an alternative pathway occurs by which HDL enriched with effluxed cholesterol acquires apolipoprotein (apo) E which targets the particle for hepatic uptake [4,5]. Using a novel rat double-perfusion system, Mindham et al. [6,7] demonstrated that most of the [³H]cholesterol released from loaded spleen cells was taken up by the liver from an intermediate-density lipoprotein fraction (*d* 1.085–1.125 g/ml) which was apoE-poor. There was relatively little uptake from the *d* 1.050–1.085 g/ml fraction which contained apoE as the only apolipoprotein.

In addition, uptake has been reported to take place via receptors specific for apoA-I and apoA-II [8,9]. Evidence has been presented both for [8–10] and against [11–13] the existence of conventional receptors for the uptake of HDL. It has been demonstrated that an appreciable proportion of the HDL cholesterol delivered directly to the liver is taken up selectively. In this process, cholesteryl ester is taken up directly by the liver without uptake of the HDL particle [12,14,15].

Although apoE plays an important role in mediating HDL cholesterol uptake [16], it has been shown not to be a major determinant of HDL cholesterol clearance by selective uptake [12,14,15]. However, many of the studies reported have employed subfractions of HDL (HDL₁, HDL₂ and HDL₃) from animal

species separated on the basis of their different densities; these particles contain either apoE as their sole apolipoprotein, as in the case of the rat, or mixtures of particles with poorly defined apoprotein compositions. In addition, the use of membranes and lipoprotein fractions from different species in some studies may have influenced the degree of binding, as species specificity has been demonstrated [17,18]. A direct comparison of the hepatic uptake, by either selective or receptor-specific mechanisms, of apoE-rich and apoE-poor HDL subfractions prepared from homologous species and separated according to their apoE content has not been reported. In the present study, the properties of these HDL subfractions of known composition, separated by heparin–Sepharose affinity chromatography and labelled in their cholesterol and apoprotein moieties, were examined. We investigated both the binding of rabbit HDL subfractions to isolated rabbit liver membranes and the uptake of the human counterparts, prepared from the plasma of normal healthy male subjects, by human hepatoma HepG2 cells. The rabbit was selected as the animal model because it has a similar distribution of lipoproteins and HDL subfractions to those of the human [19,20].

MATERIALS AND METHODS

Materials

[1a,2a-³H]Cholesteryl linoleate and [U-¹⁴C]sucrose were obtained from Amersham International, Amersham, Bucks., U.K. Heparin (sodium salt; from bovine intestinal mucosa), cyanuric chloride and materials for perfusion were obtained from Sigma, Poole, Dorset, U.K. Cell culture media and dishes were from Life Technology, Paisley, Scotland, U.K. Other chemicals were purchased from BDH/Merck, Lutterworth, Leics., U.K.

Separation of lipoproteins

Blood samples were obtained by venepuncture of the marginal ear veins of fed unanaesthetized New Zealand White rabbits into

Abbreviations used: HDL, high-density lipoprotein; apoE, apolipoprotein E; CETP, cholesteryl ester transfer protein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.

* To whom correspondence should be addressed.

EDTA (to give a final concentration of 1.3 mg/ml). The procedures used were approved by the Home Office. VLDL, LDL and HDL were prepared from rabbit plasma by ultracentrifugation at densities 1.020, 1.055 and 1.250 g/ml for 16, 16 and 40 h respectively at 105 400 *g* (40 000 rev./min) in a Beckman preparative ultracentrifuge model XL-80 with a 50Ti rotor. These densities differ from those normally used for the separation of human lipoproteins and have been shown to produce a more effective separation of rabbit lipoprotein fractions [20]. Human HDL was obtained from the plasma of normal healthy volunteers by the same ultracentrifugation procedure at a density of 1.063–1.21 g/ml.

Heparin–Sepharose affinity chromatography

Heparin–Sepharose was prepared by covalent coupling of heparin to Sepharose 4B activated by CNBr as previously described [21,22] and equilibrated in a column (1 cm × 12.5 cm) with 50 mM Tris/HCl/50 mM NaCl, pH 7.4. Approx. 15 ml of the above buffer, containing 25 mM MnCl₂, was passed through the column immediately before application of the sample. Samples of HDL, prepared by ultracentrifugation, were dialysed exhaustively against 50 mM Tris/HCl/50 mM NaCl, pH 7.4, and solid MnCl₂ was added just before application to the column to give a final Mn²⁺ concentration of 25 mM [22,23]. A volume of 1.5 ml containing between 2.5 and 3.5 mg of HDL protein was applied to the column and allowed to equilibrate for 16 h before elution of the unbound material with the same buffer. When all of this material had been removed from the column (as assessed by reduction in *A*₂₈₀ to the baseline value), the bound fraction of HDL was eluted with 5 mM Tris/HCl/0.1 M NaCl, pH 7.4. Finally, the column was washed with 5 mM Tris/HCl/0.6 M NaCl to remove small traces of impurities.

Previous studies showed that for normal healthy male subjects (*n* = 100), the unbound and bound fractions accounted for 66.8 ± 6.32 and 33.2 ± 6.32 % respectively (means ± S.D.) of the total HDL on the basis of *A*₂₈₀ measurements [24]. The bound material contained the major portion of the HDL subfraction of largest particle size, HDL_{2b}, with a small proportion of smaller particles (HDL_{2a} and HDL_{3a}) [21]. From the known distribution and apoE content of the different HDL subfractions [20,21], the bound material is estimated to contain approx. 84 % of the total apoE content of HDL and is therefore referred to as apoE-rich HDL. In contrast, the unbound material contains only approx. 16 % of the apoE content of HDL and is designated apoE-poor HDL. The concentration of apoE associated with HDL has been shown to be 1.9 ± 0.5 mg/dl of plasma in normal subjects (*n* = 3), whereas that of apoA-I and apoA-II was 103.6 ± 28.4 and 50.0 ± 12.5 respectively [24].

Preparation of ¹⁴C- and ³H-labelled lipoproteins

Total HDL and its subfractions, separated by heparin–Sepharose chromatography, were labelled with [¹⁴C]sucrose by a modification of the method of Pittman and Taylor [25]. Briefly, 1 MBq of dried [U-¹⁴C]sucrose (20.74 GBq/mmol) was dissolved in 200 μl of 10 mM 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in acetone. The commercial preparation of cyanuric chloride was recrystallized twice in toluene before use. A volume of 150 μl of 20 mM NaOH was added and, after 15 s, 150 μl of 2.5 mM acetic acid was added to neutralize the reaction mixture. To the resulting [¹⁴C]sucrose–dichlorotriazine was added HDL (or its subfraction) containing 2 mg of protein in 1 ml of 150 mM NaCl/20 mM NaH₂PO₄/1 mM EDTA, pH 7.4. The reaction was allowed to proceed at room temperature for 6 h in the presence of 200 μl of 20 mM NaH₂PO₄/1.5 mM 5,5'-dithiobis-

(2-nitrobenzoic acid), pH 7.4. The sample was then dialysed for 12 h against incubation buffer (see below). The distribution of subfractions of [¹⁴C]sucrose-labelled HDL, determined by gradient gel electrophoresis, was identical with that of the subfractions of unlabelled HDL. In addition, the distribution of label between apoproteins, separated by SDS/PAGE, paralleled the distribution of the concentrations of the individual apolipoproteins. These observations demonstrated that radioactivity was distributed in proportion to the distribution of apolipoproteins, as previously reported by other investigators [26]. The mean specific radioactivity of the [¹⁴C]sucrose-labelled HDL was 125 Bq of ¹⁴C/μg of HDL protein. HDL and LDL were labelled with [³H]cholesteryl linoleate as described by Brown et al. [27]. HDL or LDL containing 60–100 μg of protein in a volume of 1 ml of 150 mM NaCl/1 mM EDTA, pH 7.4, was incubated with 100 μCi (3.7 × 10⁶ MBq) of [³H]cholesteryl linoleate for 6 h at 37 °C in the presence of 10 % DMSO. The sample was then dialysed against a minimum of 6 litres of 150 mM NaCl/0.3 mM EDTA, pH 7.4. The absence of free non-lipoprotein-bound [³H]cholesteryl linoleate in the final preparation was confirmed by TLC [20].

Preparation of rabbit liver membranes

Membranes were prepared by several different procedures. Initially, they were obtained by methods involving homogenization of portions of the intact liver as described by Fidge [8] and Arnold et al. [28]. As these membranes failed to bind either of the HDL subfractions, a procedure based on that of Wishart and Mackinnon [26] was used. A New Zealand White rabbit was exsanguinated under anaesthesia by cardiac puncture, and heparin (500 units in 1 ml of 150 mM NaCl) was injected into the heart. The thorax and abdomen were opened, and the inferior vena cava and right atrium were cannulated. The system was perfused using a peristaltic pump with Hanks Ca²⁺-free buffered saline solution containing EGTA (0.25 mM) at 10 ml/min for approx. 15 min, until the liver appeared yellow. The perfusion buffer was changed to Hanks buffered saline containing collagenase (0.16 unit/ml), trypsin inhibitor (50 μg/ml) and 0.3 mM CaCl₂, and perfusion was continued for a further 15 min. The liver was then excised and the cells combed away from the organ and placed in 150 mM NaCl/10 mM Tris/HCl/1 mM CaCl₂/0.5 mM PMSF, pH 7.4. The cell suspension was then centrifuged at 50 *g* for 2 min at 4 °C and the hepatocytes were carefully removed from the bottom of the tube after removal of a thin layer of blood cells that formed between the hepatocyte layer and the supernatant. The hepatocyte preparation was then stirred with 12.5 mM Tris/HCl, pH 8.0, for 20 min at 4 °C. The cells were observed under the microscope to be completely disrupted. The membrane suspension was then centrifuged at 500 *g* for 10 min at 4 °C, and the supernatant re-centrifuged at 105 400 *g* for 1 h at 4 °C. The pellet was suspended in 150 mM NaCl/10 mM Tris/HCl/1 mM CaCl₂/0.5 mM PMSF, pH 7.4.

Measurement of binding of labelled HDL and LDL to liver membranes

Approx. 0.5 ml of the above suspension, containing 0.5 mg of membrane protein, and approx. 0.2 ml of [¹⁴C]sucrose-labelled HDL or LDL, containing 125 μg of protein, were added to 0.8 ml of 150 mM NaCl/1 mM CaCl₂/5 mM Tris/HCl, pH 7.4 (incubation medium) in 10 ml polyallomer centrifuge tubes and incubated at 0 °C for the times indicated. Further binding was inhibited by the addition of 200 μl of 100 mM EDTA and 10 ml of 150 mM NaCl with centrifugation of the mixture at 105 400 *g* for 1 h at 4 °C. The pellet was suspended in 0.5 ml of water, and

0.5 ml of hexane and 1 ml of Optiphase hisafe II were added. Radioactivity was measured in an LKB 1219 Rackbeta scintillation counter, the data being processed on the Ultratherm 2, programmed for double-labelled counting. All double-labelled samples contained an excess of ^3H . In a preliminary series of experiments, a similar degree of binding was obtained over a time course in the presence and absence of EDTA, added at the end of the incubation period, demonstrating that binding was not reversed by the presence of EDTA.

Cell culture experiments

HepG2 cells were plated at a density of $10^6/\text{ml}$ with a total of 7 ml per dish with Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum. After an overnight incubation at 37°C with a 5% $\text{CO}_2/95\%$ air-humidified atmosphere, the medium was replaced with Dulbecco's modified Eagle's medium containing [^3H]cholesteryl ester/[^{14}C]sucrose-labelled HDL with a protein content of $100\text{ }\mu\text{g}$.

At times 0, 7, 24 and 48 h, the medium was removed from individual dishes and the cells harvested after two washes with PBS. The cells were then scraped from each dish using two portions of 1 ml of PBS and frozen until the end of the time course. The packed cells were then washed with 2 vol. of 1 ml of

PBS and mixed with 2 ml of Optiphase hisafe II for scintillation counting. In further sets of experiments, the above procedure was repeated with the addition of unlabelled apoE-poor HDL in the incubation mixture.

RESULTS

Binding of rabbit LDL, HDL, apoE-rich HDL and apoE-poor HDL to rabbit liver membranes

When rabbit liver membranes, prepared by the method of Wishart and Mackinnon [26], were incubated at 4°C with [^{14}C]sucrose-labelled LDL, the amount of radioactivity recovered in the membranes increased over the time course shown in Figure 1(a). Competitive inhibition produced by increasing concentrations of unlabelled LDL is illustrated in Figure 1(b). These results demonstrate that the membranes apparently contained functional LDL receptors after the procedures used for their preparation.

Incubation of the same membrane preparations with either total rabbit HDL (of which approx. 33% was apoE-rich) or its isolated apoE-rich or apoE-poor subfractions labelled with [^{14}C]sucrose failed to produce any detectable binding of label to the membranes over the same time course. The results for total HDL are shown in Figure 2. Essentially identical results were obtained for the two subfractions (results not shown). The recovery of ^{14}C in the membrane pellet was not significantly greater than in the pellet obtained when membranes were omitted from the incubation mixture (Figure 2a). Omission of Ca^{2+} ions

Figure 1 Binding of [^{14}C]sucrose-labelled LDL to rabbit hepatocyte membranes: (a) incorporation of label by membranes with time; (b) competitive inhibition by unlabelled LDL

Each tube contained $500\text{ }\mu\text{g}$ of membrane protein isolated from rabbit hepatocytes (suspended in 0.5 ml of 150 mM NaCl/10 mM Tris/HCl/1 mM $\text{CaCl}_2/0.5\text{ mM}$ PMSF, pH 7.4), 0.2 ml of [^{14}C]sucrose-labelled LDL (containing $125\text{ }\mu\text{g}$ of protein) and 0.8 ml of 150 mM NaCl/1 mM $\text{CaCl}_2/5\text{ mM}$ Tris/HCl, pH 7.4 (incubation mixture). After incubation at 4°C for the time interval indicated (a) or in the presence of unlabelled LDL (added to the incubation buffer) containing the amounts of LDL protein indicated (b), $200\text{ }\mu\text{l}$ of 100 mM EDTA and 10 ml of 150 mM NaCl were added. The total amount of [^{14}C]sucrose-labelled LDL bound to the membranes was determined as described in the Materials and methods section.

Figure 2 Binding of [^{14}C]sucrose-labelled HDL to hepatocyte membranes: (a) incorporation of label by the membranes with time; (b) competitive inhibition by unlabelled ligand

Membranes (containing $500\text{ }\mu\text{g}$ of protein in 0.5 ml) and 0.8 ml of incubation buffer, as detailed in the legend to Figure 1, together with 0.2 ml of [^{14}C]sucrose-labelled HDL (containing $125\text{ }\mu\text{g}$ of protein; specific radioactivity $125\text{ Bq}/\mu\text{g}$ of HDL protein) were incubated at 4°C (a) for the time intervals indicated, and (b) in the presence of increasing amounts of unlabelled HDL as indicated. ●, Incubation in the presence of membranes as described above; □, incubation in which the membrane suspension was replaced with 0.5 ml of buffer.

Figure 3 Incorporation of [^{14}C]sucrose-labelled HDL and [^3H]cholesteryl ester into human hepatoma HepG2 cells from subfractions of human HDL

HepG2 cells were grown as described in the Materials and methods section. Cell suspension (7 ml containing 10^6 cells/ml in Dulbecco's modified Eagle's medium with 10% foetal bovine serum) were left overnight at 37 °C with 5% $\text{CO}_2/95\%$ air in tissue culture dishes. The medium was then replaced with Dulbecco's modified Eagle's medium containing [^{14}C]sucrose/[^3H]cholesteryl ester-labelled HDL subfractions (containing 100 μg of protein). At the time points indicated, the medium was removed and the cells were washed twice with PBS. Radioactivity was determined as described in the Materials and methods section. The points represent the uptake of [^{14}C]sucrose-labelled HDL (○) and [^3H]cholesteryl ester (□) from apoE-poor HDL (containing 2.5 $\mu\text{Ci}/\text{mg}$ ^{14}C and 33 $\mu\text{Ci}/\text{mg}$ [^3H]cholesteryl ester) and the uptake of [^{14}C]sucrose-labelled HDL (●) and [^3H]cholesteryl ester (■) from apoE-rich HDL (containing 3.75 $\mu\text{Ci}/\text{mg}$ ^{14}C and 50 $\mu\text{Ci}/\text{mg}$ cholesteryl ester). Each value represents the mean of duplicate measurements.

Figure 4 Effect of increasing concentration of unlabelled apoE-poor HDL on the binding and uptake of double-labelled HDL by HepG2 cells grown in culture

HepG2 cells were grown and incubated with the labelled HDL subfractions as described in the legend to Figure 3 in the presence of increasing concentrations of unlabelled apoE-poor HDL. The designation of the points is the same as in Figure 3. Each point represents the mean value of duplicate experiments.

and modifications to the composition of the incubation medium and to the temperature [29] failed to produce binding.

Uptake of ^3H and ^{14}C from double-labelled HDL and its subfractions by HepG2 cells

Double-labelled apoE-rich and apoE-poor subfractions of HDL were incubated with HepG2 cells for 48 h. Over this time period, considerable and increasing amounts of ^3H were present in the cells incubated with both HDL subfractions, with up to 30 % of the added label being present in the cells after this period. The amount of ^3H present in the cells at each time point was similar

(Figure 3). In contrast, only a small amount of ^{14}C (associated with the apoprotein moiety of the HDL) was present in the cells after incubation with either of the two subfractions during the same period and amounted to less than 4 % of the added ^{14}C . Addition of increasing amounts of unlabelled apoE-poor HDL to the incubation assay resulted in a progressive decrease in the amount of label in all cases (Figure 4).

DISCUSSION

It has long been known that apoE is involved in the transport and tissue uptake of cholesterol [16]. Cholesterol feeding produces a species of HDL that is rich in apoE in addition to increased concentrations of other lipoproteins such as β -VLDL [30]. The recent finding by several laboratories that a subfraction of HDL enriched in apoE (HDL_{2b}) and apoE-rich HDL prepared by affinity chromatography were lower than normal in patients with coronary heart disease [24,31–33] further suggests a role for apoE-rich HDL in reverse cholesterol transport. The findings imply that apoE-rich HDL is taken up by the liver, although the mechanism is not understood. Although some investigators have demonstrated the presence of receptors for apoE-rich HDL on isolated liver membranes [4], strong evidence has been presented that selective uptake is the major route of entry of HDL cholesteryl ester into the liver [12,14,15].

In the present study, no binding of [^{14}C]sucrose-labelled HDL or the apoE-rich or apoE-poor subfractions to rabbit liver membranes could be detected. The observation that [^{14}C]sucrose-labelled LDL showed characteristic binding properties with the same preparation of membranes and that binding of ^{14}C was competitively inhibited by unlabelled LDL demonstrated that the membrane preparations contained functional receptors. The binding of HDL to membranes has been shown to be species-specific [18], and significantly lower levels of binding were observed when human HDL or LDL were used in binding studies with animal tissues [17,18,34]. However, Fidge and co-workers [8,9,35] have reported successful binding of human HDL to rat liver membranes. In the present investigations, both rabbit HDL and rabbit membranes were used and therefore species-specificity will not have affected the results. These experiments therefore suggest that, in the rabbit under the conditions used, delivery of cholesterol to the liver by HDL is not dependent on binding to the cell surface and that other mechanisms are operative, as suggested by others [14,26].

To investigate the effect of apoE on the selective uptake of HDL cholesterol, we used human hepatoma HepG2 cells with HDL subfractions of known composition separated from human plasma on the basis of their apoE content, as few binding studies have been performed using specific subfractions of HDL of defined apoprotein composition. The apoE-rich fraction consists of particles mainly of the HDL_2 class, with apoE comprising approx. 1.1 % of their total apoprotein content. The apoE-poor HDL contains mainly HDL_3 but also some HDL_2 and less than 0.2 % of its apoprotein as apoE [21,36]. The apoE-rich HDL used in these studies is a fraction that is normally present in human plasma [24] and is distinct from HDL_c separated from the plasma of cholesterol-fed animals which contains apoE as its only apoprotein constituent [37]. In contrast with the apoE-rich HDL used in the present study, HDL_c has been shown to bind with high affinity to the LDL (B,E) receptor [37]. The rabbit subfractions used in the present study have been shown to have very similar properties and compositions to those of their human counterparts [20]. These human HDL subfractions differ considerably from those from both human and animal species, such

as the rat and dog, and from other human subfractions which have been used in previously reported investigations.

The present studies with double-labelled HDL subfractions demonstrated that for both apoE-rich and apoE-poor fractions there was a rapid uptake of [³H]cholesterol with only very small amounts of [¹⁴C]apoprotein entering the cells. This demonstrates that selective uptake is the major route of uptake of cholesterol from both HDL fractions, although it does not preclude the possibility of a small amount of uptake by a conventional receptor. The observation that the uptake of label from both fractions was competitively inhibited to the same extent by the addition of unlabelled apoE-poor HDL indicates that uptake is not directly dependent on the presence of apoE.

Exchange of cholesteryl esters between labelled and unlabelled particles during the 48 h incubation period seems unlikely as the incubations were performed with isolated HDL subfractions devoid of CETP activity which is necessary for such processes [38]. The use of labelled sucrose covalently attached to the apolipoproteins ensured that the radioactive catabolic products remained trapped within the cells and thereby provided a quantitative measure of their rate of uptake [39,40]. Although efflux of labelled cholesterol during the incubation period in the presence of excess labelled lipoprotein cannot be precluded, the extent of such a process would be expected to be small, as the large HDL particles present are known to be poor acceptors of cellular cholesterol [41].

The above findings with apoE-rich and apoE-poor HDL are in general agreement with previous observations on animal membranes using other lipoprotein fractions. Using reconstituted HDL, Pittman et al. [42] showed that uptake by cultured rat hepatocytes and adrenal cells was independent of apoprotein composition but was dependent on particle size. The selective uptake of HDL₃ by HepG2 cells was demonstrated to be stimulated by CETP over a prolonged time course, suggesting transfer of HDL cholesteryl ester to secreted lipoproteins that are then taken up again [43,44]. *In vivo* studies on rats showed that cholesteryl ester was taken up by the liver at a more rapid rate from HDL without apoE than from the apoE-rich HDL₁ fraction [15]. Kilsdonk et al. [45], however, reported that LpA-I (i.e. lipoproteins containing only apoA-I), which contains the apoE-rich particles, was both bound and taken up by HepG2 cells to a greater extent than LpA-I/A-II (i.e. lipoproteins containing both apoA-I and apoA-II) which is largely devoid of apoE. Several investigators, however, have reported the binding of HDL₃, but not of HDL₂, to liver membranes [8,9,29].

It has been shown that large apoE-containing particles are formed from smaller HDL devoid of apoE [46] and that these particles are important in the clearance of HDL cholesterol by the liver. This is supported by clinical data [2,24,32,33]. In the present study, the direct uptake by the liver of these particles by means of a conventional receptor could not be demonstrated. The avid accumulation of cholesterol by HepG2 cells from these particles by selective uptake was, however, independent of their apoE content. These findings suggest that alternative mechanisms may operate under different conditions, such as high cholesterol loading, and that further work is required to clarify the mechanism involved in the role of apoE in the clearance of HDL cholesterol.

This work was supported by The Wellcome Trust. We are grateful to Mrs. I. Wood and Mrs. C. Watt for skilled technical assistance.

REFERENCES

- 1 Glomset, J. A. (1968) *J. Lipid Res.* **9**, 155–167
- 2 Reichl, D. and Miller, N. E. (1989) *Arteriosclerosis* **9**, 785–797
- 3 Barter, P. J. (1993) *Curr. Opin. Lipidol.* **4**, 210–217
- 4 Hui, D. Y., Brecht, W. J., Hall, E. A., Friedman, G., Innerarity, T. L. and Mahley, R. W. (1986) *J. Biol. Chem.* **261**, 4256–4267
- 5 Sherrill, B. C., Innerarity, T. L. and Mahley, R. W. (1982) *J. Biol. Chem.* **255**, 1804–1807
- 6 Mindham, M. A., Mayes, P. A. and Miller, N. E. (1990) *Biochem. J.* **268**, 499–505
- 7 Mindham, M. A. and Mayes, P. A. (1991) *Biochem. J.* **279**, 503–508
- 8 Fidge, N. H. (1986) *FEBS Lett.* **199**, 265–268
- 9 Tozuka, M. and Fidge, N. (1989) *Biochem. J.* **261**, 239–244
- 10 Ghosh, D. K. and Menon, K. M. J. (1987) *Biochem. J.* **244**, 471–479
- 11 Tabas, I. and Tall, A. R. (1984) *J. Biol. Chem.* **259**, 13897–13905
- 12 Glass, C., Pittman, R. C., Civen, M. and Steinberg, D. (1985) *J. Biol. Chem.* **260**, 744–750
- 13 Mendel, C. M., Kunitake, S. T., Kane, J. P. and Kempner, E. S. (1986) *J. Biol. Chem.* **263**, 1314–1319
- 14 Goldberg, D. I., Beltz, W. F. and Pittman, R. C. (1991) *J. Clin. Invest.* **87**, 331–346
- 15 Richard, B. M. and Pittman, R. C. (1993) *J. Lipid Res.* **34**, 571–579
- 16 Mahley, R. W. (1982) *Med. Clin. N. Am.* **66**, 375–402
- 17 Drevon, C. A., Attie, A. D., Pangburn, S. H. and Steinberg, D. (1981) *J. Lipid Res.* **22**, 37–46
- 18 Fernandez, M. L. and McNamara, D. J. (1990) *Biochim. Biophys. Acta* **1042**, 142–145
- 19 Chapman, J. M. (1986) *Methods Enzymol.* **128**, 70–143
- 20 Fragoso, Y. D. and Skinner, E. R. (1993) *Comp. Biochem. Physiol.* **B105**, 699–706
- 21 Wilson, H. M., Griffin, B. A., Watt, C. and Skinner, E. R. (1992) *Biochem. J.* **248**, 477–481
- 22 Skinner, E. R. (1992) in *Lipoprotein Analysis: A Practical Approach* (Converse, C. A. and Skinner, E. R., eds.), pp. 85–118, IRL Press, Oxford
- 23 Weisgraber, K. H. and Mahley, R. W. (1980) *J. Lipid Res.* **21**, 316–325
- 24 Wilson, H. M., Patel, J. C., Russell, D. and Skinner, E. R. (1993) *Clin. Chim. Acta* **220**, 175–187
- 25 Pittman, R. C. and Taylor, C. A. (1986) *Methods Enzymol.* **129**, 622–628
- 26 Wishart, R. and Mackinnon, M. (1990) *Biochim. Biophys. Acta* **1044**, 375–381
- 27 Brown, M. S., Dana, S. E. and Goldstein, J. L. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2925–2929
- 28 Arnold, K. S., Innerarity, T. L., Pitas, R. E. and Mahley, R. W. (1992) in *Lipoprotein Analysis: A Practical Approach* (Converse, C. A. and Skinner, E. R., eds.), pp. 145–168, IRL Press, Oxford
- 29 Chacko, G. K. (1982) *Biochim. Biophys. Acta* **712**, 129–141
- 30 Mahley, R. W. and Holcombe, K. S. (1977) *J. Lipid Res.* **18**, 314–324
- 31 Wilson, H. M., Patel, J. C. and Skinner, E. R. (1990) *Biochem. Soc. Trans.* **18**, 1175–1176
- 32 Johansson, J., Carlson, L. A., Landou, C. and Hamsten, A. (1991) *Arterioscler. Thromb.* **11**, 174–182
- 33 Williams, P. T., Krauss, R. M., Vranizan, K. M., Stefanik, M. L., Wood, P. D. S. and Lindgren, F. T. (1992) *Arterioscler. Thromb.* **12**, 332–334
- 34 Chapman, J. M., Loisy, F. Y. M., Forgez, P. and Cadman, H. (1985) *Biochim. Biophys. Acta* **835**, 258–272
- 35 Allan, C. M., Fidge, N. H. and Kanellos, J. (1992) *J. Biol. Chem.* **267**, 13257–13261
- 36 Skinner, E. R. (1994) *Curr. Opin. Lipidol.* **5**, 241–247
- 37 Weisgraber, K. H. and Mahley, R. W. (1986) *Methods Enzymol.* **129**, 145–166
- 38 Fielding, C. J. (1993) *Curr. Opin. Lipidol.* **4**, 218–222
- 39 Pittman, R. C., Attie, A. D., Carew, T. E. and Steinberg, D. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5345–5349
- 40 Pittman, R. C., Green, S. R., Attie, A. D. and Steinberg, D. (1979) *J. Biol. Chem.* **254**, 6876–6879
- 41 Franceschini, G., Maderna, P. and Sirtori, C. S. (1991) *Atherosclerosis* **88**, 99–107
- 42 Pittman, R. C., Glass, C. K., Atkinson, D. and Small, D. M. (1987) *J. Biol. Chem.* **262**, 2435–2442
- 43 Granot, E., Tabas, I. and Tall, A. R. (1987) *J. Biol. Chem.* **262**, 3482–3487
- 44 Rinninger, F. and Pittman, R. C. (1989) *J. Biol. Chem.* **264**, 6111–6118
- 45 Kilsdonk, E. P. C., van Gent, T., Dorsman, N. R. D. and van Tol, A. (1992) *Atherosclerosis* **97**, 131–142
- 46 Gavish, D., Oschry, Y. and Eisenberg, S. (1987) *J. Lipid Res.* **28**, 257–267