Effect of apolipoproteins E and C-III on the interaction of chylomicrons with parenchymal and non-parenchymal cells from rat liver

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1. [^3H]Triacylglycerol-labelled chylomicrons were isolated from intestinal lymph, obtained from rats made hypolipidaemic by treatment with pharmacological amounts of 17 α -ethinyloestradiol. Oestrogen treatment results in a large reduction in the content of apolipoproteins (apo) E and C of lymph chylomicrons. Upon incubation *in vitro* with freshly isolated parenchymal and non-parenchymal cells the apo E-, apo C-poor chylomicrons became readily cell-associated. With increasing chylomicron concentrations this cell-association was saturable and half-maximal cell-association was achieved at about 0.55 mg of triacylglycerol/ml. The cell-association was time- and temperature-dependent. A more than 90% inhibition of the cell-association of the [^3H]triacylglycerol moiety was observed with both parenchymal and non-parenchymal cells when pure apo C-III (12.6 $\mu\text{g}/\text{mg}$ of triacylglycerol) was incorporated into the chylomicrons. These data indicate that apo E-, apo C-poor chylomicrons are bound to both parenchymal and non-parenchymal liver cells at a high-affinity site of limited capacity and that binding to this site is strongly inhibited by apo C-III. 2. With apo C-III-enriched chylomicrons simultaneous determination of the cell-association of the ^{125}I -apo C-III and the [^3H]triacylglycerol moiety indicated that more ^{125}I -apo C-III becomes associated to the cells than expected on the basis of [^3H]triacylglycerol radioactivity measurements. It is suggested that upon cell-association of apo C-III its binding to the chylomicron particles is lost. Consequently the occupation of the cellular recognition site by apo C-III prevents further chylomicron binding and thus leads to a decrease of the cell-association level of the [^3H]triacylglycerol moiety. 3. Apo E enrichment of the chylomicrons led to an increased cell-association rate with parenchymal cells and to a marked increase of the cell-association level with non-parenchymal cells. The cell-association of the apo E radioactivity followed closely the [^3H]triacylglycerol radioactivity, indicating that the particle-apo E complex is bound as a unity. The apo E effects were opposed by apo C-III. With apo E-, apo C-III-enriched chylomicrons more ^{125}I -apo E became associated with the cells than could be expected on the basis of the [^3H]triacylglycerol measurements. It is concluded that apo C-III can weaken the interaction of apo E with the chylomicrons leading to the cell-association of free apo E. It appears that subtle changes in the apo E and/or apo C-III content of chylomicrons can influence the interaction with both parenchymal and non-parenchymal liver cells. The quantitatively different effects observed with the parenchymal and non-parenchymal cells may influence the relative importance of these cell types in lipoprotein metabolism inside the liver.

Abbreviations used: apo E, apolipoprotein E; apo C, apolipoprotein C; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein.

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The liver plays a major role in the removal of lipoproteins from blood. Recent evidence indicates that various distinct types of high-affinity receptors are responsible for this uptake. The receptors are tentatively named (1) the remnant (apo E) receptor

(Van Berkel *et al.*, 1980, 1981; Hui *et al.*, 1981; Mahley *et al.*, 1981), (2) the LDL (apo B, E) receptor (Mahley *et al.*, 1981; Harkes & Van Berkel, 1982) and (3) the acetyl-LDL (scavenger) receptor (Van Berkel *et al.*, 1982).

The remnant (apo E) receptor from rats interacts with rat VLDL-remnants, rat LDL and rat HDL but not with apo E-free human LDL (Van Berkel *et al.*, 1981). Mahley *et al.* (1981) indicated that in dogs, swine and man the remnant receptor is metabolically independent from the apo B, E receptor and Kita *et al.* (1982) showed its distinct genetic origin in WHHL-rabbits.

The high apparent affinity of the remnant receptor for VLDL-remnants (Van Berkel *et al.*, 1981) and chylomicron-remnants (Cooper *et al.*, 1982a) was suggested to be the consequence of the presence of apo E on the remnant particles. In a rat liver perfusion system, studies have been performed that showed the stimulatory action of apo E and the inhibitory action of apo C-III on the hepatic uptake of chylomicrons [obtained from 17α -ethynyoestradiol-treated rats] (Windler *et al.*, 1980a,b,c) or synthetic emulsions (Shelburne *et al.*, 1980; Quarfordt *et al.*, 1980, 1981, 1982; Havel *et al.*, 1980).

However, besides parenchymal cells the liver contains non-parenchymal cell types contributing 25–30% of the total amount of cell membranes (Blouin *et al.*, 1977). Previously it was shown that the relative uptake *in vivo* of chylomicron- or VLDL-remnants by parenchymal and non-parenchymal cells can be explained by the presence of a 5–8-fold higher concentration of the remnant receptor in non-parenchymal cells as compared with parenchymal cells (Van Berkel *et al.*, 1981). The present study was undertaken to determine the influence of apo E and apo C on the interaction of lipoproteins with the various types of liver cells. We used chylomicrons from 17α -ethynyoestradiol-treated rats rather than chylomicron-remnants because defined amounts of labelled apolipoproteins can be incorporated in the apo E-, apo C-poor lymph chylomicrons and so the individual effect of apo E and apo C on the interaction with the liver can be investigated. Furthermore the isolated cell-system permits a closer study of the underlying mechanism of the effect of the individual apolipoproteins upon the association of lipoproteins with the liver.

Experimental

Materials

Intralipid labelled with glycerol tri[9,10- ^3H]oleate was purchased from Vitrum, Stockholm, Sweden, and [^{125}I]iodide from NEN Chemicals, Dreieich, Germany. Ham's F-10 medium was obtained from Gibco (Europe), Paisley, Scotland, U.K. Albumin (fraction V, defatted), ovalbumin, collagenase (type

I) and chloroquine were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals were reagent grade.

Isolation of triacylglycerol-labelled chylomicrons

Donor animals were male Wistar rats (body weight about 250g) pretreated for 5 days with a pharmacological dose of 17α -ethynyoestradiol (5mg/kg body wt. per day) to suppress the lymphatic concentration of apo E (Davis & Roheim, 1978; Windler *et al.*, 1980b). On the 5th day a cannula was placed in the duodenum and the main mesenteric lymph vessel, and animals were kept in a restraining cage. Intralipid labelled with glycerol tri[9,10- ^3H]oleate (sp. radioactivity 0.1 Ci/mol) was infused intraduodenally (1.5 ml/h, 75 mg of triacylglycerol/h). The intestinal lymph was collected overnight in a vessel chilled on ice. Fibrin clots were removed and chylomicrons were isolated by flotation from 0.9% NaCl/2.5 mM-EDTA (pH 7.4) ($1.8 \times 10^6 g_{av} \cdot \text{min}$), resuspended in the same medium by injection through a 27-gauge hypodermic needle and re-isolated ($3.4 \times 10^6 g_{av} \cdot \text{min}$). This step was repeated once. The ^3H -label distribution in the final preparation was determined by t.l.c. More than 93% of the label was associated with glycerol trioleate. The remaining radioactivity was associated with diacylglycerol (about 4%) and monoacylglycerol plus phospholipids (about 2%).

The apolipoprotein composition of chylomicrons from oestrogen-treated rats and control animals was investigated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis by the method of Weber & Osborn (1969). Chylomicrons were delipidated with diethyl ether and a mixture of ethanol and diethyl ether (3:1, v/v) essentially as described previously (Imaizumi *et al.*, 1978). Oestrogen treatment of rats results in a large reduction in the content of apo E (less than 0.5% of the apolipoprotein content; results not shown) and apo C of lymph chylomicrons (Windler *et al.*, 1980b).

Isolation and labelling of apo E and apo C-III

Human apo C-III (containing a single sialic acid residue) was isolated as described previously (Groot *et al.*, 1978). Rat apo E was isolated from rat HDL ($1.050 < \rho < 1.21 \text{ g/ml}$). Delipidated rat apo-HDL was solubilized in 5 M-urea/0.05 M-NaCl/2 mM-sodium phosphate buffer, pH 7.4, and apo E was isolated by affinity chromatography on Sepharose 4-B columns containing covalently attached heparin by a procedure essentially the same as described previously (Shelburne & Quarfordt, 1977) for the isolation of human apo E. Both apolipoprotein preparations showed only one protein band on urea/polyacrylamide gels (apo C-III) or sodium dodecyl sulphate/polyacrylamide gels (apo E).

Apolipoproteins were labelled with ^{125}I using the

ICI method of McFarlane (1963). The labelling procedure was performed in 0.4 M-glycine/NaOH buffer (pH 10) (apo E) or 0.06 M-sodium borate buffer (pH 8.5) (apo C-III). Free ^{125}I was removed by extensive dialysis against 0.9% NaCl/10 mM-Tris/HCl (pH 7.4)/2.5 mM-EDTA. The molar ratio of iodine incorporated/protein varied between 0.8–1.8 (apo E) and 0.1–0.3 (apo C-III). Labelled and unlabelled apolipoprotein solutions were mixed. The specific radioactivity of apo E and C-III used in these studies varied between 26–135 and 7–23 c.p.m./ng of protein.

Preparation of triacylglycerol-labelled chylomicrons enriched in apo E or apo C-III

^3H -Labelled chylomicrons from oestrogen-treated rats were incubated in the presence of ^{125}I -labelled apolipoprotein in 0.9% NaCl/2 mM-sodium phosphate buffer (pH 7.4) in a metabolic shaker at 37°C for 30–60 min. The reaction mixture (3–5 ml) contained chylomicrons (15–45 mg of triacylglycerol) and apo E or apo C-III in quantities of 2 and $20\text{ }\mu\text{g}/\text{mg}$ of triacylglycerol respectively. Under the conditions employed 40–70% of the added apolipoproteins became associated with the chylomicrons. Double-enriched chylomicrons (^{125}I -apo E plus unlabelled apo C-III or unlabelled apo E plus ^{125}I -apo C-III) were prepared by a slightly different procedure. As apo C-III decreased the efficiency of apo E incorporation, chylomicrons were first exposed to apo E (30 min at 37°C) before apo C-III was added (total incubation time 60 min). More apo E (1.5 times) and less apo C-III (0.1 times) were added in these incorporation incubations.

Apolipoprotein-enriched chylomicrons were isolated from the incubation mixture by centrifugation ($3.8 \times 10^6 g_{\text{av}}$, -min), suspended in 0.9% NaCl/2.5 mM-EDTA (pH 7.4) and re-isolated. The quantities of apo E and apo C-III associated with the chylomicrons were calculated from ^{125}I -radioactivity measurements and the known specific radioactivities of these apolipoproteins.

Cell isolation procedure and cell-association and degradation measurements

Parenchymal and non-parenchymal cells were isolated from rat liver by collagenase perfusion and differential centrifugation exactly as described in detail previously (Van Berkel *et al.*, 1982). Incubations of freshly isolated cells with the indicated amounts of chylomicrons were performed in Ham's F-10 medium (modified) containing 2% (w/v) defatted albumin. In pilot studies no difference in cell-association was noticed when albumin was replaced by ovalbumin or deleted from the medium, although the cell-viability during the long-term incubations (up to 4 h) was maintained better in the presence of albumin. For this reason albumin was

included routinely and the cell viability during incubation remained higher than 85% (see also Van Berkel *et al.*, 1982).

The incubations were carried out either in plastic tubes in a total volume of 1 ml or in 25 ml Erlenmeyer flasks stoppered with rubber caps with a total incubation volume of 6 ml. At the indicated times 1 ml samples were withdrawn and the cell-associated ^{125}I -radioactivity was determined after three washings in the presence of albumin as described previously (Van Berkel *et al.*, 1982). Degradation of the apolipoproteins was measured by the method of Bierman *et al.* (1974) as described previously (Van Berkel *et al.*, 1982). The degradation values represent radioactivity present in the acid-soluble water phase. In the corresponding

Fig. 1. Effect of temperature on the cell-association of chylomicrons and apo C-III-enriched chylomicrons from oestrogen-treated rats to parenchymal (PC) and non-parenchymal (NPC) cells

The cells were incubated for 1 h at 0.19 mg of triacylglycerol/ml. Δ and $\blacktriangle$, Chylomicrons; $\square$ and $\blacksquare$, apo C-III-enriched chylomicrons ($12.2\text{ }\mu\text{g}$ of apo C-III/mg of triacylglycerol). The cell-association is expressed in μg of triacylglycerol/mg of cell protein.

blanks the chylomicrons or apolipoproteins were incubated in the absence of cells. The cell-association of [^3H]triacylglycerol was determined after extraction of the lipids by the method of Bligh & Dyer (1959).

Results

Effect of temperature on the cell-association

The cell-association of the chylomicrons isolated from 17 α -ethynyoestradiol-treated rats was strongly dependent on the temperature (Fig. 1). After 1 h of incubation the cell-association at 0°C is for parenchymal cells only 10% of the value at 37°C, whereas for non-parenchymal cells this relative percentage is 23%. The cell-association at 0°C did not increase further by extending the incubation time to 2 or 3 h (results not shown). Irrespective of the incubation temperature a strong inhibition of the cell-association was observed by preloading the chylomicrons with 12.5 μg of apo C-III/mg of triacylglycerol.

Effect of incubation time and substrate concentration

The rate of association of the chylomicrons to both liver cell types at 37°C is initially rapid and slows down at 30 min for non-parenchymal cells and 60 min for parenchymal cells (Fig. 2). The amount of cell-associated triacylglycerol with non-parenchymal cells was about twice as high per mg of cell protein as that with parenchymal cells. The cell-

association of [^3H]triacylglycerol was, with parenchymal and non-parenchymal cells, nearly completely blocked by preloading the chylomicrons with apo C-III.

With increasing chylomicron concentrations the cell-association approaches saturation both at 10 min and 60 min of incubation (Fig. 3). Replotting of the 10 min data by the Michaelis-Menten method reveals a linear double-reciprocal relationship and, with both parenchymal and non-parenchymal cells, an apparent K_m value of 0.55 mg of triacylglycerol/ml is obtained. The 60 min values (equilibrium association) can be plotted by the Scatchard method and reveal a monoexponential linear relationship from which a dissociation constant (K_d) for parenchymal cells of 0.50 mg of triacylglycerol/ml and for non-parenchymal cells of 0.20 mg of triacylglycerol/ml can be deduced. No evidence for a second low-affinity or unspecific component was obtained.

The specificity of the cell-association is further indicated by more than 90% inhibition by low amounts of apo C-III. Even at the highest triacylglycerol concentration (1 mg) only 12 μg of apo C-III exerts this inhibition in the presence of 20 mg of albumin (an excess of 1600-fold).

Relationship between the cell-association of [^3H]triacylglycerol and apo C-III

The labelling of both the triacylglycerol (with ^3H) and the apolipoprotein (with ^{125}I) moieties of chylomicrons allows the simultaneous measurement of cell-association of both components. Further-

Fig. 2. Time course of the cell-association of chylomicrons and apo C-III-enriched chylomicrons from oestrogen-treated rats to parenchymal (PC) and non-parenchymal cells (NPC)

The cells were incubated with 0.17 mg of triacylglycerol/ml. Values are means of three different chylomicron and apo C-III-enriched chylomicron preparations incubated with three different cell preparations. The results are given as means $\pm$ s.e.m. (indicated by the bars). The chylomicrons contained $5.9 \pm 0.4 \mu\text{g}$ of protein/mg of triacylglycerol (Δ). The apo C-III-enriched chylomicrons contained an additional $12.6 \pm 0.2 \mu\text{g}$ of apo C-III/mg of triacylglycerol ($\square$).

Fig. 3. Relation of the concentration of chylomicrons and apo C-III-loaded chylomicrons from oestrogen-treated rats to the extent of cell-association with parenchymal (PC) and non-parenchymal cells (NPC)

The amount of triacylglycerol was varied and the cell-association was determined after 10 min (Δ) or 60 min ($\circ$ and $\bullet$) of incubation. Δ and $\circ$, Chylomicrons ($5.4\mu\text{g}$ of protein/mg of triacylglycerol); $\bullet$, apo C-III-loaded chylomicrons ($12.2\mu\text{g}$ of apo C-III/mg of triacylglycerol).

Fig. 4. Time course of the cell-association and degradation of ^{125}I -apo C-III present on ^3H -triacylglycerol-labelled chylomicrons from oestrogen-treated rats

$\blacksquare$, ^{125}I radioactivity (representing actual amount of ^{125}I -apo C-III associated to the cells); $\square$, calculated on the basis of the ^3H -triacylglycerol radioactivity (representing the expected amount of apo C-III that should be associated with the cells). The apo C-III-enriched chylomicrons contained $12.5\mu\text{g}$ of ^{125}I -apo C-III/mg of triacylglycerol. The amount of triacylglycerol added to the incubation was 0.08 mg/ml . $\blacktriangledown$, Apo C-III degradation (^{125}I radioactivity present in the acid-soluble water phase of the incubation medium).

more, the degradation of a single apolipoprotein (in this case apo C-III) can be monitored by measurement of the trichloroacetic acid-soluble water phase. Since the amount of apo C-III associated with the chylomicrons is known, the expected amount of cell-associated apo C-III can be calculated from the triacylglycerol radioactivity (Fig. 4). It appears that

the actual amount of ^{125}I -apo C-III that becomes cell-associated is much higher than that expected on the basis of the measurement of ^3H -triacylglycerol radioactivity, indicating that upon binding of the apo C-III-enriched chylomicrons to the cells the triacylglycerol moiety is lost, whereas apparently free apo C-III remains associated with the cells.

The cell-association of apo C-III to non-parenchymal cells is about 5–6-fold higher than to parenchymal cells per mg of cell protein. Furthermore an effective degradation of apo C-III occurs with both parenchymal and non-parenchymal cells and especially with non-parenchymal cells a lag phase is observed before the degradation products are formed at a constant rate.

Relation between the cell-association and degradation of apo C-III from apo C-III-enriched chylomicrons

The cell-association of apo C-III from apo C-III-enriched chylomicrons is dependent on temperature in a similar way to that of the control chylomicrons (Fig. 5): with non-parenchymal cells

Fig. 5. Effect of temperature on the cell-association and degradation of ^{125}I -apo C-III present on apo C-III-enriched chylomicrons from oestrogen-treated rats

Abbreviations used: PC, parenchymal cells; NPC, non-parenchymal cells. The cells were incubated for 1 h with 0.19 mg of triacylglycerol/ml enriched with $12.2\mu\text{g}$ of ^{125}I -apo C-III/mg of triacylglycerol.

Fig. 6. Concentration-dependent cell-association and degradation of ^{125}I -apo C-III present on apo C-III-loaded chylomicrons from oestrogen-treated rats to parenchymal (PC) and non-parenchymal cells (NPC)

The cells were incubated for 1 h with various concentrations of triacylglycerol loaded with $12.2\mu\text{g}$ of ^{125}I -apo C-III per mg of triacylglycerol.

Fig. 7. Time course of cell-association of chylomicrons enriched with various amounts of apo E and/or apo C-III to parenchymal (PC) and non-parenchymal cells (NPC)

(a) The chylomicrons from oestrogen-treated rats contained $6.4 \mu\text{g}$ of protein/mg of triacylglycerol (Δ). The ^{125}I -apo E-enriched chylomicrons contained in addition $1.3 \mu\text{g}$ of ^{125}I -apo E/mg of triacylglycerol (O). The apo E, apo C-III-enriched chylomicrons contained in addition $2.5 \mu\text{g}$ of ^{125}I -apo E and $1.9 \mu\text{g}$ of apo C-III/mg of triacylglycerol (∇). The apo C-III-enriched chylomicrons contained, in addition, $12.5 \mu\text{g}$ of apo C-III/mg of triacylglycerol ($\square$). The triacylglycerol concentration present during the incubation was 0.18 mg/ml for all preparations. (b) and (c) The filled symbols ($\bullet$, $\blacktriangledown$ and $\blacklozenge$) represent the iodine measurements [actual amount of apo E associated to ($\bullet$ and $\blacktriangledown$) or degraded by ($\blacklozenge$) the cells]. The open symbols (O and ∇) represent the expected amount of apo E that should be associated to the cells on basis of the ^{125}I -triacylglycerol radioactivity (from a). (b) shows ^{125}I -apo E-loaded chylomicrons; (c) shows ^{125}I -apo E, apo C-III-loaded chylomicrons.

at 0°C only 19% of the cell-association at 37°C is found, whereas this percentage for parenchymal cells is 5%. The effect of temperature on the degradation of apo C-III is, however, markedly different from the cell-association data and a marked increase in degradation occurs with an increment in the temperature from 25°C to 37°C. When increasing amounts of apo C-III-enriched chylomicrons are added to the cells at 37°C, both the cell-association and degradation are clearly saturable and a similar concentration-dependency for both processes is observed (Fig. 6). This provides strong evidence that these phenomena are related. The degradation of apo C-III was not influenced by addition of 100 μ M-chloroquine ($100 \pm 1\%$ of the control; $n = 4$; mean $\pm$ S.E.M.; 2 h incubation) or 10 mM-NH₄Cl ($92 \pm 6\%$ of the control; $n = 4$; mean $\pm$ S.E.M.; 2 h incubation).

Effect of apo E and opposing effect of apo C-III

The influence of apo E on the cell-association of chylomicrons from 17 α -ethnyloestradiol-treated rats to parenchymal and non-parenchymal cells is quantitatively different. With parenchymal cells the incorporation of apo E leads to an increased cell-association rate and the equilibrium level of cell-association is reached at 30 min of incubation. No effect on the cell-association level is observed (Fig. 7a). With non-parenchymal cells the level of cell-association of the chylomicrons is clearly increased (nearly 3-fold).

The effect of apo E is opposed by apo C-III, leading to a lower rate of cell-association with parenchymal cells (even lower than with the native chylomicrons). With non-parenchymal cells a cell-association curve is found that is intermediate between the solely apo E-containing and native chylomicrons. Under suboptimal conditions for cell-association (0°C) it is clear that apo E does influence the interaction level of the chylomicrons with both cell-types, an effect also opposed by apo C-III (Fig. 8). By simultaneous labelling of the triacylglycerol moiety (with ³H) and the apo E (with ¹²⁵I) it was possible to follow both constituents. A comparison between the theoretical amount of apo E that will become associated with the cells on the basis of [³H]triacylglycerol radioactivity, and the actually measured amount of ¹²⁵I-apo E indicate that with the solely apo E-containing chylomicrons both values agree closely (Fig. 7b). This indicates that during the total time course of incubation apo E remains associated with the particles. With apo E-, apo C-III-containing chylomicrons, however, more ¹²⁵I-apo E is found associated with the cells (Fig. 7c), as expected on the basis of [³H]triacylglycerol radioactivity. This implies that in the presence of apo C-III, non-particle-associated apo E is attached to the cells. Similarly, as with apo C-III,

Fig. 8. The cell-association of chylomicrons enriched with various amounts of apo E and/or apo C-III to parenchymal (PC) and non-parenchymal cells (NPC) at 0°C

The chylomicron preparations and incubation conditions were identical with those used in Fig. 7 except that incubation was performed for 1 h at 0°C. The left (unlabelled) columns represent the cell-association of the untreated chylomicrons from oestrogen-treated rats.

it is clear that apo E is degraded. The time course of degradation shows a clear lag phase and the degradation by the non-parenchymal cells is about 11–12-fold higher (per mg of cell protein) as by parenchymal cells. Similarly, as with apo C-III no effect of 100 μ M-chloroquine ($97 \pm 6\%$; $n = 4$; mean $\pm$ S.E.M.; 2 h incubation) or of 10 mM-NH₄Cl ($93 \pm 7\%$; $n = 4$; mean $\pm$ S.E.M.; 2 h incubation) on the degradation of apo E was observed.

Discussion

The use of isolated and purified parenchymal and non-parenchymal cells allows the cellular localization and elucidation of the mechanism of lipoprotein interaction with the liver. Previously we showed that the relative uptake *in vivo* of rat LDL, HDL and VLDL-remnants in the different liver cell types can be explained by the cellular localization of a lipoprotein receptor that recognizes apo E, and it was suggested that the interaction with this receptor could also be influenced by apo C (Van Berkel *et al.*, 1980, 1981). This receptor, now tentatively named remnant (apo E) receptor (see Mahley *et al.*, 1981; Cooper *et al.*, 1982a), shows no interaction with human apolipoprotein B (Van Berkel *et al.*, 1981). Recently, Cooper *et al.* (1982a) described properties of the chylomicron remnant binding to total rat liver membranes. The characteristics of this binding were similar to those described for the VLDL-remnant binding by isolated rat liver cells (Van Berkel *et al.*, 1981). Moreover, Cooper *et al.* (1982b)

reported a direct competition between chylomicron remnant and VLDL-remnant binding, indicating the similar identity of the receptor. The binding of lipoproteins to the remnant (apo E) receptor is highly temperature-dependent; with rat LDL or HDL at 0°C we obtained only 10–25% of the binding observed at 37°C (Van Berkel *et al.*, 1980). Similarly Cooper *et al.* (1982a) observed sub-optimal binding at low temperature, whereas Kita *et al.* (1982) concluded that the chylomicron-remnant uptake mechanism *in vivo* is not measured adequately *in vitro* by the current membrane-binding assays (performed at low temperatures) (Kovanen *et al.*, 1979). The present results clearly show that the interaction with the remnant (apo E) receptor is only optimally exerted at physiological temperature as suggested previously (Van Berkel *et al.*, 1980).

The properties of the remnant (apo E) receptor in both parenchymal and non-parenchymal cells were studied using chylomicrons, obtained from the intestinal lymph of rats made hypolipoproteinaemic by administration of pharmacological doses of 17 α -ethynyoestradiol (Chao *et al.*, 1979; Windler *et al.*, 1980b). These chylomicrons contain as the sole form of apo B the so-called apo B-48 form (Kane *et al.*, 1980; Krishnaiah *et al.*, 1980) and greatly reduced amounts of C apoproteins and apo E (Windler *et al.*, 1980b; Elovson *et al.*, 1981; see Experimental section). The present paper shows that these chylomicrons are still readily recognized by both isolated parenchymal and non-parenchymal cells as found before in a perfused rat liver system (Windler *et al.*, 1980b). The cell-association-versus-substrate-concentration curve is indicative of a high affinity and saturable interaction. A half-maximal cell-association is obtained at about 0.55 mg of triacylglycerol/ml, corresponding to an apoprotein concentration of 3 μ g/ml. The amount of apo E present in these chylomicrons is less than 0.5%, which indicates an apo E concentration of less than 0.015 μ g/ml at half-maximal cell-association. This suggests either a very high affinity of the recognition site for apo E associated with chylomicrons (see Hui *et al.*, 1981) or more probably that other chylomicron constituents are responsible for this high affinity interaction. This component may be apo B-48, as also suggested by Windler *et al.* (1980b), or chylomicron surface lipids (Borensztajn *et al.*, 1980; Pattnaik & Zilversmit, 1980; Nilsson *et al.*, 1981). The mechanism of the inhibitory effect of apo C-III on the interaction of the chylomicrons with the cells was investigated by simultaneous radioactivity measurements of the triacylglycerol moiety and the apo C-III itself. The data indicate that more apo C-III becomes cell-associated than would be expected on the basis of the cell-association of the triacylglycerol moiety. As the apo C-III was originally firmly associated with the

chylomicrons (unbound apo C-III was removed by two chylomicron isolation steps), this suggests that upon cell-association of apo C-III the binding to the particle is lost, although binding of free apo C-III, in equilibrium with chylomicron-associated apo C-III, is another possibility. Both mechanisms, the cell-association of the original particle-bound apo C-III or free apo C-III, would prevent the stable association of the triacylglycerol moiety with the cells. The competition of apo C-III with the chylomicrons implies that both are bound to the same site, presumably the remnant receptor, and that the preferential binding of apo C-III to this receptor prevents the further interaction of the triacylglycerol moiety with the cells. The cell-association of apo C-III is accompanied by a degradation, which, after a short lag phase, occurs at a fairly constant rate with time. This degradation is highly temperature-dependent and the concentration dependency of the degradation closely follows the cell-association. This suggests a close relation between the cell-association and degradation. The sharp increase in degradation rate with an increase in incubation temperature from 25°C to 37°C might indicate that a complex sequence of steps is involved in the degradation process.

In contrast with the degradation of acetyl-LDL and native LDL, which are inhibited by chloroquine and NH₄Cl (Van Berkel *et al.*, 1982), no effect of these lysosomotropic agents on the degradation of apo C-III is found. This might be cited as evidence for an extralysosomal degradation pathway for apo C-III.

In agreement with previous data on the effect of apo E on the interaction of triacylglycerol-rich emulsions (Havel *et al.*, 1980; Shelburne *et al.*, 1980; Quarfordt *et al.*, 1981, 1982) or lipoproteins (Windler *et al.*, 1980a,b,c) with rat liver, we find an increase of the cell-association rate with pure parenchymal cells and furthermore a marked increase in the cell-association level with non-parenchymal cells when chylomicrons are preloaded with apo E. Such an effect can also be observed at suboptimal binding conditions at low temperatures. The data show that upon cell-association of the particle the apo E radioactivity closely follows the lipid moiety, indicating that the particle-apo E complex is bound as a unity. Addition of apo C-III to the apo E-containing chylomicrons *in vitro* leads to a less efficient incorporation of apo E in the particles (see the Experimental section). Apparently the interaction of apo E with the particles is influenced by the presence of apo C-III. A destabilizing effect of apo C-III on the apo E association with chylomicrons may also explain the observation that with the apo E-, apo C-III-enriched chylomicrons more apo E becomes associated with the cells than would be expected on the basis of the

[³H]triacylglycerol measurements. So, apparently apo C-III can inhibit the interaction of the chylomicrons with the cells by a second mechanism through weakening of the apo E interaction with the particle leading to a cell-association of free apo E. Both mechanisms obviously lead to a similar result, i.e. the inhibition of the interaction of the triacylglycerol moieties with the cells. Similarly, as with apo C-III, the cell-association of apo E is accompanied by a degradation, although at a much lower rate than for apo C-III. Also similarly to apo C-III the degradation of apo E occurs apparently extralysosomally. After degradation of the apo E or C-III the recognition site may be re-exposed, resulting in only a temporary effect, as is observed in rat liver perfusion studies (Windler *et al.*, 1980b).

The qualitatively similar effects of apo E and C-III on the interaction of chylomicrons with both parenchymal and non-parenchymal cells strengthen the previous conclusions that a lipoprotein (remnant) receptor is present in parenchymal and non-parenchymal cells with similar characteristics (Van Berkel *et al.*, 1981). Quantitatively the apo E effect is most pronounced with non-parenchymal cells and the inhibitory effect of apo C-III appears to be more complete on parenchymal cells. To what extent these quantitative differences may influence the relative importance of these cell types in lipoprotein metabolism inside the liver remains to be elucidated.

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