Cellular uptake and catabolism of high-density-lipoprotein triacylglycerols in human cultured fibroblasts: degradation block in Neutral Lipid Storage Disease

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High-density lipoprotein (HDL)-[^{3}H]triolein (i.e. [^{3}H]triolein incorporated into reconstituted HDL) was taken up by cultured fibroblasts through an apparently saturable process, competitively inhibited by non-labelled HDL and independent of the LDL receptor. Using ^{125}I-HDL and HDL-[^{3}H]triolein, binding experiments (at 0 °C) followed by a short-time 'chase' at 37 °C showed that ^{125}I radioactivity was rapidly released in the culture medium (as trichloroacetic acid-precipitable material), whereas ^{3}H radioactivity remained associated with the cell. The cell-associated HDL-[^{3}H]triolein was rapidly degraded in normal fibroblasts, and the liberated [^{3}H]oleic acid was incorporated into newly biosynthesized phospholipids. In Wolman-disease fibroblasts HDL-[^{3}H]triolein was degraded at a normal rate, and thus independently of the lysosomal compartment. In contrast, the degradation of HDL-[^{3}H]triolein was blocked in fibroblasts from Neutral Lipid Storage Disease (NLSD), similarly to that of endogenously biosynthesized triacylglycerols [Radom, Salvayre, Nègre, Maret and Douste-Blazy (1987) Eur. J. Biochem. **164**, 703–708]. Trypsin-treated HDL-[^{3}H]triolein was also taken up by cells and degraded quite similarly to HDL-[^{3}H]triolein. In conclusion, all these data taken together suggest that HDL-[^{3}H]triolein is: (i) associated with the cell through a process independent of intact apolipoprotein (apo) As, thus probably independent of an apoA-receptor-mediated uptake; (ii) internalized by cells, whereas ^{125}I-apoAs are released in the culture medium; (iii) directed to the same non-lysosomal catabolic pool (blocked in NLSD) as for endogenously biosynthesized triacylglycerols.

INTRODUCTION

Neutral Lipid Storage Disease (NLSD), first described by Dorfman et al. [1] and Chanarin et al. [2], is also known as 'ichthyosiform dermatosis with systemic lipidosis' [1] or 'Multisystemic Lipid Storage Myopathy' [3] or Chanarin–Dorfman syndrome [4]. NLSD is a rare inherited metabolic disease generally characterized by the association of muscular weakness, ichthyosis, multisystemic triacylglycerol storage and Jordans' anomaly (fat vacuoles in leucocytes) [2,5], without deficiency of carnitine or carnitine acyltransferase and without major impairment of the mitochondrial lipid metabolism [3,6]. We have previously demonstrated that the triacylglycerol accumulation in cultured cells (fibroblasts and immortalized lymphoid cells) of NLSD results from a severe defect in the degradation of endogenously biosynthesized triacylglycerols containing natural or fluorescent long-chain fatty acids [6–10]. This has been confirmed on cultured fibroblasts from other NLSD patients by Di Donato et al. [11].

Wolman disease and cholesteryl ester storage disease are two rare autosomal disorders of lipid metabolism characterized by a severe deficiency of the acid lipase and a subsequent lysosomal storage of triacylglycerol and cholesteryl esters [12–14]. Previous studies have shown that the major part of the neutral lipids stored in lysosomes of acid-lipase-deficient fibroblasts originated in exogenous lipoproteins [low-density (LDL) and/or very-low-density (VLDL)] taken up by the cells [15].

A comparative study on acid-lipase-deficient and NLSD cells has provided evidence for two distinct and independent catabolic pools of intracellular triacylglycerols: (i) a cytoplasmic pool containing endogeneously biosynthesized triacylglycerols; (ii) a lysosomal pool constituted by exogenous triacylglycerols from LDL or VLDL taken up by the cells [16].

The cellular metabolism of neutral lipids contained in high-density lipoprotein (HDL) is only partly understood. It is now admitted that the cellular metabolism of [apolipoprotein (apo)E-free-] HDL particles is fundamentally different from the apoB/E-receptor-mediated pathway (and more generally from the lysosomal endocytotic pathway) [17–21]. The selective uptake of cholesteryl esters is now well established [20,21], and seems to occur without parallel uptake of HDL apoprotein [18,21], in agreement with the general view that the intracellular traffic of HDL-lipids and HDL-apoproteins seems to be different [22,23]. Since, to our knowledge, the uptake, the intracellular route and the catabolic pools of (apoE-free-) HDL-triacylglycerols have not been reported, we investigated this metabolism comparatively in cultured fibroblasts from Wolman disease and NLSD.

MATERIALS AND METHODS

Chemicals

[^{3}H]Triolein (26 Ci/mmol) and ^{125}I (17 Ci/mg) were purchased from New England Nuclear (Les Ulis, France). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], fatty-acid-free BSA, triolein, chloramine-T and trichloroacetic acid (TCA) were from Sigma (St. Louis, MO, U.S.A.), silica-gel G t.l.c. analytical plates were from Merck (Darmstadt, Germany).

Abbreviations used: HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins; NLSD, neutral lipid storage disease; FH, familial hypercholesterolaemia; apo, apolipoprotein; T-HDL, trypsin-treated HDL; TCA, trichloroacetic acid.

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RPMI 1640, Phenol Red-free RPMI 1640, fetal-calf serum, glutamine, streptomycin, penicillin and trypsin/EDTA were from Gibco (Cergy-Pontoise, France). Ultroser G was from IBF (Villeneuve-la-Garenne, France). Cell culture flasks and multiwell culture plates were from Nunc (distributed by Polylabo-Block, Strasbourg, France). Hydragel was from Sebia (Issy-les-Moulineaux, France), Picofluor from Packard (Rungis, France), and the other reagents and organic solvents were from Merck or Prolabo (Paris, France).

Cell culture

Skin fibroblasts were obtained from normal individuals (n) and from patients affected with NLSD (Bo. and Dem.), Wolman Disease (GM1606) or familial hypercholesterolaemia (FH) (GM486, apoB/E-receptor negative). Bo. fibroblasts were kindly provided by Dr. J. M. Mussini and Dr. S. Billaudel (CHU Nantes, France) and Dem. cells by Dr. B. Winchester (Institute of Child Health, London, U.K.); GM1606 and GM486 cells were purchased from the NIGMS Human Genetics Mutant Cell Repository (Camden, NJ, U.S.A.), and normal fibroblasts (n) were from our laboratory. Fibroblasts were grown at 37 °C in air/CO_2 (19:1) in RPMI 1640 medium with penicillin (100 units/ml), streptomycin (100 μg/ml) and glutamine (2 mM), supplemented with 10% heat-inactivated fetal-calf serum or 2% Ultroser G (a lipoprotein-free serum substitute) under the conditions previously used [6,7]. Cell viability was assessed by the MTT test [24].

Lipoprotein isolation, labelling with [^{3}H]triolein and/or ^{125}I, and trypsin treatment

LDL and HDL from human pooled sera were isolated by sequential ultracentrifugation (Beckman L8-70 ultracentrifuge) as described by Havel et al. [25], dialysed extensively (against 0.15 M NaCl containing 0.3 mM EDTA), and their purity was monitored by electrophoresis on agarose gel (Hydragel). Lipoprotein solutions were sterilized by filtration (0.4 μm Millipore membrane) and stored at 4 °C under N_2 (up to 3 weeks). ApoB and apoA-I concentrations were determined by immunonephelometry (Behring system).

HDL were labelled with [^{3}H]triolein and unlabelled triolein by the procedure of Krieger et al. [26], and LDL were labelled with tracer doses of [^{3}H]triolein (by the procedure of Roberts et al. [27]), isolated again by ultracentrifugation and dialysed before use. HDL-[^{3}H]triolein was iodinated by the procedure of Weech et al. [28]. Briefly, 100 μl of HDL-[^{3}H]triolein (containing 2 mg of apoA-1 in 0.2 M phosphate buffer, pH 7.5) was mixed with 0.5 mCi of ^{125}I and 2.5 μg of chloramine-T (3 times every 30 s). The reaction was stopped by adding 250 μg of tyrosine, and the lipoproteins were extensively dialysed as indicated above. Some 95% of the radioactivity was TCA-precipitable, and less than 5% of the radioactivity was extracted in chloroform/methanol (2:1, v/v). The ^{3}H radioactivity was determined by liquid-scintillation counting in 5 ml of Picofluor in a Packard counter (Tricarb 4530) and the ^{125}I radioactivity by a γ-counter (Packard Minaxi 5000).

HDL-[^{3}H]triolein was trypsin-treated by the procedure of Tabas and Tall [29], modified as follows: 100 μl of trypsin was added to 500 μl of HDL-[^{3}H]triolein solution (1 mg of apoA-1 in 0.15 M NaCl containing 0.3 mM EDTA) and incubated for 1 h at 37 °C. Then the reaction was stopped by adding RPMI 1640 containing 2% Ultroser G (which inhibits trypsin activity), and the trypsin-treated HDL-[^{3}H]triolein (T-HDL-[^{3}H]triolein) was sterilized by filtration and used immediately.

Binding, uptake and metabolism of ^{125}I-HDL-[^{3}H]triolein or HDL-[^{3}H]triolein

The cellular binding of ^{125}I-HDL-[^{3}H]triolein or HDL-[^{3}H]triolein was determined under the conditions described by Briesbroeck et al. [17] and modified as follows. Cells were washed twice with cold RPMI, chilled at 0 °C and incubated with ^{125}I-HDL-[^{3}H]triolein or HDL-[^{3}H]triolein (20 μg of apoA/ml) for 2 h at 0 °C. Experiments with ^{125}I-HDL-[^{3}H]triolein or HDL-[^{3}H]triolein were done in parallel, because ^{125}I extractable in organic solvents interfered with the liquid-scintillation counting of ^{3}H. After 2 h at 0 °C, the incubation medium was removed and cells were washed six times with cold PBS containing 2 mg/ml BSA and once with PBS alone. After the final wash, two sets of dishes were used to determine bound ^{125}I-HDL or HDL-[^{3}H]triolein, and the other sets of dishes were incubated for the indicated time at 37 °C in RPMI containing non-labelled HDL (20 μg of apoA-1/ml). At each time, cells were washed seven times as indicated above and incubated with trypsin plus EDTA for 10 min at 37 °C. Cells were pelleted by centrifugation (800 *g* for 5 min) and the ^{125}I and ^{3}H radioactivity of the supernatant (trypsin-releasable) and of the cell pellet (trypsin-resistant) was determined. At the indicated time, the culture medium was collected, BSA and TCA (final concns. 10 mg/ml and 150 mg/ml respectively) were added successively, left to react for 30 min at 0 °C, and the mixture was centrifuged (300 *g* for 10 min). The TCA-soluble (supernatant) and TCA-insoluble (pellet solubilized in 0.1 M NaOH containing 20 mg/ml SDS for 10 h at 37 °C) were counted for ^{125}I or ^{3}H radioactivity.

For studying the cellular degradation of LDL-[^{3}H]triolein and HDL-[^{3}H]triolein, cells were incubated for 12 h in the presence of the indicated concentration of radiolabelled lipoproteins. At the end of this pulse, cells were carefully washed (twice with PBS containing 10 mg/ml BSA), harvested by scraping with a rubber policeman, homogenized in water by sonication (MSE sonicator) and used for determining the cell-associated ^{3}H radioactivity by liquid-scintillation counting.

Lipid extraction and analysis

Cell pellets were suspended in 1 ml of distilled water, sonicated (2 × 15 s; Soniprep 150), and cellular lipids were extracted with chloroform/methanol (2:1, v/v) by the procedure of Folch et al. [30]. Lipids were separated by t.l.c. on silica-gel G plates, in the following solvent systems: light petroleum (b.p. 40–65 °C)/diethyl ether/acetic acid (80:20:1, by vol.) for neutral lipids, and chloroform/methanol/water (50:21:3, by vol) for phospholipids. Lipid spots were detected by iodine vapour or by carbonization (by spraying a solution of 10% H_2SO_4 in methanol and heating at 160 °C for 20 min). Radiolabelled lipids was detected and counted for radioactivity directly on the t.l.c. plate by using a t.l.c. radiochromatoscanner (Berthold model LB-285). Alternatively (when using low levels of radiolabelled lipids), triacylglycerols (and other neutral hydrophobic lipids) were separated from polar lipids and non-esterified fatty acids by solvent partitioning in the biphasic system of Dole [31], and the radioactivity was determined by liquid-scintillation counting as indicated above.

Protein concentrations were determined by the method of Lowry et al. [32].

RESULTS

Uptake of HDL-[^{3}H]triolein and LDL-[^{3}H]triolein

HDL-[^{3}H]triolein, i.e. HDL partially delipidated and reconstituted with [^{3}H]triolein by a procedure derived from Krieger et

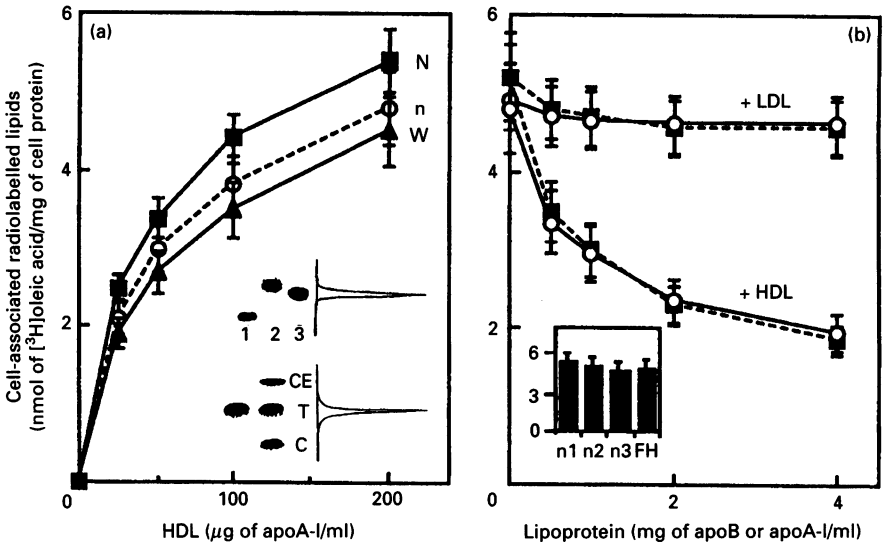

Figure 1 Uptake of HDL-[^{3}H]triolein by cultured fibroblasts from normal subjects (n; ○) and patients with NLSD (N; ■) and Wolman disease (W; ▲)

(**a**) Uptake in 8 h of increasing concentration (up to 200 μg of apoA-I/ml) of HDL-[^{3}H]triolein (32000 d.p.m. of [^{3}H]triolein and 0.9 nmol of triolein/μg of apoA-I). Cells were washed and harvested, and the cell-associated radioactivity was determined by liquid-scintillation counting. Inset upper part, electrophoresis of HDL-[^{3}H]triolein (lane 3) reconstituted by the procedure of Krieger et al. [26], by comparison with native LDL (lane 1) and HDL (lane 2). The peak of radioactivity of lane 3 (detected by Berthold radiochromatoscanner) showed that [^{3}H]triolein co-migrated with HDL. Inset lower part, t.l.c. of triolein marker (left lane) and of the neutral lipids extracted from the reconstituted HDL-[^{3}H]triolein (right lane) (CE, cholesteryl esters; T, triacylglycerols; C, cholesterol). The radioactivity of the t.l.c. right lane detected by radiochromatoscanner showed a single peak of [^{3}H]triolein (without any hydrolysis products). (**b**) Competitive uptake in 8 h of fixed concentration (100 μg of apoA-I/ml) of HDL-[^{3}H]triolein in the presence of increasing concentrations of HDL or LDL (up to 4 mg of apoA-I or apoB/ml) by normal (○) and NLSD (■) fibroblasts. Inset of (**b**), comparative uptake in 8 h of a fixed concentration of HDL-[^{3}H]triolein (100 μg of apoA-I/ml) by fibroblasts from normal subjects (n) and from FH patients. Results are means ± S.E.M. of 3 experiments.

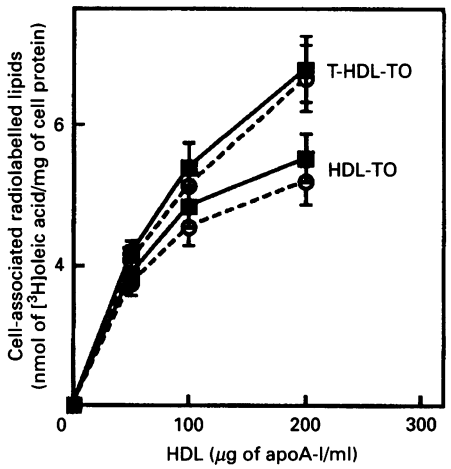

Figure 2 Comparative uptake of HDL-[^{3}H]triolein (HDL-TO) and T-DHL-[^{3}H]triolein (T-HDL-TO)

HDL-[^{3}H]triolein (32000 d.p.m.; 1 nmol of triolein/μg of apoA-I) was trypsin-treated as indicated in the Materials and methods section. Normal (○) and NLSD (■) fibroblasts were grown for 8 h in the presence of increasing concentrations of HDL-[^{3}H]triolein (HDL-TO and T-HDL-TO) under the conditions of Figure 1, and after washing, the cell-associated radioactivity was determined. Results are means ± S.E.M. of 3 experiments.

al. [26], were used for studying the cellular uptake and the subsequent degradation of [^{3}H]triolein incorporated into HDL. As shown by the co-migration of the radioactivity with reconstituted HDL on agarose-gel electrophoresis (upper part of the inset of Figure 1a), [^{3}H]triolein was associated with HDL. The analysis of the neutral lipids of reconstituted HDL-[^{3}H]triolein showed that [^{3}H]triolein was not hydrolysed before their uptake (lower part of the inset of Figure 1a). The cellular uptake of HLD-[^{3}H]triolein was apparently saturable (Figure 1a), it was inhibited by high HDL concentrations but not by LDL (Figure 1b), and was similar in control and in FH cells (inset of Figure 1b). These data suggest that HDL-[^{3}H]triolein is taken up by fibroblasts through a pathway independent of apoB/E-receptor-mediated endocytosis and of fluid-phase pinocytosis (Figures 1a and 1b).

To investigate a hypothetical role for the apoA receptor, we have compared the uptake of HDL-[^{3}H] triolein and of T-HDL-[^{3}H]triolein. Trypsin treatment of HDL-[^{3}H]triolein did not inhibit the cellular uptake of [^{3}H]triolein, since, when the concentration of T-HDL-[^{3}H]triolein or HDL-[^{3}H]triolein was increased in the culture medium, the amount of cell-associated T-HDL-[^{3}H]triolein was higher than that of HDL-[^{3}H]triolein (Figure 2). Therefore, the cellular uptake of HDL-[^{3}H]triolein seems to be independent of the integrity of apoAs, and thus probably independent of a receptor-mediated interaction between ApoAs and cells.

Experiments on binding at 0 °C, followed by a 'chase' at 37 °C for variable periods of time, showed that the routing of ^{125}I-apoAs is entirely different from that of [^{3}H]triolein. The cell-bound ^{125}I radioactivity (bound to the cells during the incubation at 0 °C) was rapidly released during the 'chase' at 37 °C and was recovered in the culture medium in 15–30 min (as a TCA-precipitable material) (Figure 3a). In contrast, under the same conditions, almost all the ^{3}H radioactivity bound to cells at 0 °C remained associated with the cell during the 'chase' at 37 °C (and the amount of ^{3}H radioactivity released into the culture medium was less than 15%) (Figure 3b). All these data strongly suggest that cell-bound ^{125}I-HDL-[^{3}H]triolein was dissociated,

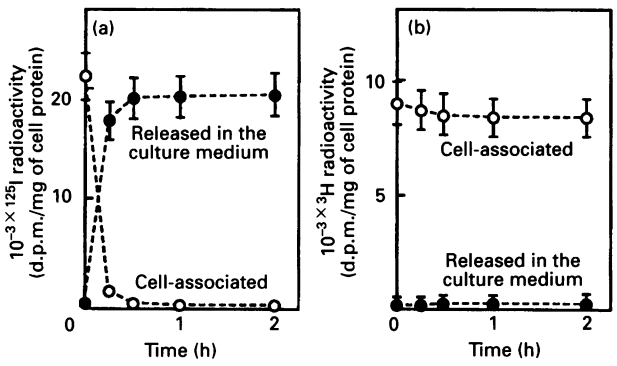

Figure 3 Fate of ^{125}I-HDL-[^{3}H]triolein and HDL-[^{3}H]triolein bound to fibroblasts to 0 °C and subsequently incubated at 37 °C

Fibroblasts were incubated for 2 h at 0 °C with 20 μg of apoA-I/ml or HDL-[^{3}H]triolein (32000 d.p.m.; 0.9 nmol of triolein/μg of apoA-I) or ^{125}I-HDL-[^{3}H]triolein (70000 d.p.m. of ^{125}I/μg of apoA-I) and thoroughly washed, as described in the Materials and methods section. After the final wash, two sets of dishes were used for determining the cell-bound ^{125}I-HDL or HDL-[^{3}H]triolein and the other sets of dishes were incubated for the indicated time at 37 °C in RPMI containing non-labelled HDL (20 μg of apoA-I/ml). At each time, cells were washed seven times and incubated with trypsin plus EDTA for 10 min at 37 °C. Cells were pelleted by centrifugation (800 ***g*** for 5 min) and the ^{125}I and ^{3}H radioactivities of the supernatant (trypsin-releasable) and of the cell pellet (trypsin-resistant) were determined. The culture medium was collected, and BSA and TCA (final concns. 10 mg/ml and 150 mg/ml respectively) were added successively, left to react for 30 min at 0 °C, and the mixture was centrifuged (3000 ***g*** for 10 min). Cells and medium samples were counted for ^{125}I or ^{3}H radioactivity. Results are means ± S.E.M. of 3 experiments.

the ^{125}I-apoAs being rapidly released into the culture medium, whereas the HDL-[^{3}H]triolein associated with the cell was internalized and metabolized (see below).

To confirm the specificity of the results obtained with HDL-[^{3}H]triolein with the cell lines used (from normal subjects, Wolman disease and NLSD), we have investigated comparatively the uptake of LDL-[^{3}H]triolein in these cells. As shown in the inset of Figure 4(a), the procedure, derived from that of Roberts et al. [27], permitted association of [^{3}H]triolein with LDL (as shown by detection of radioactivity in the LDL spot on Hydragel electrophoresis; upper part of the inset) without any appreciable hydrolysis of [^{3}H]triolein (as shown by radioactivity detection t.l.c. of the lipid extract from LDL-[^{3}H]triolein; lower part of the inset). As expected, LDL-[^{3}H]triolein was taken up through the apoB/E receptor pathway in normal, Wolman-disease and NLSD cells, as suggested by the saturation curve, by the competition by non-labelled LDL and by the deficient uptake in FH fibroblasts (Figure 4b). This also demonstrates that [^{3}H]triolein was really incorporated into LDL.

Degradation of HDL-[^{3}H]triolein, T-HDL-[^{3}H]triolein and LDL-[^{3}H]triolein

HDL-[^{3}H]triolein taken up by the cells was rapidly degraded by normal and Wolman-disease fibroblasts, whereas its degradation was severely deficient in NLSD fibroblasts (Figure 5a). At the same time, radiolabelled phospholipids were biosynthesized in control fibroblasts (by recycling [^{3}H]oleic acid liberated by [^{3}H]triolein hydrolysis), but not in NLSD fibroblasts (Figure 5b). Moreover, during the 'chase' period of Figure 5, we detected only low levels of radioactivity released from the cells into the culture medium, suggesting that there was no or only little efflux of radiolabelled lipids (containing [^{3}H]oleic acid) from the cells. Quite similar results were obtained with T-HDL-[^{3}H]triolein. This suggests that internalization, intracellular routing and metabolism of HDL-[^{3}H]triolein are independent from those of apoAs.

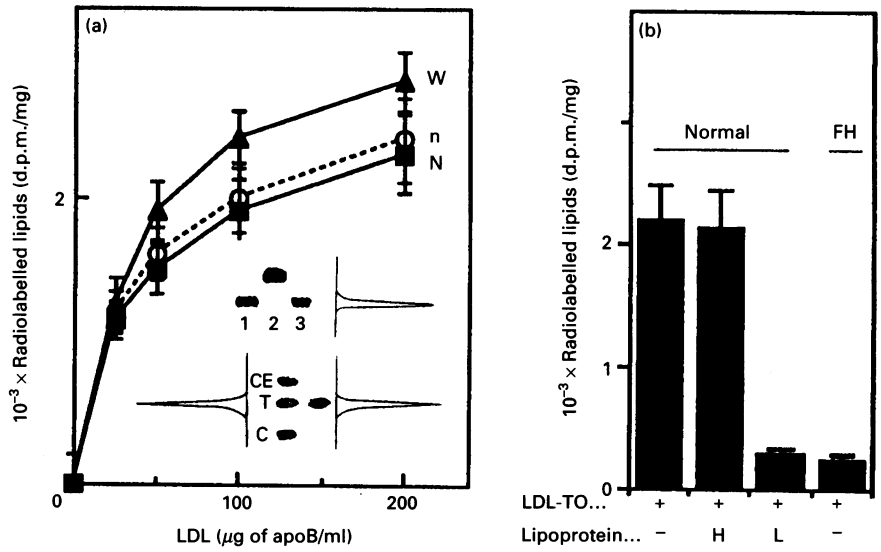

Figure 4 Uptake of LDL-[^{3}H]triolein by cultured fibroblasts

In (**a**), fibroblasts from normal subjects (n; ○) and patients with NLSD (N; ■) and Wolman disease (W; ▲) were incubated for 12 h in the presence of increasing concentrations (up to 200 μg of apoB/ml) of LDL-[^{3}H]triolein (400 d.p.m.; 8 pmol/μg of apoB), washed twice, and the cell-associated radioactivity was determined under the conditions of Figure 1(a). Inset upper part, electrophoresis of LDL-[^{3}H]triolein (lane 3) compared with native LDL (lane 1) and HDL (lane 2), and the peak of ^{3}H radioactivity in lane 3 was detected on the gel by radiochromatoscanner: the radioactivity co-migrated with LDL, thus suggesting that [^{3}H]triolein is associated with LDL particles. Inset lower part, t.l.c. of neutral lipids extracted from reconstituted LDL-[^{3}H]triolein (left lane) and of [^{3}H]triolein marker (right lane); all the radioactivity (peaks of radiochromatoscan) showed no degradation products of [^{3}H]triolein. Same abbreviations as in Figure 1(a) are used. In (**b**), uptake of LDL-[^{3}H]triolein (LDL-TO; 100 μg of apoB/ml) by normal and FH fibroblasts, in the absence of any additional lipoprotein (−) or in the presence of a large excess of LDL (L) or HDL (H) (2000 μg of apoB or apoA-1/ml). Results are means ± S.E.M. of two separate experiments, each point being done in triplicate.

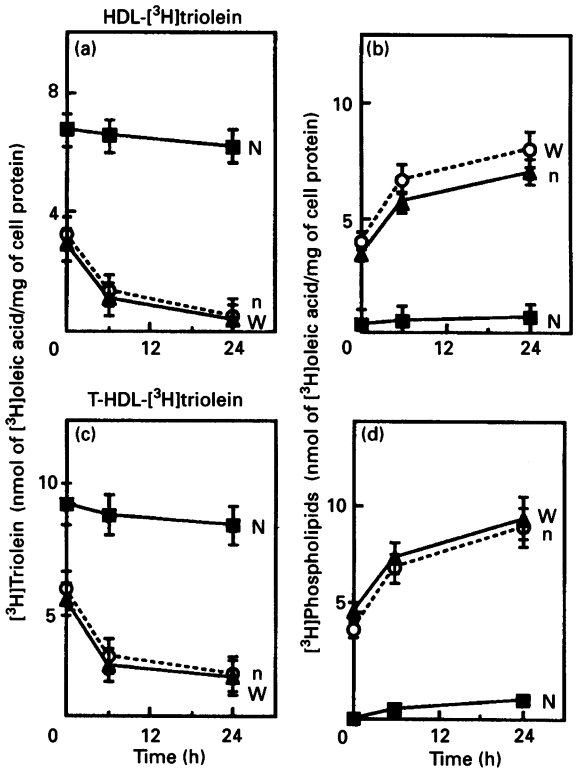

Figure 5 Metabolism of HDL-[^{3}H]triolein (a, b) or T-HDL-[^{3}H]triolein (c, d) by cultured fibroblasts from normal subjects (n; ○) and from patients with NLSD (N; ■) and Wolman disease (W; ▲)

Cells were pulsed for 12 h with a fixed amount (200 μg of apoA-I/ml) of HDL-[^{3}H]triolein (**a, b**) or T-HDL-[^{3}H]triolein (**c, d**) (each 32000 d.p.m. of [^{3}H]triolein; 0.9 nmol of triolein/μg of apoA-I) under the conditions of Figure 1. Then cells were washed and 'chased' in the standard culture medium. At the indicated time, cells were harvested, and lipids were extracted and analysed as indicated in the Materials and methods section. Degradation of [^{3}H]triolein (**a** and **c**) and incorporation of the liberated [^{3}H]oleic acid into phospholipids (**b** and **d**) are shown. Results are means ± S.E.M. of three experiments.

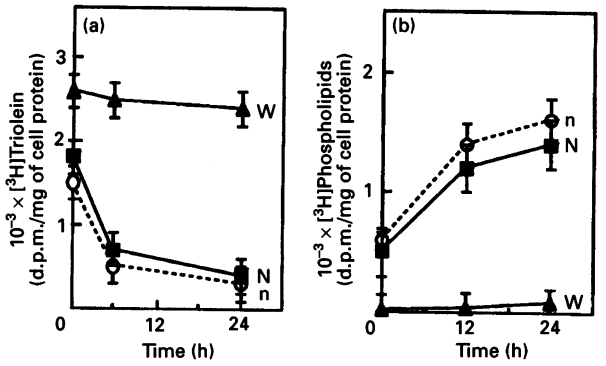

Figure 6 Metabolism of LDL-[^{3}H]triolein by cultured fibroblasts from normal subjects (n; ○) and from patients with NLSD (N; ■) and Wolman disease (W; ▲)

Cells were pulsed for 12 h with a fixed amount (200 μg of apoA-I/ml) of LDL-[^{3}H]triolein (400 d.p.m. of [^{3}H]triolein; 8 pmol of triolein/μg of apoB). Then cells were washed, 'chased' in the standard culture medium and harvested under the conditions of Figure 5, and lipids were extracted and analysed as indicated in the Materials and methods section. (**a**) Degradation of [^{3}H]triolein; (**b**) incorporation of the liberated [^{3}H]oleic acid into cellular phospholipids. Results are means ± S.E.M. of three experiments.

All these results suggest that: (i) HDL-[^{3}H]triolein is effectively taken up and internalized in cultured fibroblasts; (ii) the internalized HDL-[^{3}H]triolein is degraded in normal fibroblasts and [^{3}H]oleic acid liberated is re-utilized for lipid biosynthesis (mainly incorporated in phospholipids, under the experimental conditions used here); (iii) HDL-[^{3}H]triolein degradation is normal in Wolman-disease fibroblasts, and thus is independent of the lysosomal lipase; (iv) HDL-[^{3}H]triolein degradation is blocked in NLSD fibroblasts, thus suggesting that HDL-[^{3}H]triolein is degraded in the same catabolic pool as endogenously biosynthesized triacylglycerols.

In contrast with HDL-[^{3}H]triolein, LDL-[^{3}H]triolein (expressed as radioactivity, because only a tracer dose of [^{3}H]triolein was used for labelling LDL) was degraded at a normal rate by NLSD (half-life estimated as less than 1 day, under the experimental conditions used), whereas a typical block of triolein degradation was observed in Wolman-disease fibroblasts (half-life longer than 10–15 days) (Figure 6a). These data were confirmed by the study of the radiolabelled phospholipid biosynthesized during the 'chase' (Figure 6b). As expected, LDL-[^{3}H]triolein taken up through the apoB/E receptor pathway is exclusively degraded by the lysosomal acid lipase (since their degradation was almost completely deficient in Wolman-disease cells) and is independent of the catabolic pool of HDL-[^{3}H]triolein taken up by the cells.

DISCUSSION

The data reported here strongly suggest that triacylglycerols contained in HDL are taken up by fibroblasts independently from the apoB/E-receptor-mediated pathway and degraded in a cytoplasmic compartment completely independent from lysosomes.

The cellular metabolism of HDL seems to be puzzling, because of separate intracellular pathways for lipids and apoproteins of HDL [20,22,33,34] or direct transfer of lipids from HDL by lipid-exchange proteins (such as transfer of cholesteryl esters by cholesteryl ester transfer protein) into certain cultured cells [35]. Almost all the studies were focused on cholesteryl esters (or phospholipids), and, to our knowledge, the metabolism of HDL-triacylglycerols is only poorly documented.

The interaction of native HDL and reconstituted HDL-[^{3}H]triolein with cells seems to be similar, as shown by competition experiments. These results and others concerning the binding and the uptake of T-HDL-[^{3}H]triolein are consistent with previous reports and suggest that the intracellular routing and metabolism of HDL-[^{3}H]triolein is not an artefact due to the reconstitution procedure.

Experiments with T-HDL-[^{3}H]triolein suggest that intact apoAs (and thus probably the apoA receptor) are not necessary for the uptake and metabolism of HDL-[^{3}H]triolein, in agreement with the conclusions of Tabas and Tall [29], who demonstrated that the association of ^{125}I-HDL with cells is not mediated by specific ligand and receptor proteins but may involve the interaction between HDL surface lipids with cell-surface lipids.

The results of binding experiments followed by a 'chase' at 37 °C suggest that the components of HDL pre-bound to the cell plasma membrane are dissociated: ^{125}I-apoAs was released to the culture medium, whereas [^{3}H]triolein remained associated with the cell and was subsequently taken up and metabolized by the cell. Our results are consistent with those of Oram et al. [36], who reported that most of the ^{125}I radioactivity of pre-bound ^{125}I-HDL was released in the culture medium. No data are available

about the fate of pre-bound HDL-[^{3}H]triolein, but it may be compared with the 'selective uptake' of HDL-cholesteryl esters [22,37]. This suggests that the highly hydrophobic lipids (triacylglycerols and cholesteryl esters) constituting the core of the HDL particle can be taken up by cells, possibly by a process involving cholesteryl ester transfer protein (or other lipid-exchange proteins) [35] or apoE localized on the cell surface [38].

The question of the cellular metabolism of HDL-lipids taken up by cells has been highly debated, since it was reported to be either lysosome-dependent [33,35,39,40] or lysosome-independent [21,23,41–44]. Experiments with Wolman-disease fibroblasts demonstrate that the lysosomal compartment (in which the degradation of LDL-triacylglycerols is severely deficient) is not involved in the degradation of the taken-up HDL-triacylglycerols. Moreover, the decrease in HDL-[^{3}H]triolein taken up by cells was not due to a reverse transport outside the cell (i.e. retro-endocytosis), since (i) no significant amount of radiolabelled lipid (particularly undegraded [^{3}H]triolein) was recovered in the culture medium during the chase under the culture conditions employed here and (ii) the radioactivity was recovered in the cellular phospholipids, suggesting that radiolabelled [^{3}H]oleic acid liberated by hydrolysis of HDL-[^{3}H]triolein was re-utilized in the cellular biosynthesis of phospholipids.

In NLSD fibroblasts the degradation of HDL-[^{3}H]triolein is almost completely blocked, whereas the degradation of LDL-[^{3}H]triolein is in the normal range. These results constitute additional evidence that the HDL-triacylglycerol degradation pathway is completely independent of the lysosome. In NLSD fibroblasts, the concomitant catabolic block of the taken-up HDL-triacylglycerols (the present paper) and of endogenously biosynthesized triacylglycerols [6–11] strongly suggests that the endogenously biosynthesized and HDL-triacylglycerols are degraded by the same lipase system, and thus belong to the same extra-lysosomal catabolic pool of cellular triacylglycerols (termed cytoplasmic triacylglycerols).

Although no deficiency of the neutral lipase was detected in NLSD fibroblasts by assays *in vitro* [9,11,16], it is probable that the catabolic block of the triacylglycerol cytoplasmic pool (detected *in situ*) results from a defect of a component of the lipase system. Several possibilities could explain these apparently puzzling results: (i) the mutation of the enzyme affects the activity *in situ*, but not *in vitro* (for instance, by modifying enzyme–substrate interactions or by altering the conformation of the enzyme *in situ*); (ii) the mutation of the enzyme did not affect the activity, but alters its intracellular routing (by a partial analogy with I-cell-disease fibroblasts, in which the abnormal routing of lysosomal enzymes induces their loss into the extracellular medium and their deficiency in cells) [45], or its localization, leading to a lack of enzyme–substrate contact; (iii) the mutation affects another molecule (different from the enzyme), for instance a natural cofactor necessary to the activity *in situ* but not *in vitro* (by analogy with the co-lipase for the pancreatic lipase [46] or sphingolipid activator proteins of some lysosomal hydrolases) [47–49]; (iv) the mutation affects a protein directing the substrate to the enzyme (for instance, a hypothetical intracellular triacylglycerol-transport protein, leading to a loss of enzyme–substrate contact.

In conclusion, as summarized in Figure 7, the reported data demonstrate that the catabolic pool of cytoplasmic triacylglycerols is supplied by (at least) two separate pathways, the first one being the intracellular pathway (pathway 1) of endogenously biosynthesized triacylglycerols [6,16], and the second one being the extracellular HDL-triacylglycerols pathway (pathway 2). The HDL-triacylglycerol pathway converges to the same (non-lysosomal) catabolic pool as the endogenous triacylglycerol pathway, since the degradation of both endogenous and HDL-triacylglycerol is blocked in NLSD fibroblasts. The HDL-triacylglycerol pathway (not deficient in Wolman-disease cells) is also different from the well-known LDL-receptor pathway (pathway 3) which is connected to lysosomes where triacylglycerols are degraded by the acid lipase in normal cells.

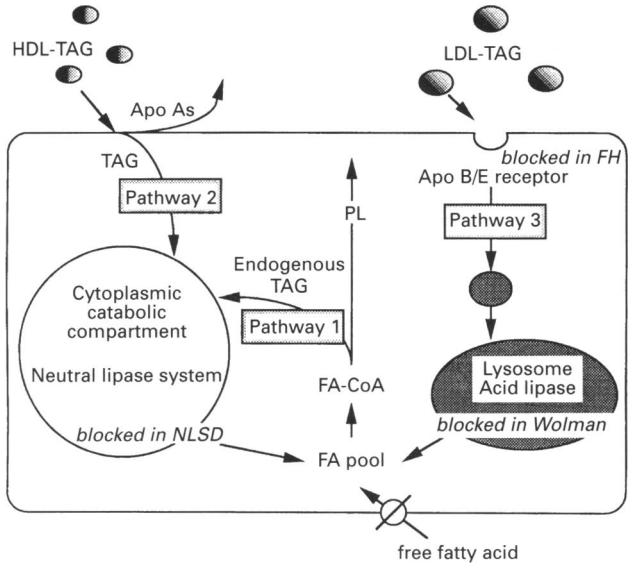

Figure 7 Schematic representation of the possible pathways for catabolic pools of triacylglycerols (TAG)

TAG originating in HDL-TAG taken up by cells (pathway 2) are degraded in the same cytoplasmic (i.e. non-lysosomal) catabolic pool as endogenously biosynthesized TAG (pathway 1) which is blocked in NLSD. The lysosomal catabolic pool, supplied by LDL-TAG (pathway 3), is blocked in Wolman-disease cells and is completely independent of the cytoplasmic pool. Abbreviations: FA, fatty acid; PL, phospholipid.

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