Differential Effects of Low-Density Lipoprotein and Chylomicron Remnants on Lipid Accumulation in Human Macrophages

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The effects of low-density lipoprotein (LDL) and chylomicron remnants on lipid accumulation in human monocyte–derived macrophages (HMDMs) and in macrophages derived from the human monocyte cell line THP-1 were compared. The HMDMs or THP-1 macrophages were incubated with LDL, oxidized LDL (oxLDL), chylomicron remnant–like particles (CMR-LPs), or oxidized CMR-LPs (oxCMR-LPs), and the amount and type of lipid accumulated were determined. As expected, the lipid content of both cell types was increased markedly by oxLDL but not LDL, and this was due to a rise in cholesterol, cholesteryl ester (CE), and triacylglycerol (TG) levels. In contrast, both CMR-LPs and oxCMR-LPs caused a considerable increase in cellular lipid in HMDMs and THP-1 macrophages, but in this case there was a greater rise in the TG than in the cholesterol or CE content. Lipid accumulation in response to oxLDL, CMR-LPs, and oxCMR-LPs was prevented by the ACAT inhibitor CI976 in HMDMs but not in THP-1 macrophages, where TG levels remained markedly elevated. The rate of incorporation of [^{3}H]oleate into CE and TG in THP-1 macrophages was increased by oxLDL, CMR-LPs, and oxCMR-LPs, but incorporation into TG was increased to a greater extent with CMR-LPs and oxCMR-LPs compared with oxLDL. These results demonstrate that both CMR-LPs and oxCMR-LPs cause lipid accumulation in human macrophages comparable to that seen with oxLDL and that oxidation of the remnant particles does not enhance this effect. They also demonstrate that a greater proportion of the lipid accumulated in response to CMR-LPs compared with oxLDL is TG rather than cholesterol or CE and that this is associated with a higher rate of TG synthesis. This study, therefore, provides further evidence to suggest that chylomicron remnants have a role in foam cell formation that is distinct from that of oxLDL. Exp Biol Med 229:528–537, 2004

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Introduction

The earliest lesion to occur during atherogenesis is the formation of the fatty streak, which is initiated following the invasion of monocyte-macrophages and the transport of lipoproteins into the subendothelial space of the artery wall (1). The macrophages scavenge the lipoproteins and store the lipid intracellularly, becoming lipid-laden foam cells (2). Extensive studies have shown that low-density lipoprotein (LDL) has a major role in foam cell formation, although it is now well established that oxidized (oxLDL) rather than native LDL is mainly responsible for this effect (3). More recently, however, it has become clear that chylomicron remnants, which carry lipids of dietary origin in the blood, are also able to induce macrophages to form foam cells and that in this case prior oxidation of the particles is not required (4, 5).

Dietary lipid is transported via chylomicrons, which are synthesized in the intestine and enter the blood via the thoracic duct (6). These particles are rapidly catabolized to smaller triacylglycerol (TG) and cholesterol-rich remnants, which are then taken up by the liver. There is now a large amount of evidence to indicate that these remnant particles are atherogenic. They have been shown to be taken up by the aorta (7–9) as efficiently as LDL (10), and remnant-like particles containing apolipoprotein E (apoE) have been isolated from human aortic intima and atherosclerotic plaque (11, 12). In addition, delayed clearance of remnants from the circulation is correlated with the development of atherosclerotic lesions (13, 14).

We have shown previously that chylomicron remnants induce extensive lipid accumulation in the murine cell line, J774 (4), and a similar effect has been reported in experiments with human monocyte–derived macrophages

(HMDMs; Ref. 5). In both these studies, nonoxidized lipoprotein particles were used, highlighting an important difference between foam cell induction by chylomicron remnants and oxLDL. Another difference may be in the type of lipid accumulated, since there is evidence to suggest that other TG-rich lipoproteins such as very low density lipoprotein (VLDL) or β-VLDL cause the accumulation of TG rather than cholesterol (15, 16). Thus, at least some of the mechanisms involved in macrophage lipid accumulation by chylomicron remnants and LDL appear to be different. Most previous studies, however, have focused on the role of oxLDL, and although the induction of macrophage lipid accumulation by chylomicron remnants has been clearly demonstrated (4, 5), little further information about the process is available. Moreover, the effects of oxLDL and chylomicron remnants have not been directly compared. Since oxidation of LDL is known to take place within the artery wall (3), it is likely that chylomicron remnants that penetrate the subendothelial space will also become oxidized. Despite this, almost nothing is known about how the oxidation of chylomicron remnants influences their induction of macrophage foam cell formation.

The aims of the present study are to characterize the effects of chylomicron remnants on lipid accumulation in macrophages of human origin in terms of the amount and type of lipid accumulated and changes in lipid synthesis in the cells, to investigate how these parameters are affected by prior oxidation of the remnant particles, and to compare the effects of chylomicron remnants and oxidized chylomicron remnants directly with those of LDL and oxLDL. The LDL was obtained from human blood, but it is difficult to obtain from this source chylomicron remnants uncontaminated with lipoproteins of similar density, such as chylomicrons and VLDL. For this reason, artificial chylomicron remnant-like particles (CMR-LPs) containing human apoE with a size, density, and lipid composition similar to that of physiological remnants were used (17). Accumulation of lipid in HMDMs and macrophages derived from the human monocyte cell line THP-1 was assessed by oil red O staining and by quantitative assay of cholesterol, cholesteryl ester (CE), and TG. In addition, the effects of the four types of lipoprotein on cholesterol esterification and TG synthesis were assessed in THP-1 macrophages by measuring the incorporation of [^{3}H]-oleate into CE and TG.

Materials and Methods

Materials. RPMI-1640 medium, fetal bovine serum, glutamine (Glutamax), penicillin/streptomycin, and β-mercaptoethanol were supplied by Gibco (Paisley, Scotland). Fetal bovine serum was heat inactivated by incubation at 56°C for 30 mins before use. Fatty acid–free bovine serum albumin, phospholipids, cholesterol, cholesteryl oleate, phorbol 12-myristate 13-acetate (PMA),Trypan blue, 1,1,3,3-tetraethoxypropane, Chelex 100, Histopaque-1077, and oil red O were obtained from Sigma Chemical Company (Poole, Dorset, England). The fluorescent probe 1,1'-dioctadecyl-3,3,3'3'-tetramethylindo-carbocyanine perchlorate (DiI) was supplied by Cambridge Bioscience (Cambridge, England). Radioisotopes ([9,10(n)-^{3}H]oleate and cholesteryl -[1-^{14}C]oleate) were purchased from Amersham International (Aylesbury, Bucks).

Preparation and Oxidation of Lipoproteins. The LDL was prepared from fresh human serum obtained from blood donated by healthy volunteers or from the National Blood Service, North London Centre, London, England. Serum was layered under 0.9% NaCl (d = 1.006 g/ml) and centrifuged for 5 hrs at 65,000 *g* (4°C), and the top fraction was discarded. The density of the bottom layer was raised to 1.063 g/ml with potassium bromide (KBr), layered under KBr (d = 1.063 g/ml), and centrifuged for 24 hrs at 23,000 *g* (4°C), and LDL was collected from the top fraction by tube slicing. The LDL was dialyzed against phosphate-buffered saline (PBS) (72 hrs, 15 l, pH 7.4) at 4°C before use.

The CMR-LPs were prepared by sonication of a lipid mixture followed by density gradient centrifugation and binding to human apoE as described (18). Briefly, a mixture containing 70% trilinolein, 2% cholesterol, 5% CE, and 25% phospholipids in 0.9% NaCl in Tricine buffer (20 m*M*, pH 7.4) was sonicated at 22–24 μm for 20 mins at 56°C. The resulting emulsion was brought to a density of 1.21 g/ml with KBr, layered under a stepwise density gradient (19), and centrifuged at 17,000 *g* for 20 mins at 20°C. The upper layer of grossly emulsified lipids was then removed and replaced with an equal volume of 0.9% NaCl (d = 1.006 g/ml) and centrifuged at 70,000 *g* for 1 hr (20°C). The CMR-LPs were harvested from the top layer. These particles were incubated with the dialyzed (18 hrs, 4°C) d > 1.006 g/ml fraction of human plasma in a ratio of 1:2.5 and incubated at 37°C with shaking for 18 hrs. The CMR-LPs were then reisolated by ultracentrifugation (45,000 *g*, 18 hrs, 12°C), harvested from the top layer, and stored at 4°C under nitrogen until required. For the preparation of DiI-labeled CMR-LPs, DiI (1 mg/25 mg of total lipid) was added to the lipid mixture before sonication.

The LDL and CMR-LPs were oxidized by incubation with $CuSO_4$ (10 μ*M*) with shaking for 18–23 hrs at 37°C. The $CuSO_4$ was subsequently removed by dialysis against 0.9% NaCl for 16 hrs at 4°C or, alternatively, by the addition of the chelating resin, Chelex 100. The extent of oxidation was determined by measuring the level of thiobarbituric acid–reactive substances (TBARSs) in the preparations before dialysis. All lipoproteins were filtered (0.22 μm) before addition to the cell culture and used within 48 hrs of preparation.

Culture of HMDMs and THP-1 Cells and Experimental Protocols. Mononuclear cells were isolated from fresh human blood collected from healthy volunteers in the presence of EDTA (4 m*M*). Histopaque-1077 (15 ml) was added to the upper chamber of Accuspin tubes (Sigma Chemical) and centrifuged into the lower chamber (800 *g*, 5 mins). Blood samples (30 ml) were then pipetted into the

upper chamber, the tubes were centrifuged (2000 *g*, 20 mins), and the plasma layer was aspirated. The mononuclear cells in the opaque interface were washed with PBS (3 × 30 ml)-and finally resuspended in RPMI-1640 culture medium supplemented with 5% human serum and 1% glutamine. The cells were then incubated in 24-well plates in culture medium at 37°C in 5% CO_2 and 95% air at a density of 0.5 × 10^6 cells/well for up to 8 days to enable differentiation to the macrophage phenotype. The purity of the monocytic fraction was estimated by differential counts of DiffQuik (Porvair Sciences Ltd., Shepperton UK) stained cell preparations and was 90% or higher. The THP-1 monocytes were maintained in suspension in RPMI-1640 culture medium containing 10% FBS, 2 m*M* glutamine, 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 50 m*M* β-mercaptoethanol at a density of 3–9 × 10^5 cells/ml at 37°C in 5% air and 95% CO_2. The cells were induced to differentiate into macrophages by incubation in 24-well plates at a density of 0.5–1 × 10^6cells/well for 72 hrs in the presence of PMA (200 ng/ml). After this time, the cells adhered to the culture plates, and the medium containing the PMA and any remaining monocytes were removed. Viability of HMDMs and THP-1 macrophages as assessed by Trypan blue exclusion was more than 95%.

For uptake studies, DiI-labeled CMR-LPs (30 μg/ml of cholesterol) were incubated with fully differentiated macrophages for 4 or 18 hrs, and the cells were then viewed with Zeiss LMS 510 laser scanning confocal microscope. For studies on lipid accumulation, human LDL, oxLDL, CMR-LPs, or oxCMR-LPs (30 μg/ml of cholesterol) were added to the macrophages, and the incubation was continued for a further 48 hrs. For determination of the incorporation of [^{3}H]oleate into CE and TG, THP-1 macrophages were incubated in 24-well plates at a density of 1 × 10^6 cells/well and exposed to lipoproteins for the times indicated in the text. The medium was then replaced with culture medium (1 ml) containing [9,10-^{3}H] oleic acid (37 KBq/ml, 55,000 dpm/μ*M*) and 2% fat-free bovine serum albumin in RPMI-1640, and the incubation was continued for 1 hr. The cells were then washed with PBS (2 × 1 ml), and lipids were extracted with 5 ml of chloroform-methanol (2:1 v/v) in the presence of [1-^{14}C] cholesteryl oleate (2500 dpm) as a recovery marker and separated by thin-layer chromatography (hexane–diethyl ether–formic acid, 80:20:2 v/v/v). Radioactivity in the bands corresponding to CE and TG was then determined by liquid scintillation counting. Preliminary experiments showed that [^{3}H]oleate incorporation into CE and TG was linear for a period of 2 hrs in all the conditions used.

Analytical Methods. The diameter of the CMR-LPs was measured after electron microscopy (20). Images were captured using a Gatan MSC 791 TEM CCD camera, and the mean diameter of CMR-LPs and oxCMR-LPs was determined using a randomly chosen sample of 60 particles taken from four different fields. The total cholesterol, TG, and phospholipid content of the lipoproteins was determined using commercially available kits (Boehringer Mannheim, Mannheim, Germany), and cholesterol, CE, and TG in macrophages were assayed by high-performance thin-layer chromatography (21) using hexane–heptane–diethylether–acetic acid (63:18.5:18.5:1, v/v/v/v) as the mobile phase. Lipoprotein oxidation was determined by measuring the TBARS content of the preparations as described by Steinbrecher *et al.* (22). Tetraethoxypropane, which yields malondialdehyde, was used as a standard. The apolipoprotein composition of CMR-LPs was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (23).

The toxicity of LDL, oxLDL, CMR-LPs, and oxCMR-LPs in HMDMs and THP-1 macrophages was assessed using a (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based toxicology assay kit (Sigma Chemical) according to the manufacturer's instructions. Lipid accumulation in THP-1 monocyte–derived macrophages and HMDMs was assessed by oil red O staining. The cells were washed twice with PBS and once with 60% propan-2-ol, and oil red O (0.5%, w/v) in 40% propan-2-ol/H_2O (v/v) was added. After 15 mins, the stain was removed and cells were washed twice with PBS. Images of the cells were captured using a Canon microscopically mounted camera and the extent of staining analyzed using Media Cybernetics LP Optimas software, version 6.5. Proteins were determined using the method described by Lowry *et al.* (24).

Significance limits were calculated using analysis of variance. Where necessary, the *P* value taken to indicate statistical significance was adjusted using Dunnet's post hoc test to simultaneously test the control with each treatment group and to take account of the multiple comparisons made.

Results

Characteristics of Native and Oxidized LDL and CMR-LPs. The total cholesterol and TG content of the LDL, oxLDL, CMR-LPs, and oxCMR-LPs used is given in Table 1. The ratio of total cholesterol to TG was similar in all CMR-LP preparations and was not significantly changed after oxidation of the particles. The diameter of the CMR-LP particles was within the range reported for human chylomicron remnants (50–150 nm; Ref. 25) and was unaffected by oxidation (CMR-LPs, 136 ± 8.5 nm; oxCMR-LPs, 133 ± 5.8 nm). In terms of total lipid mass, the lipid composition of the CMR-LPs (percent of total lipid:total cholesterol, 9% ± 0.5%; TG, 78% ± 1%; phospholipid, 13% ± 1%) was similar to that found in physiological chylomicron remnants (percent of total lipid to total cholesterol, 13%; TG, 75%; phospholipid, 12%; Ref. 6). Analysis of the apolipoprotein composition of CMR-LPs by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that they contained apoE, and no other apolipoproteins were detected. The extent of oxidation of the lipoproteins as measured by the levels of TBARS present was markedly increased after incubation with

Table 1. TC, TG, and TBARS Content of Native and Oxidized LDL and CMR-LPs[a]

Parameter	LDL	oxLDL	CMR-LPs	oxCMR-LPs
TG (m*M*)	0.27 ± 0.05 (10)	0.23 ± 0.04 (10)	3.71 ± 0.53 (15)	2.29 ± 0.37 (15)
TC (m*M*)	2.65 ± 0.34 (10)	1.96 ± 0.13 (10)	1.23 ± 0.26 (15)	0.74 ± 0.16 (15)
TG:TC (molar ratio)	0.11 ± 0.01 (10)	0.11 ± 0.02 (10)	4.2 ± 0.49 (15)	3.9 ± 0.44 (15)
TBARS (n*M* malondialdehyde/μ*M* TC)	0.87 ± 0.31 (10)	6.72 ± 0.62 (10)	1.67 ± 0.22 (14)	7.78 ± 0.25 (14)

[a] LDL and CMR-LPs were prepared and oxidized by exposure to $CuSO_4$ (10 μ*M*) as described in the "Materials and Methods" section, and the TC, TG, and TBARS levels were determined. Data shown are the mean ± SEM from the number of preparations shown in parentheses. TC, total cholesterol; TG, triacylglycerol; TBARS, thiobarbituric acid–reactive substance; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.

$CuSO_4$ by approximately 7.7-fold for LDL and 4.7-fold for CMR-LPs (Table 1).

Uptake of CMR-LPs by Macrophages. DiI-labeled CMR-LPs were incubated with THP-1 macrophages and viewed by confocal microscopy after 4 and 18 hrs (Fig. 1). The amount of fluorescence associated with the cells was relatively small after 4 hrs (Fig. 1A) but increased markedly with time (Fig. 1B), indicating that the fluorescence-labeled particles were taken up by the cells. This pattern was observed consistently in six experiments with separate CMR-LP preparations.

The Effects of LDL and CMR-LPs on Lipid Accumulation in Macrophages. Before experiments on the effects of the lipoproteins on lipid accumulation, their toxicity toward HMDMs and THP-1 cells was tested. After 48-hr incubation with the cells at a level of 30 μg/ml of cholesterol, which was used in all subsequent experiments, the viability of the cells as assessed by Trypan blue exclusion was more than 95% in all cases, and there was no evidence of increased numbers of dead cells in the culture medium. Furthermore, neither LDL nor CMR-LPs showed significant toxicity toward either type of cells as assessed by an MTT-based toxicology assay kit, irrespective of the oxidation state of the particles (Table 2).

In initial experiments, the effects of LDL and CMR-LPs on lipid accumulation in HMDMs and THP-1 cells were assessed by staining with oil red O. Quantitative determination of the extent of staining showed that exposure of HMDMs to oxLDL for 48 hrs led to a marked rise in the cells, whereas LDL had little effect (Table 3). In contrast, both CMR-LPs and oxCMR-LPs caused a striking increase in the staining. Similar results were obtained in experiments with THP-1 macrophages (Table 3).

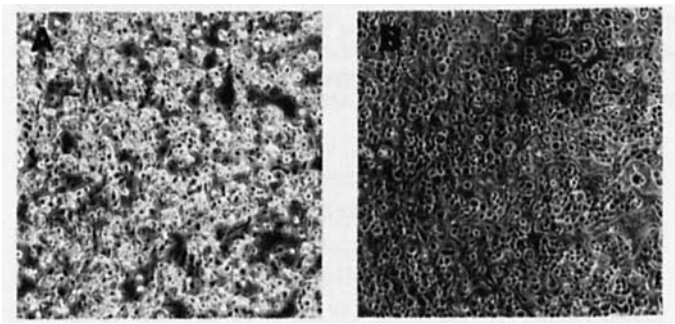

Figure 1. 1,1'-dioctadecyl-3,3,3'3'-tetramethylindo-carbocyanine perchlorate–labeled chylomicron remnant–like particles (CMR-LPs) (30 μg/ml of cholesterol) were incubated with THP-1 macrophages for 4 hrs (A) or 18 hrs (B), and the cells were then viewed by confocal microscopy. Images shown are from a typical experiment of six performed with separate CMR-LP preparations.

When the lipid content of the cells was measured, the results were consistent with those obtained from oil red O staining. A modest increase of approximately 59% in total lipid (total cholesterol + TG) was observed after incubation of THP-1 cells with LDL (Table 4), and this was mainly due to a significant increase in TG (Table 5). More marked rises in total lipid levels, however, were found with oxLDL, CMR-LPs, and oxCMR-LPs in both types of cells. The oxLDL increased the cholesterol, CE, and TG content of the macrophages to approximately similar extents, whereas CMR-LPs and oxCMR-LPs tended to raise TG levels preferentially. This effect was particularly pronounced in THP-1 cells, where increases of more than 400% were seen in TG levels, whereas cholesterol and CE concentrations were raised by 40%–200%. In control HMDMs and THP-1 cells, the total cholesterol (cholesterol + CE) mass was 1.5-fold (HMDMs) or 2.1-fold (THP-1 cells) higher than that of

Table 2. Toxicity of Native and oxLDL and CMR-LPs in HMDMs and THP-1 Macrophages[a]

Treatment	HMDMs	THP-1 cells
LDL	84.6 ± 3.0	92.6 ± 2.4
oxLDL	100.9 ± 5.7	108.0 ± 5.8
CMR-LPs	89.0 ± 5.7	94.5 ± 6.3
oxCMR-LPs	91.2 ± 6.7	90.8 ± 2.0
CI976	86.5 ± 4.6	92.7 ± 4.5
LDL + CI976	82.5 ± 2.9	91.6 ± 2.9
oxLDL + CI976	91.8 ± 3.9	98.5 ± 3.5
CMR-LPs + CI976	84.2 ± 3.9	91.0 ± 3.8
oxCMR-LPs + CI976	92.6 ± 4.8	97.4 ± 5.5

[a] HMDMs or THP-1 macrophages were incubated with LDL, oxLDL, CMR-LPs or oxCMR-LPs (30 μg/ml of total cholesterol) for 48 hrs in the presence or absence of the ACAT inhibitor CI976 (10 μ*M*), and the toxicity of the lipoproteins was determined using an MTT-based toxicology assay kit. The results are the mean ± SEM from six separate experiments and are expressed as a percentage of the mean optical density (O.D.) found in untreated (control) macrophages (control value, 1.06 ± 0.03 O.D. units). HMDMs, human monocyte–derived macrophages; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.

Table 3. Effect of LDL and CMR-LPs on Lipid Accumulation in HMDMs and THP-1 Macrophages Assessed by Oil Red O Staining[a]

Lipoprotein	HMDMs		THP-1 cells	
	-CI976	+CI976	-CI976	+CI976
None	13.6 ± 1.1	12.1 ± 1.1	9.4 ± 6.4	9.0 ± 1.9
LDL	15.8 ± 1.3	15.3 ± 0.6	11.4 ± 1.3	8.5 ± 1.0
oxLDL	33.1 ± 2.1*	16.3 ± 1.9	34.2 ± 3.0*	11.3 ± 1.7
CMR-LPs	52.7 ± 2.9*	21.9 ± 3.3**	44.5 ± 3.7*	25.0 ± 3.2**
oxCMR-LPs	59.5 ± 6.4*	20.8 ± 2.9**	45.1 ± 2.8*	27.5 ± 3.1**

[a] HMDMs or THP-1 macrophages were incubated with LDL, oxLDL, CMR-LPs, or oxCMR-LPs (30 μg/ml of total cholesterol) for 48 hrs in the presence or absence of the ACAT inhibitor CI976 (10 μ*M*), and the cells were stained with oil red O. Data show the total region stained by oil red O as a percentage of the total cell area in each image and are the mean ± SEM from 10 separate experiments. HMDMs, human monocyte–derived macrophages; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.
* $P < 0.05$; ** $P < 0.01$ compared with untreated macrophages.

Table 4. Effect of LDL and CMR-LPs on the Total Lipid Content of HMDMs and THP-1 Macrophages[a]

Lipoprotein	HMDMs		THP-1 cells	
	-CI976	+CI976	-CI976	+CI976
LDL	114.8 ± 9.8	107.4 ± 12.9	159.1 ± 8.7	104.5 ± 9.4
oxLDL	225.9 ± 14.3**	107.4 ± 3.3	254.5 ± 17.9*	122.7 ± 11.9
CMR-LPs	240.1 ± 5.3**	103.7 ± 6.1	331.8 ± 8.9**	218.2 ± 11.6**
oxCMR-LPs	318.5 ± 70.8**	114.8 ± 6.8	322.7 ± 65.1**	209 1 ± 22.4**

[a] HMDMs or THP-1 macrophages were incubated with LDL, oxLDL, CMR-LPs, or oxCMR-LPs (30 μg/ml of total cholesterol) for 48 hrs in the presence or absence of the ACAT inhibitor CI976 (10 μ*M*), and the total lipid content (cholesterol + cholesteryl ester + TG) of the cells was determined. Data are expressed as a percentage of the value found in untreated macrophages and are the mean + SEM from three separate experiments. HMDMs, human monocyte–derived macrophages; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.
*$P < 0.05$; ** $P < 0.01$ compared with untreated macrophages.

Table 5. Effect of LDL and CMR-LPs on the TC, CE, and TG Content of HMDMs and THP-1 Macrophages[a]

Treatment	HMDMs			THP-1 cells		
	TC	CE	TG	TC	CE	TG
LDL	111.1 ± 5.6	114.3 ± 10.7	118.2 ± 14.3	112.5 ± 8.9	128.6 ± 11.4	242.8 ± 32.4**
oxLDL	222.2 ± 8.2**	271.4 ± 32.2**	200.0 ± 11.9**	200.0 ± 42.6**	300.0 ± 5.7**	271.4 ± 15.3**
CMR-LPs	211.1 ± 26.1**	185.7 ± 18.9**	300.0 ± 19.7**	250.0 ± 8.0**	185.7 ± 11.3**	571.4 ± 26.2**
oxCMR-LPs	288.9 ± 7.6**	271.4 ± 13.0**	372.7 ± 28.3**	312.5 ± 21.3**	142.9 ± 6.7**	514.3 ± 14.8**
LDL +CI976	98.9 ± 3.9	85.7 ± 7.6	127.3 ± 11.0	87.5 ± 12.7	57.1 ± 19.3	171.4 ± 23.5
oxLDL +CI976	122.2 ± 4.9	71.4 ± 1.3	118.2 ± 6.0	125.0 ± 6.3	57.1 ± 23.6	185.7 ± 13.3*
CMR-LPs + CI976	100.0 ± 15.8	100.0 ± 12.2	109.1 ± 46.9	87.5 ± 3.4	114.3 ± 4.9	471.4 ± 29.7**
oxCMR-LPs + CI976	122.2 ± 9.5	128.6 ± 4.7	100.0 ± 10.1	87.5 ± 3.7	114.3 ± 3.4	442.8 ± 19.5**

[a] HMDMs or THP-1 macrophages were incubated with LDL, oxLDL, CMR-LPs, or oxCMR-LPs (30 μg/ml of total cholesterol) for 48 hrs in the presence or absence of the ACAT inhibitor CI976 (10 μ*M*), and the cholesterol, CE, and TG content of the cells was determined. Data are expressed as a percentage of the value found in untreated macrophages and are the mean ± SEM from three separate experiments. TC, total cholesterol; CE, cholesteryl ester; TG, triacylglycerol; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.
* $P < 0.05$, ** $P < 0.01$ compared with untreated macrophages.

TG. As a result of the changes described above, however, the ratio of total cholesterol to TG was decreased in experiments with CMR-LPs or oxCMR-LPs to 0.8–1.1 but remained at 1.5–2 in experiments with oxLDL. In THP-1 cells incubated with LDL, however, the observed rise in TG concentrations caused the ratio to decrease to 1.1.

The effects of inhibition of cholesterol esterification on lipid accumulation in HMDMs and THP-1 cells were investigated using the ACAT inhibitor, CI976 (26). Inclusion of CI976 in the incubation medium in the presence or absence of LDL or CMR-LPs had little toxic effect (Table 2). However, quantitative analysis of oil red O–stained cells showed that in HMDMs and THP-1 macrophages staining was clearly decreased in the presence of the inhibitor in response to all four lipoproteins tested. In experiments with oxLDL, the level was similar to that seen

Table 6. Effect of LDL, CMR-LPs, and CI976 on the Incorporation of [^{3}H]oleate Into CE and TG in THP-1 Macrophages[a]

Treatment	CE	TG
None	1.26 ± 0.07	5.24 ± 1.51
LDL	1.64 ± 0.23	11.62 ± 0.67*
oxLDL	2.43 ± 0.16*	13.14 ± 0.78*
None	1.35 ± 0.14	9.45 ± 0.45
CMR-LPs	2.16 ± 0.09*	28.07 ± 2.73**
oxCMR-LPs	2.74 ± 0.24*	27.53 ± 1.72**
None	1.35 ± 0.14	6.08 ± 0.48
CI976	0.16 ± 0.05**	6.62 ± 0.42

[a] THP-1 macrophages were incubated with LDL, oxLDL, CMR-LPs, or oxCMR-LPs (30 μg/ml of total cholesterol) for 48 hrs in the presence or absence of the ACAT inhibitor CI976 (10 μ*M*), and the incorporation of [3H]oleate into CE and TG was determined. Data are expressed as pmol of oleate incorporated/min/mg of cell protein and are the mean ± SEM from six separate experiments. CE, cholesteryl ester; TG, triacylglycerol; LDL, low-density lipoprotein; oxLDL, oxidized LDL; CMR-LPs, chylomicron remnant–like particles; oxCMR-LPs, oxidized CMR-LPs.
* $P < 0.05$, ** $P < 0.01$ compared with corresponding untreated macrophages.

in control cells (incubated without lipoproteins or CI976), but with CMR-LPs and oxCMR-LPs, the amount of staining remained significantly higher than that observed in control cells in both HMDMs and THP-1 cells (Table 3).

CI976 abolished the increase in total lipid content of HMDMs caused by exposure to oxLDL, CMR-LPs, or oxCMR-LPs, and this was reflected in the levels of cholesterol, CE, and TG, which were not significantly different from those in cells incubated without lipoproteins (Tables 4 and 5). The increases in total lipid in THP-1 macrophages caused by LDL and oxLDL were also prevented by the inhibitor, with the levels of cholesterol, CE, and TG all reduced. Compared with cells incubated in the absence of lipoproteins, however, the amount of TG remained 70%–85% higher, whereas the content of CE was reduced by 43%. Inclusion of CI976 in the medium of THP-1 cells incubated with CMR-LPs led to a decrease in total lipid accumulation (Table 4), but the levels remained higher than those found in cells not exposed to lipoproteins. Further analysis showed that this was due to raised TG levels, since cholesterol and CE values were similar to those observed in control cells, but TG concentrations remained 4- to 5-fold higher (Table 5).

The Effect of LDL and CMR-LPs on the Incorporation of [^{3}H]oleate Into CE and TG in THP-1 Cells. The rate of cholesterol esterification and TG synthesis in THP-1 macrophages exposed to LDL, oxLDL, CMR-LPs, or oxCMR-LPs was measured by the incorporation of [^{3}H]oleate into CE and TG, respectively. After 48-hr incubation, the amount of label incorporated into CE was significantly increased in cells incubated with oxLDL but not LDL, and an increase was also observed after incubation with CMR-LPs and oxCMR-LPs (Table 6). Incorporation of the label into TG was increased to a similar extent by LDL compared with oxLDL and by CMR-LPs compared with oxCMR-LPs, but the rate found with the CMR-LPs after 48-hr incubation was approximately 2-fold greater than that observed with LDL (Table 6). Treatment of the cells with the ACAT inhibitor CI976 (10 μ*M*) led to a decrease of approximately 85% in incorporation of [^{3}H]oleate into CE but had no significant effect on the amount of radioactivity found in TG (Table 6).

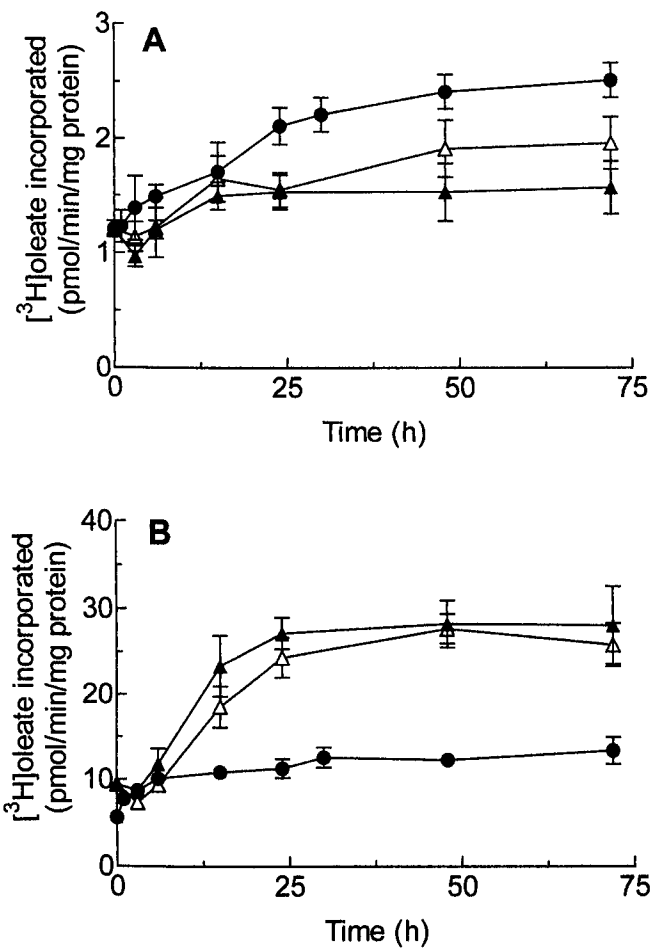

Figure 2. Incorporation of [^{3}H]oleate into cholesteryl ester (A) or triacylglycerol (B) in THP-1 macrophages incubated with oxidized low-density lipoprotein (closed circles), chylomicron remnant–like particles (CMR-LPs) (closed triangles), or oxidized CMR-LPs (open triangles) (30 μg/ml of cholesterol) for periods up to 72 hrs. Data shown are the mean from six separate experiments and error bars show the SEM.

The course of the effects of oxLDL, CMR-LPs, and oxCMR-LPs on the incorporation of [^{3}H]oleate into CE and TG is shown in Figure 2. After a short lag phase, oxLDL caused a steady increase in incorporation into CE, which continued up to 72 hrs, although the rate of increase was slower after approximately 24 hrs, and experiments with CMR-LPs and oxCMR-LPs showed a similar pattern (Fig. 2A). Incorporation of the label into TG increased rapidly following the initial addition of oxLDL to the culture medium, but the rate of increase slowed markedly after 6 hrs (Fig. 2B). When CMR-LPs were used, however, the amount

of radioactivity in TG did not begin to increase until 6 hrs and reached a plateau after approximately 24 hrs (Fig. 2B).

Because the CMR-LPs deliver significant quantities of TG to the cells, it is possible that the fatty acids released may dilute the substrate pools for cholesterol esterification and TG synthesis, leading to an underestimation of the increases caused. To investigate this possibility, double-label experiments were performed using CMR-LPs or oxCMR-LPs labeled with [^{3}H]trilinolein and [^{14}C]oleate. The results showed that fatty acids from CMR-LP TG make a small contribution to the substrate pools of approximately 7%–9% for cholesterol esterification and 5.5%–8.5% for TG synthesis.

Discussion

In this study, we aimed to investigate the influence of chylomicron remnants and oxidized chylomicron remnants on lipid accumulation in human macrophages and to compare their effects with those of LDL and oxLDL. Since homogeneous chylomicron remnants cannot be obtained easily from human blood, it was necessary to use CMR-LPs to mimic their action. The CMR-LPs used were similar in size, density, and lipid composition to physiological remnants (17, 27) and also contained human apoE; thus, they differ from physiological remnants only in that they lack apoB48. Extensive previous studies in both humans and experimental animals, however, have shown that chylomicron and CMR-LPs without apoB48 are cleared from the blood and metabolized in a similar way to the corresponding physiological lipoproteins (28–31). CMR-LPs that lacked apoB48 but contained apoE from the appropriate species have also been found to have effects that mimic those of physiological remnants in rat hepatocytes and pig endothelial cells (17, 32–34). Furthermore, in earlier work (4), we have demonstrated that physiological chylomicron remnants from rats cause extensive lipid accumulation in J774 cells comparable to that seen in the present study with CMR-LPs and THP-1 macrophages. In addition, our experiments with CMR-LPs labeled with the fluorescent probe DiI clearly demonstrate that the particles are taken up by THP-1 macrophages (Fig. 1). The CMR-LPs used, therefore, provide a suitable model for the investigation of the role of chylomicron remnants in macrophage foam cell formation.

Lipid accumulation in response to LDL or CMR-LPs was evaluated in HMDMs and in the human monocyte cell line THP-1, which can be induced to differentiate into macrophages by treatment with phorbol ester (35). In agreement with the previously reported effects of LDL and oxLDL in macrophages (36–38), native LDL did not cause any change in lipid accumulation in HMDMs or THP-1 cells as assessed by quantitative evaluation of histological staining with oil red O (Table 3), whereas oxLDL caused a marked increase. In contrast, when the macrophages were incubated with either CMR-LPs or oxCMR-LPs, extensive lipid accumulation was observed (Table 3). Since it has been calculated that chylomicron remnants carry approximately 40 times more cholesterol per particle than LDL (39) and the cholesterol content of the LDL used in our experiments was approximately 2-fold that of the CMR-LPs (Table 1), it is clear that considerably fewer CMR-LP than oxLDL particles are required to produce an equivalent level of lipid accumulation. Yu and Mamo (5) have demonstrated previously that chylomicron remnants induce lipid accumulation in HMDMs, and we have found a similar effect in J774 macrophages (4) and after short-term incubation (8 hrs) of this murine cell line with both native and oxidized chylomicron remnants (40). This is the first report, however, of the effects of oxidized remnants on lipid accumulation in human macrophages.

The type of lipid accumulated in human macrophages exposed to chylomicron remnants has not been studied in earlier work, although we have found that levels of both TG and total cholesterol are raised in J774 cells after short-term (8-hr) incubation with this type of particle (40). In addition, substantially increased TG levels have been reported after exposure of HMDMs to VLDL (16), and β-VLDL has been shown to cause increases in both the CE and TG components in rat peritoneal macrophages (41). Liposomes containing phospholipid and cholesterol have also been found to lead to the massive accumulation of lipid droplets containing CE and TG in mouse macrophages (42). On the other hand, oxLDL has been shown to load macrophages with CE (38), but previous studies have paid little attention to any possible effects of oxLDL on TG accumulation. Recent evidence from experiments on the effects of TG depletion on the physical state of CE and measurements of TG mass in THP-1 macrophages and HMDMs exposed to acetylated LDL, however, indicates that accumulation of TG and CE occurs in these cases (43, 44). Indeed, in the study of Kritharides *et al.* (43), the levels of TG found in HMDMs and THP-1 cells exceeded those of total cholesterol both before and after their exposure to acetylated LDL.

Measurement of the total lipid mass (total cholesterol + TG) in HMDMs and THP-1 macrophages in the present work (Table 4) gave results broadly similar to those obtained by determining the percentage of oil red O staining per cell; thus, a considerable increase was observed with oxLDL, CMR-LPs, and oxCMR-LPs. As expected, oxLDL caused a large rise (approximately 3-fold) in the CE content in both types of macrophages, and in agreement with the findings of Aviram with THP-1 cells (38), this was accompanied by a marked rise (2- to 2.5-fold) in intracellular cholesterol levels (Table 5). In addition, however, we also observed a 2- to 3-fold increase in TG in both HMDMs and THP-1 cells, and this had the effect of maintaining the ratio of total cholesterol to TG at a level (1.5–2) similar to that found in macrophages incubated without lipoproteins. In THP-1 cells, although not in HMDMs, unmodified LDL also caused a significant increase in TG mass (Table 5), and this led to a decrease

in the ratio of total cholesterol to TG to 1.1. We conclude, therefore, that oxLDL and, to a much more limited extent, LDL have a similar effect to acetylated LDL (43, 44) in causing mixed lipid droplets containing CE and TG to accumulate in macrophages during foam cell formation. Our findings also show that, in contrast to oxLDL, CMR-LPs and oxCMR-LPs induced greater increases in the TG content of macrophages than in the cholesterol or CE levels, particularly in THP-1 cells (Table 5), resulting in a decrease in the ratio of total cholesterol to TG from 1.5 to 0.9 (CMR-LPs) or 1.1 (oxCMR-LPs) in HMDMs and from 2.1 to 0.8 (CMR-LPs) or 1.0 (oxCMR-LPs) in THP-1 cells. These findings are in agreement with our previous, more limited study with chylomicron remnants and J774 macrophages (35). Thus, these results clearly show that macrophage foam cells induced by chylomicron remnants differ from those induced by oxLDL in that TG makes up a greater proportion of the accumulated lipid.

To further investigate the influence of oxLDL and chylomicron remnants on cholesterol esterification and TG synthesis during foam cell formation, experiments were performed to measure lipid accumulation in the presence of the ACAT inhibitor CI976. CI976 is a potent inhibitor of hepatic and intestinal ACAT activity (45), and in our experiments a concentration of 10 μM reduced cholesterol esterification by almost 90% but had no effect on TG synthesis (Table 6). Histological staining with oil red O clearly demonstrated that lipid accumulation in response to oxLDL, CMR-LPs, and oxCMR-LPs was considerably reduced in the presence of the inhibitor (Table 3). The finding that control levels were reached in the case of oxLDL but not CMR-LPs or oxCMR-LPs, in both HMDMs and THP-1 macrophages (Table 3), however, provides further evidence to suggest that TG accumulation is quantitatively more important than CE accumulation in foam cell induction by chylomicron remnants compared with oxLDL.

Inhibition of cholesterol esterification might be expected to lead to increased accumulation of cholesterol within the cells, particularly since no cholesterol acceptor was present in the medium in our experiments. In fact, although the mass of CE was reduced to or below control levels in both HMDMs and THP-1 cells, the cholesterol content was also decreased compared with that seen in macrophages exposed to oxLDL, CMR-LPs, or oxCMR-LPs in the absence of the inhibitor. These findings contrast with those reported for the ACAT inhibitors E5324, HL-004, and NTE-122, which reduced CE mass in macrophages loaded with acetylated LDL but increased the mass of cholesterol in the absence of a cholesterol acceptor (46–48). Inhibition of ACAT using 58-035, however, was found to increase cholesterol efflux from HMDMs after treatment with acetylated LDL (49), and it was suggested that apoE secreted by the cells may act as an acceptor.

Although CI976 did not appear to affect TG synthesis in the absence of lipoproteins (Table 6), TG levels were reduced when HMDMs were exposed to lipoproteins, with values falling to control levels with both oxLDL and CMR-LPs (Table 5). This effect was less marked in THP-1 macrophages, particularly with CMR-LPs and oxCMR-LP; nevertheless, a small decrease in TG levels was recorded in the presence of the inhibitor. These results suggest that the metabolism of the extra lipid taken up by the macrophages from the lipoproteins is affected by CI976, possibly by diversion of the fatty acids derived from the TG into other substrate pools, such as those for the blocked cholesterol esterification pathway or for phospholipid synthesis.

ACAT converts excess cholesterol taken up by macrophages to CE, which is stored in the cytosol, but undergoes a continuous cycle of hydrolysis by CE hydrolase and re-esterification by ACAT (50). Measurement of the incorporation of radiolabeled fatty acid into CE, therefore, gives an indication of the overall rate of cholesterol esterification. Previous work using this technique has shown that acetylated LDL increases cholesterol esterification in THP-1 macrophages (11, 12, 41) and in HMDMs, whereas LDL has a lesser effect (51). In our experiments, incubation of THP-1 macrophages with oxLDL, but not LDL, led to a significant increase in cholesterol esterification, and this is consistent with the relative changes in cellular CE content induced by the two types of lipoprotein (Tables 5 and 6). Similarly, CMR-LPs and oxCMR-LPs caused a significant increase in cholesterol esterification, and both types of particles caused CE to accumulate in the cells (Tables 5 and 6). Yu and Mamo (52) have also reported that chylomicron remnants increase the rate of cholesterol esterification in rabbit alveolar macrophages. In contrast, in our previous work with J774 cells, we found no effect of remnants on cholesterol esterification (40); however, in these experiments the cells were exposed to the particles for only 4 hrs rather than 48 hrs as in the present study.

[^{3}H]oleate may be incorporated into TG and CE, and this can be used to give an indication of the rate of TG synthesis from fatty acids. Our experiments demonstrate that LDL, oxLDL, CMR-LPs, and oxCMR-LPs all increased TG synthesis in THP-1 macrophages and, furthermore, that the level of [^{3}H]oleate incorporated into TG compared with CE was many fold higher. These results indicate that the TG pool is more metabolically active than the CE pool in these cells and are in agreement with the findings of Kritharides *et al.* (43) in similar studies using THP-1 macrophages loaded with acetylated LDL. The TG synthesis was increased to a similar extent by LDL and oxLDL, accounting for the similar levels of TG accumulation induced by the two lipoproteins in THP-1 cells (Table 5). In addition, the greater increment in TG synthesis observed with CMR-LPs and oxCMR-LPs compared with LDL and oxLDL is also in accord with the greater increases in TG mass found (Table 5).

Although the course of the stimulation of cholesterol esterification in the macrophages was similar when oxLDL, CMR-LPs, or oxCMR-LPs were used, that for TG synthesis

differed in that oxLDL caused an immediate sharp rise, whereas with CMR-LPs or oxCMR-LP there was a lag phase of approximately 6 hrs (Fig. 2). These differences may reflect different pathways of uptake of the two types of particles. The oxLDL is taken up by macrophages via a number of different receptors, including class A and B scavenger receptors and the leptin-like oxLDL receptor-1 (53). The receptors involved in macrophage chylomicron remnant uptake, however, are not yet clearly defined but may be different from those used for oxLDL. The LDL receptor may be involved (54, 55), and Elsegood *et al.* (55) have found that chylomicron remnants bind to unidentified 43-kDa proteins in THP-1 macrophages. A receptor expressed on macrophages reported to recognize apoB48 has also been described (56).

In this study, the response of HMDMs and THP-1 macrophages to CMR-LPs was found to be similar in most respects, indicating that the THP-1 cell line provides a suitable model for study of the effects of chylomicron remnants on macrophage lipid accumulation. The major novel findings from the results obtained with the two types of human macrophages are that CMR-LPs, without prior oxidation, cause lipid accumulation in human macrophages to an extent that is comparable to that caused by oxLDL; a greater proportion of the lipid accumulated in response to the CMR-LPs is in the form of TG rather than CE, which accumulates in response to oxLDL; TG synthesis is increased to a greater extent by CMR-LPs compared with oxLDL; and cellular TG levels after exposure to oxLDL or CMR-LPs are decreased when cholesterol esterification is inhibited, although TG synthesis was unaffected, suggesting that fatty acids are diverted into other substrate pools in these circumstances. In addition, the experiments demonstrate that oxidation of CMR-LPs does not change their effects on the amount or type of lipid accumulated or on CE or TG synthesis in the cells. Thus, in contrast to LDL, oxidation of chylomicron remnants does not appear to enhance their induction of foam cell formation. We conclude, therefore, that the characteristics of lipid accumulation in human macrophages exposed to chylomicron remnants differ in many important respects from those found in cells exposed to oxLDL. Overall, these findings provide good evidence to support the idea that chylomicron remnants have a role in foam cell formation that is distinct from that of oxLDL and highlight the need for further studies of their effects.

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809, 1993.
2. Libby P, Geng YJ , Aikawa M, Schoenbeck U, Mach F, Clinton SK, Sukhova GK, Lee RT. Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 78:330–335, 1996.
3. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 272:20963–20966, 1997.
4. Napolitano M, Rivabene R, Avella M, Botham KM, Bravo E. The internal redox balance of cells influences the metabolism of lipids of dietary origin by J774 macrophages: implications for foam cell formation. J Vasc Res 38:350–360, 2001.
5. Yu KC, Mamo JC. Chylomicron remnant-induced foam cell formation and cytotoxicity: a possible mechanism of cell death in atherosclerosis. Clin Sci (London) 98:183–192, 2000.
6. Redgrave TG. Formation and metabolism of chylomicrons. Int Rev Physiol 28:103–130, 1983.
7. Mamo JC, Proctor SD, Smith D. Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. Atherosclerosis 141(suppl 1):S63–S69, 1998.
8. Proctor SD, Mamo JCL. Retention of fluorescent-labelled chylomicron remnants within the intima of the artery wall - evidence that plaque cholesterol may be derived from post-prandial lipoproteins. Eur J Clin Invest 28:497–503, 1998.
9. Grieve DJ, Avella MA, Elliott J, Botham KM. Influence of chylomicron remnants on endothelial cell function in the isolated perfused rat aorta. Atherosclerosis 139:273–281, 1998.
10. Mamo JCL, Wheeler JR. Chylomicrons or their remnants penetrate rabbit thoracic aorta as efficiently as do smaller macromolecules, including low density lipoprotein, high density lipoprotein and albumin. Coronary Artery Dis 5:695–705, 1994.
11. Yla-Herttuala S, Jaakkola O, Enholm C, Tikkanen MJ, Solakivi T, Sarkioja T, Nikkari T. Characterisation of two lipoproteins containing apolipoproteins B and E from lesion-free human aortic intima. J Lipid Res 29:563–572, 1988.
12. Rapp JH, Lespine A, Hamilton RL, Colyvas N, Chaumeton AH, Tweedie-Harman J, Kotite L, Kunitake ST, Havel RJ, Kane JP. Triglyceride-rich lipoproteins isolated by selective affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. Arterioscler Thromb 14:1767–1774, 1994.
13. Benlian P, De Gennes PL, Foubert L, Zhang H, Gagne SE, Hayden M. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. N Engl J Med 335:848–854, 1996.
14. Groot PHE, van Stiphout WAH, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofmann A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary heart disease. Arterioscler Thromb 11:653–662, 1991.
15. Gianturco SH, Brown SA, Via DP, Bradley WA. The β-VLDL receptor pathway of murine P388D1 macrophages. J Lipid Res 27:412–420, 1986.
16. Milosavljevic D, Kontush A, Griglio S, Le Naour G, Thillet J, Chapman MJ. VLDL-induced triglyceride accumulation in human macrophages is mediated by modulation of LPL lipolytic activity in the absence of change in LPL mass. Biochim Biophys Acta 1631:51–60, 2003.
17. Diard P, Malewiak M-I, Lagrange D, Griglio S, Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant–like particles by isolated rat hepatocytes. Biochem J 299:889–894, 1994.
18. Napolitano M, Batt KV, Avella M, Bravo E, Botham KM. Lipid synthesis in macrophages derived from the human cell line THP-1: modulation of the effects of native and oxidised chylomicron remnant–like particles by estrogen. Clin Sci (London) 101:403–413, 2001.
19. Lenzo NP, Martins I, Mortimer IBC, Redgrave TG. Effects of phospholipid composition on the metabolism of triacylglycerol, cholesteryl ester and phosphatidylcholine from lipid emulsions injected intravenously into rats. Biochim Biophys Acta 960:111–118, 1998.
20. Anderson LJ, Boyles JK, Hussain MM. A rapid method for staining large chylomicrons. J Lipid Res 30:1819–1824, 1989.
21. Schmitz G, Assmann G, Bowyer DE. A quantitative densitometric method for the rapid separation and quantitation of the major tissue and lipoprotein lipids by high-performance thin-layer chromatography, I: sample preparation, chromatography, and densitometry. J Chromatogr 307:65–79, 1984.
22. Steinbrecher UP, Parthasarathy S, Leake DS, Witzum JL, Steinberg D.

Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci U S A 81:3883–722, 1984.
23. Laemmli UK. Cleavage of structural protein during assembly of the head of bacteriophage T4. Nature 227:680–685, 1970.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the protein phenol reagent. J Biol Chem 58:4606–4611, 1951.
25. Rye KA, Clay MA, Barter PJ. Overview of plasma lipid transport. In: Barter PJ, Rye KA, Eds. Plasma Lipids and Their Role in Disease. Australia: Harwood Academic Publishers, pp1–16, 1999.
26. Krause BR, Anderson M, Bisgaier CL, Bocan T, Bousley R, DeHart P, Essenburg A, Hamelehle K, Homan R, Kieft K, McNally W, Stanfield R, Newton RS, In vivo evidence that the lipid-regulating activity of the ACAT inhibitor CI-976 in rats is due to inhibition of both intestinal and liver ACAT. J Lipid Res 34:279–294, 1993.
27. Redgrave TG, Fidge NH, Yin J. Specific, saturable binding and uptake of rat chylomicron remnants by rat skin fibroblasts. J Lipid Res 23:638–644, 1982.
28. Redgrave TG, Ly HL, Quintao CR, Ramberg CF, Boston R. Clearance from plasma of triacylglycerol and cholesteryl ester after intravenous injection of chylomicron-like lipid emulsions in rats and man. Biochem J 290:843–847, 1993.
29. Maranhao RC, Fers MC, Martins MT, Mesquita CH, Toffoletto O, Vinagre CGC, Gianinni SD, Pileggi F. Plasma kinetics of a chylomicron-like emulsion in patients with coronary artery disease. Atherosclerosis 126:15–25, 1996.
30. Oliveira HC, Hirata MH, Redgrave TG, Maranhao RC. Competition between chylomicrons and their remnants for plasma removal: a study with artificial emulsion models of chylomicrons. Biochim Biophys Acta 958:211–217, 1988.
31. Martins IJ, Vermeulen R, Redgrave TG. Relative roles of mitochondrial and peroxisomal fatty acid oxidation in the metabolism of chylomicron remnants in rats and mice as assessed by a stable-isotope breath test. Atherosclerosis 150:13–20, 2000.
32. Sultan F, Lagrange D, Le Liepvre X, Griglio S. Chylomicron remnant uptake by freshly isolated hepatocytes: effects of heparin and of hepatic triacylglycerol lipase. Biochem J 258:587–594, 1989.
33. Goulter AB, Avella MA, Elliott J, Botham KM. Chylomicron remnant–like particles inhibit receptor-mediated endothelium-dependent vasorelaxation in porcine coronary arteries. Clin Sci (London) 103:450–460, 2002.
34. Thuren T, Sisson P, Waite M. Hydrolysis of lipid mixtures by rat hepatic lipase. Biochim Biophys Acta 1083:217–220, 1991.
35. Auwerx J. The human leukaemia cell line, THP-1: a multifaceted model for the study of monocyte-macrophage differentiation. Experientia 47:22–31, 1991.
36. Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci U S A 7:333–337, 1979.
37. Heinecke JW. Free radical modification of low-density lipoprotein: mechanisms and biological consequences. Free Radic Biol Med 3:65–73, 1987.
38. Aviram M. Low density lipoprotein modification by cholesterol oxidase induces enhanced uptake and cholesterol accumulation in cells. J Biol Chem 267:218–225, 1992.
39. Proctor SD, Vine DF, Mamo JCL. Arterial retention of apolipoprotein B48- and B100-containing lipoproteins in atherogenesis. Curr Opin Lipidol 13:461–470, 2002.
40. Napolitano M, Avella M, Botham KM, Bravo E. Chylomicron remnant induction of lipid accumulation in J774 macrophages is associated with up-regulation of triacylglycerol synthesis which is not dependent on oxidation of the particles. Biochim Biophys Acta 1631:255–264, 2003.
41. Ishii I, Onozaki R, Takahashi E, Takahashi S, Fujio N, Harada T, Morisaki N, Shirai K, Saito Y, Hirose S. Regulation of neutral cholesterol esterase activity by phospholipases containing negative charges in substrate liposome. J Lipid Res 36:2303–2310, 1995.
42. Nishikawa K, Arai H, Inoue K. Scavenger receptor-mediated uptake and metabolism of lipid vesicles containing acidic phospholipids by mouse peritoneal macrophages. J Biol Chem 265:5226–5231, 1990.
43. Kritharides L, Christian A, Stoudt G, Morel D, Rothblat G. Cholesterol metabolism and efflux in human THP-1 macrophages. Arterioscler Thromb Vasc Biol 18:1589–1599, 1998.
44. Lada AT, Willingham MC, Clair RW. Triglyceride depletion in THP-1 cells alters cholesteryl ester physical state and cholesterol efflux. J Lipid Res 43:618–628, 2002.
45. Sinz MW, Black AE, Bjorge SM, Holmes A, Trivedi BK, Woolf TF. In vitro and in vivo disposition of 2,2-dimethyl-N-(2,4,6 trimethoxyphenyl) dodecanamide (CI-976): identification of a novel five-carbon cleavage metabolite in rats. Drug Metab Dispos 25:123–130, 1997.
46. Kogushi M, Tanaka H, Kobayashi H, Yamada T, Ohtsuka I, Kimura T, Saito I. Effect of E5324, a novel inhibitor of acyl-CoA: cholesterol acyltransferase, on cholesteryl ester synthesis and accumulation in macrophages. Jpn J Pharmacol 68:191–199, 1995.
47. Murakami S, Yamagishi I, Asami Y, Sato M, Tomisawa K. Effect of the ACAT inhibitor, HL-004, on cholesterol metabolism in macrophages. Cell Mol Biol 42:865–872, 1996.
48. Azuma Y, Kawasaki T, Ikemoto K, Ohno K, Yamada T, Yamasaki M, Nobuhara Y. Effects of NTE-122, a novel acyl-CoA: cholesterol acyltransferase inhibitor, on cholesterol esterification and high-density lipoprotein-induced cholesterol efflux in macrophages. Jpn J Pharmacol 79:159–167, 1999.
49. Rodriguez A, Bachorik PS, Wee SB. Novel effects of the acyl-coenzyme A: cholesterol acyltransferase inhibitor 58-035 on foam cell development in primary human monocyte-derived macrophages. Arterioscler Thromb Vasc Biol 19:2199–2206, 1999.
50. Brown MS, Ho YK, Goldstein JL. The cholesteryl ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cytoplasmic cholesteryl esters. J Biol Chem 255:9344–9352, 1980.
51. Knight BL, Soutar AK, Patel DD. Non-saturable degradation of LDL by monocyte-derived macrophages leads to a reduction in HMG-CoA reductase activity with little synthesis of cholesteryl esters. Atherosclerosis 64:131–138, 1987.
52. Yu K C-W, Mamo JCL. Regulation of cholesterol synthesis and esterification in primary cultures of macrophages following uptake of chylomicron remnants. Biochem Mol Biol Int 41:33–39, 1997.
53. Kume N, Kita T. Roles of lectin-like oxidized LDL receptor-1 and its soluble forms in atherogenesis. Curr Opin Lipidol 12:419–423, 2001.
54. Ellsworth JL, Kraemer FB, Cooper AD. Transport of β-very low density lipoproteins and chylomicron remnants by macrophages is mediated by the low density lipoprotein receptor pathway. J Biol Chem 262:2316–2325, 1987.
55. Elsegood CL, Pal S, Roach PD, Mamo JC. Binding and uptake of chylomicron remnants by primary and THP-1 monocyte-derived macrophages: determination of binding proteins. Clin Sci (London) 101:111–119, 2001.
56. Brown ML, Ramprasad MP, Umeda PK, Tanaka A, Kobayashi Y, Watanabe T, Shimoyamada H, Kuo WL, Li R , Song R, Bradley WA, Gianturco SH. A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis. Proc Natl Acad Sci U S A 97:7488–7493, 2000.