Uptake and degradation of low density lipoproteins in atherosclerotic rabbit aorta: role of local LDL modification

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Abstract The uptake of native and modified low density lipoprotein (LDL) in foam cells in atherosclerotic tissue was studied in an in vitro perfusion system for rabbit aorta. Experimental atherosclerosis was induced in rabbits by a combination of cholesterol feeding and mechanical injury. The aorta was perfused in an incubation chamber. A trace-label, radioiodinated tyramine-cellobiose, was used to study cellular uptake of lipoproteins. After perfusion, the tissue was digested and cells were isolated by centrifugation in a density gradient. About 40 times more LDL per cell was accumulated in the foam cell fraction than in the smooth muscle cell fraction. When the cellular uptake of LDL and acetylated LDL (AcLDL) was compared, about 4 times more AcLDL than LDL was taken up by the foam cells, suggesting that the scavenger receptor is expressed in these cells. In a competition experiment, the uptake of LDL into foam cells was reduced by 70% when a tenfold excess of AcLDL was added. This experiment suggests that native LDL is taken up by the same mechanism as AcLDL. The accumulation of radiolabeled LDL in plaques and in foam cells was reduced by 30–55% by adding vitamin E (0.1 mg/ml) to the system. These studies show an uptake of LDL by foam cells in the atherosclerotic tissue. Furthermore, these cells seem to express the scavenger receptor. The competition experiment would suggest that native LDL is taken up by the scavenger receptor. The observation that an antioxidant, vitamin E, may decrease this uptake suggests that oxidative modification of LDL is of importance for this process. —**Wiklund, O., L. Mattsson, T. Björnheden, G. Camejo, and G. Bondjers.** Uptake and degradation of low density lipoproteins in atherosclerotic rabbit aorta: role of local LDL modification. *J. Lipid Res.* 1991. **32:** 55–62.

Supplementary key words modified lipoproteins • foam cells • atherosclerosis • alpha-tocopherol • macrophages • oxidation of lipoproteins

The intracellular and extracellular deposition of lipids, mainly cholesteryl ester, is one of the major characteristics of the atherosclerotic plaque. The alleged early lesion, the fatty streak, is dominated by lipid-filled "foam cells," while in the more advanced lesion these cells are seen surrounding the central necrotic core. Although the origin of these cells has been disputed, most available data today suggest that a majority of them originate from monocyte-derived macrophages (1–5). The understanding of the mechanisms for the formation of foam cells in the arterial intima is incomplete (6, 7). In several studies, formation of foam cells from macrophages has been observed under cell culture conditions. The uptake of native LDL by macrophages is subject to feed back control (8, 9). On the other hand, an unregulated uptake of modified LDL has been shown to create foam cells from macrophages in cell culture (6, 7). A variety of modifications have been utilized for foam cell induction in vitro, such as acetylation (8) and malondialdehyde treatment (9). More recently, interest has been focused on the oxidative modification of LDL, obtained by exposure of LDL to cells (10–12) or copper ions (13, 14). These modifications can be inhibited by the presence of an antioxidant such as alpha-tocopherol or probucol in the medium (13–15).

The role of modified lipoproteins for foam cell formation in vivo in atherogenesis is still unclear (7). It is not known whether macrophages and foam cells, in the atherosclerotic plaques, have lipoprotein-receptor expression or lipoprotein metabolism similar to that observed in cell culture. The handling of native and modified lipoproteins by the arterial wall may be affected by the tissue architecture. It is therefore important that studies are carried out in systems where the tissue architecture is preserved.

Abbreviations: LDL, low density lipoproteins; TC, tyramine-cellobiose; ^{125}I-TC-LDL, LDL labeled with ^{125}I-labeled TC; AcLDL, acetylated LDL; TCA, trichloroacetic acid; LPDS, lipoprotein-deficient serum; SMC, smooth muscle cells; TBAR, thiobarbituric acid reactivity; FC, foam cells; MDA, malondialdehyde.

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In the present study, an in vitro perfusion system for rabbit aorta was utilized to study the uptake of native and modified LDL in different cell populations of atherosclerotic tissue. Since local oxidative modification of LDL might be a significant mechanism for foam cell formation, we studied the effect of a biological antioxidant (alpha-tocopherol) on the cellular uptake and degradation of LDL as well.

METHODS

Isolation and labeling of lipoproteins

Fresh human EDTA-plasma, obtained after overnight fasting, was used for the preparation of lipoproteins. LDL_2 was isolated by sequential ultracentrifugation in the presence of 0.01% EDTA as described (16). Conventional iodination (^{125}I-labeled TC), was performed with ICl-technique as described by McFarlane (17) and modified by Shepherd (18). To study cellular uptake, a tracer label, ^{125}I-labeled tyramine-cellobiose (^{125}I-labeled TC), was used. LDL labeled with ^{125}I-labeled TC as described by Pittman et al. (19). With both iodination procedures, the specific activity was 100–200 cpm/ng. Ninety-five–99% of the radioactivity could be precipitated with 15% TCA. Several of the experiments were performed as double-label experiments using ^{131}I as a second label. Labeling with ^{131}I was performed with the same methods. When the two different labels were analyzed by 2–16% gradient-SDS polyacrylamide electrophoresis, the staining pattern and location of radioactivity were identical. On isoelectric focusing, no change in isoelectric point was seen when labeled lipoproteins were compared with unlabeled ones.

To evaluate whether a modification of LDL of biological importance might have been induced during the preparation or labeling, ^{125}I-TC-LDL was incubated with: *1*) receptor-induced fibroblasts; *2*) receptor-repressed fibroblasts; and *3*) mouse peritoneal macrophages (which express only the modified LDL-receptor (8). Significant internalization of LDL was observed only in the receptor-induced fibroblasts.

Acetylation of LDL was performed as described (20). To evaluate the degree of aggregation of the LDL or AcLDL preparations, the incubation medium was run on a polyacrylamide gradient gel 2–16% (PAA 2/16, Pharmacia, Uppsala, Sweden). After electrophoresis, the major bands were cut and counted in a gamma-counter. There was 11.1–14.4% of the radioactivity in LDL retained in the origin, while 74.4–76.1% of total radioactivity was recovered from the major LDL band. For AcLDL, 13.2–22.2% was recovered from the origin and 60.9–72.7% of total radioactivity was recovered from the main AcLDL band.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS), and isoelectric focusing were performed in a Phast System, an integrated system for horizontal electrophoresis in small gels (Pharmacia).

Cellular lipoprotein uptake

Human fibroblasts were obtained from skin biopsies of healthy volunteers (21). At the fourth or fifth passage, during their logarithmic growth phase, the cells were exposed to a medium with 10% lipoprotein-deficient human serum. After 24 h in this medium, the cells were incubated in Eagle's minimum essential medium containing ^{125}I-TC-LDL. These incubations were carried out for 6 h at 37°C. The cells were then washed and collected after trypsinization and centrifugation. Radioactivity in the pellet and the supernate was evaluated separately.

Mouse peritoneal macrophages were obtained from BALB/C mice. The cells were seeded into 24-well dishes, 10^6 cells/well, and allowed to attach to the well overnight. Nonadherent cells were removed by gentle washing. The adherent cells were then incubated in Eagle's minimum essential medium, containing ^{125}I-TC-LDL. Incubation was performed for 6 h at 37°C. The cells were then washed and cell-associated radioactivity was counted.

Induction of atherosclerosis in rabbits

In the present study, experimental atherosclerosis was induced in New Zealand White rabbits either by cholesterol feeding (1%) or by combining endothelial injury and cholesterol feeding. In order to induce endothelial injury, rabbits were anesthetized with Ketamine (25 mg/kg) and Rompuran (10 mg/kg). An arterial embolectomy catheter (size 4f, Shirley Inc., Irvine, CA) was introduced through the right femoral artery up to the aortic arch. The balloon was filled with 0.3 ml saline and drawn through the entire aorta three times. From the day of injury, the rabbits were given a diet consisting of regular rabbit chow supplemented with 1% cholesterol. After 8–22 weeks the rabbits were killed and their aortas removed for the perfusion experiments.

With cholesterol feeding, a mild to moderate localized atherosclerosis was obtained. With the combination of injury and cholesterol feeding, a severe atherosclerosis with fibrous plaques along the entire aorta was obtained in most cases. During cholesterol feeding the serum level of cholesterol varied between 33 and 103 mM and triglycerides between 0.8 and 7.9 mM.

In vitro perfusion of rabbit aorta

The in vitro perfusion system used in the present study has been characterized in detail (22) and used for studies of arterial wall/blood cell interactions (23), oxygen consumption (24), and the tissue uptake and cellular degradation of LDL (21).

Under general anesthesia (Ketamine + Rompuran) the descending thoracic aorta was removed, dissected free from adventita and mounted in the incubation chamber, as described by Björnheden et al. (22).

For tissue incubations, radiolabeled LDL or AcLDL was added to Eagle's minimum essential medium with Earle's salt, 0.8 mg/ml sodium bicarbonate, and 1% nonessential fatty acids. In addition, the medium contained: 100 μg/ml streptomycin and 100 IU/ml penicillin, 20 mM HEPES buffer, pH 7.4 (Flow Laboratories, Herts, England), 60 mg/ml bovine serum albumin (Sigma Chemical Company, St. Louis, MO) and 10% rabbit lipoprotein-deficient serum (LPDS) prepared by ultracentrifugation at d > 1.21 g/ml. Radiolabeled lipoproteins were added to the medium through a filter (0.22 μm, Millipore Divisions, Bedford, MA). In experiments using one label, the final concentration of apoB in the medium was 0.5 mg/ml. In double labeling experiments we used 0.25 mg/ml of each of the labeled lipoproteins.

In some experiments alpha-tocopherol (vitamin E) (Sigma Chemical Company) was added to the medium at the start of the incubation in a small volume (100 μl) of ethanol. Vitamin E was added to a final concentration of 0.1 mg/ml of medium. To the medium of parallel control incubations, the same volume of ethanol was added. The specific activity of the lipoproteins during the incubations was 22–84 cpm/ng of LDL protein.

In the first experiments (where atherosclerosis had been induced by cholesterol feeding only), the tissue was incubated for about 42 h. In later experiments, the incubation was decreased to 20 h. Preservation of the tissue after dissection and the viability after perfusion was checked by transmission electron microscopy. No evidence of cellular injury was observed.

Peroxidation products in the incubation medium were determined as thiobarbituric acid reactivity (TBAR) and expressed as malondialdehyde (MDA) equivalents (25). During incubation, the TBAR values of the medium did not increase (1.52 at the start of the incubation vs. 1.42 nmol MDA/ml medium at the end). The amount of TCA-precipitable radioactivity from the medium and the electrophoretic mobility was the same before and after incubations.

After incubation, the tissue was taken out of the chamber, cut open, and rinsed twice in saline for 10 min. After rinsing, wet weight and total tissue radioactivity was determined in a gamma-counter (Compugamma, LKB, Pharmacia). The plaques were dissected from the underlying media and analyzed separately for cell isolation.

Isolation of cells from tissue

In order to study the cellular uptake of LDL or AcLDL in foam cells or medial smooth muscle cells, the tissue samples were cut into 1-mm^3 cubes, which were washed twice in 20 ml Hank's medium. The tissue pieces were then incubated in a proteolytic solution as described (26) and modified in our laboratory (24). The proteolytic solution contained: 450 U/ml collagenase C-5138 Type IV (Sigma Chemical Company); 47 U/ml of elastase E-0127 (Sigma Chemical Company); and 1 mg/ml trypsin inhibitor (Sigma Chemical Company) in Hank's solution without Ca^{2+} or Mg^{2+}. After 20 min of digestion, a supernatant with cells was separated from the tissue debris. The digestion was repeated until the tissue samples were completely dissolved. The cell pellets were combined and fractionated on Percoll as described by Björnheden and Bondjers (24). In most of the experiments in the present study atherosclerotic plaques were dissected from the tissue before the digestion and the isolation of foam cells.

The cell fractions were recovered from the gradient with a syringe and the radioactivity in the cells was measured in a gamma-counter (LKB, Bromma, Sweden. A fraction of the cells was then collected on a 0.45-μm filter (SMWP; Millipore, Molsheim, France) and stained with Mayer's hämalun and oil red O. The cells were counted on the filter under light microscopy in order to evaluate the uptake of LDL per cell. Cells were also counted in a cell counter (Cellcounter 134, Analysinstrument, AB, Stockholm, Sweden). Between 600 and 3000 foam cells were obtained per mg wet weight. The cell recovery with this technique has earlier been estimated as approximately 5–15% (24). In the present study an average of 0.7% of the radioactivity in the plaque was recovered in the foam cell fraction.

RESULTS

Cellular uptake of LDL and acetylated LDL in atherosclerotic aorta

In three experiments (aortas II, IV, V, **Table 1**), foam cells (FC) from plaques and smooth muscle cells (SMC) from underlying media were isolated simultaneously. On the average, about 40 times more LDL was taken up by the foam cells.

In six experiments (aortas IV–IX, Table 1), cellular uptake of LDL and AcLDL was studied by a double label technique. In two experiments, AcLDL and LDL were labeled with ^{125}I-labeled TC and ^{131}I-labeled TC, respectively, and in the other two experiments the labels were reversed. Aortas IV and V were obtained from cholesterol-fed rabbits, while aortas VI–IX were obtained from rabbits with atherosclerosis induced by a combination of cholesterol-feeding and endothelial injury (see Methods). The latter aortas showed more fibrosis and more diffuse delineation between the plaques and the underlying media. In cell preparations from these aortas

TABLE 1. Uptake of LDL and AcLDL by foam cells and medial smooth muscle cells isolated from atherosclerotic rabbit aorta

Aorta No.	Incubation Time	LDL		AcLDL		Ratio LDL/AcLDL	
		FC	SMC	FC	SMC	FC	SMC
	h	*nl medium/10⁶ cells/24 h*					
I	36	973					
II	34	717	8.3				
III	32	449					
IV	16	326	24.0	1133	61.5	0.29	0.39
V	17	671	31.1	1687	65.9	0.40	0.47
VI	42	39.1		469		0.08	
VII	20.5	140		447		0.31	
VIII	20.5	74		496		0.15	
IX	20.5	96		257		0.37	

Atherosclerosis was induced by cholesterol-feeding (rabbits I–V) or by a combination of endothelial injury and cholesterol-feeding (rabbits VI, VII). The rabbit aortas were perfused with a medium containing ^{125}I-TC-LDL or, in double-label experiments, LDL and AcLDL labeled with different radioiodine isotopes (^{125}I-TC or ^{131}I-TC). After perfusion, the tissue was digested and the cells were isolated by gradient centrifugation. Data are given as nl medium/10⁶ cell per 24 h; FC, foam cells; SMC, smooth muscle cells. Concentrations of lipoproteins in rabbits I–V were 0.5 mg/ml of LDL and/or AcLDL; in rabbits VI–IX, 0.25 mg/ml of both LDL and AcLDL.

we were not able to recover a pure smooth muscle cell fraction. Even the foam cell fraction was more heterogenous and contained 5–10% elongated, lipid-containing cells, presumably of SMC origin. In general, the cellular uptake of lipoproteins was lower in aortas where atherosclerosis was induced by a combination of endothelial injury and cholesterol feeding as compared with cells isolated from aortas of rabbits that were only cholesterol-fed.

In the foam cell fraction, there was a higher uptake of AcLDL, with a ratio between the uptakes of LDL and AcLDL varying between 0.08 and 0.40 (average 0.27). In the SMC fraction there was a higher uptake of AcLDL (ratio LDL/AcLDL 0.39 and 0.47) as well.

A competition experiment was performed in order to determine whether LDL and AcLDL were taken up by foam cells via the same mechanism. Segments from the same aorta were incubated in parallel incubation chambers. In both chambers, ^{125}I-TC-LDL was added to the medium (0.1 mg/ml). In one of the chambers, unlabeled acetylated LDL was added in tenfold excess (1 mg/ml). The addition of AcLDL reduced the uptake of LDL by about 70% (**Fig. 1**). To control the specificity of this inhibition, the same lipoprotein preparations were incubated with fibroblasts under cell culture conditions (Fig. 1). The cells were incubated with ^{125}I-TC-LDL, 20 μg/ml, after receptor induction. A tenfold excess of AcLDL did not compete with the uptake of LDL into fibroblasts. The addition of a tenfold excess of unlabeled LDL almost completely inhibited the uptake of radiolabeled LDL. The results suggest that LDL is taken up by the foam cells in the tissue via the same mechanism as AcLDL.

As a control to the competition experiments, ^{125}I-TC-AcLDL was added to two incubation chambers, and a tenfold excess of unlabeled AcLDL was added to one of the chambers. The uptake of radiolabel was reduced by 45% in presence of unlabeled AcLDL (Fig. 1).

To study cellular degradation in relation to nonspecific cell adherence or tissue trapping of lipoproteins, we used conventional labeling (ICl-method) and the TC-labeling in double-label experiments. As has been described by Carew et al. (27) this technique can be used to quantify LDL degradation in the tissue. LDL (or AcLDL) was labeled both by direct iodination using the ICl-technique (ICl-label), and by conjugation with radiolabeled tyramine-cellobiose (TC). Atherosclerotic rabbit aortas were incubated with either a combination of ^{125}I-AcLDL and ^{131}I-TC-AcLDL or with a combination of ^{125}I-LDL and ^{131}I-TC-LDL. The experiment is summarized in **Fig. 2**. Radioactivity was first determined in dissected whole plaques. For acetylated LDL, the accumulation of TC-label was about twice that of the conventional label (ratio

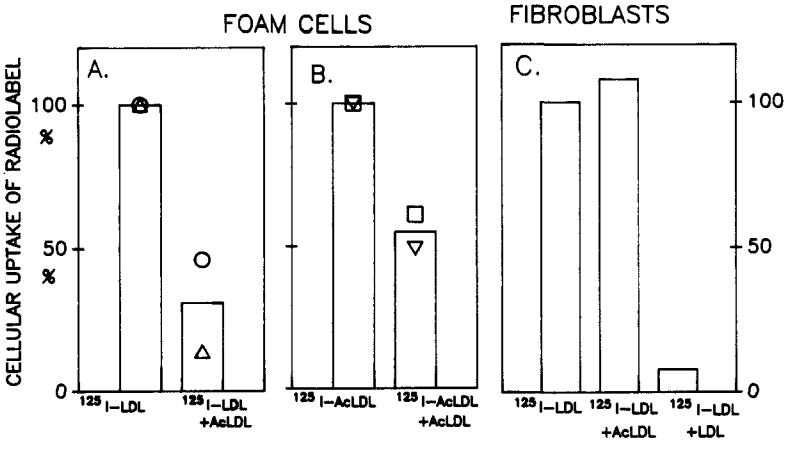

Fig. 1. Uptake of LDL by foam cells in atherosclerotic rabbit aorta and displacement by AcLDL. A: Segments of the same aorta were incubated in two chambers for 20 h: one with ^{125}I-TC-LDL, 0.05 mg/ml, and one with ^{125}I-TC-LDL plus a tenfold excess of AcLDL. The figure shows data from two separate experiments (○ and △, bars = average). C: The same lipoprotein preparations were also incubated with fibroblasts in cell culture under three different conditions: ^{125}I-TC-LDL 20 μg/ml, ^{125}I-TC-LDL plus a tenfold excess of AcLDL, and ^{125}I-TC-LDL plus a tenfold excess of unlabeled LDL. Samples were run in triplicate with a coefficient of error of 3.4%. B: In a separate set of experiments (two aortas □, ▽) the segments of the aortas were incubated in two chambers for 20 h: one with ^{125}I-TC-AcLDL, 0.05 mg/ml and one with ^{125}I-TC-AcLDL plus a tenfold excess of unlabeled AcLDL.

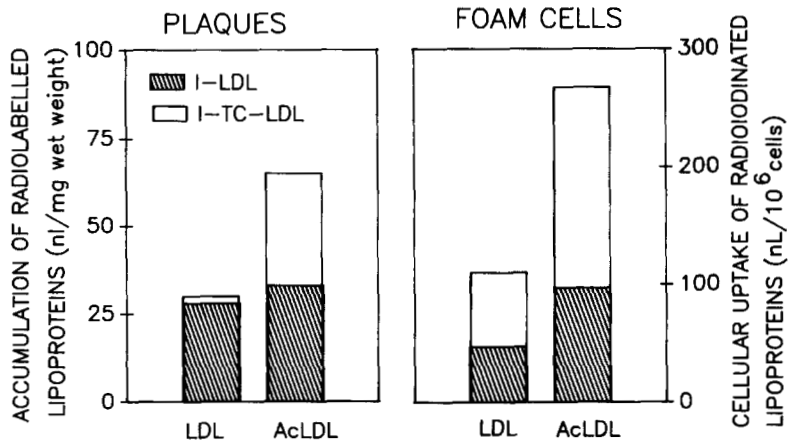

Fig. 2. Uptake and accumulation of radiolabeled lipoprotein after perfusion in vitro of atherosclerotic rabbit aorta. Segments of the aorta were incubated for 21 h with a combination of ^{125}I-LDL (0.25 mg/ml) and ^{131}I-TC-LDL (0.25 mg/ml) or ^{125}I-AcLDL (0.25 mg/ml) and ^{131}I-TC-AcLDL (0.25 mg/ml). Data are given for the dissected plaques as nl/mg wet weight and for the cellular uptake in isolated foam cells as nl/10^6 cells; I-LDL, ^{125}I-labeled LDL or AcLDL; I-TC-LDL, ^{131}I-labeled TC-LDL or AcLDL.

ICl-label/TC-label = 0.51). For native LDL, the difference between labels was smaller with only 5% lower uptake of the ICl-label (ratio = 0.95).

In the foam cell fraction, the ratio between ICl-label and TC-label was 0.36 for AcLDL and 0.43 for native LDL. This experiment would suggest that only a small fraction of the LDL present in the plaque is degraded, while about 50% of the AcLDL is degraded. In the isolated FC fraction, 43% of the LDL was undegraded, while 36% of the cell-associated AcLDL was undegraded.

Cellular uptake of LDL in the presence of alpha-tocopherol

To explore the effect of an antioxidant on the uptake of LDL into foam cells, we incubated pieces of the same aorta in parallel incubation chambers. One was incubated with ^{125}I-TC-LDL in the presence of alpha-tocopherol (0.1 μg/ml), while the other was incubated with ^{125}I-TC-LDL without alpha-tocopherol. When accumulation of radioactivity was analyzed in the plaques, there was a 60% (range 43–72, n = 6) lower accumulation of radioactivity in the presence of alpha-tocopherol. In the underlying media there was actually a tendency towards a higher uptake of LDL in the presence of alpha-tocopherol (mean 122%, range 91–137, n = 4).

In three experiments, foam cells from the dissected plaques were isolated and the accumulated radioactivity in the cell fractions was determined. The accumulation was 30 to 55% lower in the presence of alpha-tocopherol (**Fig. 3**). The result was the same when the calculations were based on cell numbers obtained by cell counter.

The possibility that alpha-tocopherol had other effects on cellular lipoprotein metabolism, which might explain the effects on LDL uptake, was further explored. In these experiments tissue segments were incubated with ^{125}I-TC-AcLDL, in the presence or absence of alpha-tocopherol. No reduction of AcLDL uptake in the presence of alpha-tocopherol was seen. In fact, in both experiments the cellular uptake of AcLDL was higher in the presence of alpha-tocopherol (48 and 168% higher, respectively). Therefore, we conclude that direct effects of alpha-tocopherol on the cellular uptake of modified LDL could not explain our previous observations.

To find out whether any modification of LDL took place in the medium during the incubation, mouse perioneal macrophages were incubated with medium sampled before and after tissue incubation, in the presence or in the absence of alpha-tocopherol (**Fig. 4**). Cells were also incubated with AcLDL. There was no difference in the cellular accumulation of LDL between the media before or after incubation, or between the media with or without alpha-tocopherol.

DISCUSSION

The perfusion system used in the present study was characterized in detail earlier (21, 22); in this system tissue has been shown to be metabolically stable for at least 3 days. Most of the present incubations were shorter than 24 h and in no case was the incubation period longer than 42 h. In the first experiments, experimental atherosclerosis was induced by feeding cholesterol to rabbits. This gave a mild atherosclerosis with fatty streaks and occasional plaques. Due to the variable degree of atherosclerosis, we later combined the cholesterol feeding with mechanical injury. With this method, more severe changes along the total length of the descending aorta were obtained in most cases. The different characteristics of the lesions induced by the two methods might explain the lower cellular uptake of lipoproteins after cholesterol feeding combined with endothelial injury, than after cholesterol feeding alone. In general, the variation among aortas is large, which presumably reflects different degrees of atherosclerosis as well as variations in other structural characteristics of the tissues.

To study the cellular uptake of lipoproteins, we used radioiodinated tyramine-cellobiose as an intracellulary

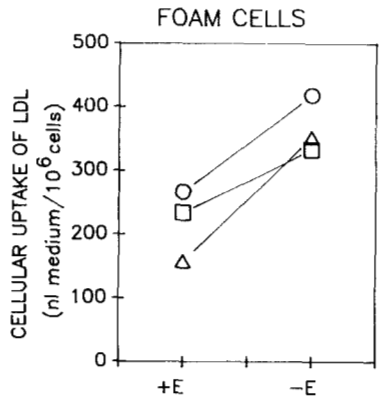

Fig. 3. Cellular uptake of LDL in atherosclerotic rabbit aorta. The tissue was incubated in vitro with ^{125}I-TC-LDL (0.5 mg/ml) for 20 h. In parallel chambers, tissue segments from the same aortas were incubated in the presence (+E) or in the absence (−E) of alpha-tocopherol (vitamin E, 0.1 mg/ml). Each symbol represents one aorta. Cell number was evaluated by counting cells under a microscope.

trapped ligand. By comparing conventional (ICl) iodination and TC-labeling, Carew et al. (27) have estimated the cellular degradation of lipoproteins. In a similar experiment, we found that 60–70% of the cell-associated radioactivity represented lipoprotein degradation. The undegraded 30–40% could represent surface-associated lipoproteins or intracellularly located, undegraded lipoproteins. Shifts in the distribution between degraded and undegraded lipoproteins should be considered as one potential explanation for some of the changes in the lipoprotein uptake by the foam cells observed in the present study. However, the pool of undegraded lipoproteins is too small to explain a major portion of the results. It is also unlikely that the displacement of LDL by AcLDL can be explained by the blocking of extracellular binding sites, since the negative charge of AcLDL makes it less prone to interact with proteoglycans (Hurt-Camejo, E., personal communication).

It has been concluded from cell culture studies that, since LDL uptake is regulated by a negative feed-back mechanism, macrophages or foam cells do not take up native LDL at all, or only to a very limited extent (6, 7). On the other hand, various types of modified LDL are taken up in an unregulated way, and foam cells can be created by the incubation of macrophages with modified LDL in vitro. Such modifications of LDL include acetylation, maleylation, malondialdehyde-modified LDL, oxidatively modified LDL, aggregated LDL, and LDL reacted with proteoglycans (6–10, 28, 29). Maleylated, acetylated, malondialdehyde-modified LDL, and oxidatively modified LDL are all taken up by a common receptor on macrophages, the scavenger, or AcLDL-receptor (6). The presence of this receptor in vivo has been deduced from studies where AcLDL was injected into animals. AcLDL is cleared from plasma by cells of the reticulo-endothelial system within minutes (30–32). Due to the very fast clearance of AcLDL from plasma, it has not been possible to study uptake of AcLDL into arterial macrophages or foam cells, in vivo. The in vitro perfusion system used in the present study allows for the exposure of atherosclerotic tissue to constant concentrations of lipoproteins. To study the uptake of native LDL and AcLDL simultaneously, we used a double-label technique with different radioiodine isotopes on the two lipoproteins. The present study suggests that modified LDL (AcLDL) is taken up more actively than native LDL by the foam cells of the atherosclerotic intima. Thus, about four times more AcLDL than native LDL was taken up by the foam cells. An active uptake of modified LDL by foam cells has also been suggested from studies on cells from explants of rabbit aorta (33).

The uptake of lipoproteins into the smooth muscle cell fraction was low in the model used in this study. Smooth muscle cells are mainly derived from the media, while foam cells primarily are derived from the intima. Since we do not know the concentrations or specific activity of the lipoproteins in the two compartments, the cellular uptake cannot be compared in quantitative terms. Even in the smooth muscle cell fraction the uptake of AcLDL was higher than the uptake of LDL. However these data have to be interpreted with caution since even a small contamination of foam cells or macrophages within the smooth muscle cells could explain these results.

LDL-modification as a cause of foam cell formation is an attractive hypothesis, and there is substantial experimental support for this mechanism. The presence of

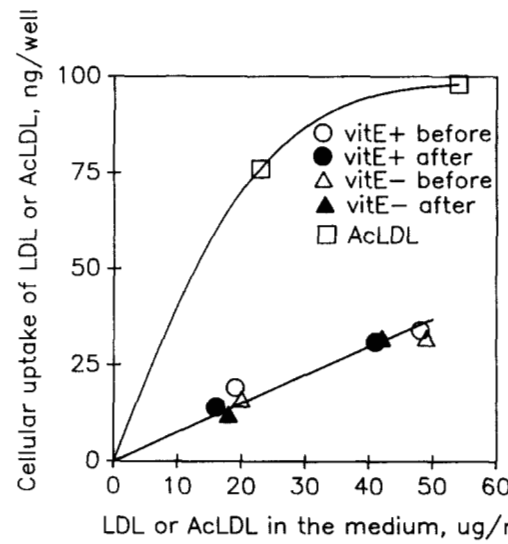

Fig. 4. Effect of incubation of LDL with tissue on uptake of LDL by mouse peritoneal macrophages. Mouse peritoneal macrophages were incubated with ^{125}I-TC-LDL which was sampled from the tissue incubation medium. Samples were taken before or after incubation of tissue for 20 h, both in the presence and in the absence of vitamin E. Cells were also incubated with ^{125}I-TC-AcLDL. Each point represents an average of triplicate determinations with a coefficient of variation between triplicates of 3.6%.

modified lipoproteins is strongly suggested from immunochemical studies of rabbit lesions (34–36) and from studies on lipoproteins eluted from atherosclerotic tissue (35, 37). A role of oxidative modification in atherogenesis is supported by the studies of Carew, Schwenke, and Steinberg (38) where they were able to inhibit plaque formation in hypercholesterolemic rabbits by treating them with probucol, an effective antioxidant. The present study suggests that LDL is taken up by macrophage-derived foam cells via the scavenger receptor, presumably after local modification. The lipoprotein modification could be mediated by exposure to free oxygen radicals from macrophages, granulocytes, or endothelial cells. Such a mechanism is supported by our observation of a 30–55% lower uptake of LDL into foam cells in the presence of alpha-tocopherol. In addition, a 30–60% lower uptake was seen in plaques when the accumulation of radioactivity in whole tissue was determined. In theory, the modification of LDL could take place in the oxygenized medium or in the tissue by interaction with tissue components. We found no evidence of a modification in the medium, and we therefore conclude that, if LDL modification is the basis for the effects of vitamin E, the modification probably takes place locally in the tissue.

In conclusion, these studies showed an active uptake of LDL into foam cells of the atherosclerotic rabbit aorta. We also observed a more active uptake of AcLDL than of native LDL, suggesting an expression of scavenger receptors on the foam cells of atherosclerotic plaques. Alpha-tocopherol, an efficient antioxidant, decreased the uptake of LDL into plaques and into foam cells. This suggests that the local oxidative modification plays a role in the uptake of LDL by foam cells.

We gratefully acknowledge the excellent technical assistance of Aira Lidell, Ulla-Britt Rignell, and Margareta Evaldsson, and thank Maria Stern for revising the English text. This work was supported by research grants from the Swedish Heart-Lung Foundation and the Swedish Medical Research Council (projects 4531, 8457, and 8708). We obtained tyramine-cellobiose as a generous gift from Dr. Ray Pittman, UCSD, La Jolla. A part of this manuscript was presented at the 62nd annual Scientific Session of the American Heart Association, New Orleans, 1989.

Manuscript received 30 April 1990 and in revised form 26 September 1990.

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