The low-density-lipoprotein pathway of native and chemically modified low-density lipoproteins isolated from plasma incubated *in vitro**

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(Received 21 May 1984/Accepted 21 August 1984)

Normal fasting human plasma was incubated for 24 h at 37°C in the presence or absence of lecithin :cholesterol acyltransferase (LCAT) inhibitors. The low-density lipoprotein (LDL) fractions of incubated plasma (control LDL and LCAT-modified LDL) were studied with respect to their chemical and functional properties. LCAT-modified LDL differed from control LDL by a decreased phospholipid and free-cholesterol content, but increased cholesteryl esters. Furthermore, an increase of the relative protein content in LDL by 16–20% was found. Apolipoproteins of LCAT-modified LDL exhibited a 10-fold increase of apo AI, a 4–5-fold increase of apo E, and a 2-fold increase of apo C. All these apolipoproteins resided together with apo B on the same particles. LCAT-modified LDL displayed a higher electrophoretic mobility, a higher hydrated density, a decreased flotation constant and a smaller diameter. Cultured human fibroblasts bound and internalized LCAT-modified LDL to a lower extent than control LDL. The degradation, however, was faster. Modified LDL suppressed 3-hydroxy-3-methylglutaryl-CoA reductase activity to a lower extent than did control LDL. Our results demonstrate that LCAT action, together with lipid transfer and exchange processes, markedly alters the chemical and physicochemical properties of LDL. This in turn significantly influences LDL catabolism *in vitro*.

The cholesteryl esters found in human plasma are mainly produced by the action of LCAT [lecithin (phosphatidylcholine) :cholesterol acyltransferase; EC 2.3.1.43]. It has been shown that this enzyme is synthesized in the liver (Glomset, 1973, 1979), released into the circulation and bound to its major substrate, the high-density lipoproteins (HDL) (Rose, 1981). LCAT utilizes free cholesterol and lecithin (phosphatidylcholine) as substrates, which are abundant in HDL or supplied by other lipoproteins. The cholesteryl esters formed are continuously exchanged with the core lipids of other serum lipoproteins by means of specific transfer and exchange proteins (Barter *et*

al., 1982a,b; Fielding & Fielding, 1980). Thus the action of both LCAT and the specific transfer and exchange proteins appear to alter the chemical composition and the physicochemical properties of all serum lipoprotein classes. A possible involvement of LCAT has been suggested in the formation of HDL₂ from HDL₃ during incubations *in vitro* of human plasma at 37°C (Nichols *et al.*, 1981; Schmitz *et al.*, 1981). Little, however, is known about the influence of LCAT on the chemical and biological properties of low-density lipoproteins (LDL).

Structural or compositional changes of LDL may be of importance for the catabolism of LDL *in vivo*, as they have been shown to influence the binding behaviour to the apo B/E receptor (Steinberg *et al.*, 1978). We report here that incubations of human plasma *in vitro* cause profound alterations in the lipid and apolipoprotein composition of LDL. Because of the possible significance for atherogenesis, we also studied the changes in the catabolism of LCAT-modified LDL via the receptor-dependent pathway in cultured human fibroblasts.

Abbreviations used: LCAT, lecithin (phosphatidylcholine) :cholesterol acyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; apo, apolipoprotein; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); LPDS, lipoprotein-deficient serum; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; SDS, sodium dodecyl sulphate.

* This paper is dedicated to Professor Dr. J. Schurz on the occasion of his 60th birthday.

Experimental

Plasma preparations and incubation conditions

Three portions of blood were drawn from normolipaeic fasting male and female blood donors. EDTA (0.15%) was present in all samples to prevent coagulation. LCAT was completely inhibited in two of three samples obtained, by using iodoacetate or DTNB at concentrations of 5 mM. Plasma samples were routinely mixed with NaN_3 (0.5 mg/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), and penicillin (50 units/ml) to avoid microbial contamination. In control experiments these three preservatives were omitted without significant differences in the results. Immediately after plasma preparation one LCAT-active and one LCAT-inhibited sample were incubated for 24 h at 37°C under an N_2 atmosphere and in darkness. A second LCAT-inhibited sample was stored during the incubation period at 4°C, also under the same conditions.

To terminate LCAT action after 24 h, LCAT inhibitors were added at the same concentration as in LCAT-inactive samples.

Preparation of LDL

The plasma samples were dialysed at 4°C against phosphate-buffered saline (0.02 M-phosphate/0.15 M-NaCl), density of 1.020 g/ml, pH 7.4. Preparative ultracentrifugation was performed in a Beckman L-8 ultracentrifuge (60 Ti rotor) for 22 h at 15°C at 120 000 g, to remove VLDL and IDL. The infranatant was adjusted to a density of 1.063 g/ml with solid NaCl, and further centrifuged for 24 h at 15°C and at 150 000 g. The LDL fractions obtained were purified by re-centrifugation for 24 h at 15°C and 200 000 g in a linear salt gradient (1.020–1.060 g/ml) using a Beckman SW 41 rotor. After dialysis against buffered 0.15 M-NaCl, pH 7.4, LDLs were stored in darkness at 4°C. LDL fractions not older than 1 week were used throughout the present study.

LCAT-modified LDL was obtained from the LCAT-active plasma incubation.

LDL prepared from samples incubated at 37°C in the presence of LCAT inhibitors served as control LDL. Reference LDL was isolated from LCAT-inactive plasma stored at 4°C for 24 h.

Lipid composition of LDL

Non-esterified and esterified cholesterol were determined by the cholesterol reagent from E. Merck (Darmstadt, Germany). Non-esterified cholesterol was assayed by omitting the cholesterol esterase in the assay. Triacylglycerols were determined by an enzymic test system (Boehringer, Mannheim, Germany), and phospholipids by the enzyme kit from Bio-Merieux (Charbonnières, France). The phosphatidylcholine/sphingomyelin

ratio was estimated by on silica-gel 60 glass plates (E. Merck), using a solvent mixture of chloroform/methanol/acetic acid/water (25:15:4:2, by vol.). To reveal the lipid spots, plates were soaked in 10% (v/v) H_2SO_4 and then heated for 20 min at 160°C. The relative ratio was calculated after scanning the plates on a Vernon (France) densitometer.

Analysis of apolipoprotein composition

Protein was measured as described by Lowry *et al.* (1951), with human serum albumin as standard. Turbid solutions were cleared by the addition of 100 μl of 2.5% (w/v) Triton X-100. LDLs were delipidated by treatment with 10% (w/v) SDS in the presence of 5% (v/v) mercaptoethanol and separated by SDS/polyacrylamide-gel electrophoresis in 3.5% (w/v) polyacrylamide gels as described by Laemmli (1970). For the separation of the different apo C peptides, 10% polyacrylamide gels containing 8 M-urea were used (Davis, 1964). Gels were stained with Coomassie Brilliant Blue after fixation with 10% trichloroacetic acid. In some experiments mercaptoethanol was added to reduce disulphide bonds.

Agarose-gel electrophoresis was performed in 0.5% (w/v) agarose gels, whereas 1% agarose gels were used for immunoelectrophoresis. The antisera against different apolipoproteins were the same as used previously (Kostner *et al.*, 1974). Tetramethylurea-soluble protein was estimated from the difference between protein concentration before and after apo B precipitation (Kane *et al.*, 1979). Apo AI, apo AII and apo E were quantified by Laurell rocket electrophoresis of the tetramethylurea-soluble fraction of LDL as described previously (Kostner *et al.*, 1979). The difference between the protein concentration and that of the total tetramethylurea-soluble protein was considered to be attributable to apo C.

Physicochemical methods

Flotation constants were determined in a Beckman Model E analytical ultracentrifuge at 52 000 rev./min. LDL samples with concentrations of 15 mg/ml were dialysed overnight against an NaCl solution with a density of 1.063 g/ml, pH 7.5. Photographs of schlieren patterns were taken every 32 min and analysed by common procedures (Lindgren *et al.*, 1972). Any concentration discrepancies would be minimal, since the samples were analysed at nearly the same concentrations.

The hydrated density of the particles was measured with a high-precision densitometer produced by A. Paar and Co. (Graz, Austria).

Electron micrographs were taken after negative staining with sodium phosphotungstate at sample concentrations between 0.2 and 0.4 mg/ml in

0.15M-ammonium acetate, pH 7.5. The diameters of LDL were obtained by measuring 300 particles.

Iodination of LDL

LDL samples were iodinated by the method of Bilheimer *et al.* (1972). Specific radioactivities varied from 300 to 600 c.p.m./ng of LDL protein. More than 98% of the radioactivity resided on the protein part. Non-specific radioactivity remaining in the supernatant after precipitation of LDL with 50% (w/v) trichloroacetic acid was less than 3%.

Cell culture

Human fibroblasts from the foreskin of healthy newborns were established by routine techniques. Cells were used between the passage 10 and 15. Stock cultures were grown in Eagle's basal medium supplemented with Earle's salts, 10% (v/v) foetal-calf serum, 2 mM-glutamine and streptomycin (1 µg/ml) as previously described (Roscher *et al.*, 1980). For experiments, subcultures were initiated with approx. 0.5×10^6 cells/well in six-well multi-dish trays (Nunc).

Normal growth medium was removed on day 5, the cell monolayers were washed with phosphate-buffered saline and further incubated for 48 h with Eagle's basal medium containing 5% (v/v) LPDS.

Cell studies

Binding, internalization and degradation studies were performed essentially by the method of Brown & Goldstein (1976). Cell monolayers were incubated in an air/CO₂ (19:1) atmosphere with different amounts of ¹²⁵I-LDL in 1 ml of medium containing 5% LPDS, for 6 h at 37°C. A 0.9 ml portion of medium was then removed and mixed with 0.5 ml of ice-cold 50% trichloroacetic acid. ¹²⁵I-LDL degradation was determined by the amount of ¹²⁵I-labelled acid-soluble material formed by the cells and released into the medium. Binding refers to the total amount of ¹²⁵I-LDL that had been bound to the cell surface and was released by heparin into the medium. The amount of ¹²⁵I radioactivity that remained associated with the cells after heparin treatment was defined as internalized ¹²⁵I-LDL.

HMG-CoA reductase assay

HMG-CoA reductase activity was determined by the method of Brown *et al.* (1973). Confluent monolayers were incubated for 16 h with 1.5 ml of medium containing 5% LPDS. After addition of various concentrations of LDL, dishes were further incubated for 12 h. Medium was then removed, the cell monolayers were washed and cell extracts were prepared by addition of KRYO-EOB, a non-ionic detergent (Gianturco *et al.*, 1978). Further treatment of samples and calculation of HMG-CoA

reductase activity was performed exactly as described by Brown *et al.* (1973).

Results

Changes in the chemical composition of LCAT-modified LDL

In preliminary experiments we compared the composition of LDL isolated from samples that have been stored for 24 h at 4°C in the presence of 5 mM-iodoacetate with LDL isolated immediately after blood drawing in the presence or absence of LCAT inhibitors. Since no significant differences between these samples were found, reference LDLs were subsequently isolated from samples stored at 4°C. Control experiments also revealed that all results obtained in the present study were not dependent on the type of inhibitors used (iodoacetate or DTNB). Either inhibitor inactivated LCAT by over 95%, the confidence limit of our assays. This has been confirmed by performing the LCAT assay with several freshly drawn human plasma samples in the presence or absence of 5 mM-iodoacetate or -DTNB.

As shown in Table 1, incubation of plasma at 37°C in the presence of iodoacetate caused a significant increase of the acylglycerol content in LDL as compared with reference LDL. This increase is due to the incubation temperature and is independent of the action of LCAT. The mean levels of cholesteryl esters slightly decreased. No changes were observed in the relative amount of non-esterified cholesterol, phospholipids and protein. In LCAT-modified LDL, marked alterations ($P < 0.001$) of these parameters occurred. Protein and acylglycerols increased, whereas non-esterified cholesterol and phospholipids decreased. This led to a marked increase in the ratio cholesteryl ester/non-esterified cholesterol and to a decrease in the ratio non-esterified cholesterol/phospholipids. The lecithin/sphingomyelin ratio also decreased during LCAT action. The protein/lipid ratio increased by a factor of 1.22 in the presence of active LCAT. This increase was only in part due to a decrease in total lipid. Table 2 shows that tetramethylurea-soluble proteins increased during LCAT action in LCAT-modified LDL, whereas no difference in the apolipoprotein pattern between reference LDL and control LDL was observed. On the other hand, LCAT action caused a marked increase of apo AI, apo AII, apo C and apo E in LCAT-modified LDL.

It was demonstrated by immunoelectrophoresis that all these apolipoproteins co-migrated with the apo B-containing lipoproteins in LDL (Fig. 1). The fact that all LDLs were isolated from a narrow density region after gradient ultracentrifugation and also behaved uniformly in immunoelectro-

Table 1. Chemical composition of LCAT-modified LDL, control LDL and reference LDL

LDLs were isolated from normal human plasma that was incubated at 37°C for 24h in the presence (control LDL) and in the absence of 5 mM-idoacetate (LCAT-modified LDL). LDL isolated from plasma stored at 4°C served as reference LDL. The chemical composition was determined as described in the Experimental section. Results were obtained from nine independent experiments and are expressed as means $\pm$ s.d. Values are given as percentages of total mass. Abbreviation used: n.s., not significant; * $P < 0.001$.

Parameter	Control LDL (I)	Change % (I versus II)	LCAT-modified LDL (II)	Change (%) (III versus II)	Reference LDL (III)	Change (%) (I versus III)
Protein	22.0 $\pm$ 3.1	+19*	26.2 $\pm$ 3.0	+23*	21.3 $\pm$ 3.3	n.s.
Cholesteryl ester	36.6 $\pm$ 4.1	+9*	40.0 $\pm$ 3.6	n.s.	40.8 $\pm$ 2.0	+10*
Non-esterified cholesterol	8.7 $\pm$ 1.1	-48*	4.5 $\pm$ 0.8	-51*	9.1 $\pm$ 1.2	n.s.
Phospholipids	21.5 $\pm$ 1.4	-19*	17.4 $\pm$ 1.7	-18*	21.3 $\pm$ 2.7	n.s.
Acylglycerols	11.2 $\pm$ 6.1	n.s.	11.9 $\pm$ 4.5	+58*	7.5 $\pm$ 4.1	-40*
Cholesteryl ester/non-esterified cholesterol	4.2	+112*	8.9	+98*	4.5	n.s.
Non-esterified cholesterol/phospholipid	0.40	-39*	0.26	-33*	0.40	n.s.
Phosphatidylcholine/sphingomyelin	2.7	-12*	2.4	-12*	2.7	n.s.

Fig. 1. Immunoelectrophoresis of LCAT-modified LDL in 1% agarose gels using different antisera
After electrophoresis, antibodies were allowed to diffuse for 48 h. Gels were washed with 0.15 M-NaCl for 24 h and stained with Sudan Black B. The antibody wells contain: 1, anti-(lipoprotein B); 2, anti-(apo C1); 3, anti-(apo A1); 4, anti-(apo E).

Fig. 2. Schlieren pattern obtained from analytical ultracentrifugation of various LDLs
The solvent density was 1.063 g/ml, adjusted by dialysis against a NaCl solution of appropriate density. Runs were performed in a Beckman model E ultracentrifuge (speed 52000 rev./min; temperature 20°C; schlieren angle 70°). The picture was taken 48 min after the centrifuge had reached full speed. A, reference LDL; B, control LDL; C, LCAT-modified LDL.

Table 2. *Apolipoprotein composition of LCAT-modified LDL compared with control LDL and reference LDL*
Non-apo B protein in LDL was determined after tetramethylurea precipitation of apo B. Apo AI, apo AII, and apo E were determined by rocket electrophoresis using monospecific antibodies against these proteins. Apo C and apo B were calculated from the difference with respect to tetramethylurea (TMU)-soluble protein or total protein respectively. Results are the means $\pm$ s.d. obtained from three experiments. Values are given as percentages of total mass.

Apolipoprotein	LCAT-modified LDL	Control LDL	Reference LDL
TMU-soluble protein	17.5 $\pm$ 4.2	5.5 $\pm$ 1.3	5.0 $\pm$ 1.0
Apo AI	2.5 $\pm$ 0.20	0.25 $\pm$ 0.1	0.25 $\pm$ 0.2
Apo AII	1.5 $\pm$ 0.5	<0.1	<0.1
Apo E	2.6 $\pm$ 0.15	0.43 $\pm$ 0.1	0.60 $\pm$ 0.3
Apo CII + CIHI	10.9 $\pm$ 4.1	4.8 $\pm$ 1.0	4.1 $\pm$ 0.9
Apo B	82.5 $\pm$ 5.3	94.5 $\pm$ 1.0	95.0 $\pm$ 1.0

Table 3. *Physicochemical properties of LCAT-modified LDL compared with control and reference LDL*
LDL was isolated after incubation of plasma for 24 h at 37°C in the absence (LCAT-modified LDL) and in the presence of iodoacetate (control LDL). Reference LDL represents LDL isolated after storage for 24 h at 4°C. Results were obtained from nine independent experiments and are given as means $\pm$ s.d.. Significance: * P < 0.05; ** P < 0.02; *** P < 0.001.

Parameter	Control LDL	LCAT-modified LDL	Reference LDL
Flotation constant ($s_{1,063}^{app}$)	5.68 $\pm$ 0.35	4.95 $\pm$ 0.30	5.45 $\pm$ 0.47
Hydrated density (g/ml)	1.033 $\pm$ 0.002	1.045 $\pm$ 0.003	1.037 $\pm$ 0.002
Particle size (nm)	22.27 $\pm$ 0.51	20.74 $\pm$ 0.78	21.83 $\pm$ 0.42

phoresis strongly suggests a homogeneous LDL population.

Physicochemical properties

LCAT-modified LDL migrated faster in 0.5%-agarose-gel electrophoresis as compared with reference LDL and control LDL. Whereas control LDL and reference LDL had exactly the same migration velocity, LCAT-modified LDL migrated faster by 60–70%. This change was not due to major changes in the apo B moiety, as demonstrated by identical apo B migration behaviour of all three LDL samples on SDS/polyacrylamide-gel electrophoresis using 3.5% gels. Table 3 lists some other physicochemical properties of LCAT-modified LDL. The hydrated density of LCAT-modified LDL increased from 1.033 to 1.045 g/ml, whereas the flotation constant decreased by 13% as compared with control LDL. The flotation patterns of the three LDL samples are shown in Fig. 2.

The LCAT action also caused a significant decrease in the LDL-particle diameter, as verified by electron microscopy. LCAT-modified LDL was approx. 8% smaller than reference LDL, corresponding to a difference in diameter of about 1.5 nm (15 Å) (Table 3).

Interaction of LCAT-modified LDL with cultured human fibroblasts

Binding, internalization and degradation of LCAT-modified LDL, as well as of control LDL

(plasma incubated at 37°C in the presence of LCAT inhibitors) and reference LDL (stored at 4°C in the presence of LCAT inhibitors), were studied in cultured human fibroblasts. Preliminary control experiments revealed that these functional parameters were not dependent on the type of inhibitor used. Furthermore, no significant differences were obtained between control LDL and reference LDL. Thus LDL isolated after plasma incubation at 37°C in the presence of 5 mM-iodoacetate was chosen as LCAT-inactive control.

Fig. 3 shows the result of one characteristic experiment. Binding capacity and internalization of LCAT-modified LDL was markedly decreased as compared with control LDL. Degradation of LCAT-modified LDL, however, was faster than that of control LDL. This was a consistent finding in all our experiments. Lineweaver–Burk analysis of data from nine independent experiments is summarized in Table 4. LCAT modification of LDL caused a significant decrease of the apparent V_{max} by 32% for binding and 27% for internalization respectively. The Michaelis constant (K_m) decreased by 38%, but this was not statistically significant. On the other hand, V_{max} for degradation increased by 94% and K_m decreased by 36% for LCAT-modified LDL. In order to exclude the possibility that iodoacetate might influence LDL binding, internalization or degradation, we compared in control experiments freshly isolated LDL without LCAT inhibition with LDL isolated from

Fig. 3. Binding (●), internalization (■) and degradation (▼) of LCAT-modified LDL (closed symbols) and control LDL (open symbols) in cultured human fibroblasts as a function of concentration

Cell monolayers were incubated for 6 h at 37°C in the presence of medium containing 5% LPDS and increasing amounts of ^{125}I -LDL. After incubation, the medium from each dish was removed and the content of trichloroacetic acid-soluble material was measured (degradation). Heparin-releasable radioactivity was determined after incubation of the cells for 30 min at 4°C with 1.5 ml of 1% sodium heparin solution, and refers to binding. Radioactivity remaining associated with the cells was defined as internalized ^{125}I -LDL.

Table 4. Kinetic data of binding, internalization and degradation of LCAT-modified LDL and control LDL in cultured human fibroblasts

Apparent K_m and V_{max} values were derived from Lineweaver-Burk analysis, calculated from saturation curves for binding, internalization and degradation in cultured human fibroblasts. Values are given as means $\pm$ S.E.M. Significance: n.s., not significant; * $P < 0.02$.

	K_m (μg of protein/ml)			V_{max} (ng of protein/ μg of cell protein)		
	Control LDL	LCAT-modified LDL	Significance	Control LDL	LCAT-modified LDL	Significance
Binding	33.9 ± 10.3	46.7 ± 9.5	n.s.	0.59 ± 0.13	0.40 ± 0.11	*
Internalization	30.6 ± 8.1	42.8 ± 12.6	n.s.	2.6 ± 0.4	1.9 ± 0.3	*
Degradation	32.4 ± 8.3	20.7 ± 5.9	*	6.7 ± 2.1	13.0 ± 5.3	*

iodoacetate-incubated plasma. No significant differences could be observed. Also, further incubation with iodoacetate for 24 h at 37°C after an incubation with LCAT being active exerted no additional effect. The lower binding and internalization of LCAT-modified LDL was also reflected in its decreased ability to suppress HMG-CoA reductase activity. Maximal suppression of HMG-CoA reductase activity was reached with 20 μmol of LCAT-modified LDL/ml, whereas only half of this amount of control LDL was needed to obtain the same suppression (Fig. 4).

Discussion

The LDL fraction of human plasma represents a dynamic pool that is regulated by the continuous transfer and exchange of lipids from or to other lipoprotein classes, or by the specific receptor-

mediated LDL catabolism. In a given individual the dynamics of these processes appear to be reflected by the composition and the physico-chemical properties of LDL. This is supported by a recent report demonstrating that, under conditions of fasting, the LDL pattern is stable but heterogeneous, whereas dietary and drug interferences may lead to significant changes (Fisher, 1983). The receptor-specific metabolism of LDL plays a dominant role in the LDL homeostasis (Brown & Goldstein, 1977). The binding affinity to the B/E receptor can be influenced by artificial alterations of the LDL structure (Steinberg *et al.*, 1978). In a preliminary report (Zechner *et al.*, 1982) we demonstrated that the binding affinity of LCAT-modified LDL to the specific surface receptors is decreased. In this study we further investigated the functional consequences of LCAT modification on LDL metabolism in the Brown & Goldstein (1976)

Fig. 4. Effects of LCAT-modified LDL and control LDL on HMG-CoA reductase activities in cultured human fibroblasts

Confluent cells were incubated for 15h with 1.5ml of medium containing 5% LPDS. Indicated amounts of LDL were added and dishes were further incubated for 12h. Reductase activity was measured as described in the Experimental section. The results are expressed as pmol of mevalonate formed/min per ml. Data represent averages for three independent experiments. ■, LCAT-modified LDL; ●, control LDL.

pathway. In addition, more detailed information is provided on changes in the lipid and apolipoprotein composition of LCAT-modified LDL.

LCAT seems to be responsible for the delivery of most of the core constituents to LDL by means of transfer and exchange processes (Barter & Hopkins, 1983). In addition, it has been postulated recently that LCAT may act directly on LDL without using HDL₃ as substrate (Chen & Albers, 1982).

After incubation of plasma for 24h at 37°C the whole system was equilibrated. If plasma was incubated while LCAT was active, the amount of non-esterified cholesterol and phospholipids in LDL diminished. These changes in the free cholesterol/phospholipid ratio in LDL may be of importance *in vivo*, since it has been suggested that a low non-esterified cholesterol/phospholipid ratio represents an indicator of risk for ischaemic vascular diseases (Kuksis *et al.*, 1982).

The relative amount of protein in LDL increased and the cholesteryl ester content stayed virtually constant in our experiments. The LCAT inhibitors used in the present study apparently did not affect transfer and exchange processes, since the triacylglycerol content of LDL increased at the expense of cholesteryl esters, whereas surface constituents remained constant in control LDL. At 4°C, LCAT and transfer and exchange processes were inactive.

This has been verified by the finding that LDL isolated from freshly drawn plasma had the same chemical composition as that isolated from plasma stored for 48h at 4°C.

The LDL fractions used in our experiments were homogeneous by electrophoresis and flotation behaviour in the analytical ultracentrifuge, and we proved by immunoelectrophoresis that the apo A, apo C and apo E peptides resided together with apo B on the same particle. The uptake of different non-apo B apolipoproteins during incubation with LCAT may also be responsible for the faster migration of LCAT-modified LDL on agarose-gel electrophoresis. The studies with cultured human fibroblasts revealed a marked decrease in both binding and internalization of LCAT-modified LDL. This was rather unexpected, since the apo E content of LCAT-modified LDL was higher and apo E is known to have a higher affinity to the B/E receptor as compared with apo B (Innerarity *et al.*, 1979). It is feasible that the decrease in surface lipids may have caused these effects by altering the surface exposure of apo B. An alternative explanation is that the apo C content influences the binding, a process that is known to alter the chylomicron-remnant uptake by the liver (Windler *et al.*, 1980).

Surprisingly, LCAT-modified LDL was degraded markedly faster than control LDL. This was a consistent finding with LDL preparations from a total of nine individuals and indicates that binding and internalization processes are not necessarily linked to the degradation system. The finding that control LDL is more potent than LCAT-modified LDL in suppressing the activity of HMG-CoA reductase is a further indication that binding and internalization are the rate-limiting step in the overall suppression of cholesterol biosynthesis, as previously suggested by Goldstein *et al.* (1975).

In a control experiment, plasma that has been incubated for 24h in the presence of active LCAT was incubated for additional 24h after admixture of iodoacetate. The control LDL, incubated for 24h in the presence of LCAT inhibitors at 37°C, was stored in the meantime at 4°C. Subsequent binding studies clearly indicated that a prolonged contact of LDL with iodoacetate, occurring in reference LDL preparations, is without influence on the whole system.

Within the limitation of studies *in vitro*, these observations may be useful in understanding the functional impact of gradual LDL modifications that may occur *in vivo* (Havekes *et al.*, 1976).

We thank Mrs. E. Zenzmaier and Miss R. Moser for technical assistance, and Miss F. Schlacher for secretarial help. The work was supported by grants from the Österreichischen Fonds zur Förderung der wissenschaftlichen Forschung (Projects nos. 5144 and 4654).

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