Low-Density Lipoproteins of the Postprandial State Induce Cellular Cholesteryl Ester Accumulation in Macrophages

Monika Lechleitner, Fritz Hoppichler, Bernhard Foger, Josef R. Patsch

Abstract  Chemically or biologically modified low-density lipoproteins (LDL) but not native unmodified LDL lead to foam cell formation in monocyte-derived macrophages. Since the magnitude of postprandial lipemia after a challenge test seems to be associated with coronary artery disease, we tested the hypothesis that in the course of postprandial lipemia, LDL appear in plasma that are capable of leading to foam cell formation even without prior modification. We incubated the macrophage-like cell line P388 with unmodified postabsorptive and postprandial LDL from 17 healthy donors and measured the cellular cholesterol and triglyceride contents and amounts of exogenous[^1] oleic acid incorporated into the cholesteryl ester fraction. Postprandial LDL induced a significantly more pronounced cholesteryl ester accumulation than did postabsorptive LDL (477±286% versus 212±173%, respectively; P<0.003). The increase in cellular total cholesterol was significantly higher as a result of cell incubation with postprandial LDL (107±61%) than with postabsorptive LDL (54±40%, P<0.003), whereas no increase in triglyceride content was observed (P<0.589) in either case. After CuSO₄ incubation and incubation with P388 cells, postprandial LDL revealed more thiobarbituric acid–reacting substances than did postabsorptive LDL (55±10 versus 28±9 nmol/mg protein, P<0.018; 28±4 versus 20±3 nmol/mg protein). The increase in cellular cholesteryl ester synthesis caused by postprandial LDL was reduced by more than 50% when lipoproteins and cells were incubated in the presence of ascorbic acid (P<0.007). In competition studies, cellular binding and degradation of[^2] labeled postprandial LDL were reduced 30% by cold acetyl-LDL, and coincubation of postprandial LDL with acetylated-LDL resulted in no further increase in cellular cholesteryl ester accumulation. Our results are consistent with the view that both lipoproteins may share the same cellular uptake mechanism and that this mechanism might be the scavenger receptor. From our results the reason for the increased atherogenicity of postprandial LDL appears to be a greater susceptibility to chemical modification such as oxidation during incubation with cells in culture. (Arterioscler Thromb. 1994; 14:1799-1807.)

Key Words  • postprandial LDL • postprandial LDL • cellular lipid accumulation

E levated levels of plasma low-density lipoprotein (LDL) are correlated with an increased risk of developing atherosclerotic vascular disease.[1,2] The major pathway for LDL removal from plasma is adsorption and endocytosis through the cellular LDL receptor. Saturation of this pathway leads to a longer half-life of LDL,[3] resulting in a greater susceptibility of the LDL particle to modification such as oxidation. The chemically modified LDL particles are cleared from plasma by the scavenger receptor expressed primarily by macrophages,[4] leading to intracellular cholesteryl ester (CE) accumulation and potentially to foam cell formation.[5]

The postprandial state, with an elevated plasma triglyceride pool, increases the triglyceride content of high-density lipoprotein (HDL) via neutral lipid exchange so that the metabolic fate of HDL is affected.[6] The same can be expected for LDL because the triglyceride content of LDL has been shown to be a function of the plasma triglyceride level.[7,8] Most of the published data on the effect of LDL and its subfractions on cholesterol metabolism relate to LDL in the postabsorptive state[9-14]; virtually nothing is known regarding LDL occurring in the postprandial state. The triglyceride content of LDL affects its affinity for the LDL receptor on HepG2 cells and fibroblasts.[8]

We have recently demonstrated that plasma triglyceride levels when measured in the postprandial state are independent risk factors for coronary artery disease (CAD).[14] We therefore became interested as to whether in the postprandial state LDL occur with altered biological activities that could help to explain the atherogenicity of postprandial hypertriglyceridemia. We report here that LDL from healthy donors in the postprandial state compared with the postabsorptive state cause a more pronounced cellular CE accumulation in macrophages.

Methods

Study Subjects

LDL was prepared from the plasma of 17 healthy volunteers (7 women, 10 men; aged 22 to 66 years; mean, 49 years) who came to the laboratory in the morning after a 14-hour overnight fast, ie, in the postabsorptive state. The first blood sample was obtained with subjects in the postabsorptive state. Immediately thereafter, subjects ingested a fatty liquid test meal with a composition described earlier.[15] The second blood sample was obtained 4 hours after intake of the test

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meal for the isolation of postprandial LDL. In 2 test subjects, samples were taken 2, 4, 6, 8, 10, and 24 hours after the test meal to determine a possible time dependency of the athero-
genetic effect of postprandial LDL. Blood was collected into tubes containing EDTA to give a final concentration of 1 mg/mL, and plasma was separated immediately by centrifugation at 4°C.

Lipid Analysis

Serum cholesterol, triglyceride, and HDL cholesterol levels were determined in the postabsorptive and postprandial states. Plasma cholesterol was measured by an enzymatic colorimetric assay with cholesterol oxidase and amionophenazon (cholesterol PAP, MA Kit 100; Roche). Triglycerides were determined by a colorimetric reaction with ionion-
trotriazolium chloride after enzymatic hydrolysis (triglyceride PAP, Uni Kit II; Roche). HDL cholesterol, HDL₃, and HDL₄ cholesterol were measured after a stepwise precipitation procedure with heparin–manganese chloride.¹⁶ LDL cholesterol was calculated according to the Friedewald formula.¹⁷

Lipoprotein Isolation and Characterization

LDL was prepared from freshly obtained EDTA-plasma by rate zonal ultracentrifugation using Beckman L-8 ultracentri-
fuges and Beckman Ti-14 zonal rotors.¹⁸ An NaBr density gradient, linear with respect to rotor volume, was constructed using a Beckman pump model 141. Ultracentrifugation was performed at 41,000 rpm and 15°C for 140 minutes. The LDL obtained by this procedure elute from the rotor in volume fraction 180 to 250 mL.¹⁸ These zonally isolated LDL were concentrated by pressure filtration using Amicon cells. Subse-
quenty, they were dialyzed exhaustively against 0.9% NaCl and 1 mmol/L EDTA, pH 7.4, in the dark at 4°C, filtered through a 0.45-µm Millipore filter, and stored at 4°C under nitrogen. LDL subfractions were used within 1 week after preparation.

Cholesterol and triglyceride levels of isolated LDL were quantified by use of an enzymatic kit (cholesterol CHOD-PAP, triglycerides GPO-PAP; Boehringer Mannheim). Lipid phosphorus was measured by the method of Bartlett,¹⁹ ex-
pressed as phosphatidylcholine equivalent, and protein con-
tent was determined by the method of Lowry et al.¹⁰ using bovine serum albumin as a standard. Electrophoretic mobility of the lipoproteins was determined by electrophoresis at pH 8.6 in 0.05 mol/L barbital buffer in agarose gels.¹¹ The particle diameters in postabsorptive and postprandial LDL subfractions were measured by nondenatur-
ing polyacrylamide gradient gel electrophoresis.⁹

The lipid peroxide content of LDL was determined by the thiobarbituric acid–reacting substances (TBARS) assay of Buege and Aust;²² dilutions of tetraethoxypropane were used to generate standards. EDTA-free LDL (200 µg protein/mL) was modified by oxidation in a cell-free system in the presence of 2.5 µmol CuSO₄ in phosphate-buffered saline at 37°C for 24 hours.²² For the determination of cell-mediated oxidation, EDTA-free LDL (100 µg protein/mL) was incubated with P388 cells or cell-free wells in RPMI medium for 24 hours at 37°C. In both systems, oxidation was arrested by addition of 200 µmol EDTA and refrigeration. The fatty acid pattern and α-tocopherol content of postab-
sorptive and postprandial LDL were kindly analyzed by Drs. W. Sattler and G.M. Kostner (Department of Medical Bio-
chemistry, University of Graz [Austria]) using gas chromatog-
raphy²⁴ and high-performance liquid chromatography.²⁵ Ace-
tyl-LDL was prepared by the method of Basu et al.²⁴ Labeling of postabsorptive, postprandial, and acetyl-LDL with [¹³C] (Am-
ersham) was performed as described previously by Bilheimer et al.²⁷ The specific activity of labeled preparations was approximately 200 cpm/µg protein.

Cells and Cell Culture

The murine macrophage cell line P388 (lymphoid neoplasm of DBA/2 mice treated with 3-methylcholanthrene) was ob-
tained from the Salk Institute Cell Repository. The cell culture medium was HEPES-buffered RPMI 1640 supplemented with 1 mmol/L glutamine, 10% fetal calf serum, 100 µg/mL peni-
cillin, and 100 U/mL streptomycin. Cells were maintained in 100-mm dishes in a humidified incubator (5% CO₂) at 37°C.

For control procedures, human monocytes were isolated by density gradient centrifugation from blood derived from fast-
ning normolipidemic subjects. Blood (20 mL, anticoagulated with 10 U/mL heparin) was layered over 15 mL Ficoll-paque (Pharmacia) and centrifuged at 500g for 30 minutes at 23°C. The mixed mononuclear cell band was removed by aspiration, and the cells were washed twice in RPMI medium. The cells were plated at 10⁴ cells per 35-mm dish, and nonadherent cells were removed after 2 hours of incubation at 37°C by three washes with serum-free medium. Monocyte-derived macro-
phages were used within 7 to 10 days of plating. In all experi-
ments, 10⁶ cells per dish were seeded in 1 mL complete medium in triplicate. After 24 hours, when the cells had reached approximately 70% confluence, they were carefully washed with saline and refed with 1 mL serum-free RPMI 1640 containing the respective LDL concentrations indicated in the figure legends. Control cultures contained lipoprotein-
deficient serum (LPDS).²⁸ After 24 hours, lipids were ex-
tracted and measured as described below.

Cholesterol Esterification Assay

For determination of CE synthesis, [¹⁴C]oleic acid (55 mCi·mmol⁻¹, Amersham) was complexed with bovine serum albumin,²⁹ and cells were incubated with lipoproteins and 0.01 mol/L of the [¹³C]oleic acid–albumin complex. After 24 hours of incubation, cell monolayers were washed and extracted with hexane/isopropanol (3:2, vol/vol) containing 10 µg·mL⁻¹ carrier cholesterol, cholesterol oleate, and [¹³C]cholesterol for estimation of recovery. Labeled lipids were taken to dryness, redissolved in hexane, and separated by thin-layer chromatog-
raphy on Whatman MK-6F microslides in heptane/diethyl-
ether/acetic acid (75:25:2, vol/vol). Areas containing lipids were visualized with iodine vapor and transferred into vials for counting of radioactivity. Cell monolayers were dissolved in 0.1N NaOH for protein determination by the method of Lowry et al.³⁰ Aliquots of unlabeled lipid extracts were used to determine cholesterol and triglyceride mass using an enzy-
matic kit (cholesterol CHOD-PAP, triglycerides GPO-PAP; Boehringer Mannheim) supplemented with Triton X-100 (10 µL/mL reagent).

Metabolism of [¹²⁵I]-LDL

After a 48-hour preincubation period in which cell cultures were grown in medium containing 10% LPDS, P388 cells were incubated for 5 hours at 37°C in the presence of [¹²⁵I]-labeled postabsorptive or postprandial LDL. For estimation of lipo-
protein degradation, the medium was removed and brought to 2 mL ice-cold medium containing various concentrations of [¹²⁵I]-labeled LDL for 2 hours. In these experiments performed at 4°C and 37°C, respectively, cells were washed extensively after the incubation period at 4°C with buffer containing bovine serum albumin before being dissolved in 0.1N NaOH to quantify bound and internalized LDL.³¹ Protein was deter-
minal by the method of Lowry et al.³²
Statistical Analysis

Data are expressed as mean±SD. Data from paired observations were analyzed using the Wilcoxon signed rank test employing the STATVIEW SE+ graphics statistical package (Abacus Concepts).

Results

Screening for the Most Atherogenic LDL

In two subjects, postabsorptive and postprandial LDL obtained 2, 4, 6, 8, 10, and 24 hours after ingestion of the fat meal were examined for their ability to affect cellular CE accumulation in P388 cells. As illustrated in Fig 1 (top), this ability increased over the first 4 hours and decreased subsequently until, at approximately 8 hours postprandially, it was similar to that in the postabsorptive state. Cellular cholesterol accumulation paralleled this pattern (Fig 1, middle). Similar kinetics in the amount of [\(^{14}\)C]oleic acid incorporation into cellular CE were found in both P388 cells and human monocyte-derived macrophages (Fig 1, bottom). After conducting this series of pilot studies, we used for further experiments only postabsorptive and postprandial LDL obtained 4 hours after the test meal.

The Postprandial State Alters LDL Composition

Table 1 shows the postabsorptive and postprandial lipid levels of the study subjects. Along with the postprandial elevation of triglycerides, HDL became enriched with triglycerides at the expense of CE. The reduction in HDL cholesterol averaged 10%, in excellent agreement with previous reports.

Table 2 gives the lipid-protein composition of LDL. There was a small but consistent, and therefore statistically highly significant, increase in the percent content of triglyceride at the expense of CE. LDL from the postprandial state contained also more phospholipid at the expense of unesterified cholesterol. The overall lipid-protein ratio of LDL, however, remained unchanged from the postabsorptive to the postprandial state.

Table 3 shows the fatty acid composition and \(\alpha\)-tocopherol content of postabsorptive and postprandial LDL. There was no difference between postabsorptive and 4-hour and 8-hour postprandial LDL.

The LDL from the postabsorptive and postprandial states could not be distinguished by their electrophoretic behavior on agarose gels; they all demonstrated \(\beta\)-electrophoretic mobility. The particle sizes of postabsorptive and postprandial LDL subfractions were evaluated in nondenaturing polyacrylamide gradient gels. Five study subjects exhibited a pattern B distribution and the remainder pattern A, and no change in the respective distribution pattern was observed from the postabsorptive to the postprandial state.

Binding and Degradation of LDL

After an incubation period of 5 hours at 37°C, \(^{125}\)I-postprandial LDL was degraded by P388 cells to a significantly larger extent than \(^{125}\)I-postabsorptive LDL (Fig 2, top). This higher affinity of postprandial LDL was also found in binding experiments, which showed a twofold higher amount of postprandial LDL bound to the cell surface of P388 cells (after incubation with 100 µg/mL LDL at 37°C, 85±6 ng/mg cell protein; at 4°C, 39±4 ng/mg cell protein) than for postabsorptive LDL (37±4 ng/mg cell protein at 37°C and 18±3 ng/mg cell protein at 4°C).
Competition studies were conducted to establish whether postabsorptive LDL, postprandial LDL, or acetyl-LDL is able to interfere with the degradation of \(^{125}\text{I}-\)postprandial LDL (Fig 2, middle). Cold postprandial LDL was by far the most effective competitor, reducing degradation of \(^{125}\text{I}-\)postprandial LDL by 95%, followed by postabsorptive LDL. Acetyl-LDL also showed some influence on the degradation of \(^{125}\text{I}-\)postprandial LDL. Competition studies for cell binding (Fig 2, bottom) were consistent with the degradation studies (Fig 2, middle).

**Effect of LDL on Cellular Lipid Content**

LDL from the postabsorptive state induced only a small increase in the amount of \([^{14}\text{C}]\)oleic acid incorporation into cellular CE (mean increase, 212±173%) compared with basal values obtained from cell cultures incubated with LPDS containing medium without LDL. This result is in agreement with the well-established phenomenon of unmodified LDL causing a small and saturable CE accumulation in macrophages and macrophage-like cell lines.\(^{4,5,28}\) In contrast, LDL from the postprandial state caused a more pronounced increase in cellular cholesterol esterification, averaging 477±286% (\(P<.003\)). This result was very consistent in that it was observed with every subject studied (Fig 3).

When cellular cholesterol mass was measured instead of radioactivity, the values of cholesterol accumulation were again higher after incubation with postprandial LDL (+107±61%) compared with postabsorptive LDL (+54±40%; \(P<.003\)) (Fig 4). No significant difference was found in triglyceride accumulation in cell cultures incubated with postabsorptive or postprandial LDL (postabsorptive, 48±35% increase; postprandial, 53±42%; \(P=.589\)).

Neither postabsorptive nor postprandial LDL showed any cytotoxic effect on P388 cells over an incubation period of up to 24 hours and a lipoprotein concentration of up to 100 \(\mu\text{g/mL}\) as evidenced by trypan blue dye exclusion.\(^{29}\) Cell viability and protein content did not differ between experiments performed with LPDS alone or with LDL added to the culture medium.

**Coincubation Studies of Postprandial LDL With Acetyl-LDL or With Ascorbic Acid**

When the postprandial LDL subfractions were incubated in the presence of acetyl-LDL, no further increase in cellular CE synthesis was observed, and the results were similar to those obtained with acetyl-LDL or postprandial LDL alone (Table 4).

Addition of the antioxidant ascorbic acid (40 \(\mu\text{mol/mL}\))\(^{30-32}\) together with postprandial LDL to the cell cultures resulted in a significant reduction in the rate of \([^{14}\text{C}]\)oleic acid incorporation (\(P<.007\)) (Table 4).

**Determination of Lipid Peroxidation Products**

The values of TBARS were very low in the LDL when determined immediately after preparation and before our typical tissue culture experiments. These findings are consistent with normal \(\beta\)-mobility of postprandial LDL and suggest that no major lipid peroxidation processes had occurred during our LDL preparation.

After incubation for 2 to 24 hours with P388 cells or in a cell-free system with CuSO\(_4\), EDTA-free postprandial LDL showed higher levels of TBARS compared with postabsorptive LDL, indicating that postprandial LDL are more prone to oxidation (Table 5).

**Discussion**

LDL have been heavily implicated in the pathogenesis of atherosclerosis, the underlying cause of CAD and stroke.\(^{1,3,33}\) Physiologically, LDL particles function as the major carriers of plasma cholesterol to cells that express the LDL receptor\(^{3,34}\) to satisfy the requirement for cellular cholesterol. Chemical modification of LDL, such as oxidation,\(^{35,36}\) leads to a cellular uptake through the scavenger receptor,\(^{37,38}\) resulting in the formation of foam cells. Oxidized LDL were found in atherosclerotic lesions\(^{39,39}\) but not in the normal arterial wall.\(^{40}\) During the development of atherosclerotic lesions, cells of the

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**Table 1. Lipid Values of 17 Study Subjects Before and 4 Hours After Standard Fatty Test Meal**

<table>
<thead>
<tr>
<th>State</th>
<th>Cholesterol</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C (HDL-TG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postabsorptive</td>
<td>199±31</td>
<td>114±48</td>
<td>113±23</td>
<td>61±21 (25±11)</td>
</tr>
<tr>
<td>Postprandial</td>
<td>205±31</td>
<td>238±149</td>
<td>108±24</td>
<td>54±19 (34±15)</td>
</tr>
</tbody>
</table>

\(P<.025\)* \(P<.003\) \(P=NS\) \(P<.003\)

\(\text{TG}\) indicates serum triglycerides; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol. Values are mean±SD and are given in milligrams per deciliter.

*Probability values are for comparison between postabsorptive and postprandial lipid values.

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**Table 2. Lipid Composition of Postabsorptive and Postprandial Low-Density Lipoprotein**

<table>
<thead>
<tr>
<th>State</th>
<th>%CE</th>
<th>%UC</th>
<th>%TG</th>
<th>%PL</th>
<th>%Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postabsorptive</td>
<td>38.1±3.6</td>
<td>8.3±0.6</td>
<td>4.8±1.5</td>
<td>24.5±2.3</td>
<td>24.5±2.3</td>
</tr>
<tr>
<td>Postprandial</td>
<td>37.0±3.6</td>
<td>8.1±0.9</td>
<td>5.8±1.7</td>
<td>25.2±2.2</td>
<td>24.5±1.6</td>
</tr>
</tbody>
</table>

\(P<.001\) \(P<.046\) \(P<.004\) \(P<.019\) \(P<.698\)

\(\text{CE}\) indicates cholesteryl ester; \(\text{UC}\), unesterified cholesterol; \(\text{TG}\), triglycerides; \(\text{PL}\), phospholipids; and \(\text{Pr}\), protein. Values are relative chemical composition (percent dry mass, mean±SD obtained from 17 people).
arterial wall become stimulated to oxidize LDL,\textsuperscript{11} an effect triggered by minimally modified LDL.\textsuperscript{41} Among other cell types, mouse peritoneal macrophages,\textsuperscript{38} human monocytes,\textsuperscript{42} and monocyte-derived macrophages\textsuperscript{43} have been shown to oxidize LDL.

The cell line P388 used in our experiments is a well-defined murine macrophage cell line\textsuperscript{27,44,45} and serves as a model for in vitro foam cell formation because it expresses scavenger receptors in a way comparable to human macrophages.\textsuperscript{28} P388 cells contain low levels of LDL receptors and express receptors for hypertriglyceridemic very-low-density lipoproteins (VLDL).\textsuperscript{44,45}

Our data suggest that the scavenger receptor pathway may be responsible for the increased cellular CE loading effect of postprandial LDL, because acetyl-LDL, the most excessively modified form of LDL,\textsuperscript{26} competed with cellular degradation and binding of postprandial LDL. Furthermore, no additive effect on cellular CE accumulation was found in coinubation studies using postprandial LDL and acetyl-LDL, indicating that both lipoproteins, postprandial LDL, possibly modified during cell incubation, and acetyl-LDL might share the same cellular uptake mechanism. It should be kept in mind, however, that the lack of an additive effect between postprandial LDL and acetyl-LDL could result not only from a competition for binding and internalization but from an interference in intracellular pathways, ie, competition for lysosomal hydrolysis or reesterification.\textsuperscript{3,4}

With respect to lipoprotein binding and uptake, minimally modified LDL have been reported to be internalized by the LDL receptor,\textsuperscript{41} whereas acetyl-LDL and oxidized LDL are recognized by the scavenger receptor.\textsuperscript{5} The degree of LDL oxidation and thus its receptor specificity depend on the time of incubation with cells or CuSO\textsubscript{4}.\textsuperscript{12,43,46} The suggested occurrence of an oxidative modification of postprandial LDL during cell incubation could explain why acetyl-LDL interacted with cellular binding and degradation of \textsuperscript{125}I-postprandial LDL, determined after 5 hours, to a lower extent than with the cellular cholesterol esterification rate, determined after 24 hours. The results of our competition studies are in accordance with previously published data about the interaction of acetyl-LDL and endothelial cell-modified LDL: Whereas unlabeled acetyl-LDL could compete for only 50% of degraded \textsuperscript{125}I-cell-modified LDL, cell-oxidized LDL competed for approximately 80% of degraded \textsuperscript{125}I-acetyl-LDL.\textsuperscript{47} Because oxidized LDL and acetyl-LDL did not serve as identical ligands with respect to macrophage recognition and uptake, more than one macrophage receptor for modified lipoproteins was suggested.\textsuperscript{37,47} Freeman et al\textsuperscript{48} demonstrated in their studies that nonidentical but partially interacting sites on the scavenger receptor account for the nonreciprocal cross competition of these modified lipoproteins.

In our study, postprandial LDL were found to be more prone to CuSO\textsubscript{4}-mediated oxidation than postabsorptive LDL, but cell-mediated oxidation did not appear to be substantially different, despite the statistical significance. These different results of CuSO\textsubscript{4} and cell-mediated oxidation could be due to an immediate uptake of the oxidized LDL by the cells as soon as it is formed.

The addition of ascorbate to postprandial LDL resulted in a markedly lower accumulation of cellular CE. Ascorbate is known to act as a strong antioxidative substance, especially by free radical scavenging and by saving the tocopherol content of lipoproteins.\textsuperscript{30,32} We found no decrease in the \(\alpha\)-tocopherol content of LDL particles from the postabsorptive to the postprandial state. This finding is consistent with previous studies in which differences in oxidation rates among LDL subfractions did not correlate with their \(\alpha\)-tocopherol content.\textsuperscript{12,13}

The mechanism by which peroxidation of LDL particles is initiated remains uncertain. The fatty acid pattern of LDL could be of importance because lipid peroxidation depends on the formation of various aldehydes arising from chain cleavage of polyunsaturated fatty acids.\textsuperscript{25} However, we found no significant difference in the fatty acid pattern among postabsorptive and postprandial LDL.

An increased oxidizability of LDL has been ascribed by some investigators to a deficiency in free cholesterol-
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Fig 2. Top, Line graph shows degradation of \(^{125}\)I-labeled postabsorptive (pa) and \(^{125}\)I-labeled postprandial (pp) low-density lipoprotein (LDL) by P388 cells. Cell monolayers were incubated with increasing concentrations of \(^{125}\)I-labeled postabsorptive and postprandial LDL for 5 hours at 37°C before analysis of \(^{125}\)I-labeled degradation products. Results are representative of two experiments and are the average of duplicates that varied by less than 5%. Middle and bottom, line graphs show competition for degradation (middle) and binding (bottom) of \(^{125}\)I-LDL metabolism by lipoproteins. Cultures were incubated for 4 hours at 37°C with 25 \(\mu\)g/mL \(^{125}\)I-postprandial LDL and the indicated concentrations of unlabeled postprandial LDL, postabsorptive LDL, and acetyl-LDL (Ac LDL). Medium was processed for determination of lipoprotein degradation products and monolayers for cellular binding of \(^{125}\)I-LDL as detailed in "Methods."

Fig 3. Bar graph shows \(^{14}\)C-oleic acid incorporation into the cholesteryl ester fraction of P388 cells after incubation with low-density lipoprotein (LDL) subfractions (100 \(\mu\)g/mL) from 17 healthy subjects. Data indicate mean levels of percent increase (experiments in triplicate) in the amount of cellular cholesteryl ester synthesis compared with cell cultures with lipoprotein-deficient serum. pa indicates postabsorptive; pp, postprandial.

ol,\(\text{13,14}\) which was suspected to alter properties of the LDL surface monolayer, thereby increasing its permeability to oxidants.\(\text{15}\) Our postprandial LDL exhibited a decreased abundance of free cholesterol compared with postabsorptive LDL. The triglyceride content of our LDL increased in the postprandial state, which was also described recently to increase the proneness of LDL to oxidative modification.\(\text{50}\) Furthermore, the lag phase of triglyceride-enriched LDL for oxidative modification\(\text{46}\) was shown to be inversely related to the severity of CAD.\(\text{50}\)

The correlation between triglyceride content and oxidizability of LDL affords several novel aspects of the atherogenic effect of hypertriglyceridemia, which is most pronounced in the postprandial state. Because plasma triglyceride levels determine the triglyceride content of LDL,\(\text{7,8}\) the hypertriglyceridemic postpran-
TABLE 4. Coincubation of Postprandial LDL With Acetyl-LDL and With Ascorbic Acid

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
<th>[*C]Oleic Acid Incorporation Into CE, nmol/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-LDL</td>
<td>100 µg/mL</td>
<td>5.6±0.9*</td>
</tr>
<tr>
<td>pp LDL</td>
<td>100 µg/mL</td>
<td>4.5±1.0</td>
</tr>
<tr>
<td>Acetyl-LDL+pp LDL</td>
<td>100+100 µg/mL</td>
<td>5.1±1.1</td>
</tr>
<tr>
<td>pa LDL</td>
<td>100 µg/mL</td>
<td>2.3±0.2*</td>
</tr>
<tr>
<td>pa LDL+ascorbic acid</td>
<td>40 µmol/mL</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>pp LDL</td>
<td>100 µg/mL</td>
<td>4.7±1.3</td>
</tr>
<tr>
<td>pp LDL+ascorbic acid</td>
<td>40 µmol/mL</td>
<td>2.2±0.6</td>
</tr>
<tr>
<td>Acetyl-LDL</td>
<td>100 µg/mL</td>
<td>5.5±1.0</td>
</tr>
<tr>
<td>Acetyl-LDL+ascorbic acid</td>
<td>40 µmol/mL</td>
<td>4.8±1.0</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; pp, postprandial; and pa, postabsorptive. Acetyl-LDL+pp LDL vs pp LDL, P<.17; acetyl-LDL+pp LDL vs acetyl-LDL, P<.7; pp LDL+ascorbic acid vs pp LDL, P<.007. *Mean±SD of three experiments performed in triplicate.

TABLE 5. Thiobarbituric Acid-Reacting Substances

<table>
<thead>
<tr>
<th></th>
<th>Post-absorptive LDL</th>
<th>Post-prandial LDL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>After lipoprotein preparation*</td>
<td>0.7±0.2</td>
<td>0.9±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>After CuSO₄ incubation†</td>
<td>4.3±0.7</td>
<td>8.0±0.3</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>2 Hours</td>
<td>16.4±0.6</td>
<td>31.8±2.3</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>8 Hours</td>
<td>27.3±1.6</td>
<td>52.0±3.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>24 Hours</td>
<td>28.0±9.1</td>
<td>55.2±9.8</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>After cell incubation†</td>
<td>4.7±0.4</td>
<td>6.2±0.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>2 Hours</td>
<td>6.3±0.7</td>
<td>12.4±0.8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>8 Hours</td>
<td>12.4±0.9</td>
<td>18.3±1.2</td>
<td>&lt;.04</td>
</tr>
<tr>
<td>24 Hours</td>
<td>20.2±3.2</td>
<td>28.4±3.9</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein. Values are expressed as nanomoles per milligram protein. Significance is shown for postabsorptive vs postprandial state. *Mean±SD of 10 experiments done in triplicate. †Mean±SD of three experiments done in triplicate.

dial state could be expected to increase the proneness of LDL to oxidative modification.

Postprandial triglycerides have been shown to constitute a risk factor for CAD and stroke. In one of these studies, it was found that a multivariate analysis was performed, postprandial but not postabsorptive triglycerides figured as independent risk factors for CAD whose strength equalled that of the most powerful fasting lipid risk factor, ie, HDL cholesterol.

As a mechanism for the atherogenicity of triglycerides, a scenario was developed in which HDL cholesterol is transferred via the action of CE transfer protein into the augmented pool of triglyceride-rich lipoproteins. According to this notion, the increased CE content in triglyceride-rich lipoproteins disturbs the lipolysis of the particles such that they accumulate, enter macrophages, and cause atherosclerosis. Indeed, CE-enriched β-VLDL and VLDL from certain hypertriglyceridemic subjects can cause, without prior modification, foam cell formation. In our scenario, HDL cholesterol is misdirected by triglyceride from its centripetal path from tissues to the liver into triglyceride-rich lipoproteins and ends up in foam cells in the arterial wall.

The increased ability of postprandial LDL to enrich macrophages with cholesterol demonstrated in the present study could provide an additional mechanism that might explain the vascular risk associated with high postprandial lipemia, ie, a possible increase in the proneness of postprandial LDL to oxidation. This mechanism could be of considerable importance because it occurs on a daily basis. Indeed, dietary antioxidant combinations were found to protect LDL from oxidation. However, further studies are required to answer the question of whether, in addition to controlling the magnitude of postprandial lipemia, the use of antioxidants could help to reduce the atherosclerotic risk.

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