Low Density Lipoprotein Subfractions and [Ca$^{2+}$]$_i$ in Vascular Smooth Muscle Cells

Burkhard Weisser, Rudolf Locher, Jacqueline de Graaf, Wilhelm Vetter

Several studies have established that plasma low density lipoprotein (LDL) consists of various discrete subfractions. Using a variety of techniques (analytical ultracentrifugation, equilibrium density gradient ultracentrifugation, and gradient gel electrophoresis), LDL has been fractionated into a maximum of seven subclasses that differ in particle size, density, and physicochemical composition. Recently, a predominance of smaller denser LDL particles has been associated with an increased risk of coronary artery disease. However, other lipoprotein changes, such as elevated triglycerides and lower HDL cholesterol levels, have been shown in patients with a predominance of the smaller denser LDL subfractions. Thus, it is unclear whether the enhanced atherogenic potential is induced by the LDL subfraction pattern per se or by concomitant lipoprotein changes. Because intracellular free Ca$^{2+}$ is an important second messenger involved in atherogenesis and regulation of vascular tone, we studied the influence of three LDL subfractions (very light [LDL$_1$], 1.030 to 1.033 g/mL; light [LDL$_2$], 1.033 to 1.040 g/mL; and dense [LDL$_3$], 1.040 to 1.045 g/mL) on [Ca$^{2+}$]$_i$ in vascular smooth muscle cells (VSMCs) cultured from rat aorta. LDL subfractions were isolated by density gradient ultracentrifugation from human EDTA-plasma (n=15). [Ca$^{2+}$]$_i$ was measured by fura 2 fluorescence. Basal [Ca$^{2+}$]$_i$ was 77±6 nmol/L. Stimulation of VSMCs with dense LDL$_3$ caused a significantly (P<.05) more pronounced increase (+71±13 nmol/L) compared with LDL$_4$ (+38±8 nmol/L) and LDL$_3$ (+36±9 nmol/L). To further investigate the mechanisms leading to the stimulation of [Ca$^{2+}$]$_i$ by LDL subfractions, we incubated VSMCs with the Ca$^{2+}$ antagonists nifedipine, diltiazem, and verapamil in concentrations up to 10 μmol/L. The LDL-induced increases in [Ca$^{2+}$]$_i$ remained unaffected. Furthermore, the inhibitor of intracellular Ca$^{2+}$ mobilization (TMB-8) did not prevent the increases in [Ca$^{2+}$]$_i$. The effects were not mediated by the LDL receptor because [Ca$^{2+}$]$_i$ was equally stimulated by LDL subfractions in LDL-receptorless fibroblasts isolated from patients with familial hypercholesterolemia. Our results indicate that the LDL subfraction with the highest density, LDL$_3$, has a more pronounced effect on [Ca$^{2+}$]$_i$ in VSMCs, which is an important second messenger involved in atherogenesis. These results suggest a possible mechanism that may contribute to the enhanced biological activity of dense LDL per se. (Circulation Research 1993;73:118-124)

**KEY WORDS** • low density lipoprotein subfractions • vascular smooth muscle cells • cytosolic Ca$^{2+}$ • atherogenesis

It is now generally accepted that low density lipoproteins (LDLs) are among the most important risk factors for coronary artery disease. However, many subjects who develop atherosclerosis have normal LDL cholesterol concentrations. It is well recognized that human LDL comprises discrete subfractions, varying in size, density, and chemical composition and that subjects with similar LDL levels may have differences in their LDL subfraction distribution. Recently, a predominance of small dense LDL subfractions has been associated with an atherogenic lipoprotein profile and an increased risk of coronary artery disease. Although the pathophysiological importance of smaller denser LDL particles in coronary artery disease has been suggested by several investigators (for a review, see Reference 6), the mechanisms by which dense LDL particles lead to an enhanced atherogenesis remain unclear. It has been shown that small dense LDL subfractions have an enhanced susceptibility to in vitro oxidation, which may contribute to the increased atherogenic potential of dense LDL per se, since oxidative modification of LDL plays an important role in the atherosclerotic process.

The epidemiological significance of LDL subfraction patterns and the differences in physical and biochemical properties between the subfractions are well investigated, but the cellular mechanisms of action of the LDL subfractions in cells of the vascular wall responsible for the postulated increased atherogenic effect of small dense LDL subfractions are unclear. In the present study, LDL from healthy volunteers was fractionated into three subfractions by density gradient ultracentrifugation (very light [LDL$_1$], 1.030 to 1.033 g/mL; light [LDL$_2$], 1.033 to 1.040 g/mL; and dense [LDL$_3$], 1.040 to 1.045 g/mL). The influence of these subfractions on [Ca$^{2+}$]$_i$ in vascular smooth muscle cells (VSMCs) was...
investigated. This parameter is an important second-
messenger system involved in many processes leading to
atherosclerosis.12 We have previously shown that both
native and oxidized LDLs are able to elevate [Ca2+]i in
VSMCs.13,14 In addition, [Ca2+]i is also involved in the
regulation of vascular tone, and serum lipids might
influence this parameter by increasing [Ca2+]i in
VSMCs.13 The present study was undertaken to further
elucidate possible cellular mechanisms of action of LDL
subfractions using cultured VSMCs isolated from rat
aortas.

Materials and Methods

Experimental Design

LDL subfractions were isolated from 15 different
plasma preparations. Blood samples were drawn from
apparently healthy volunteers (aged between 27 and 55
years) taking no medication influencing lipid metabo-
lim. Subfractions were isolated either from individual
subjects (n=6) who had three clearly detectable sub-
frations or from pools of two samples (n=9). Using
pooled plasma frequently facilitates the detection of the
different subfractions. The results obtained using indi-
vidual or pooled plasma preparations were not different
throughout the study. The blood was collected into
EDTA (1 mg/mL)–containing tubes. Plasma was iso-
lated by low-speed centrifugation, and the antioxidant
butylated hydroxytoluene (BHT, 5 μg/mL) was added.
The influence of the different LDL subfractions (n=15)
on intracellular free Ca2+ in VSMCs was tested at a
dose of 20 μg LDL protein/mL. Five dose-response
curves (5 to 20 μg LDL protein/mL) were determined.
Experiments were performed in the presence of 1 mM
extracellular Ca2+, and control experiments were per-
formed without extracellular Ca2+ (n=4). Furthermore,
the influence of the inhibitor of intracellular Ca2+
mobilization (N,N-diethyleniminioctyl-3,4,5-trimethoxy-
benzoate (TMB-8) and of the Ca2+ antagonists dilitia-
zem, verapamil, and nifedipine in concentrations up to
10 μmol/L was tested. To clarify whether the effects of
LDL subfractions on intracellular free Ca2+ were medi-
ated through the LDL receptor, we used fibroblasts
from patients with familial hypercholesterolemia with a
genetic deficiency of LDL receptors.15

Materials

Fura 2/pentaacetoxymethyl ester (fura 2-AM) was
purchased from Calbiochem, Zurich, Switzerland. Dul-
becco's modified Eagle’s medium (DMEM) and Dul-
becco's phosphate-buffered saline (PBS) were obtained
from Amimed, Basel, Switzerland. EDTA was bought at
Merck-Schuchardt, Zurich, Switzerland. Antidimesin
antibody and anti-mouse immunoglobulin G–fluores-
cine isothiocyanate antibody were purchased at Boe-
ringer Mannheim, Rotkreuz, Switzerland. All other
chemicals were obtained from Sigma Chemical, Zurich,
Switzerland. Fibroblasts with and without LDL recep-
tors were purchased at Corell Institute, Camden, NJ.

Isolation of LDL Subfractions

For the isolation of LDL subfractions, density gradi-
ent ultracentrifugation was used as previously de-
scribed.310 LDL subfractions were prepared immedi-
ately after blood collection. EDTA (1 mg/mL) and BHT
(5 μg/mL, previously dissolved in absolute ethanol)
were added to the plasma. It has been shown that LDL
isolated in the presence of either EDTA or BHT contains
no detectable oxidation products, such as thiobarbituric acid reactive substances.16

From each plasma preparation, 3.0 mL was pipetted
into six polycarbonate centrifuge tubes. The density of
the plasma was adjusted to 1.10 g/mL by adding 0.42 g
KBr to each tube. The plasma in one of the tubes was
stained with 20 μL freshly prepared 15 g/L aqueous
solution of Coomassie brilliant blue R-250 as a refer-
ence for the position of the LDL subfractions. After
the dissolution of KBr, the plasma was then slowly overlay-
ered by four solutions of decreasing density also con-
taining the antioxidants (2 mL of 1.065 g/mL solution, 3
mL of 1.035 g/mL solution, 3 mL of 1.020 g/mL solution,
and 1.5 mL of 1.006 g/mL solution). For optimal stain-
ing, the density solutions used in the reference tube
were adjusted to pH 4.5 to 5.0 with 1 M HCl. The tubes
were then centrifuged at 37 000 rpm (160 000g) at 20°C
for 19 hours using a rotor (model TST 4114, Beckman
Instruments, Inc, Fullertoon, Calif) in an ultracentrifuge
(model T 2050, Kontron, Zurich, Switzerland). After
ultracentrifugation (low acceleration rate and no use of
brakes), the LDL subfractions (LDL1, 1.030 to 1.033
g/mL; LDL2, 1.033 to 1.040 g/mL; LDL3, 1.040 to 1.045
g/mL) could be detected as distinct bands in the middle
of the tubes. The bands could be seen best in the refer-
ce tube that was placed in the middle of the rack
when the subfractions were sequentially isolated by
aspiration with a rubber bulb pipette from the tubes to
which no stain had been added.

LDL subfractions were then separately dialyzed at
4°C for 48 hours against three changes of 1.5 L of 0.15
M NaCl and 1 mM EDTA at pH 7.4. After dialysis, LDL
subfractions were concentrated to a concentration of 3
to 4 mg LDL protein/mL by using membrane separation
(Cenitrasart 1 tubes, Sartorius, Göttingen, Germany)
with a cutoff at a molecular mass of 20 000 D.

Determination of LDL protein was performed using
Lowry’s method.17 Concentrations of LDL are reported
as micrograms protein per milliliter throughout this
study. LDL was sterilized by the use of 0.45-μm filters
(Millipore Corp, Bedford, Mass) and stored in the dark
at 4°C for a maximum of 1 week before the experiments
were performed.

Measurement of Oxidation of LDL Subfractions

Oxidation of LDL subfractions was measured by
determination of absorption at a wavelength of 234 nm
as described previously.10 Absorption increases if LDL
is oxidized, indicating an increase in diene formation of
fatty acids.10 This was done because the higher biologi-
cal activity of oxidized lipids is known,13,14 and the aim
of the study was to determine the potential to increase
[Ca2+]i in VSMCs of native LDL subfractions indepen-
dent of their state of oxidation.

Vascular Smooth Muscle Cells

VSMCs were cultured from rat aorta (female, Wistar-
Kyoto strain, 6 to 8 weeks old) and grown over several
passages according to the method of Ross.18 Smooth
muscle cells were identified according to morphological
criteria18 and immunologically with an antidimesin anti-
body plus a second fluorescent anti–immunoglobulin G
antibody. The cells were allowed to grow for 4 or 5 days in 5% CO2–95% air at 37°C. Cells were cultured in DMEM supplemented with 2 mM L-glutamine, 50 U/mL penicillin-streptomycin, 1% nonessential amino acids, and 10% fetal calf serum. VSMCs were used after 5 to 10 passages. Confluent cells were detached by trypsinization (0.05% trypsin) and subsequently suspended in PBS supplemented with 1% bovine serum albumin. After detachment, cells were washed twice and suspended in PBS (approximately 2×106 cells/mL) and incubated with 2 μM fura 2-AM at 37°C for 20 minutes as previously reported.13,14 Time provided for deesterification of fura 2-AM was 60 minutes.

**Measurement of [Ca2+]i**

[Ca2+]i was measured as previously described.13,14,20 Briefly, after loading the cells with 2 μM fura 2-AM and 60 minutes for deesterification, 1-mL aliquots of the cell suspensions were spun down at 100g and the supernatant was removed. Cells were resuspended in PBS buffer immediately before use. Fluorescence of cells was measured at 37°C under stirring in a cuvette placed in an SLM-Amino SPF-500C spectrofluorometer (SLM Instruments Inc, Urbana, Ill). This fluorometer allows computer-sustained rapid alterations of excitation wavelength. For determination of intracellular free Ca2+, excitation wavelengths of 340 and 380 nm and an emission wavelength of 505 nm were chosen. Excitation bandwidth was set at 7.5 nm, and the emission bandwidth was set at 5 nm. Fluorescence was corrected for cellular autofluorescence (<12%). Fluorescence signals were calibrated using 0.5% Triton X-100 for maximum, followed by the addition of 1.0 M Tris buffer and 300 mM EGTA, pH 8.8, for minimum fluorescence. More than 85% of total fluorescence (after cell lysis by Triton) was quenchable by Mn2+. Computer-assisted calculation of the ratio of the emitted fluorescence signals permits calculation of [Ca2+]i. According to Grynkiewicz et al.,20 the dissociation constant (Kd) between fura 2 and Ca2+ was assumed to be 224 nM. However, as with every other Kd, absolute Ca2+ levels should be interpreted with caution. Therefore, results in this study are mainly expressed as the percentage of relative increases in [Ca2+]i. All data reported throughout the study are peak Ca2+ levels of the transients obtained after stimulation of VSMCs.

**Statistical Analysis**

Values are given as mean±SEM. The Wilcoxon paired rank test was used for statistical comparison. Unless otherwise stated, differences were considered to be of statistical significance at P<.05.

**Results**

**LDL Subfraction Pattern**

With the methods used in this study, three LDL subfractions were observed. They were isolated by aspiration only if they were separated by a clear interface and were used for measurement of oxidation and the stimulation of the VSMCs.

**Oxidation of LDL Subfractions**

Mean absorbances at a 234-nm wavelength of 0.25 mg/mL LDL protein aqueous solution were 0.546±0.145 for LDL1, 0.479±0.142 for LDL2, and 0.459±0.152 for LDL3 (mean±SD), indicating no significant difference in oxidation between the three subfractions. These values are similar to the absorption obtained with nonoxidized whole LDL preparations in a previous study from our laboratory.14 Thus, if LDL subfractions are prepared as described in “Materials and Methods,” differences in oxidation between the LDL subfractions are not responsible for possible differences in cellular effects of these subfractions in vitro.

**Stimulation of Intracellular Ca2+ by LDL Subfractions**

In Fig 1, representative original tracings of intracellular Ca2+ as assessed by the fura 2 method are shown. In this figure, [Ca2+]i values induced by LDL subfractions (20 μg protein/mL) are compared with a Ca2+-transient induced by angiotensin II (100 nmol/L). Angiotensin II induced an increase in [Ca2+]i from 85 to 197 nmol/L as opposed to smaller increases seen after the addition of LDL subfractions (from 84 to 111 nmol/L for LDL1, from 92 to 121 nmol/L for LDL2, and from 89 to 171 nmol/L for LDL3). In the experiments shown in Fig 1, peak [Ca2+]i was reached at approximately 20 seconds after LDL subfractions (20 μg/mL) were added to the VSMC suspensions.

In Fig 2, the effect of the three LDL subfractions on [Ca2+]i in VSMCs (mean±SEM, n=15) is illustrated. LDL1 induced the most pronounced elevation in [Ca2+]i. Mean relative increase in [Ca2+]i was significantly higher than the values obtained after stimulation with the lighter subfractions, whereas there was no significant difference between LDL1 and LDL2.

**Dose-Response Relation**

Increasing doses of LDL subfractions were added to VSMCs. The starting dose was 5 μg protein/mL. There-
after, doses of 10 and 20 μg/mL were used (n=5). A significantly (P<.05) more pronounced elevation of intracellular Ca\(^{2+}\) was induced by the higher doses of LDL₁, as compared with the other LDL fractions. The dose-response curves are shown in Fig 3. Data are given as percent change in [Ca\(^{2+}\)]. The configuration of both curves is similar. After an initial sharp increase, higher concentrations of LDL fractions did not produce substantial further Ca\(^{2+}\) elevations. Even with higher doses of LDL₁ and LDL₂, the increase in [Ca\(^{2+}\)], did not reach the levels obtained with LDL₃.

Influence of Ca\(^{2+}\) Antagonists and TMB-8

Different concentrations of the Ca\(^{2+}\) antagonists diltiazem, verapamil, and nifedipine (0.1, 1, and 10 μmol/L, respectively) were tested on their ability to prevent the LDL subtraction-induced increase in [Ca\(^{2+}\)]. Neither addition of Ca\(^{2+}\) antagonists immediately before the stimulation of VSMCs nor incubation of the muscle cell suspensions for up to 1 hour with these concentrations changed the increases in [Ca\(^{2+}\)], by either one of the subtraction. In Table 1, it is demonstrated that increases in [Ca\(^{2+}\)] induced by LDL subtraction were not altered by the Ca\(^{2+}\) antagonists (only the highest concentration of Ca\(^{2+}\) antagonists is shown). In contrast, increases in intracellular free Ca\(^{2+}\) induced by angiotensin II (100 nM) and thrombin (1 U/mL) were inhibited by nifedipine, diltiazem, and verapamil at doses of 1 μM. Fig 4 illustrates that addition of the intracellular Ca\(^{2+}\) blocker TMB-8 (blockade of sarcoplasmatic reticulum Ca\(^{2+}\) release) did not alter the increases in [Ca\(^{2+}\)], induced by LDL subtraction, whereas TMB-8 reduced angiotensin II–induced changes by 32%.

Extracellular Ca\(^{2+}\)

Control experiments were performed in Ca\(^{2+}\)-free buffer. VSMCs were washed twice in PBS buffer with-

![Graph showing mean relative increase in [Ca\(^{2+}\)] after exposure of vascular smooth muscle cell suspensions to low density lipoprotein (LDL) subfractions.](#)

**TABLE 1. Influence of the Ca\(^{2+}\) Antagonists Nifedipine, Verapamil, and Diltiazem on Increases in [Ca\(^{2+}\)], in Vascular Smooth Muscle Cells Obtained by Stimulation With Low Density Lipoprotein Subfractions**

<table>
<thead>
<tr>
<th>LDL Subfractions</th>
<th>Relative [Ca(^{2+})] increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nifedipine (10 μM)</td>
</tr>
<tr>
<td>LDL₁</td>
<td>65±5</td>
</tr>
<tr>
<td>LDL₂</td>
<td>71±6</td>
</tr>
<tr>
<td>LDL₃</td>
<td>105±6</td>
</tr>
</tbody>
</table>

LDL₁, very light low density lipoprotein (LDL₁); LDL₂, light LDL; LDL₃, dense LDL. Values are mean±SEM (n=4).

LDL subfractions are 20 μg protein/mL.

![Graph showing mean relative increase in [Ca\(^{2+}\)] after exposure of vascular smooth muscle cell suspensions to low density lipoprotein (LDL) subfractions.](#)
TABLE 2. [Ca\(^{2+}\)] in Vascular Smooth Muscle Cells Before and After Stimulation With Low Density Lipoprotein Subfractions and Angiotensin II in the Presence and Absence of Extracellular Ca\(^{2+}\)

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>1 mM Ca(^{2+}) _</th>
<th>1 mM EGTA without Ca(^{2+}) _</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>67±6 nM</td>
<td>55±4 nM</td>
</tr>
<tr>
<td>LDL(_1)</td>
<td>95±9 nM</td>
<td>60±6 nM</td>
</tr>
<tr>
<td>LDL(_2)</td>
<td>99±10 nM</td>
<td>60±7 nM</td>
</tr>
<tr>
<td>LDL(_3)</td>
<td>137±11 nM</td>
<td>61±8 nM</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>198±13 nM</td>
<td>112±11 nM</td>
</tr>
</tbody>
</table>

LDL\(_1\), very low density lipoprotein (LDL); LDL\(_2\), light LDL; LDL\(_3\), dense LDL. Values are mean±SEM for maximum increases (n=5).

Vascular smooth muscle cells were loaded with fura 2. Cells were stimulated at 37°C with 20 μg LDL protein/mL. The time course of the increase in [Ca\(^{2+}\)] is shown in Fig 1.

out Ca\(^{2+}\), and experiments were performed without external Ca\(^{2+}\) and in the presence of 1 mM EGTA. As shown in Table 2, basal [Ca\(^{2+}\)] was slightly reduced, and the angiotensin II–induced rise in [Ca\(^{2+}\)] was smaller than that obtained in the presence of Ca\(^{2+}\) in the buffer. Whereas there was still a substantial rise in [Ca\(^{2+}\)], after angiotensin II, increases induced by LDL subfractions were almost completely blunted in the Ca\(^{2+}\)-free buffer (Table 2). Thus, the increases in [Ca\(^{2+}\)] in VSMCs induced by LDL subfractions appear to be dependent on extracellular Ca\(^{2+}\).

**Fibroblasts With and Without LDL Receptor**

Increases in [Ca\(^{2+}\)] induced by LDL subfractions in cultured fibroblasts were comparable with those seen in VSMCs. There was no difference between normal fibroblasts and fibroblasts without LDL receptor from patients with familial hypercholesterolemia (Fig 5). The results obtained with LDL are shown; the other subfractions yielded the same pattern.

**Discussion**

Previous studies have shown that LDLs contain a varying number of subfractions in human plasma. These subfractions differ not only in their chemical composition, molecular weight, and cholesterol to protein ratio but might also have a different biological activity and atherogenic potential. These different LDL subfractions can be isolated by different techniques, such as density gradient ultracentrifugation and gradient gel electrophoresis. Epidemiological studies using these techniques have shown a close association between the smaller denser LDL subfraction and risk of myocardial infarction. The reason for this association remains unknown. There is evidence for genetic influences on the LDL subfraction patterns, as recently demonstrated in large studies. In the literature, the term subclass pattern B is used for the prevalence of small LDL subfractions obtained by gradient gel electrophoresis. By use of density gradient ultracentrifugation as in the present study, LDL\(_3\) is defined by its density, but it has been shown that there is a strong correlation between LDL particle size and density. Although there is convincing evidence concerning a correlation between LDL subclass pattern B (increased levels of the small LDL subfraction) and an increased cardiovascular risk, this concept has been questioned by findings on concomitant lipoprotein changes in individuals with LDL subclass pattern B. Increased serum triglyceride levels and reduced HDL concentrations have been demonstrated in pattern B, and statistical adjustment for triglyceride concentration decreased the risk ratio of LDL subclass pattern B to a nonsignificant level.

Thus, there is still controversy over whether there is a causal relation between increased levels of LDL\(_3\) and higher cardiovascular risk. Several mechanisms have been proposed for the atherogenic potential of LDL\(_3\). The differences in the cholesterol to protein ratio and content of saturated cholesteryl esters might produce changes in LDL-particle surface conformation and affect its intravascular metabolism and interactions with the artery wall.

Another proposed difference between the subfractions might be the decreased carbohydrate content of the heavier LDL subfractions. Again, it has been speculated that these alterations might influence the metabolic fate of LDL particles. In addition, differences in glycosylation seem to influence the sialic acid content of LDL and thereby alter the binding of LDL to tissue proteoglycans. This process has been suggested to be involved in atherogenesis.

Furthermore, the dense LDL subfraction has been shown to be more susceptible to oxidative modification in vitro. This could be of pathological importance since oxidative modification of LDL is a key event in atherogenesis. Increased oxidation of LDL\(_3\) might result in excessive uptake by the scavenger receptor of macrophages, thereby converting them into foam cells, an important step in atherogenesis.

However, these proposed mechanisms are only indirect explanations for an increased activity of LDL\(_3\) in processes leading to atherosclerosis. Only few data exist on direct effects of LDL subfractions on a cellular level. Differences in metabolism of LDL subfractions in human hepatoma cells have been demonstrated, but data on the effects of LDL subfractions on cells of the vascular wall are scarce.

![Graph showing mean relative increases in [Ca\(^{2+}\)] in percent of baseline value after exposure of fibroblasts with and without low density lipoprotein (LDL) receptor to dense LDL. (LDL 3). Percent mean±SEM values of four experiments are shown.](image_url)
Because atherogenesis and atherosclerosis have been linked to changes in $[\text{Ca}^{2+}]$, we investigated the influence of LDL subfractions on $[\text{Ca}^{2+}]$ in VSMCs. Several processes involved in atherogenesis, such as membrane permeability, secretion of extracellular matrix proteins, cell migration, and cellular damage, among others, are regulated by changes in $[\text{Ca}^{2+}]$. Thus, not only end-stage atherosclerotic disease, such as calcifications of plaques and necrosis, but also the initial steps leading to vascular damage are associated with alterations of intracellular Ca$^{2+}$ metabolism.

Our results indicate that LDL$_1$ has an increased potential to raise $[\text{Ca}^{2+}]$, compared with the lighter subfractions. These findings demonstrate that the subfraction pattern might have a direct effect, at least in vitro, on cells of the vascular wall, which is independent of the concomitant changes in lipoprotein profile mentioned above. It is not known what concentration of LDL is likely to exist in the neighborhood of the smooth muscle cells in the arterial wall, but our results were obtained with concentrations substantially lower than those physiologically occurring in serum (85 to 130 mg per 100 mL). Under physiological conditions, lipoproteins are transported through the endothelial layer by transcytosis and come into direct contact with VSMCs. Under pathophysiological conditions, there is a migration of VSMCs through the internal elastic lamina to form a neointima. Thus, there is the possibility of a direct exposure of VSMCs to LDL both in normal blood vessels and in atherosclerotic lesions, and the involvement of this process in atherogenesis is likely.

Although Ca$^{2+}$-chelating agents and Ca$^{2+}$ antagonists have been shown to have an antiatherogenic potential in animal models, our study revealed no effect of the major Ca$^{2+}$ antagonists on increases in $[\text{Ca}^{2+}]$. Thus, Ca$^{2+}$ antagonist–sensitive channels do not seem to be involved in the effects seen in the present study. One can only speculate about the mechanisms responsible for the increases in $[\text{Ca}^{2+}]$. Since intracellular Ca$^{2+}$ blocker TM-B did not alter the increase in $[\text{Ca}^{2+}]$, the elevations in $[\text{Ca}^{2+}]$ seem to be caused by an influx of calcium ions from the extracellular space. This is confirmed by the results obtained in the absence of external Ca$^{2+}$.

In addition, the LDL receptor does not mediate the effects of LDL subfractions on $[\text{Ca}^{2+}]$, because the same results were obtained with fibroblasts without LDL receptors isolated from patients with familial hypercholesterolemia.

In conclusion, an increased biological activity of the LDL$_1$ subfraction was demonstrated. A direct action of LDL subfractions on cells of the vascular wall was shown. Based on the assumption that $[\text{Ca}^{2+}]$ seems to be a second messenger involved in many processes in atherogenesis, these results may indicate that LDL$_1$ per se has a higher atherogenic potential than the other subfractions. Therefore, the higher incidence in coronary artery disease among individuals with a small dense LDL subclass pattern might be the result not only of concomitant changes in the lipoprotein profile such as hypertriglyceridemia and decreased high density lipoprotein cholesterol levels but also of an enhanced direct atherogenic action of LDL$_1$. Our results may thus contribute to the explanation of the increased risk of coronary artery disease among subjects with a small dense LDL subfraction profile.

References


Low density lipoprotein subfractions and \([Ca^2+]_i\) in vascular smooth muscle cells.

B Weisser, R Locher, J de Graaf and W Vetter

_Circ Res._ 1993;73:118-124
doi: 10.1161/01.RES.73.1.118

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/73/1/118