Preparation of apoE-free rat low density lipoprotein for catabolic studies

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Summary The use of serum from rat fetuses instead of serum from adult rats for preparation of LDL by preparative ultracentrifugation leads to an LDL fraction containing apoB-100 and apoB-95 as the only protein moieties without need for any further purification. The yield of LDL is five times greater compared to the use of adult rat serum. Lipid composition and particle size of LDL from fetal and adult rats are quite similar. The method described allows a simple way for preparation of sufficient amounts of apoE-free LDL for use in metabolic studies. — Schlag, B., L. Winkler, D. Plonneé, U. Dürer, and R. Dargel. Preparation of apoE-free rat low density lipoprotein for catabolic studies. J. Lipid Res. 1987. 28: 1521-1524.

Supplementary key words serum • fetal rat • preparation • ultracentrifugation • apoB

The use of rat LDL in studies of receptor-mediated catabolic pathways meets with many difficulties. These arise not only from the very small amount of LDL in serum of the adult rat but also from the fact that rat LDL obtained by ultracentrifugation is essentially contaminated with HDL$_1$ (1). Therefore, additional purification steps are necessary in order to remove the concomitant apoE-containing HDL species (2, 3). The aforementioned difficulties can be overcome by using serum from fetal rats instead of serum from adult animals for preparation of LDL. The present study demonstrates that LDL prepared by the commonly used flotation procedure represents an apoE-free particle fraction that can be obtained in sufficient amounts for metabolic studies.

MATERIALS AND METHODS

Fetal Wistar rats were delivered by cesarian section on day 22 of gestation. After an incision in the throat, the
carotid vessels were cut through and fetal blood was collected by glass capillaries and pooled. Approximately 1.5 ml of serum was obtained from the fetuses of one dam (10 fetuses on average).

LDL was prepared by means of ultracentrifugation (4) using only the density range of 1.020-1.050 g/ml. The crude fractions were centrifuged twice at a density of 1.050 g/ml followed by dialysis against 10 mM ammonium bicarbonate (pH 7.4).

The following methods were included in the characterization of the prepared LDL. Determination of protein content was according to the method of Lowry et al. (5) with bovine serum albumin as standard and inclusion of SDS to avoid turbidity. Electrophoretic separation of apoB variants in 3.75% PAG in the presence of SDS was performed as reported by Wu and Windmueller (6). Isoelectric focusing of apolipoproteins after delipidization by acetone-ethanol treatment was as described by Warnick et al. (7) and by Yamamura et al. (8). Lipid composition was determined as previously described (9). Negative staining of LDL particles for ascertainment of the size distribution pattern was according to Forte et al. (10).

RESULTS AND DISCUSSION

Measurement of the distribution of apoB in several density fractions between 1.020 and 1.060 g/ml showed that the bulk of LDL from serum of rat fetuses floated at the density range of 1.020-1.050 g/ml, which corresponds to the flotation behavior of LDL from adult rats. Using the preparation procedure described above, 3 mg of LDL protein could be obtained from the fetuses of five dams. The size distribution patterns and main particle diameters

![Fig. 1. Negatively stained LDL particles (d 1.020-1.050 g/ml) from serum of fetal (a) and adult (b) rats (bar > 0.1 μm) and the corresponding size distribution patterns.](image)

**TABLE 1. Composition and concentration of the LDL fraction from serum of fetal (day 22 of gestation) and of adult Wistar rats**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Fetal LDL (n = 13)</th>
<th>Adult LDL (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Total LDL content</td>
<td>156 ± 23</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>9.1 ± 1.5*</td>
<td>6 ± 3.3*</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>16.7 ± 1.7</td>
<td>20.6 ± 6.5</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>19.2 ± 4.7*</td>
<td>14.9 ± 5.3*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>28.5 ± 2.8</td>
<td>26.2 ± 7.3</td>
</tr>
<tr>
<td>Protein</td>
<td>26.7 ± 6.1*</td>
<td>32.6 ± 8.6*</td>
</tr>
</tbody>
</table>

*A Data obtained for fetal and adult rat LDL were compared statistically by using Student's t-test. The composition is given as percentage values. Results are means ± SD.
*Significant difference (P < 0.01) between fetal and adult rat LDL.
*Significant difference (P < 0.05) between fetal and adult rat LDL.

(20-23 nm) evaluated by morphometric analysis of negatively stained LDL fractions were essentially the same for LDL isolated either from blood of fetal or from blood of adult rats (Fig. 1). Furthermore, LDL from fetuses exhibited a composition quite similar to that of LDL from adults. However, in the fetuses the serum concentration of LDL was five times higher than in adult animals (Table 1).

The apoB components of LDL from adult and fetal rats were separated in 3.75% PAG (Fig. 2). ApoB of LDL from adults consisted of 85% of the two high molecular weight species (B-100 and B-95) and 15% of the low molecular weight (B-48) subspecies, whereas only the high molecular forms were detectable in fetal LDL. In 10% gels, no other protein components were present in fetal LDL. In contrast, LDL from adult rats showed some protein bands within the apoE region, as well as traces of apoA-I and apoC (Fig. 3). This indicates a contamination of adult LDL with HDL₁. The lack of apoE in LDL from...
fetal rat serum is in contrast to the findings with respect to LDL from either newborn humans (12, 13) or piglets (14).

Since fetal LDL from rat serum is free of apoE even after the first flotation, the data presented here do not seem to be an artificial finding caused by its loss during prolonged ultracentrifugation. ApoE was found, however, in the d < 1.020 g/ml and d 1.050–1.064 g/ml fractions, the latter containing only traces of apoE but marked amounts of apoA-I. The d < 1.020 g/ml fraction from fetal rat serum had a large amount of apoE, which is in accordance with studies of other species.

Studies on LDL catabolism and, in particular, studies of the apoB,E receptor-mediated pathway require highly purified LDL. This necessitates LDL particles with only the specific apoB moiety and completely free of apoE-containing subspecies. These requirements are fulfilled by LDL isolated from fetal rat serum using the usual flotation techniques. The lack of the low molecular weight variant of apoB in fetal LDL may be an additional advantage. Furthermore, the high content of LDL in fetal blood favors the possibility of obtaining sufficient amounts of uncontaminated rat LDL in this simple way so that binding experiments, including competition studies with an excess of unlabeled LDL, can be performed easily (15).

Fig. 2. SDS-PAG electrophoresis (0.2% SDS, 3.75% polyacrylamide) of apoB variants from adult (a) and fetal (b) rat LDL; load: 40 µg of LDL protein. Designations are according to Kane et al. (12).

Fig. 3. Apolipoprotein pattern of lipoprotein particles from fetal and adult rats obtained by isoelectric focusing (pH 3.5–10.0); a) fetal rat, d < 1.020 g/ml; b) fetal rat, d 1.020–1.050 g/ml; c) fetal rat, d 1.050–1.064 g/ml; d) fetal rat, d 1.064–1.21 g/ml; e) adult rat, d 1.020–1.064 g/ml. Amounts of protein for delipidization: 700 µg (b, e), 300 µg (c), 100 µg (a, d). The total urea-soluble material was loaded.

REFERENCES