Hyperlipoproteinemia in Nagase analbuminemic rats: effects of pravastatin on plasma (apo)lipoproteins and lecithin:cholesterol acyltransferase activity


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Abstract The present study demonstrates very high levels of plasma lipids and high density lipoprotein (HDL) apolipoproteins (apoA-I and apoE) in female Nagase analbuminemic rats (NAR) fed a semi-synthetic diet in order to further increase the hyperlipidemia present in this strain. Plasma apoB-containing lipoproteins (very low, intermediate, and low density lipoproteins) were also elevated in NAR. Plasma cholesterol was mainly present in lipoprotein particles with a density between 1.02 and 1.12 g/ml. Separation of lipoprotein classes by gel filtration showed that the major cholesterol-carrying lipoprotein fractions in NAR plasma are apoE-rich HDL and apoA-I-rich HDL. The high HDL levels in NAR are explained, at least partly, by the two- to threefold elevated activity of plasma lecithin:cholesterol acyltransferase (LCAT). The lysophosphatidylcholine generated in the LCAT reaction, as well as plasma free fatty acids, are bound to lipoproteins in NAR plasma. A study was carried out to determine whether the elevated LDL and apoE-rich HDL levels could be corrected by administration of the HMG-CoA reductase inhibitor pravastatin (at a dose of 1 mg/kg per day). Pravastatin treatment results in a 43% decrease in plasma triglycerides in NAR, but not in Sprague-Dawley (SDR) rats, and had no significant effect on plasma total cholesterol, phospholipids, apolipoproteins A-I, A-IV, B, or E, as well as on plasma LCAT activity levels in NAR or SDR.

Hyperlipidemia in the Nagase analbuminemic rat (NAR) (1, 2) is characterized by increased plasma levels of both cholesterol and triglycerides, the latter being mostly present in very low density lipoproteins (VLDL) (3). High density lipoproteins (HDL), including an apoE-rich subfraction, are the major cholesterol-carrying lipoproteins in normal rats, as well as in NAR (4–6).

Lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) is a key enzyme for the esterification of cholesterol in plasma (7–12). As rats have no measurable plasma cholesteryl ester transfer protein activity, LCAT is a very important determinant for plasma HDL-cholesterol concentrations in this species (9, 12). It was reported that the plasma cholesterol esterification rate by LCAT is decreased in patients with hypoalbuminemia and hyperlipidemia due to the nephrotic syndrome (13, 14). This coincides with the low levels of plasma HDL often found in this disease (15). However, two siblings with analbuminemia demonstrated increases in HDL3, as well as LCAT activity (16). In order to find out whether analbuminemia per se has specific effects on HDL and LCAT, we measured HDL subfractions, LCAT activity levels, and rates of cholesterol esterification in NAR plasma.

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis, have been given to hypoalbuminemic patients with the nephrotic syndrome. This treatment resulted in decreases in plasma total cholesterol, LDL-cholesterol and apoB, as well as in small increases in HDL-cholesterol and plasma apoA-I (17, 18). Administration of an HMG-CoA reductase inhibitor to patients

Supplementary key words analbuminemia • apoA-I • apoA-IV • apoB • apoE • cholesterol • free fatty acids • HMG-CoA reductase inhibitor • lysophatidylcholine • triglycerides
with familial hypercholesterolemia caused a twofold increase in LCAT activity (19).

As we found that the female NAR, fed a semi-synthetic diet, is one of the most severe hypercholesterolemic rat models available (mean plasma cholesterol level of 10 mM), we tested the effect of a reductase inhibitor on the hypercholesterolemia and the LCAT reaction in NAR. Pravastatin was chosen because it is water-soluble; it was administered by a subcutaneously implanted osmotic pump.

METHODS

Animals

Adult female NAR from our own colony (which was founded with animals generously donated by Dr. S. Nagase, Tokyo, The Netherlands) and adult female SDR (Harlan-CPB, Zeist, The Netherlands) were housed three to a cage under conventional conditions. The animals were fed a semi-synthetic diet (Hope Farms, Woerden, The Netherlands) containing 20% casein-protein, 10% corn starch, 5% cellulose, 53% glucose monohydrate, and 5% soybean oil (by weight). The remaining 7% consisted of salts, vitamins, and trace elements. Six animals of each strain were treated with pravastatin, dissolved in isotonic saline at a concentration of 4 mg/ml. The pravastatin solution was administered using a subcutaneously implanted osmotic pump (Alzet®, 2ML4; volume ~2 ml). These pumps, which administer their contents at a constant rate over 28 days, delivered a dose of 1 mg pravastatin/kg per day. The untreated rats (six of each strain) had osmotic pumps containing isotonic saline only. Rats treated with pravastatin or saline did not differ in body weight. On the 28th day after pump implantation, the animals were weighed in the fed state (body weights were between 250 and 300 g) and subsequently exsanguinated by puncture of the abdominal aorta under fentanyl-diazepam anesthesia. Blood was collected in chilled K-EDTA-coated tubes and immediately centrifuged at 4°C for 10 min at 1000 g. The plasma was pooled into two samples per subgroup. Each pooled sample was derived from equal amounts of plasma obtained from three rats. Plasma lipoproteins in this pooled sample were separated by density gradient ultracentrifugation (25) into six fractions (very low density lipoprotein, VLDL, d < 1.006 g/ml; intermediate density lipoprotein, IDL, d 1.006-1.019 g/ml; low density lipoprotein, LDL, d 1.019-1.063 g/ml; high density lipoprotein 2; HDL2, d 1.063-1.125 g/ml; HDL3, d 1.125-1.21 g/ml; and an infranatant with d > 1.21 g/ml). Lipoprotein TC, TG, and PL were measured as described above. Lipoprotein protein concentration was measured colorimetrically with a kit from Bio-Rad Laboratories (Munich, Germany).

Lipid and apolipoprotein analyses

Enzymatic methods were used for the determination of total plasma cholesterol (TC) and triglycerides (TG). The kits were obtained from Boehringer GmbH (Mannheim, Germany). Unesterified cholesterol (UC) and phospholipids (PL) were also assayed enzymatically, using kits from Wako Chemicals GmbH (Neuss, Germany). Cholesteryl esters (CE) were calculated as the difference between TC and UC. Free fatty acids (FFA) were assayed in plasma and isolated lipoprotein fractions using a kit from Wako Chemicals GmbH. Plasma apolipoproteins A-I, A-IV, and E were measured by electro-immunoassay as described previously (20). Plasma apoB was determined by radial immunodiffusion as described (21), using a specific antiserum raised in rabbits against purified rat LDL (22). Plasma apoB concentrations, expressed in arbitrary units (A.U.), were calculated as percentages of a rat serum standard pool (obtained from 50 rats) run simultaneously on the plates with the plasma samples. Six different dilutions of the serum standard pool were run on each plate. All samples were run in triplicate. The standard pool was stored at −80°C in batches of 0.25 ml. The data are expressed in A.U. because of the insolubility of purified apoB and the lack of sufficient quantities of pure apoB for use as an absolute standard. The within-day and between-day coefficients of variation of the apoB assay were 2% and 6%, respectively. Plasma levels as low as 5 A.U. could be detected.

LCAT activity assays

Plasma levels of LCAT activity were measured using excess exogenous substrate, as described (23). In addition, the rate of cholesterol esterification was determined with endogenous substrates by incubating total plasma and assaying the decrease in UC at 37°C, as described (24). The rate was constant during the first hour of incubation. This rate may be given as the absolute rate (in nmol/ml plasma per h), or as the fractional esterification rate (% of plasma UC converted to CE/h).

Lipoprotein isolation by density gradient ultracentrifugation

The plasma was pooled into two samples per subgroup. Each pooled sample was derived from equal amounts of plasma obtained from three rats. Plasma lipoproteins in this pooled sample were separated by density gradient ultracentrifugation (25) into six fractions (very low density lipoprotein, VLDL, d < 1.006 g/ml; intermediate density lipoprotein, IDL, d 1.006-1.019 g/ml; low density lipoprotein, LDL, d 1.019-1.063 g/ml; high density lipoprotein 2; HDL2, d 1.063-1.125 g/ml; HDL3, d 1.125-1.21 g/ml; and an infranatant with d > 1.21 g/ml). Lipoprotein TC, TG, and PL were measured as described above. Lipoprotein protein concentration was measured colorimetrically with a kit from Bio-Rad Laboratories (Munich, Germany).

Apolipoprotein composition of total plasma and of each lipoprotein fraction was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS), as described previously and evaluated with an image analysis system (26). The polyacrylamide gradient gels consisted of a linear gradient of acrylamide from 4 to 10% and a constant 2.6% N,N'-bis-methylene-acrylamide. Standardization was performed using a mix-
ture of two commercial preparations (Bio-Rad, Richmond, CA, and Serva, Heidelberg, Germany) containing proteins with the following molecular masses: 6.5, 12.5, 21.0, 29.0, 45.0, 66.2, 92.5, 116, and 200 kDa. The unknown proteins were characterized by their molecular masses using linear extrapolation between the two next standard proteins in the gel. Plasma albumin was determined immunoturbimetrically using rabbit anti-rat albumin obtained from Nordic (Tilburg, The Netherlands). As a reference we used rat albumin (Fraction V, Sigma, St. Louis, MO). The detection limit of this assay is 0.05 g/ml.

**Lipoprotein separation by gel filtration**

Plasma pools from either SDR or NAR (three to six animals/group) were analyzed by gel filtration using an FPLC system (27), with some modifications. A Superose 6 and a Superose 12 prep-grade column (2 x 100 ml, Pharmacia, Uppsala, Sweden) were used in sequence and 2-ml plasma samples were applied. The lipoproteins were eluted at a flow of 0.12 ml/min (during 1000 min). Subsequently, the non-lipoprotein components were washed from the columns at a flow of 1.33 ml/min (90 min). Lipoprotein collection started after 500 min in 30 fractions of 2.0 ml each. The elution buffer was 150 mM NaCl, containing 2 mM Na-phosphate, 5 mM EDTA, and 0.02% NaN3, (pH 7.4). In some experiments, with special emphasis on lipoprotein-deficient plasma fractions, 36 fractions of 2.4 ml each were collected. The fractions were analyzed for TG, TC, PL, FFA, and apolipoproteins A-I, B, and E. Average recoveries were 88% (TG, n=2), 101% (E, n=2), 98% (PL, n=3), 86% (apoA-I, n=2), 101% (apoB, n=2), 74% (apoE, n=2), and 129% (FFA, n=2). The FFA levels measured in the isolated fractions were not corrected for turbidity occurring in the assay of the void volume fractions (chylomicrons/VLDL); this resulted in high values, which caused the >100% recovery.

**Statistical analysis**

Values were subjected to a completely randomized two-way analysis of variance. If a variance ratio (F) reached statistical significance (P < 0.05), the differences between the means were analyzed by the least significant difference test (28), using the interaction mean square error to calculate the least significant difference. Results are expressed as arithmetic means with their standard errors (SEM).

**RESULTS**

Plasma TC, UC, CE, TG, and PL were all much higher in the NAR than in the SDR (P < 0.01; Table 1). Pravastatin treatment in NAR reduced TG levels to 3.0 ± 0.3 mM, as compared to 5.3 ± 0.3 mM in the saline-treated animals (P < 0.01), but did not affect the low TG level of ~0.7 mM found in the SDR. Plasma cholesterol and PL levels were not affected by pravastatin in either rat strain.

Plasma apolipoproteins A-I, B, and E were all increased in NAR (P < 0.01), the most striking difference being in the apoA-I levels, which were 4–5 times higher in the NAR than in the SDR. Plasma apoA-IV was not different between the two strains. Plasma apolipoprotein levels were not affected by pravastatin treatment (Table 1).

Density gradient ultracentrifugation showed that cholesterol levels were elevated in all lipoprotein fractions in the NAR, when compared with the SDR. The fraction with density 1.02-1.063 g/ml contained 62% of total plasma cholesterol in the NAR, as compared to 51% in

**TABLE 1. Effects of pravastatin on plasma lipid and apolipoprotein concentrations in female Nagase analbuminemic rats (NAR) and Sprague-Dawley rats (SDR)**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Saline (n = 6)</th>
<th>SDR (n = 6)</th>
<th>Pravastatin (n = 6)</th>
<th>SDR (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mM)</td>
<td>9.9 ± 0.3*</td>
<td>2.8 ± 0.1</td>
<td>9.1 ± 0.7*</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Unesterified chol. (mM)</td>
<td>3.3 ± 0.1*</td>
<td>1.0 ± 0.1</td>
<td>3.3 ± 0.2*</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol ester (mM)</td>
<td>6.6 ± 0.2*</td>
<td>1.8 ± 0.0</td>
<td>5.7 ± 0.6*</td>
<td>1.7 ± 0.0</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>5.3 ± 0.3*</td>
<td>0.7 ± 0.1</td>
<td>3.0 ± 0.3*</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Phospholipid (mM)</td>
<td>7.2 ± 0.2*</td>
<td>2.4 ± 0.1</td>
<td>6.4 ± 0.5*</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>ApoA-I (mg/100 ml)</td>
<td>145 ± 5*</td>
<td>31 ± 2</td>
<td>142 ± 4*</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>ApoA-IV (mg/100 ml)</td>
<td>15.2 ± 0.4</td>
<td>16.6 ± 1.2</td>
<td>15.8 ± 1.8</td>
<td>16.6 ± 0.9</td>
</tr>
<tr>
<td>ApoE (mg/100 ml)</td>
<td>31.1 ± 1.1*</td>
<td>19.4 ± 0.6</td>
<td>31.3 ± 2.2*</td>
<td>20.5 ± 4.2</td>
</tr>
<tr>
<td>ApoB (arbitrary units)</td>
<td>166 ± 5*</td>
<td>83 ± 2</td>
<td>165 ± 6*</td>
<td>85 ± 8</td>
</tr>
</tbody>
</table>

The duration of pravastatin treatment was 28 days. Pravastatin was dissolved in isotonic saline and administered at a dose of 1 mg/kg body weight per day. Untreated rats were administered saline only. Values given are means ± SEM.

*P < 0.01, NAR versus SDR.

**TABLE 1. Effects of pravastatin on plasma lipid and apolipoprotein concentrations in female Nagase analbuminemic rats (NAR) and Sprague-Dawley rats (SDR)**

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the SDR (Fig. 1). Pravastatin administration had no significant effect on cholesterol distribution.

TG, located mostly in the fraction with density <1.006 g/ml in both NAR and SDR, were much higher in the NAR (Fig. 2). In NAR considerable amounts of TG were also located in the IDL and LDL density fractions. Pravastatin decreased the plasma TG in NAR (see Table 1). This effect was located specifically in the d<1.006 g/ml and LDL density fractions (triglycerides decreased from 45% to 34%, and from 29% to 20% of total plasma TG in the d<1.006 g/ml fraction and in LDL, respectively). Pravastatin had no major effects on TG distribution in SDR.

The distribution of PL (Fig. 3) was practically identical with that of cholesterol, with the exception of the PL content of the lipoprotein-deficient fraction (d>1.21 g/ml), where the PL content was markedly decreased in NAR, as compared to SDR (3 ± 1% vs 28 ± 2%; P < 0.01). The PL profile was not affected by pravastatin.

SDS-polyacrylamide gel electrophoresis of total plasma demonstrated a markedly altered plasma protein profile in the NAR (Figs. 4A and 4B, lane 1). Note the absence of albumin with molecular weight (MW) 66,000 in the NAR. Apolipoprotein distribution in plasma lipoprotein fractions was qualitatively quite similar in both strains (Fig. 4), but quantitatively very different. The HDL₃ fraction was not evaluated due to contamination with the protein-rich infranatant. Proteins were tentatively identified using molecular weight values from the literature (29). Small amounts of protein were observed in the NAR (even less in the control SDR) at molecular weights of about 350,000 and 215,000 in VLDL, LDL, and IDL (these bands are likely to represent apoBs). Increases in protein content were observed in the NAR at MW of about 26,000 a major apolipoprotein was seen in both NAR and SDR (apoA-I). It was increased in NAR, as compared to SDR, in all density fractions, and was also visible in total plasma (Fig. 4, lane 1). Increases of proteins with molecular weights of about 10,000 and less were also observed in NAR (apoCs and apoA-II) throughout the gradient. A protein with an MW of about 43,000 (possibly apoA-IV) was present in HDL₂ in similar quantities in both NAR and SDR. HDL₂ in the SDR was slightly contaminated with albumin (MW 66,000), a phenomenon that was more marked in HDL₃ (not shown). Plasma proteins were also noted in NAR HDL₁ at molecular weights of 81,000 and 54,000 (possibly transferrin and α-1-acid glycoprotein, respectively). Pravastatin had no significant effects on the electrophoretic patterns of total plasma and lipoprotein fractions (data not shown).
Fig. 4A. SDS-polyacrylamide gel electrophoresis of total plasma proteins and apolipoproteins from lipoprotein fractions isolated from saline-treated female Nagase analbuminemic rats (NAR). The lipoprotein fractions were obtained by density gradient ultracentrifugation. Lane 1: total plasma. The approximate molecular masses (kDa) of the major protein bands in total plasma are indicated on the left. Lane 2: VLDL density range; Lane 3: IDL density range; Lane 4: LDL density range; Lane 5: HDL2 density range. The approximate molecular masses (kDa) of the major protein bands in the lipoprotein fractions of various densities (lanes 2–5) are indicated on the right. Volumes of 0.5, 37.5, 37.5, 37.5, and 37.5 µl of total plasma or lipoprotein fraction were applied to the gels for samples 1–5, respectively. This resulted in 27, 3.4, 4.9, 75, and 47 µg of protein applied, respectively.

Fig. 4B. SDS-polyacrylamide gel electrophoresis of total plasma proteins and apolipoproteins from lipoprotein fractions isolated from saline-treated female Sprague-Dawley rats (SDR). Volumes of 0.5, 37.5, 37.5, 37.5, and 37.5 µl of total plasma or lipoprotein fractions were applied to the gels for samples 1–5, respectively. This resulted in 24, 0.4, 0.2, 12.4, and 18.8 µg of protein applied, respectively. For further details see the legend to Fig. 4A.

Lipoprotein profiles obtained after gel filtration of SDR and NAR plasma are shown in Fig. 5 (TG and apoB), Fig. 6 (TC), Fig. 7 (apoE), and Fig. 8 (apoA-I). The data in Fig. 5 show that TG are present mostly in particles with the size of VLDL or larger (all present in the void volume) in both SDR and NAR. Both TG and apoB were strongly elevated in this fraction from NAR plasma. Fig. 5 also shows that plasma apoB is mainly present in LDL-sized particles of molecular masses >10^3 kDa. The vertical line in Figs. 5–10 indicates the peak elution volume of IgM with a molecular mass of 1x10^3 kDa. The increased levels of plasma TC in NAR were recovered in the same three fractions that also occur in normal rat plasma (particles with the size of chylomicrons/VLDL, large apoE-rich HDL, and smaller apoA-I-rich HDL (compare Figs. 6–8). Fig. 7 shows that the increase in total plasma apoE (Table 1) in NAR is located mostly in large-sized, apoE-rich HDL (fractions 15–20). The apoA-I-rich HDL of relatively small size was depleted of apoE and further enriched in apoA-I in NAR (see Figs. 7 and 8). Only a relatively small amount of total plasma cholesterol was present in lipoproteins with the size of apoB-containing LDL (particle mass

Fig. 5. Triglyceride and apoB profiles obtained after gel filtration of plasma from NAR (closed symbols) and SDR (open symbols). Plasma samples (2 ml) were chromatographed on a Superose 6 and a Superose 12 column connected in sequence. Column fractions were assayed for TG (squares) and apoB (circles). TG contents are given in lmmol/ml of injected plasma (summation of all fractions results in total plasma concentration in mM). ApoB is given in arbitrary units (A.U., see Methods). The vertical line represents the peak elution volume of IgM with molecular mass 10^3 kDa. The column was further calibrated as described in ref. 27.
Fig. 6. Total cholesterol profiles obtained after gel filtration of plasma from NAR (closed symbols) and SDR (open symbols). Column fractions were assayed for TC, given in \( \mu \text{mol/ml} \) of injected plasma (summation of all fractions results in total plasma concentration in mM). For further details see the legend to Fig. 5.

Fig. 7. ApoE profiles obtained after gel filtration of plasma from NAR (closed symbols) and SDR (open symbols). Column fractions were assayed for apoE, using a quantitative electroimmunoassay (20). ApoE levels are expressed in \( \mu \text{g/ml} \) of injected plasma (summation of all fractions results in total plasma concentration in \( \mu \text{g/ml} \) plasma).

The phospholipid profiles, shown in Fig. 10, confirm the data obtained by density gradient ultracentrifugation (Fig. 3). In the lipoprotein-deficient plasma (fractions 27–35) we observed a decreased PL level in NAR. The various phospholipid classes present in HDL subfractions and in the lipoprotein-deficient plasma fraction (molecular mass <100 kDa), obtained from SDR or NAR, are shown in Fig. 11. The lipids were visualized with iodine after separation by thin-layer chromatography on silica gel plates. It can be seen that the lipoprotein-deficient fraction from NAR contains very little LPC, when compared with the same fraction from SDR. On the other hand, fractions 27–35 do contain more phosphatidylcholine in NAR than in SDR, due to the smaller average size of apoA-I-rich HDL in NAR, when compared to SDR.

Fig. 8. ApoA-I profiles obtained after gel filtration of plasma from NAR (closed symbols) and SDR (open symbols). For further details see the legend to Fig. 7.

Fig. 9. Free fatty acid profiles obtained after gel filtration of plasma from NAR (closed symbols) and SDR (open symbols). FFA levels in total plasma were 0.14 ± 0.07 mM for NAR and 0.25 ± 0.15 mM for SDR (n=6). Thirty-six fractions (of 2.4 ml each) were assayed for FFA, expressed in \( \mu \text{mol/ml} \) of injected plasma. For further details see the legend to Fig. 5.
(see Figs. 6 and 10). It is clear that extra LPC is recovered in the lipoprotein fractions from NAR.

LCAT activity levels, as well as absolute plasma cholesterol esterification rates, were about twice as high in NAR as in SDR \((P < 0.01; \text{Table } 2)\). The high plasma UC levels in NAR gave rise to a reduced fractional esterification rate, despite the elevated absolute rates. LCAT activities, measured in various ways, were not significantly affected by pravastatin treatment (Table 2).

DISCUSSION

Plasma lipid levels were markedly higher in the NAR than in the SDR, as expected (2, 3). TG \((-7\text{-fold})\), cholesterol \((-4\text{-fold}; \text{both the esterified and the unesterified form})\), and PL \((-3\text{-fold})\) levels were all extremely elevated in the female animals fed the semi-synthetic diet used in the present study. Mean plasma apoA-I, apoB, and apoE levels were 4.7-, 2.0-, and 1.6-fold increased in the NAR, respectively.

**TABLE 2.** Effect of pravastatin on plasma LCAT activity in female Nagase analbuminemic rats (NAR) and Sprague-Dawley rats (SDR)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>Pravastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAR ((n = 6))</td>
<td>SDR ((n = 6))</td>
</tr>
<tr>
<td>LCAT activity with exogenous substrate (arbitrary units)</td>
<td>130 ± 7*</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>Absolute esterification rate (nmol/ml/h)</td>
<td>324 ± 36*</td>
<td>187 ± 12</td>
</tr>
<tr>
<td>Fractional esterification rate (%/h)</td>
<td>9.9 ± 2.7*</td>
<td>19.3 ± 1.9</td>
</tr>
</tbody>
</table>

Plasma LCAT activity (expressed as enzyme activity with exogenous substrate) and endogenous cholesterol esterification rate (nmol/ml/h) in NAR and SDR after 28 days of pravastatin administration. Pravastatin was dissolved in isotonic saline and given at a dose of 1 mg/kg body weight per day. Untreated rats were administered saline only. Values given are means ± SEM.

\[^*P < 0.01, \text{NAR versus SDR.}\]

The lipoprotein profile in the NAR, as determined by density gradient ultracentrifugation, was characterized by very high cholesterol and phospholipid levels in the fraction with a density between 1.019 and 1.063 g/ml. The assessment of total plasma PL, TC, and apolipoproteins, as mentioned above, suggests an increase in HDL levels, whereas the largest increase in cholesterol was found in the LDL density range (see Figs. 1 and 3). This apparent contradiction may be explained by the notion that the bulk of rat lipoproteins in the LDL density range carry HDL apolipoproteins (apoE, apoA-I, and apoCs; see Fig. 4). The ultimate proof that the extreme hypercholesterolemia in NAR was due to elevated HDL levels was obtained.
Only a minor part of the plasma cholesterol in NAR was present on LDL-sized, apoB-containing lipoproteins, while the bulk was present on apoE-rich HDL and apoA-I-rich HDL, a situation comparable to normalalbuminemic rats (4, 9, 30-32). Several arguments are in accordance with elevated levels of apoE-rich HDL in the NAR. First, Fig. 4A shows only small amounts of high molecular mass proteins (>200 kDa), and relatively large amounts of proteins with molecular masses <50 kDa in the LDL density range. Second, the cholesterol peak in Fig. 6 (particles with average masses of 730 kDa) does not coincide with LDL-apoB (see Fig. 5), but with the major peak of apoE in the NAR profile (see Fig. 7). It is clear, however, that the plasma levels of typical LDL (particle mass 2.10³ kDa and apoA-I-rich HDL (particle mass 325 kDa) are also increased in NAR (see Figs. 5 and 8, respectively). The presence of small amounts of apoA-I in the VLDL density fraction (see Fig. 4) indicates the presence of chylomicrons, which is not surprising because the rats were used in the post-absorptive state.

The TG distribution (Fig. 2) indicates considerable amounts of TG in the IDL and LDL fractions of NAR plasma, whereas TG were practically restricted to the d<1.006 g/ml fraction in SDR plasma. This is in agreement with the increased amounts of TG-rich particles with sizes in the VLDL-LDL range, observed after gel filtration (Fig. 5). Consequently, the lipoprotein composition of IDL and LDL are quite different between the two strains, e.g., the LDL density fraction from NAR has a molar ratio of TC/TG of 4.6, versus a ratio of 18.1 in the LDL density fraction from SDR plasma. This may be connected with reduced lipoprotein lipase activities in NAR, measured in postheparin plasma (33), as well as in adipose tissue (3). However, normal fractional catabolic rates for chylomicron- and VLDL-TG were reported (33).

Striking findings of the present study are the very high rates of plasma cholesterol esterification (coinciding with high plasma LCAT activity levels) and the altered distribution of plasma LPC and FFA between lipoproteins and other plasma proteins (albumin) after density gradient ultracentrifugation and gel filtration in NAR, as compared with control SDR. The PL distribution (Figs. 3 and 10) was similar to the cholesterol distribution in both strains, with the exception of the lipoprotein-deficient fractions, with d>1.21 g/ml and molecular mass <100 kDa. The PL concentration in the d>1.21 g/ml fraction of plasma from NAR was extremely low (see Fig. 3). The major component of this PL fraction in normal rats was LPC, a product of the LCAT reaction, bound to albumin (7-10). The data in Fig. 11 indeed indicate that the NAR has a decreased concentration of LPC in its lipoprotein-deficient plasma fraction. It has been suggested that low albumin levels and low LCAT activity in the nephrotic syndrome are linked by depletion of the acceptor of the reaction product LPC (13). However, Cohen et al. (13) reported only a decreased fractional esterification rate. Their data indicate that the patients probably had normal absolute esterification rates. They also measured normal LCAT activity levels in their patients, using excess exogenous substrate. In the present study we report a high level of plasma CE, as well as increased LCAT activity levels and absolute rates of plasma cholesterol esterification in analbuminemic rats. Similarly, in two analbuminemic human siblings, high levels of LCAT activity have been described (16).

The absence of albumin presents potential problems related to hemolysis. LPC is a potent hemolytic agent (34) and indeed small increases in unbound LPC may account for the increased in vitro hemolysis and slight anemia observed in NAR (35). Obviously the NAR is protected effectively in vivo against severe hemolytic effects. This may be due to the observed binding of LPC (and FFA) to lipoproteins (Figs. 9 and 11).

Continuous subcutaneous administration of the hydrophilic HMG-CoA reductase inhibitor, pravastatin, was tolerated well by the rats. In a previous study (Joels, J. A., N. Willekes-Koolschijn, H. A. Koomans, A. Van Tol, M. M. Geelhoed-Mieras, D. Crommelin, L. Van Bloois, M. Kraijnc-Franken, L. H. Cohen, A. M. Griffioen, and D. W. Erkelens, unpublished results), we demonstrated increases in hepatic microsomal HMG-CoA reductase activity in NAR and SDR after similar subcutaneous treatment with pravastatin, confirming uptake of the drug. Pravastatin did not affect any lipid parameter in SDR. The only change effected by pravastatin in the present study was a 43% decrease in plasma TG in the NAR. It has been reported that pravastatin decreases VLDL synthesis in rats (36). The reduction in VLDL-TG and LDL-TG, observed in the pravastatin-treated NAR in the present study, may therefore be due to a reduction in hepatic VLDL synthesis. Reduction in plasma TG has been described after oral administration of pravastatin, using much higher doses than those used in the present study (37). The same study reports the absence of hypocholesterolemic effects in rats. Pravastatin decreases plasma LDL, TG levels, and VLDL-apoB synthetic rates in patients with primary hypercholesterolemia (38).

In summary, the present study demonstrates very high levels of plasma lipids and HDL-apolipoproteins in the female analbuminemic rat fed a semi-synthetic diet. Plasma cholesterol is mainly present in lipoprotein particles with a density between 1.02 and 1.12 g/ml. Separation of lipoprotein classes by gel filtration showed that the major cholesterol-carrying lipoprotein fractions in NAR plasma are apoE-rich HDL and apoA-I-rich HDL. The synthesis of CE for the assembly of the core of these HDL particles is catalyzed by a high plasma activity of LCAT.
The apolipoprotein assays, density gradient ultracentrifugation, electrophoresis, gel filtration, and LCAT activity measurements were performed by M. M. Geelhoed-Mieras, N. Willekes-Koolschijn, C. Laan, T. van Gent, and L. M. Scheek, respectively, whom we thank for their expert technical assistance. This research was supported by the Netherlands Heart Foundation, grant no. 88.223 and Squibb B.V., Rijswijk, The Netherlands. Pravastatin was provided by Squibb Inc., Princeton, NJ.

REFERENCES

agarose columns and sequential immunoprecipitation. 

**J. Lipid Res. 27**: 1035-1043.


