

# Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency<sup>1</sup>

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**Abstract** Apolipoprotein(apo) E deficiency is an inherited disease characterized by type III hyperlipoproteinemia and <1% normal plasma apoE concentration. The role of apoE in LDL metabolism was investigated by quantitating the metabolism of radiolabeled normal and apoE-deficient LDL in both normal and apoE-deficient subjects. ApoE deficiency resulted in an accumulation of plasma IDL, and a decreased synthesis of LDL consistent with a block in the conversion of IDL to LDL. The LDL isolated from the apoE-deficient patient was similar to normal LDL in hydrated density, size, and composition. However, the apoE-deficient LDL was kinetically abnormal with delayed catabolism in both normal subjects and the apoE-deficient patient. In addition, the catabolism of normal LDL in the apoE-deficient subject was increased. These results were interpreted as indicating that apoE is necessary for the conversion of IDL to LDL and the formation of kinetically normal LDL. The rapid catabolism of normal LDL in the apoE-deficient patient suggests an up-regulation of the hepatic LDL receptor pathway. Based on these results, apoE is proposed to play an important role in the conversion of IDL to LDL, the formation of kinetically normal LDL, and the regulation of LDL receptor function. —Gabelli, C., R. E. Gregg, L. A. Zech, E. Manzato, and H. B. Brewer, Jr. Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency. *J. Lipid Res.* 1986. 27: 326–333.

**Supplementary key words** lipoprotein remnants • type III hyperlipoproteinemia • LDL receptor

Apolipoprotein E (apoE), a glycoprotein of 299 amino acids with a molecular weight of approximately 34,000, is present predominately in VLDL and HDL (1–3). ApoE rapidly exchanges between VLDL and HDL depending on the physiologic state of the individual (4). ApoE has been shown to facilitate the hepatic removal of remnants of triglyceride-rich lipoproteins or triglyceride emulsions (5–7) and to interact with the hepatic LDL receptor (B<sub>2</sub>E receptor) as well as a specific apoE receptor (8, 9). It has been proposed that the primary function of apoE in lipoprotein metabolism is to facilitate the removal of remnants of triglyceride-rich lipoproteins (chylomicrons and VLDL) by interaction with the specific high affinity hepatic apoE receptor (1–9).

A number of apoE variants (apoE<sub>1</sub>, apoE<sub>2</sub>, apoE<sub>Bethesda</sub>, and apoE deficiency) are associated with abnormal metabolism of triglyceride-rich lipoproteins (3, 10–13). Patients with these apoE variants manifest type III HLP, accelerated atherosclerosis, and elevated plasma concentrations of remnants of triglyceride-rich lipoproteins, as well as decreased LDL levels. While several studies on the metabolism of triglyceride-rich lipoproteins in type III HLP have been reported (14, 15), LDL metabolism has not been extensively studied in type III subjects or in subjects with the different apoE phenotypes.

Apolipoprotein E deficiency is an inherited disease characterized by premature cardiovascular disease, tuberous xanthomatosis, type III hyperlipoproteinemia, and less than 1% of the normal apoE concentration in plasma or lipoprotein fractions by radioimmunoassay (13). ApoE deficiency provides a unique opportunity to investigate LDL metabolism in the absence of apoE and to gain new insights into the role of apoE in lipoprotein metabolism. In this report we describe the results of a kinetic study in which the metabolism of normal LDL and LDL from the apoE-deficient subject were assessed in both normal and apoE-deficient subjects.

## METHODS

### Subjects

Four adult subjects, three normal volunteers and one apoE-deficient patient, were studied while inpatients on

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; HLP, hyperlipoproteinemia; SDS, sodium dodecyl sulfate; apoE, apolipoprotein E; CH, cholesterol; TG, triglycerides.

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the metabolic ward of the National Institutes of Health Clinical Center. All subjects were free of renal, hepatic, hematologic, and thyroid abnormalities, and all medications were discontinued 4 weeks before admission to the Clinical Center. An iso-weight diet containing 42% fats, 42% carbohydrate, 16% protein, polyunsaturated to saturated fat ratio of 0.2, and 200 mg of cholesterol/1000 kcal was given in three meals per day starting 10 days before the injection of the radiolabeled lipoproteins and continuing throughout the study. Nine hundred mg of potassium iodide was given orally in three equal doses per day beginning 3 days before the injection and continuing for the course of the study. The study protocol was approved by the Internal Review Board, National Heart, Lung, and Blood Institute, and informed consent was obtained from all of the subjects.

### Preparation of radioiodinated lipoproteins

Fasting plasma was obtained by plasmapheresis from a normal subject (E3/3 phenotype) and the apoE-deficient patient using EDTA (0.1%) as anticoagulant. LDL of d 1.030–1.050 g/ml from each of the two subjects were isolated by sequential ultracentrifugation at 4°C in a Beckman L2-65B ultracentrifuge (Beckman, Palo Alto, CA) using a 60 Ti rotor (16). The LDL were dialyzed against 50 mM sodium phosphate–100 mM saline (pH 7.4), and concentrated in dialysis tubing using Aquacide 1-A (Calbiochem-Behring Co., La Jolla, CA) to obtain a final protein concentration ranging from 4 to 8 mg/ml. One ml of each isolated LDL fraction was filtered through a 0.20- $\mu$ m filter (Gelman Acrodisc, Ann Arbor, MI) and radioiodinated (5 mCi, New England Nuclear, Boston, MA) using a modification of the iodine monochloride method of McFarlane (17, 18) utilizing  $^{131}$ I for normal LDL and  $^{125}$ I for apoE-deficiency LDL. The quantity of ICl added was calculated to yield one mole of iodine per two moles of LDL protein, assuming all of the protein within LDL was apoB-100 with a molecular weight of 300,000. Unbound iodine was removed from the LDL preparations by dialysis against 0.15 M NaCl, 0.1 M Tris-HCl (pH 7.4), and 0.01% EDTA at 4°C. Ninety-nine percent of the radioactivity remaining after dialysis was associated with the lipoproteins as determined by descending paper chromatography. The efficiency of iodination was 15 to 20% and lipid labeling determined by lipid extraction (19) was less than 4%. Human serum albumin (1%) was added to the radiolabeled lipoproteins, and the samples were sterilized with a 0.20- $\mu$ m filter followed by testing for pyrogenicity and sterility.

### Lipoprotein metabolism

At 8 AM, 23  $\mu$ Ci of normal  $^{131}$ I-labeled LDL and 21  $\mu$ Ci of apoE-deficient  $^{125}$ I-labeled LDL were injected simultaneously into each subject. Blood samples were

collected at 10 min, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, and daily for 1 week. The blood was collected in EDTA (0.1%), cooled in crushed ice, and plasma was separated by low speed centrifugation (2000 rpm) for 20 min at 4°C. Sodium azide (0.05%) and aprotinin (1000 KIU/ml) were added to prevent apolipoprotein degradation. LDL (d 1.030–1.050 g/ml) were obtained by sequential ultracentrifugation using a 40.3 rotor (Beckman, Palo Alto, CA). Radioactivity was quantitated using a Packard 5260 Autogamma spectrometer.

### Quantitation of apoB-100 radioactivity

Separation of apoB-100 was performed by SDS agarose-acrylamide gel electrophoresis (0.5% agarose, 2.0% acrylamide) using a modification of the method of Peacock and Dingman (20).<sup>3</sup> Each LDL sample was dialyzed against 0.1 M EDTA (pH 8.2) at 4°C and the protein was quantitated by the method of Lowry et al. (21). Five hundred  $\mu$ g of LDL protein was lyophilized, delipidated twice with chloroform-methanol 2:1 (v/v), and dissolved in sample buffer (3.0% SDS, 1.2% dithiothreitol, 1.08% Tris-HCl pH 8.0, and 0.5% boric acid). The samples were incubated at 100°C for 3 min and applied to a 3-mm thick three-well slab gel utilizing a Hoefer SE 500 slab gel apparatus (Hoefer, San Francisco, CA). Each gel was fixed and stained in methanol-water-acetic acid 10:10:1 (v/v) containing 0.1% Coomassie Brilliant Blue R-250 (Bio-Rad, Richmond, CA) for 30 min and destained in 5% methanol-7.5% acetic acid. The bands corresponding to apoB-100 were sliced and the radioactivity was quantified. The data were analyzed by computer curve fitting using a SAAM 27 program (22) on a VAX-11/780 computer system (Digital Equipment Corp.).

### Lipoprotein and apolipoprotein determinations

Lipid and lipoprotein determinations were performed by the Lipid Research Clinic methodology (23), except that a Gilford system 3500 analyzer (Gilford Instruments, Inc., Oberlin, OH) was used to determine cholesterol and triglyceride levels and HDL-cholesterol was quantitated after dextran sulfate precipitation (24). Cholesterol determinations in the effluent from the zonal rotor were performed as previously described (25). Phospholipids were quantified using an enzymic colorimetric assay kit by the method of Takayama et al. (26) (Wako, Ltd., Osaka, Japan). ApoB in plasma and lipoprotein fractions was quantitated by radial immunodiffusion as previously described (27). ApoE concentrations were ascertained by a double antibody radioimmunoassay (28), and apoE phenotypes were determined as previously described (12, 29).

<sup>3</sup>Gabelli, C., D. G. Stark, R. E. Gregg, and H. B. Brewer, Jr. 1986. Separation of apolipoprotein forms by agarose-acrylamide gel electrophoresis. *J. Lipid Res.* In press.

## Other procedures

Zonal ultracentrifugation was performed on plasma or the 1.006 g/ml infranate obtained from 9 ml of plasma under rate flotation conditions using a linear gradient (d 1.00–1.30 g/ml) at 98,000 *g* at 15°C for 140 min with a Ti-14 zonal rotor in an L5-65 Beckman ultracentrifuge as previously described (25, 30, 31). Electron microscopy was performed on the LDL samples negatively stained with 1% carbonized phosphotungstic acid (pH 6.5) and analyzed with a Siemens Elmiskop IA microscope at 80 KV (27).

## RESULTS

The clinical characteristics and lipid as well as lipoprotein values for each patient are summarized in **Table 1**. The apoE-deficient patient had a slightly elevated plasma cholesterol, normal plasma triglycerides, and an increased plasma VLDL cholesterol level. The VLDL cholesterol/plasma triglyceride ratio was extremely elevated and the IDL-LDL and HDL cholesterol levels were normal in the apoE-deficient patient (Table 1).

The lipid and protein composition of LDL isolated by preparative sequential ultracentrifugation from a normal subject and from the apoE-deficient patient is shown in **Table 2**. The protein/cholesterol ratio was 0.76 in the apoE-deficiency LDL and 0.63 in normal LDL. ApoE was < 0.01 mg/dl in the apoE-deficiency LDL and 0.04 mg/dl in the normal LDL. By SDS gel electrophoresis, all of the protein in the normal LDL (d 1.03–1.05 g/ml) was apoB-100, while the protein in the apoE-deficient LDL was approximately 5% apoB-48 with the remainder being apoB-100. The small amount of apoB-48 should not affect the results of the kinetic studies though, since apoB-48 and apoB-100 reside on different particles and the radioactivity was quantitated from isolated apoB-100 for the determination of the kinetic parameters. Analysis of the morphology and size of the LDL particles by electron microscopy revealed that there were no major differences in the LDL isolated from normal and from apoE-deficient

subjects, although the apoE-deficiency LDL were somewhat more heterogeneous in size (**Fig. 1** and **Fig. 2**).

The absorbance profiles obtained after zonal ultracentrifugation of normal plasma and lipoproteins (d > 1.006 g/ml) from the apoE-deficient subject are illustrated in **Fig. 3**. The profile of the lipoproteins from the apoE-deficient patient revealed two peaks (**Fig. 3B**). The first peak coincides with the position of IDL (31) and is labeled LpIII while the second peak coincides with the elution position of normal LDL<sub>2</sub> (**Fig. 3**). The cholesterol concentrations quantitated in the LpIII and LDL<sub>2</sub> of the apoE-deficient subject were 124 mg/dl and 29 mg/dl, respectively, indicating that the majority of the IDL-LDL cholesterol (d 1.006–1.063 g/ml) as shown in Table 1 is in IDL and not LDL<sub>2</sub>. The composition of LDL<sub>2</sub> isolated by zonal ultracentrifugation from the apoE-deficient patient is similar to that obtained from the 1.030–1.050 g/ml lipoproteins isolated by sequential ultracentrifugation (Table 2). In addition, the lipoproteins (d 1.03–1.05 g/ml) isolated by sequential ultracentrifugation from the apoE-deficient patient contained only LDL<sub>2</sub> and no LpIII when analyzed by zonal ultracentrifugation.

Representative catabolic curves of normal and apoE-deficient LDL in a normal subject and in the apoE-deficient patient are illustrated in **Fig. 4** and **Fig. 5**. In the normal subject at 7 days, 8% of the normal <sup>131</sup>I-labeled LDL apoB-100 radioactivity remained in plasma, while the apoE-deficient <sup>125</sup>I-labeled LDL apoB-100 was more slowly catabolized with 20% of the initial radioactivity remaining in plasma (**Fig. 4**). In the apoE-deficient patient, the decay of normal <sup>131</sup>I-labeled LDL apoB-100 was very fast and after 5 days only 3% of normal <sup>131</sup>I-labeled LDL apoB-100 injected remained in plasma (**Fig. 5**). In contrast, 15% of the apoE-deficient <sup>125</sup>I-labeled LDL apoB-100 remained in plasma at 5 days in the apoE-deficient patient.

Determination of the residence times gave additional information regarding the kinetic behavior of the LDL in the subjects under investigation (**Table 3**). Normal LDL apoB-100 in normal subjects had a mean residence time of 2.0 ± 0.3 days, which is similar to previous results of

TABLE 1. Clinical characteristics of the study subjects

| Subjects               | Age | Sex | Weight | Height | Plasma <sup>a</sup> |     | Cholesterol |           |     | VLDL-CH/<br>Plasma-TG<br>Ratio | ApoE<br>Phenotype |
|------------------------|-----|-----|--------|--------|---------------------|-----|-------------|-----------|-----|--------------------------------|-------------------|
|                        |     |     |        |        | TG                  | CH  | VLDL        | IDL + LDL | HDL |                                |                   |
|                        | yr  |     | kg     | cm     | mg/dl               |     | mg/dl       |           |     |                                |                   |
| Normal                 |     |     |        |        |                     |     |             |           |     |                                |                   |
| 1                      | 22  | F   | 65     | 171    | 112                 | 219 | 21          | 145       | 53  | 0.18                           | E3/4              |
| 2                      | 22  | M   | 78     | 180    | 75                  | 164 | 17          | 102       | 45  | 0.22                           | E3/3              |
| 3                      | 21  | M   | 72     | 186    | 116                 | 141 | 17          | 87        | 37  | 0.14                           | E3/3              |
| ApoE-deficient patient | 63  | F   | 72     | 161    | 112                 | 297 | 112         | 140       | 45  | 1.00                           |                   |

<sup>a</sup>Lipid and lipoprotein determinations were performed by the Lipid Research Clinic Methodology (23).



TABLE 2. Composition of lipoproteins isolated from a normal subject and patient with apoE deficiency

| Lipoprotein Fraction                     | Cholesterol     | Triglycerides | Phospholipids | Protein | Protein/<br>Cholesterol<br>Ratio | ApoE<br>Concentration |
|--|-----------------|---------------|---------------|---------|----------------------------------|-----------------------|
|  | % of total mass |               |               |         |                                  | mg/dl                 |
| Sequential ultracentrifugation           |                 |               |               |         |                                  |                       |
| Normal LDL<br>(d 1.03–1.05 g/ml)         | 43              | 7             | 22            | 28      | 0.65                             | 0.04                  |
| ApoE-deficient LDL<br>(d 1.03–1.05 g/ml) | 43              | 6             | 18            | 33      | 0.77                             | <0.01                 |
| Zonal ultracentrifugation                |                 |               |               |         |                                  |                       |
| ApoE-deficient LpIII                     | 55              | 8             | 23            | 14      | 0.25                             | <0.01                 |
| ApoE-deficient LDL <sub>2</sub>          | 42              | 3             | 20            | 35      | 0.83                             | <0.01                 |

LDL metabolic studies in normal human subjects (14). The apoE-deficient LDL injected into normal subjects had a slower catabolism with a mean residence time of  $2.8 \pm 0.5$  days. When normal  $^{131}\text{I}$ -labeled LDL were injected into the apoE-deficient patient, the residence time was half the normal value (1.0 day), while the apoE-deficient  $^{125}\text{I}$ -labeled LDL in the apoE-deficient patient had a residence time of 2.5 days. In these studies, the most striking difference is the shortened residence time of normal LDL in the apoE-deficient patient. In addition, the residence time of the apoE-deficient LDL was prolonged compared to normal LDL both in normals and in the apoE-deficient patient. The slow catabolism of the apoE-deficient LDL particle and an up-regulated LDL catabolic pathway in the apoE-deficient patient resulted in a nearly normal residence time for apoE-deficient LDL in the apoE-deficient subject (Table 3).

The production rate of LDL<sub>2</sub> apoB-100 in the apoE-deficient patient was substantially decreased, being 20% of the production rate obtained in normal subjects (Table 4). Therefore, the decreased plasma LDL<sub>2</sub> concentration is due primarily to a decreased production rate. In addition, the LDL<sub>2</sub> that is synthesized is kinetically abnormal and catabolized at a slower than normal rate (Tables 3 and 4).

## DISCUSSION

The analysis of patients with apoE variants and type III HLP and in vitro cell culture studies have provided insights into the physiological role of apoE in lipoprotein metabolism (1–15, 32, 33). The importance of apoE in the catabolism of remnants of triglyceride-rich lipoproteins

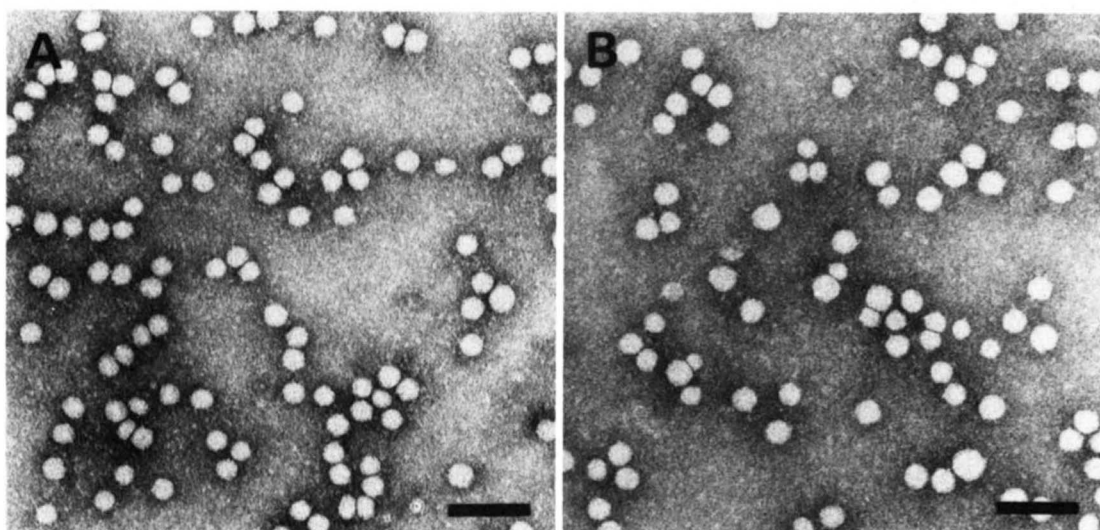
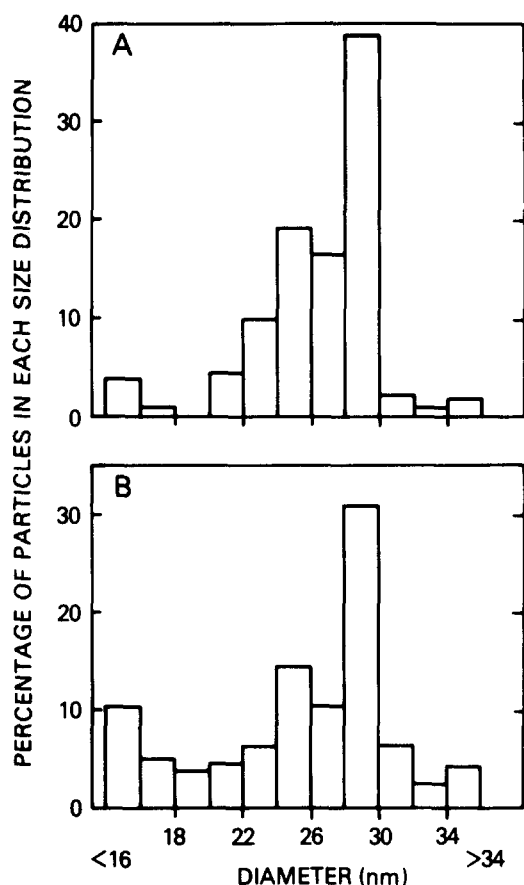


Fig. 1. Electron micrographs of LDL (d 1.03–1.05 g/ml) isolated by sequential ultracentrifugation from a normal subject (A) and an apoE-deficient patient (B). The bar represents 100 nm.



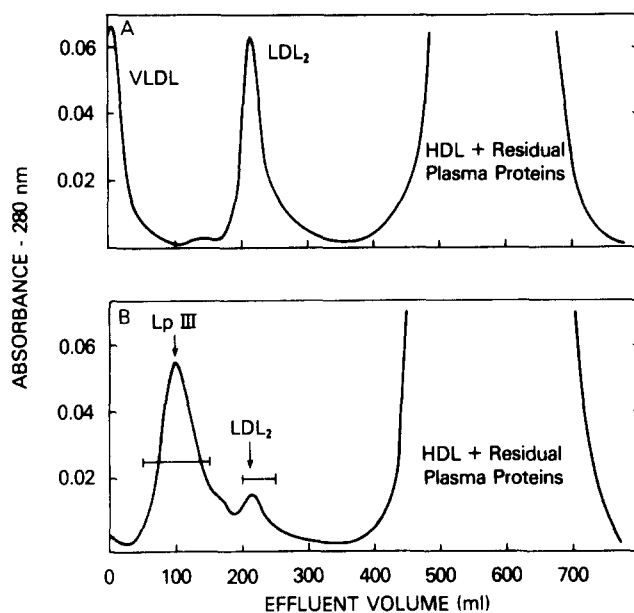
**Fig. 2.** Size distribution determined following electron microscopic analysis of LDL ( $d$  1.03–1.05 g/ml) isolated by sequential ultracentrifugation and injected in the LDL kinetic study. Panel A, normal LDL; Panel B, apoE deficiency LDL. A total of 243 normal LDL and 258 apoE-deficient LDL particles were analyzed.

by the hepatic apoE receptor has been established (1, 5–9, 11). Kinetic studies have also indicated that the elevated plasma level of IDL in type III HLP is due to a defect in conversion of IDL to LDL (14, 15).

To date, detailed studies on LDL metabolism in type III HLP have not been reported. The patient with apoE deficiency provides the unique opportunity to evaluate LDL metabolism without modulation by the E apolipoprotein. The plasma lipoproteins of the apoE-deficient patient are similar to those from patients with type III HLP (33); however, the lipoprotein remnants are more cholesteryl ester-enriched than those from type III HLP patients with the apoE2 phenotype (13, 33). In addition, separation of the lipoproteins by zonal ultracentrifugation demonstrated a marked increase in IDL or LpIII and a reduced level of LDL. Kinetic analysis of apoE-deficient LDL apoB-100 metabolism in the apoE-deficient patient revealed a significant decrease in synthesis of LDL apoB-100. Thus the absence of apoE results in the marked

accumulation of plasma IDL and a decrease in synthesis of LDL consistent with a block in conversion of IDL to LDL.

The LDL present in the apoE-deficient patient had a hydrated density as well as lipid and protein composition similar to normal LDL<sub>2</sub>. Of particular importance, however, was the finding of a decreased rate of catabolism of the LDL isolated from the apoE-deficient patient in both normal subjects and the apoE-deficient patient. Thus the apoE-deficient LDL was kinetically abnormal. This decreased rate of catabolism, when compared to normal LDL, was more significant in the apoE-deficient patient than the normal subject, suggesting that some metabolic correction of the apoE LDL may occur in the normal subject (e.g., the kinetic abnormality in the apoE-deficient LDL may be able to be corrected in the normal subject but not in the apoE-deficient patient). An additional interesting result was the increased rate of catabolism of normal LDL in the apoE-deficient patient compared to normal subjects. This result is consistent with an up-regulation of the LDL receptor pathway. Injection of the apoE-deficient LDL, which was slowly catabolized in normal subjects, into the apoE-deficient patient with an up-regulated LDL catabolic pathway resulted in a near normal residence time for LDL catabolism. Thus, the decreased LDL level in the apoE-deficient patient is due



**Fig. 3.** Absorbance profile (280 nm) obtained after zonal ultracentrifugation using a linear density gradient (1.00–1.30 g/ml) of normal plasma (A), and apoE deficiency 1.006 g/ml infranate (B). LDL<sub>2</sub> in both samples eluted in the same effluent volume. VLDL in the panel B were not visualized due to the previous separation of plasma by ultracentrifugation at 1.006 g/ml. The bar represents the fractions pooled for the analyses presented in Table 2.

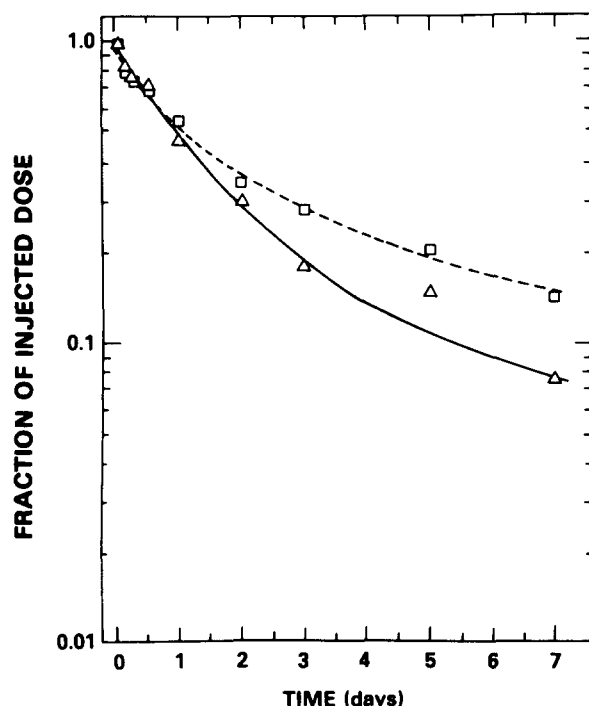


Fig. 4. Catabolism of normal  $^{131}\text{I}$ -labeled LDL apoB-100 ( $\triangle$ — $\triangle$ ) and apoE deficiency  $^{125}\text{I}$ -labeled LDL apoB-100 ( $\square$ — $\square$ ) following injection into a normal subject. Five ml of plasma was obtained at various time intervals and LDL were separated by sequential ultracentrifugation. ApoB-100 was separated by agarose-acrylamide gel electrophoresis and radioactivity in the corresponding band was quantitated (see text for details). All values are normalized to 1.0 at the first time point.

primarily to a decreased LDL production rate with a relatively normal rate of LDL catabolism.

The combined results from this study have been interpreted as indicating that, in addition to its role in hepatic clearance of remnants of triglyceride-rich lipoproteins, apoE is important in the metabolic conversion of IDL to LDL. This conversion may be mediated by a modulation of a lipase system (34, 35) or cellular receptor interaction (1, 33). The defect in conversion to LDL results in an increased plasma level of IDL and decreased flux into LDL. The LDL synthesized in the apoE-deficient patient is kinetically abnormal in both normal and apoE-deficient subjects, indicating that apoE is necessary for the synthesis of normal LDL. The difference between normal and apoE-deficient LDL could not be ascertained by chemical composition or hydrated density and may reflect a subtle difference in lipid composition, structural organization of the lipid and apoB-100 constituents, or a conformational change in apoB-100. The changes in lipoprotein metabolism induced by the deficiency of apoE have resulted in a probable up-regulation of the LDL receptor based on the results of the increased catabolic rate of normal LDL in the apoE-deficient patient. This may be a compensatory

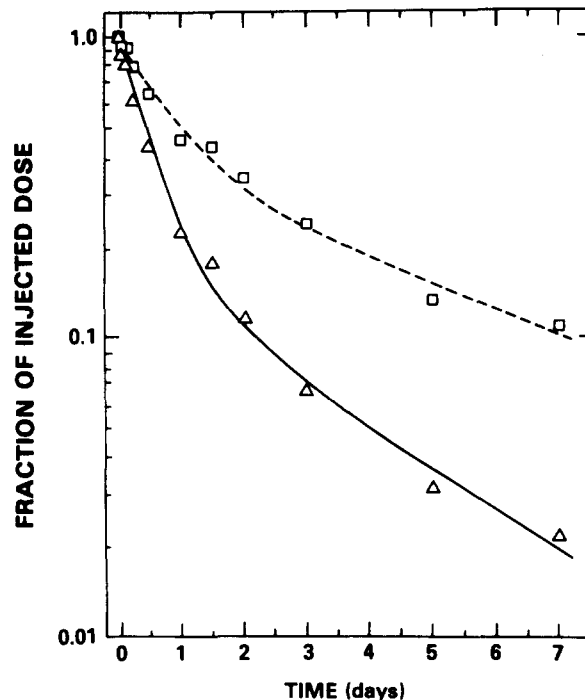


Fig. 5. Catabolism of normal  $^{131}\text{I}$ -labeled LDL apoB-100 ( $\triangle$ — $\triangle$ ) and apoE deficiency  $^{125}\text{I}$ -labeled LDL apoB-100 ( $\square$ — $\square$ ) following injection into an apoE-deficient patient. LDL were separated by sequential ultracentrifugation from 5 ml of plasma at each time point and apoB-100 radioactivity was quantitated following separation by agarose-acrylamide gel electrophoresis (see text for details). Values are expressed as fraction of the first time point.

response, and one may speculate that the decreased hepatic uptake of triglyceride-rich remnants due to the deficiency of apoE leads to a relative deficiency of intracellular cholesterol and/or triglycerides which results in the up-regulation of the LDL receptor pathway to restore the intracellular lipid composition. An up-regulation of the LDL receptor would also tend to normalize LDL catabolism in the apoE-deficient patient by increasing the catabolism of the kinetically abnormal LDL present in the apoE-deficient patient. Based on these conjectures, it would be anticipated that metabolic conditions that result

TABLE 3. Residence times of normal and apoE-deficient LDL apoB-100 in three normal subjects and in an apoE-deficient patient

| Subject                | Residence Time of Normal LDL ApoB-100 | Residence Time of ApoE-deficient LDL ApoB-100 |
|------------------------|---------------------------------------|---|
|                        | <i>days</i>                           |   |
| Normal (n = 3)         | $2.0 \pm 0.3^a$                       | $2.8 \pm 0.5$                                 |
| ApoE-deficient (n = 1) | 1.0                                   | 2.5   |

<sup>a</sup>Values are mean  $\pm$  SD.



TABLE 4. Kinetic parameters of LDL apoB-100 metabolism

| Subject                | LDL ApoB-100<br>Concentration | Residence <sup>a</sup><br>Time | Fractional<br>Catabolic<br>Rate | Production<br>Rate |
|------------------------|-------------------------------|--------------------------------|---------------------------------|--------------------|
|                        | mg/dl                         | days                           | days <sup>-1</sup>              | mg/kg-day          |
| Normal (n = 3)         | 43 ± 1.0 <sup>b</sup>         | 2.0 ± 0.3                      | 0.50 ± 0.08                     | 10 ± 4             |
| ApoE-deficient (n = 1) | 11                            | 2.5                            | 0.40                            | 2                  |

<sup>a</sup>Kinetic values are for normal LDL in normolipidemic control subjects and apoE-deficient LDL in the apoE-deficient patient.

<sup>b</sup>Values are mean ± SD.

in down-regulation of the LDL receptor (e.g., hypothyroidism) (36) would be associated with an increase in the hyperlipoproteinemia in patients with apoE deficiency or type III HLP. Future studies will be directed toward elucidating the apoE-mediated mechanisms involved in the conversion of IDL to LDL and the structural change in the LDL present in the apoE-deficient patient that leads to delayed clearance from the plasma. ■

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