# Dynamics of apolipoprotein E metabolism in humans

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**Abstract** The dynamics of human apoE metabolism were explored by examining the effects of alimentary lipemia and postheparin lipolysis on the plasma level and lipoprotein distribution of apoE. In the studies of alimentary lipemia, fasting and postprandial plasma samples were obtained from five normal adult males, each of whom drank 100 g of corn oil. Although no change in the plasma concentration of apoE accompanied alimentary lipemia, a major redistribution of apoE among lipoproteins occurred. The portion of apoE associated with triglyceride-rich lipoproteins as assessed by agarose column chromatography increased by a mean of 44%. Furthermore, in the two subjects in whom multiple postprandial samples were taken, there were striking linear correlations between plasma triglyceride concentrations and the fraction of apoE in triglyceride-rich lipoproteins ($r$ = 0.96 and 0.73). In contrast, the plasma concentration of apoE fell in each of the seven studies of postheparin lipolysis. The fall averaged 17% of the control plasma apoE level. In hypertriglyceridemic patients, the decline in plasma triglyceride concentration preceded the decline in apoE concentration, suggesting that the decline in apoE was due to removal of remnants of triglyceride-rich lipoproteins. Lipoprotein fractionation demonstrated substantial loss of apoE from triglyceride-rich lipoproteins; the data suggested that this loss of apoE from triglyceride-rich lipoproteins was due both to removal of apoE from plasma and to transfer of apoE to an HDL fraction. During the recovery phase, as plasma triglyceride levels rose, opposite changes occurred: the plasma apoE level rose, apoE in triglyceride-rich lipoproteins increased in concentration, and apoE in HDL decreased in concentration. Furthermore, it became apparent during the recovery phase that apoE in triglyceride-rich lipoproteins was composed of two discrete subfractions. The first subfraction consisted of apoE on larger, probably recently synthesized lipoproteins; the second consisted of apoE on much smaller lipoproteins. These studies provide evidence in intact humans for a dynamic traffic of apoE between triglyceride-rich lipoproteins and high density lipoprotein. This traffic is a prominent phenomenon of normal alimentary lipemia and of lipolysis. By modulating the lipoprotein distribution of apoE, it probably plays a key functional role in lipoprotein metabolism.—**Blum, C. B.** Dynamics of apolipoprotein E metabolism in humans. *J. Lipid Res.* 1982. **23:** 1308–1316.

**Supplementary key words** postheparin lipolysis • alimentary lipemia

Apolipoprotein E, a glycoprotein with molecular weight approximately 35,000, is a quantitatively important protein constituent of the triglyceride-rich lipoproteins of plasma and a minor constituent of the high density lipoprotein fraction of plasma (1–7). This apoprotein has attracted considerable interest in recent years because of its ability to interact with at least two different sorts of cell surface receptors, thereby delivering lipid to cells (8–10). In particular, apoE appears to be of critical importance in the process of hepatic catabolism of chylomicron remnants (11–13). ApoE-rich high density lipoprotein (HDL) seems to be an important modulator of cholesterol metabolism in patients with abetalipoproteinemia (14), and abnormalities of apoE seem central to the pathogenesis of type III hyperlipoproteinemia (15–17).

However, the current literature on the dynamics of the metabolism of apoE in intact humans is limited to two reports. A brief paper by Gregg et al. (18) suggests that human apoE may be removed from the plasma much more rapidly than other apolipoproteins; they reported a mean plasma residence time for apoE of 0.35 days in a group of normal volunteers who underwent tracer kinetic studies. They did not assess the possibility of exchange or transfer of apoE between lipoprotein classes. Falko et al. (19) have reported a redistribution of apoE from HDL to very low density lipoprotein (VLDL) concomitant with the hypertriglyceridemia of a chronic high carbohydrate diet, raising the possibility of a metabolic traffic of apoE between triglyceride-rich lipoproteins and high density lipoprotein.

This report describes experiments in which short-term perturbations of lipoprotein metabolism were used in an attempt to demonstrate some of the dynamic features of plasma apoE metabolism. In particular, the effects of alimentary lipemia and postheparin lipolysis on the plasma level and lipoprotein distribution of apoE were studied in a group of normal and hypertriglyceridemic humans.

Some of the findings presented here have previously been reported in abstract form (20).

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

## METHODS

### Subjects

Normal volunteers were healthy males aged 30–47 years with plasma cholesterol and triglyceride concentrations below the age- and sex-specific 95th percentile limits (21). They were in good health and were taking no medications. The three hypertriglyceridemic patients were being followed in the Lipid Clinic of the Columbia University Specialized Center of Research in Arteriosclerosis. They were men aged 35–50 years. They were not diabetic, and they had normal renal, hepatic, and thyroid function. They were taking no medications when studied. Subjects for study of alimentary lipemia are designated 1F–5F. Subjects for study of postheparin lipolysis are designated 1H–7H. One individual underwent both studies and he is designated 2F and 4H.

### Radioimmunoassay of apolipoprotein E

The procedure for double antibody radioimmunoassay of apoE has been described in detail (5). In brief, standards or unknowns were preincubated overnight in a solution of 50 mM sodium phosphate, 100 mM NaCl, 0.02% sodium azide, 50 mM sodium decyl sulfate, pH 7.4. The assay was performed in the presence of a final concentration of 5 mM sodium decyl sulfate. Following a 48-hr incubation of standards or unknowns with $^{125}$I-labeled apoE and rabbit anti-apoE antiserum, sheep or goat antiserum against rabbit gamma globulin was added. The precipitate was harvested by centrifugation 24 hr later. The within-assay coefficient of variation was 9%, and the coefficient of variation for systematic between-assay variability was 3%.

### Fractionation of plasma lipoproteins

*Agarose column chromatography.* Whole plasma (1–2 ml) was applied to a 1.0 × 120 cm or 1.5 × 90 cm column of 6% agarose (BioGel A5M, BioRad Laboratories, Richmond, CA) and was eluted at 4°C with a solution of 0.2 M NaCl, 1 mM EDTA, 2 mM sodium phosphate, 0.02% sodium azide, pH 7.4. All plasma samples were applied to the columns within 45 min of the time of venipuncture.

*Preparative ultracentrifugation.* Aliquots of plasma were adjusted to densities 1.019 and 1.21 g/ml by addition of NaCl or KBr. Each aliquot underwent a single ultracentrifugation at 4°C and 40,000 rpm in a Beckman 40.3 rotor in a Beckman L5-75 ultracentrifuge. The aliquot at 1.019 g/ml was centrifuged for 18 hr; the aliquot at 1.21 g/ml was centrifuged for 48 hr. Top and bottom fractions were separated by tube slicing. ApoE in these fractions was measured by radioimmunoassay as described above.

### Protocols for perturbation studies

Studies of alimentary lipemia were performed in five normal volunteers after a 12-hr fast. A baseline blood sample was obtained after which the subjects drank 100 g of corn oil. In all cases, blood was taken 5 hr later. In two subjects (4F and 5F), multiple serial samples were taken after the oral dose of corn oil. Blood was collected in EDTA (1 mg/ml) and was immediately cooled on crushed ice. Plasma obtained by low speed centrifugation in a refrigerated centrifuge was assayed for cholesterol, triglyceride, and apoE content. Aliquots were immediately fractionated by ultracentrifugation and by agarose column chromatography.

Studies of the effects of postheparin lipolysis were performed in three hypertriglyceridemic patients and four normal male volunteers after a 12-hr fast. A baseline blood sample was obtained, after which the subjects received an intravenous injection of sodium heparin (50 u/kg). Serial blood samples were obtained over the ensuing 8 hr. Blood was anticoagulated with EDTA (1 mg/ml) and was immediately cooled by passing it through 2 meters of polyethylene tubing which had been submerged in ice water. Plasma was obtained by centrifugation for 15 min at 2,000 rpm at 4°C. To a 10-ml aliquot of plasma, 1.11 ml of 5 M NaCl was immediately added, producing a background salt density of 1.019 g/ml and a NaCl concentration of approximately 0.6 M. This would be expected to inhibit lipoprotein lipase activity nearly completely (22). This aliquot was used for cholesterol and triglyceride determinations and for lipoprotein fractionation by ultracentrifugation. Aliquots for cholesterol and triglyceride determination were immediately frozen and were thawed just prior to extraction with isopropanol. Another aliquot, to which NaCl had not been added, was used for lipoprotein fractionation by agarose column chromatography and for determination of total plasma apoE concentration. Samples were chromatographed from volunteers 5H and 7H only. They were applied to the columns within 30 min of the time of venipuncture and were eluted in about 18 hr.

### Analytical techniques

Cholesterol and triglyceride concentrations in plasma were measured by Auto-Analyzer I methodology (23, 24). Cholesterol concentrations in extracts of agarose column fractions were measured by the method of Chiamori and Henry (25).

## RESULTS

### Alimentary lipemia

The results of the five studies of alimentary lipemia are depicted in **Table 1.** The postprandial values, rep-

TABLE 1. Effects of alimentary lipemia on concentration and lipoprotein distribution of apoE

| Sub. | | Triglyceride | ApoE | % of ApoE in Triglyceride-Rich Lipoproteins | |
|---|---|---|---|---|---|
| | | | | By Column | By Ultracentrifugation |
| | | *mg/dl* | *μg/ml* | | |
| 1F | Fasting | 70 | 32 | 35 | 23 |
| | Postprandial | 188 | 32 | 60 | 43 |
| 2F | Fasting | 123 | 41 | 51 | 37 |
| | Postprandial | 183 | 38 | 61 | 51 |
| 3F | Fasting | 133 | 31 | 42 | 23 |
| | Postprandial | 207 | 35 | 65 | 52 |
| 4F | Fasting | 76 | 35 | 47 | 21 |
| | Postprandial | 133 | 34 | 63 | 35 |
| 5F | Fasting | 46 | 31 | 20 | 5 |
| | Postprandial | 78 | 31 | 28 | 12 |
| Mean Fasting (±SEM) | | 90 ± 17 | 34 ± 2 | 39 ± 5 | 22 ± 5 |
| Postprandial | | 158 ± 23 | 34 ± 1 | 55 ± 7 | 39 ± 7 |

For all subjects except 5F, the postprandial sample was taken 5 hr after ingestion of 100 g of corn oil. In subject 5F a biphasic triglyceride curve was seen with a nadir at 5 hr, and peaks at 3 hr and 7 hr. Since the 3-hr sample was associated with the highest observed triglyceride concentration, it was used to indicate postprandial values in this table.

resenting samples taken 5 hr after ingestion of 100 g of corn oil (3 hr for subject 5F whose triglyceride/time curve was biphasic with peaks at 3 and 7 hr), demonstrate a mean increase in plasma triglyceride concentration of 84% (22% SE). The total plasma cholesterol concentration remained quite constant, undergoing a mean increment of 1.0% (1.5% SE) compared to control values. Similarly, there was no consistent change in plasma apoE levels; the mean ratio of postprandial to fasting levels being 1.01 (1.034 SE).

Fractionation of the plasma lipoproteins revealed striking changes associated with alimentary lipemia. A marked increase in the fraction of plasma apoE associated with triglyceride-rich lipoproteins attended alimentary lipemia (Table 1). This was seen to be the case when plasma was fractionated by agarose column chromatography or by ultracentrifugation. The mean increment in the fraction of plasma apoE associated with triglyceride-rich lipoproteins was 44% when assessed by agarose column chromatography; the mean increment was 92% when assessed by ultracentrifugation, a method more likely to introduce artifact (5).

**Fig. 1** depicts agarose column chromatograms of fasting and lipemic plasma from subject 1F. The areas under the two apoE curves were similar; hence the total mass of apoE eluting from the column in the fasting sample was not substantially different from that eluting in the lipemic sample. In the lipemic curve, however, the first peak (triglyceride-rich lipoproteins) was seen to have grown substantially compared to fasting values; this growth was entirely at the expense of the size of the second peak (a subfraction of HDL). Thus, with alimentary lipemia, the total plasma concentration of apoE did not change, although a major transfer from HDL to triglyceride-rich lipoproteins was apparent.

In two subjects (4F and 5F), multiple samples were obtained during the 8 hr following the fatty meal. In both cases, a strong linear correlation between the plasma triglyceride concentration and the fraction of plasma apoE present in triglyceride-rich lipoproteins was found ($r = 0.96$ for subject 4F, $r = 0.73$ for subject 5F) (**Fig. 2**).

### Post-heparin lipolysis

The effects of post-heparin lipolysis on plasma triglyceride and apoE concentrations are shown in **Table 2.** In each case, the plasma triglyceride concentration fell promptly after injection of heparin. In all four subjects studied for more than 4 hr after the injection of heparin, plasma triglyceride concentration eventually rose to levels exceeding the baseline.

Plasma apoE concentrations were also seen to fall following intravenous heparin injection. In hypertriglyceridemic patients (1H–3H), the decline in apoE levels was delayed compared to the fall in triglyceride levels. Thus, in these patients, the minimum plasma tri-

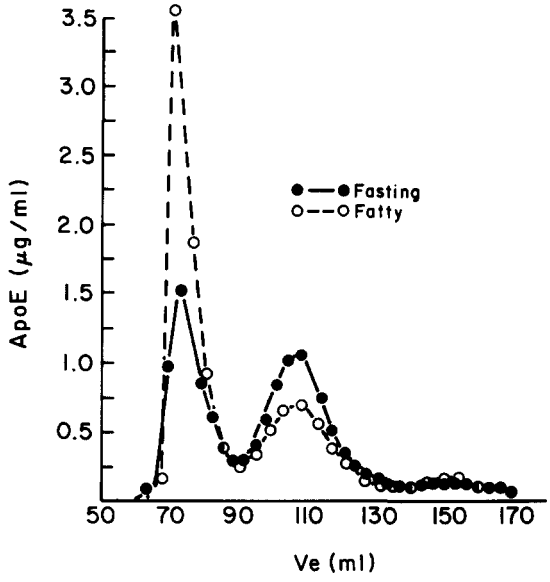


**Fig. 1.** Effect of alimentary lipemia on the lipoprotein distribution of apoE. Fasting and 5-hr postprandial blood samples were obtained from subject 1F who drank 100 g corn oil. Plasma, 2-ml aliquots, was fractionated by gel filtration on a 1.5 × 90 cm column of 6% agarose. ApoE concentration in column eluates was measured by radioimmunoassay. The first peak of apoE corresponds to the elution volume of triglyceride-rich lipoproteins; the second peak corresponds to the elution volume of large HDL particles.

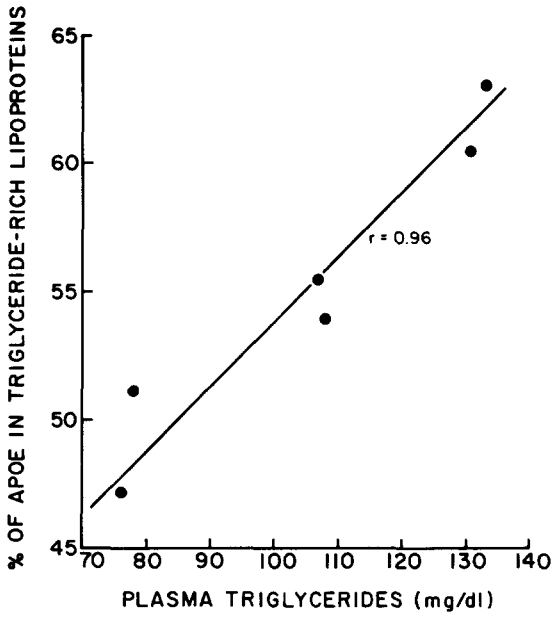


**Fig. 2.** Effect of alimentary lipemia on the lipoprotein distribution of apoE. A fasting blood sample and multiple postprandial samples were taken over the 8-hr period after subject 4F drank 100 g of corn oil. The lipoprotein distribution of apoE was determined by gel filtration over 1 × 120 cm columns of 6% agarose.

glyceride value was observed 45 min after heparin injection, while the minimum apoE level was seen 1 to 3 hr after heparin injection. In three of the four normal volunteers, no delay was seen in the apoE curve compared to the triglyceride curve; the minimum apoE value occurred simultaneously with or even earlier than the minimum triglyceride value.

In all of the studies of postheparin lipolysis, the distribution of apoE among lipoproteins was assessed by ultracentrifugation at 1.019 and 1.21 g/ml. **Table 3** indicates that in each case the portion of plasma apoE in d < 1.019 g/ml fell with postheparin lipolysis. In five of the seven subjects, however, the fall in apoE in the d < 1.019 g/ml fraction was exceeded by an increase in apoE in the nonlipoprotein fraction of d > 1.21 g/ml. On average, a rise in apoE in d > 1.21 g/ml exceeded the fall in apoE in d < 1.019 g/ml by 30%. In previous studies it had been found that in the absence of postheparin lipolysis apoE in d > 1.21 g/ml represents an artifact due to ultracentrifugation (5) and that when plasma was fractionated by agarose column chromatography all apoE appeared in association with lipoproteins. Thus, in the present studies it could not be determined with certainty on the basis of ultracentrifugation whether postheparin lipolysis removed apoE from triglyceride-rich lipoproteins to the nonlipoprotein fraction of plasma or resulted in its being more susceptible to artifactual separation from lipoproteins by ultracentrifugation. Consequently, in two of the subjects (5H and 7H), serial samples were fractionated by agarose column chromatography (**Fig. 3, Table 4**). In these experiments, all plasma apoE eluted from the columns in association with lipoproteins. Therefore, postheparin lipolysis did not itself remove apoE to the nonlipoprotein fraction of plasma. However, it did enhance the ability of ultracentrifugation to remove apoE from lipoproteins.

In Table 4, data from both subjects (5H and 7H) are presented numerically. The plasma apoE concentration was measured and was apportioned between triglyceride-rich lipoproteins and high density lipoprotein ac-

TABLE 2. Time course of plasma apoE and triglyceride concentrations during postheparin lipolysis

| | | | Percent of Control Value at Indicated Times (Hours) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sub. | | Control[a] | 0.25 | 0.50 | 0.75 | 1.00 | 1.50 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 | 7.00 | 8.00 |
| 1H | Triglyceride | 341 | 84.7 | | 70.4 | | | 75.4 | | 90.0 | | 100.9 | | 97.7 |
| | ApoE | 70.6 | 95.0 | | 78.6 | | | 72.7 | | 82.2 | | 88.2 | | 85.0 |
| 2H | Triglyceride | 285 | 81.1 | | 64.2 | | | 84.9 | 112.6 | 118.9 | 138.9 | 121.1 | | |
| | ApoE | 53.6 | 99.4 | | 91.8 | | | 85.1 | 95.9 | 86.6 | 122.9 | 121.3 | | |
| 3F | Triglyceride | 414 | 67.4 | | 63.8 | | | 66.4 | 63.8 | 92.0 | 105.6 | 106.0 | 111.6 | 116.4 |
| | ApoE | 70.8 | 112.3 | | 112.4 | | | 83.8 | 83.8 | 87.3 | 97.0 | 117.5 | 118.9 | 113.3 |
| 4H | Triglyceride | 113 | 82.3 | 69.0 | 61.9 | 63.7 | | 68.1 | 98.2 | 115.0 | | 116.8 | | |
| | ApoE | 38.0 | 97.6 | 95.8 | 90.0 | 86.3 | | 91.3 | 105.3 | 113.2 | | 110.5 | | |
| 5H | Triglyceride | 104 | 78.7 | 60.6 | 54.3 | | 70.2 | | 107.4 | | | | | |
| | ApoE | 39.7 | 89.2 | 84.6 | 83.9 | | 90.4 | | 89.2 | | | | | |
| 6H | Triglyceride | 61 | 72.7 | 72.7 | 65.5 | | 72.7 | | 87.3 | | | | | |
| | ApoE | 38.0 | 82.9 | 81.1 | 82.9 | | 84.7 | | 87.1 | | | | | |
| 7H | Triglyceride | 135 | 82.0 | 64.8 | 59.0 | | 56.6 | | 68.0 | | | | | |
| | ApoE | 27.5 | 103.2 | 96.0 | 98.3 | | 89.1 | | 92.7 | | | | | |

[a] Plasma triglyceride concentration is in mg/dl; plasma apoE concentration is in μg/ml.

TABLE 3. Lipoprotein distribution of apoE assessed by ultracentrifugation during postheparin lipolysis

| | Percent of ApoE in d < 1.019 g/ml Lipoproteins at Indicated Times (Hours) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 | 1.50 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 | 7.00 | 8.00 |
| | | | | | | | % | | | | | | |
| 1H | 55.8 | 41.6 | | 40.6 | | | 40.1 | | 48.9 | | 56.2 | | 54.8 |
| 2H | 50.9 | 42.8 | | 35.8 | | | 42.4 | 52.4 | 60.3 | 65.4 | 60.7 | | |
| 3H | 66.7 | 38.4 | | 36.5 | | | 40.6 | 54.0 | 64.2 | 60.6 | 67.8 | | 74.5 |
| 4H | 23.5 | 14.5 | 11.7 | 7.6 | 7.1 | | 9.1 | 22.9 | 29.7 | | 39.9 | | |
| 5H | 19.0 | 12.5 | 5.6 | 2.5 | | 3.6 | | 20.4 | | | | | |
| 6H | 3.5 | 0.0 | 0.0 | 0.0 | | 1.2 | | 0.7 | | | | | |
| 7H | 53.5 | 30.2 | 30.3 | 27.7 | | 13.3 | | 27.3 | | | | | |

cording to the elution of apoE immunoreactivity from 6% agarose columns. In both subjects, an immediate fall in the apoE content of VLDL occurred. This was in qualitative agreement with the results of ultracentrifugal fractionation of plasma during postheparin lipolysis. Fifteen minutes after heparin injection, the fall in VLDL apoE averaged 24% of the baseline concentration, and it was greatest (49% of baseline) 45 min after the onset of the study. Associated with the fall in VLDL apoE were a large decrease in the total plasma concentration of apoE and a smaller increase in HDL apoE. The increases in HDL apoE were very striking when expressed as percent of baseline concentration: 25% for subject 5H and 83% for subject 7H. These data are consistent with the interpretation that some VLDL apoE was transferred to HDL and that some was directly removed from the plasma consequent to intravascular lipolysis. Opposite changes were seen at later times when a fall in HDL apoE was associated with a rise in VLDL apoE; this suggested transfer of apoE from HDL to newly synthesized VLDL as those newly formed particles began to accumulate in the circulation.

To determine whether the redistribution of apoE among lipoprotein classes in the course of postheparin lipolysis may have been an artifact of the interaction of heparin with lipoproteins rather than a result of lipolysis, aliquots of control plasma and plasma incubated in vitro with heparin (100 u/ml) were chromatographed on agarose columns. The concentration of heparin used was 100 times the peak plasma concentration during the in vivo studies of postheparin lipolysis. Control and heparin-treated plasma samples gave identical profiles of apoE immunoreactivity in agarose column eluates. Thus, direct interaction of heparin with lipoproteins is not capable of simulating the altered lipoprotein distribution of apoE seen in the in vivo studies of postheparin lipolysis.

Fig. 3 presents the data from subject 5H graphically. By 15 min after heparin injection, the amplitude of the peak of VLDL apoE was much diminished; a shoulder in the ascending limb of that peak had been abolished and the area under the HDL peak was clearly increased. Furthermore, the peak of VLDL apoE eluted earlier than in the control sample. This last finding indicated that those apoE molecules initially on the smallest VLDL particles had been removed from the circulation (perhaps with these particles), transferred to slightly larger VLDL particles, or transferred to HDL. As time passed, the elution volume of the peak of VLDL apoE increased. Furthermore, in both of the studies in which column fractionation was used (subjects 5H and 7H) a discrete, very early eluting peak of apoE on large VLDL particles became apparent. This peak had the same elution volume and was of the same amplitude as the shoulder on the ascending limb of VLDL apoE in the control sample. However, in the last samples it was evident as a distinct peak because the second portion of the peak of VLDL apoE never approached its initial amplitude during the 3-hr course of this study. This incomplete regeneration of the second part of the apoE peak may have resulted from incomplete repletion of endothelial lipoprotein lipase activity during the 3 hr following intravenous heparin injection. The observation that plasma triglyceride levels rose to greater than control values supports this explanation. Such incomplete regeneration of tissue lipolytic activity might reduce the rate of formation of small VLDL from larger particles, thereby increasing the ratio of large to small VLDL.

## DISCUSSION

Previous studies have suggested a metabolic relationship of apoE in triglyceride-rich lipoproteins with apoE in high density lipoprotein. Exchange of rat apoE between VLDL and normal plasma HDL subfractions has been demonstrated in tracer kinetic studies (26, 27). Moreover, experiments of several sorts have suggested that under certain conditions, mass transfer of apoE from HDL to triglyceride-rich lipoproteins may occur, while under other circumstances mass transfer in the opposite direction may occur. Thus, in the rat, apoE is

a major protein of discoidal HDL in liver perfusates, but it is relatively less prominent in plasma HDL, suggesting that loss of apoE from hepatic HDL may occur following its secretion (28, 29). In the hereditary human disease lecithin:cholesterol acyltransferase deficiency, the abnormal discoidal apoE-rich HDL release their apoE to triglyceride-rich lipoproteins on in vitro incubation with lecithin:cholesterol acyltransferase (30). With rat or human lipoproteins, in vitro incubation of lymph chylomicrons, which contain little if any apoE, with normal HDL has resulted in a relative enrichment of the apoE content of the chylomicrons as assessed by polyacrylamide gel electrophoresis (31, 32). These experiments demonstrated in vitro transfer of apoE from HDL to chylomicrons.

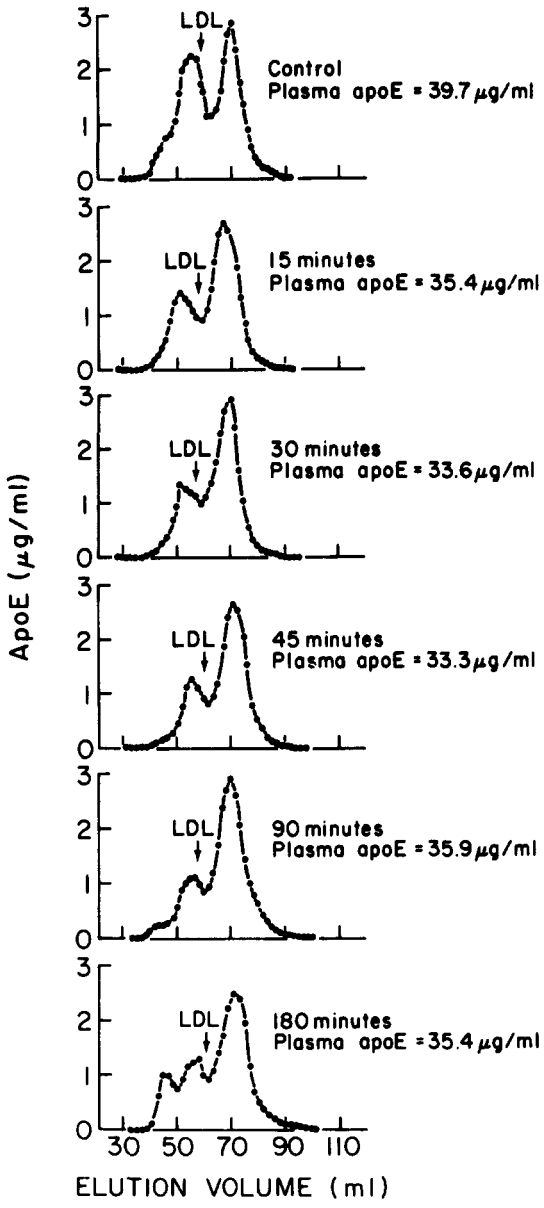


**Fig. 3.** Effect of postheparin lipolysis on lipoprotein distribution of apoE. Multiple blood samples were obtained from subject 5H who received intravenous heparin, 50 U/kg. The blood was immediately chilled, and plasma was promptly obtained and applied to 1 × 120 cm columns of 6% agarose. The concentation of apoE in the column eluate is plotted. The elution volume of the peak of low density lipoprotein (LDL) cholesterol is indicated by an arrow. The ranges of elution volumes of ultracentrifugally isolated lipoproteins are as follows: VLDL (d < 1.006 g/ml) 35–55 ml, IDL (d 1.006–1.019 g/ml) 47–60 ml, LDL (d 1.019–1.063 g/ml) 52–64 ml, $HDL_2$ (d 1.063–1.125 g/ml) 65–75 ml, and $HDL_3$ (d 1.125–1.21 g/ml) 67–78 ml.

TABLE 4. Lipoprotein distribution of apoE by agarose column chromatography during postheparin lipolysis

| | Subject 5H | | | Subject 7H | | |
|---|---|---|---|---|---|---|
| Time | Plasma | VLDL | HDL | Plasma | VLDL | HDL |
| *hr* | *apoE, μg/ml* | | | | | |
| 0.00 | 39.7 | 19.1 | 20.6 | 27.5 | 18.9 | 8.6 |
| 0.25 | 35.4 | 12.4 | 23.0 | 28.4 | 16.6 | 11.8 |
| 0.50 | 33.6 | 10.1 | 23.2 | 26.4 | 13.3 | 13.1 |
| 0.75 | 33.3 | 9.3 | 24.0 | 25.8 | 10.1 | 15.7 |
| 1.50 | 35.9 | 10.2 | 25.7 | 24.5 | 10.8 | 13.6 |
| 3.00 | 35.4 | 13.7 | 21.6 | 25.5 | 12.0 | 13.5 |

Transfer of apoE in the opposite direction, from triglyceride-rich lipoproteins to HDL, has been seen to occur in the functionally hepatectomized rat (33). In this model, ongoing peripheral lipolysis occurs in the absence of hepatic uptake of remnants of chylomicrons or VLDL. One might speculate that under those conditions, with remnant removal blocked, transfer of apoE from triglyceride-rich lipoproteins undergoing catabolism might occur even if it would not occur normally. However, those experiments did establish a precedent for the occurrence of such transfer in vivo in the rat even if they did not prove it to be a normal occurrence.

In previous work, we demonstrated that in normal humans a subfraction of HDL bears the majority of plasma apoE, while in hypertriglyceridemic patients most plasma apoE was in triglyceride-rich lipoproteins (5). This suggested an analogy between apoE and the C apoproteins, for which exchange and transfer between triglyceride-rich lipoproteins and HDL occur and for which a functional role in triglyceride-rich lipoproteins is most important (34).

The experiments described here were designed to illustrate some of the dynamics of apoE metabolism in the course of two major processes involved in the normal physiology of fat transport in plasma: alimentary lipemia and lipolysis. These experiments, by utilizing marked perturbations from the steady state, have permitted inferences regarding the effects on apoE of alimentary lipemia and lipolysis.

The experiments involving fat feeding are straightforward in their interpretation, and those experiments demonstrate normal events occurring regularly in hu-

mans. They showed a marked redistribution of apoE from HDL to triglyceride-rich lipoproteins during alimentary lipemia. There was a strong linear relationship between the plasma triglyceride concentration and the portion of plasma apoE residing in triglyceride-rich lipoproteins. In the course of these studies, there was no significant change in the total plasma concentration of apoE, a finding consistent with the concept that the observed redistribution of apoE between lipoprotein classes represents mass transfer from HDL to triglyceride-rich lipoproteins. Furthermore, this finding of constant total plasma apoE levels in the course of the experiments with alimentary lipemia is consistent with data generated by others indicating that apoE is primarily a synthetic product of the liver and is not synthesized by the intestine (28, 29, 31, 35–37). In contrast to our findings with apoE, apoA-I levels increase and apoA-I is transferred to HDL during alimentary lipemia (32, 38).

Experiments involving postheparin lipolysis should be interpreted with circumspection because of known differences between postheparin lipolysis and normal physiological lipolysis. Postheparin lipolysis occurs very rapidly and in plasma, while physiological lipolysis occurs more slowly and at the capillary endothelium (39). Postheparin lipolysis has been found to result in the production of morphologically unusual lipoproteins (40) including large (400–1200 Å) flattened structures in the 1.006–1.019 g/ml and 1.019–1.063 g/ml density ranges, and lamellar structures on the surface of chylomicron-like particles. Furthermore, a suppression of LCAT activity has been reported to occur for 30 min after heparin injection (41); thus, studies of postheparin lipolysis may show the results of transient LCAT deficiency as well as accelerated lipolytic activity. Finally, in such experiments lipolysis may continue to occur in vitro producing discrepancies between experimental observations and conditions in vivo. We have attempted to avoid this last source of artifact by cooling blood samples to 0°C as soon as they were drawn, by adding NaCl (to 0.6 M) to plasma, and by beginning chromatography of plasma within 30 min of venipuncture. Hepatic triglyceride lipase would not be expected to have activity in the samples because it is inhibited by low concentrations of HDL (42). Despite the potential artifacts involved in studies of postheparin lipolysis, in many important respects such studies, and conceptually similar studies of in vitro lipolysis, have produced results closely analogous to those obtained with physiological systems, i.e., perfused organs or intact animals. Thus, in both kinds of lipolytic systems, LDL-like particles are formed, and there is a transfer of C apoproteins, phospholipids, and unesterified cholesterol from triglyceride-rich lipoproteins to HDL (43–48). Finally, the expected artifacts in studies involving postheparin lipolysis involve the lipolytic phase of those studies, and phenomena occurring during the subsequent return to a steady state are more likely to reflect normal physiological events. Thus interpretation of events of the recovery phase may be more straightforward.

The finding of a fall in the plasma concentration of apoE in the course of postheparin lipolysis was of considerable interest. The mean maximum measured fall in plasma apoE concentrations was 17% of the baseline value. That the fall in plasma apoE concentration followed the fall in plasma triglyceride concentration in hypertriglyceridemic patients suggests that apoE was removed from the circulation in the process of remnant removal: remnants must *first* be formed by lipolysis and *then* they may be removed by specific hepatic receptors. This delay was not generally seen in the normal volunteers. The absence of a delay in normal volunteers may reflect more rapid generation of remnants in normals; consequently, our sampling may not have been early enough or frequent enough in the normal volunteers to document a delay in the fall of apoE relative to the fall in plasma triglyceride levels. The fall in the plasma apoE concentration with postheparin lipolysis is consistent with the concept that receptors for apoE are responsible for uptake of remnants of triglyceride-rich lipoproteins (11–13). Furthermore, it suggests that apoE is removed from the circulation concurrently with remnant removal, rather than being quickly transferred to circulating lipoproteins and being immediately reutilized as a ligand for cell-surface receptors.

Agarose column fractionation of plasma samples obtained in the course of postheparin lipolysis demonstrated two discrete apoE-containing fractions within VLDL, one fraction in very large particles and the other in much smaller particles. Because the peak of apoE in larger sized particles appears most distinctly during the phase of recovery of plasma triglyceride concentrations, when intravascular lipolysis has waned, it seems likely that this peak represents apoE on newly synthesized VLDL. The second peak of apoE immunoreactivity within triglyceride-rich lipoproteins probably represents catabolic remnants.

Quantitation of the distribution of apoE between triglyceride-rich lipoproteins and high density lipoproteins demonstrated a simultaneous increase in apoE in the HDL fraction and a decrease in the apoE content of plasma and of VLDL; this suggested a transfer of apoE from VLDL to HDL. Other interpretations consistent with these data seem highly unlikely. Thus, if the increase in HDL apoE during the first 15 min after heparin injection represented new synthesis or represented transfer of apoE from a nonplasma pool, the rate of this

new synthesis or transfer would be 25–50% of the plasma pool per hr, values far too high to be credible. During the recovery phase of these studies, as plasma triglyceride levels increased, a transfer of apoE in the opposite direction, from HDL to VLDL, seemed to take place.

In summary, these studies provide evidence in intact humans for a dynamic traffic of apoE between triglyceride-rich lipoproteins and high density lipoprotein. The influx of triglyceride-rich lipoproteins into the circulation and their removal from the circulation appear to be important determinants of the net direction and amplitude of this traffic. This phenomenon is prominent in normal alimentary lipemia and in lipolysis, and by modulating the lipoprotein distribution of apoE, it may play a key functional role in lipoprotein metabolism.

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## REFERENCES

1. Shore, V. G., and B. Shore. 1973. Heterogeneity of plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry.* **12:** 502–507.
2. Shelburne, F. A., and S. H. Quarfordt. 1974. A new apoprotein of human plasma very low density lipoproteins. *J. Biol. Chem.* **249:** 1428–1433.
3. Havel, R. J., and J. P. Kane. 1973. Primary dysbetalipoproteinemia: predominance of a specific apoprotein species in triglyceride-rich lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70:** 2015–2019.
4. Utermann, G. 1975. Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very low density lipoproteins: apolipoprotein E. *Hoppe-Seyler's Z. Physiol. Chem.* **356:** 1113–1121.
5. Blum, C. B., L. Aron, and R. Sciacca. 1980. Radioimmunoassay studies of human apolipoprotein E. *J. Clin. Invest.* **66:** 1240–1250.
6. Havel, R. J., L. Kotite, J. L. Vigne, J. P. Kane, P. Tun, N. Phillips, and G. C. Chen. 1980. Radioimmunoassay of human arginine-rich apolipoprotein, apolipoprotein E. Concentration in blood plasma and lipoproteins as affected by apoprotein E-3 deficiency. *J. Clin. Invest.* **66:** 1351–1362.
7. Weisgraber, K. H., and R. W. Mahley. 1978. Apoprotein (E-A-II) complex of human plasma lipoproteins. I. Characterization of this mixed disulfide and its identification in a high density lipoprotein subfraction. *J. Biol. Chem.* **253:** 6281–6288.
8. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1979. Binding of arginine-rich (E) apoprotein after recombination with phospholipid vesicles to the low density lipoprotein receptors of fibroblasts. *J. Biol. Chem.* **254:** 4186–4190.
9. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes. Metabolically distinct apoE and apo-B, E receptors. *J. Biol. Chem.* **256:** 5646–5655.
10. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. *J. Clin. Invest.* **68:** 1197–1206.
11. Sherill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of canine lipoproteins containing only the E apoprotein by a high affinity receptor (identity with the chylomicron remnant transport process). *J. Biol. Chem.* **255:** 1804–1807.
12. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652–658.
13. Windler, E., Y. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat: opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* **255:** 8303–8307.
14. Blum, C. B., R. Deckelbaum, L. Witte, and M. Fainaru. 1980. Apolipoprotein E in abetalipoproteinemia. *Circulation.* **62(Part II):** 43.
15. Utermann, G., M. Jaeschke, and J. Mangel. 1975. Familial hyperlipoproteinemia type III: deficiency of a specific apolipoprotein (apoE-III) in the very low density lipoproteins. *FEBS Lett.* **56:** 352–355.
16. Zannis, V. I., and J. L. Breslow. 1980. Characterization of a unique human apoE variant associated with type III hyperlipoproteinemia. *J. Biol. Chem.* **255:** 1759–1762.
17. Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1240.
18. Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia: defective metabolism of an abnormal apolipoprotein E. *Science.* **211:** 584–586.
19. Falko, J. M., G. Schonfeld, J. L. Witztum, J. B. Kolar, S. W. Weidman, and R. Steelman. 1980. Effects of diet on apoprotein E levels and on the apoprotein E subspecies in human plasma lipoproteins. *J. Clin. Endocrinol. Metab.* **50:** 521–528.
20. Blum, C. B. 1980. Dynamics of apolipoprotein E metabolism in humans. *Circulation.* **62(Part II):** 194.
21. The Lipid Research Clinics Program Epidemiology Committee. 1979. Plasma lipid distributions in selected North American populations: The Lipid Research Clinics Program Prevalence Study. *Circulation.* **60:** 427–439.
22. Krauss, R. M., H. G. Windmueller, R. I. Levy, and D. S. Frederickson. 1973. Selective measurement of two different triglyceride lipase activities in rat postheparin plasma. *J. Lipid Res.* **14:** 286–295.
23. Technicon Instruments. Total cholesterol procedure: N-24b. 1964. *In* Auto-Analyzer Manual. Chauncy, New York. 345.
24. Lipid Research Clinics Program. 1974. Manual of laboratory operations. Volume I. Lipid and lipoprotein analysis. National Institutes of Health, Department of Health, Education, and Welfare Publication No. (NIH) 75-628. 74–81.

25. Chiamori, N., and R. J. Henry. 1959. Study of the ferric chloride method for determination of total cholesterol and cholesterol esters. *Am. J. Clin. Pathol.* **31:** 305–309.
26. Weisgraber, K. H., R. W. Mahley, and G. Assmann. 1977. The rat arginine-rich apoprotein and its redistribution following injection of iodinated lipoproteins into normal and hypercholesterolemic rats. *Atherosclerosis.* **28:** 121–140.
27. Van't Hooft, F., and R. J. Havel. 1981. Metabolism of chromatographically separated rat serum lipoproteins specifically labeled with $^{125}$I-apolipoprotein E. *J. Biol. Chem.* **256:** 3963–3968.
28. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
29. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.
30. Glomset, J. A., C. D. Mitchell, W. C. King, K. A. Applegate, T. Forte, K. R. Norum, and E. Gjone. 1980. In vitro effects of lecithin:cholesterol acyltransferase on apolipoprotein distribution in familial lecithin:cholesterol acyltransferase deficiency. *Ann. NY Acad. Sci.* **348:** 224–241.
31. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.
32. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins: studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
33. Davis, R. A., A. D. Hartman, L. Dory, B. J. van Lenten, and P. S. Roheim. 1981. Metabolic fate of VLDL apolipoproteins B and E in hepatectomized rats. *Biochim. Biophys. Acta.* **665:** 154–164.
34. Schaefer, E. J., S. Eisenberg, and R. I. Levy. 1978. Lipoprotein apoprotein metabolism. *J. Lipid Res.* **19:** 667–687.
35. Riley, J. W., R. M. Glickman, P. H. R. Green, and A. R. Tall. 1980. The effect of chronic cholesterol feeding on intestinal lipoproteins in the rat. *J. Lipid Res.* **21:** 942–952.
36. Davis, R. A., S. C. Engelhorn, S. H. Pangburn, D. B. Weinstein, and D. Steinberg. 1979. Very low density lipoprotein synthesis and secretion by cultured rat hepatocytes. *J. Biol. Chem.* **254:** 2010–2016.
37. Zannis, V. I., D. M. Kurnit, and J. L. Breslow. 1982. Hepatic apoA-I and apoE and intestinal apoA-I are synthesized in precursor isoprotein forms by organ cultures of human fetal tissues. *J. Biol. Chem.* **257:** 536–544.
38. Tall, A. R., C. B. Blum, G. P. Forester, and C. A. Nelson. 1982. Changes in the distribution and composition of plasma high density lipoproteins after ingestion of fat. *J. Biol. Chem.* **257:** 198–207.
39. Blanchette-Mackie, E. J., and R. O. Scow. 1971. Sites of lipoprotein lipase activity in adipose tissue perfused with chylomicrons: electron microscope cytochemical study. *J. Cell Biol.* **51:** 1–25.
40. Forte, T. M., R. M. Krauss, F. T. Lindgren, and A. V. Nichols. 1979. Changes in plasma lipoprotein distribution and formation of two unusual particles after heparin-induced lipolysis in hypertriglyceridemic subjects. *Proc. Natl. Acad. Sci. USA.* **76:** 5934–5938.
41. Homma, Y., and P. J. Nestel. 1975. Changes in plasma lipoprotein constituents during constant infusions of heparin. *Atherosclerosis.* **22:** 551–563.
42. LaRosa, J. C., R. I. Levy, H. G. Windmueller, and D. S. Fredrickson. 1972. Comparison of the triglyceride lipase of liver, adipose tissue, and postheparin plasma. *J. Lipid Res.* **13:** 356–363.
43. Chajek, T., and S. Eisenberg. 1978. Very low density lipoprotein: metabolism of phospholipids, cholesterol, and apolipoprotein C in the isolated perfused rat heart. *J. Clin. Invest.* **61:** 1654–1665.
44. Deckelbaum, R. J., S. Eisenberg, M. Fainaru, Y. Barenholz, and T. Olivecrona. 1979. In vitro production of human plasma low density lipoprotein-like particles: a model for very low density lipoprotein catabolism. *J. Biol. Chem.* **254:** 6079–6087.
45. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
46. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. *Biochim. Biophys. Acta.* **326:** 361–377.
47. Schonfeld, G., W. Patsch, B. Pfleger, J. L. Witztum, and S. W. Weidman. 1979. Lipolysis produces changes in the immunoreactivity and cell reactivity of very low density lipoproteins. *J. Clin Invest.* **64:** 1288–1297.
48. Glangeaud, M. C., S. Eisenberg, and T. Olivecrona. 1977. Very low density lipoprotein: dissociation of apolipoprotein C during lipoprotein lipase induced lipolysis. *Biochim. Biophys. Acta.* **486:** 23–35.