Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype[1]

Bernard J. Brenninkmeijer, Paul M. J. Stuyt, Pierre N. M. Demacker, Anton F. H. Stalenhoef, and Albert van 't Laar

Department of Medicine, Division of General Internal Medicine, University of Nijmegen, Nijmegen, The Netherlands

Abstract The role of the various apolipoprotein E isoproteins in the removal of chylomicrons and their remnants from plasma was studied in 16 normolipidemic subjects with various apoE phenotypes: 5 homozygous for apoE-2, 6 heterozygous for apoE-2 (phenotype E3/2), and 5 without apoE-2 (phenotypes E3/3, E4/4, and E4/3). The subjects were given an oral fat load as cream (50 g/m²). Retinyl palmitate was added as a marker for chylomicrons and their remnants. Blood was sampled at regular time intervals for 8 hr. Remnant particles were isolated from the $d < 1.019$ g/ml fraction by heparin-Sepharose chromatography (heparin-bound fraction) after removing the large chylomicrons by flotation at $7{,}8 \times 10^5$ *g*-min. All groups showed a rise in triglycerides in serum and in the chylomicron fraction between 3 and 6 hr to about twice the basal value, followed by a decrease to nearly fasting values. In the homozygous E-2 subjects, fasting lipids in the remnant fraction were increased. In all three groups the fat load did not induce a significant rise in the lipids of the remnant fraction. The homozygous E-2 group showed a strong continuing rise in the retinyl palmitate concentration in the chylomicron and remnant fractions up to 8 hr, whereas in the other groups its maximum was already reached at 5 hr at a much lower level. At 8 hr similar retinyl palmitate concentrations were found in both fractions in the heterozygous E-2 subjects compared to the non-E-2 subjects. These results indicate a delayed removal of chylomicrons and chylomicron remnants in normolipidemic homozygous E-2, but not in heterozygous E-2 subjects. —**Brenninkmeijer, B. J., P. M. J. Stuyt, P. N. M. Demacker, A. F. H. Stalenhoef, and A. van 't Laar.** Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype. *J. Lipid Res.* 1987. **28:** 361–370.

Supplementary key words apoE polymorphism • retinyl palmitate

Type III hyperlipoproteinemia (familial dysbetalipoproteinemia) is a genetic disorder of lipoprotein metabolism with a strong predisposition to coronary and peripheral artery disease, caused by an accumulation of remnants of both chylomicrons and very low density lipoproteins (β-VLDL) (1–3). This disorder is characterized by a seriously delayed uptake of remnants in the liver because of a defective recognition of these particles by specific lipoprotein receptors (2, 3). Apolipoprotein (apo)E, an important surface component of remnants and critical to this process of recognition, is functionally abnormal in type III hyperlipoproteinemia (2–6). In general, three major apoE isoproteins, apoE-2, E-3, and E-4 are known (7). The synthesis of these isoproteins is genetically controlled by three co-dominant alleles (ε2, ε3, and ε4) on one polymorphic genetic locus (8). Homozygosity for apoE-3 (phenotype E3/3) occurs most frequently (about 60% of the general population), whereas homozygosity for the E-2 isoprotein (phenotype E 2/2) is relatively rare (about 1% of the population); heterozygosity for apoE-2 (phenotypes E3/2 and E4/2) occurs in about 18% (8–11). These apoE isoproteins differ from each other by single amino acid substitutions in the primary structure of the molecule (12). In general, ε2 and ε4 are associated with hypertriglyceridemic and hypercholesterolemic states, respectively (13–16). Homozygosity for apoE-2 (phenotype E2/2) is associated with type III hyperlipoproteinemia (8, 9, 17). However, only 1% of all subjects with this phenotype develops hyperlipidemia. Accompanying factors, increasing the VLDL production, are required for the development of this condition, such as several genetic forms of hyperlipidemia, hypothyroidism, glucose intolerance, and obesity (17). Although the great majority of subjects with phenotype E2/2 are normolipidemic, at least in the fasting state, these subjects also possess

Abbreviations: apoE, apolipoprotein E; HDL, high density lipoproteins, ($d > 1.063$ g/ml); LDL, low density lipoproteins ($1.019 < d < 1.063$ g/ml); RP, retinyl palmitate; VLDL + IDL, very low density lipoproteins plus intermediate density lipoproteins ($d < 1.019$ g/ml).

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β-VLDL, as is evident from the cholesterol enrichment and β-mobility on agarose electrophoresis of their VLDL fraction (17). In addition, the structural and receptor binding defects of apoE-2 are identical in normo- and hyperlipidemics (18). E-2 heterozygous subjects also have the defective E-2 isoform and some of them may have β-VLDL as well. Rarely, E-2 heterozygosity is associated with type III hyperlipoproteinemia (16, 19).

Thus, in normolipidemics, heterozygosity as well as homozygosity for apoE-2 might be associated with a defective removal of remnants. This may become particularly apparent in the postprandial state, when the lipoprotein receptor system is challenged to a greater extent. Therefore, we administered oral fat loads to healthy normolipidemic subjects with different apoE phenotypes and studied the metabolic fate of chylomicrons and their remnants. For this purpose retinyl palmitate (RP) was added to the fat load as a marker for the chylomicron remnants, which were isolated by means of heparin-Sepharose chromatography of the lipoprotein fraction with density less than 1.019 g/ml.

MATERIALS AND METHODS

Subjects and study protocol

Sixteen normolipidemic volunteers participated in the study. All were healthy male hospital workers or medical students and were selected after apoE phenotype screening. Two of the five E-2 homozygous subjects, however, were detected by screening relatives of probands with type III hyperlipoproteinemia (18). They were divided into three groups, according to their apoE phenotype: *1*) the non-E-2 group, consisting of five subjects without the apoE allele ε2 (three subjects with apoE phenotype E 3/3, one with E4/3, and one with E4/4); *2*) the heterozygous E-2 group, consisting of six subjects, all with phenotype E3/2; and *3*) the homozygous E-2 group. Some clinical characteristics are summarized in **Table 1.** All subjects were on a normal diet and did not take drugs or excessive alcohol. After informed consent had been obtained from each subject, they were studied in the outpatient clinic. After an overnight fast of 12 hr, the subjects were given an oral fat load of 50 g/m² as cream (per 100 g cream: 35 g of fat, 2 g of protein, and 2.8 g of carbohydrates), containing 150,000 IU of RP. Blood samples were taken by venipuncture into vacutainer tubes at 0, 2, 3, 4, 5, 6, and 8 hr after the fat load and were spun within 2 hr to separate the serum. During this time the subjects did not take meals. The sera were stored in the dark for 16–23 hr at 4°C under nitrogen before lipoprotein isolation was carried out. In all samples cholesterol and tryglycerides were determined in total serum and lipoprotein fractions; RP was measured in chylomicrons and remnants, which were isolated by heparin-Sepharose chromatography of the fraction with $d < 1.019$ g/ml.

METHODS

Chylomicrons were isolated by carefully overlayering 3 ml of serum at room temperature with 5 ml of saline of density 1.006 g/ml. The tubes were centrifuged for 30 min

TABLE 1. Clinical characteristics and fasting plasma lipid levels of the non-E-2 group (apoE phenotypes E 4/4, E 4/3, or E 3/3), the heterozygous E-2 group (apoE phenotype E 3/2), and the homozygous E-2 group (apoE phenotype E 2/2)

	Non-E-2	E-2 Heterozygotes	E-2 Homozygotes
		mean ± SD	
Number[a]	5	6	5
Age (yr)	25 ± 4	28 ± 7	32 ± 12
Height (cm)	184 ± 4	183 ± 6	181 ± 6
Rel. weight (%)[b]	97 ± 4	113 ± 13[d]	103 ± 9
Serum chol[c]	4.54 ± 0.62	4.39 ± 0.85	3.58 ± 0.52
Serum TG[c]	1.16 ± 0.33	1.06 ± 0.47	1.34 ± 0.31
Chyl-TG	0.14 ± 0.14	0.19 ± 0.15	0.14 ± 0.07
VLDL + IDL-chol	0.49 ± 0.12	0.54 ± 0.31	1.00 ± 0.25[e]
VLDL + IDL-TG	0.64 ± 0.24	0.47 ± 0.26	0.83 ± 0.14[e]
Ratio $\frac{\text{VLDL + IDL-chol}}{\text{serum TG}}$	0.43 ± 0.07	0.55 ± 0.21	0.75 ± 0.15[e]
LDL-chol	2.83 ± 0.64	2.53 ± 0.45	1.47 ± 0.40[e]
HDL-chol	1.13 ± 0.29	1.30 ± 0.38	1.11 ± 0.09

[a] All subjects are males.
[b] $\frac{\text{Actual body weight}}{\text{ideal body weight}} \times 100\%$ (based on 1983 Metropolitan height and weight tables).
[c] Total serum and lipoprotein lipid concentrations are in mmol/l; chol, cholesterol (mg/dl = 38.8x mmol/l); TG, triglycerides (mg/dl = 88.5x mmol/l).
[d] $P < 0.05$ vs non E-2.
[e] $P < 0.05$ vs non E-2 and E-2 heterozygotes.

at 14,000 rpm (7.8×10^5 *g*-min) in the I.E.C. B-60 ultracentrifuge (Damon I. E. C., Needham Heights, MA) using the SW 41 swinging bucket rotor at 20°C. The chylomicrons were recovered by aspirating the first 4.5 ml with a Pasteur pipette. The infranatant was mixed and quantitatively transferred into a different ultracentrifuge tube. The density was then raised to 1.019 g/ml by addition of D_2O and the tubes were spun overnight for 16 hr at 40,000 rpm (168,000 *g*) in the fixed SW angle rotor no. 468 at 14°C. The top layer, containing the VLDL + IDL fraction, was isolated and then fractionated by heparin-Sepharose chromatography according to Shelburne and Quarfordt (20) with commercially available columns (Lipotype™ Biolab, Amersfoort, the Netherlands) into two subfractions: one, not binding to heparin-Sepharose and eluting with 0.05 M NaCl, and a second fraction, binding to heparin-Sepharose and eluting with 1.0 M NaCl. The cholesterol concentration in the heparin-Sepharose-bound fraction was taken as an estimate for the chylomicron- and VLDL-remnant concentration. The coefficient of variation for replicate analyses of cholesterol in the heparin-Sepharose bound- and unbound fractions of a VLDL + IDL pool was 6.0% and 7.1%, respectively (n = 10); the recovery of lipoprotein-associated cholesterol after heparin-Sepharose chromatography ranged from 95 to 105% (n = 10). This method of estimating the concentration of remnants was validated in a preliminary study by analyzing VLDL fractions (d < 1.006 g/ml) from fasting serum of normolipidemic subjects and patients with type III and type IV hyperlipoproteinemia. In all subjects the concentration of cholesterol relative to triglycerides was about twice as high in the heparin-Sepharose-bound fraction as in the unbound fraction **(Table 2).** Furthermore, the apoE/apoC ratio, determined by densitometric scanning of isoelectric focusing gels (21), was also considerably higher in the heparin-Sepharose-bound fraction (Table 2). On agarose gel, the electrophoretic mobility of the heparin-Sepharose-bound fraction was predominantly between the β and pre-β position, whereas the unbound fraction migrated mainly to the pre-β position.

The high density lipoprotein (HDL) fraction was isolated in whole serum using the polyethylene glycol-6000 precipitation method (22). Cholesterol and triglycerides were determined by enzymatic methods (CHOD-PAP reagent no. 237574, Boehringer, Mannheim-GFR, and sera-PAK triglycerides, Milan, Italy) using a centrifugal analyzer (Multistat III). LDL-cholesterol was calculated as the difference between the cholesterol content in the d 1.019 g/ml infranatant and HDL-cholesterol.

One-dimensional isoelectric focusing of VLDL apoproteins and determination of apoE phenotypes was carried out as previously described (21).

Retinyl palmitate assay

RP was determined in extracts of chylomicrons as well as of the VLDL + IDL (d < 1.019 g/ml) subfractions, separated by heparin-Sepharose chromatography, by means of straight phase high-pressure liquid chromatography (Si-60; Varian 8500). For quantitation, 20 μl of syn-all-*trans* retinal oxime (3.06 nmol) was added as an internal standard to 1 ml of each lipoprotein fraction (23). Extraction was performed twice with 4 ml of acetone–diethylether 1:1 (v/v). The recovery of RP after extraction was 85–95% (n = 10). The extracts were combined, dried under nitrogen, and redissolved in 15 μl of dioxane. This solution was diluted with 100 μl of hexane, and 40 μl of this mixture was applied to the Si-60 column and eluted isocratically. Eluting peaks were detected at 328 nm (Schoeffel SF 770) and peak areas were determined with an HP 3353 data analysis system and converted by means of an internal standard program into absolute amounts.

The hexane–dioxane system that was used does not separate the various long-chain retinyl esters. Upon analysis with 0.4% diethylether as eluent, RP was observed to be the predominant component (more than 90%) of the total retinyl ester population, in agreement with other reports (24, 25). Since the hexane–dioxane system strongly reduces analysis time (8 min), this system has been applied routinely and the resulting retinyl ester peak was

TABLE 2. Composition of two subfractions of VLDL (d < 1.006 g/ml) separated by heparin-Sepharose chromatography in serum of fasting normolipidemic subjects and patients with type III and type IV hyperlipoproteinemia

Heparin-Sepharose Fractions		Normal (n = 4)	Type III (n = 3)	Type IV (n = 2)
			mean ± SD	
Bound	ratio chol/TG	0.59 ± 0.28	1.67 ± 0.24	0.70 ± 0.08
	ratio apoE/C	0.94 ± 0.38	0.72 ± 0.46	1.01 ± 0.21
Unbound	ratio chol/TG	0.25 ± 0.10	0.87 ± 0.06	0.49 ± 0.03
	ratio apoE/C	0.12 ± 0.08	0.30 ± 0.03	0.11 ± 0.04

Chol, cholesterol; TG, triglyceride.

considered to represent RP. The RP concentrations measured in the chylomicron fraction and in the heparin-Sepharose-bound part of the VLDL + IDL fraction were taken as estimates of the amount of chylomicron particles and remnants of chylomicrons, respectively. The recovery of RP after ultracentrifugation in the chylomicrons, heparin-Sepharose-bound and unbound fractions was over 85%.

Study of the transfer of retinyl palmitate from chylomicrons to lipoproteins with higher densities in vitro

RP-containing chylomicrons were harvested by ultracentrifugation from 48 ml of plasma from a normolipidemic donor 4 hr after a fat load of 275 g of cream and 150,000 IU of RP. Two ml of the chylomicron fraction (triglycerides, 3.92 mmol/l) isolated from 4 ml of plasma was incubated for 5 hr at 37°C with 3 ml of plasma from two non-E-2, two E-2 heterozygous, and two E-2 homozygous acceptors (24). To correct for slight shifts in the hydrated densities of the lipoprotein particles during ultracentrifugation, blank experiments were performed by incubating the same amount of RP-rich chylomicrons with 4 ml of lipoprotein-deficient plasma ($d > 1.25$ mg/dl, dialyzed against saline). After incubation, 30 mg of sucrose was added to each ultracentrifugation tube in order to increase the viscosity of the incubation sample to the same serum conditions and to facilitate the layering procedure. The chylomicrons and VLDL + IDL were again isolated as described above. The density was then raised to 1.063 g/ml to isolate the LDL. In these lipoprotein fractions and in the LDL infranatant, the RP concentrations were subsequently measured. The results were corrected for the RP content already present in the lipoprotein fractions by incubating 4 ml of the acceptor plasma with 2 ml of saline.

Statistical methods

Mean lipid values and RP concentrations in the various lipoprotein fractions were compared by the unpaired and paired Student's *t* test. *P*-values less than 5% were considered to be significant.

RESULTS

Concentrations of serum lipids and lipoproteins before and after the fat load

The non-E-2 and heterozygous E-2 groups had similar mean basal levels of serum lipids and lipoproteins (Table I). The homozygous E-2 group, however, had significantly lower mean total serum and LDL-cholesterol concentrations and significantly higher VLDL + IDL cholesterol and triglyceride concentrations than the other groups; also, the ratio VLDL + IDL-cholesterol/serum triglycerides was increased in this group.

After the fat load was administered there was in all groups a rise in the mean serum triglyceride concentrations to about twice the basal value between 3 and 6 hr, followed by a decrease to nearly fasting values at 8 hr **(Fig. 1).** The serum triglyceride response was comparable in the three groups and proportional to the fasting triglyceride levels. The changes in the triglyceride concentration of the chylomicron fraction were similar to those in whole serum and no changes were observed in the concentration of chylomicron cholesterol after the fat load. In the homozygous E-2 group, mean VLDL + IDL cholesterol concentrations were at all time points significantly higher than in both other normolipidemic groups **(Fig. 2).** Eight hours after the fat load, the E-2 homozygous group had a significantly higher VLDL + IDL-triglyceride concentration also.

The cholesterol concentration in the heparin-Sepharose-bound fraction was twice as high in the homozygous E-2 group as in both other groups **(Fig. 3).** All E-2 homozygous subjects demonstrated a slight increase in heparin-Sepharose-bound cholesterol; however, the mean increase just failed to reach significance at any time point. In contrast, only one out of five non-E-2 and one out of six heterozygous E-2 subjects showed an increase in this parameter. The heparin-Sepharose-bound triglyceride

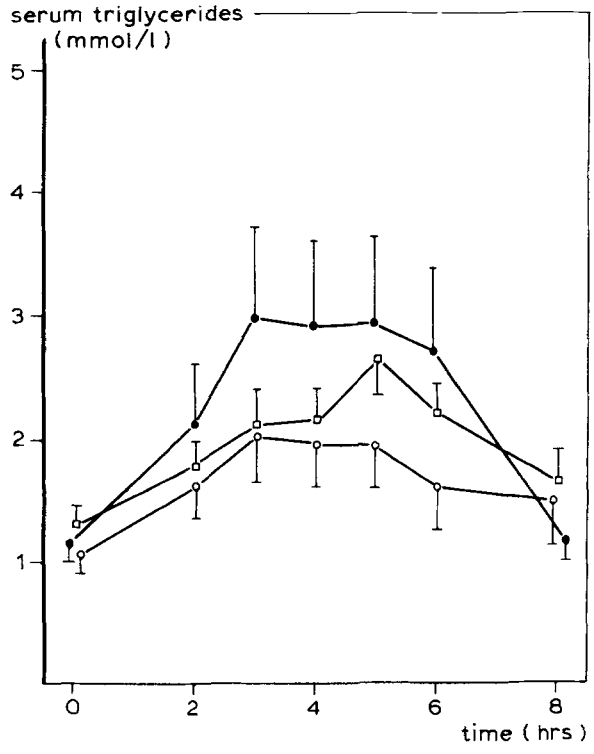

Fig. 1. Mean levels (± SEM) of serum triglyceride concentrations before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups.

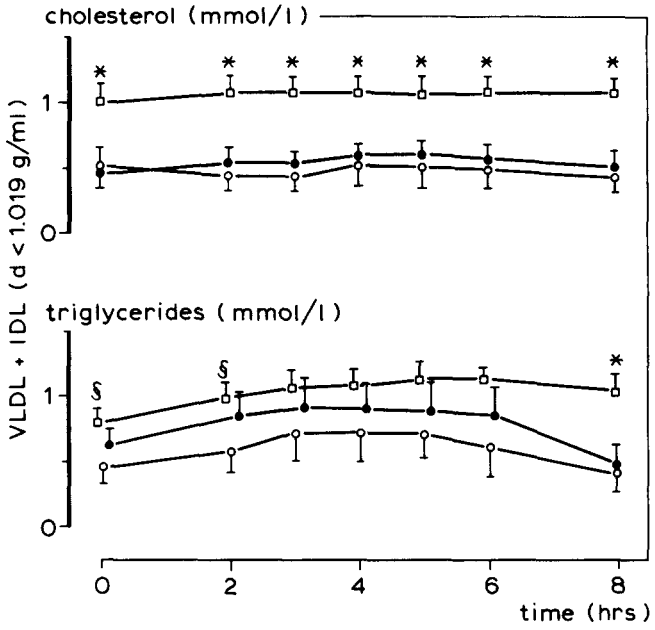

Fig. 2. Mean levels (± SEM) of VLDL + IDL (d < 1.019 g/ml) cholesterol and triglyceride concentrations before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups. *$P < 0.05$ versus the non-E-2 and the heterozygous groups; §$P < 0.05$ versus the heterozygous E-2 group.

concentration at 8 hr was significantly higher in the homozygous E-2 group than in both other groups (Fig. 3). There were no significant changes in heparin-Sepharose unbound cholesterol and triglycerides **(Fig. 4).**

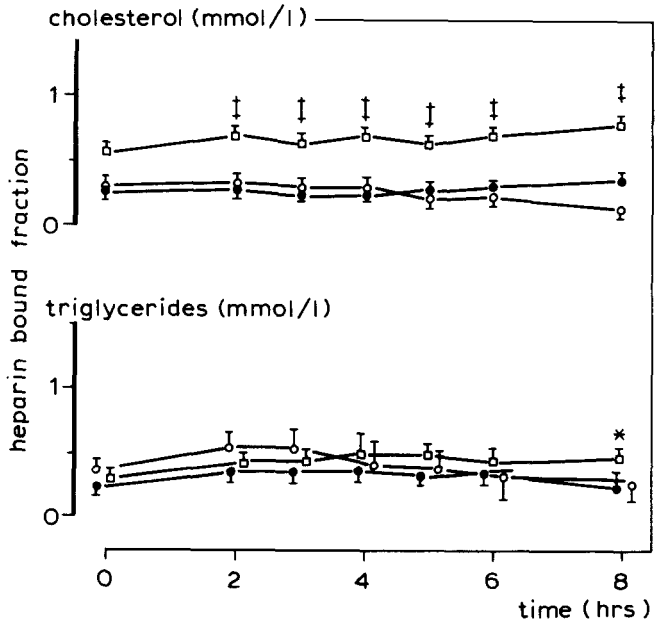

Fig. 3. Mean levels (± SEM) of heparin-Sepharose-bound cholesterol and triglycerides before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups. *$P < 0.05$ versus the non-E-2 and the heterozygous groups; ‡$P < 0.01$ versus the non-E-2 and the heterozygous E-2 groups.

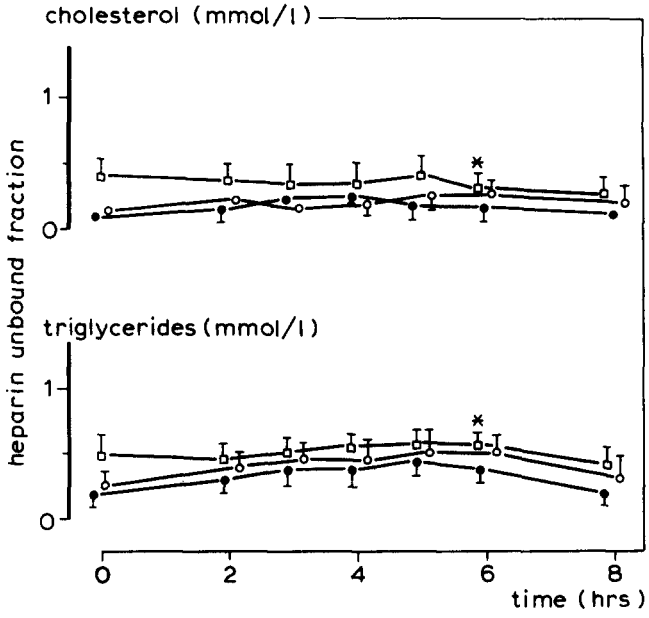

Fig. 4. Mean levels (± SEM) of heparin-Sepharose unbound cholesterol and triglycerides before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups. *$P < 0.05$ versus the non-E-2 group.

The mean serum cholesterol and LDL- and HDL-cholesterol concentrations did not change significantly after the fat load. In the homozygous E-2 group, the mean LDL-cholesterol concentration was significantly lower at all time points **(Fig. 5).**

Changes in the retinyl palmitate concentrations after the fat load

In all groups the mean chylomicron-RP increased after the fat load, but, in contrast to a decline in the concentration of chylomicron-RP between 6 and 8 hr in the non-E-2 and heterozygous E-2 subjects, there was a continuous rise during this time interval in the homozygous E-2 group **(Fig. 6).** At 8 hr after the fat load, the mean chylomicron-RP concentration was twice as high in the latter group ($P = 0.01$). The curves in the heterozygous E-2 and the non-E-2 groups were similar. In these latter groups the mean heparin-Sepharose-bound-RP showed a minimal rise at 5 and 6 hr after the fat load, followed by a slight decline at 8 hr **(Fig. 7).** The homozygous E-2 group, however, showed a much greater increase in heparin-Sepharose-bound-RP, which still continued to rise between 6 and 8 hr to a level that was nearly four times as high as in the two other groups ($P < 0.01$ at 5, 6, and 8 hr). In the three groups, there was a slight increase of RP in fractions not bound by heparin-Sepharose that was twice as high in the homozygous E-2 group as in the other groups, but these differences just failed to reach significance **(Fig. 8).**

The possible influence of an increased pool size of chylomicrons or VLDL remnants was also studied by per-

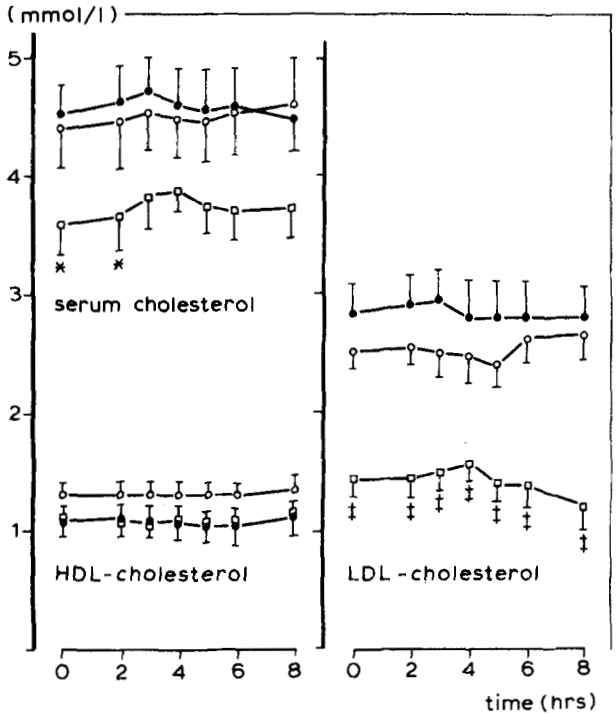

Fig. 5. Mean levels (± SEM) of serum cholesterol (chol), HDL- and LDL-cholesterol concentrations before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups. *$P<0.05$ versus the non-E-2 and the heterozygous E-2 groups; ‡$P<0.01$ versus the non-E-2 and the heterozygous E-2 groups.

forming the same fat load studies in three moderately hyperlipidemic homozygous E-2 subjects (serum cholesterol, 5.35–7.38 mmol/l; serum triglycerides, 2.50–3.60 mmol/l). The changes in the RP concentration in the different lipoprotein fractions were comparable to those in the normolipidemic E-2 subjects **(Fig. 9).**

Study of the transfer of RP from chylomicrons to lipoproteins with higher densities in vitro

The above results on the clearance of chylomicrons and their remnants are valid only if there is no significant transfer of RP from chylomicrons to other lipoproteins in vivo. This possible transfer was studied by incubating RP-rich chylomicrons with fasting plasma obtained from normolipidemic volunteers with different apoE phenotypes as described in the Methods section. The levels of heparin-bound cholesterol in the E-2 homozygous acceptor sera were 0.69 and 0.80 mmol/l, respectively, which was more than twice the values in the non-E-2 and the E-2 heterozygous acceptors. After 5 hr of incubation, 11.9 ± 1.9% (mean ± SD) of total RP (range 9.4–14.0%) was recovered in the VLDL + IDL and 2.9 ± 0.5% (range 2.1–3.5%) in the LDL fraction (n = 6). No differences between the various apoE phenotypes were observed. In the incubation experiments (n = 2) with lipoprotein-deficient plasma, 12.8% of total RP was found in the VLDL + IDL density range and 1.1% in LDL.

DISCUSSION

An increase of remnant particles in the plasma, as is evident in patients with type III hyperlipoproteinemia and in animals fed high cholesterol diets, is closely linked with accelerated atherosclerosis. Homozygosity for apoE-2 is thought to be the basic defect in type III hyperlipoproteinemia (8, 9, 17). It is of interest to learn whether normolipidemic individuals, who possess the same apoE2/2 phenotype or who are heterozygous for apoE-2, are able to clear remnants normally. If not, they might, at least temporarily, be exposed to increased levels of atherogenic particles (26). Recently, however, no increased risk was found either in homozygous E-2 or heterozygous E-2 subjects in the absence of hypercholesterolemia, and it was even suggested that the $\epsilon 2$ allele might have a protective effect by lowering the LDL-cholesterol (10, 27). Although homozygous E2/2 subjects generally are normolipidemic, they appear to have a remnant removal defect, because a certain amount of cholesterol enriched VLDL (β-VLDL) is present, as has been previously published (11, 19, 28, 29); this was also found in the present study (Table 1). Studies in first degree relatives of patients with type III

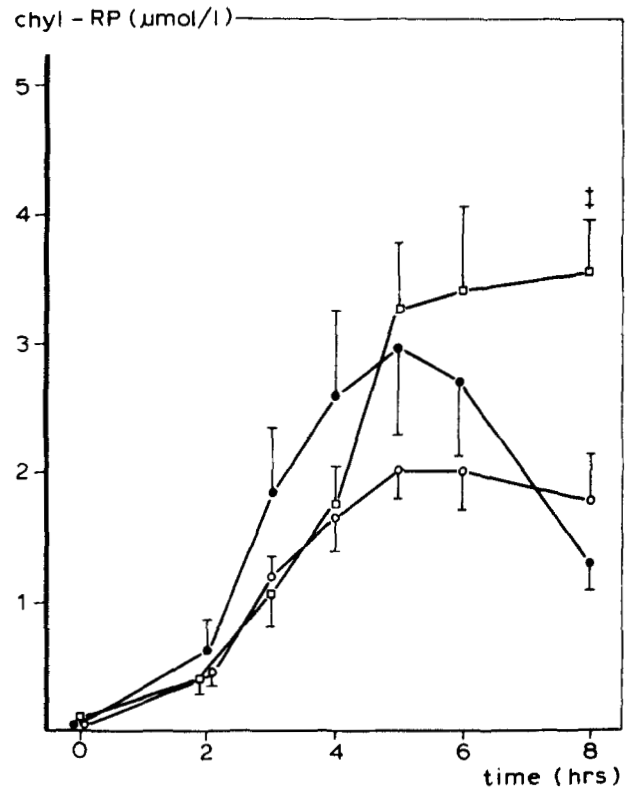

Fig. 6. Mean levels (± SEM) of the retinyl palmitate concentrations in the chylomicron fraction (chyl-RP) before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups. ‡$P<0.01$ versus the non-E-2 and the heterozygous E-2 groups.

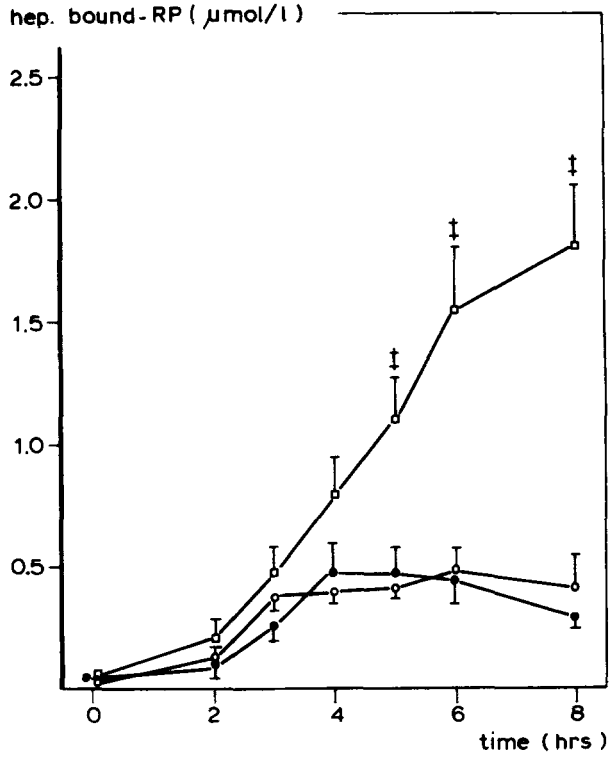

Fig. 7. Mean levels (± SEM) of heparin-Sepharose-bound retinyl palmitate (RP) concentrations before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups; ‡$P < 0.001$ versus the non-E-2 and the heterozygous E-2 groups.

hyperlipoproteinemia or in subjects detected by population studies suggested that a partial removal defect may also exist in subjects heterozygous for apoE-2. In some studies, cholesterol-enriched VLDL (β-VLDL) (28, 30, 31) and apoE concentrations (30) also indicated the presence of remnants in normolipidemic E-2 heterozygotes. Others, however, documented only a low value for serum cholesterol (11) and low density lipoprotein (LDL)-cholesterol concentrations (19, 29, 32). In our study, the ratio VLDL + IDL cholesterol/serum triglycerides was found to be normal (Table 1).

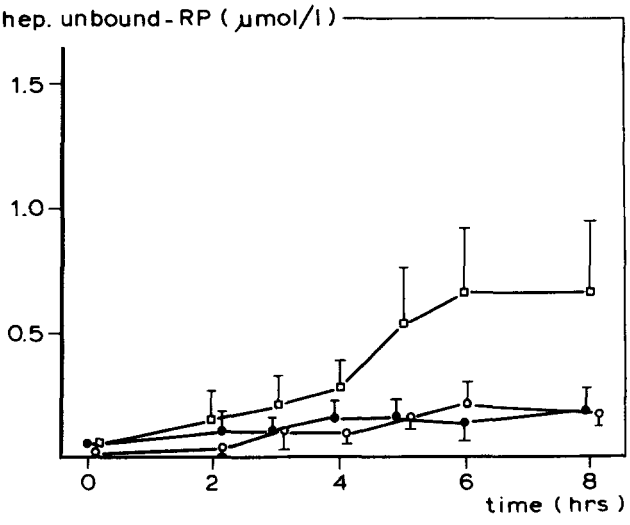

Fig. 8. Mean levels (± SEM) of heparin-Sepharose-unbound retinyl palmitate (RP) concentrations before and after a fat load in the non-E-2 (●), the heterozygous E-2 (○), and the homozygous E-2 (□) groups.

To study whether the apoE phenotype is an important determinant in the removal of chylomicron remnants, we used a combination of several techniques for specific determination of these particles during an oral fat load in normolipidemic subjects. First, a density fraction which preferentially may contain the chylomicron remnants was prepared by removing the large chylomicrons at the arbitrarily chosen centrifugal force of 7, 8×10^5 *g*-min and the LDL at 1.019 g/l. However, after a fat load, there is a wide overlap in density distribution between chylomicrons, VLDL, and their remnants. Ultracentrifugal separation on the basis of flotation characteristics can only be considered as a rough measurement of these particles. So, additionally, we performed heparin-Sepharose chromatography to isolate VLDL and chylomicron remnants. Shelburne and Quarfordt (20) discriminated two subfractions of VLDL, one that was rich in cholesterol and apoE and was bound to heparin-Sepharose and another, poor in cholesterol and apoE, that was not bound. Nestel et al. (33) provided evidence that VLDL particles in different stages of catabolism can be isolated with heparin-Sepharose chromatography. They found a precursor–product relationship between the heparin-Sepharose unbound fraction (containing mainly nascent VLDL) and the heparin-Sepharose-bound fraction (containing mainly chylomicron and VLDL remnants). With this method we were also able to separate two subfractions with different ratios of VLDL-cholesterol/VLDL triglycerides and apoE/apoC in normal, type III, and type IV subjects (Table 2).

Furthermore, retinyl palmitate was added to the cream as a marker of chylomicrons and chylomicron remnants. In the intestinal mucosal cells, RP is incorporated in the apolar core of chylomicrons and remains in the particle during transport in the lymphatic and vascular spaces. After hydrolysis in the capillary beds by lipoprotein-lipase, chylomicrons are taken up as chylomicron remnants in the parenchymal liver cells (34). In several studies the suitability of this method to label chylomicrons has been demonstrated (24, 25, 35). A small transfer of between 5 and 10% of retinyl esters from chylomicrons to lipoproteins with densities higher than 1.019 g/ml has been reported in humans in vivo as well as in vitro (24, 25, 36). Taking into account our in vitro transfer experiments, we found no significant transfer of total RP to VLDL + IDL and LDL. These results agree well with similar in vitro experiments by Berr and Kern (24).

The total serum triglyceride responses after the oral fat load were not significantly different in the E2/2, E3/2, and

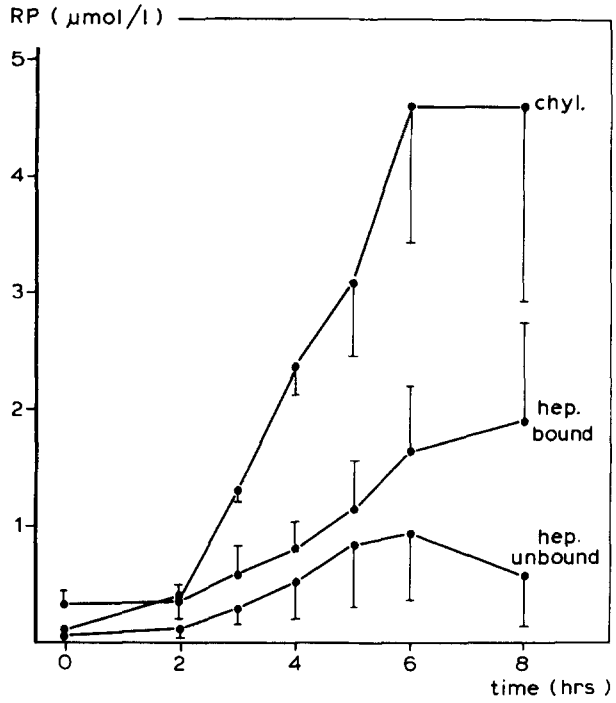

Fig. 9. Mean levels (± SEM) of retinyl palmitate (RP) concentrations in the chylomicron and the heparin-Sepharose fractions in moderately hyperlipidemic E 2/2 subjects (n = 3) after a fat load.

non-E-2 groups. It is known that the serum triglyceride concentrations after a fat load are also determined by other factors, such as the concentration of fasting triglycerides (37) and HDL (38). The three phenotype groups had comparable basal serum triglycerides and HDL-cholesterol concentrations. In hyperlipidemic subjects with the apoE2/2 phenotype, a normal lipoprotein lipase activity has been reported (39) and a normal conversion of large VLDL (S_f 100–400) to smaller particles (S_f 20–60) (40). Nevertheless, a special class of chylomicron-like particles present in the VLDL fraction of patients with type III hyperlipoproteinemia has been shown to be resistant to lipolysis in vitro; that was found to be caused by the absence of the normal apoE-3 or E-4 (41, 42). In our study, the similar triglyceride responses in total serum and in the chylomicron fraction suggest that in normolipidemic subjects the initial hydrolysis of chylomicrons is independent of the apoE phenotype. Only slight effects of the fat load on the lipid concentrations were seen in the heparin-Sepharose fractions. This could be a consequence of the in- and outflow of a wide spectrum of partially delipidated particles, with no net differences of lipid concentrations in these fractions.

The results of the RP measurements in the different fractions are more informative. A persistent rise in chylomicron-RP was seen between 6 and 8 hr in the homozygous E-2 subjects (Fig. 6). Plasma triglycerides had already decreased at this time, which indicates a normal initial hydrolysis of chylomicron triglycerides but a delay in removal of partially degraded chylomicrons or remnants. This is also evident from the persistent rise of RP in the remnant fraction isolated by heparin-Sepharose. In contrast, the chylomicron-RP in the heterozygous E-2 subjects showed a decrease within 8 h after the fat load (Fig. 6). At 8 hr similar chylomicron-RP concentrations were measured and compared with the non-E-2 group. In addition, the pattern of the RP concentration in the heparin-bound fraction (Fig. 7) appeared to be highly comparable with that observed in the non-E-2 group. Furthermore, the retinyl palmitate/triglycerides ratios in the chylomicron and the heparin-bound fractions at 8 hr were comparable in the non-E-2 and E-2 heterozygous groups (5.7 ± 2.1 and 0.8 ± 0.5 vs. 4.5 ± 1.7 and 1.3 ± 1.2, respectively) but were distinctly lower in comparison with the E-2 homozygotes (11.9 ± 5.6 and 3.7 ± 1.4). Therefore, the conclusion that in E-2 heterozygotes the clearance of chylomicron remnants is normal is justified even when the removal mechanism is challenged by a fat load.

Results similar to those in the normolipidemic E2/2 subjects were obtained in moderately hyperlipidemic E2/2 subjects (Fig. 9), which demonstrates that the distribution of RP is not affected by the pool size of the remnant particles. Our results agree well with the report by Hazzard and Bierman (35) who gave vitamin A-containing oral fat loads to patients with type III and type IV hyperlipoproteinemia and found that the removal of chylomicron remnants was delayed in type III patients, as was indicated by a slow disappearance rate of vitamin A in the S_f 20–30 fraction. Our study is an extension of this observation to normolipidemic E2/2 subjects.

Recently, Stalenhoef et al. (43, 44) described studies using a different approach to investigate separately the metabolism of chylomicrons, VLDL, and their remnants. They injected nascent chylomicrons and large VLDL, obtained from a donor with lipoprotein lipase deficiency, and followed the removal of radiolabeled intestinal apoB-48 and hepatic apoB-100 in normal individuals and in three subjects with type III hyperlipoproteinemia, one of whom was normolipidemic at the time of the study. The removal of apoB-48 (and apoB-100) was markedly and comparably delayed in all three E2/2 subjects, even in the one with normal lipid levels. This finding again supports our results in normolipidemic homozygous E-2 subjects and also suggests that the clearance of VLDL remnants is delayed in these subjects.

We conclude from our in vivo studies that, in normolipidemics, the presence of two $\epsilon 2$ alleles, which determines the apoE phenotype E2/2, leads to a temporary accumulation of chylomicron remnants up to 8 hr after a fat load, while the presence of one $\epsilon 2$ allele, which determines the E-2 heterozygous apoE phenotypes, apparently does not affect the catabolism of chylomicron remnants.

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