Type III Hyperlipoproteinemia: Defective Metabolism of an Abnormal Apolipoprotein E

Abstract. *The apolipoprotein E isolated from plasma of individuals with type III hyperlipoproteinemia (HLP) shows an abnormal pattern when it is examined by isoelectric focusing. Compared to apolipoprotein E from normal subjects, apolipoprotein E isolated from subjects with type III HLP had a decreased fractional catabolic rate in vivo in both type III HLP patients and normal individuals. The delayed catabolism of apolipoprotein E in type III HLP patients may be responsible for the lipid and lipoprotein abnormalities characteristic of these patients.*

Patients with type III hyperlipoproteinemia (HLP) have increased plasma concentrations of triglyceride and cholesterol and a ratio of very low density lipoprotein (VLDL) cholesterol to total plasma triglyceride of more than 0.3. Other characteristics of this disease, which is also known as dysbetalipoproteinemia, include the presence of lipoproteins with a hydrated density of less than 1.006 g/ml that migrate in the β position on electrophoresis (floating β lipoproteins), a decreased concentration of low density lipoproteins (LDL), an increased plasma apolipoprotein E (apoE) concentration, and premature cardiovascular disease (*1*). The apoE from patients with type III HLP migrates abnormally on gel electrophoresis (*2, 3*), and when Utermann *et al.* examined the apoE by isoelectric focusing (IEF), they found that one polymorphic form, $apoE_3$, which is present in normal individuals, is absent in type III HLP patients (*2*). This abnormal IEF pattern is now considered to be the most characteristic biochemical feature of type III HLP (*4*).

Most of the lipoprotein abnormalities in type III HLP may be caused by a defect in the catabolism of lipoprotein remnants (*5*). Chylomicrons and VLDL are triglyceride-rich lipoproteins produced in the small intestine and liver. Lipoprotein lipase hydrolyzes the triglycerides of these lipoprotein particles, so that lipoprotein remnant particles are produced that are cholesterol-rich and triglyceride-poor (*6*). Studies in humans indicate that most hepatic VLDL remnants are metabolized to LDL in normal individuals (*7, 8*), and that chylomicron remnants appear to be removed primarily by the liver (*9, 10*), with only a small fraction of the remnants being metabolized to LDL (*11*). The removal of lipoprotein remnants by the liver is modulated by apoE (*10*), presumably through the interaction with an apoE receptor on the hepatocyte membrane. Zilversmit (*12*) has hypothesized that chylomicron remnants are atherogenic, and disease states in which chylomicron remnants are elevated, such as type III HLP, are associated with an increased incidence of cardiovascular disease. To investigate the metabolic difference between normal apoE (which we designate $apoE_3^+$) and apoE from type III HLP patients ($apoE_3^-$) we conducted the following studies.

We isolated $apoE_3^+$ from either normal subjects or type V HLP patients and $apoE_3^-$ from patients with type III HLP. The apoE was purified from lipoproteins of density less than 1.019 g/ml by ultracentrifugation (*13*), separation of tetramethylurea-soluble apolipoproteins (*14*), and isolation of apoE by heparin Sepharose 4B affinity chromatography (*15*). The apoE purified by this method migrated as a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (*16*) and was free of any detectable lipid (< 1 percent). The $apoE_3^+$ from normal subjects and type V patients had the same polymorphic forms on IEF and the same amino acid composition, whereas $apoE_3^-$ from type III patients was lacking the $apoE_3$ band on IEF (*4*).

The purified apoE was radioactively labeled with either ^{125}I or ^{131}I by a modification of the iodine monochloride method (*17*). One mole of iodine was incorporated for every two moles of apoE. Uptake of the label by $apoE_3^+$ and $apoE_3^-$ appeared to be equal. Greater than 90 percent of the radioactivity migrated with purified apoE on Sephadex G-50 column chromatography or SDS-PAGE, and more than 95 percent of it precipitated with 20 percent trichloroacetic acid. The labeled apoE was incubated for 30 minutes at 37°C with normal plasma which contained a 20- to 50-fold excess of unlabeled apoE. Lipoproteins of density less than 1.21 g/ml were then isolated (*13*), sterilized by filtration through a 0.45-μm Millipore filter, tested

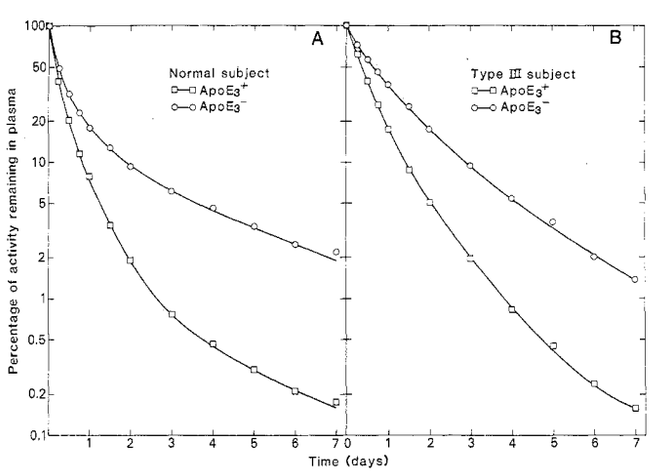

Fig. 1. The clearance of simultaneously injected $apoE_3^+$ (□) and $apoE_3^-$ (○) from the plasma of (A) a normal subject and (B) a type III HLP subject. All activity values have been normalized to 100 percent at day 0. The points represent actual data, whereas the curves are computer-generated. Each curve is the best multiexponential curve to fit the data points (*19*).

Table 1. Lipid and lipoprotein characterization of normal and type III hyperlipoproteinemic subjects. The values shown are the range for each group of subjects.

Subject	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	VLDL cholesterol (mg/100 ml)	Ratio of VLDL-cholesterol to plasma triglyceride	ApoE (mg/100 ml)	Floating β lipoprotein	$apoE_3$ by IEF
Normal (N = 10)	119 to 239	60 to 141	2 to 26	0.03 to 0.22	2.8 to 15.0	Absent	Present
Type III (N = 5)	255 to 748	275 to 1521	132 to 657	0.34 to 0.48	25.6 to 54.7	Present	Absent
Normal range	130 to 250	50 to 200	5 to 40	0.05 to 0.30	2 to 18	Absent	Present

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for pyrogenicity, and used within 36 hours.

Plasma from the subjects with type III HLP showed all of the lipid and lipoprotein abnormalities characteristic of the disease (Table 1). All subjects gave their informed consent to the experiments. The patients were taken off hypolipidemic medications at least 4 weeks before the study. At the beginning of the study the subjects were placed on a weight-maintaining diet (42 percent fat, 42 percent carbohydrate, 16 percent protein, and 200 mg of cholesterol per 1000 Kcal; the ratio of polyunsaturated to saturated fats was 0.1 to 0.2) for approximately 7 days. Beginning 3 days before the patients were given the radioactively labeled apoE, they were given 1 g of potassium iodide per day, and the meal schedule was altered so that each individual continued to receive the same diet, but it was given in four equal meals every 6 hours as liquid formula.

The radioactively labeled apoE-lipoprotein complex was injected intravenously (7:00 to 7:30 a.m.) and blood was collected in EDTA (final concentration of 0.1 percent) at the following times: 10 minutes after the injection, approximately 30 minutes later (at 8 a.m.), then 6, 12, 18, 24, and 36 hours later, and then daily at 8 a.m. through day 7. Plasma was separated by centrifugation and radioactivity measured in a Packard 5260 Autogamma counter. Plasma apoE was quantitated by electroimmunoassay (*18*). The residence time of apoE (residence time = 1 per fractional catabolic rate) was obtained by using the SAAM 27 computer program to fit a multiexponential curve to the data points and then determining the residence time (*19*). Cholesterol and triglyceride measurements, lipoprotein electrophoresis, and lipoprotein fractionation and quantification were performed by standard methods (*20*).

The clearance of both $apoE_3^+$ and $apoE_3^-$ from the plasma of normal subjects was multiexponential, with $apoE_3^+$ being cleared much more rapidly than $apoE_3^-$ (Fig. 1A). In type III HLP subjects $apoE_3^+$ was also cleared much more rapidly from the plasma than $apoE_3^-$ (Fig. 1B). In normal subjects the residence time of the $apoE_3^+$ was approximately half of that of $apoE_3^-$, a difference that was statistically significant (Table 2).

In further studies of apoE metabolism we used a double-labeling technique, administering simultaneously ^{125}I- and ^{131}I-labeled $apoE_3^+$ and $apoE_3^-$ to two normal and two type III HLP subjects. In the two normal subjects the $apoE_3^+$ residence times were 0.37 day and 0.36 day, whereas the $apoE_3^-$ residence times were 0.83 day and 0.81 day, respectively. In the two type III HLP subjects the $apoE_3^+$ residence times were 0.66 day and 0.42 day, whereas the $apoE_3^-$ residence times were 1.22 days and 0.92 day, respectively. These results confirm the difference in metabolism between $apoE_3^+$ and $apoE_3^-$ in both normal and type III HLP subjects (Table 2).

Table 2. Residence times of radioactively labeled $apoE_3^+$ and $apoE_3^-$ in the plasma of normal and type III hyperlipoproteinemic subjects. The results shown are the mean residence times in days ± 1 standard deviation. Using Student's *t*-test, we calculated two-tailed *P* values for the following comparisons: (A) versus (B), $P < .001$; (C) versus (D), $P < .017$; (A) versus (C), $P < .001$; and (B) versus (D), $P < .102$.

Subject	Type of apoE	
	$apoE_3^+$	$apoE_3^-$
Normal	(A) 0.35 ± 0.04 (N = 10)	(B) 0.77 ± 0.08 (N = 3)
Type III	(C) 0.59 ± 0.15 (N = 5)	(D) 1.07 ± 0.21 (N = 2)

We interpret our results as indicating that there is a decreased fractional catabolic rate of $apoE_3^-$ in type III HLP patients. Since apoE is important in lipoprotein remnant uptake by the liver, we propose that patients with $apoE_3^-$ have delayed lipoprotein remnant catabolism. This defect, possibly accompanied by other compensatory changes in lipoprotein metabolism (*7*), then leads to the characteristic plasma lipid and lipoprotein abnormalities found in this syndrome (*1*).

Estrogens have been reported to normalize the lipoprotein abnormalities in females with type III HLP (*5, 21*). On the basis of these reports and our results, we suggest that the $apoE_3^-$ and lipoprotein remnant catabolic defect in type III HLP patients is a partial defect with $apoE_3^-$ still retaining some of its normal function. It has been suggested that estrogens increase the uptake of lipoprotein remnants in rat liver perfusion studies by a saturable apoE receptor-mediated process (*22*). We speculate that estrogens also increase the affinity or number of apoE receptors on hepatocyte membranes in humans, and that this overcomes the partial catabolic defect of $apoE_3^-$ and lipoprotein remnants in type III HLP patients, correcting their plasma lipid and lipoprotein abnormalities.

The molecular basis for the catabolic defect of $apoE_3^-$ is unknown. It may be related either to an alteration of the primary amino acid sequence of apoE or to a defect in post-translational modification of apoE resulting in the failure of formation of the $apoE_3$ isoform.

The prolonged residence times of both $apoE_3^+$ and $apoE_3^-$ in type III HLP patients compared to normal subjects may be the result of an increased pool of apoE in type III HLP patients competing for a limited number of apoE catabolic sites. Since $apoE_3^-$ is catabolized more slowly than $apoE_3^+$, it may also have reduced affinity for the apoE catabolic site. Therefore, $apoE_3^-$ may not be as effective in competing for the $apoE_3^+$ catabolic site and the increased pool size may only partially explain the prolonged residence time for $apoE_3^+$. An alternative explanation is that the prolonged catabolism of apoE in type III HLP patients depends on the number of apoE molecules per lipoprotein particle. If apoE-containing lipoprotein particles are catabolized as particles, the relative proportion of $apoE_3^+$ to $apoE_3^-$ on each particle might regulate the lipoprotein particle uptake and also the apoE catabolism.

Thus apoE appears to be necessary for lipoprotein remnant catabolism and type III HLP is a lipoprotein remnant catabolic disease. The slower catabolism of the abnormal $apoE_3^-$ from type III HLP patients compared to that of the normal $apoE_3^+$ may be responsible for the defect in lipoprotein metabolism found in type III HLP patients.

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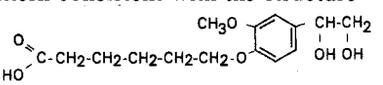
Specific and Sensitive Radioimmunoassay for 3-Methoxy-4-hydroxyphenylethyleneglycol (MOPEG)

Abstract. *Antibodies that specifically bind the norepinephrine metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) were produced in rabbits after injection of a derivative of MOPEG conjugated with bovine thyroglobulin. A sensitive radioimmunoassay was devised with this antiserum, in which as little as 0.5 nanogram of MOPEG can be accurately measured with a final antibody dilution of 1:180. The antibody appears to be specific for MOPEG, since tritiated MOPEG was not displaced from the antibodies by norepinephrine, epinephrine, dopamine, serotonin, or their major metabolites, including MOPEG-sulfate (333 nanograms each).*

The major terminal metabolite of norepinephrine in the central nervous system of the rat (*1*) and man (*2*) is 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), and changes in the concentration of MOPEG and its sulfate conjugate (MOPEG-sulfate) faithfully reflect changes in the amount of norepinephrine released by nerve activity in the brain (*3-6*). On the basis of observations of this type (*3-6*), we wished to use changes in the regional concentrations of MOPEG in rat brain to gauge the effects of pharmacological agents on central noradrenergic neurotransmission. However, each of the methods of analysis used in the studies cited above presented problems. The measurement of MOPEG by either fluorometry (*3*, *4*) or gas-liquid chromatography (*5*, *6*) requires large amounts of tissue, so that the same anatomical region from several rat brains would have to be pooled for assay. The quantification of MOPEG by gas chromatography combined with mass spectrometry is extremely specific and sensitive (*1*, *2*), but the instrumentation is expensive and not widely available. We therefore undertook the development of a specific and sensitive radioimmunoassay for MOPEG.

Because MOPEG is a small molecule that occurs naturally in the body, it is not antigenic; furthermore, MOPEG has no free carboxyl or amino groups that can be used to couple it to a carrier protein. To obtain a derivative of MOPEG that has a free carboxyl group and an aliphatic bridge to position the 3-methoxyphenylethyleneglycol portion of MOPEG some distance from the surface of the carrier protein, we synthesized 3-methoxy-4-(5-carboxypentoxy)-phenylethyleneglycol by reacting MOPEG with 6-bromohexanoic acid (Fig. 1). The equivalent of 400 mg of free MOPEG as the piperazine salt was added to 4 ml of water, and the *p*H of the solution was adjusted to 6 with 1*N* H_2SO_4. Free MOPEG was extracted four times with 3 ml of water-saturated ethyl acetate. After evaporation of the pooled ethyl acetate fractions with a stream of nitrogen, 199 mg (49 percent) of the MOPEG was recovered. It is important that MOPEG be extracted from the aqueous solution of its piperazine salt at a *p*H of 5 or above; below *p*H 4, even for short exposures, a dimerization reaction occurs, and less free MOPEG is obtained from the extract. The 199 mg of MOPEG was dissolved in 1 ml of water, and the *p*H was adjusted to 10 with concentrated NaOH. In a separate test tube, an equimolar amount (210 mg) of 6-bromohexanoic acid (Aldrich) was added to 1 ml of water, and the *p*H was raised to 11 with concentrated NaOH. After the two solutions were mixed (final *p*H, 10.0), a stream of nitrogen was bubbled through the reaction mixture for 1 minute. The reaction vessel (an acid-washed 13-ml glass centrifuge tube) was stoppered, sealed with Parafilm, and placed in a 100°C oil bath. After 1 hour, the *p*H of the reaction mixture had dropped to 5.85. At this point, 214 mg of 6-bromohexanoic acid was added to the reaction mixture, and the *p*H was again adjusted to 11 with concentrated NaOH. After the reaction mixture was degassed with nitrogen, the reaction vessel was sealed and the mixture was again incubated for 1 hour at 100°C. As before, the *p*H decreased to 5.6 during this incubation. The *p*H was raised to 7.7 with concentrated NaOH, and the mixture was washed four times with 3 ml of chloroform. The *p*H of the reaction solution was then adjusted to 2.3 with 2*N* HCl, and the reaction product was extracted three times with 3 ml of water-saturated ethyl acetate. After evaporation of the ethyl acetate under a stream of nitrogen, 70 mg of a viscous oily material was recovered. Mass spectral analysis revealed the presence of a compound with a molecular weight of 298 and a fragmentation pattern consistent with the structure



Little unreacted MOPEG was detected, but unreacted 6-bromohexanoic acid (and possibly 6-hydroxyhexanoic acid) was present. No further attempt was made to purify the 3-methoxy-4-(5-carboxypentoxy)phenylethyleneglycol or to corroborate the proposed structure. This derivative of MOPEG was coupled to bovine thyroglobulin with a water-soluble carbodiimide. The above reaction product (5 mg) was dissolved in 200 μl of water, and 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (Sigma) was dissolved in 200 μl of water. To each of these solutions was added 150 μl of 0.1*M* $NaHCO_3$. The two solutions were then mixed together, and 25 mg of bovine thyroglobulin (Sigma), dissolved in 300 μl of water, was added to the mixture. The mixture was stirred constantly for 24 hours at room temperature and then dialyzed for 2 days (at 4°C) against 4 liters of 0.1*M* phosphate buffer, *p*H 7.5, containing 0.1*M* NaCl. After 3.8 ml of fresh dialysis buffer was added to the dialyzed mixture, the protein-hapten complex was stored in 300-μl portions at −20°C.

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