Metab

Medical Grand Rounds
February 17, 1983

FAMILIAL TYPE 3 HYPERLIPOPROTEINEMIA

(Familial Dysbetalipoproteinemia)
(Broad Beta Disease)

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Contents

Introduction

Familial Type 3 Hyperlipoproteinemia (Type 3 HLP) is a relatively rare clinical syndrome characterized by the presence of abnormal lipoproteins in the plasma and an association with premature atherosclerosis. In the medical literature, this syndrome is sometimes called Familial Dysbetalipoproteinemia or Broad Beta Disease.

The study of this disease has produced important new information about lipoprotein and apolipoprotein metabolism, and the nature of lipoproteins that appear to promote atherosclerosis. Since this information has broad application in the general population, it will be reviewed today.

Clinical Picture

The first clinical description of this disease was presented by Gofman and co-workers (1). They described a series of 23 patients who were identified because they had xanthoma tuberosum. Gofman reported that characteristic cutaneous lesions appeared on (1) the extensa surfaces of the elbows and knees, (2) the buttocks, (3) over the ankle malleoli, especially laterally, and (4) on the hands, especially the area surfaces. Dr. Gofman's laboratory, using the analytical ultracentrifuge, also characterized the lipoprotein abnormalities present in these patients (Table 1).

Table 1

Current Terminology	LDL_2	LDL_1	VLDL	VLDL
Svedberg units of flotation	S_f 0-12	S_f 12-20	S_f 20-100	S_f 100-400
	concentration in mg%			
Patients with xanthoma tuberosum (n=23)	206	128	616	650
Controls	358	74	105	72

From Ref 1.

By this methodology, these patients had elevated levels of VLDL and LDL_1 (or IDL), but low levels of LDL_2. These early findings have been confirmed by a number of subsequent studies.

Because of the complexity and cost of analytical ultracentrifugation, little further work on lipoprotein disorders was conducted until an inexpensive electrophoretic method was developed to study plasma lipoproteins.

Dr. Fredrickson and co-workers, using the preparative ultracentrifuge along with paper electrophoresis, were the first to identify a "marker" for Type 3 HLP in the form of "floating beta lipoprotein" (Fig 1) (2,3). This lipoprotein is also referred to a beta-migrating very low density lipoprotein, or β-VLDL.

Figure 1

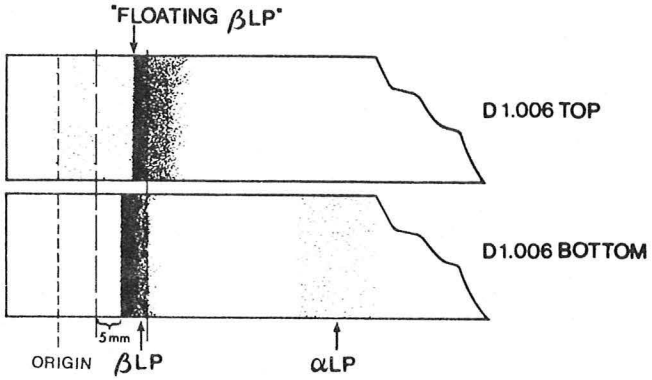


Figure 1. Diagram illustrating the definition of "floating beta" lipoproteins used in this study. Paper electrophoresis is performed on the supernatant (D 1.006 top) and infranatant (D 1.006 bottom) fractions obtained by preparative ultracentrifugation of plasma without adjustment of density (5). The strips are stained with a lipid-binding dye (oil-red-O), dried, and the origins aligned. The low-density lipoproteins (βLP) and high-density lipoproteins (αLP) are found in the bottom fractions. Normally very low-density (pre-beta) lipoproteins are present in the top fraction and migrate such that their trailing edge is beyond the leading edge of the βLP band. The presence of a band in the top fraction that begins between the indicated lines is considered to be floating beta.

Floating beta lipoprotein is a lipoprotein of abnormal composition that accumulates in the plasma of patients with Type 3 HLP. It is noteworthy that Type 3 HLP is the only hyperlipoproteinemic disorder associated with the accumulation of grossly abnormal lipoprotein particles in the plasma. Using this diagnostic approach, Dr. Fredrickson and co-workers accumulated a large series of Type 3 patients at the National Institutes of Health (NIH) and a summary of their experience with these patients is given below (4).

I. Lipid and lipoprotein levels in patients with Type 3 HLP.
The lipid and lipoprotein levels in 47 patients with Type 3 HLP are given in Table 2.

Table 2

Table 1. Lipid and Lipoprotein Concentrations* in 47 Patients with Untreated Type III Hyperlipoproteinemia

	Number	Plasma Cholesterol	C_{HDL}†	C_{VLDL}†	C_{LDL}†	Plasma Triglycerides
		← *mg/100 ml* →				
Type III						
All subjects	47	452.7 ± 20.9	37.7 ± 2.5	286.9 ± 24.7	121.3 ± 8.3	698.6 ± 76.9
All men	27	440.3 ± 24.9	36.5 ± 2.6	267.8 ± 31.6	112.7 ± 13.5	694.0 ± 104.2
All women	20	469.6 ± 36.3	39.1 ± 4.5	307.0 ± 38.6	131.3 ± 8.5	704.8 ± 116.5
Controls‡						
Men, age 30-39	50	210 ± 5	48 ± 2	21 ± 2	143 ± 4	78 ± 6
Women, age 40-49	44	217 ± 5	62 ± 2	14 ± 1	130 ± 4	80 ± 6

* Values cited are mean ± SEM.
† C_{HDL} = high-density lipoproteins; C_{VLDL} = very low-density lipoproteins; C_{LDL} = low-density lipoproteins.
‡ Control values are derived from Reference 5.

Note that there is both hypercholesterolemia and hypertriglyceridemia. HDL- and LDL-cholesterol levels are lower than normal while VLDL-cholesterol (and triglyceride) levels are clearly elevated. Also note that the total cholesterol and triglyceride levels are elevated to a similar extent, though

there is often more hypertriglyceridemia; this quantitative relationship reflects the accumulation of the abnormal lipoprotein in the plasma. Ordinarily the accumulation of VLDL of normal composition would result in hypertriglyceridemia far in excess of hypercholesterolemia. It should be recognized that severe hypertriglyceridemia (>1000 mg/dl) is fairly common and that wide swings in plasma lipids may occur with treatment (lability of lipid levels) (3,4).

II. Xanthoma formation and atherosclerosis in Type 3 HLP (Table 3)(4).
The types of xanthomas observed in patients with Type 3 HLP as well as the clinical manifestations due to atherosclerosis are presented in Table 3 and are compared with the findings of two smaller published series (Borrie, Ref 5; Mishkel, Ref 6).

Table 3

Table 3. Comparison of Clinical and Biochemical Data on Patients with Type III Hyperlipoproteinemia

	Present Study	Borrie (6)	Mishkel (7)
Patients			
Total, *no.*	47	18	16
Age range, *yrs*	23-70	24-53	26-59
Mean cholesterol, *mg/100 ml*	453	429	465
Mean triglyceride, *mg/100 ml*	699	600	733
	← *% of patients* →		
Xanthomas			
Palmar xanthomas (xanthoma striata palmaris)	64	72	44
Tendinous xanthomas	23	17	38
Tuberous xanthomas (xanthoma tuberosum)	51	100	88
Eruptive xanthomas	4	28	
Xanthelasma	6	6	25
Corneal arcus	6	11	31
Ischemic heart disease	37	28	31
Peripheral vascular disease	29	11	31

Note that palmar xanthomas (also called xanthoma striata palmaris) and tuberous (or tubero-eruptive) xanthomas are most commonly observed. Tendon xanthomas characteristic of familial hypercholesterolemia are much less common, as are eruptive xanthomas, xanthelasmas, and the corneal arcus.

Atherosclerosis appears to be generalized and 43% of the NIH patients had detectable vascular disease (4). Ischemic heart disease was present in one-third of the patients and a similar overall prevalence was seen for peripheral vascular disease; Eleven of the 47 patients (22%) had both. Cerebrovascular disease was present in 5 of the 47 patients. The age of onset of vascular disease in these patients is given in Table 4.

Table 4

Table 4. Age of Onset of Vascular Disease in Patients with Type III Hyperlipoproteinemia*

	Ischemic Heart Disease	Peripheral Vascular Disease	Cerebral Vascular Disease
Men	38.1 ± 3.4 (11)	38.1 ± 4.8 (8)	57.5 ± 0.5 (2)
Women	49.7 ± 2.9 (7)	50.5 ± 3.9 (4)	53.7 ± 7.9 (3)

* Numbers in parentheses represent number of patients in each group. Age is in years (mean ± SEM).

The pathology of the atherosclerosis in patients with Type 3 HLP has been studied in 9 patients (7-11). In two patients, the primary component of the atherosclerotic plaque was lipid-laden foam cells (7,10). In addition, foam cells were observed in the spleen, liver, lymph nodes, bone marrow, and renal glomeruli (7,10). However, in the remaining patients, including 5 with extensive and diffuse coronary artery disease (11), the atherosclerotic plaques contained very few, if any, foam cells and were composed primarily of fibrous tissue.

It has been suggested that Type 3 HLP may not necessarily be associated with premature vascular disease (12) because the Type 3 patients detected in a Dermatology Clinic because of characteristic xanthomas had many fewer cardiovascular symptoms than did patients with Type 2 hyperlipidemia (hypercholesterolemia) (13,14). However, even in these Dermatology Clinic patients, symptomatic atherosclerotic cardiovascular disease was present in 23% of Type 3 patients but in only 14% of Type 4-5 patients, suggesting that the Type 3 phenotype may be associated with accelerated atherosclerosis (14).

III. Other Clinical Abnormalities (4).

A variety of other clinical abnormalities have been identified in the NIH patients. These are listed with their frequency in Table 5.

Table 5: Other Clinical Abnormalities in Type 3 Hyperlipoproteinemia (NIH Study)

Disorder	Number Affected Over Total Tested	Percentage
Hyperuricemia	20/46	40%
Abnormal GTT	19/36	55%
Fasting Hyperglycemia	2/46	4%
Body Mass Index		
Male	26.3 ± 3.4	-
Female	29.9 ± 1.4	-
Hypertension	7/47	15%
Nephrolithiasis	4/47	9%
Gout	2/47	4%
Peptic Ulcer	2/47	4%
Diabetes Mellitus	2/47	4%
Cholelithiesis	6/47	13%

In summary, the Type 3 patients had more hyperuricemia and abnormal glucose tolerance than expected in the general population. They were also significantly more obese than the general population in which the average body mass index is only 24.5. In 5 Type 3 patients recently evaluated for pancreatic alpha and beta cell function, there was no evidence for primary insensitivity to either insulin or glucagon (15).

IV. Age of onset: Type 3 HLP is rarely present before adult life. Only 7 cases have been reported in patients less than 20 years of age (16-18) and the youngest patient was 10 years old (18). In the NIH series, the mean age of onset for men was 39.8 ± 2.3 years while that for women was 49.1 ± 2.2 years. The reason for this disparity in the age of onset between the sexes is unknown.

The Nature of the Abnormal Lipoprotein in Type 3 HLP

I. Chemical Composition: As noted above, the detection of floating beta lipoprotein (or β-VLDL) in the sera of patients with Type 3 HLP was the first clue that an abnormal lipoprotein was present in this clinical syndrome. It was initially thought that the floating beta lipoprotein could be a useful diagnostic marker for the disease but subsequent studies have shown that it can be detected in several other hyperlipidemic syndromes (3); it is therefore not a specific marker for Type 3 HLP. Similarly, the presence of a "broad beta" band on paper or agarose electrophoresis is only observed in about 50% of the plasma samples that contain the abnormal lipoprotein as shown by other criteria (3). Thus, lipoprotein electrophoresis of the plasma is not a useful procedure for detecting Type 3 HLP.

A variety of techniques have now been employed to isolate and characterize the abnormal particles. The general finding, irrespective of the methods used, is that both chylomicrons and VLDL from patients with Type 3 HLP are relatively poor in triglyceride and enriched in cholesterol when compared to similar lipoproteins isolated from either normal subjects or patients with other forms of hyperlipoproteinemia (19-28).

For example, Hazzard and co-workers detected chylomicrons in the sera of patients by polyvinyl pyrollidone (PVP) flocculation and demonstrated that Type 3 patients often exhibited some degree of fasting hyperchylomicronemia (19). When the triglyceride/cholesterol ratio (TG/C) on chylomicrons was measured in the fasting state in several groups of patients with different types of hyperlipidemia, the TG/C ratio varied considerably, depending on the state of the patient (Table 6) (19).

Table 6: Triglyceride/Cholesterol ratios of chylomicrons in different forms of hyperlipidemia before and after a dietary fat challenge.

Diagnosis	TG/C of Chylomicrons		
	Fasting	4-10 hr after eating fat meal	14-24 hr after eating fat meal
Familial Hypercholesterolemia(LDL)	ND*	15.5	4.0
Type 3 HLP	2.4	14.0	2.4
Endogenous Lipemia(VLDL)	ND	22.0	10.0
Mixed Lipemia (VLDL + Chylomicrons)	10	15.0	10.5

* ND = None Detected.

These authors concluded that the chylomicrons first secreted after a fat meal challenge in Type 3 patients were of normal composition but that they were cleared more slowly and consequently became cholesterol-rich because of

exaggerated lipid exchange and lipolysis that take place during prolonged circulation in the plasma (19).

Quarfordt and co-workers subjected the VLDL from Type 3 patients and patients with endogenous hypertriglyceridemia (Type 4 phenotype) to starch block electrophoresis (21). They noted that "usual VLDL" migrated primarily as one family of particles whereas the Type 3 VLDL migrated as two separate groups of particles (Fig. 2) (21).

Figure 2

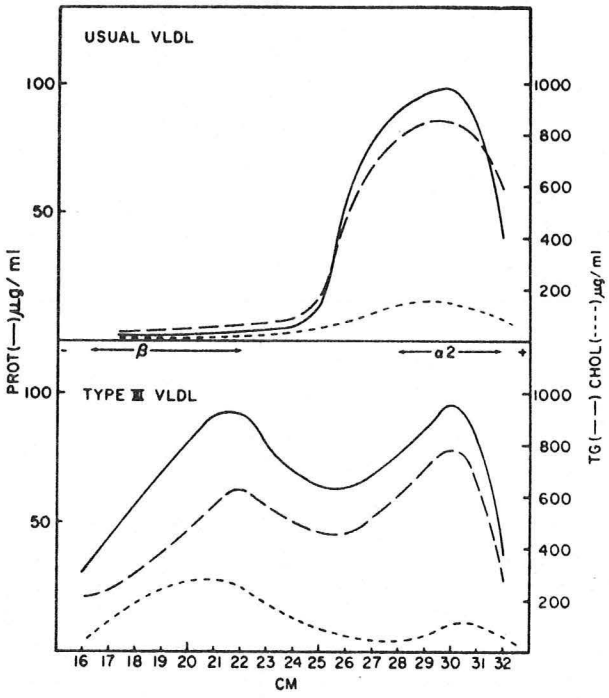


FIGURE 1 Starch block electrophoresis of VLDL from a patient with type IV hyperlipoproteinemia (top panel, usual VLDL) and VLDL from a Type III patient (bottom panel). Both were in-patients on a balanced diet. PROT, protein; TG, triglyceride; CHOL, cholesterol.

When the composition of these particles was examined, they noted that the cholesterol content of Type 3 VLDL was significantly higher and the triglyceride content significantly lower than that observed in "usual" VLDL (Table 7) (21). These findings were similar to those reported earlier for chylomicrons (19).

Table 7

TABLE III

*Composition of Ultracentrifugal Subfractions of VLDL in Type III and Type IV Hyperlipoproteinemia**

	Protein		Phospholipid		Cholesterol		Triglyceride	
	Type III	Type IV	Type III	Type IV	Type III	Type IV	Type III	Type IV
				mg per 100 mg				
S_f^0 20-400	10	7	20	15	29	10	42	68
S_f^0 20-60	13	15	14	23	31	13	33	58
S_f^0 60-100	8	10	22	17	29	14	42	59
S_f^0 100-400	5	5	16	19	30	7	48	69

* All values represent the mean of duplicate determinations.

Furthermore when heparin was given to Type 3 and Type 4 patients to induce rapid lipolysis of VLDL, the lipoprotein patterns observed by analytical ultracentrifugation were distinctly different in these two types of patients (Fig. 3) (21).

Figure 3

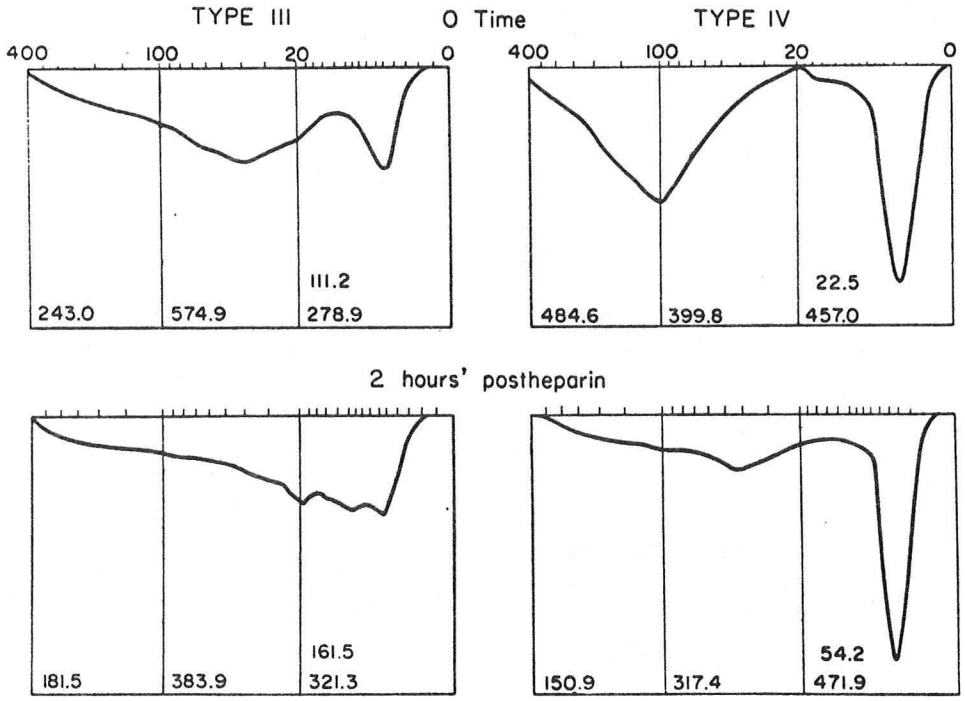


FIGURE 4 Ultracentrifugal patterns of plasma lipoproteins in type III and type IV hyperlipoproteinemia before and 2 hr after heparin administration. The numbers in the boxes under each ultracentrifugal pattern represent the absolute concentration of lipoprotein in mg/100 ml. In each box the three frames, from left to right represent the lipoproteins of S_f 100–400, 20–100, and 0–20. The numbers in the S_f 0–20 box represent the concentrations of S_f 0–12 (below) and 12–20 (above) lipoproteins.

Note that the initial pattern for the Type 3 patient differs significantly from that in the Type 4 patient in that there is much less S_f 0-20 material and more S_f 20-100 material in the Type 3 subject. After heparin was given, S_f 20-400 material dropped while S_f 0-20 material increased in the Type 4 patient but there was only a modest shift in the initial pattern observed in the Type 3 patient.

After it was clear that the triglyceride-rich lipoproteins of Type 3 patients were abnormal in composition, attempts were made to use this information to devise a diagnostic index. Hazzard and co-workers calculated cholesterol/triglyceride ratios on total plasma and on VLDL isolated by ultracentrifugation from the plasma of 5 different groups of patients and their results are summarized in Table 8.

Table 8. Cholesterol/triglyceride ratios of whole plasma and of VLDL in normal and hyperlipoproteinemic patients.

Diagnosis	Number of Patients	C/TG	
		Whole Plasma	VLDL
Normal	10	2.4 ± 0.9	0.35 ± 0.08
Familial Hypercholesterolemia	5	3.8 ± 1.1	0.39 ± 0.05
Type 3 HLP	9	0.83 ± 0.19	0.60 ± 0.11
Endogenous Lipemia (Type 4)	14	0.85 ± 0.37	0.27 ± 0.06
Endogenous Lipemia with Chylomicrons (Type 5)	5	0.37 ± 0.14	0.25 ± 0.08

These authors suggested that a VLDL cholesterol/triglyceride ratio of > 0.42 (the upper limit of the range observed among subjects with endogenous lipemia) be considered diagnostic of Type 3 HLP in patients with hypertriglyceridemia (22)

A similar analysis on a large number of patients was performed by Fredrickson and co-workers at the NIH. They reviewed their data on lipid and lipoprotein determinations in over 3100 plasma samples from 182 hyperlipidemic subjects. In their assay procedure, they usually measured the total plasma cholesterol and the cholesterol content of the individual lipoproteins but not the triglyceride content of the individual lipoproteins. Consequently they expressed their data in terms of ratio of VLDL-cholesterol to the total plasma triglyceride level. Their findings are summarized in Table 9 (3).

Table 9. Criteria for diagnosis of Type 3 HLP based on the VLDL-C/plasma TG ratio.*

VLDL-C/Plasma TG Ratio	Conclusion
< 0.25	Type 3 HLP not present.
0.25 - 0.30	Suggestive of Type 3 HLP
> 0.30	Diagnostic of Type 3 HLP

* Applies only to plasma samples with plasma triglyceride levels between 150 and 1000 mg/dl.

Fredrickson and co-workers noted that this chemical index was better than the attempts to detect floating betalipoprotein for the diagnosis of Type 3 HLP but they clearly recognized that this index was only a temporary definition

and they were correct. As we shall see later, the apolipoprotein E isoforms of VLDL have become the key marker for Type 3 HLP.

Mishkel and co-workers (29) noted that the above chemical index persisted over periods of up to 2.5 years, even when patients were successfully treated with diet and/or drug therapy. Thus the abnormal lipoproteins appeared to persist despite marked shifts in the lipoprotein levels of individual patients.

II. Metabolism of VLDL in Patients with Type 3 HLP

During the time that the lipid composition of the lipoproteins in Type 3 HLP was being defined, information about the protein content of the lipoproteins was also being gathered, and this information was used to evaluate the metabolism of VLDL using radiolabeled lipoproteins.

The protein component of VLDL actually consists of a mixture of proteins now identified by letters of the alphabet. The major protein constituents of the lipoproteins are listed in Table 10 (30).

Table 10. Apolipoprotein composition of the lipoprotein classes.

Apolipoprotein	Chylomicrons	VLDL	LDL	HDL
A-I	Major*	Minor	Trace	Major
A-II	Major	Minor	Trace	Major
A-IV	Major	Trace	Absent	Minor
B-100	Minor, if present.	Major	Major	Absent
B-48	Major	Trace	Absent	Absent
C-I	Major	Major	Trace	Minor
C-II	Major	Major	Trace	Minor
C-III	Major	Major	Trace	Minor
D	Unknown	Minor, if present.	Trace	Minor
E	Minor	Major	Minor	Minor

* Major refers to proteins comprising 5% or more of the total protein in a lipoprotein.

When VLDL is radioiodinated and injected intravenously into a normal subject, it is rapidly catabolized but the protein constituents have different metabolic fates (31,32). VLDL apoB is converted unilaterally to LDL apo B but portions of the VLDL are probably cleared from the plasma before this conversion takes place. The apoB in this case is B-100, the larger molecular weight form of apoB that is the major component of VLDL and LDL. As VLDL is metabolized, its triglyceride core is depleted and it loses excess surface phospholipid, free cholesterol and apolipoproteins. It also gains cholesteryl ester by exchange with other lipoproteins with the help of lecithin:cholesterol acyltransferase (LCAT) (33,34). The net effect of these changes is a restructing of the VLDL through a series of intermediates called intermediate density lipoprotein (or IDL) to yield LDL.

When radioiodinated VLDL in injected intravenously into a Type 3 patient, the normal metabolic relationship is distorted (Fig. 4) (35) such that apoB radioactivity accumulates in the IDL region and is not converted to LDL. This change is not due to hypertriglyceridemia alone since similar aberrant metabolic relationships are not observed in patients with endogenous hypertriglyceridemia (Fig 4) (35).

Figure 4

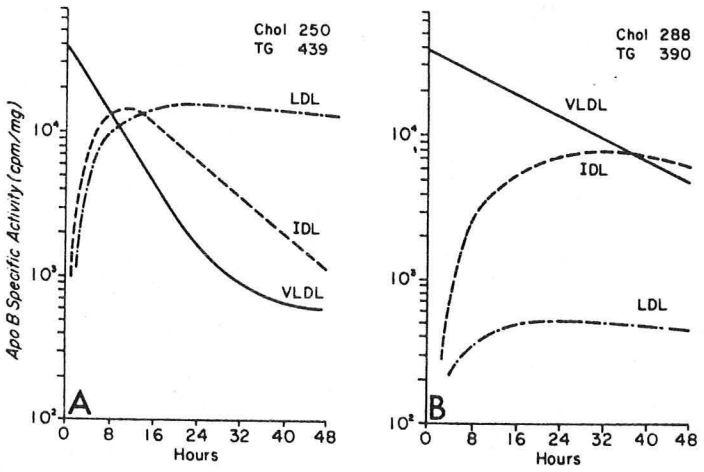
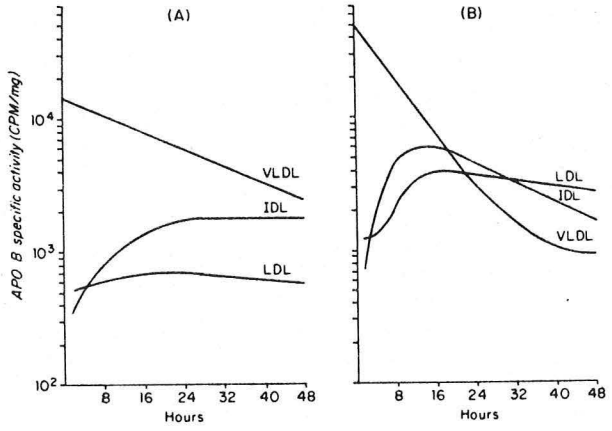


Fig. 1. Apoprotein B specific activity curves following the injection of ^{125}I-labeled VLDL. ——— = VLDL; - - - = IDL, ····· = LDL. (A) In a subject with endogenous hypertriglyceridemia, (B) in a subject with broad beta disease.

Furthermore, a reduction in lipoprotein levels with treatment fails to fully restore this aberrant metabolism of VLDL to normal (Fig 5) (32,36). Finally, it has been shown that the failure to convert VLDL properly to LDL is not due to an abnormality in lipoprotein lipase activity (37).

Figure 5



Apoprotein-B specific activity in a subject with type-III hyperlipoproteinæmia after injection of I^{125} very-low-density lipoprotein from a donor with endogenous hypertriglyceridæmia.

(A) Before œstrogen therapy (B) during therapy with ethinylœstradiol 1 μg/kg/day.

Thus, these metabolic studies suggest that in Type 3 patients there is a block in the normal catabolism of VLDL such that intermediate density lipoprotein accumulates and LDL is not formed. These data help to explain the fact that LDL levels are typically lower than normal in Type 3 patients. Furthermore, the block in conversion of VLDL to LDL was actually suggested by the heparin experiments of Quarfordt, et al. (Fig. 3) (21). Finally, the block in conversion of VLDL to LDL would help to explain the accumulation of an abnormal particle in the plasma of patients with Type 3 HLP. Ordinarily the IDL is rapidly converted to LDL but in Type 3 HLP, it accumulates to high levels and remains in the plasma for longer than normal periods. As a consequence, this particle is partially catabolized and contains less triglyceride than VLDL but more than LDL. It is also partially converted to LDL so it contains more cholesterol than VLDL but less than LDL. These results led to the concept that VLDL "remnants" accumulated in Type 3 HLP. Detailed kinetic analysis of these turnover curves in a few patients also led to the conclusion that VLDL apoB synthesis was 2- to 3-fold greater than normal (32). This conclusion is tentative because the studies were performed before it was appreciated that the apoB content of VLDL and IDL in Type 3 plasma consists of B-100 and B-48. The B-48 presumably accumulates in the plasma of Type 3 patients because chylomicron remnants are also not properly catabolized.

While this series of studies provided some insight into the pathogenesis of the hyperlipidemia in Type 3 HLP, the biochemical defect that produced these changes was not discovered until apolipoprotein E became the focus of attention.

III. Apolipoprotein E and Type 3 HLP.

The importance of apolipoprotein E in Type 3 HLP was first recognized when Havel and Kane reported that the average content of apolipoprotein E (then called the arginine-rich peptide) in the VLDL from 7 patients with Type 3 HLP was more than 2-fold greater than that in VLDL from 10 appropriate control subjects (39% vs 17% of densitometric area on gel scanning, respectively (38). Within a short while, several laboratories developed immunoassays for apoE and reported that the total apoE level in the plasma of Type 3 patients was considerably higher then it was in both control subjects and in patients with other forms of hyperlipoproteinemia (39,40). It was initially felt that the immunoassay of apoE in plasma would serve as a useful test for the diagnosis of Type 3 HLP (39) but as described later, the study of apoE by electrophoretic methods led to an even better marker for the disease.

Nevertheless, the immunoassay techniques were used to learn more about the distribution and metabolic behavior of apoE in human plasma. Blum co-workers reported that when lipoproteins were separated by agarose column chromatography, nearly all the apoE was found associated with lipoproteins. In normal subjects and hypercholesterolemic patients, apoE was found principally in VLDL and HDL_2. In hypertriglyceridemic subjects, nearly all the apoE was in triglyceride-rich particles (41). Blum also looked at the dynamics of apoE metabolism in humans during fat feeding and following the injection of heparin (42). In normal subjects, fat feeding did not alter the concentration of apoE in the plasma but the distribution shifted such that the apoE associated with triglyceride-rich lipoproteins increased by 44% with a corresponding decrease in HDL. When patients were given heparin to induce rapid lipolysis, apoE levels in the plasma dropped by 17% but there was also a shift of the remaining apoE in plasma such that it was reduced in triglyceride-rich lipoproteins but correspondingly increased in HDL particles (42). These studies pointed out that the distribution of apoE among the triglyceride-rich lipoproteins and HDL was dynamic and probably underwent repeated shifts during the day, depending on both the timing and the composition of individual meals.

Subsequently it was pointed out that apoE was also not firmly attached to lipoproteins because up to 40% of the apoE could be dissociated from lipoproteins during ultracentrifugation (43).

The ability of apoE to shift from HDL to VLDL and chylomicrons during a fat meal suggested that apoE may have some role to play in the metabolism of triglyceride-rich lipoproteins. Sherrill and co-workers demonstrated that canine lipoproteins containing only apoE (apoE HDLc), when perfused thru a rat liver, displayed high affinity, receptor mediated uptake and saturation kinetics (Fig 6) (44).

Figure 6

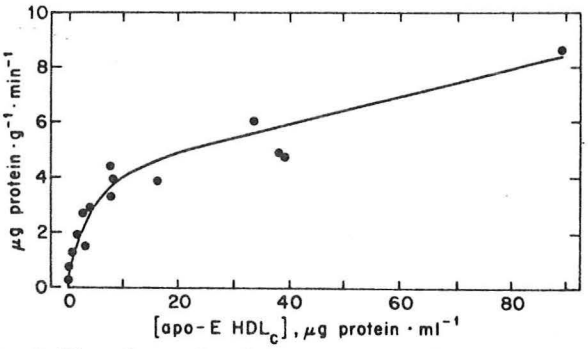


FIG. 2. **Hepatic uptake of canine apo-E HDL$_c$ (micrograms of protein per g of liver per min of perfusion) by rat livers as a function of protein concentration (μg/ml).** Each *point* represents six determinations per perfused liver. This concentration course represents hepatic uptake values from three different apo-E HDL$_c$ preparations.

Chylomicron remnants competed with this uptake process, suggesting that the uptake mechanism for apoE HDL$_c$ and chylomicron remnants was identical (44).

Havel and co-workers explored the mechanism of this uptake process in greater detail (45-47). Their studies focussed on the regulation of uptake of triglyceride-rich lipoproteins in perfused rat livers as a function of the apoproteins on the surface of the lipoproteins. In one series of experiments they observed that the uptake of large (newly secreted) chylomicrons from the perfused rat liver was slow whereas the rate of uptake of small chylomicrons (partially metabolized) and VLDL was more rapid (45). When small chylomicrons and VLDL were first treated in such a way as to increase the surface apoC content, then uptake was significantly retarded. In remnants that were depleted of apoC but not apoE, hepatic uptake was rapid (45).

These experiments were further refined by incubating VLDL with purified apoC peptides to determine how they individually affected hepatic uptake (Fig 7) (46).

Figure 7

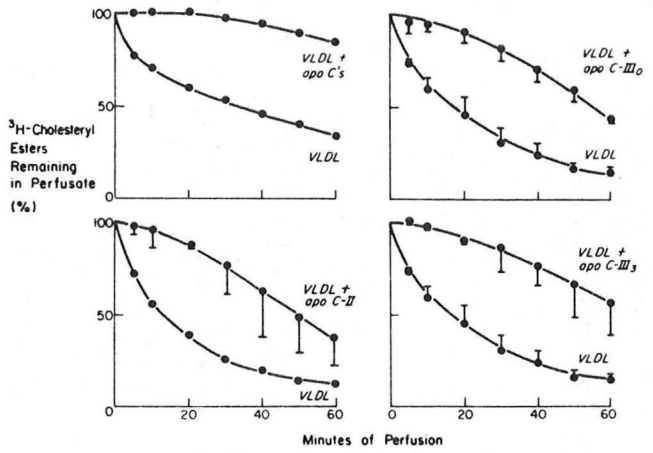


FIG. 3. **Removal of [^{3}H]cholesteryl esters of hepatic VLDL from the medium of perfused rat livers.** *Upper left,* hepatic VLDL (13 mg, n = 1) and hepatic VLDL incubated with unfractionated C apoproteins (11 mg, n = 1) (values in parentheses are milligrams of triglyceride in VLDL added to perfusate and numbers of experiments); *lower left,* hepatic VLDL (3.5 mg, n= 1) and hepatic VLDL incubated with apo C-II (1.5 mg, n = 2); *upper right,* hepatic VLDL (1.2 and 3.5 mg, n = 2) and hepatic VLDL incubated with apo C-III-0 (1.5 and 3.0 mg, n = 2); *lower right,* hepatic VLDL (1.2 and 3.5 mg, n = 2) and hepatic VLDL incubated with apo C-III-3 (1.0 and 4.0 mg, n = 2).

They observed clearly that all the apoC peptides, when added to VLDL, retarded the clearance of this lipoprotein by the perfused liver.

Similar experiments were performed with small chylomicrons but in this case, apoE was also added to the particles (Fig 8) (46).

Figure 8

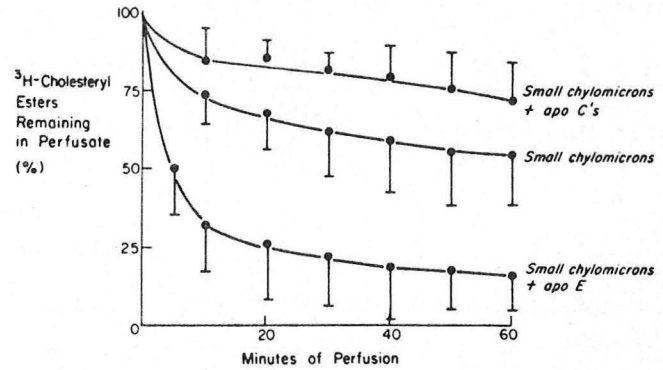


FIG. 6. **Removal from the medium of perfused rat livers of [^{3}H]cholesteryl esters of small chylomicrons from estrogen-treated rats.** Small chylomicrons added to perfusates contained 0.6 to 6.0 mg of triglyceride (n = 4); small chylomicrons that had been incubated with C apoproteins contained 3.0 to 6.0 mg of triglyceride (n = 3); small chylomicrons that had been incubated with apo E contained 0.6 to 6.0 mg of triglyceride (n = 5).

This figure shows that the addition of apoC's retard hepatic clearance of small chylomicrons while addition of apoE enhanced hepatic clearance.

Even when estrogen was given to rats to stimulate the formation of hepatic receptors that recognize apoB- and apoE-containing lipoproteins, addition of apoC to VLDL particles retarded their uptake by the estrogen-primed perfused livers (47).

Thus, these studies indicated that the hepatic uptake of triglyceride-rich lipoproteins was partly regulated by the peptide content on their surface. When newly secreted VLDL and chylomicrons enter the plasma they contain, or rapidly acquire by exchange, a complete complement of both apoE and apoC apoproteins. At this time the particles do not exhibit much affinity for hepatic receptors. During lipolysis, the triglyceride core is lost along with excess surface material, most of the apoC but only some of apoE. It appears that the loss of apoC (or the relative enrichment of apoE) results in a particle with increased affinity for hepatic receptors. Thus, apoE in some way serves as a recognition site for uptake of remnants by hepatocytes, but only after the appropriate loss of apoC.

IV. The abnormalities of apoE isoforms in Type 3 HLP

Dr. Gerd Utermann and co-workers were the first to investigate the isoforms of apoE and to determine that Type 3 HLP was associated with an abnormality in one of those isoforms. They reported their findings in a series of important papers that led the way toward a better understanding of the genetic and clinical expression of Type 3 HLP (48-53).

In brief, Utermann applied the technique of isoelectric focusing to the apoproteins of VLDL. Earlier work from several laboratories indicated that apoE was a single protein species when separated by polyacrylamide gel electrophoresis which separates proteins as a function of both charge and molecular size (Fig 10) (48).

Figure 10

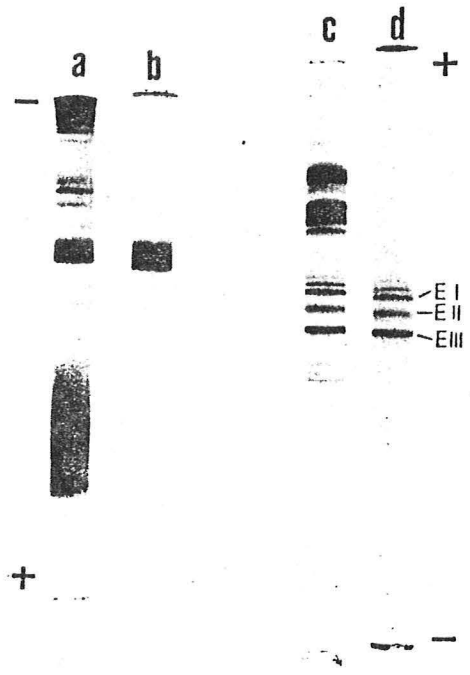


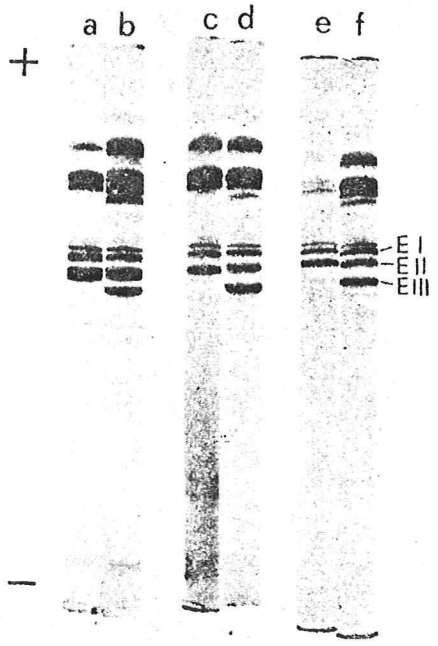
Fig.1. (Left) SDS-PAGE of apo VLDL (a) and purified Apo E (b). (Right) Analytical isoelectric focusing of urea soluble apo VLDL in polyacrylamide gels at pH 3.5–10 (c) and the same apo E preparation as in gel b (d).

However, with isoelectric focusing (IEF), the proteins migrate through an electric field until they reach their isoelectric point where their net charge is zero. A genetic mutation causing a single amino acid substitution

may alter the total charge on the molecule and cause it to have a slightly different isoelectric point. In the case of apoE, the IEF patterns in different individuals varied but 3 major bands were usually detected (Fig. 10) (48).

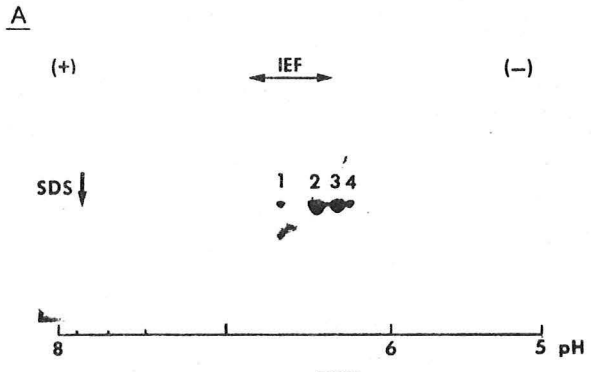
When Utermann compared the IEF patterns of normal subjects with those of patients with Type 3 HLP, he noted that the E-III band was missing and concluded that Type 3 HLP was in some way due to a deficiency of apoE-III (Fig 11) (48-51).

Figure 11



The fundamental observation of an abnormality in apoE is correct but Utermann's interpretation that there was a deficiency in apoE-III had to be modified when Zannis and Breslow determined, using two dimensional electrophoresis, that the apparent missing apoE-III band observed in single dimension IEF was actually present but shifted in position on the gel due to a charge shift; this charge shift was presumably due to a mutation affecting the primary sequence of the protein (56-59). In two dimensional electrophoresis, the protein or proteins in question are separated first in one dimension by isoelectric focusing. The entire gel is then placed horizontally atop a second polyacrylamide gel and the proteins are subjected to SDS gel electrophoresis (54, 55) (Fig 12). The SDS polyacrylamide gel electrophoresis (SDS-PAGE) separates the proteins according to molecular weight. One benefit of this approach is that it provides a way to detect proteins whose charge has been changed by missense mutations (54).

Figure 12



When Zannis and Breslow published their work, they introduced a system of nomenclature that was cumbersome and also confusing because it failed to account for the prior work of Utermann. Fortunately this problem was quickly resolved when a group of interested investigators proposed a uniform nomenclature for apoE isoproteins, apoE genotypes and phenotypes (60). The subsequent discussion of the work of Zannis and Breslow will be in terms of this uniform nomenclature.

These investigators used 2-dimensional electrophoresis to study the apoE of a large group of individuals and noted that the patterns observed consisted of either a single predominant apoE isoprotein or two major types along with minor bands (56,57). The minor bands were shown to represent post-translational modifications of the major apoE isoforms by sialic acid since they were largely eliminated when the sialic acid residues were removed by pre-treatment with neuraminidase (57) (Fig 13). Altogether there were 3 major apoE isoforms.

Figure 13

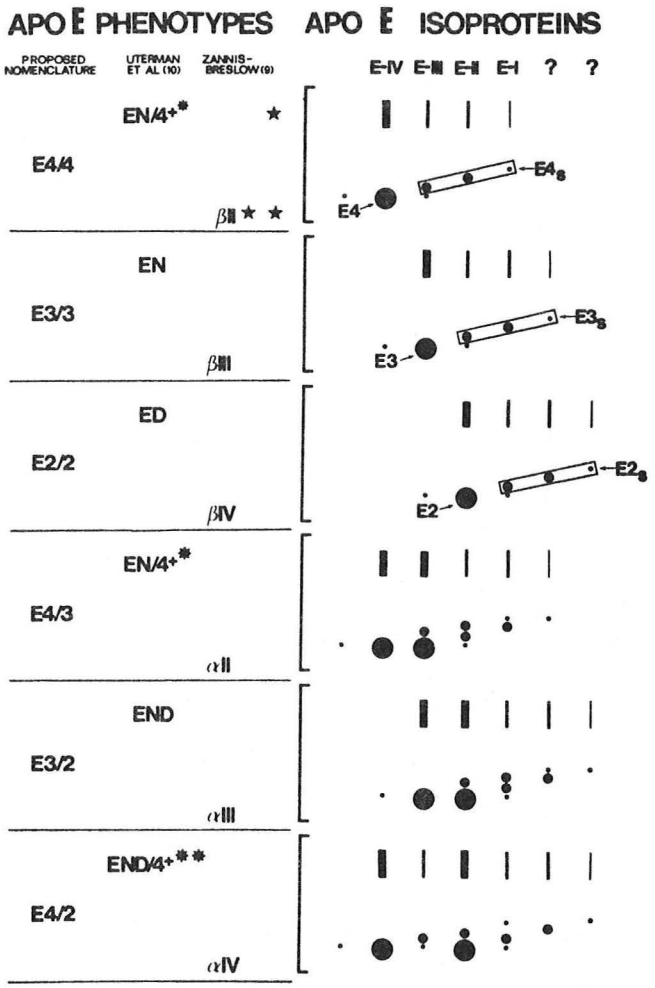


Fig. 3. Schematic presentation and proposed nomenclature of apoE isoproteins and phenotypes seen by one-dimensional isoelectric focusing and two-dimensional gel electrophoresis. *, Phenotypes E4/4 and E4/3 were not differentiated in the original work of Utermann, et al. (10). Both were collectively designated EN/4$^+$ **Phenotype E4/2 (αIV) ought to correspond to Utermann's hypothetical apoE phenotype ED/4$^+$ (10). Due to interference of the sialo apoE isoprotein E4$_{sl}$ (13), the phenotype observed was not originally recognized as such, but was designated as END/4$^+$ (10). ★, Phenotypes and isoproteins observed by one-dimensional isoelectric focusing. ★★, Phenotypes and isoproteins observed by two-dimensional gel electrophoresis.

The phenotyping patterns observed on 2-dimensional electrophoresis appeared to result from the interaction of 3 alleles operating at a single gene locus (58) and subsequent analyses have supported this conclusion (61,62). The three allele model and the different nomenclatures are shown in Fig 14 (60).

Figure 14

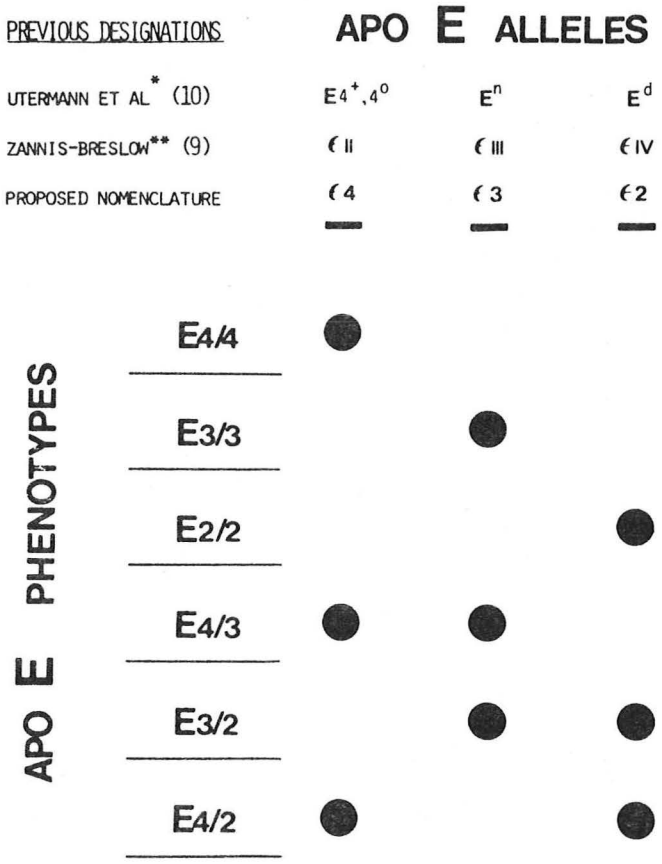


Fig. 2. Schematic presentation of the three-allele model of apoE inheritance and nomenclature of the apoE alleles and phenotypes. *, E^n, E^d, and $E^{4+,4o}$ were thought to be regulatory or structural genes at different, but closely linked, loci which were assumed to control apoE phenotypes (10). Previous haplotypes $E^n/E4^+$, $E^n/E4^o$, and $E^d/E4^o$ (10) correspond to alleles $\epsilon 4$, $\epsilon 3$ and $\epsilon 2$. **, The original ϵII, ϵIII, ϵIV, or the corresponding $\epsilon 4$, $\epsilon 3$, and $\epsilon 2$ alleles represent three alleles at the structural apoE gene locus (9). The closed circles represent the major asialo apoE isoproteins.

Thus if an individual is homozygous for the $\varepsilon 4$ allele, his phenotype will show only the E4/4 pattern reflected in the presence of only one principal apoE isoform on the 2 dimensional electrophoretic pattern. Homozygosity for the other 2 alleles is also found along with the expected heterozygosity (E4/3, E3/2, and E4/2). With regard to Type 3 HLP, Utermann's apoE-III deficiency actually equated to E2/2 homozygosity. The effect of the charge shift on the single dimension isoelectric focusing pattern is shown clearly in Fig 15 (61).

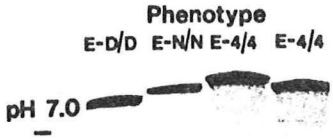


Figure 15

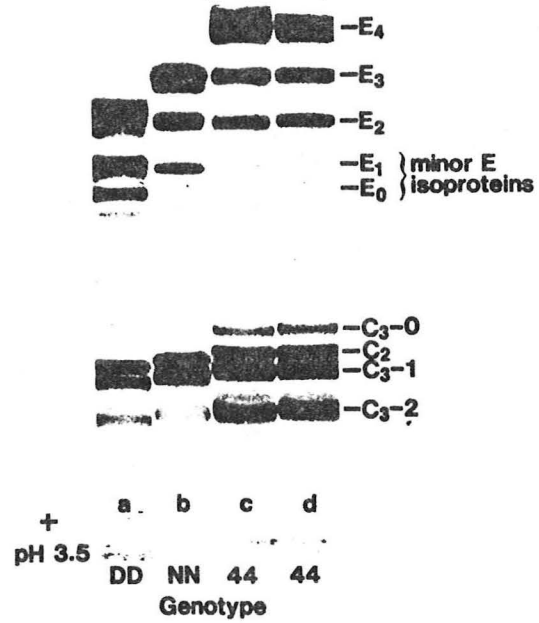


Fig. 2. Analytical isoelectric focusing in polyacrylamide gels of urea-soluble apo-VLDL isolated by ultracentrifugation. The three homozygous states are shown to demonstrate the entire apoE pattern shift. a, A subject with type III hyperlipoproteinemia homozygous for the ε2(D) allele. b, A subject homozygous for the ε3(N) allele. c and d, Two brothers homozygous for the ε4(4) allele.

Sufficient population studies have been performed to estimate phenotype frequencies (53,58, 61) (Table 11).

Table 11. ApoE Phenotype Frequencies in Population Studies*

Phenotype	Population Frequency		
	Ref 53 (n = 717)	Ref 58 (n = 61)	Ref 61 (n = 426)
E 3/3	55.92	49	51.41
E 4/3	24.12	15	25.12
E 4/4		2	0.94
E 3/2	15.62	31	19.95
E 4/2	3.09	3	1.17
E 2/2	1.25	0	1.41

* Modified from Reference 61.

Thus the most common phenotype is E3/3, followed by E4/3 and E3/2. Note that the E2/2 phenotype, which is characteristic of Type 3 HLP, is far more frequent than is the clinical syndrome. The reasons for this discrepancy will be mentioned later.

V. The Biochemical basis for apoE heterogeneity

Dr. Robert Mahley and co-workers have established the biochemical basis for the heterogeneity of apoE by determining the primary amino acid sequences of the purified isoforms (63-66). In the process, they also discovered a 4th allele (66). Their findings are summarized in figures 16 and 17 (Ref 66).

Figure 16

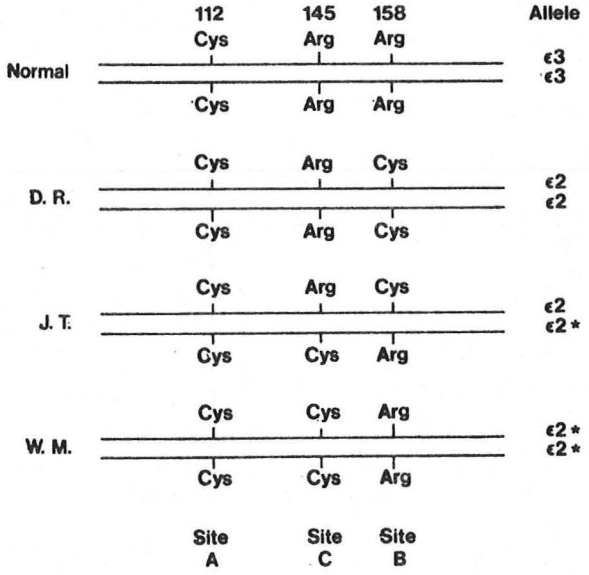


FIG. 2. Summary of the apo-E2 sequence and allelic (genotypic) relationships among subjects D.R., J.T., and W.M. The normal apo-E3 is given for comparison. The numbers 112, 145, and 158, corresponding to sites A, C, and B, respectively, are residue numbers in the apo-E polypeptide chain (14).

Figure 17

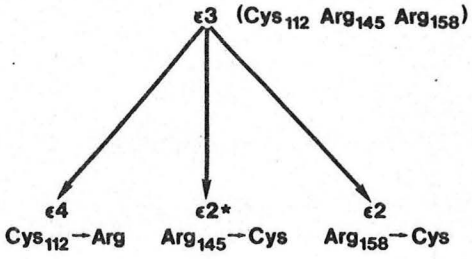


FIG. 3. Probable genetic relationship among the four known human apo-E alleles—*ε3*, *ε4*, *ε2**, and *ε2*. The single amino acid substitution found for alleles *ε4*, *ε2**, and *ε2*, compared to *ε3*, is given below each allele.

These investigators first determined that the heterogeneity of the 3 major isoforms of human apoE was due to cysteine-arginine interchanges in their primary structure. Amino acid analyses revealed that E-4 had no cysteine, E-3 had 1 cysteine residue and E-2 had 2 cysteine residues (63). Sequence data and amino acid analyses of the critical fragments of these isoforms indicated that E-3 was Cys112 Arg145 Arg158. E-4 was Arg at position 112, E-2 was Cys at position 158 and the 4th allele, E-2* was Cys at position 145 (Fig. 17) (66).

These various amino acid substitutions significantly alter the ability of lipoproteins or lipid emulsions containing the different apoE isoforms to bind to the LDL receptor (or B,E receptor) on cultured cells (66) and provide an explanation for the accumulation of remnants in patients with Type 3 HLP. This information will now be reviewed.

VI. The structural basis for the heterogeneity of receptor binding of apoE isoforms. The first evidence that apoE from Type 3 patients might result in abnormal metabolism of lipoproteins was obtained by Havel and co-workers (68). These investigators prepared lamellar complexes with egg lecithin and different preparations of apoE and then measured the uptake of these complexes in perfused rat livers. They observed that the lamellar complexes made with apoE-2 from patients with Type 3 HLP were not taken up nearly as rapidly as were complexes made with either apoE-3 or apoE-4 (68).

Shortly thereafter Gregg and co-workers isolated apoE from either normal or severely hypertriglyceridemic (Type 5) patients and from patients with Type 3 HLP. These apoE preparations were radioiodinated and were then re-injected into the plasma of normal subjects and patients with Type 3 HLP (69). These workers observed that the apoE from Type 3 subjects was cleared more slowly from the plasma of normal subjects then was normal apoE. Similar differences were observed when these apoE preparations were injected into a Type 3 subject (Fig 18) (69).

Figure 18

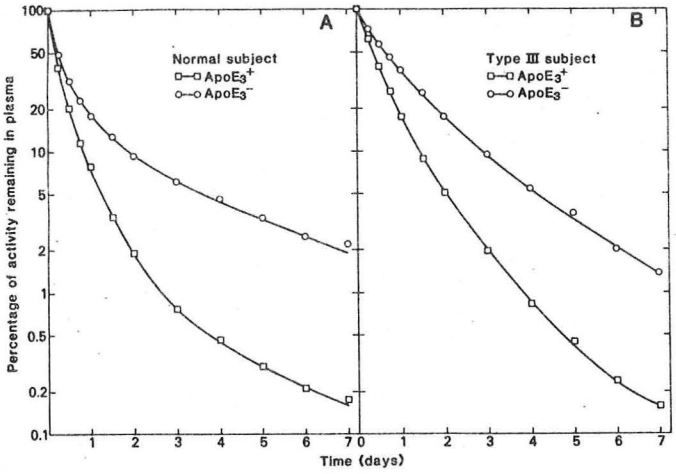


Fig. 1. The clearance of simultaneously injected $apoE_3^+$ (□) and $apoE_3^-$ (○) from the plasma of (A) a normal subject and (B) a type III HLP subject. All activity values have been normalized to 100 percent at day 0. The points represent actual data, whereas the curves are computer-generated. Each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

These results suggested that a defect in apoE isolated from Type 3 subjects was responsible for the accumulation of apoE in this syndrome since normal apoE was cleared at a much faster rate from the plasma of Type 3 subjects.

The direct binding of apoE from different patients with Type 3 HLP was compared in an extensive multicenter collaborative study reported by Schneider et al. (70). These investigators measured the ability of unlabeled

apoE incorporated into phospholipid complexes to compete with ^{125}I-LDL for binding to the LDL receptors of cultured fibroblasts. They noted that the apoE complexes from 6 patients with Type 3 HLP were not able to successfully compete with ^{125}I-LDL for binding to the LDL receptor but that the apoE from three others competed quite well. A representative experiment from this paper is shown in Fig 19 (70).

Figure 19

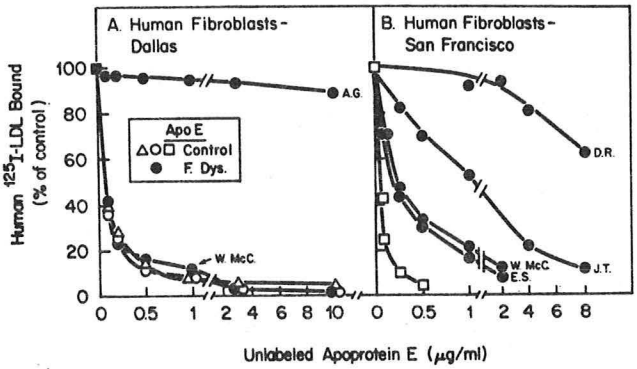


FIGURE 1 Ability of apo E/phospholipid complexes from control subjects (○, △, □) and F. Dys. subjects (●) to compete for the binding of human ^{125}I-LDL to monolayers of human fibroblasts at 4°C. The binding experiments were performed in semi-confluent cells (35-mm petri dishes) that had been incubated for the preceeding 48 h in 10% human lipoprotein-deficient serum (26). Experiment A (Dallas): each monolayer received 0.6 ml of ice-cold growth medium containing 10% human lipoprotein-deficient serum, 3 μg/ml of ^{125}I-LDL (316 cpm/ng protein), and the indicated concentration of unlabeled apo E/phosphatidylcholine complexes prepared by method B. After incubation for 2 h at 4°C, the cells were washed extensively, and the total amount of ^{125}I-LDL bound to the cells was determined (26). The "100% of control" value was 103 ng/mg protein. Experiment B (San Francisco): each monolayer received 0.6 ml of ice-cold growth medium containing 10% human lipoprotein-deficient serum, 2 μg/ml of ^{125}I-LDL (345 cpm/ng protein), and the indicated concentration of unlabeled apo E/dimyristoylphosphatidylcholine complexes prepared by method C. After incubation for 2 h at 0°C, the cells were washed extensively and the total amount of ^{125}I-LDL bound to the cells was determined (25). The "100% of control" value was 157 ng/mg protein.

Mahley and co-workers were able to show that the binding heterogeneity was a function of the amino acid substitutions in apoE-2 (64,66). They first demonstrated that apoE/phospholipid complexes made with apoE-3 or E-4 effectively competed with ^{125}I-LDL for receptor binding to cultured fibroblasts but apoE-2 complexes did not (Fig 20). Thus, E-2 was unable to bind to the LDL receptor because of the Arg 158 → Cys substitution (64).

Figure 20

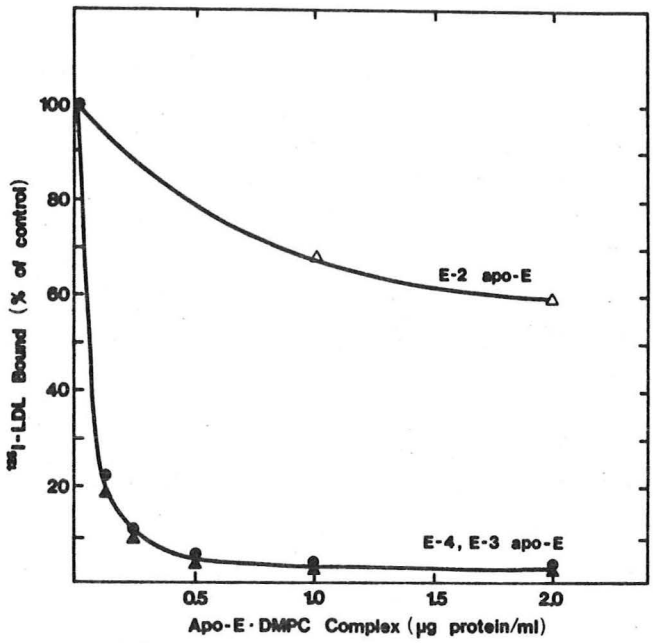


FIG. 2. Ability of apo-E•DMPC complexes from subjects homozygous for E-2 (△; subject D. R.), E-3 (▲), and E-4 (●) to compete with human ^{125}I-LDL for binding to normal human fibroblasts. Cells previously incubated for 48 h in Dulbecco's modified Eagle's medium containing 10% human lipoprotein-deficient serum received 1 ml of the same medium with 2 μg/ml of ^{125}I-LDL and the indicated concentrations of apo-E·DMPC complexes. After a 2-h incubation on ice at 4 °C, the cells in 35-mm Petri dishes were extensively washed and the ^{125}I-LDL bound to the cells was determined (10). The 100% control value was 111 ng of ^{125}I-LDL protein bound/mg of cellular protein.

When apoE-2 was chemically treated with cysteamine ($HS-CH_2-CH_2-NH_2$), a disulfide link was formed between the cysteine and cysteamine, and the amino acid side chain at position 158 again resembled arginine in that a free amino group was available. Cysteamine-reacted E-2 was able to bind much more effectively to the LDL receptor as shown in Fig 21 (61).

Figure 21

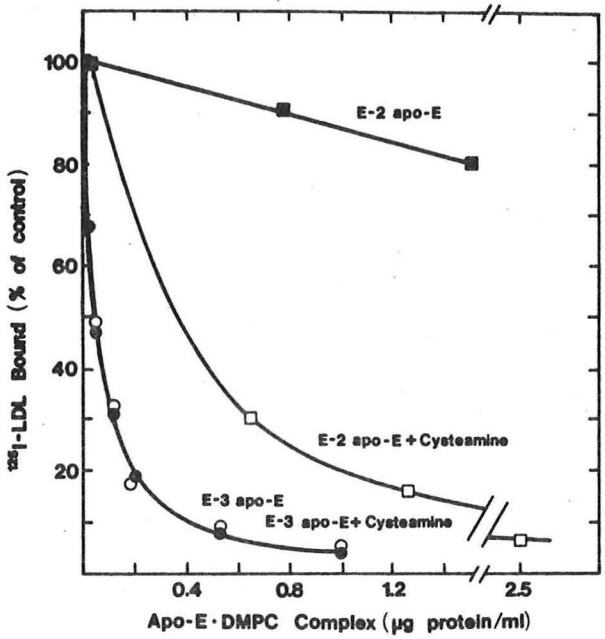


FIG. 4. **Comparison of the ability of control (β-mercaptoethanol-treated) and cysteamine-modified apo-E·DMPC complexes to compete with ^{125}I-LDL for binding to normal human fibroblasts.** Control E-2 apo-E·DMPC (■); cysteamine-treated E-2 apo-E·DMPC (□); control E-3 apo-E·DMPC (●); cysteamine-treated E-3 apo-E·DMPC (○). The E apoproteins were treated with β-mercaptoethanol or cysteamine as described under "Experimental Procedures" before they were complexed with DMPC. The binding experiments were performed as described in Fig. 2. The 100% control value was 143 ng of ^{125}I-LDL protein bound/mg of cellular protein.

In additional studies, Mahley and co-workers demonstrated that a 4th allele (E-2*) showed a different affinity for the LDL receptor than did E-2 (Fig 22) (66).

Figure 22

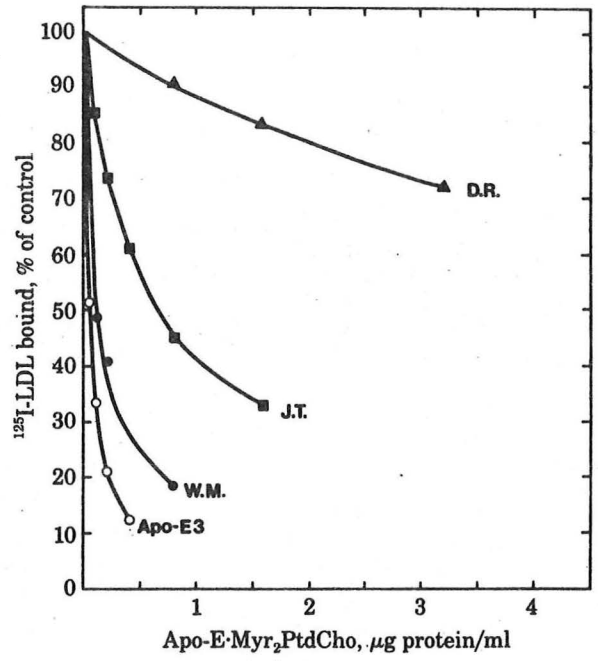


FIG. 1. **Ability of apo-E·Myr_2PtdCho complexes to compete with human ^{125}I-LDL for binding to normal human fibroblasts.** Cells (average, 208 μg of cellular protein per 35-mm plate) were incubated for 2 hr on ice in the presence of ^{125}I-LDL (2 μg/ml) and the Myr_2PtdCho complex. The 100% control value was 74 ng of ^{125}I-LDL protein bound per mg of cellular protein. Complexes were prepared from the apo-E of subjects D.R. (▲), J.T. (■), W.M. (●), and from the apo-E of an individual homozygous for E3 (○).

In this figure patient D.R. is E2/E2, J.T. is E2/E2*, and W.M. is E2*/E2*. Thus, the substitution of cysteine for arginine at position 145 did not impair binding as much as when this same shift occurred at position 158. These results helped to explain the earlier heterogeneity of binding observed by Schneider et al. (70).

Since cysteamine treatment of apoE from Type 3 patients partially restores the binding of apoE to LDL receptors, it has been proposed that cysteamine be used in the treatment of this disease (67). Unfortunately cysteamine is rather toxic and, as will be reviewed later, a reasonable treatment for this disease is available.

This series of experiments provides a biochemical explanation for the impaired catabolism of chylomicron and VLDL remnants in Type 3 HLP.

Not all classic Type 3 patients are E2/E2 homozygotes. Ghiselli and co-workers recently described a kindred with typical Type 3 HLP in which the proband had no apoE in the plasma (71). In this kindred the affected individuals had fasting chylomicronemia, characteristic floating betalipoprotein in the plasma, tubero-eruptive xanthomas, and premature coronary disease. They also had lower triglyceride levels and higher LDL levels than did classic Type 3 patients. The authors concluded that apoE deficiency represented a new disease (71).

The genetics and clinical spectrum of Type 3 HLP.

Since the pattern of inheritance of the apoE phenotypes is now better understood, our view of Type 3 HLP is changing. It was noted earlier that E2/E2 homozygotes were detected in about 1% of the population whereas the full-blown clinical syndrome of Type 3 HLP was observed relatively rarely (approximately 1 in 10,000 individuals) (72). Utermann was the first to note this and actually observed that while patients with E2/E2 phenotypes have the abnormal lipoproteins in their plasma, they often have plasma cholesterol levels lower than normal (Table 12) (50,52).

Table 12

Table 1 Clinical data on the five probands of phenotype Apo E-D

Subject	Age/sex	Serum Cholesterol (mg per 100 ml)	Serum Triglycerides (mg per 100 ml)	VLDL* Cholesterol (mg per 100 ml)	VLDL* Triglycerides (mg per 100 ml)	$VLDL_{ch/TG}$	Ch_{VLDL}/TG_{serum}	β-VLDL
B.Z.	24/M	89	77	23	61	0.38	0.3	+
B.Th.	26/M	128	157	64	83	0.77	0.41	+
M.B.	28/F	113	78	42	45	0.94	0.54	+
G.W.	60/M	196	141	41	66	0.61	0.27	+
A.W.	65/F	177	175	42	74	0.53	0.24	+
Mean ± s.d.		141 ± 45	128 ± 47	42 ± 15	66 ± 14	0.65 ± 0.22	0.35 ± 0.12	
Controls ± s.d.		191 ± 31	102 ± 34	17 ± 6	59 ± 39	0.32 ± 0.15	0.17 ± 0.07	

A group of six age- and sex-matched blood donors of phenotype Apo E-N served as controls.
*VLDL was isolated from 200–250 ml of serum to get reliable data and lipid values are given as mean of direct and indirect determinations.

This low cholesterol level actually reflects low LDL levels that presumably result from the failure of conversion of VLDL remnants to LDL.

In any case, since nearly all patients with clinical Type 3 HLP were E2/E2 phenotypes while not all E2/E2 phenotypes had hyperlipidemia, it was suggested that the E2/E2 homozygous state was required but not sufficient to cause Type 3 HLP.

It was also noted by several investigators that many family relatives of Type 3 patients had elevated VLDL levels (Type 4 patterns) (4,73), variable lipoprotein patterns (74) or co-existent familial hypercholesterolemia (75,76). Furthermore, it was observed that patients with hypothyroidism and Type 3 HLP improved markedly when they were rendered euthyroid (4, 72).

These observations led Utermann to speculate that E2/E2 homozygosity ordinarily rendered people slightly hypocholesterolemic. Patients exhibited clinical Type 3 HLP only if a second factor was involved such as the co-inheritance of a gene for another form of hyperlipidemia (Familial Hypercholesterolemia, Familial Multiple Lipoprotein Type hyperlipidemia or Familial Hypertriglyceridemia) or the development of hypothyroidism, obesity or diabetes (Fig 22) (51,52).

Figure 22

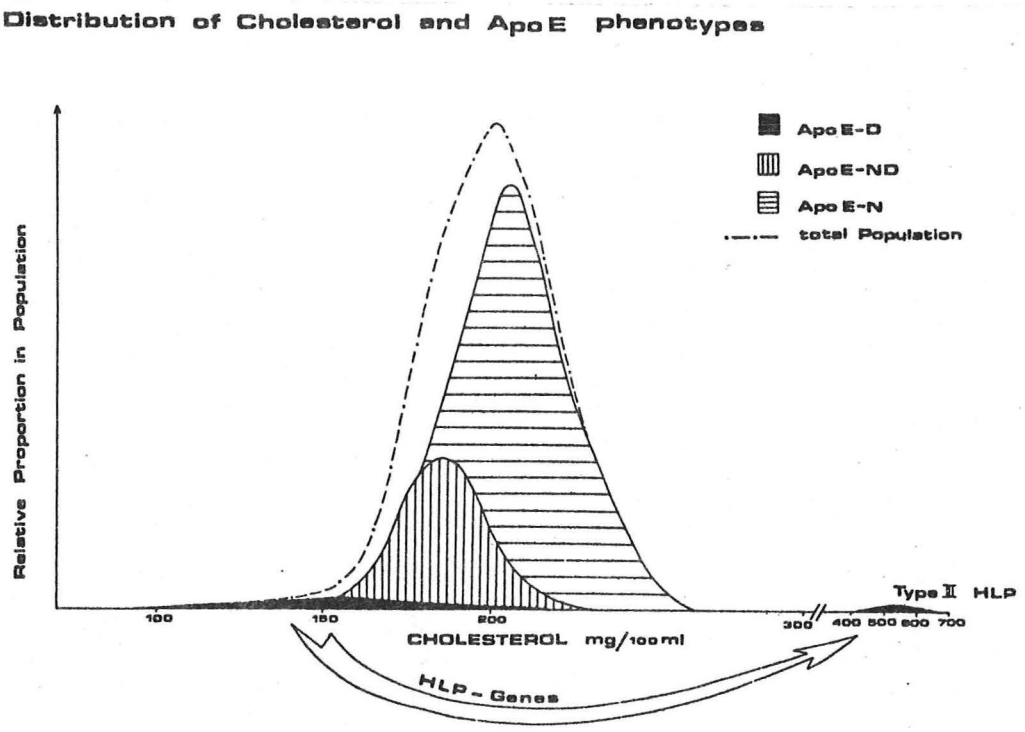


Fig. 3. Schematic representation demonstrating the presence of the three overlapping cholesterol distributions in the population that are determined by the Apo E phenotypes, and of the interaction of phenotype Apo E-D and "hyperlipidemia genes" in producing severe hyperlipoproteinemia type III.

Breslow et al (62) have extended these observations and reported that the relatives of Type 3 patients have normal cholesterol and HDL cholesterol levels, slightly low LDL cholesterol levels and almost twice elevated triglyceride levels. They also observed Type 3 HLP in two individuals with E4/2 heterozygosity. Thus apoE phenotypes affect plasma lipid levels but other genetic and environmental factors are also important.

A variety of pedigrees studied by Breslow et al. are illustrative of the inheritance of Type 3 HLP (Fig 23) (62).

Figure 23

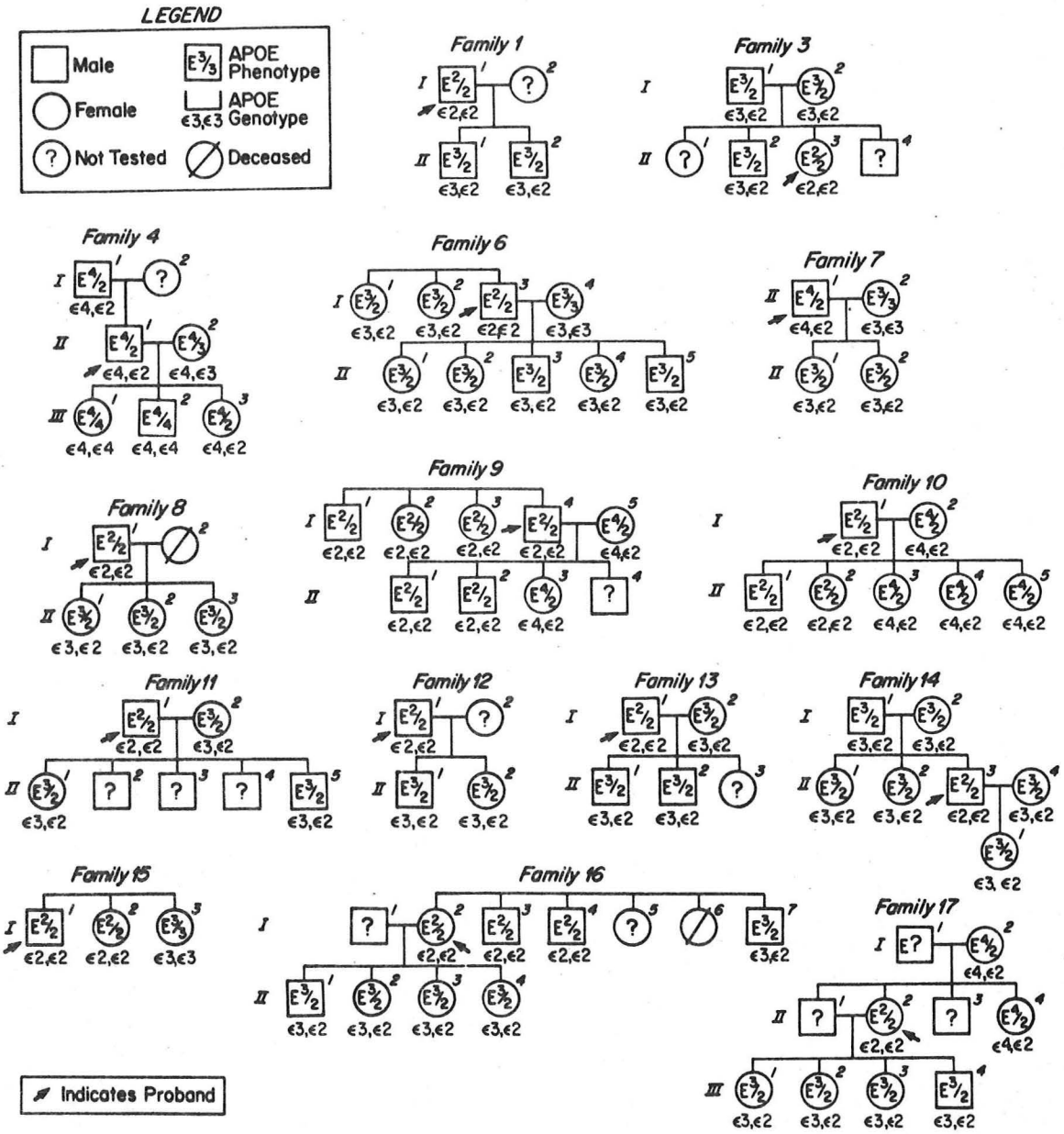


Fig. 2. Pedigrees of 15 families showing the transmission of type III HLP, as well as the transmission of the apoE phenotypes.

In Family 3, the disease appears for the first time in an offspring of two E3/E2 heterozygotes. In Family 10, the disease appears to be transmitted in a dominant fashion but this only appears to be so because the E2 allele is relatively common in the population; this is actually an example of

pseudodominant inheritance. These examples illustrate why the genetics of Type 3 HLP were so difficult to understand until the genetics of the apoE isoforms was worked out.

ApoE isoforms in other forms of hyperlipidemia

Since apoE has been shown to influence plasma lipoprotein and lipid levels, the distribution of apoE isoforms in other forms of hyperlipidemia have been examined (77-79). Studies from the NIH indicate that patients with lipoprotein phenotypes 1, 2a, 2b, and 4 have apoE phenotypic distributions similar to those of normal subjects (tables 13 and 14) (78).

Tables 13 and 14

TABLE II—ApoE PHENOTYPE DISTRIBUTION IN NORMAL AND HYPERLIPOPROTEINAEMIC SUBJECTS*

Phenotype	Normals	Type I	Type IIa	Type IIb	Type III	Type IV	Type V
E_4	4·1% (*3*)	0·0% (*0*)	14·0% (*7*)	5·4% (*4*)	0·0% (*0*)	6·7% (*3*)	31·4% (*11*)
$E_{3/4}$	21·6% (*16*)	30·0% (*3*)	18·0% (*9*)	13·5% (*10*)	0·0% (*0*)	17·8% (*8*)	37·2% (*13*)
$E_{2/4}$	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	5·7% (*2*)
E_3	55·4% (*41*)	40·0% (*4*)	60·0% (*30*)	54·1% (*40*)	0·0% (*0*)	46·6% (*21*)	5·7% (*2*)
$E_{2/3}$	18·9% (*14*)	30·0% (*3*)	8·0% (*4*)	27·0% (*20*)	25·0% (*3*)	28·9% (*13*)	17·1% (*6*)
E_2	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	0·0% (*0*)	75·0% (*9*)	0·0% (*0*)	2·9% (*1*)
Total	100% (*74*)	100% (*10*)	100% (*50*)	100% (*74*)	100% (*12*)	100% (*45*)	100% (*35*)

*Nos. in parentheses refer to number of individuals studied.

TABLE III—ApoE ALLELE FREQUENCY IN NORMAL AND HYPERLIPOPROTEINAEMIC SUBJECTS

Allele	Normals	Type I	Type IIa	Type IIb	Type III	Type IV	Type V
E^4	14·9%	15·0%	23·0%	12·2%	0·0%	15·6%	52·9%
E^3	75·6%	70·0%	73·0%	74·3%	12·5%	70·0%	32·9%
E^2	9·5%	15·0%	4·0%	13·5%	87·5%	14·4%	14·2%

Among the Type 3 subjects, 75% are E2/E2 and 25% are E3/E2. This finding agrees with that of Breslow and co-workers (62). In Type 5 patients (those with both increased VLDL and chylomicron levels) there is a clear enrichment of apoE phenotypes E4/E4 and E3/E4. The significance of these findings is unknown and not all laboratories have observed this E4 enrichment in Type 5 patients (79).

Although the E2/E2 state, when occurring with hyperlipidemia, is associated with premature atherosclerosis, it is not clear if either normolipidemic E2/E2 homozygotes or E3/E2 heterozygotes are also susceptible to premature atherosclerosis. Preliminary data from Utermann et al. indicates that normolipidemic E2/E2 or E3/E2 subjects are not enriched in a pool of coronary artery disease victims (53). However Davignon and co-workers (80) have reported an increased frequency of E3/E2 heterozygotes in normolipidemic patients with xanthelasmas. Although the numbers of patients in their series is small, they argue that the E3/E2 heterozygous state may not be benign and that tissue deposition of lipid in xanthelasmas may also be accompanied by the development of atherosclerosis. This remains a speculative point but extensive xanthomatosis has been described in a normolipidemic patient with E2/E2 homozygosity (81). These results imply that remnant deposition in tissues may be significant even in the normolipemic state.

The biological importance of lipoprotein remnants

The fact that Type 3 hyperlipidemia is characterized by the accumulation in plasma of both chylomicron and VLDL remnants and that it is also associated with the development of premature atherosclerosis has focussed attention on the atherogenic potential of lipoprotein remnants. Zilversmit reviewed the

evidence that both chylomicron remnants and VLDL particles accumulate in the artery wall and suggested that atherogenesis may be in part a postprandial phenomenon since that is the period during which chylomicron remnants are at their peak concentration (82).

Although these issues remain speculative, mounting evidence from cell culture work and experimental atherogenesis suggest that certain lipoprotein remnants are important in the formation of atherosclerosis.

Cholesterol-feeding in animals and man leads to the accumulation of remnant-like particles in the blood and these have been termed β-VLDL (83,84,85).

These lipoproteins (β-VLDL) are known to stimulate cholesteryl ester synthesis in macrophages (Fig 24) and this event is considered important in the genesis of foam cells in atheromas (86).

Figure 24

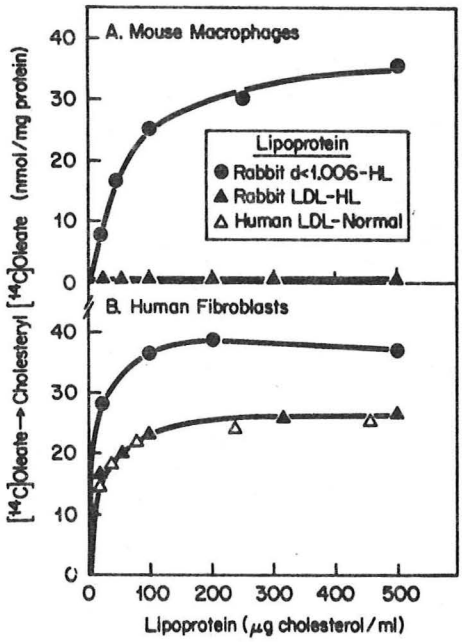


Fig. 4. Stimulation of cholesteryl ester formation in mouse macrophages (A) and human fibroblasts (B) by hyperlipidemic rabbit lipoproteins. Exp. A: each monolayer of mouse macrophages received 0.6 ml of DMEM containing 0.2 mM [^{14}C]oleate–2.4 mg/ml albumin and the indicated concentration of either d < 1.006 g/ml lipoproteins from hyperlipidemic rabbit No. 1 (●) or LDL from hyperlipidemic rabbit No. 1 (▲). After incubation for 6 hr at 37°C, the cellular content of cholesterol [^{14}C]oleate was determined. Exp. B: on day 7 of cell growth, after the cells had been incubated for 48 hr in lipoprotein-deficient serum, each monolayer of human fibroblasts received 1.5 ml of medium A containing 0.2 mM [^{14}C]oleate–albumin and the indicated concentration of one of the following lipoproteins: ●, d < 1.006 g/ml from hyperlipidemic rabbit No. 1; ▲, LDL from hyperlipidemic rabbit No. 1; or △, normal human LDL. After incubation for 6 hr at 37°C, the cellular content of cholesteryl [^{14}C]oleate was determined. The content of cholesteryl [^{14}C]oleate in cells incubated without lipoproteins was 0.8 and 0.4 nmol/mg protein for Exps. A and B, respectively. (Abbreviation in Fig. 4: HL, hyperlipidemic).

Furthermore Pitas et al. have recently demonstrated that foam cells in explants of atherosclerotic rabbit aortas possess receptors for β-VLDL and chemically modified LDL (87). Thus the cells actually contained in atheromas are able to take up these lipoprotein particles.

But do these β-VLDL particles induced by cholesterol feeding actually have anything to do with the abnormal particles that accumulate in Type 3 HLP? It appears that there are many structural similarities between β-VLDL and the remnants from Type 3 patients (27,28) with the exception that abnormal apoE is not found in β-VLDL. Fainaru et al. have recently shown that the abnormal human lipoprotein isolated from Type 3 patients stimulate cholesteryl ester in a manner similar to that for β-VLDL (Table 15) (27).

Table 15

TABLE 4. Stimulation of cholesteryl ester formation in mouse peritoneal macrophages by β-VLDL fractions of cholesterol-fed dogs and Type III hyperlipidemic patients[a]

Lipoprotein Fraction Added to Medium	Concentration of Lipoprotein in Medium		Cholesteryl [^{14}C]oleate Synthesis
	Protein	Cholesterol	
	μg/ml		*nmol/mg cellular protein*
Dog lipoproteins			
Experiment I[b]			
None			0.37
β-VLDL	9.3	50	7.4
Fraction I	3.7	50	14.6
	7.4	100	25.5
Fraction II	12.4	50	4.8
	24.8	100	10.1
Experiment II[c]			
None			0.37
Fraction I	5.0	66.7	30.6
Fraction II	5.0	20.2	1.8
Human lipoproteins (S.B.)[b]			
β-VLDL	28.0	150	3.0
	56.0	300	4.3
Fraction I	11.2	150	23.5
	22.4	300	26.2
Fraction II	37.5	150	6.2
	75.0	300	5.1
Human lipoproteins (D.R.)[d]			
None			1.2
d < 1.006	59	100	2.0
VLDL	70	100	2.7
β-VLDL	31	100	5.0
Fraction I	19	100	13.3
Fraction II	43	100	2.7

[a] Each monolayer (16-mm plastic Petri dishes) received 0.5 ml of DMEM containing 0.2 mM [^{14}C]oleate with 2.4 mg/ml albumin (sp act 18,000 dpm/nmol) and the indicated concentration of the lipoprotein fractions (cholesterol-fed dog and human Type III [S.B.] β-VLDL and its fractions separated by gel filtration, Figs. 1 and 5). After incubation for 16 hr at 37°C, the cellular content of cholesteryl [^{14}C]oleate was determined and the results expressed per mg of cellular protein.

[b] The studies with the dog lipoproteins (Experiment I) and the human Type III lipoproteins were performed with the same batch of cells on the same day. The lipoproteins were added at equal cholesterol concentrations.

[c] In this experiment, cholesterol-fed dog lipoprotein fractions were added at equal lipoprotein concentrations.

[d] A separate experiment performed as described in Footnote *a*. The β-VLDL and VLDL (pre-β lipoproteins) were isolated from the d < 1.006 g/ml fraction by Geon-Pevikon block electrophoresis.

However remnant particles that stimulate cholesteryl ester synthesis in macrophages have also been isolated from the blood of some individuals fed a high fat, high cholesterol diet. Normal individuals on a typical American diet do not appear to have β-VLDL in their blood but no one has yet excluded the possibility that β-VLDL occur transiently after meals and deposit slowly over years as a post-prandial event (85).

It is noteworthy that remnant-like particles have been observed in several diseases characterized by atherosclerosis and/or xanthomatosis. Remnants have been described in uremic patients treated with hemodialysis (88-90), in patients with systemic mastocytosis (91), and in patients with multiple myeloma (92-94).

Whether or not remnant formation in response to the standard American diet is a significant contributor to atherosclerosis in the general population remains a speculative issue.

Treatment of Type 3 hyperlipoproteinemia

Fortunately, Type 3 hyperlipidemia usually responds well to both diet and drug therapy (94-96). The NIH laboratory noted that switching from a regular diet to a low cholesterol diet enriched in polyunsaturated fat for a 3-month period produced a mean decline of 52% in plasma cholesterol and 70% in plasma triglyceride levels (Fig 25 A, solid lines) (95).

Figure 25 A and B

A

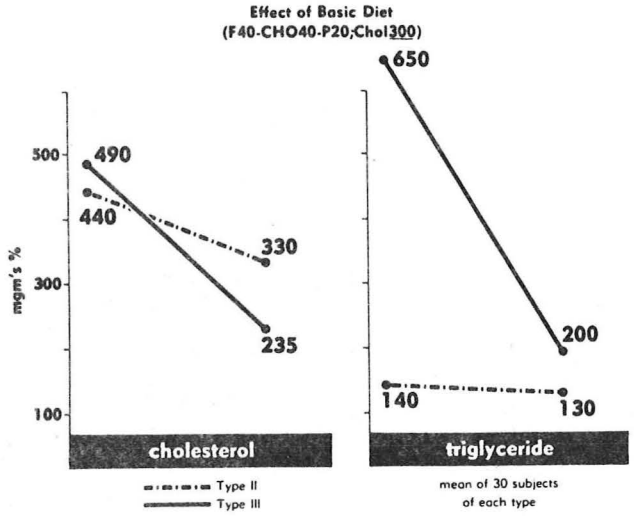


Figure 9. The effect of a low-cholesterol, balanced diet in patients with type II and type III hyperlipoproteinemia. F = fat; CHO = carbohydrate; P = protein; Chol = cholesterol.

B

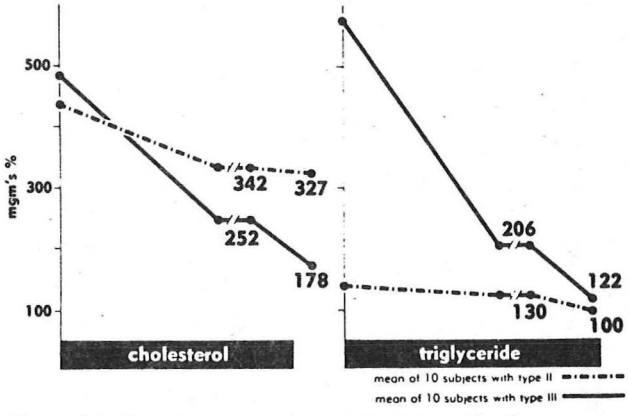


Figure 14. The effect of weight reduction on basic diet response in patients with type II and type III hyperlipoproteinemia.

When these patients were then switched to a 1000 calorie, low cholesterol, balanced diet for an additional month to promote weight loss, the mean serum cholesterol and triglyceride levels declined a further 29% and 40%, respectively (Fig 25 B, solid lines after the slash marks) (95).

This group also demonstrated that these patients responded well to therapy with clofibrate (Atromid-S) (Table 17) (95).

Table 17

Table 9. Effect of Clofibrate (2 g/day) Versus Placebo on Plasma Lipids and Lipoproteins in 11 Type III Patients

Medication Period	Plasma* Cholesterol	Plasma Triglyceride*	HDL* (as Cholesterol)	LDL* (as Cholesterol)	VLDL* (as Cholesterol)
	← mg/100 ml →				
I (diet only)	260 ± 57	288 ± 102	38 ± 13	92 ± 34	129 ± 37
II (diet + placebo)†, week 4	275 ± 67	332 ± 209	37 ± 11	105 ± 22	133 ± 55
III (diet + clofibrate)‡, week 4	186 ± 38	187 ± 77	41 ± 17	84 ± 27	61 ± 17
IV (diet only)	257 ± 50	300 ± 93	40 ± 13	95 ± 22	122 ± 31

* Mean ± standard deviation. HDL = high-density lipoproteins; LDL = low-density lipoproteins; VLDL = very low density lipoproteins.
† Placebo values are the mean values ± SD regardless of sequence.
‡ Clofibrate values are the mean values ± SD regardless of sequence.

Clofibrate lowers the total cholesterol, LDL-cholesterol and VLDL-cholesterol. HDL-cholesterol increases slightly. Total triglyceride is also reduced.

Similar observations have been reported by others (96,97) and clofibrate has remained effective therapy for up to 7 years in individual patients (97). The long-term efficacy of clofibrate treatment with regard to the reversal of atherosclerosis is still unknown. However, Falko et al. (98) treated 6 women and 7 men with Type 3 HLP with clofibrate and/or diet therapy for 2-8 months and reported visible regression of cutaneous xanthomas in the 6 patients who presented with them. Intermittent claudication diminished in 5 of 5 patients and angina pectoris diminished in 2 of 2 patients with these symptoms. Zelis and co-workers reported that therapy with diet and clofibrate in 6 Type 3 patients produced a significant improvement in peak reactive hyperemic blood flow in the lower extremity (Fig 26) (99).

Figure 26

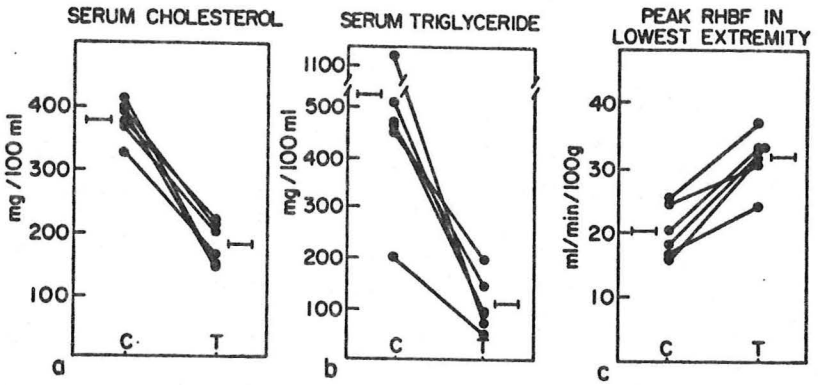


FIGURE 6 Response of six patients with type III hyperlipoproteinemia to treatment. Panel *a* serum cholesterol, panel *b* serum triglyceride, panel *c* the lowest peak reactive hyperemia blood flow in any extremity. C, control observation; T, treatment.

Although the safety of clofibrate for routine use has been questioned (98), the use of this drug in the treatment of Type 3 HLP has been approved by the FDA.

Nicotinic acid is also reported to be effective in Type 3 HLP but its many side-effects make it a second line drug.

Unlike other forms of hypertriglyceridemia in which estrogen therapy worsens the hyperlipidemia, estrogens have a significant hypolipidemic effect in Type 3 HLP (101, 102). Ethinyl estradiol at a dose of 1 μg/kg/day lowers the total, VLDL and LDL cholesterol levels and the total triglyceride. The response in women is fairly predictable but the response in men is uncertain since one patient improved (101) while another got much worse (102). In any case, since therapy with diet and clofibrate is so satisfactory, it is unlikely that estrogen therapy will be employed to any significant degree, particularly since it can be associated with thromboembolic phenomena (103).

In summary treatment of Type 3 HLP should consist of (1) weight loss to ideal weight, (2) maintenance of a low cholesterol, low saturated fat diet, and (3) clofibrate 1 gm b.i.d. if the diet and weight loss have not significantly lowered the lipid levels. A new drug, gemfibrozil (LOPID), is chemically related to Atromid-S and may prove useful in patients with Type 3 HLP. The dose of gemfibrozil is 600 mg b.i.d.

Conclusions

1) The classic form Type 3 hyperlipidemia (palmar and tubero-eruptive xanthomas with floating betalipoproteinemia and atherosclerosis) is a relatively rare disorder occurring with an estimated frequency of one in 10,000.

2) Metabolically, the disorder is characterized by the accumulation of both chylomicron and VLDL remnants that are cleared more slowly than normal from the plasma.

3) The disorder is associated with increased levels of apoE and the apoE phenotype is almost always E2/E2 in the triglyceride-rich lipoproteins.

4) Lipoprotein remnants from Type 3 patients do not bind properly to the LDL receptor and this inability can be related to amino acid mutations in selected portions of the apoE molecule.

5) The remnants found in Type 3 patients are structurally similar to those found in the plasma of a variety of cholesterol-fed animals that develop atherosclerosis. These remnants induce cholesteryl ester formation in macrophages and such a process may lead to foam cell formation in vitro and in vivo.

6) Remnants are found in several human diseases associated with accelerated atherosclerosis. The degree to which normal individuals form remnants post-prandially is unknown but a low-fat, low cholesterol diet probably generates the least number of remnants.

7) Type 3 HLP responds well to diet therapy and treatment with clofibrate.

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Appendix

Figures 27-29 are included here for reference. It will be helpful to refer to them particularly when reviewing pages 5-14 and pages 27-30. These figures outline our present conceptions about the dynamic interplay of apolipoproteins among the lipoproteins during normal lipoprotein metabolism. These schemes also indicate our latest ideas about remnant formation, receptor uptake of lipoproteins and the cholesterol shifts that occur between lipoproteins during their catabolism (Figure 29). These figures are taken from an article by RJ Havel entitled "Approach to the Patient with Hyperlipidemia" (Medical Clinics of North America 66:319, 1982).

Figure 27

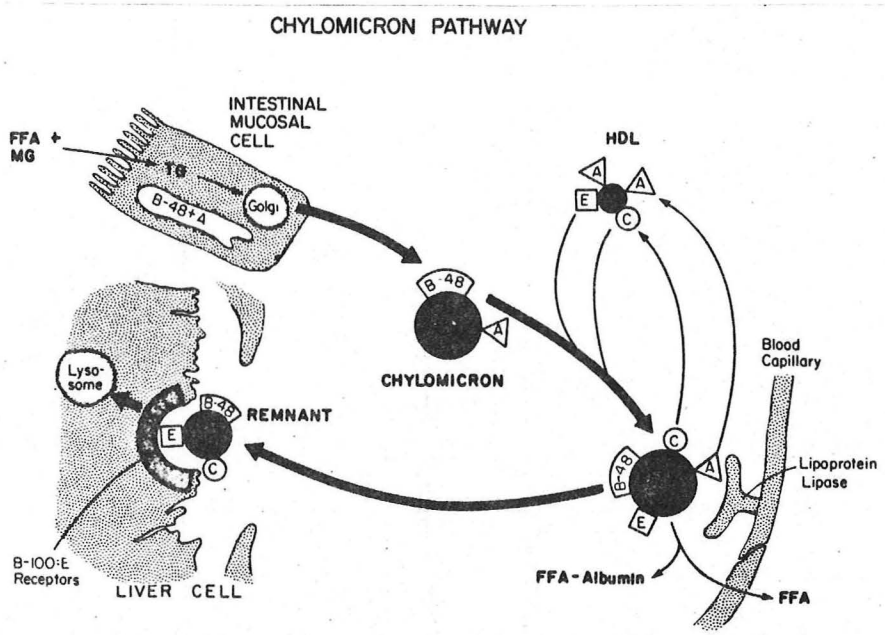

Figure 1. The pathways for transport of dietary fat in chylomicrons, depicted here, have been deduced mainly from studies in rats, but appear, on less direct evidence, to be similar in humans. Dietary cholesterol, like fatty acids and monoglycerides, is esterified in the mucosal cells and is transported in the nonpolar core of chylomicron particles (black area) with triglycerides (dark gray area). Whereas most of the triglycerides are removed in extrahepatic tissues, almost all of the cholesteryl esters are taken up by the parenchymal cells of the liver during endocytosis of chylomicron remnants (see text). As remnants are formed, not only are certain proteins, as shown, transferred to HDL, but also a substantial fraction of the surface lipids, mainly phosphatidylcholine (lecithin). Uptake of chylomicron remnants by the liver appears to be mediated primarily by a receptor that recognizes only the E apoprotein.

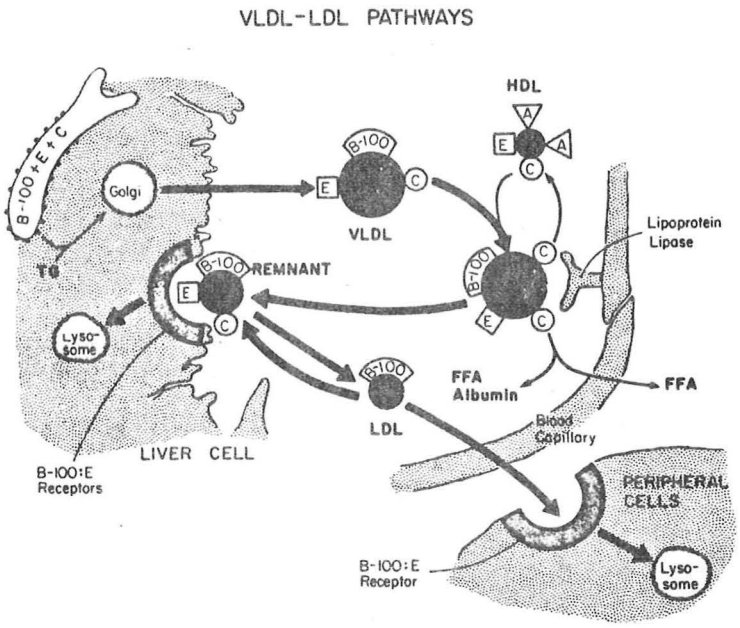

Figure 28

Figure 2. The pathway of VLDL transport from the liver, shown here, resembles in several respects that of chylomicrons, but note that the protein components of newly secreted VLDL differ from those of chylomicrons. After remnants are formed in extrahepatic tissues, both VLDL and chylomicrons contain C and E apoproteins, but different B apoproteins. The B-100 protein, secreted by the liver, unlike B-48 of chylomicrons, is recognized by an hepatic lipoprotein receptor, which also recognizes apoprotein E. However, most VLDL remnants are not normally taken up by endocytosis and catabolized in the liver; rather, they are mainly catabolized further with loss of all proteins except B-100 to yield LDL. LDL are metabolized slowly by interaction with B-100, E receptors in extrahepatic tissues and liver, and also by less well defined mechanisms (see text). Whereas chylomicron cholesteryl esters are derived mainly from the diet, the cholesteryl esters of VLDL, as well as those of LDL and HDL (black areas in core of particles), are derived from the action of lecithin-cholesterol acyltransferase (see also Figure 3).

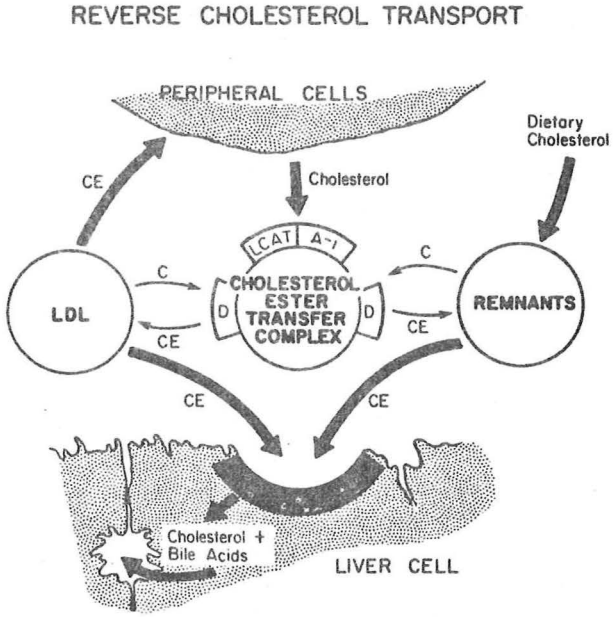

Figure 29

Figure 3. This diagram depicts the current concept of the major pathway by which cholesterol in the surface coat of plasma lipoproteins and in cells is transported to the liver for excretion in the bile. The enzyme, LCAT, exists in blood plasma and probably in extravascular spaces as part of a subraction of HDL, the cholesteryl ester transfer complex, which also contains the cofactor protein for the enzyme (apoprotein A-I) and apoprotein D. The cholesteryl ester-product of the transferase reaction is rapidly transferred by apoprotein D to other lipoproteins. The conversion of polar cholesterol to nonpolar cholesteryl esters creates a gradient which permits cholesterol to be transferred continually to the complex from cells and other lipoproteins, resulting in the movement of cholesterol to cells that have active lipoprotein receptors.