CAUSATION AND TREATMENT OF HYPERLIPIDEMIA: A MECHANISTIC APPROACH

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One of the most controversial issues in internal medicine is whether and how to treat patients with hyperlipidemia. Although great advances have been made in our understanding of lipoprotein metabolism and the mechanisms of hyperlipoproteinemia, the treatment of these disorders seemingly has lagged behind. There is at present little agreement on how best to treat the different forms of hyperlipidemia. Indeed, many physicians have the tendency to be negative towards specific therapy. Many doubt whether treatment will offer any benefit at all. Also, a sizable group are of the opinion that if any treatment is to be used, the only type should be dietary.

On the other hand, there is a growing body of evidence that a rational approach to the therapy of hyperlipoproteinemia (HLP) is possible. Two facts stand out. First, we have learned much about the mechanisms responsible for HLP. And second, we have made significant advances in our understanding of the ways in which drugs act--in their mechanisms of action. With this new knowledge it may be possible to develop a rationale for the therapy of HLP. This rationale will be based not only on the most effective means of therapy, but also on estimated risk associated with particular forms of HLP.

Before beginning a discussion of the treatment of the different forms of hyperlipidemia it will be necessary to review the primary pathways of lipoprotein metabolism. Each of the major lipoproteins in plasma can be considered. Also, the potential of each lipoprotein to produce atherosclerosis can be discussed.

LIPOPROTEIN METABOLISM

Since plasma lipids are insoluble in aqueous solutions, they do not circulate freely in plasma but are complexed with specialized proteins called apoproteins (Table 1). The resulting lipid-apoprotein complexes are named lipoproteins. The lipoproteins are composed of a central core of neutral lipids (cholesterol esters and triglycerides) and a membranous coating of unesterified

Table 1
Plasma Apolipoproteins

Apolipoprotein	Lipoprotein	Origin	Function
B-100	VLDL, LDL	Liver	Binds LDL receptor
B-48	Chylomicron	Intestine	Binds hepatic chylomicron remnant receptor(?)
C-II*	Chylomicrons VLDL	Liver	Activates lipoprotein lipase
C-III*	Chylomicrons VLDL	Liver	Inhibits lipoprotein lipase(?)
E	Chylomicrons VLDL	Liver	Binds hepatic apo E receptor
A-I	Chylomicrons HDL	Intestine and liver	Activates LCAT
A-II	Chylomicrons HDL	Intestine and liver	?

*Apoprotein C-II and C-III are tranferred and "stored" in HDL during lipolysis of chylomicrons and VLDL they are transferred back to newly secreted, triglyceride-rich lipoproteins.

cholesterol, phospholipids, and apoproteins. The lipoproteins, or their precursors, are produced both in the liver and gut. In the following discussion the metabolism of each major lipoprotein will be considered along with available evidence of their role in atherogenesis.

Chylomicrons. The intestine produces lipoproteins called chylomicrons. Most of the lipids in chylomicrons are derived from the digestion of dietary fats (triglycerides). Dietary triglycerides are hydrolyzed in the intestinal lumen to fatty acids and monoglycerides; these in turn are taken up by the intestinal mucosa and resynthesized into triglycerides. Mucosal triglycerides, along with any absorbed cholesterol, are incorporated into large lipoproteins, or chylomicrons. The major "structural" apoprotein of chylomicrons is apoprotein B-48 (apo B-48), but most soluble apoproteins also have been identified on the surface coat of these lipoproteins (Table 1).

The metabolism of chylomicrons is outlined in Figure 1. These particles are secreted from the intestine following ingestion of dietary fat. They contain mainly triglycerides (TG) and small amounts of cholesterol ester (CE). In their surface coat are unesterified cholesterol (Ch), phospholipids (PL), and apoproteins A and B. The apoprotein B of chylomicrons, designated B-48 (1) appears to be unique to this species of lipoprotein. In plasma, chylomicrons acquire apoproteins E and C. The apoproteins are shown in small enclosed circles. As chylomicrons circulate in plasma, they come in contact with an enzyme, lipoprotein lipase, located on the surface of capillary endothelial cells. The triglycerides are hydrolyzed, releasing fatty acids and glycerol; in this process, apoproteins C, E, and A are released and enter the surface coat of high density lipoproteins (HDL). After lipolysis is almost complete, a chylomicron remnant returns to the circulation and is rapidly removed from the liver. The recognition of the chylomicron remnant for hepatic uptake may be mediated by specific receptors on liver cells for apo B-48.

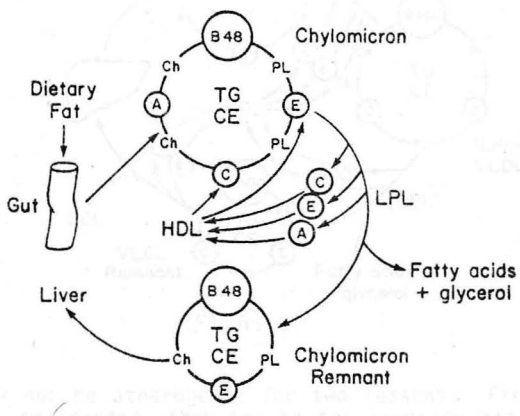

Figure 1

The atherogenic potential of chylomicrons is a matter of dispute, but possible mechanisms have been postulated (23). For example, as chylomicrons undergo lipolysis on the surface of vascular endothelial cells, some cholesterol could be released into the arterial intima. Also, cholesterol-rich chylomicron remnants might be atherogenic in man as they are in cholesterol-fed rabbits (2). On the other side, however, patients who have isolated hyperchylomicronemia appear not to be at unusually high risk for coronary heart disease (CHD). Furthermore, if chylomicrons or their remnants are atherogenic, CHD should be greater in populations with a high intake of fat. It is true that CHD is uncommon in countries where total fat ingestion is low, but in some countries where consumption of fat is relatively high, as in Crete, the incidence of CHD also is quite low (4). In these latter countries, a lack of CHD seems related more to low total plasma cholesterol than to the level of fat intake.

Very low density lipoproteins (VLDL). Like the gut the liver secretes a triglyceride-rich lipoprotein called VLDL. The metabolism of VLDL is outlined in Figure 2. VLDL resemble chylomicrons except that they are smaller. Most plasma triglyceride in the fasting state are carried in VLDL. As VLDL enter plasma they contain another structural apoprotein (apo B-100) (1). Since this is the major form of apoprotein B in fasting plasma, it will be designated simply "apo B". Other apoproteins on circulating VLDL are of the C and E species, and this type are soluble in aqueous solutions. As VLDL circulate in plasma, they acquire apoproteins C and E from high-density lipoproteins (HDL) along with cholesterol ester (CE). In this process, nascent VLDL are transferred into "native" VLDL, which in turn interact with lipoprotein lipase (LPL); this interaction leads to release of fatty acids and glycerol from TG and partial loss of apoproteins C and E to HDL. A smaller VLDL remnant is the product. Further catabolism of VLDL remnants results in transformation to low density lipoprotein (LDL) or, in some circumstances, into direct uptakes of remnants by the liver.

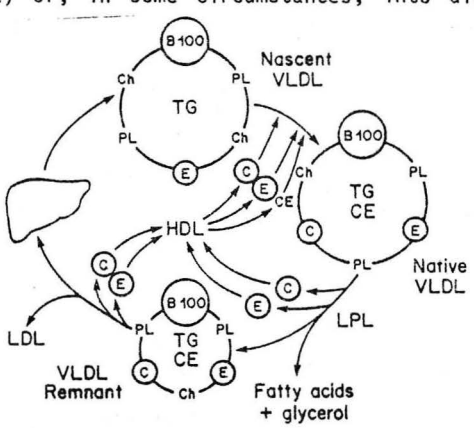

Figure 2

Normal VLDL may not be atherogenic for two reasons. First, they are not rich in cholesterol, and second, they may be too large to easily penetrate the arterial intima. However, VLDL from hypertriglyceridemic and diabetic patients

behave abnormally in tissue culture in that they tend to deposit lipids in fibroblasts, endothelial cells and macrophages. Thus, even if normal VLDL are not atherogenic, those from hyperlipidemic or diabetic patients may be.

VLDL remnants are smaller than native VLDL and contain more cholesterol, either of which may enhance their atherogenic potential. Evidence for the atherogenicity of VLDL remnants comes from three sources. First, many patients with CHD seem to have an increase in this class of lipoproteins. Second, in the genetic disorder, familial dysbetalipoproteinemia, VLDL remnants accumulate in plasma, and premature atherosclerosis is common; and third, cholesterol-fed animals, which rapidly develop atherosclerosis, have an increase in lipoproteins resembling VLDL remnants (2).

Low density lipoproteins (LDL). Pathways of formation and removal of LDL are presented in Figure 3. As indicated above, LDL are derived largely from catabolism of VLDL. They are the major cholesterol-carrying lipoproteins in man. Their core contains mainly cholesterol ester and very little triglyceride, and their surface coat has only one apoprotein, apo B (B-100). Recent evidence suggest that some LDL may be secreted "directly" by the liver (5-7), but this pathway may involve newly-secreted, very small VLDL as precursors of LDL.

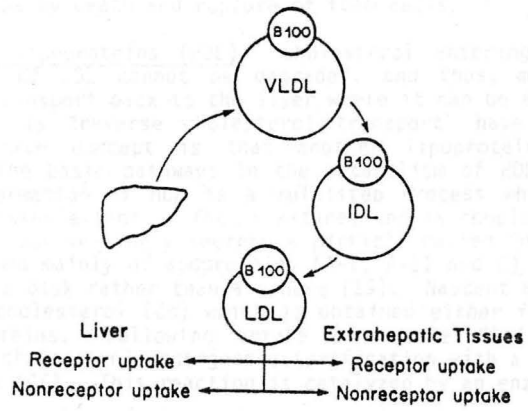

Figure 3

The means of removal of LDL from plasma have been a subject of great interest, and the basic mechanisms, as worked out in tissue culture, are outlined in Figure 3 (8). One pathway is a high-affinity, receptor-mediated uptake of LDL by cells. By this mechanism, the rate of uptake of LDL is a function of the number of binding sites; the lipoprotein is first bound to the LDL receptor and then is internalized and digested by lysosomes; the cholesterol esters are hydrolyzed with release of cholesterol into the cytoplasm for use in cell membranes. When amounts of unesterified cholesterol exceed those needed for cell membranes, the excess will both inhibit the cells' own synthesis of cholesterol and be stored in the cell as inert cholesterol ester. Also, if a cell accumulates an excess of cholesterol because of large amounts of LDL in the media, synthesis of receptors is reduced, thereby reducing uptake of cholesterol.

Besides the uptake of LDL via the receptor mechanism, LDL also can be degraded by other means. One example is bulk-phase phagocytosis of LDL. This pathway has been called the "scavenger" pathway because it may consist in part of phagocytic cells of the reticuloendothelial system (8). This mechanism for LDL degradation is particularly important when the specific-receptor pathways actually may be involved in LDL catabolism, and some of these probably are not part of the reticuloendothelial system.

Recent studies indicate that circulating LDL can be removed from the circulation by both peripheral tissues and the liver (8). Peripheral catabolism of LDL is of interest because one site of uptake could be the arterial wall. There is increasing evidence that most lipid in atherosclerotic plaques, which consist mostly of cholesterol esters, is derived from LDL. Indeed, LDL appears to be the most atherogenic of all the plasma lipoproteins. The contribution of LDL to the arterial wall cholesterol might occur by at least four ways: (a) LDL-cholesterol could enter arterial smooth muscle cells via the receptor-mediated process, (b) LDL may be incorporated in arterial-wall macrophages via the "scavenger" path, (c) LDL might become entrapped in extracellular spaces by interaction of LDL-apo B with mucopolysaccharide ground substance of the arterial intima, or (d) cholesterol may be released into extracellular spaces by death and rupture of foam cells.

High density lipoproteins (HDL). Cholesterol entering peripheral cells during catabolism of LDL cannot be degraded, and thus, mechanisms must be provided for its transport back to the liver where it can be excreted. Although the pathways of this "reverse cholesterol transport" have not been defined fully, an attractive concept is that another lipoprotein, HDL, plays an important role. The basic pathways in the metabolism of HDL are presented in Figure 4. The formation of HDL is a multistep process which begins in the liver, and to a lesser extent in the intestine, and is completed in the plasma. Both the liver and gut seemingly secrete a particle called "nascent" HDL. This particle is composed mainly of apoproteins (A-I, A-II and E) and phospholipids; it is shaped like a disk rather than a sphere (19). Nascent HDL has an affinity for unesterified cholesterol (Ch) which is obtained either from cell membranes or other lipoproteins. Following uptake of unesterified cholesterol into nascent HDL, this cholesterol undergoes esterification with a fatty acid to form cholesterol esters (CE). This reaction is catalyzed by an enzyme called

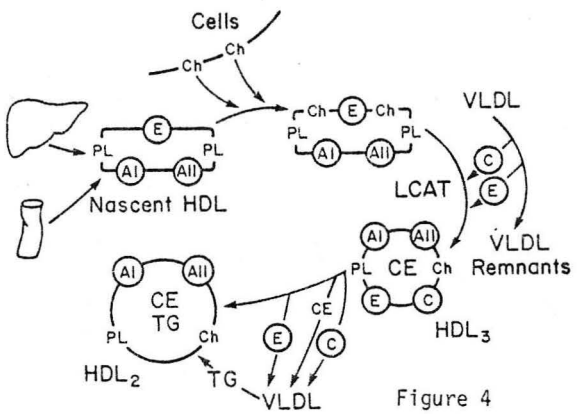

Figure 4

lecithin-cholesterol acyltransferase (LCAT); it is so named because it transfers an acyl group (fatty acid) from lecithin to cholesterol. LCAT is activated by apo A-I. As cholesterol ester is formed, it enters the core of the lipoprotein, and the resulting particle takes on a spherical shape. The product of this sequence of steps appears to be a small lipoprotein designated HDL_3.

Finally, HDL_3 may undergo interaction with other lipoproteins of plasma. In the catabolism of triglyceride-rich lipoproteins (chylomicrons and VLDL), surface components (unesterified cholesterol, phospholipids, and C and E apoproteins) may be transferred to the surface coat of HDL. Also, HDL can exchange some of its cholesterol ester for triglycerides of VLDL (10). The net result of these changes is the transformation of HDL_3 into a larger particle, HDL_2. The fate of HDL is poorly understood; whether it is removed intact from the circulation has not been determined.

The role of HDL in atherogenesis has become a subject of great interest in recent years. As will be discussed below, HDL are thought to be protective against development of atherosclerosis. Epidemiological studies (11) have shown that low concentrations of HDL are associated with greater rates of CHD than are high concentrations. Although the mechanisms by which HDL exerts its protective effects are not well understood, an attractive hypothesis is that these lipoproteins mobilize excess cholesterol from the arterial wall.

CLASSIFICATION OF HYPERLIPIDEMIAS

1. <u>According to plasma lipid abnormality</u>. Hyperlipidemia can be defined as an elevation in either plasma cholesterol or triglyceride (TG). The former is called hypercholesterolemia, the latter hypertriglyceridemia. Mixed forms also can occur. The precise levels of plasma cholesterol and TG that constitute hyperlipidemia however have been a matter of dispute. One definition of hyperlipidemia is a plasma concentration exceeding the 90th and 95th percentile for the population. The best available data for the distribution of plasma lipids in the United States was obtained by the Lipid Research Clinic Survey. These distributions are shown in Tables 2 and 3.

Table 2

Plasma Total Cholesterol (Population Distribution) (mg/dl)

AGE	White Males PERCENTILES							White Females PERCENTILES						
(Years)	5	10	25	50	75	90	95	5	10	25	50	75	90	95
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	125	131	141	153	168	183	189	131	136	151	164	176	190	197
10-14	124	131	144	160	173	188	202	125	131	142	159	171	191	205
15-19	118	123	136	152	168	183	191	118	126	140	157	176	198	207
20-24	118	126	142	159	179	197	212	121	132	147	165	186	220	237
25-29	130	137	154	176	199	223	234	130	142	158	178	198	217	231
30-34	142	152	171	190	213	237	258	133	141	158	178	199	215	228
35-39	147	157	176	195	222	248	267	139	149	165	186	209	233	249
40-44	150	160	179	204	229	251	260	146	156	172	193	220	241	259
45-49	163	171	188	210	235	258	275	148	162	182	204	231	256	268
50-54	157	168	189	211	237	263	274	163	171	188	214	240	267	281
55-59	161	172	188	214	236	260	280	167	182	201	229	251	278	294
60-74	163	170	191	215	237	262	287	172	186	207	226	251	282	300
65-69	166	174	192	213	250	275	288	167	179	212	233	259	282	291
70+	144	160	185	214	236	253	265	173	181	196	226	249	268	280

Table 3

Plasma Triglycerides (mg/dl)

Age	White Males PERCENTILES							White Females PERCENTILES						
(Years)	5	10	25	50	75	90	95	5	10	25	50	75	90	95
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	28	34	39	48	58	70	85	32	37	45	57	74	103	126
10-14	33	37	46	58	74	94	111	39	44	53	68	85	104	120
15-19	38	43	53	68	88	125	143	36	40	52	64	85	112	126
20-24	44	50	61	78	107	146	165	37	42	60	80	104	135	168
25-29	45	51	67	88	120	171	204	42	45	57	76	104	137	159
30-34	46	57	76	102	142	214	253	40	45	55	73	104	140	163
35-39	52	58	80	109	167	250	316	40	47	61	83	115	170	205
40-44	56	69	89	123	174	252	318	45	51	66	68	116	161	191
45-49	56	65	89	123	174	252	318	44	55	71	94	139	180	223
50-59	63	75	94	128	178	244	313	53	58	75	103	144	190	223
55-59	60	70	85	117	167	210	261	59	65	80	111	163	229	279
60-64	56	65	84	111	150	193	240	57	66	78	105	143	210	256
65-69	54	61	78	108	164	227	256	56	64	86	118	158	221	260
70+	63	71	87	115	152	202	239	60	68	83	110	141	189	289

Another approach to the definition of hyperlipidemia is to consider any level that promotes the development of atherosclerosis abnormally high. When this approach is taken, particularly for the plasma total cholesterol, concentrations well below the 90th percentile have been implicated in atherogenesis. The best available evidence suggests that rates of atherogenesis are accelerated when plasma cholesterol levels exceed 200-220 mg/dl (12). Thus, as seen in Table 2, at least 50 per cent of the adult population of the U.S.A. have cholesterol levels above 200 mg/dl and therefore may be in the zone of accelerated atherogenesis. For this reason many investigators believe that cholesterol concentrations between 200-220 mg/dl and the 95th percentile (which is approximately 280 mg/dl) should be called "mild hypercholesterolemia". While this conception probably has merit, in the present presentation, the terms hypercholesterolemia and hypertriglyceridemia will be reserved for patients whose lipids levels exceed the 95th percentile for the population.

2. According to the lipoprotein abnormality. With the discovery that different forms of hyperlipidemia can vary according to which lipoprotein is abnormally elevated, the attempt has been made to define hyperlipidemia according to the lipoprotein abnormality. The best known classification is that of Fredrickson, Levy, and Lees (13). This classification has been called lipoprotein phenotyping, and different patterns of lipoprotein abnormality are designated by numbers 1 through 5. Type 1 hyperlipoproteinemia (type 1 HLP) represents an increase only in chylomicrons. Type 4 HLP is an increase restricted to VLDL, type 3 to VLDL remnants, and type 2a to LDL. An increase in both VLDL and chylomicrons is called type 5 HLP, while increases in VLDL and LDL constitute type 2b. The distribution of concentrations of VLDL-cholesterol (VLDL-C), LDL-C and HDL-C are given in Tables 4, 5, and 6. These distributions are useful in the diagnosis of different types of HLP.

Table 4

Plasma VLDL-Cholesterol (mg/dl) (Population Distribution)

Age	White Males PERCENTILES							White Females PERCENTILES						
(Years)	5	10	25	50	75	90	95	5	10	25	50	75	90	95
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	0	2	4	7	11	15	18	1	1	4	9	13	19	24
10-14	1	2	5	9	13	18	22	2	3	6	10	15	20	23
15-19	2	3	8	12	17	23	26	2	3	6	11	15	22	24
20-24	1	5	8	12	18	24	28	2	4	8	13	18	24	28
25-29	3	6	9	15	22	31	36	2	3	7	11	19	24	29
30-34	1	5	8	11	26	36	48	1	3	6	11	17	21	27
35-39	3	7	12	19	30	46	56	2	3	8	13	21	29	36
40-44	5	8	14	21	30	43	56	3	5	8	13	20	28	32
45-49	5	8	13	20	31	40	51	2	4	9	15	22	33	41
50-54	8	10	14	23	33	49	62	2	5	9	15	23	32	37
55-59	3	6	11	19	28	39	49	2	4	9	18	28	37	49
60-64	3	4	9	16	23	35	44	1	3	6	13	20	29	39
65-69	0	3	8	16	23	40	45	0	3	7	13	21	36	41
70+	0	3	7	15	23	31	38	0	1	6	13	19	32	48

Table 5

Plasma LDL-Cholesterol (mg/dl) (Population Distribution)

Age	White Males PERCENTILES							White Females PERCENTILES						
(Years)	5	10	25	50	75	90	95	5	10	25	50	75	90	95
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	63	69	80	90	103	117	129	68	73	88	98	115	125	14
10-14	64	72	81	94	109	122	132	68	73	81	94	110	126	13
15-19	62	68	80	93	109	123	130	59	65	78	93	111	129	13
20-24	66	73	85	101	118	138	147	57	65	82	102	118	141	15
25-29	70	75	96	116	138	157	165	71	77	90	108	126	148	16
30-34	78	88	107	124	144	166	185	70	77	91	109	128	147	15
35-39	81	92	110	131	154	176	189	75	81	96	139	161	172	
40-44	87	98	115	135	157	173	186	74	84	104	122	146	165	17
45-49	98	106	120	141	163	186	202	79	89	105	127	150	173	18
50-54	89	102	118	143	162	185	197	88	94	111	134	160	186	20
55-59	88	103	123	145	168	191	203	89	97	120	145	168	199	21
60-64	83	106	121	143	165	188	210	100	105	126	149	168	191	22
65-69	98	104	125	146	170	199	210	92	99	125	151	184	205	22
70+	88	100	119	142	164	182	186	96	108	127	147	170	189	20

Table 6

Plasma HDL-Cholesterol
(mg/dl)
(Population Distribution)

Age	White Males PERCENTILES							White Females PERCENTILES						
(Years)	5	10	25	50	75	90	95	5	10	25	50	75	90	95
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	38	42	49	54	63	70	74	36	38	47	52	61	67	73
10-14	37	40	46	55	61	71	74	37	40	46	52	58	64	70
15-19	30	34	39	46	52	59	63	35	38	43	51	61	68	74
20-24	30	32	38	45	51	57	63	33	37	44	51	62	72	79
25-29	31	32	37	44	50	58	63	37	39	47	55	63	74	83
30-34	28	32	38	45	52	59	63	36	40	46	55	64	73	77
35-39	29	31	36	43	49	58	62	34	38	44	53	64	74	82
40-44	27	31	36	43	51	60	67	34	39	48	56	65	79	88
45-49	30	33	38	45	52	60	64	34	41	47	58	68	82	87
50-54	28	31	36	44	51	58	63	37	41	50	62	73	85	91
55-59	28	31	38	46	55	64	71	37	41	50	60	73	85	91
60-64	30	34	41	49	61	69	74	38	44	51	61	75	87	92
65-69	30	33	39	49	62	74	78	35	38	49	62	73	85	98
70+	31	33	40	48	56	70	76	33	36	48	60	71	82	92

Although this classification has helped to focus our attention on the role of lipoproteins in development of HLP, it also has been misleading to many. Unfortunately, it has not been widely understood that the phenotyping system does not define disease entities but merely denotes which lipoproteins are present in excess. There are multiple causes of each type of HLP. Therefore, the phenotyping system is an oversimplification in classification, and it must eventually be superceded by a more precise definition of disease entities.

3. <u>According to pattern of inheritance</u>. Although it has been recognized for many years that many forms of hyperlipidemia have a genetic basis, an extremely important advance was made by Goldstein and coworkers (14, 15)in their genetic classification of hyperlipidemia (Table 7). These investigators recognized three common monogenic forms of hyperlipidemia which they designated familial hypertriglyceridemia, familial hypercholesterolemia, and familial combined hyperlipidemia. In addition, a polygenic form of hypercholesterolemia was recognized along with several forms in which the genetics could not be defined precisely; these were called sporadic hyperlipidemias.

Table 7
Genetic Classification of Hyperlippidemia

Monogenic
- Familial hypercholesterolemia
- Familial hypertriglyceridemia
- Familial combined hyperlipidemia

Polygenic
- Hypercholesterolemia

Sporadic
- Hypertriglyceridemia

4. According to mechanism of hyperlipidemia. Another approach to the classification of hyperlipidemia is to categorize disorders according to the mechanisms of their abnormalities, or their metabolic defects. In some instances the specific molecular abnormality responsible for the elevation in a lipoprotein species has been defined exactly. In other cases, it has been possible to demonstrate the general area in which the defect occurs. In other words, a high concentration in plasma can be the result of either an overproduction of a lipoprotein or a decrease in its clearance. Sometimes both abnormalities are present in the same patient. The major thrust of this presentation will be to classify HLP according to the primary physiological defect (i.e. either overproduction or decreased clearance), and where possible to define the biochemical defect (when it is known). Not only will the different HLP's be defined according to their physiological defects, but when a treatment is available, it also can be considered in terms of its physiological mode of action.

DRUG VS. DIETARY TREATMENT OF HYPERLIPIDEMIA

In the past, the use of diet for treatment of hyperlipidemia has received much attention, and there is a widely held belief that dietary change is the preferred form of therapy. There is very little danger of adverse reactions from diet. This is not true for drug therapy. All drugs are associated with some side effects or potential adverse reactions, and the use of drugs has been received with considerable skepticism.

There is little doubt that dietary therapy is preferred in most forms of mild hyperlipidemia. Indeed, most mild hyperlipidemias probably are due in large part to diet--to excess intakes of saturated fatty acids, cholesterol, and total calories (obesity). But dietary hyperlipidemia is not the object of the current paper. Instead, the genetic hyperlipidemias will be the major focus of attention. In many of these, dietary change alone is not sufficient to normalize the lipid levels. It often is necessary to turn to drugs. A reasonable concept is that dietary hyperlipidemias, which are usually mild hyperlipidemias, should be treated with diet. Genetic hyperlipidemias, on the other hand, often requires drugs, and sometimes drugs in combination.

MECHANISMS OF HYPERLIPIDEMIA

An elevation of a plasma lipoprotein can be due to its overproduction or to a defective clearance. Three major types of clearance defects can occur (Figure 5). First, lipolysis of TG in TG-rich lipoproteins (chylomicrons or VLDL) can be

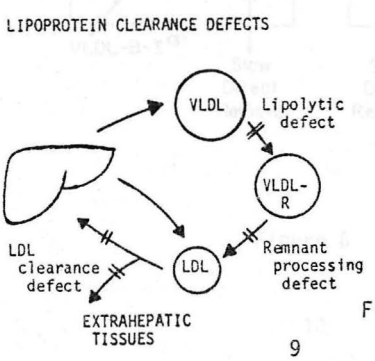

Figure 5

defective. Second, the processing of VLDL remnants can be impaired; and third, the clearance of LDL can be retarded. Genetic defects have been discovered that can cause each of these clearance defect. In some notable cases, the molecular abnormality responsible for the defect is known. Recent work also demonstrates that two of the major lipoproteins--VLDL, LDL--can be produced in excessive amounts. The biochemical defects responsible for lipoprotein overproduction are not known, but overproduction of lipoproteins remains an important contributor to HLP.

The determination of the physiological defects responsible for development of HLP has presented a great challenge to clinical investigators. This work has been greatly facilitated in the past few years by the use of isotope kinetics and multicompartmental analysis. Research in multicompartmental analysis of lipoprotein kinetics was pioneered by the late Dr. Mones Berman at the National Institutes of Health. In the past six years we have collaborated closely with the laboratory of Dr. Berman in development of kinetic methods for determination of the physiological defects producing HLP. Our investigations have concentrated on the metabolism of two major constitutents of lipoproteins, namely, triglycerides and apolipoprotein B (apo B). We have developed an integrated multicompartmental model for simultaneous analysis of the kinetics of TG and apo B. This model is shown in Figure 6. Through the use of tritiated glycerol as a precursor of VLDL-TG and radioiodinated VLDL and LDL as exogenous markers for apo B, it is possible to estimate production and clearance rates of VLDL-TG, VLDL-apo B, and LDL-apo B. These methods have been employed both for determining abnormalities in VLDL and LDL in various forms of hyperlipidemia as well as for elucidating mechanisms for altering lipoprotein concentrations by different diets and drugs. In the tables to follow kinetic data will include concentrations of VLDL-TG, VLDL-apo B, and LDL-apo B plus their synthetic rates and fractional catabolic rates (FCR). The FCR will be taken as a measure of clearance capacity.

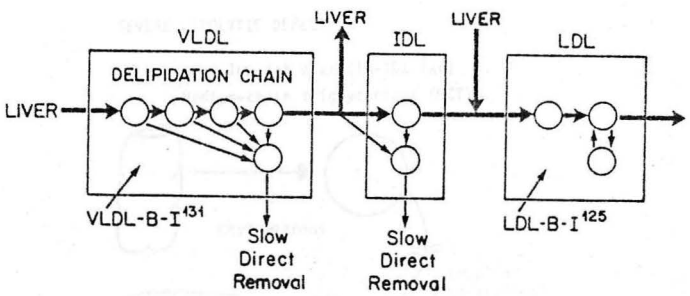

Figure 6

DEFECTIVE CLEARANCE OF TRIGLYCERIDE-RICH LIPOPROTEINS.

Lipolysis of TG in chylomicrons and VLDL occurs by interaction of the lipoprotein with lipoprotein lipase. Defective lipolysis can be the result of abnormalities in either the lipoprotein or the lipase; it also can be of varying degrees of severity--mild, moderate, or severe. A variety of clinical pictures can result depending on the severity of the defect, and the best therapy also will depend on the severity. In the following discussion each type of lipolytic defect will be considered.

Severe lipolytic defects

At least two and possibly three conditions are associated with a severe defect in lipolysis (Figure 7). They typically produce type 1 HLP. One disorder is congenital deficiency of lipoprotein lipase (16). It is characterized by a marked increase in chylomicrons, but not in VLDL. Chylomicronemia is present from birth. In the complete absence of lipoprotein lipase, fat ingestion produces accumulation of enormous quantities of chylomicrons in plasma; TG levels often are as high as 2000 to 10,000 mg/dl. A second cause of severely defective lipolysis is congenital deficiency of apolipoprotein C-II (apo C-II) (17); this apoprotein is required for activation of lipoprotein lipase. And finally, some patients with severe insulin deficiency have very low lipoprotein lipase and can present with a picture of type 1 HLP.

Severe elevations of plasma TG's can produce the chylomicronemia syndrome (18). When TG's exceed 2000 mg/dl, patients are prone to this syndrome which includes abdominal pain (63%) and/or acute pancreatitis (28%), eruptive xanthomata (40%), lipemia retinalis (23%), recent memory loss (85%), objective dyspnea (46%), and flushing with alcohol (25%). An elevation of chylomicrons per se does not necessarily predipose to premature atherosclerosis. An increased incidence of coronary heart disease has not been documented in such patients. The greatest risk is for acute pancreatitis which can be life threatening.

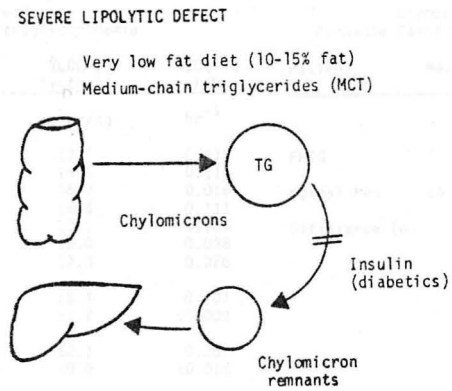

Figure 7

Treatment of severe chylomicronemia is manditory. The aim is to lower chylomicrons to a safe range; to reduce total TG to about 1000 mg/dl is a reasonable goal. Drugs are not effective in these conditions. Treatment requires a reduction in fat intake. Dietary fats should be restricted to less than 20% of total calories; intakes as low as 10% may be required to avoid pancreatitis. A low fat diet can be supplemented with medium-chain triglycerides (MCT) which are not absorbed via chylomicrons; medium chain fatty acids are absorbed directly into the portal vein.

Moderate lipolytic defects

There is little question that less severe defects in lipolysis occur. They produce moderate hypertriglyceridemia with TG levels in the range of 400 to 1000 mg/dl. Most of the increase in fasting plasma resides in VLDL-TG, but clearance of chylomicrons is impaired to some extent. Again, the defect may reside in either lipoprotein lipase or in the lipoprotein particle itself. Unfortunately, it has not been possible to elucidate the specific defect in most patients. Decreases of lipoprotein lipase (but not complete absence) have been reported. Relative deficiencies also have been claimed for apo C-II. These claims have not been substantiated, and in most instances, the causes of moderate defects in lipolysis are unknown.

Defective lipolysis of this type is probably responsible for the condition known as familial hypertriglyceridemia (14, 15). In families with this disorder, only plasma TG are elevated among affected family. LDL concentrations usually are normal in the patient and other family members. Studies from our laboratory (19) indicate that most patients of this type have reduced clearance of VLDL-TG (Table 8 and Figure 8). In these patients, VLDL-TG concentrations were distinctly elevated; synthetic rates were slightly above the mean of normal, but none exceeded the upper limit of normal (20 mg/hr/kg); FCRs for VLDL-TG on the other hand were markedly reduced. Our studies also suggest that they may have a reduction in available lipoprotein lipase (Table 9), but more investigations are needed.

Table 8
VLDL-TG Kinetics
Probable Familial Hypertriglyceridemia

Patient	VLDL-TG Conc.	VLDL-TG Synthesis	VLDL-TG FCR
	mg/dl	mg/hr/kg	hr^{-1}
1	308	19.7	0.112
2	278	14.5	0.116
3	336	16.7	0.016
4	427	18.6	0.111
5	248	10.1	0.105
6	422	18.0	0.098
7	590	17.3	0.076
mean±SEM	377 ±44	16.4 ±1.2	0.107 ±0.006
Normal (n=27)	136 ±7	12.1 ±0.8	0.207 ±0.016

Table 9
Lipoprotein Lipase Activity
Probable Familial Hypertriglyceridemia (FHTG)

Patient	No.	Postheparin Lipoprotein Lipase Activity micromoles fatty acid released per hr per ml ± SEM
FHTG	7	9.3 ± 09
Normal Men	16	15.4 ± 1.3
Difference (p)		<0.001

FAMILIAL HYPERTRIGLYCERIDEMIA

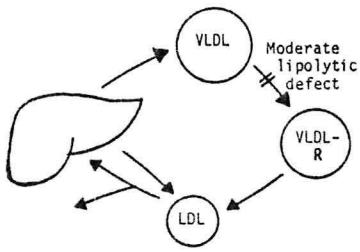

Figure 8

There is some dispute whether moderate hypertriglyceridemia associated with defective lipolysis causes an increased risk for CHD. One report suggests that such patients are free of increased risk (20). Unfortunately, information is limited, and before we can be certain, a rapid and simple method for diagnosis of defective lipolysis is needed. At the present time a decision to use drugs in familial hypertriglyceridemia probably should be restricted to patients with known atherosclerotic disease or the presence of such disease in hyperlipidemic relatives.

Most patients with defective lipolysis producing moderate hypercholesterolemia will respond with TG lowering to a fibric acid derivative, either clofibrate or gemfibrozil. These drugs enhance availability of lipoprotein lipase (21), and thereby promote the clearance of VLDL-TG (22). Therefore, if a decision is made to treat a patient with moderate lipolytic defect, one of the fibric acids is the drug of choice.

Mild lipolytic defects

Clearance capacity for plasma TG seemingly varies from person to person over the normal range of TG levels. This variability may account in part for differences in TG levels in the general population. Some people whose plasma TG falls into upper normal or mildly elevated ranges may have mild abnormalities in lipolysis of their TG-rich lipoproteins (23). Under most circumstances, mild defects in lipolysis are of no clinical significance. There is no evidence that they contribute significantly to enhanced atherogenesis, although this possibility has not been completely ruled out. Still, mild defects can assume importance in patients who have overproduction of VLDL; in these cases, which will be discussed below, hypertriglyceridemia will be accentuated by any reduction in capacity to lipolyze plasma TG.

Remnant processing defects.

In another type of clearance defect, hypertriglyceridemia can result from an abnormal processing of VLDL remnants (Figure 9). Chylomicron remnants can accumulate in plasma, but VLDL remnants usually predominate. The latter are beta-VLDL, hence the term "dysbetalipoproteinemia". The metabolic defect

usually resides in apolipoprotein E (apo E) (24). Apo E occurs in three major forms called E-4, E-3, and E-2. Patients with dysbetalipoproteinemia generally have only E-2 in VLDL, the result of inheriting E-2 from both parents. They are said to have the E 2/2 phenotype. Apo E-2 seemingly is less effective than E-3 or E-4 for mediating catabolism of remnant lipoproteins. Approximately 1% of the total population has the E 2/2 phenotype. This one percent has an increase in circulating remnants, but not enough to produce hyperlipidemia. Although there is a slight increase in VLDL remnants in patients in this category, no data are currently available to indicate that this condition enhances the risk for atherosclerotic disease. On the contrary, patients with dysbetalipoproteinemia without hyperlipidemia may actually be slightly protected from atherosclerosis because of relatively low levels of LDL. Therefore, without hyperlipidemia, no treatment for dysbetalipoproteinemia is needed. If the patient develops definite hyperlipidemia there may in fact be increased risk, and treatment may be indicated, as will be discussed below.

FAMILIAL DYSBETALIPOPROTEINEMIA

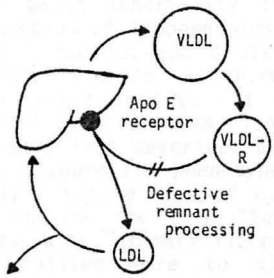

Figure 9

PRIMARY OVERPRODUCTION OF LIPOPROTEINS

A second major cause of hyperlipidemia is overproduction of lipoproteins, especially of VLDL and LDL. In our laboratory, an overproduction can be detected by an increased secretion of VLDL-TG, VLDL-apo B, or LDL-apo B. The number of apo B molecules per lipoprotein particle seems to be fixed at two, and a quantitative measurement of production rates of apo B gives a good indication of the number of lipoprotein particles being secreted into plasma. In other words, an overproduction of apo B signifies an overproduction of lipoproteins.

Excessive input of VLDL and LDL can be either primary or secondary to other diseases. The major secondary causes are obesity (with increased caloric intake) and diabetes mellitus. The primary form can be either familial or sporadic, although most primary sporadic cases probably have a familial basis. In this review, major attention will be given to the primary form.

An overproduction of lipoproteins may or may not lead to hyperlipidemia. The level of a particular lipoprotein in plasma is determined by both production and clearance rates. In the presence of a compensatory increase in clearance of lipoproteins, a high input into plasma will not necessarily cause high concentrations. The secretion of apo B-containing lipoproteins can be into VLDL or directly into LDL. Under most circumstances, the input will be mainly into VLDL. Likewise, most VLDL is converted to LDL. Therefore, in the usual course

of events overproduction of VLDL should result in a high input of LDL. Thus, there is the potential for a high VLDL, a high LDL, or both. An increase in VLDL remnants also is a possibility. There is increasing evidence that several forms of hyperlipidemia may have a primary overproduction of lipoproteins as their base. The following is a consideration of some of these forms.

Familial combined hyperlipidemia (multiple lipoprotein type HLP)

This important entity was described by Goldstein et al (14,15). It is characterized by increase of one or more lipoproteins in members of a single family, and thus has been called multiple-type HLP. Some family members have increases in VLDL alone (type 4 HLP). Others have increases only in LDL (type 2a HLP), and still others have high levels of both VLDL and LDL (type 2b HLP). The hyperlipidemia rarely becomes manifest until adulthood, and is made worse by obesity or diabetes. It has been estimated that about 10% of patients with CHD have familial combined hyperlipidemia (14,15).

The basic abnormality in familial combined hyperlipidemia seemingly is overproduction of lipoproteins containing apo B (Figure 10). Synthesis of both VLDL-apo B and LDL-apo B appear to be increased (25-28). In some patients, overproduction causes an increase predominately in VLDL; in others, the major increase occurs in LDL. It is our view that there are several clinical and familial variants of this disorder, and the family inheritance pattern need not be precisely that described by Goldstein et al (14,15). Not only may there be multiple lipoprotein phenotypes in the same families, but other families may show only a single form of hyperlipidemia (e.g. type 4 HLP). Nonetheless, we propose to use the term "familial combined hyperlipidemia" to be taken as synonymous with "primary lipoprotein overproduction". The former is a term that can be related more to clinical medicine, whereas the latter requires measurement of lipoprotein turnover to name a definite diagnosis. However, in using the term familial combined hyperlipidemia we are changing the definition somewhat to indicate abnormalities in metabolism of both VLDL and LDL, and not necessarily an increase in one or the other. By this definition a family with pure type 4 HLP could have familial combined hyperlipidemia. With this change in definition in mind, several different variants of primary lipoprotein overproduction (or familial combined hyperlipidemia) can be considered.

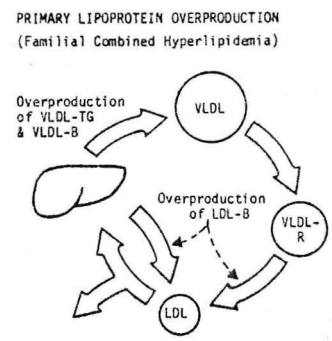

Figure 10

Type 4 HLP variant

One variant of primary lipoprotein overproduction is type 4 HLP. Previous studies from our laboratory (19) showed that this variant is associated with overproduction VLDL-TG (Table 10), and other workers have found an overproduction of VLDL-apo B. These defects have been observed in type 4 patients from families with multiple lipoprotein phenotypes and in those with only type 4 HLP. Our recent investigations have demonstrated that type 4 patients with premature CHD have an overproduction of LDL-apo B (Table 11). This finding is consistent with a combined defect in lipoprotein metabolism when only VLDL is elevated (Figure 11). The degree of hypertriglyceridemia in patients with this disorder may be determined in part by lipolytic capacity. If the patient has a concomitant mild defect in lipolysis of VLDL-TG, plasma levels of TG may become considerably higher. For example with overproduction of VLDL alone, the plasma TG may be in the range of 200 to 400 mg/dl; if a mild lipolytic defect is present in addition, levels may rise to 600-1000 mg/dl.

Table 10
VLDL-TG Kinetics
Familial Combined Hyperlipidemia (FCHL)

	VLDL-TG	Transport	FCR
	mg/dl	mg/hr/kg IW	hr^{-1}
FCHL (16 pts)	388 ± 57	25 ± 4	0.150 ± 0.013
Normal (27 men)	136 ± 7	12 ± 1	0.207 ± 0.016
Difference	p<0.01	p<0.001	p<0.01

Table 11
LDL-apo B Kinetics
Type 4 Hyperlipoprotenemia with
Premature Coronary Heart Disease

Patient	Triglyceride	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
	mg/dl	mg/dl	mg/kg/day	/day
1	761	51	16.5	0.79
2	1082	75	27.1	0.86
3	1014	85	22.2	0.66
4	966	119	19.9	0.37
5	300	95	26.0	0.61
6	307	125	18.3	0.31
mean ± SEM	738±144	92±11	21.7±1.7	0.60±0.09
Normal men (n=7)	166±24	92±4	11.8±1.1	0.31±0.02

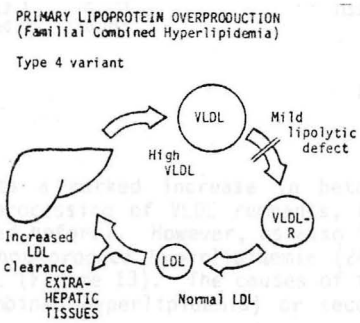

Figure 11

Type 5 HLP variant

Type 5 HLP is characterized by an increase in both VLDL and chylomicrons. The mechanisms for an increase in both lipoproteins have been a matter of some dispute. Some workers have assumed that the major abnormality is defective

clearance of TG-rich lipoproteins, while other have favored overproduction. Our studies (29) however have shown that patients with type 5 HLP usually have two abnormalities, i.e., both overproduction of VLDL-TG and decreased clearance of TG-rich lipoproteins. Kinetics of VLDL-TG were compared with two group of patients with type 4 HLP, one groups with increased synthesis of VLDL-TG, and another with low clearance rates (Table 12). Our type 5 patients had production rates similar to the former group, and low clearance rates as the latter. Thus, either defect alone appears to produce only type 4 HLP, whereas the presence of both abnormalities causes type 5 HLP. In other words, type 5 HLP seemingly is the result of the simultaneous occurrence of two disorders--primary overproduction of lipoproteins and a moderate lipolytic defect (Figure 12). The hyperlipidemia in type 5 HLP can be marked; severe accumulation of chylomicrons can occur, and the risk for pancreatitis is high if TG concentrations exceed 2000 mg/dl.

Table 12
VLDL-TG Kinetics in
Type 5 Hyperlipoproteinemia

Group	No	VLDL-TG Conc.	VLDL-TG Synthesis	VLDL-TG FCR
		mg/dl	mg/hr/kgIW	hr^{-1}
Type 5 HLP	8	1172 ±266	31.1 ±3.0	0.072 ±0.012
Type 4 HLP (overproduction)	9	454 ±38	29.2 ±1.5	0.150 ±0.010
Type 4 HLP (clearance defect)	8	393 ±68	15.6 ±1.4	0.095 ±0.009
Normal	27	136 ±7	12.1 ±0.8	0.207 ±0.016

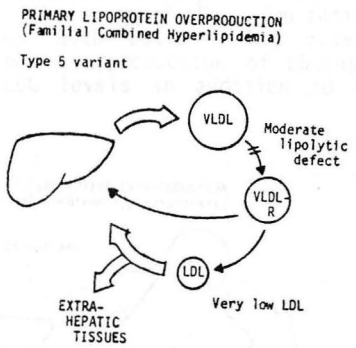

Figure 12

Type 3 HLP variant

This disorder reflects a marked increase in beta-VLDL. The underlying abnormality is defective processing of VLDL remnants, due to abnormalities in apo B isoforms (as discussed before). However, as also indicated before, an apo E abnormality alone does not produce hyperlipidemia (24). Patients also must have overproduction of VLDL (Figure 13). The causes of the latter can be either primary (e.g. familial combined hyperlipidemia) or secondary (e.g. obesity or diabetes mellitus).

Type 2 HLP variants

Patients with primary lipoprotein overproduction can present with either type 2a (increased LDL) or type 2b (increased LDL + VLDL). The latter is more common. As mentioned before, patients with type 4 HLP and premature CHD also have excessive conversion of VLDL to LDL producing a high flux rate of LDL-apo B. Under two circumstances this high synthesis of LDL can produce

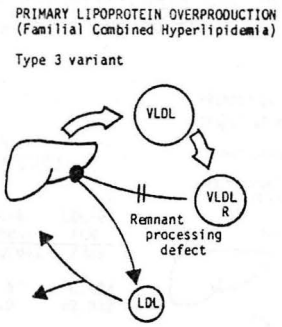

Figure 13

hypercholesterolemia: (a) with extreme overproduction or (b) with the usual high input associated with a relative defect in clearance of LDL. Two patients of the former kind are shown in Table 13. Both patients had moderate hypertriglyceridemia, but both also had extreme overproduction of LDL-apo B (Figure 14). This led to an elevation of LDL levels in addition to high concentrations of VLDL.

Table 13
Type 2 B Hyperlipoproteinemia

Patient	Plasma Chol	Plasma TG	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
	mg/dl	mg/dl	mg/dl	mg/kg/day	/day
1	367	449	239	33.1	0.44
2	285	225	113	27.1	0.63
Normal men (n=7) (mean ± SEM)	221 ±12	166 ±4	92 ±4	11.8 ±1.1	0.31 ±0.02

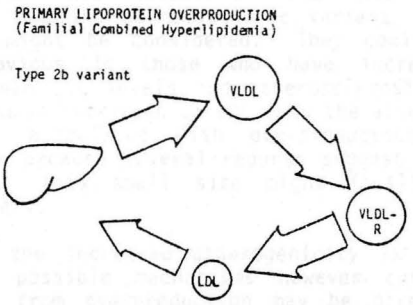

Figure 14

Normolipidemic variant

In addition to the various forms of hyperlipidemia described above, we recently described another variant of primary lipoprotein overproduction in which hyperlipidemia was completely absent (30). Patients with this pattern were identified by screening of lipoprotein kinetics of patients with premature CHD. In our measurements of LDL turnover, a significant portion had elevated production rates of LDL even though concentrations of both VLDL and LDL were relatively normal (Table 14). The discovery of this group has led us to the conclusion that excessive production of lipoproteins can occur as a primary defect without the presence of hyperlipidemia (Figure 16). The same basic

abnormality is seen secondarily in patients with marked obesity (31). The apparent association of the normolipidemic variant to CHD has led us to ask about the relationship between overproduction of lipoproteins and rates of atherogenesis.

Table 14
LDL-B Kinetics
Normolipidemic Patients with
Premature Coronary Heart Disease (CHD)

Patients	N	Plasma Chol	Plasma TG	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
		mg/dl		mg/dl	mg/kg/day	/day
CHD	8	209 ±8	139 ±6	109 ±3	21.5 ±1.9	0.427 ±0.032
Control	7	221 ±32	166 ±63	92 ±20	11.8 ±2.8	0.313 ±0.053

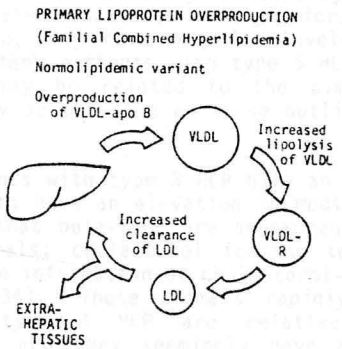

Figure 15

Relation between primary lipoprotein overproduction and atherosclerosis

In all conditions in which a primary overproduction of lipoproteins has been identified CHD appears to be common. This includes all the lipoprotein phenotypes including the type 4 variant and the normolipidemic variant. The reasons for an enhanced atherogenesis might be considered. They could be multiple. The relation is fairly obvious in those who have increased concentrations of LDL. A relation between LDL levels and atherosclerosis is well established and probably results from infiltration of LDL into the arterial wall. It is possible that the LDL associated with overproduction of lipoproteins may be unusually atherogenic because several reports suggest that the LDL particle is abnormally small: this small size might facilitate filtration of the LDL into the arterial wall.

In patients with pure type 4 HLP the increased atherogenicity is more difficult to explain. At least four possible mechanisms however can be considered. First, the VLDL resulting from overproduction may be directly atherogenic; they are relatively small particles and thus are relatively rich in apo B. These particles also may be filtered through an intact endothelium of the arterial wall. Second, enhanced flux of VLDL must be accompanied by increased transport of VLDL-C, and during lipolysis of VLDL-TG, this cholesterol might be released at the endothelial surface and be transported into the arterial wall, as suggested by others (2,3). Third, increased flux of VLDL may produce transitory periods of elevated LDL which could accelerate atherosclerosis by direct transintimal flux of LDL. Fourth, a high flux of LDL theoretically could be atherogenic even in the absence of hypercholesterolemia. One possibility is the delivery of increased amounts of cholesterol to the arterial wall. Another is interruption of reverse cholesterol transport; if the mass of cholesterol transported through plasma is increased, this could interfere with the disposal of cholesterol from peripheral tissues.

When overproduction of VLDL occurs simultaneously with a moderate clearance defect for TG-rich lipoproteins the result is type 5 HLP. Is this form of HLP associated with premature atherosclerosis? This is a matter of dispute. The prevalence of CHD in type 5 HLP seems to be less than expected from the severity of elevated lipids. Two factors may lessen the severity of atherogenesis in these patients. First, they have a marked increase in chylomicrons which seemingly are not atherogenic. And second, they have very low levels of LDL. Still, despite these mitigating factors, many patients with type 5 HLP do have premature atherosclerosis. The reason may be related to the simultaneous overproduction of VLDL; the mechanisms may be the same as those outlined above for type 4 HLP.

There are several reports that patients with type 3 HLP have an increased risk for CHD (32,33). Since these patients have an elevation in modified VLDL remnants (beta-VLDL), it can be assumed that beta-VLDL are atherogenic. This concept is supported by studies in animals; cholesterol feeding to several species (e.g. dogs, and rabbits) results in information of cholesterol-rich VLDL that closely resemble human beta-VLDL (34). These animals rapidly develop atherosclerosis. Human beta-VLDL in type 3 HLP are relatively small lipoproteins, not much larger than LDL, and they seemingly have a similar atherogenic potential.

A particularly interesting observation has been the presence of premature CHD in patients with normal plasma lipids and overproduction of LDL-apo B (30). The factors contributing to acceleration of atherogenesis in patients with this picture may be related to those suggested above for type 4 HLP--increased flux of VLDL and LDL (both cholesterol and apo B), possible transitory periods of high LDL, the presence of unusually atherogenic lipoproteins, and interference with reverse cholesterol transport.

Treatment of primary lipoprotein overproduction

The ideal agent to treat overproduction of lipoproteins would be one that inhibits the synthesis of lipoproteins without causing side effects. No such ideal agent exists at present. Several available drugs, used singly or in combination, however, can be used to interfere with the synthesis of lipoproteins. These can be reviewed.

One such agent is nicotinic acid. This drug reduces the synthesis of VLDL-TG (Table 15) (35) and LDL-apo B (36). It thus lowers both VLDL and LDL.

Table 15
VLDL-TG Kinetics in
Nicotinic Acid Treatment

Period	VLDL-TG Conc.	VLDL-TG synthesis	VLDL-TG FCR
	mg/dl	mg/hr/kgIW	hr^{-1}
Control*	275 ±37	18.7 ±3.8	0.14 ±0.02
Nicotinic acid*	179T ±26	14.7T ±2.6	0.17T ±0.01

*Number = 8 patients
TDifference from control significant at $p<0.05$

The mechanisms by which this occurs are not known; it may interfere with direct secretion of LDL as well as of VLDL (Figure 16). One action of nicotinic acid is to decrease the release of free fatty acids by adipose tissue, and thereby to make less fatty acids available to the liver for synthesis of VLDL-TG. The drug also may act directly at the liver cell to interrupt the assembly of lipoproteins.

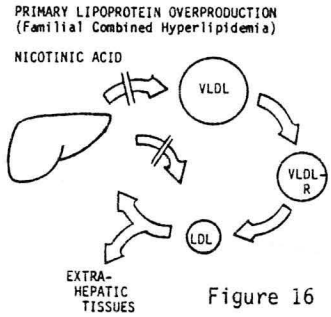

Figure 16

Side effects of nicotinic acid are flushing of the skin, pruritus, skin rashes, abdominal distress when taken between meals, occasionally hepatic dysfunction, worsening of glucose tolerance in diabetics, and a rise in uric acid. The dose required for maximum lipid lowering is in the range of 2 to 6 gm per day. The drug should be started in low doses (i.e. 100 mg times daily with meals) and increased gradually as tolerated by the patient. The flushing phenomenon often can be mitigated by low-dose aspirin.

Gemfibrozil Another drug that can be considered for lipoprotein overproduction is gemfibrozil. Our studies (37) have shown that this drug has two effects on VLDL metabolism (Table 17). It decreases the synthesis of VLDL-TG, and it promotes lipolysis (Figure 17). The drug probably decreases the synthesis of VLDL-apo B. In our laboratory, we have observed that gemfibrozil causes some reduction in synthesis of LDL-apo B in hypertriglyceridemic patients with primary lipoprotein overproduction (Table 16). Despite this apparent beneficial effect, its magnitude of decrease in production was limited, and a still excessive flux of LDL persisted. Thus, while gemfibrozil can be considered as one drug to mitigate overproduction of VLDL, it may not block direct secretion of LDL and thus may not be sufficient when used alone for normalization of LDL levels.

The fibric acid derivatives have generally proven safe, although side effects can occur. The most experience has been with clofibrate, but presumably related agents have similar actions. These drugs enhance biliary secretion of cholesterol and inhibit bile acid synthesis; this can lead to development of cholesterol gallstones in some patients. Other side effects are various gastrointestinal complaints, myalgias with elevated CPKs, and transient elevations of liver enzymes. On the whole, however, these drugs are well tolerated.

Table 16
VLDL-TG Kinetics in
Gemfibrozil Treatment

Period	VLDL-TG Conc.	VLDL-TG synthesis	VLDL-TG FCR
	mg/dl	mg/hr/kgIW	hr^{-1}
Control*	304 ±42	17.8 ±2.5	0.130 ±0.010
Gemfibrozil*	130† ±23	12.8† ±1.0	0.250† ±0.040

*Number = 7 patients
†Difference from control significant at $p<0.025$

Table 17
LDL-B Kinetics
Gemfibrozil Treatment in 7
Hypertriglyceridemic Patients with CHD

Period	Plasma Chol	Plasma TG	LDL-B Conc.	LDL-B synthesis	LDL-B FCR
	mg/dl		mg/dl	mg/kg/day	/day
Control	263 ±23	832 ±143	105 ±26	0.67 ±0.08	19.5 ±7.4
Gemfibrozil	228 ±40	181 ±9	102 ±17	0.41 ±0.03	16.9 ±2.3
Control (7 men)	229 ±17	138 ±14	100 ±9	0.298 ±0.020	14.0 ±1.4

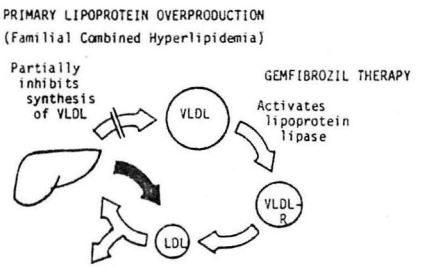

Figure 17

Neomycin. A third drug that might be employed from lipoprotein overproduction is neomycin. This drug has been shown to lower plasma LDL when given in a dose of 2 gm per day (38-41). The primary action of neomycin at this dose is to inhibit the absorption of cholesterol (40,41). The drug probably disrupts bile acid micelles in the intestinal tract and thereby makes cholesterol unavailable for absorption. At higher doses neomycin will interfere with absorption of bile acids, but at doses used to treat hypercholesterolemia, its primary action is to decrease the absorption of cholesterol. A return of less cholesterol to the liver via chylomicrons could have two effects on lipoprotein metabolism. A decreased hepatic cholesterol could cause increased synthesis of LDL receptors and thus to enhanced clearance of LDL; on the other hand, it could decrease the production of LDL. In our studies (41) on LDL turnover in patients treated with neomycin we observed the major action of this drug is to reduce the synthesis of LDL, although several patients also have shown enhanced clearance of LDL (Table 18, Figure 18). Because of inhibition of LDL production, neomycin might be useful in combination with gemfibrozil for treatment of lipoprotein overproduction (Figure 19). Gemfibrozil should inhibit synthesis VLDL, and if this causes diversion of excess lipoprotein production to LDL, the latter should be curtailed by neomycin.

Table 18
LDL-apo B Kinetics*
Effects of Neomycin

Period	LDL-C Conc. mg/dl	LDL-B Conc. mg/dl	LDL-B Synthesis mg/kg/day	LDL-B FCR /day
Control	162 ±12	124 ±12	18.4 ±3.4	0.359 ±0.049
Neomycin	121 ±7	93 ±3	13.2 ±1.5	0.361 ±0.026

*Results expressed as mean ± SEM

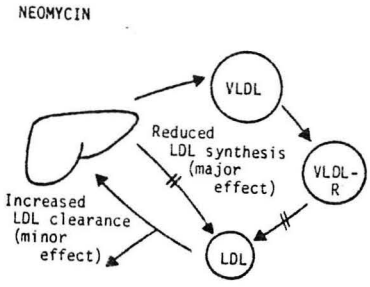

Figure 18

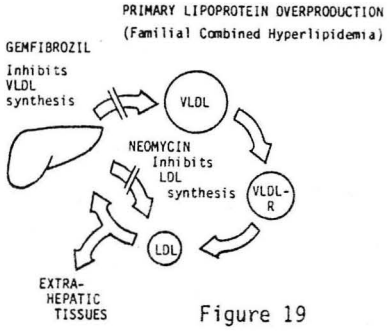

Figure 19

LDL CLEARANCE DEFECTS

Familial hypercholesterolemia (FH)

The most common abnormality in clearance of LDL is due to either a deficiency or defect in LDL receptors. This abnormality usually is manifest clinically as familial hypercholesterolemia. The incidence is about 1 in 500 people. It is characterized by severe hypercholesterolemia, tendon xanthomata, and very premature atherosclerosis. Patients often develop severe atherosclerotic disease in their teens or twenties. Total plasma cholesterol usually is in the range of 350-500 mg/dl, and most of the increase is in LDL. VLDL and HDL levels are normal or low.

Recent investigations indicate that multiple mutations in the LDL receptors on the surface of cells can interfere with the clearance of LDL from the circulation (8). Because of a reduction in uptake of LDL by the receptor pathway, a higher proportion of circulating LDL must be cleared by nonreceptor pathways. The increased uptake of LDL by nonreceptor mechanisms theoretically might promote the development of atherosclerosis. For unexplained reasons, production of LDL can be increased, and an enhanced input of LDL can accentuate

hypercholesterolemia (Table 19). Overproduction is especially marked in patients with homozygous FH which suggests that the overproduction is somehow determined by the LDL receptor defect (Figure 20) (42).

Table 19
LDL-apo B Kinetics in
Young People with Familial Hypercholesterolemia

Group	N	Age	LDL-C Conc.	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
		yrs	mg/dl		mg/kg/day	/day
Normal	6	32 ±2	76 ±11	48 ±6	8.0 ±0.7	0.45 ±0.07
Heterozygous FH	6	31 ±7	230 ±76	126 ±21	13.8 ±36	0.29 ±0.40
Homozygous FH	7	12 ±6	568 ±180	362 ±126	26.4 ±1.0	0.18 ±0.04

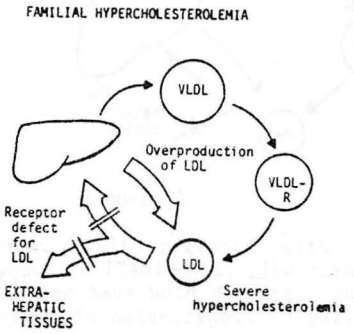

Figure 20

"Polygenic" hypercholesterolemia

If 5% of the population can be considered to have elevated LDL levels, and only 1 in 500 people have FH, then 24 of 500 must have other causes of elevated LDL. These patients were said by Goldstein et al (14, 15) to have polygenic hypercholesterolemia. What are causes of "polygenic" hypercholesterolemia? Theoretical possibilities are overproduction of LDL, reduced clearance of LDL, or both defects. The features of a high LDL on the basis of overproduction have been discussed above. For those who have a reduced clearance of LDL we can again ask about mechanisms. Three factors can be considered: (a) dietary, (b) metabolic, and (c) genetic. Diets rich in saturated fats and cholesterol may reduce hepatic receptors for LDL. Receptors may also decline with the metabolic changes associated with aging. And they may be reduced on a genetic basis. While dietary and metabolic factors may contribute to a rise in LDL levels, it seems likely that genetic factors must contribute to an LDL abnormally high levels. Without the introduction of genetics into the equation, it is difficult to explain why some people of the same age and diet develop elevated LDL while others do not. If genetic factors do contribute, the site of the abnormality is worthy of discussion. Presumably LDL receptors are at fault. Whether many people have mild structural defects in their LDL receptors is a possibility that to the present has not been explored.

In our laboratory, LDL kinetics have been studied in 7 patients with polygenic hypercholesterolemia (Table 20). These patients all had levels of LDL-cholesterol (LDL-C) over the 90th percentile for their age and sex. Their LDL-apo B (LDL-B) concentration also was increased. The LDL-apo B production in these patients was not increased; instead, almost all had a low FCR for LDL. Thus, in the patients with "polygenic" hypercholesterolemia who have been studied thus far, a reduction in clearance (and not increased LDL synthesis) appears to be the major mechanism for their high LDL levels (Figure 21).

Table 20
LDL-apo B Kinetics
in "Polygenic" Hypercholesterolemia

Patient	Plasma Chol	Plasma TG	LDL-C Conc.	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
	mg/dl		mg/dl		mg/kg/day	/day
1	332	167	231	134	11.3	0.26
2	309	207	218	133	11.4	0.29
3	273	200	184	107	10.9	0.26
4	222	80	182	108	12.1	0.25
5	246	142	199	146	12.9	0.23
6	261	113	195	112	13.1	0.26
7	235	151	186	135	14.6	0.24
Mean	268	137	199	125	12.3	0.26
±SEM	±15	±21	±7	±6	±0.5	±0.01
Control Men (n=7)	221 ±32	166 ±63	140 ±20	92 ±20	11.8 ±2.8	0.313 ±0.053

POLYGENIC HYPERCHOLESTEROLEMIA

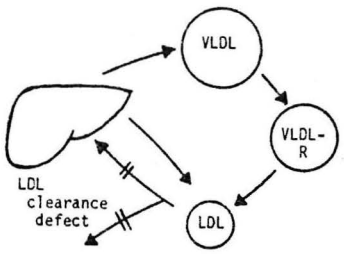

Figure 21

It is interesting to compare our adult patients with "polygenic" hypercholesterolemia with adult heterozygous FH (Table 21). The former have low FCR without overproduction of LDL; the latter have both defects. Therefore, it appears that in someway the receptor defect in heterozygous FH patients evokes overproduction of LDL (Figure 21).

Table 21
Comparison of LDL-B Kinetics
in "Polygenic" and Familial Hypercholesterolemia

Group	N	Plasma Chol.	Plasma TG	LDL-C Conc.	LDL-B Conc.	LDL-B Synthesis	LDL-B FCR
		mg/dl±SD		mg/dl	mg/dl	mg/kg/day	/day
"Polygenic" Hypercholesterolemia	7	268 ±15	137 ±21	199 ±7	125 ±6	12.3 ±0.5	0.26 ±0.01
Heterozygous Familial Hypercholesterolemia	6	321 ±15	174 ±35	262 ±19	186 ±37	19.2 ±6.4	0.30 ±0.03
Normal	7	221 ±32	166 ±63	140 ±20	92 ±20	11.8 ±2.8	0.313 ±0.053

Treatment of LDL clearance defects by induced increases in LDL receptors

Bile acid sequestrants. Interruption of the enterohepatic circulation (EHC) of bile acids is known to decrease plasma LDL levels. This approach has been used to treat severe hypercholesterolemia for many years. The EHC of bile acids can be interrupted either with bile acid binding resins or by the ileal exclusion operation. The most commonly used agents for binding bile acids are cholestyramine and colestipol. Recent investigations have provided insights into mechanisms by which LDL lowering occurs (43-45). Blockage of bile acid absorption enhances the conversion of cholesterol into bile acids; the result is a decrease in hepatic cholesterol concentrations. The fall in hepatic cholesterol is associated with an increase in LDL receptors by the liver cell.

The result is a fall in LDL concentrations (Figure 22). When bile acid sequestrants are given in doses of 16 to 20 grams per day, reductions of LDL cholesterol of 15 to 30% can be obtained. Although decreases of this magnitude may be satisfactory in patients with mild to moderately elevated LDL levels, they are insufficient for patients with heterozygous FH (46). Despite adequate intakes of sequestrants patients with FH usually remain significantly hypercholesterolemic. For this reason, other drugs have been sought for enchancing the effects of bile acid binding resins in patients with FH.

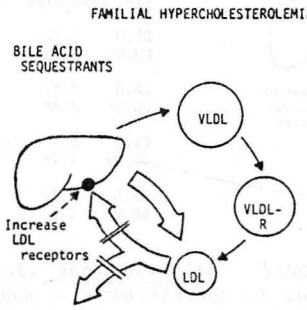

Figure 22

Cholesterol-synthesis inhibitors (compactin and mevinolin). Another approach to decreasing hepatic cholesterol concentrations has been the discovery of certain fungal metabolites that inhibit 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase), a rate-controlling enzyme in cholesterol synthesis. The prototype compound, compactin, and its analogue, mevinolin, have been shown to lower levels of LDL in normal animals (45,47), normal humans (48), and FH heterozygotes (49-51). In dogs, a lowering of plasma LDL was shown to enhance clearance of plasma LDL secondary to increased production of LDL receptors in the liver (45).

The potential for use of cholesterol-synthesis inhibitors led us to carry out a study of the mechanism of LDL lowering by mevinolin in patients with heterozygous FH. To distinguish between receptor-dependent and receptor-independent clearance of LDL, we compared the simultaneous clearance of native LDL and LDL that was modified by glucosylation in vitro. As shown by others (52), glucosylated LDL (Glc-LDL) does not bind to LDL receptors in vitro; therefore, its clearance from plasma can be used as an index of receptor-independent clearance pathways. In our study (53), turnover of LDL and Glc-LDL were determined by labelling one with 125-I and the other with 131-I. In the control period before treatment, total clearance rates of LDL were abnormally low in the FH patients. Use of Glc-LDL revealed that approximately 50% of LDL was cleared by the receptor pathway and another 50% by the nonreceptor pathway. When mevinolin was given, the mean level of plasma LDL-cholesterol in six patients fell from 262 to 191 mg/dl, a 27% decrease

(Table 22). At the same time, the clearance of LDL increased by 37%; through use of Glc-LDL the increase was shown to be via the receptors.

Table 22
LDL-B Kinetics in
Adults with Heterozygous
Familial Hypercholesterolemia

Group	N	LDL-C Conc. mg/dl±SEM	LDL-B Conc. mg/dl	LDL-B Synthesis mg/kg/day	LDL-B FCR /day
FH (No therapy)	6	262 ±19	186 ±37	19.2 ±6.4	0.30 ±0.03
FH (Mevinolin)	6	191 ±13	132 ±18	18.5 ±6.0	0.41 ±0.05
Normal (Young Adults)	6	84 ±6	48 ±9	8.5 ±1.4	0.43 ±0.06
Normal (Older Adults)	7	140 ±20	92 ±4	11.8 ±1.1	0.31 ±0.02

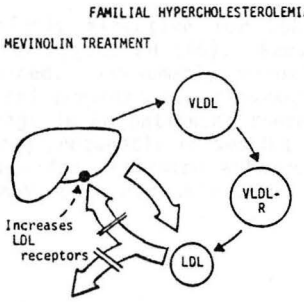

Figure 23

Combined sequestrants and mevinolin. Since neither mevinolin alone nor sequestrants alone produce a normalization of LDL levels in most patients with heterozygous FH, consideration can be given to employing both drugs simultaneously. One report (51) indicates that the combination of compactin and colestipol can produce an almost 50% reduction in LDL levels in patients with heterozygous FH. We have studied one patient in whom the combination of mevinolin and colestipol caused a 60% reduction in LDL concentrations and a 100% increase in LDL clearance rates. This combination thus appears to have the potential for normalizing LDL levels in patients with heterozygous FH (Figure 24).

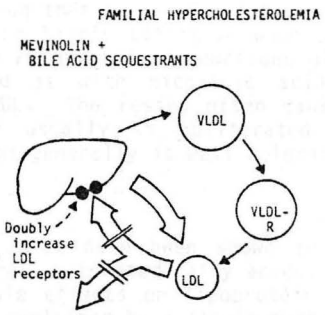

Figure 24

Treatment of LDL clearance by inhibiting LDL production

Many patients with heterozygous FH have an overproduction of LDL in addition to defective clearance. Therefore, if drugs were available to inhibit LDL synthesis, they might be useful in the treatment of this disorder. Two drugs discussed before, nicotinic acid and neomycin, have been shown to lower

production rates of LDL. These drugs might be especially valuable in combination with agents that increase LDL receptors.

Nicotinic acid has been shown to be highly effective for LDL lowering together with colestipol in patients with heterozygous FH (46). Reductions in LDL-cholesterol of 30 to 45% can be obtained. Presumably nicotinic acid decreases the production of LDL while colestipol promotes LDL clearance (Figure 25). Nicotinic acid has one potential advantage in FH patients; these patients often have low HDL concentrations, and this drug frequently raises HDL. Whether this response is a potential beneficial action for retarding atherogenesis is unknown but remains a possibility. Neither mevinolin nor colestipol causes an increase in HDL levels.

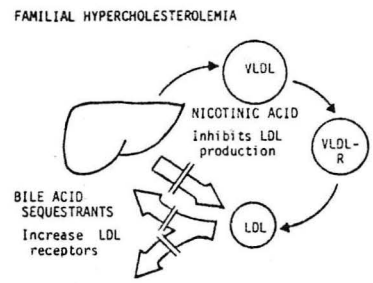

Figure 25

Neomycin is another drug that appears to inhibit the production of LDL(41). It too has been shown to be highly effective when used in combination with a bile acid sequestrant. In FH patients, reductions of LDL in the same order of magnitude can be obtained as with nicotinic acid + sequestrant, although neomycin does not raise HDL. The resins often cause constipation by binding bile acids. This action usually is obliterated by neomycin. Thus, the combination of the two drugs generally is well tolerated.

Dietary Hyperlipidemia

Three factors in the diet have been shown to raise the plasma lipids. These are dietary cholesterol, saturated fatty acids, and a high intake of total calories. Each has multiple effects on lipoprotein metabolism, and all these effects probably cannot be explained by a single mechanism. Dietary cholesterol mainly raises LDL concentrations, but it sometimes may increase the cholesterol content of VLDL and HDL. Saturated fatty acids also increase LDL, and to lesser extents VLDL and LDL. The major actions of excess calories are to raise plasma TG and to lower HDL; their actions on LDL are variable--some obese patients have high LDL, others relatively low. A large amount of research has

been done on the mechanisms of these various changes; although much remains to be learned, a general picture of their actions seem to be emerging (Figure 26).

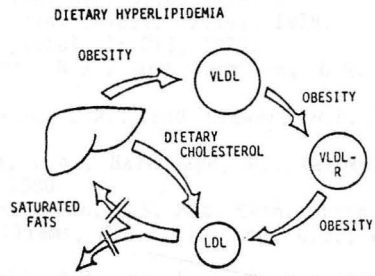

Figure 26

Whether the LDL raising action of dietary cholesterol is due mainly to stimulating secretion of LDL or to reducing LDL receptors has not been determined. However, the feeding of cholesterol to animals appears to increase the output of cholesterol in lipoproteins. One report claims the same for humans. Our finding (41) that inhibiting the absorption of cholesterol reduces LDL production suggests that increasing absorption would have the opposite effect namely, enhancing LDL output. While this may be the major action, promoting cholesterol concentrations in the liver also could reduce LDL receptors and decrease uptake from plasma.

The available data suggest that saturated fatty acids, in contrast to dietary cholesterol, heightens LDL mainly by promoting its clearance (54). In some patients, an increased production of LDL also has been noted, but reduced clearance appears to be the primary action. Saturated fatty acids could reduce LDL receptors by interfering with hepatic excretion or oxidation of cholesterol and thereby raising intracellular concentrations of cholesterol; there are reports that hepatic outputs of cholesterol and bile acids are less on diets rich in saturated fatty acids than on those containing mainly polyunsaturates. An interesting finding was that dietary cholesterol raises plasma LDL only in the presence of saturated fats in the diet, and not when the diet is rich in polyunsaturated fatty acids. Whether this reflects the presence of two separate mechanisms for raising LDL, or a combined action as one point, remains to be proven.

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