INTERNAL MEDICINE
GRAND ROUNDS
07 FEBRUARY 1991

CAUSES OF HIGH BLOOD CHOLESTEROL: IMPLICATIONS FOR TREATMENT

By

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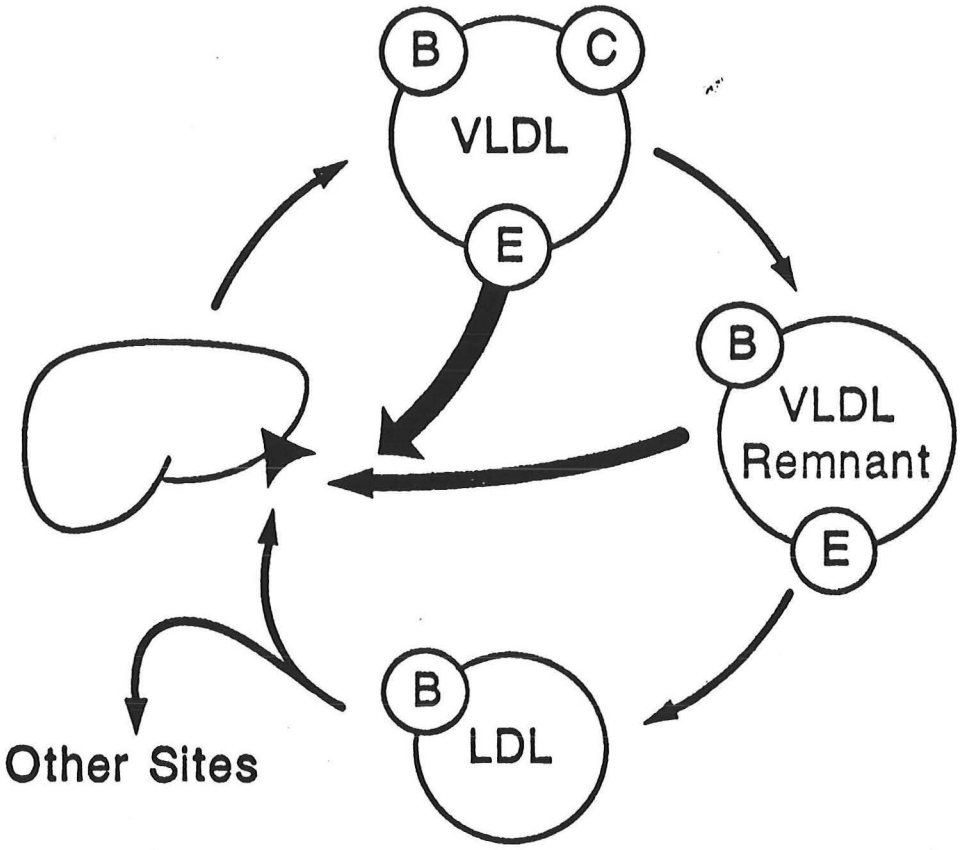


Figure 1. Origins and fates of low density lipoproteins (LDL)

High serum cholesterolemia is a major risk factor for coronary heart disease (CHD), and most cholesterol in serum circulates with low density lipoproteins (LDL). Many investigators believe that most of the atherogenecity of high serum cholesterol can be explained by relatively high levels of LDL. Although other lipoproteins--very low density lipoproteins (VLDL) and high density lipoproteins (HDL)--undoubtedly play a role of atherogenesis, LDL appear to be the primary culprit (1). This review therefore will focus on causative factors responsible for high serum levels of LDL cholesterol, and their implications for the therapeutic control of hypercholesterolemia.

REGULATION OF LDL-CHOLESTEROL LEVELS

Pathways of LDL Metabolism

The origins and fates of LDL are shown in Figure 1. LDL derives from the catabolism of triglyceride-rich VLDL, which in turn are secreted by the liver. VLDL contain three types of apolipoproteins--apo B-100, apo C's (C-I, CII, and C-III), and apo E's. The latter occur in three isoforms--E2, E3, and E4. As VLDL pass into the peripheral circulation, they encounter lipoprotein lipase (LPL), an enzyme located on the surface of capillary endothelial cells. VLDL triglycerides undergo hydrolysis, thereby converting VLDL into VLDL "remnants"; in the process, the soluble apolipoproteins, apo C's and apo E's, are progressively lost. Further hydrolysis converts VLDL remnants into LDL, and only apo B-100 remains on LDL. Although LPL may be involved in this latter hydrolysis, hepatic triglyceride lipase (HTGL) probably plays a role.

At any stage of VLDL catabolism, VLDL can be removed directly by the liver by receptor-mediated uptake. More than one receptor may participate in VLDL removal. One receptor is the well-described LDL receptor; this receptor recognizes apo B-100 of LDL, but it also binds to apo E on VLDL particles. Since VLDL have both apo E and apo B-100, they actually have a greater affinity for LDL receptors than LDL. In addition, VLDL and their remnants may be removed by other receptors that specifically recognize apo E, but differ from classical LDL receptors. To date however "apo E receptors" have not been definitely identified. One "candidate" for the apo E receptor is the LDL receptor-related protein (LRP) (3,4), although its physiological role has not been documented. The partitioning of VLDL between hepatic uptake and conversion to LDL influences the rate of formation of LDL, the latter affecting LDL levels.

LDL leave the circulation via the liver or extrahepatic tissues. By either route, uptake can occur by both receptor or nonreceptor pathways. The sole receptor for native LDL is the LDL receptor, and it accounts for about 75% of LDL removal. Nonreceptor uptake of LDL most likely occurs by bulk-phase endocytosis. In humans, the liver removes about 75% of circulating LDL.

From the above it can be seen that LDL concentrations are determined by the net balance of several pathways--hepatic secretion of VLDL, the partitioning of VLDL between direct hepatic removal and conversion to LDL, and activity of LDL receptors. The LDL-cholesterol level further is influenced by the cholesterol content of each LDL particle. In the following, the key

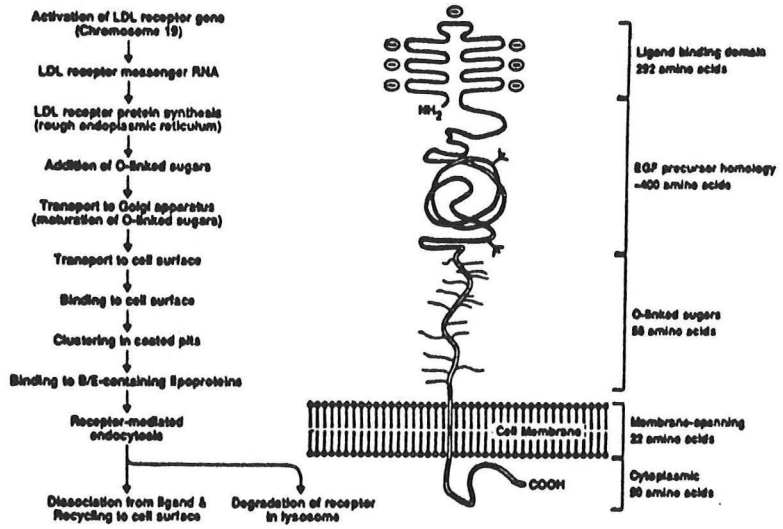
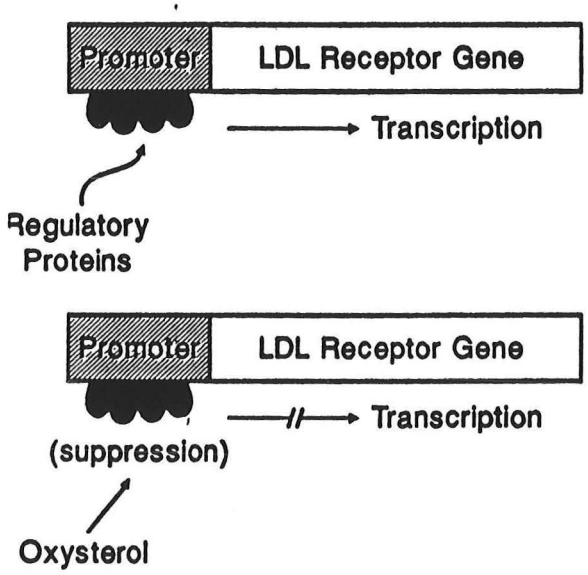


Figure 2. Schematic pathways of synthesis, intracellular transport, and degradation of LDL receptors (left) and of structure of LDL receptor (right)

Figure 3. Increasing hepatic cholesterol suppresses activity of LDL receptors



igure 4. Regulation of transcription of LDL receptor gene. Oxysterols derived from intracellular cholesterol apparently alter the conformation of regulatory proteins which suppresses transcription

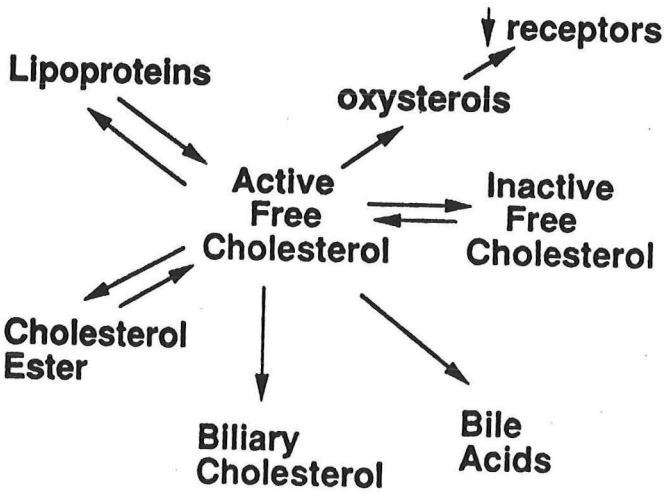


Figure 5. Factors that determine the size of the active pool of free cholesterol in the liver cell

physiological factors determining each of these processes will be examined, and then, abnormalities in this regulation will be considered.

LDL Receptor Activity

The LDL Receptor. The gene for the LDL receptor resides in chromosome 19; it occupies 45.5 kb of DNA, and encodes a 5.3-kb messenger RNA (mRNA) (21). The receptor protein contains 860 amino acids arranged in six domains (Figure 2): (a) a signal sequence at the amino terminus, (b) a ligand-binding region, (c) a domain having homology to epiderminal growth factor, (d) a clustered O-linked sugar domain, (e) a transmembrane region, and (f) a cytoplasmic tail at the carboxyterminus of the protein. LDL receptors are synthesized in the rough endoplasmic reticulum; from there they are transported to the Golgi apparatus where carbohydrate chains are added. The mature glycoprotein then moves to the cell surface and migrates to coated pits where they cluster. Here LDL receptors bind to lipoproteins containing apo B-100, apo E, or both. The resulting receptor-lipoprotein complexes undergo endocytosis. In the endosome, the receptor and lipoprotein dissociate; the lipoprotein undergoes enzymatic degradation within lysosomes, whereas receptors can have two fates: they can either recycle to the cell surface or enter lysosomes for degradation. The activity of LDL receptors at the cell surface thus depends on two factors: (a) the number of receptors synthesized, and (b) the rate of recycling to the surface.

Regulation of LDL receptor activity. The number of LDL receptors expressed is a tightly regulated process geared to maintain an optimum cellular content of cholesterol. For this purpose, the synthesis of LDL receptors can be up- or down-regulated. Cholesterol itself appears to play a key role in this regulation (5). When cellular cholesterol is increased, receptor synthesis is suppressed; conversely, a reduction in cellular cholesterol stimulates the synthesis of receptors (Figure 3). Some workers speculate that only a "metabolically active" pool of unesterified cholesterol is regulatory, although the location of this pool is not known. Still, cholesterol per se seemingly is not the regulatory sterol, but rather an oxygenated derivative of cholesterol (6). This oxysterol presumably modifies the confirmation of proteins adjacent to the promoter region of the LDL receptors and thereby suppresses transcription (Figure 4).

Regardless of the exact mechanism, factors influencing the active pool of unesterified cholesterol determine the activity of the LDL receptor gene and hence they affect LDL-receptor synthesis. Since most of the body's LDL-receptor activity is expressed by the liver, the regulation of the hepatic pool of active unesterified cholesterol must be a major factor determining LDL-receptor activity. The various factors influencing the size of this pool are outlined in Figure 5. Hepatic unesterfied cholesterol originates from (a) newly synthesized cholesterol in the liver itself, (b) newly absorbed cholesterol, entering the liver with chylomicron remnants, and (c) uptake of plasma lipoproteins carrying cholesterol derived from peripheral tissues. Cholesterol can exit the liver in three ways: (a) by secretion into plasma with lipoproteins, (b) by conversion into bile acids, and (c) by secretion into bile as cholesterol itself. It should be noted that cholesterol secreted with lipoproteins must eventually return to the liver, and this lipoprotein

Table 1

Causes of Borderline High Serum Cholesterol

Factor	Average Total Cholesterol	
	Increment	Sum
Background levels (relatively high)	0	140 mg/dl
Saturated fatty acids and cholesterol	+25 mg/dl	165 mg/dl
Weight gain with age	+25 mg/dl	190 mg/dl
Aging per se	+30 mg/dl	220 mg/dl
Loss of estrogen (women)	+20 mg/dl	240 mg/dl

cycle does not alter net cholesterol across the liver cell, except for cholesterol synthesized in peripheral tissues. Finally, the size of the active pool of unesterified cholesterol depends on intracellular events, i.e., conversion to and from (a) cholesterol ester and (b) the inactive pool of unesterified cholesterol.

Because of the effect of hepatic cholesterol content on synthesis of LDL receptors, the balance of cholesterol across the liver cell becomes a key factor determining the level of serum LDL cholesterol. Pathways of cholesterol into and out of the liver have been studied in humans by the cholesterol balance technique. The author participated in development of this technique in the laboratory of Dr. E.H. Ahrens, Jr., at the Rockefeller University in the 1960's. This method estimates the fecal excretion of neutral steroids and bile acids, and intestinal absorption of cholesterol. Whole body synthesis of cholesterol is equal to the difference between intake of cholesterol and fecal excretion of neutral steroids and bile acids. In the steady state, the excretion of bile acids equals the conversion of cholesterol into bile acids, i.e. hepatic synthesis of bile acids. Subsequently, a method was developed for determination of hepatic secretion of biliary lipids (cholesterol, bile acids, and phospholipids) (7); this technique provided a more direct estimate of exit of cholesterol and its conversion products from the liver. During development of the cholesterol balance technique, several methods were devised for estimating cholesterol absorption; these methods provide an estimate of one pathway of input of cholesterol into the liver.

It is interesting to compare parameters for cholesterol metabolism in humans with those obtained in animal studies. Values for cholesterol synthesis have been obtained in a variety of smaller laboratory animals by Dietschy et al (8), and in primates by several groups of investigators. Expressed on a per-kilogram basis, whole body synthesis of cholesterol in humans is not especially high. Smaller animals, such as the rat, have much higher synthetic rates. In contrast to these animals which transform much of their cholesterol into bile acids, however, humans convert a relatively small fraction, i.e., 30 to 40%. As a result, relatively large amounts of hepatic cholesterol must be secreted into bile as cholesterol itself. There are two adverse consequences of this low rate of conversion of cholesterol into bile acids: (a) a high output of cholesterol into bile predisposes to cholesterol gallstones, and (b) the enterohepatic circulation is enriched with cholesterol, which suppresses LDL-receptor synthesis. The latter undoubtedly contributes to relatively high serum levels of LDL cholesterol in humans compared to most laboratory animals (Table 1).

In some animals (e.g. primates), the enterohepatic circulation of cholesterol can be expanded by feeding dietary cholesterol; when this occurs, serum LDL-cholesterol levels are raised to levels typically seen in humans. In other species, e.g., the dog and rat, the feeding of dietary cholesterol has little effect on serum cholesterol concentrations because these animals have the capacity to convert most newly absorbed cholesterol into bile acids. The low efficiency of conversion of cholesterol into bile acids in humans thus appears to represent a "species defect" leading to relatively high serum cholesterol levels. Consequently, humans in general are in a state of "hepatic cholesterol overload" even on low-cholesterol diets. This explains

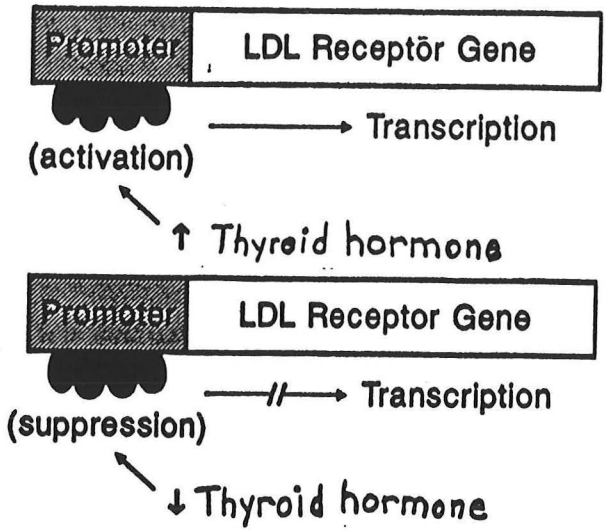


Figure 6. Mechanism whereby thyroid hormone stimulates the synthesis of LDL receptors

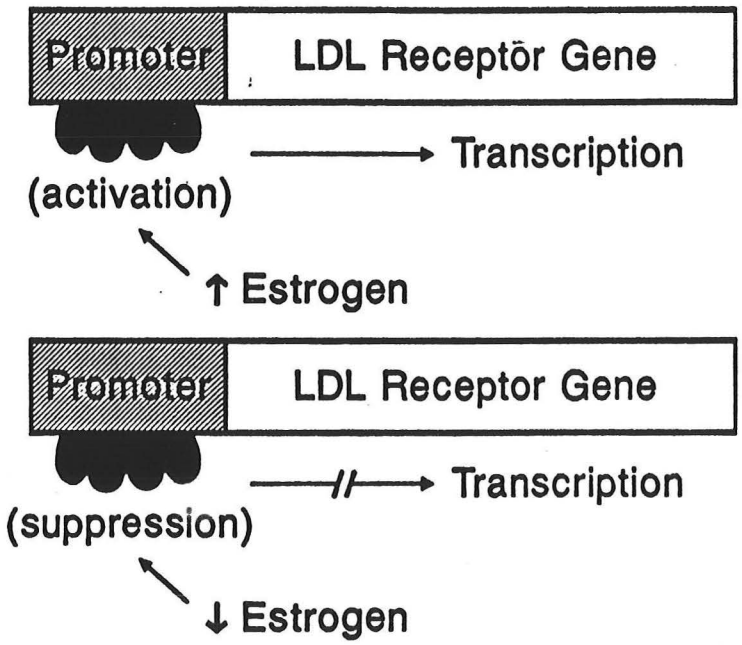


Figure 7. Mechanisms whereby estrogenic hormones stimulate the synthesis of LDL receptors

why high cholesterol intakes in humans have only a small effect to raise serum cholesterol levels. These general conclusions represent a reasonable interpretation of cholesterol-balance results in humans compared to balance data from other species.

LDL-receptor activity appears to be affected by several factors besides the cholesterol content of the liver cell. For example, thyroid hormone (9) and estrogens (10) seemingly stimulate LDL-receptor synthesis by acting on regulatory proteins in the cell nucleus (Figures 6 and 7). The fatty acid pattern of the diet is still another influence on LDL-receptor activity; its effect will be discussed later. Moreover, the transport of LDL receptors within the cell is relatively complex, and several factors could modify rates of receptor transport to the cell surface, receptor function within the cell membrane, or the rate of recycling of receptors through the endocytotic process. Finally, the "fit" between LDL receptors and apo E (or apo B-100) could influence rates of uptake of apo B-containing lipoproteins. All of these factors could modify the "activity" of LDL receptors, and hence the serum level of LDL cholesterol.

Hepatic Secretion of Apo B-Containing Lipoproteins

The major apo B-containing lipoprotein secreted by the liver is VLDL. Steps in formation of VLDL particles are incompletely understood, but in general terms, they are as follows. Apo B-100 is synthesized in ribosomes of the rough endoplasmic reticulum (RER). The triglycerides are made by membrane-bound enzymes located in the smooth endoplasmic reticulum (SER). Cholesterol esters also are formed in the SER. As apo B-100, and possibly apo E, move towards the SER, they encounter triglycerides and cholesterol ester at the RER-SER junction. The apolipoproteins in close linkage with unesterified cholesterol and phospholipids presumably wrap themselves about the neutral lipids to form nascent VLDL; these particles pass through the SER to the Golgi apparatus, and here secretory vesicles containing large numbers of nascent VLDL bud off and move to the surface of the cell for secretion into the circulation.

Several factors could influence the number of VLDL particles secreted into serum. The rate of synthesis of apo B-100 could be one factor, although recent data suggest that a sizable portion of newly synthesized apo B normally is degraded before being recruited to VLDL formation (11). Hence the fraction of apo B-100 recruited for secretion may be a more important determinant of VLDL formation and secretion than is the rate of absolute synthesis of apo B. Moreover, the rate of recruitment of apo B into lipoprotein formation could be a function of lipid metabolism. If more nonpolar lipids are produced for example, more lipoprotein particles may be formed and secreted. This could be true for either triglycerides or cholesterol ester. At present however it is not clear whether an increase in neutral-lipid synthesis in the liver enhances the total number of lipoprotein particles secreted or whether a normal number of particles are merely enriched with lipid. This crucial distinction remains to be resolved.

One technique for estimating secretion rates for apo B-containing lipoproteins in humans is to perform isotope kinetic studies. The methodology

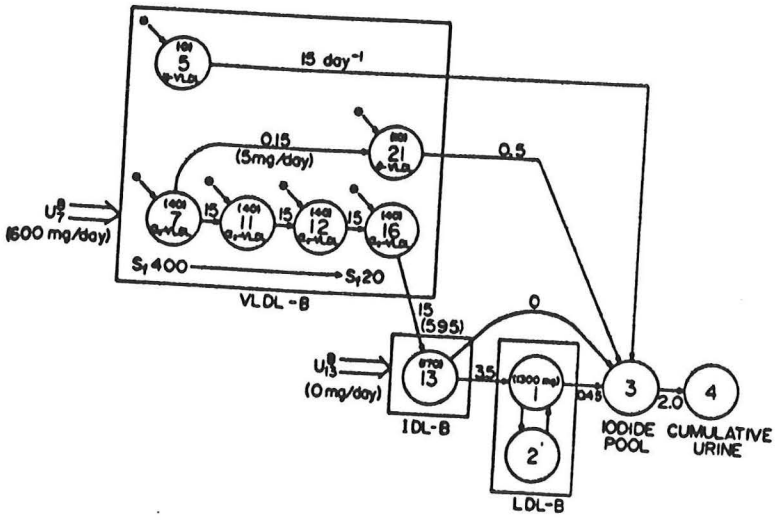


Figure 8. Multicompartmental model for apo B developed by Mones Berman and coworkers at the National Institutes of Health. Values next to arrows are rate constants in units/day. Values in parentheses are calculated or measured steady state transports of apo B containing lipoproteins in mg/day. Those in parentheses inside a circule are calculated steady state amounts of apo B in that compartment. The values are approximations for a normal individual.

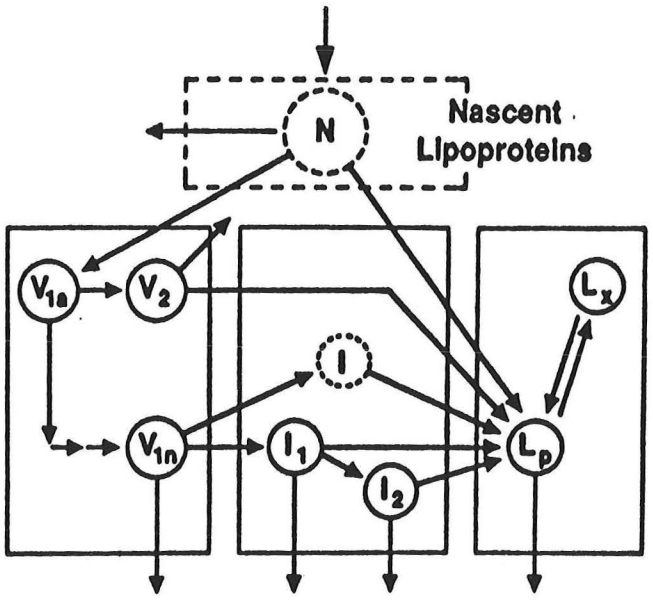


Figure 9. Alternate multicompartment model for apo B containing lipoproteins (see Figure 8). Nascent lipoproteins (N) represent a rapidly catabolized fraction. These lipoproteins can be removed irreversibly from the circulation or be transformed into either VLDL or LDL. The symbol V pertains to VLDL, I to IDL, and L to LDL.

for these studies was pioneered by investigators at the National Institutes of Health under the leadership of Dr. Mones Berman (12) and it has been employed extensively in our laboratory. In these studies, two types of isotope studies were carried out: (a) apolipoproteins on isolated lipoproteins were labeled with radioiodine and reinjected (13), and (b) labeled precursors were injected intravenously allowing the apolipoproteins or lipids to be labeled "endogenously" (14). On the basis of plasma radioactivity curves, a series of multicompartmental models were developed to define metabolism of apo B-containing lipoproteins. With these models, attempts were made to estimate secretion rates for VLDL apo B. Since each lipoprotein particle contains only one apo B molecule per particle, secretion rates of VLDL apo B should approximate the number of VLDL particles secreted into the circulation. A typical multicompartmental model for apo B metabolism developed by previous investigators is presented in Figure 8 (15). This model accounts for all of the components of isotope-kinetic curves for apo B-containing lipoproteins. Key features of this model are (a) a multicompartmental, intravascular lipolytic cascade for VLDL,and (b) a two-compartment model for LDL, with one compartment being an extravascular exchange pool. Similar models have been used by other workers.

During the course of these studies, suggestive evidence was obtained that some LDL apo B enters the circulation independently of VLDL. The total transport of LDL often exceeds that which can be derived from VLDL. This observation led to the postulation that a portion of circulating LDL arises by "direct secretion" of LDL by the liver. Although it is possible that the liver might secrete lipoprotein particles smaller than VLDL, these particles unlikely would be "mature" LDL particles since the latter contain cholesterol ester produced in plasma mainly through the action of LCAT. Further, the "direct secretion" of LDL might really represent the rapid catabolism of newly secreted VLDL into LDL, a process occurring too rapidly to be detected by typical isotope-kinetic curves. For these reasons, we modified the model to include a compartment of nascent lipoproteins out of which both VLDL and LDL could be derived (Figure 9) (13). Instead of "direct synthesis" of LDL, a better term seems to be "rapid input" of LDL, to distinguish it from the slower input through the circulating delipidation chain. Moreover, on the basis of increasing evidence we suggested that lipoproteins can exit directly from the compartment of nascent lipoproteins; if so, isotope kinetic studies will not allow for estimates of absolute hepatic secretion rates of lipoproteins, but rather only rates of transformation of nascent lipoproteins into VLDL and LDL compartments.

Conversion of VLDL to LDL

According to the model shown in Figure 9, LDL is derived from the degradation of triglyceride-rich lipoproteins, whether rapidly from nascent lipoproteins or more slowly through the VLDL lipolytic cascade. Moreover, some VLDL particles are removed directly by the liver before conversion to LDL. Thus, triglyceride-rich, apo B-containing lipoproteins undergo partitioning between hepatic uptake and transformation to LDL (Figure 1). Without question some of the hepatic uptake occurs via LDL receptors, but other receptors (e.g. the putative apo E receptor) also may be responsible. The mechanisms for conversion of triglyceride-rich lipoproteins to LDL are not

Table 2

Definitions of Serum Cholesterol Levels

Definition	Total Cholesterol	LDL Cholesterol	Relative CHD Risk
Desirable serum cholesterol	<200	<130	<1.0
Borderline-high cholesterol	200-239	130-159*	1.0-2.0
High serum cholesterol	≥240	>160†	
Moderate hypercholesterolemia	240-289	160-209	2.0-4.0
Severe hypercholesterolemia	≥290	≥210	>4.0

* Designated borderline-high LDL cholesterol

† Designated high-risk LDL cholesterol

fully understood (16). Seemingly LPL plays the major role in degrading VLDL into VLDL remnants, but whether LPL alone can convert triglyceride-rich LDL is not clear. More likely, HTGL contributes to the hydrolysis of residual triglyceride in VLDL remnants. The combined action of these two enzymes may be sufficient to complete the conversion. LDL receptors seemingly are not required for the transformation because LDL particles are made normally in patients who have a complete absence of LDL receptors.

The partitioning between hepatic uptake of triglyceride-rich lipoproteins and their conversion to LDL thus should affect LDL concentrations. The low levels of LDL cholesterol in many species, such as the rat, appears to be due to rapid removal of VLDL before its conversion to LDL. In humans, in contrast, a greater fraction of VLDL is converted to LDL, and hence LDL levels are relatively high. A relatively low activity of LDL receptors, arising by mechanisms described in the previous section, could account for the relatively high conversion of VLDL to LDL in humans, but other factors, such as a low affinity of VLDL for receptors or activities of LPL and HTGL, also could favor conversion of VLDL to LDL. In any case preferential conversion of VLDL to LDL over direct VLDL uptake should raise LDL levels.

Cholesterol Content of LDL Particles

Amounts of cholesterol carried in lipoprotein particles are not constant. In some people, the amount of cholesterol per particle is relatively low, whereas in others it is high. Estimation of the LDL-cholesterol/apo B ratio reveals the average cholesterol content per LDL particle since only one apo B molecule is present in an LDL particle. If the LDL cholesterol/apo B ratio is high, then the LDL cholesterol level will be raised. Several factors theoretically could increase this ratio: (a) a high cholesterol-ester transfer protein (CETP) activity, (b) a high LCAT activity, (c) increased cholesterol-ester content of newly secreted lipoprotein particles, (d) selective clearance of cholesterol-poor LDL by LDL receptors, and (e) delayed clearance of LDL particles (allowing for prolonged action of LCAT and CETP in plasma). The relative contributions of these different factors have not been studied systemically.

DEFINITIONS OF SERUM CHOLESTEROL LEVELS

The definitions of serum total cholesterol developed by the National Cholesterol Education Program (NCEP) are presented in Table 2 (1). Corresponding levels of LDL cholesterol and relative risk for CHD also are given. The term "hypercholesterolemia" is used synonymously with "high serum cholesterol". Most people with hypercholesterolemia have a high-risk LDL-cholesterol level although there are exceptions. In this paper, "hypercholesterolemia" will be restricted to an LDL-cholesterol exceeding 160 mg/dl. The term "moderate hypercholesterolemia" is applied to LDL-cholesterol concentrations in the range of 160 to 209 mg/dl; this range corresponds to the 75th to 95th percentile for adult Americans. "Severe hypercholesterolemia" represents LDL-cholesterol levels of 210 mg/dl or greater, i.e., over the 95th percentile. In the discussion to follow the causes of borderline-high cholesterol, moderate hypercholesterolemia, and severe hypercholesterolemia will be examined.

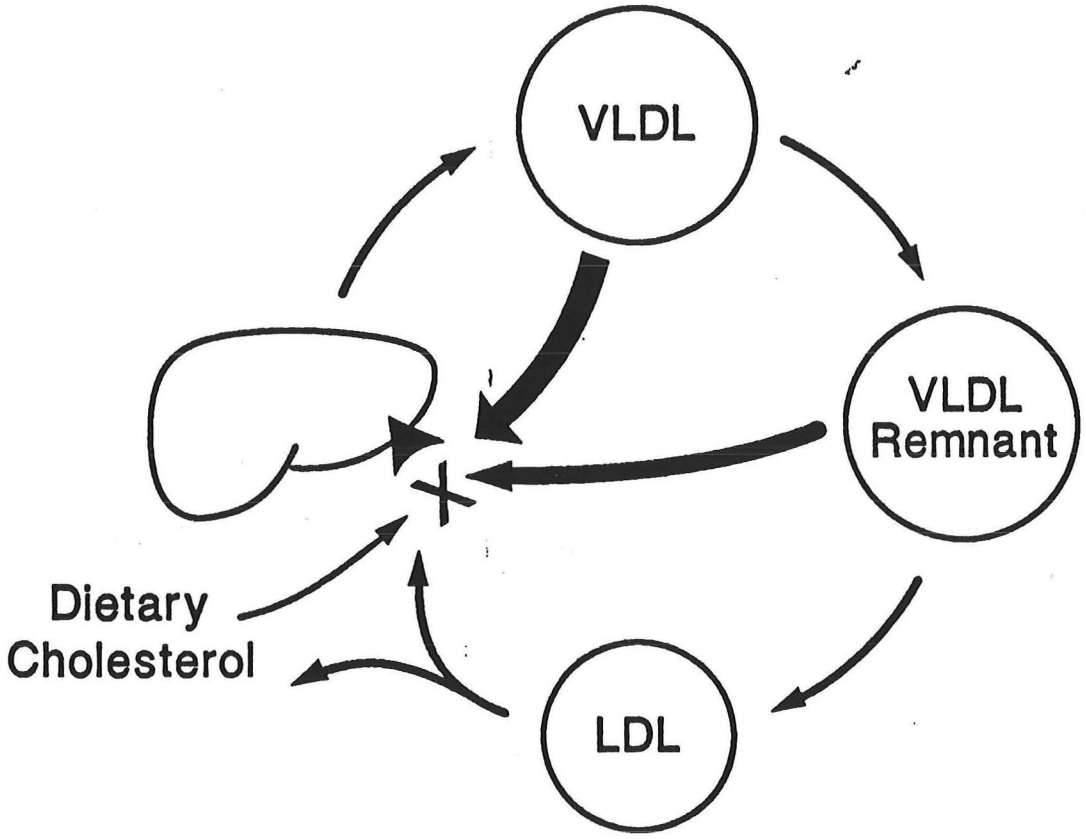


Figure 10. Dietary cholesterol suppresses the activity of LDL receptors.

CAUSES OF BORDERLINE-HIGH CHOLESTEROL LEVELS

Approximately 40% of adult Americans have total cholesterol levels in the range of 200 to 239 mg/dl, or LDL-cholesterol levels of 130 to 159 mg/dl (1). Relative risk for CHD ranges from 1.0 to 2.0, averaging about 1.5 times that of individuals with desirable cholesterol levels. Thus, borderline-high levels contribute significantly to CHD in the United States. We might therefore inquire about the causes of borderline-high serum cholesterol among American adults. Several factors have been identified as potential causes, each of which can be reviewed briefly.

Relatively High Background Cholesterol Levels

One factor contributing to borderline high cholesterol in American adults is a relatively high background cholesterol level of the human species. Humans in general have higher cholesterol levels, and especially LDL-cholesterol levels, than other species (Table 1) (17). As estimated from available epidemiologic data, a nonobese, 20-year old man consuming a diet relatively low in saturated fatty acids (<7% of calories) and cholesterol (<200 mg/day) will exhibit a serum total cholesterol about 140 mg/dl (with an LDL cholesterol ranging from 75 to 90 mg/dl). If the diet is severely restricted in cholesterol-raising nutrients, the total cholesterol level may be even lower, e.g. about 125 to 130 mg/dl, but this very low level may represent borderline malnutrition. The somewhat higher baseline value seems more realistic.

Why do humans in general have higher background cholesterol levels than other species? As indicated before, the most obvious reason is that humans have a sluggish conversion of cholesterol into bile acids in the liver; this leads to a relatively high hepatic content of cholesterol, and thus to suppression of LDL-receptor activity. Perhaps, other factors contribute to this species difference, but to date they have not been identified.

Dietary Cholesterol

Another cause of borderline high cholesterol is a relatively high cholesterol intake. For example, American men typically consume about 400 mg per day. In populations having relatively low serum cholesterol, intakes usually are below 200 mg/day. According to available estimates, increasing cholesterol intake from 200 to 400 mg/day will raise the average serum cholesterol by about 5 mg/dl (18); certainly there is individual variation in responsiveness to cholesterol in the diet, but 5 mg/dl represents a typical increase. The mechanism seemingly is suppression of LDL-receptor activity by raising hepatic cholesterol content (Figure 10). Since cholesterol intake does not change appreciably throughout life, its cholesterol-raising action should persist throughout life.

Dietary Saturated Fatty Acids

Another dietary factor known to raise the serum cholesterol is excessive intake of saturated fatty acids. Americans typically consume about 14% of their total calories as saturated fatty acids, whereas a desirable intake

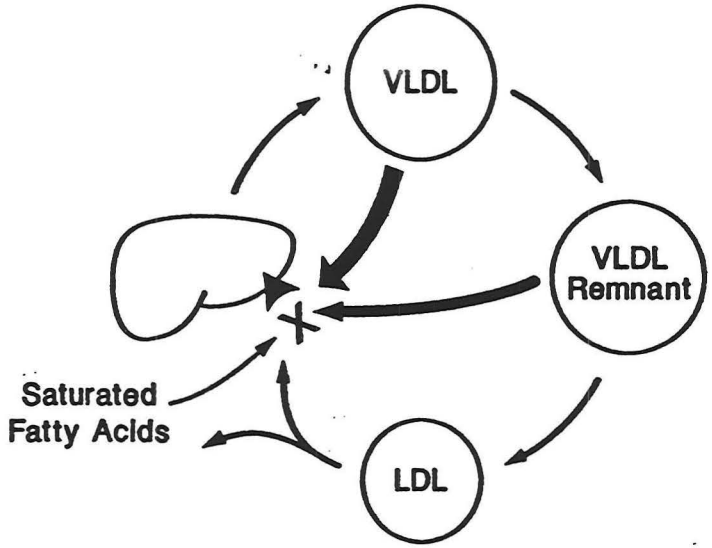


Figure 11. Dietary saturated fatty acids suppresses the activity of LDL receptors, as their primary mechanism for raising LDL cholesterol levels

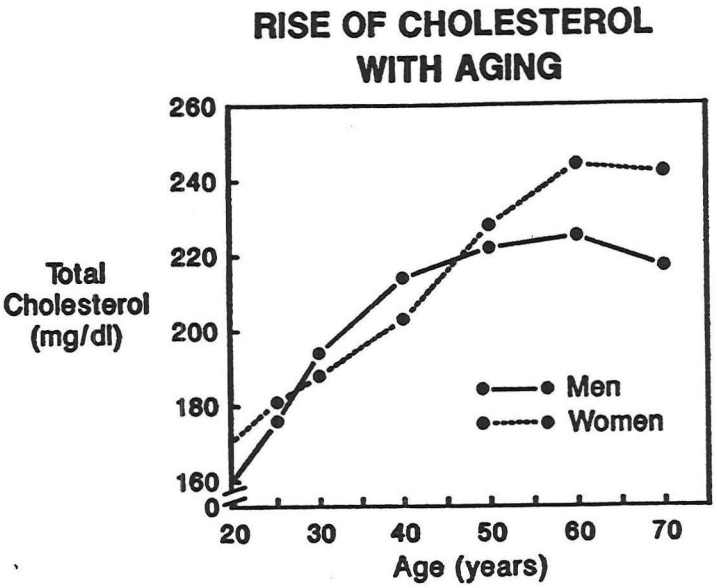


Figure 12. Rise of cholesterol with aging. Men and women.

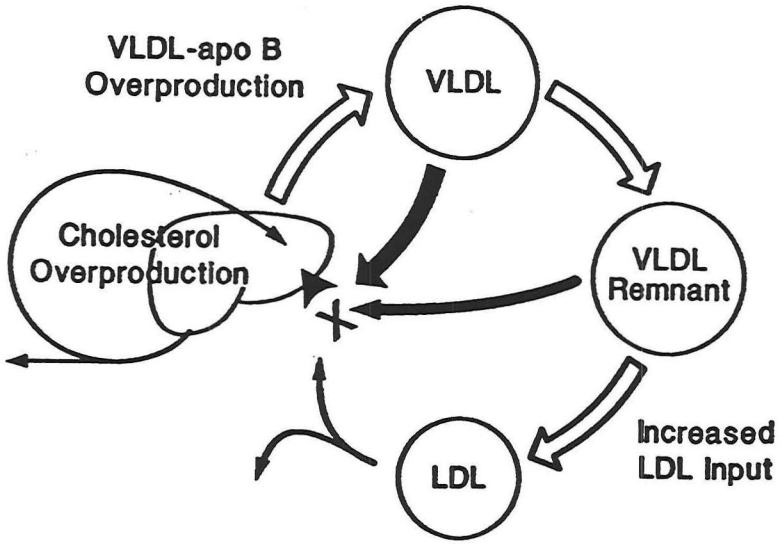


Figure 13. Possible mechanisms whereby obesity increases LDL cholesterol levels. Obesity causes overproduction of VLDL-apo B, which leads to increased input of LDL. It also causes overproduction of cholesterol, which may lead to suppression of LDL receptor activity.

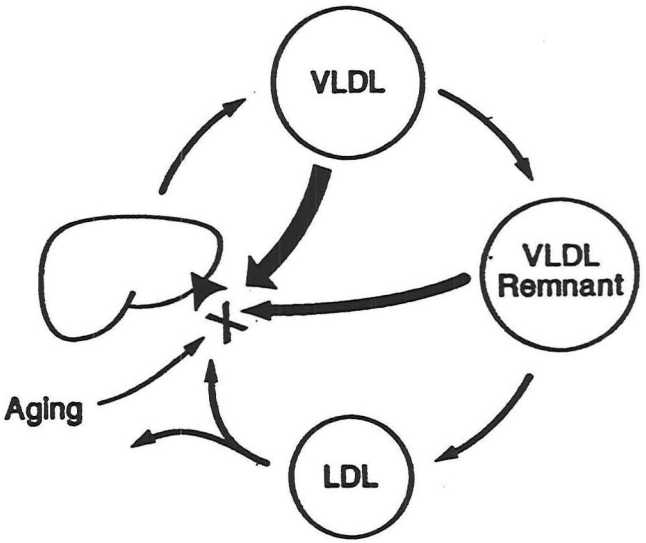


Figure 14. The rise of cholesterol with age is due in part to the decline of LDL receptor activity secondary to the aging process per se

would be in the range of 7% or less. This difference of 7% of calories as saturates can account for a further rise of serum cholesterol of about 20 mg/dl (19,20), most of which is in LDL cholesterol. The best available evidence suggests that saturated fatty acids reduce the activity of LDL receptors (Figure 11) (21-23); the precise mechanism whereby this occurs however has not been determined. Saturated fatty acids could (a) inhibit conversion of unesterified cholesterol to cholesterol ester, (b) enhance movement of inactive unesterified cholesterol into the active pool, (c) interfere with "activity" of LDL receptors at the cell surface, or (d) modify the affinity of LDL for LDL receptors (Figure 5).

The combination of high intakes of cholesterol and saturated fatty acids thus raise total cholesterol levels by about 25 mg/dl above background levels. This dietary effect explains why 20-year-old American men have total cholesterol concentrations averaging 165 mg/dl, an increment that presumably persists throughout life. On the other hand, a relatively high intake of these nutrients seemingly does account for the fact that serum cholesterol levels increase with age, as shown in Figure 12; instead, other factors must be explored, one of which may be increasing body weight.

Weight Gain (Obesity)

American adults typically put on weight with aging; on the average, they are 20 to 30 pounds heavier at age 50 than at age 20. Although data are limited, several studies (24-26) suggest that increasing obesity raises serum cholesterol levels. This increase in serum cholesterol due to obesity seeming averages about 25 mg/dl; it occurs mainly in LDL, although partly in VLDL. At least two metabolic effects may explain the influence of obesity. First, obesity apparently increases hepatic output of apo B-containing lipoproteins (27,28), and this in turn increases input of LDL (29) (Figure 13). In addition, the synthesis of cholesterol is elevated in obese people (30,31), leading to an expansion of the enterohepatic circulation of cholesterol; this response could accentuate down regulation of LDL-receptor synthesis (Figure 13). Further studies are needed to determine precisely how much obesity in American adults contributes to borderline-high cholesterol levels, but apparently it is a significant factor for the population as a whole.

Aging Per Se

Not all of the rise of cholesterol with age can be explained by obesity. Other influences appear to be involved. Previously, Miller (32) reviewed available data from LDL turnover studies carried out in several laboratories in individuals of different ages; from these studies, he concluded that part of the rise of LDL levels with aging is due to decreased activity of LDL receptors (Figure 14). Subsequently, a study from our laboratory (33) showed that FCRs for LDL decrease with aging, supporting the concept that LDL-receptor activity declines with age. The precise mechanism for this response is unknown. As indicated above, overproduction of whole-body cholesterol with increasing obesity could be one but not the only factor. One report indicates that synthesis of bile acids declines with age which could increase hepatic cellular content (Figure 5). Other events of cellular aging may occur, or there could be a decline in the body's overall metabolic rate. When available

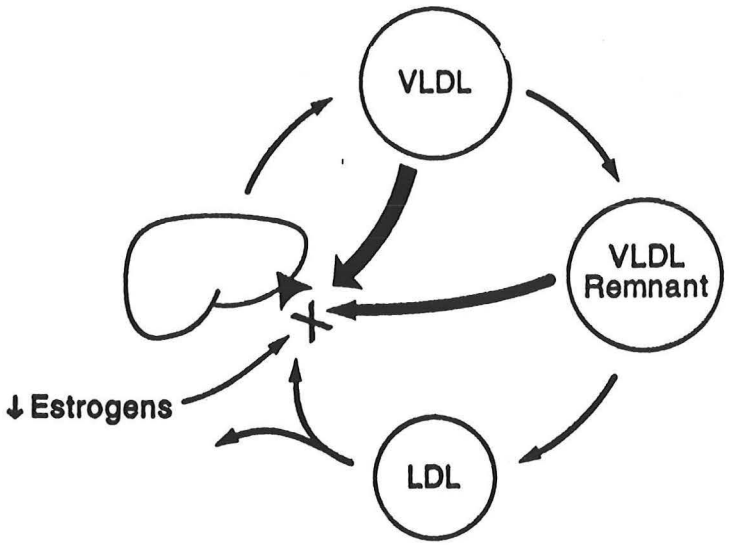


Figure 15. Loss of estrogens after the menopause leads to a decline in LDL receptor activity, which secondarily raises the LDL cholesterol level

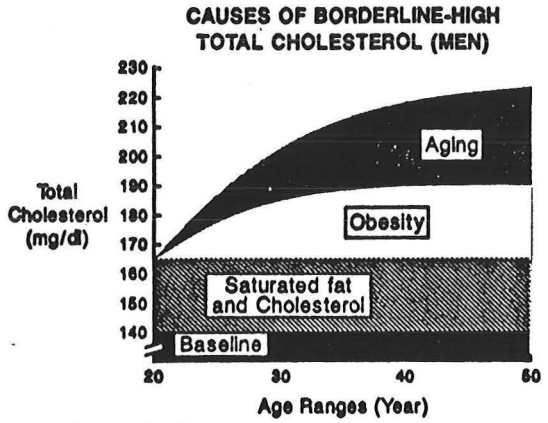


Figure 16 A.

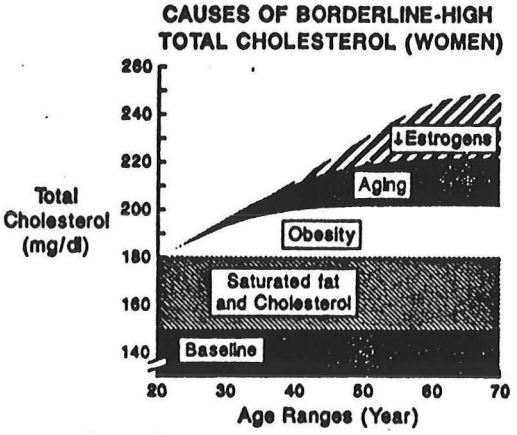


Figure 16 B.

Figure 16. Causes of borderline high cholesterol levels in men (A) and women (B)

data are taken into account, the obesity-independent factors account for approximately 30 mg/dl of the rise of serum cholesterol with aging.

Loss of Estrogens (Women)

The serum cholesterol level in women is below that of men up to age 45 to 50 years, and then it rises above that of men (Figure 12). This postmenopausal rise in women most likely can be explained by loss of estrogens, because estrogens stimulate LDL-receptor activity (Figure 15) (10). Loss of estrogens in postmenopausal women may raise total cholesterol levels by about 20 mg/dl.

Summary

Figure 16 outlines the factors responsible for borderline-high cholesterol levels, and their average contribution to raising the total level. Several of these factors seemingly reduce LDL-receptor activity: (a) an inherent sluggish conversion of cholesterol into bile acids, typical of humans in general, (b) high cholesterol intakes (Figure 10), (c) high intakes of saturated fatty acids (Figure 11), (d) obesity, leading to a high cholesterol synthesis (Figure 13), (e) aging per se (Figure 14), and (f) loss of estrogens (in women) (Figure 15). In addition, obesity leads to an overproduction of VLDL, which induces a high input of LDL (Figure 13).

PREVENTION OF BORDERLINE HIGH CHOLESTEROL

The average total cholesterol level in middle-aged American men is approximately 80 mg/dl higher than the background level; this increment enhances the risk for CHD between one- and two-fold above that of the background level. In postmenopausal women, the increment is even greater because of loss of estrogen-stimulated increase in LDL receptor activity. If borderline-high cholesterol levels could be prevented in the general public, onset CHD should be delayed substantially. Since the causes of borderline-high cholesterol levels are multiple (Figure 16), several steps will be required for mitigation of these higher levels. For example, in postmenopausal women, estrogen repletion should stimulate LDL-receptor activity. Unfortunately, the causes of the decline in LDL-receptor activity with aging per se are unknown, so this effect cannot be prevented at present. On the other hand, the fraction of the rise with aging related to weight gain should be prevented by avoiding overweight. Reducing intake of saturated fatty acids and cholesterol likewise should further decrease cholesterol levels. Overall, the composition of the typical American diet combined with substantial weight gain seemingly accounts for an average increase in total cholesterol of about 50 mg/dl, and this increment can be reduced by dietary change.

Since the recommended dietary change includes a reduction in intake of saturated fatty acids, the question arises as to which nutrients should be used in their place. If a person is overweight, foods rich in saturated fatty acids (e.g. fat-rich dairy products and meats) can be removed altogether

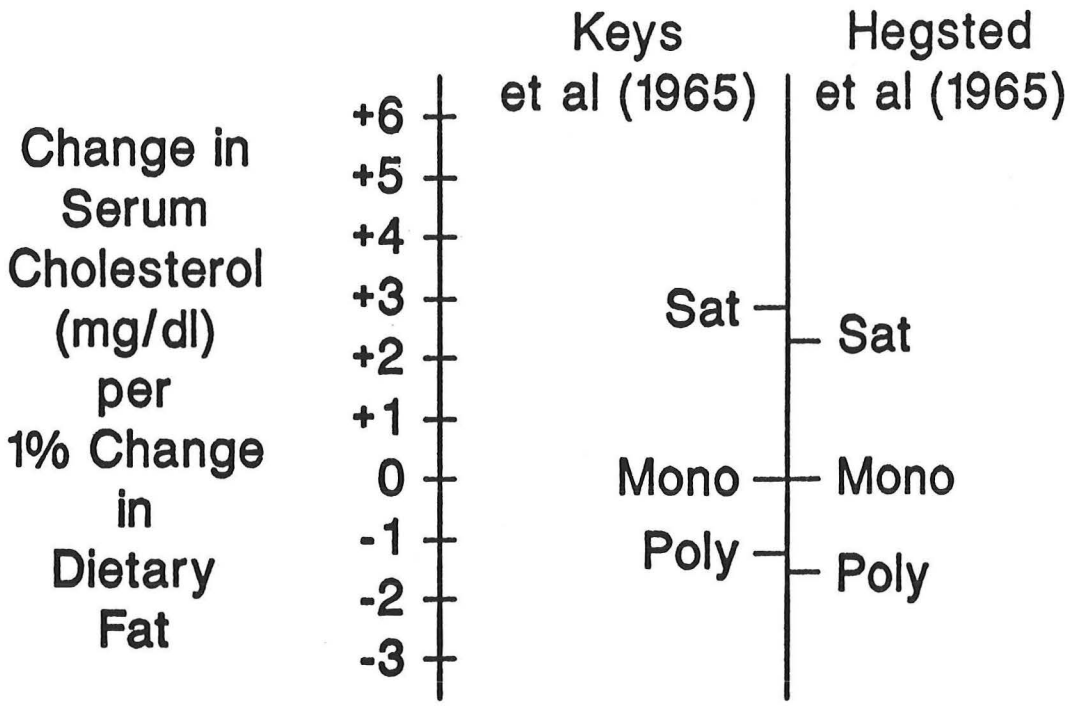


Figure 17. Effects of saturated fatty acids (SAT), monounsaturated fatty acids (MONO) and polyunsaturated fatty acids (POLY) on total cholesterol levels, according to Keys et al and Hegsted et al (in 1965)

Table 3

Possible Adverse Effects of High Intakes of polyunsaturated Fatty Acids

Promotion of carcinogenesis

Suppression of immune system

Formation of cholesterol gallstones

Lowering of HDL cholesterol

Promotion of LDL oxidation

without replacement. However, even after weight reduction and for people who are not overweight, the diet still will contain more saturated fatty acids than desirable; consequently, other nutrients must be considered in their place. What might these be?

The three major possibilities are polyunsaturated fatty acids, monounsaturated fatty acids, and carbohydrates. During the 1960's and 1970's, many investigators favored polyunsaturates for the reasons shown in Figure 17; this figure shows the relative effects of the various nutrients, as set forth by Keys et al (19) and Hegsted et al (20). According to these investigators, carbohydrates and monounsaturated fatty acids, the latter being essentially oleic acid, are "neutral" with respect to serum total cholesterol, i.e. they neither raise nor lower total cholesterol levels. In relation to these nutrients, saturated fatty acids raise cholesterol concentrations, whereas the predominant polyunsaturated fatty acid, linoleic acid, lowers the level. From these relations the preferred replacement for saturated fatty acids would appear to be linoleic acid. Consequently many researchers recommended that the linoleic-acid content of the American diet be increased, and the food industry responded with introduction of "polyunsaturated" vegetable oils for cooking oils, margarines and shortenings, and as an ingredient in other food products.

In the late 1970's however some investigators became concerned that the safety of increasing dietary linoleic acid had not been adequately substantiated. Several possible adverse effects were suggested, and since then, the list of concerns has grown longer (Table 3). For example, no large population has ever consumed large amounts of linoleic acid for prolonged periods with proven safety. Of special concern is that high intakes of linoleic acid may promote the development of cancer in humans, as it does in laboratory animals (34); recent epidemiologic evidence indeed tends to support this possibility (35). Animal studies further indicate that dietary linoleic acid can suppress the immune system (36), a possible mechanism for cancer promotion. Moreover, high intakes of linoleic acid will lower HDL-cholesterol levels (37,38). They also may raise the risk for cholesterol gallstones (39). A recent study (40) even suggests that enrichment of LDL particles with linoleic acid may enhance oxidation of LDL in the arterial wall, a possible atherogenic response. Thus, even if linoleic acid is more "hypercholesterolemic" than other nutrients, these concerns have led many workers to conclude that high intakes of linoleic acid should be avoided. Intakes above 7% of total calories seemingly cannot be advocated with prudence (41).

These concerns about the safety of dietary linoleic acid led Dr. Fred Mattson and me to consider other possible replacements for saturated fatty acids. Certainly carbohydrates are one alternative, and high-carbohydrate, low-fat diets are widely advocated. They have epidemiologic support in that such diets commonly are consumed by populations that have a low rate of CHD. On the other hand, very-low-fat diets generally have not been consumed voluntarily, but rather out of necessity. When dietary fat becomes available and affordable, most populations increase their fat intake. Moreover, in the Mediterranean region, where olive oil is consumed in large amounts, total intake of fat is relatively high, and CHD rates are low (42). Since olive oil contains mainly oleic acid, we decided to re-examine the effects of oleic acid

the Gladstone Foundation under Dr. Robert Mahley in San Francisco. In this collaboration, Dr. Thomas Innerarity and associates showed that the patient's LDL bound poorly to LDL receptors in tissue culture (Figure 22) (56), and Dr. Karl Weisgraber and coworkers (57) noted that it reacted abnormally with LDL antibodies targeted to the receptor-binding domain of the LDL receptor. These findings confirmed that the patient's LDL was abnormal. Further studies by Dr. Bryan McCarthy and associates (59) identified the abnormality as a glutamine to arginine transformation at position 3500 of apo B-100 (Figure 23). This same abnormality was identified in other relatives in this family who had hypercholesterolemia. Subsequently, the 3500 defect has been found in several other families suggesting that familial defective apo B-100 (3500 mutation) is responsible for many cases of hypercholesterolemia.

In the course of our turnover studies (55), other patients were identified in whom autologous LDL decayed more slowly than homologous LDL (Figure 24). These patients do not have the 3500 mutation, but presumably have other abnormalities in apo B-100 that interfere with normal binding of LDL to LDL receptors. We thus postulate that familial defective apo B-100 encompasses several abnormalities in the primary structure of apo B-100 that remain to be elucidated.

Increased Input of LDL

Another potential cause of moderate hypercholesterolemia is an increased input of LDL, i.e., an increased conversion of VLDL to LDL. Three possible causes are shown in Figure 25. One is a reduced activity of LDL receptors (Figure 25A); in this case, fewer VLDL particles would be removed by hepatic LDL receptors, and more would be converted to LDL. This defect should be identified by a low FCR for LDL, and has been noted in patients with familial hypercholesterolemia. Second, there could be an overproduction of apo B-containing lipoproteins by the liver (Figure 25B); here the FCR for LDL should not be markedly depressed, but only mildly decreased or within the normal range. And third, there could be a partitioning defect for VLDL, i.e., uptake of VLDL (and VLDL remnants) is reduced, allowing for a greater conversion of VLDL to LDL (Figure 25C). In this case, availability of LDL receptors for clearance of LDL should be increased because they would be less loaded with VLDL particles; hence the FCR for LDL would be relatively high. Even so, high levels of LDL should result because VLDL particles have greater affinity for LDL receptors than LDL itself, and the relatively slow uptake of LDL by receptors would result in a rise in LDL concentrations. These latter two patterns of LDL kinetics have been observed in our patients with moderate hypercholesterolemia. The potential causes of each can be considered.

An increased secretion of VLDL apo B, leading to increased conversion of VLDL to LDL was postulated before as one mechanism whereby obesity leads to borderline high cholesterol levels (Figure 13). A possible cause for a still further increment in VLDL-apo B input could be a genetic hypersensitivity to obesity, e.g., the presence of obesity may recruit the secretion of even more apo B-containing lipoproteins than typically would occur. Whether a primary hypersecretion of VLDL apo B, independent of obesity truly exists, has not been determined; such a defect has been postulated as the underlying abnormality of familial combined hyperlipidemia (60,61), but in our patients

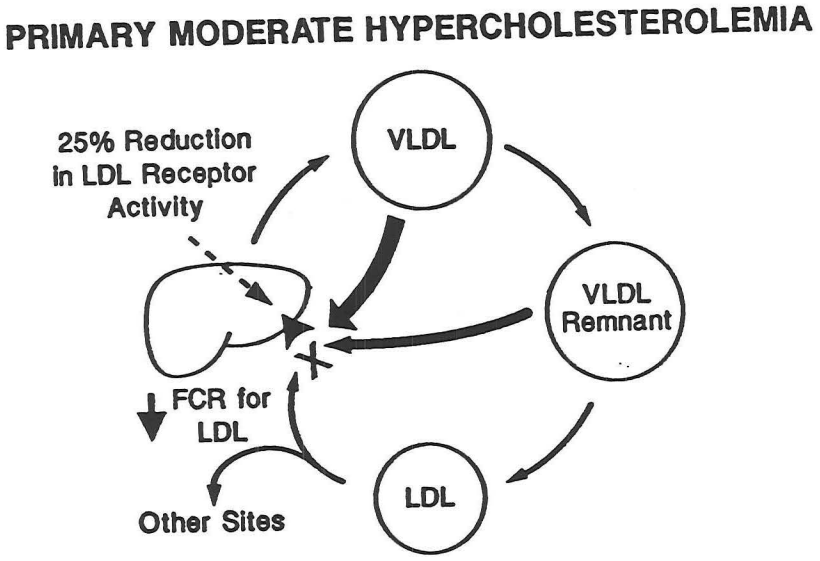


Figure 18. One cause of primary moderate hypercholesterolemia: 25% reduction in LDL receptor activity due to "metabolic" suppression of LDL-receptor synthesis

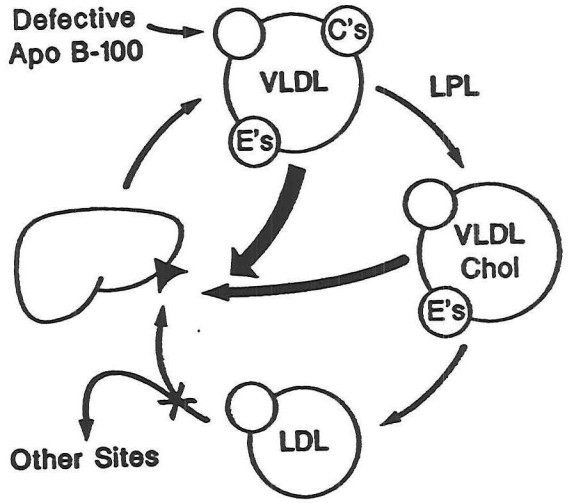


Figure 19. Hypothetical cause for primary moderate hypercholesterolemia: defective apo B-100, such that apo B-100 fails to interact normally with LDL receptors

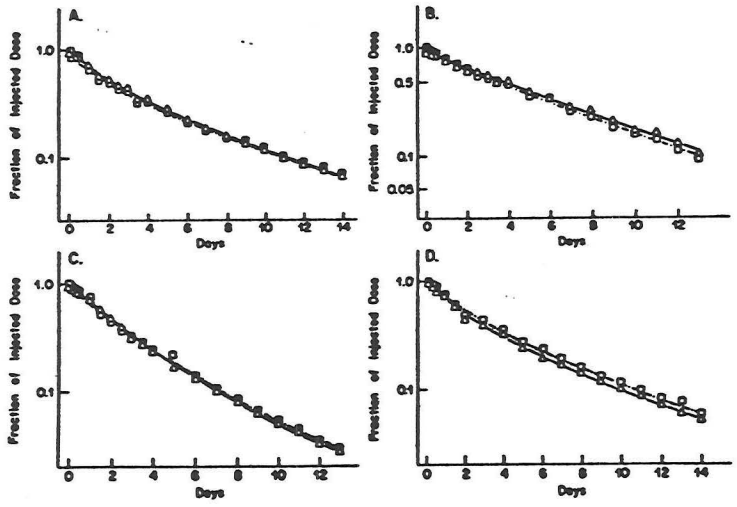


Figure 20. Plots of plasma decay curves of radiolabeled autologous LDL (△) and homologous LDL (□) for four patients with primary moderate hypercholesterolemia. All patients had relatively slow decay rates for autologous LDL, but because homologous LDL decayed at the same rate, it can be assumed that the LDL particles were not abnormal

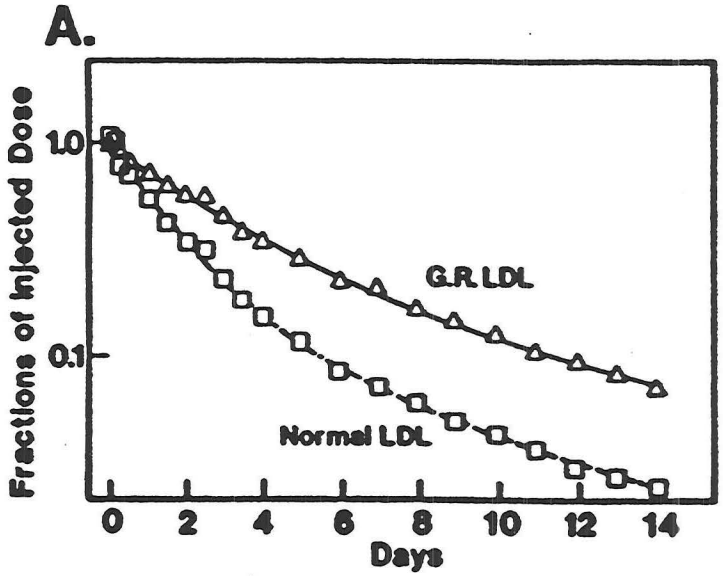


Figure 21. Plots of plasma decay curves of radiolabeled autologous LDL (△) and homologous LDL (□) in one patient (GR). In this patient, autologous LDL was cleared much more slowly than homologous LDL, suggesting that autologous LDL was defective and did not bind normally to LDL receptors.

higher cholesterol levels, other factors, either genetic or acquired, are required. However, most of the acquired factors do not raise background cholesterol levels to above the borderline zone, and hence are not responsible for still high levels in the moderate range of hypercholesterolemia. Consequently genetic abnormalities probably are required for the development of moderate hypercholesterolemia. In most instances these genetic defects have not been defined at a molecular level, but on the basis of lipoprotein kinetic studies, we have identified several categories of abnormalities that appear responsible for raising the LDL-cholesterol level from the borderline range to the moderately elevated range. These can be reviewed.

Low Clearance Rates for LDL

Our LDL turnover studies have revealed that some patients with moderate hypercholesterolemia have lower FCRs for LDL than found in patients with borderline LDL-cholesterol levels (54). Two possibilities might explain unusually low FCRs for LDL. First, some patients may have a further reduction in LDL-receptor activity, beyond that found with borderline-high levels (Figure 18). And second, LDL particles might be defective so that they bind poorly to LDL receptors (Figure 19). To distinguish between these possibilities we carried out a study in which the patients' own LDL (autologous LDL) were labeled and reinjected with labeled, normal (homologous) LDL (55). Two different isotopes of iodine--I^{125} and I^{131}--were used to label the two forms of LDL. The hypothesis of this study was that if both isotopes decayed slowly and at the same rate then the patient's own LDL was normal and hence LDL-receptor activity was reduced. If however the patient's own LDL disappeared at a slow rate, but the normal LDL at a normal rate, then the patient's own LDL was defective and thus bound poorly to LDL receptors. This combined LDL turnover technique was employed in about 25 patients.

Figure 20 shows the results in four patients. In these patients, both LDL particles decayed at the same rate; hence, they were considered to have an accentuated reduction in LDL-receptor activity. Such a defect might have had several origins. For example, the patient could have a mild form of heterozygous FH such that the LDL-cholesterol level was only moderately raised. Recently, several instances of heterozygous FH with only moderate hypercholesterolemia have been reported; presumably these patients have mitigating factors that prevent them from developing severe hypercholesterolemia. Second, reduction in LDL-receptor activity may be due to a defect in regulation of LDL receptor synthesis. Such might be secondary to excessive sensitivity to dietary cholesterol or saturated fatty acids, beyond that which is normally observed. An abnormality of this type might reside in the gut (hyperabsorption of cholesterol) or the liver (excessive sensitivity of LDL-receptive synthesis to suppressive nutrients). Limited evidence indeed suggests that some hypercholesterolemic individuals are unusually sensitive to the cholesterol-raising action of saturated fatty acids (57).

Figure 21 shows the data of another autologous/homologous LDL turnover study. This patient's own LDL disappeared at a slow rate, but the normal LDL decayed much faster, at a normal rate; seemingly the patient's LDL was abnormal. To further examine this possibility, we sought the collaboration of

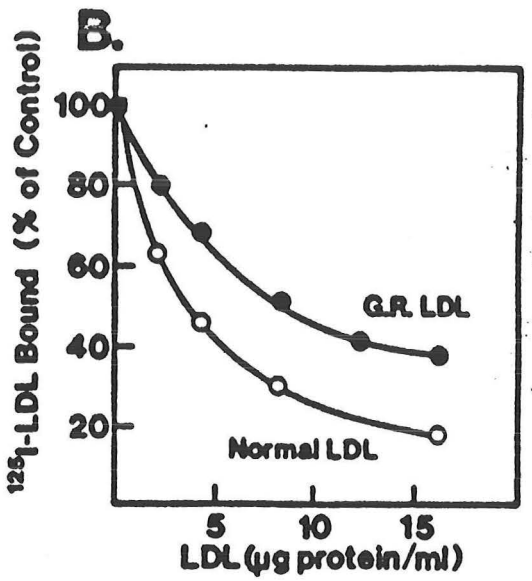


Figure 22. Defective binding of LDL from patient GR to LDL receptors in tissue culture

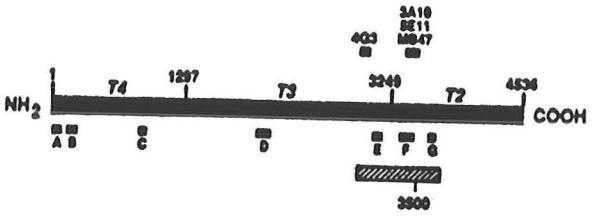
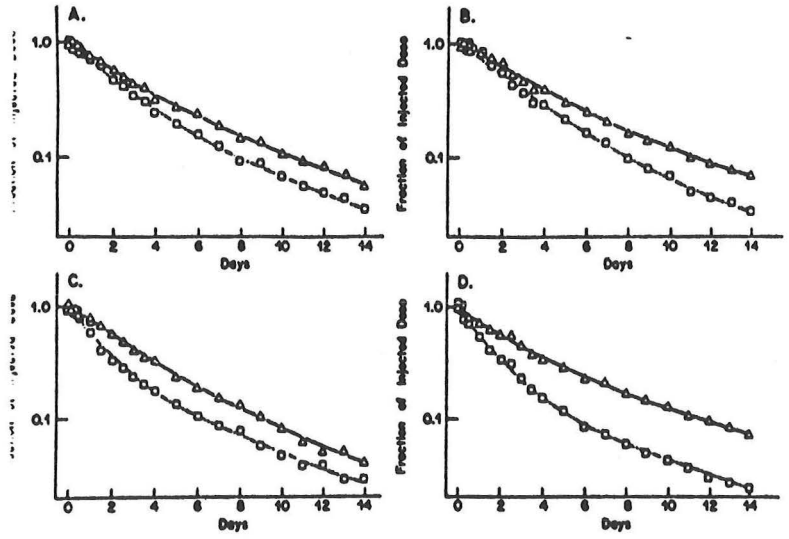


Figure 23. Linear representation of apo B-100 showing the locations of the heparing-binding sites (the boxes lettered A-G), the epitopes of four monoclonal antibodies that inhibit LDL receptor binding (4G3, 3A10, 5E11, MB47), and the putative location of the receptor-binding region (hatched box). The "3500" represents the amino acid substitution of glutamine for arginine that is believed to cause familial defective apo B-100 (FEB)



igure 24. Plots of plasma decay curves of radiolabeled autologous LDL (Δ) and homologous LDL (□) for four patients with primary moderate hypercholesterolemia. In all patients, decay rates for autologous LDL were relatively slow, whereas those for homologous LDL were normal. This suggest that the hypercholesterolemia in the patients was due to a defective LDL that did not bind normally to LDL receptors.

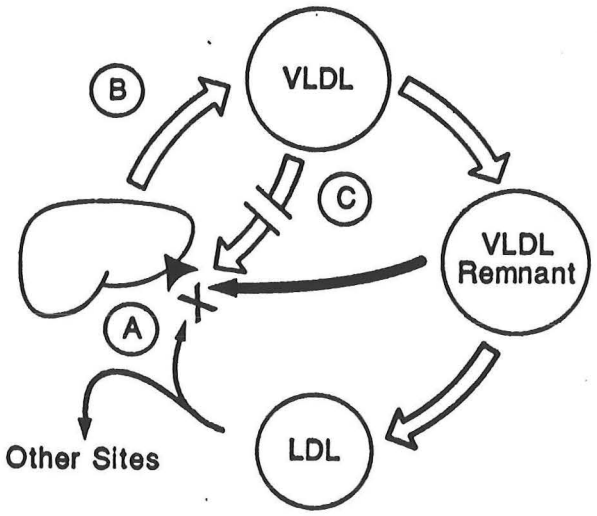


Figure 25. Schematics of mechanisms for increased production (input of LDL. These include (A) decreased activity of LDL receptors, (B) increased hepatic secretion of apo B-containing lipoproteins, and (C) decreased uptake of newly secreted VLDL.

on serum cholesterol, particularly on cholesterol levels in the various lipoprotein fractions.

In a direct comparison against palmitic acid, both oleic and linoleic acids had the same effect on LDL-cholesterol levels, i.e., both appeared to be "neutral" and did not raise the levels, whereas palmitic acid clearly increased the LDL-cholesterol concentration (43). In contrast to linoleic acid, which lowered HDL-cholesterol, oleic acid maintained a constant HDL level. Thus part of the greater total-cholesterol lowering action of linoleic acid appeared to be due to its action to reduce HDL-cholesterol levels. Several other reports (44-46) later confirmed that oleic and linoleic acids have essentially identical effects on LDL-cholesterol levels. Further, none of the adverse effects listed for linoleic acid in Table 4 have been found for oleic acid; in our view, therefore, oleic acid is preferable to linoleic acid as a replacement for saturated fatty acids.

Still, it must be asked whether oleic acid is preferable to carbohydrate as a replacement. From epidemiologic studies, carbohydrates and monounsaturates appear to have similar effects on CHD risk. In the studies of Keys et al (19) and Hegsted et al (20), both had similar effects on total cholesterol levels. We have confirmed this identity for LDL (47,48). However, several metabolic studies have shown that in contrast to a diet high in oleic acid, high-carbohydrate diets lower HDL-cholesterol and raise triglyceride levels. These latter effects have been confirmed in epidemiologic studies (49), suggesting that the theoretically adverse actions of carbohydrate on HDL and triglycerides are long lived. On the other hand, a higher intake of total fat, even if it is in the form of oleic acid, may predispose to weight gain. Higher fat diets also have been implicated in the development of cancer, although epidemiologic data taken as a whole seem to exonerate oleic acid from this effect (35). More studies thus will be required to determine whether carbohydrate or oleic acid is the preferable replacement for saturated fatty acids. In fact, both probably are satisfactory, which should allow for the introduction of more variety into cholesterol-lowering diets.

The possibility for greater variety has been increased recently by our confirmation that dietary stearic acid also is "neutral" with respect to serum cholesterol levels (50). Previous studies (51,52) had raised this possibility, and our confirmation means that not all saturated fatty acids increase cholesterol levels. Indeed, prior investigations also have shown that medium-chain fatty acids are not hypercholesterolemic (53). Thus, the cholesterol-raising saturates appears to be limited to three: lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0). The lack of hypercholesterolemic effect of several saturated acids provides the potential for still more variety for the diet.

CAUSES OF PRIMARY MODERATE HYPERCHOLESTEROLEMIA

Primary moderate hypercholesterolemia is defined as a total cholesterol level in the range of 240 to 289 mg/dl (LDL cholesterol of 160 to 209 mg/dl). Most patients with moderate hypercholesterolemia have all of the causes of borderline-high cholesterol listed in a previous section. To develop still

Table 4

Lipoprotein Kinetics in Primary Moderate Hypercholesterolemia

(Predominant Overproduction of Lipoproteins)

Group	n	LDL cholesterol (mg/dl)	LDL apo B		
			Conc (mg/dl)	FCR (pools/day)	Input (mg/kg day)
Primary moderate hypercholesterolemia (middle aged)*	13	193±2	143±10	0.47±0.02	26.7±1.9
Normal men (middle aged)†	14	144±6	101±5	0.30±0.01	13.5±0.7

Values are given as mean ±SEM. Conc, Concentrations; FCR, fractional catabolic rate; input, input rate, used synonymously with production rate.
*Patients were all men; mean age, 55±6 (±SEM) years.
†Mean age of normolipidemic men was 56±2 (±SEM) years.

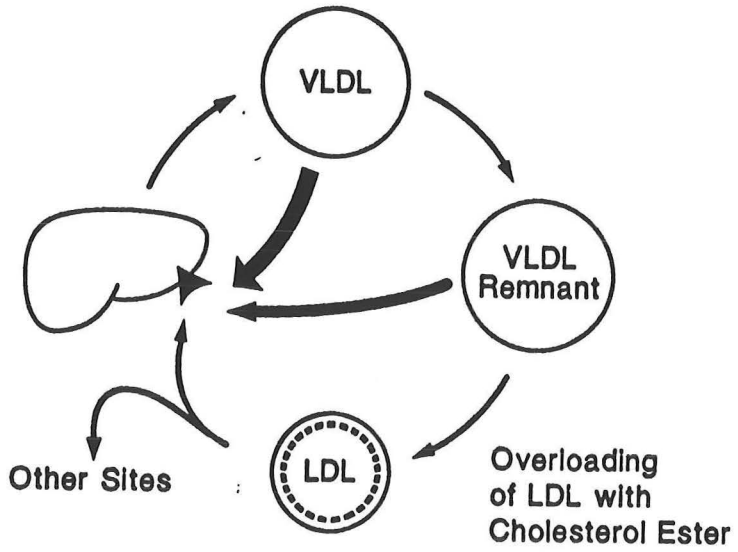


Figure 26. One mechanism for primary hypercholesterolemia: Overloading of LDL with cholesterol ester

with moderate hypercholesterolemia, the pattern of moderately increased input of LDL in the presence of a normal FCR for LDL occurred commonly and likely cannot be explained by a unique metabolic abnormality. It appears to be related more to obesity than to a genetic disorder.

The third pattern of high LDL input likewise was noted in several of our patients (Figure 25C). Table 4 shows the lipoprotein kinetics for a group of patients with this pattern. These patients had both high input rates and high FCRs for LDL. This pattern seems incompatible with an enhanced secretion of VLDL-apo B since such an abnormality should overload LDL receptors, resulting in a low-normal FCR for LDL (Figure 25B). In contrast, a high FCR for LDL suggests an increased availability of LDL receptors, secondary to decreased hepatic uptake of VLDL. The mechanisms whereby uptake of VLDL is reduced have not been determined. Overall LDL-receptor activity does not appear to be reduced because FCRs for LDL are high. The patients do not have the E2/E2 pattern typical of type 3 hyperlipoproteinemia. A deficiency of "apo E receptors" seems unlikely because LDL receptors would then be overloaded by VLDL, again leading to a low FCR for LDL. One possibility is that the affinity of VLDL is reduced even in the presence of normal apo E isoforms. Alternatively, the activities of LPL or HTGL are high, resulting in a rapid conversion of VLDL into LDL before direct uptake of VLDL can occur. Whatever the mechanism, this pattern of LDL kinetics suggests a partitioning defect between hepatic uptake of VLDL and conversion to LDL, the final result of which is hypercholesterolemia.

Enrichment of LDL with Cholesterol Ester

A final mechanism whereby LDL cholesterol levels can be raised from the borderline zone to moderately elevated is by enrichment of LDL particles with cholesterol ester (Figure 26). This abnormality is revealed by an increase in the LDL cholesterol/apo B ratio, and it is observed commonly in patients with moderate hypercholesterolemia. The mechanism is unknown, but may be related to one of the factors influencing the cholesterol ester content of LDL, e.g., LCAT, CETP, residence time of LDL, or the cholesterol content of newly secreted lipoproteins. It should be noted that most patients with overloading of LDL particles with cholesterol ester apparently do not have abnormality in apo B metabolism, but rather a defect in cholesterol metabolism.

TREATMENT OF MODERATE HYPERCHOLESTEROLEMIA

The NCEP (1) recommended that the first line of treatment for moderate hypercholesterolemia be dietary therapy, i.e., reduction in intake of saturated fatty acids, cholesterol, and total calories. Based on our previous discussion, these dietary changes could produce a decrease in total cholesterol levels of approximately 50 mg/dl, which should reduce LDL-cholesterol levels to the desirable range in some but certainly not all patients with moderate hypercholesterolemia. For the majority of patients, a reduction to the borderline zone is more likely. The exception may be patients who develop hypercholesterolemia on the basis of excessive sensitivity to diet; in these patients, dietary therapy alone may be sufficient to lower LDL-cholesterol levels to the desirable range. However,

in others who have genetic defects in LDL metabolism, a reduction of LDL levels to the desirable range will not be possible; if the desirable range is the goal of therapy, hypocholesterolemic drugs will be required. For primary prevention of CHD, a decrease of cholesterol levels to the borderline zone by dietary therapy alone may be sufficient; but for patients who already have CHD, a greater reduction of LDL levels may be indicated, and drug therapy will be required.

CAUSES OF SEVERE HYPERCHOLESTEROLEMIA

This condition is defined as a total cholesterol exceeding 290 mg/dl (LDL cholesterol $\geq$ 210 mg/dl). Severe hypercholesterolemia carries a high risk for CHD. The most dramatic example is familial hypercholesterolemia, which is the result of an inherited defect in the gene encoding for LDL receptors. In the heterozygous form, the patient exhibits half the normal number of LDL receptors, and the pattern of LDL metabolism shown in Figure 25A is present. Since heterozygous FH occurs in only one in 500 people, whereas severe hypercholesterolemia is found in five of 100 adults, most of the latter must arise from abnormalities other than FH.

Our studies have revealed that most patients with severe hypercholesterolemia have a combination of abnormalities responsible for moderate hypercholesterolemia (Figures 18,19,25A-C, and 26). Usually enrichment of LDL with cholesterol ester (Figure 26) is combined with either delayed clearance or increased input of LDL. Thus, severe hypercholesterolemia in general appears to be the result of coinheritance of two defects of LDL metabolism in addition to the causes outlined before for borderline high cholesterol.

TREATMENT OF SEVERE HYPERCHOLESTEROLEMIA

Although dietary therapy is indicated as an adjunct for management of severe hypercholesterolemia, it generally will not reduce levels to the desirable range. The use of one hypercholesterolemic drug usually will lower the level to the moderately elevated range, but two drugs typically are required to achieve desirable levels. This is because multiple metabolic defects must be overcome. The combination of a bile acid sequestrant with either nicotinic acid or an HMG CoA reductase inhibitor is adequate therapy is most patients (62,63).

SUMMARY

This review underlines the multiplicity of causation of hypercholesterolemia in the American public. Dietary factors (cholesterol, saturated fatty acids, and obesity) clearly raise the cholesterol level, and are important causes of borderline-high cholesterol. Still, the unexplained decline of LDL-receptor activity with aging contributes importantly to borderline-high levels and cannot be ignored. The loss of estrogen-stimulated LDL receptor activity after the menopause is an important contributor to elevated cholesterol in post-menopausal women. In addition, several genetic defects inherited singly appear to be responsible for moderate hypercholesterolemia. Some of these defects may represent genetic

hypersensitivity to diet, and dietary therapy alone may provide adequate cholesterol lowering. Other defects impart resistance to dietary control, and use of a single cholesterol-lowering drug may be required. With the exception of the relatively rare heterozygous FH, most cases of severe hypercholesterolemia result from coinheritance of at least two genetic defects in LDL metabolism, and in general these can be overcome only by using cholesterol-lowering drugs in combination.

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