High serum cholesterol is a major risk factor for coronary heart disease (CHD), and most cholesterol in serum circulates with low density lipoproteins (LDLs). These lipoproteins are macromolecular complexes containing a core of nonpolar lipids—mainly cholesterol ester—and a surface coat of unesterified cholesterol, phospholipids, and apolipoprotein (apo) B-100. Many investigators believe that the high levels of LDL are mainly responsible for the association between high serum cholesterol and CHD. Apparently, circulating LDL particles filter into the arterial wall where they are chemically or physically modified and are taken up by macrophages. Uptake of LDL transforms macrophages into foam cells, the hallmark of atherosclerotic lesions. Although other lipoproteins—very low density lipoproteins (VLDLs) and high density lipoproteins (HDLs)—undoubtedly participate in atherogenesis, LDL appears to be the primary culprit. This review therefore will focus on the causation of high serum levels of LDL cholesterol, and it will consider the implications of causation for therapeutic control of hypercholesterolemia.

Regulation of Low Density Lipoprotein Cholesterol Levels

Pathways of Low Density Lipoprotein Metabolism

LDLs derive from the catabolism of triglyceride-rich VLDLs, which in turn are secreted by the liver (Figure 1). VLDLs contain three types of apolipoproteins—apo B-100, apo Cs (C-I, C-II, and C-III), and apo Es. The latter occur in three isoforms—E₂, E₃, and E₄. When VLDLs enter capillaries, they encounter lipoprotein lipase (LPL), an enzyme located on the surface of endothelial cells, and VLDL triglycerides undergo hydrolysis. VLDLs thereby are converted into small VLDL "remnants"; in this process, the soluble apolipoproteins, apo Cs and apo Es, leave the surface of the lipoproteins, with apo Cs leaving more readily than apo Es. Further hydrolysis of triglycerides converts VLDL remnants to LDL; as this occurs, the remaining apo E is lost, leaving only apo B-100 on LDL. The mechanisms whereby triglycerides of VLDL remnants are hydrolyzed to yield LDL are not understood; LPL may participate, but hepatic triglyceride lipase (HTGL) probably plays a role as well.

At any stage in VLDL catabolism, VLDL particles can be removed directly by the liver. One pathway of VLDL removal is via the well-described LDL receptor; this receptor not only recognizes apo B-100 of LDL but also binds apo E on VLDL. Since VLDLs have both apo E and apo B-100, they have a greater affinity for LDL receptors than does LDL itself. It has been suggested that VLDLs and their remnants may be removed by other receptors that specifically recognize apo E, although to date physiological apo E receptors remain elusive. One candidate for this receptor is the LDL receptor-related protein, but its function has not been proven. Partitioning of VLDL between hepatic uptake and conversion to LDL influences the formation of LDL, in turn affecting LDL levels. LDLs leave the circulation via either receptor or nonreceptor pathways, both occurring in liver and extrahepatic tissues. The sole receptor for native LDL is the LDL receptor, and it accounts for about 75% of LDL clearance. Nonreceptor uptake of LDL, accounting for the rest, probably occurs by bulk-phase endocytosis. In humans, the liver removes about 75% of circulating LDL.

These pathways reveal that LDL concentrations depend on the balance among 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 may be further influenced by the cholesterol content of LDL particles. The critical physiological factors controlling each of these processes will be examined, and then abnormalities in their regulation will be considered.
cholesterol. Consequently, synthesis of LDL receptors is tightly regulated by regulatory factors also influencing receptor formation. Malformations in the gene encoding LDL receptors clearly have a greater affinity for LDL receptors than does LDL itself, and hence the former are removed at a greater rate.

**Figure 1. Basic pathways for lipoproteins containing apolipoprotein (apo) B-100.** Very low density lipoprotein (VLDL) particles are secreted by the liver and contain apo B-100 (B), apo Cs (C's), and apo Es (E's). VLDL triacylglycerides undergo lipolysis by lipoprotein lipase, converting VLDL to VLDL remnants. The final removal of triacylglycerides from VLDL remnants in their conversion to LDL may occur via hepatic triacylglyceride lipase (HTGL). At any step in this cascade, lipoproteins can be removed directly by the liver via low density lipoprotein (LDL) receptors. VLDL and VLDL remnants have a greater affinity for LDL receptors than does LDL itself, and hence the former are removed at a greater rate.

**LDL Receptor Activity**

The low density lipoprotein receptor. The gene for the LDL receptor resides in chromosome 19; it occupies 45.5 kb of DNA and encodes a 5.3-kb mRNA. The receptor protein contains 860 amino acids arranged in six domains: 1) a signal sequence at the amino terminus, 2) a ligand-binding domain, 3) a domain having homology to epidermal growth factor, 4) a clustered O-linked sugar domain, 5) a transmembrane region, and 6) a cytoplasmic tail at the carboxy terminus of the protein. LDL receptors are synthesized in the rough endoplasmic reticulum (RER) and are then transported to the Golgi apparatus where carbohydrate chains are added. Mature glycoprotein receptors next move to the cell surface and migrate to coated pits where they cluster. Here they bind to lipoproteins containing apo B-100, apo E, or both. The resulting receptor–lipoprotein complex undergoes endocytosis, and in the endosome, receptor and lipoprotein dissociate; the lipoprotein undergoes enzymatic degradation within lysosomes, whereas the freed receptor can have two fates: it can 1) recycle to the cell surface or 2) enter lysosomes for degradation. The activity of LDL receptors at the cell surface thus depends on two factors: 1) the number of receptors synthesized and 2) the rate of their recycling to the surface. Abnormalities in the gene encoding LDL receptors clearly can reduce the number of receptors synthesized, but regulatory factors also influence receptor formation.

**Regulation of low density lipoprotein receptor activity.** The expression of LDL receptors is tightly regulated and geared to maintain an optimum cellular content of cholesterol. Consequently, synthesis of LDL receptors can be upregulated or downregulated. Cholesterol itself seemingly plays a key role in this regulation. When cellular cholesterol increases, receptor synthesis is suppressed; conversely, when cellular cholesterol declines, synthesis increases. Available evidence suggests that only a "metabolically active" pool of unesterified (free) cholesterol within the cell is regulatory. The location of this pool is not known, and indeed, cholesterol itself seemingly is not the regulatory sterol; instead, the regulator appears to be an oxygenated derivative of cholesterol. This oxygenated cholesterol presumably modifies the conformation of proteins adjacent to the promoter region of the LDL receptors and thereby suppresses transcription. Regardless of the exact mechanism, factors influencing the active pool of unesterified cholesterol largely determine LDL receptor synthesis. Since most of the body's LDL receptor activity is expressed by the liver, the regulation of the active pool of unesterified cholesterol in liver cells must be a major factor determining LDL receptor activity.

The various factors influencing the size of this pool are outlined in Figure 2. The pool of active free cholesterol is derived from newly synthesized cholesterol in the liver, hepatic uptake of lipoproteins (including chylomicron remnants), hydrolysis of cholesterol ester, and the inactive pool of free cholesterol. At the same time, active free cholesterol is depleted by incorporation into lipoproteins, esterification, transfer to the inactive pool, conversion into bile acids, or direct secretion into bile. The net result of these various inputs and exits determines the quantity of active free cholesterol, which in turn regulates the activity of LDL receptors (via formation of regulatory oxysterols).

Since hepatic cholesterol content influences LDL receptor synthesis, the balance of cholesterol across the liver cell is a key determinant of serum LDL cholesterol. Pathways of cholesterol into and out of the liver can be studied indirectly in humans by the cholesterol balance technique. The author participated in the development of this technique in the laboratory of E.H. Ahrens Jr., at Rockefeller University in the 1960s. This approach includes such measurements as fecal excretion of neutral steroids and bile acids and intestinal absorption of cholesterol. Whole-body synthesis of cholesterol equals...
the difference between intake of cholesterol and fecal excretion of neutral steroids and bile acids. In the steady state, excretion of bile acids equals the conversion of cholesterol into bile acids, that is, hepatic synthesis of bile acids. A method was later developed for estimating hepatic secretion rates of biliary lipids (cholesterol, bile acids, and phospholipids); this technique provides a more direct estimate of rates of exit of cholesterol and its conversion products from the liver. In the development of the cholesterol balance technique, several methods were devised for estimating cholesterol absorption, thus defining one pathway of cholesterol input into the liver.

It is interesting to compare parameters for cholesterol metabolism in humans with those of laboratory animals. Cholesterol synthesis has been measured both in smaller animals and in primates. On a per-kilogram basis, whole-body synthesis of cholesterol in humans is not especially high; indeed, it generally is lower than in most other species. In contrast to many animals that transform much of their cholesterol into bile acids, humans convert a relatively small fraction, that is, 30–40%. Consequently, relatively large quantities of cholesterol are secreted into bile in humans. Two adverse consequences follow a relatively low fractional conversion of cholesterol into bile acids: first, a high output of biliary cholesterol predisposes to cholesterol gallstones; and second, the enterohpatic circulation is enriched with cholesterol, suppressing LDL receptor synthesis. Because of the latter, serum levels of LDL cholesterol in humans are relatively high compared with those in most laboratory animals (Table 1).

In some animals (e.g., primates), the enterohpatic circulation of cholesterol can be expanded by feeding excess dietary cholesterol; as a result, serum LDL cholesterol levels rise to levels typically seen in humans. In other species, for example, the rat and dog, excess dietary cholesterol fails to raise serum cholesterol concentrations markedly because most of the newly absorbed cholesterol is converted rapidly into bile acids. The naturally sluggish conversion of cholesterol into bile acids in humans apparently is a "species defect" that leads to relatively high serum cholesterol levels, even in the absence of excess dietary cholesterol. Thus, compared with most other animals, humans in general reside in a perpetual state of "hepatic cholesterol overload," and high intakes of cholesterol are not required to produce relatively high serum cholesterol levels. This general conclusion represents a reasonable interpretation of cholesterol-balance data from humans compared with those of other species.

LDL receptor activity, however, is affected by factors other than hepatic cholesterol content. For example, thyroid hormone and estrogens stimulate LDL receptor synthesis, seemingly by acting on regulatory proteins in the cell nucleus. The fatty acid pattern of the diet also influences LDL receptor activity, as will be discussed later. Moreover, cellular transport of LDL receptors is relatively complex; thus, several factors could modify rates of receptor transport to the cell surface, receptor function within the cell membrane, or rates of recycling of receptors through the endocytotic process. Finally, the "fit" between LDL receptors and apo B (or apo E) may affect uptake rates of apo B-containing lipoproteins. These many factors thus may modify the "activity" of LDL receptors and hence the serum LDL cholesterol level.

### Hepatic Secretion of Apolipoprotein B–Containing Lipoproteins

The major apo B-containing lipoprotein secreted by the liver is VLDL. Steps in the formation of VLDL particles are incompletely understood, but a general sequence has been elucidated. Apo B-100 originates in ribosomes of the RER. Triglycerides and cholesterol esters are made by membrane-bound enzymes located in the smooth endoplasmic reticulum (SER). As apo B-100 and possibly apo E move toward the SER, they encounter these lipids 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; the latter pass through the SER to the Golgi apparatus, where secretory vesicles containing large numbers of nascent VLDL bud off and move to the surface of the cell for secretion into the circulation.

A variety of influences probably affect the number of VLDL particles secreted into serum. The rate of synthesis of apo B-100 may be one, although recent data suggest that a sizable portion of newly synthesized apo B normally is degraded before being recruited to VLDL formation; hence, the fraction of apo B-100 actually used may affect VLDL secretion more than does the absolute synthesis rate for apo B. The rate of recruitment of apo B into lipoprotein particles may be determined in part by lipid metabolism; for example, if more nonpolar lipids are produced, more particles may be formed and secreted. Still, whether increased neutral-lipid synthesis increases the total number of lipoprotein particles se-

<table>
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<tr>
<th>Species</th>
<th>Serum concentration (mg/dl)</th>
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<tr>
<td>Humans (4–20 yr)</td>
<td>75–90</td>
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<tr>
<td>Mouse</td>
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<td>Baboon</td>
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created into plasma or merely enriches a fixed number of particles with lipid remains to be determined.

One technique employed for estimating secretion rates for apo B-containing lipoproteins in humans has been isotope-kinetic studies. This approach has been developed extensively by investigators at the National Institutes of Health under Mones Berman.47 Two techniques for labeling may be used: 1) apolipoproteins on isolated lipoproteins can be labeled with radioiodine and reinjected48 or 2) labeled precursors can be injected intravenously, allowing apolipoproteins or lipids to be labeled "endogenously."49 A series of multicompartmental models have evolved to define metabolic parameters of apo B-containing lipoproteins obtained by these techniques. With these models, attempts have been made to estimate secretion rates for VLDL apo B. Since each lipoprotein particle contains only one apo B molecule, secretion rates of VLDL apo B should approximate the number of VLDL particles secreted into the circulation. A typical multicompartmental model used by Berman et al50 is presented in Figure 3; this model accounts for all of the components of isotope-kinetic curves for apo B-containing lipoproteins. It assumes that all apo B entering the circulation is subsumed within the compartments of the model. Similar approaches have been used by other investigators.51,52

Application of this approach suggested that some LDL entered the circulation independently of VLDL.53,54 The total calculated flux of LDL often exceeded LDL derived from VLDL, which raised the possibility that some LDL was secreted directly by the liver. It is possible, however, that this apparent "direct" secretion of LDL reflects rapid conversion of newly secreted VLDL into LDL, a process occurring too rapidly to be detected by typical isotope-kinetic curves.55 We support this latter possibility and thus, have modified the model shown in Figure 3 to include a compartment of nascent lipoproteins from which both VLDL and LDL are derived (Figure 4).56 The so-called "direct synthesis" of LDL in reality may be a very rapid conversion of some VLDL to LDL, to be distinguished from the slower input of LDL through the VLDL delipidation chain. If this mechanism pertains, lipoproteins probably can exit...
directly from the compartment of nascent lipoproteins as well, and if so, currently available isotope-kinetic techniques cannot provide accurate estimates of hepatic secretion rates of lipoproteins, but rather only rates of transformation of nascent lipoproteins into VLDL and LDL compartments.

**Conversion of Very Low Density Lipoprotein to Low Density Lipoprotein**

According to Figure 4, LDL comes from the degradation of triglyceride-rich lipoproteins, whether very rapidly from nascent lipoproteins or more slowly through the VLDL lipolytic cascade. Moreover, some particles are removed directly by the liver before conversion to LDL. In other words, triglyceride-rich lipoproteins undergo partitioning between hepatic uptake and transformation to LDL. Without question, some hepatic uptake occurs via LDL receptors, but other receptors (e.g., the putative apo E receptor) also may participate. The mechanisms for conversion of triglyceride-rich lipoproteins to LDL are not fully understood. LPL undoubtedly plays the major role in degrading VLDL to VLDL remnants, but whether LPL alone can convert the latter to LDL is not clear; more likely, HTGL also participates in this conversion. LDL receptors almost certainly are not required for formation of LDL because typical LDL particles occur in patients having a complete absence of LDL receptors.

The partitioning between hepatic uptake of remnant lipoproteins and conversion to LDL thus should affect LDL concentrations. Low levels of LDL cholesterol in many species, such as the rat, apparently reflect a high removal of VLDL before conversion to LDL. Humans, in contrast, convert a greater fraction of VLDL to LDL, leading to higher LDL levels. A relatively low activity of LDL receptors in humans in general, as discussed before, could retard remnant uptake, favoring conversion to LDL, but other factors, such as a low affinity of VLDL remnants for receptors or high activities of LPL and HTGL, also could enhance conversion to LDL. In any case, preferential conversion of VLDL to LDL over direct remnant uptake should raise LDL levels.

**Cholesterol Content of Low Density Lipoprotein Particles**

Amounts of cholesterol carried in lipoprotein particles are not constant. Measurement of the LDL cholesterol to apo B ratio yields the average cholesterol content of LDL particles because every LDL particle has only one apo B molecule. If the LDL cholesterol to apo B ratio is high, then the LDL cholesterol level will be high for a given LDL apo B level. Several factors theoretically could increase this ratio: 1) a high cholesterol ester transfer protein (CETP) activity; 2) a high lecithin:cholesterol acyltransferase (LCAT) activity; 3) increased cholesterol ester content of newly secreted lipoprotein particles; 4) selective clearance of cholesterol-poor LDL by LDL receptors, and 5) delayed clearance of LDL particles (allowing for prolonged action of LCAT and CETP in plasma).

**Definitions of Serum Cholesterol Levels**

Categories of serum total cholesterol according to the National Cholesterol Education Program (NCEP) are given in Table 2. Corresponding levels of LDL cholesterol and relative risk for CHD likewise are shown. The term "hypercholesterolemia" will be used here synonymously with "high serum cholesterol." Most people with hypercholesterolemia have a high-risk LDL cholesterol level, although there are exceptions. In this article, hypercholesterolemia will be restricted to an LDL cholesterol level exceeding 160 mg/dl. The term "moderate hypercholesterolemia" will be applied to LDL cholesterol concentrations in the range 160–209 mg/dl; this range corresponds to the 75th–95th percentile for adult Americans. "Severe hypercholesterolemia" is defined as an LDL cholesterol level of 210 mg/dl or higher, that is, greater than the 95th percentile. In the following, causes of borderline-high cholesterol, moderate hypercholesterolemia, and severe hypercholesterolemia will be examined.

**Causes of Borderline-High Cholesterol Levels**

About 40% of adult Americans have borderline-high levels of total cholesterol, that is, serum cholesterol in the range 200–239 mg/dl, or LDL cholesterol levels of 130–159 mg/dl. Relative risk for CHD averages 1.0 to 2.0 times that of individuals with desirable cholesterol levels (<200 mg/dl). Borderline-high levels thus contribute significantly to CHD in the United States. We therefore can inquire about causes of borderline-high serum cholesterol among American adults. Several factors have been identified as potential causes, as will be considered.

**Relatively High Background Levels of Cholesterol**

One factor contributing to borderline-high cholesterol levels in American adults is a relatively high background level for total and LDL cholesterol for humans in general, at least as compared with other species (Table 1). As estimated from available epidemiological data, a nonobese, 20-year-old man consuming a diet relatively low in saturated fatty
acids (<7% of total calories) and cholesterol (<200 mg/day) will exhibit a serum total cholesterol of about 140 mg/dl (with an LDL cholesterol level 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, for example, about 125–130 mg/dl, but this still lower level probably reflects borderline malnutrition, whereas the somewhat higher value, about 140 mg/dl, seems more representative of the baseline level.

Why do humans have higher background levels of serum cholesterol than do most other species? As indicated before, one reason appears to be a sluggish conversion of cholesterol into bile acids; this raises the hepatic content of cholesterol and thus suppresses LDL receptor activity (Figure 2). Other factors also may contribute to higher background levels, but they have not been identified.

**Dietary Cholesterol**

Another cause of borderline-high serum cholesterol is a high cholesterol intake. American men typically consume about 400 mg/day, whereas populations having low serum cholesterol levels generally take in less than 200 mg/day. According to available estimates, raising cholesterol intake from 200 to 400 mg/day will increase the serum cholesterol by an average of about 5 mg/dl. The mechanism appears to be suppression of LDL receptor synthesis secondary to increased hepatic cholesterol content. Since cholesterol intake does not change appreciably throughout adult life, its cholesterol-raising action should persist indefinitely.

**Dietary Saturated Fatty Acids**

Another dietary factor that increases serum cholesterol is a high intake of saturated fatty acids. Americans typically consume about 14% of total calories as saturated fatty acids, whereas a desirable intake is 7% or less. According to generally accepted data, the extra 7% of calories as saturates in the American diet increases serum cholesterol by approximately 20 mg/dl, most of which is LDL cholesterol. Available evidence indicates that saturated fatty acids also suppress LDL receptor activity, but the precise mechanism is unknown. Five possibilities for LDL receptor suppression are shown in Figure 5; dietary saturates could 1) inhibit cholesterol ester synthesis in the liver, 2) enhance transfer of inactive free cholesterol into the active pool, 3) promote formation of regulatory oxysterols, 4) reduce functional activity of LDL receptors at the cell surface, or 5) decrease affinity of LDL for LDL receptors.

Based on the evidence presented above, combined high intakes of cholesterol and saturated fatty acids in the American diet should raise serum total cholesterol (and LDL cholesterol) 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. Still, this relatively high intake of cholesterol-raising nutrients cannot explain the rise in serum cholesterol levels that occurs with aging (Figure 6) because intakes of these nutrients remain constant through the years. Instead, additional factors must account for this phenomenon.

**Weight Gain (Obesity)**

One of these additional factors may be increasing body weight with aging. American adults typically gain weight with increasing years, being on the average 20–30 pounds heavier at age 50 than at age 20. Several reports indicate that serum cholesterol levels rise because of weight gain. This increase seemingly averages about 25 mg/dl, and it occurs mainly in LDL although partly in VLDL. At least two metabolic effects may explain this rise. First, increasing obesity promotes hepatic output of apo B-containing lipoproteins, which in turn enhances input of LDL. And second, whole-body synthesis of cholesterol is increased by obesity, expanding the hepatic cholesterol pool and suppressing LDL receptor synthesis.
Grundy  Etiology of Hypercholesterolemia  1625

VLDL-apo B
Overproduction

Decreased LDL
Receptor Activity

INHERENT
DIETARY CHOLESTEROL
SATURATED FATTY ACIDS
AGING
LOSS OF ESTROGENS

FIGURE 7. Graph of factors responsible for the rise of serum total cholesterol levels (mg/dl) with age (years) leading to borderline-high serum cholesterol in American men and women. The factors in the two sexes are essentially the same except that loss of estrogens after menopause in women causes the mean total cholesterol to reach higher levels.

Aging Per Se

Not all of the rise of cholesterol with age can be explained by weight gain, however. For example, Miller79 reviewed available data from LDL turnover studies performed in several laboratories on individuals of different ages; he concluded from these studies that part of the rise of LDL levels with aging is due to decreased activity of LDL receptors. Subsequently, a study from our laboratory80 showed that fractional catabolic rates (FCRs) for LDL decrease with aging, supporting the concept that LDL receptor activity declines with age. This finding has been confirmed recently by Ericsson et al.81 The precise mechanism for an age-related fall in LDL receptor activity is unknown. As indicated before, overproduction of whole-body cholesterol due to obesity could be one but not the only factor. One report82 indicates that synthesis of bile acids declines with age; this could increase hepatic cholesterol content and further suppress LDL receptors (Figure 2). Other events related to cellular aging may occur, or the body's overall metabolic rate could decline. When available data are taken into account, obesity-independent factors appear to 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 45–50 years, and then it rises above that of men (Figure 6).1 This postmenopausal increment in cholesterol levels probably can be explained largely by loss of estrogens; as noted before, estrogens enhance LDL receptor activity, both in animals85 and in humans.10,84 The postmenopausal rise in total cholesterol levels in American women averages about 20 mg/dl.1

The major factors responsible for borderline-high cholesterol levels and their average contributions are outlined in Figure 7; suggested mechanisms for each factor are summarized in Figure 8. Several of these factors apparently suppress LDL receptor activity, namely 1) an inherently slow conversion of cholesterol into bile acids, typical of humans in general; 2) a high cholesterol intake; 3) a high intake of saturated fatty acids; 4) obesity, leading to high cholesterol synthesis; 5) aging per se; and 6) loss of estrogens (in postmenopausal women). In addition, obesity stimulates overproduction of VLDL, resulting in a high input of LDL.

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 (Figure 7), enhancing risk for CHD between one- and twofold.1 In postmenopausal women, the increment is even greater because of the loss of estrogen-stimulated increase in LDL receptor activity. If borderline-high cholesterol levels could be prevented in the general public, the prevalence of CHD in the United States should be reduced substantially. Since the causes of borderline-high cholesterol levels are multiple, several steps will be necessary to mitigate these higher levels. In postmenopausal women, for example, estrogen repletion should raise LDL receptor activity. Causes of the decline in LDL receptor activity with aging per se are unknown, so this effect cannot be mitigated at present. On the other hand, the weight gain–related rise of cholesterol with aging can be prevented by avoiding overweight. Reducing intakes of saturated fatty acids and cholesterol should further decrease cholesterol levels. Overall, the current composition of the American diet, together with gain in weight with aging, accounts for an increase in total cholesterol of about 50 mg/dl, and this
increment could be eliminated by appropriate dietary change.

Since the recommended dietary change includes decreased intake of saturated fatty acids, the question must be asked which nutrients can be used in their place. If one is overweight, foods rich in saturated fatty acids (e.g., fat-rich dairy products and meats) can be eliminated altogether without replacement, and the benefit of weight reduction will be obtained. However, even after weight reduction and for those who are not overweight, the diet still will contain more saturated fatty acids than desirable; consequently, other nutrients must be considered as substitutes for saturated fatty acids.

What might these be? The foremost candidates are polyunsaturated fatty acids, monounsaturated fatty acids, and carbohydrates. During the 1960s and 1970s, many investigators favored polyunsaturates because of the studies of Keys et al.66 and Hegsted et al.67 These workers indicated that carbohydrates and monounsaturated fatty acids, the latter being essentially oleic acid, are “neutral,” that is, they neither raise nor lower total cholesterol levels. In comparison, saturated fatty acids raise the cholesterol concentration, whereas polyunsaturates, predominantly linoleic acid, lower 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 by introducing “polyunsaturated” vegetable oils for cooking oils, margarines and shortenings, and as ingredients in other food products.

In the late 1970s, however, some investigators became concerned that the high intakes of linoleic acid might not be entirely safe. 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 carcinogenesis in humans, as it does in laboratory animals.85 Recent epidemiologic evidence seems to support this possibility.86 Animal studies further revealed that dietary linoleic acid can suppress the immune system,87 a possible mechanism for cancer promotion. High intakes of linoleic acid will lower HDL cholesterol levels,88,89 and they also may increase the risk for cholesterol gallstones.90 Recent studies91,92 even suggest that enrichment of LDL particles with linoleic acid may predispose LDL particles to oxidation within the arterial wall, a possible atherogenic response. Thus, even if linoleic acid is more “hypocholesterolemic” than other nutrients, these several concerns have led many authorities to the position that high intakes of linoleic acid should be avoided. Intakes above 7% of total calories seemingly cannot be advocated with prudence.93

Concerns about the safety of dietary linoleic acid led Fred Mattson and me to consider other replacements for saturated fatty acids. Certainly, carbohydrates are one alternative, and high-carbohydrate, low-fat diets are widely advocated. They have epidemiological support in that such diets commonly are consumed by populations having a low rate of CHD.94 On the other hand, very-low-fat diets generally are not eaten voluntarily but rather out of necessity. When fat for the diet becomes available and affordable, most populations increase their fat intake. In Mediterranean countries, moreover, where olive oil is consumed in large amounts, total fat intake is relatively high, and yet age-adjusted rates of CHD and even total mortality are low.95,96 Since olive oil contains mainly oleic acid, we reexamined the effects of oleic acid on serum cholesterol, particularly on cholesterol levels in the different lipoprotein fractions.

In a direct comparison with palmitic acid, both oleic and linoleic acids in the diet had the same effect on LDL cholesterol levels, that is, both appeared to be neutral and did not raise these levels, whereas palmitic acid clearly increased the LDL cholesterol concentration.90 In contrast to dietary linoleic acid, which lowered HDL cholesterol, oleic acid maintained a constant HDL level. Thus, part of the effect of linoleic acid on total cholesterol levels appears to be due to its action to reduce HDL cholesterol levels. Several other reports97-99 later confirmed that oleic and linoleic acids have essentially identical effects on LDL cholesterol levels. Furthermore, none of the adverse effects listed for linoleic acid in Table 3 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, is oleic acid preferable to carbohydrate as a replacement? From epidemiological studies, carbohydrates and monounsaturates appear to have similar effects on CHD risk. In the studies of Keys et al.66 and Hegsted et al.67 both nutrients similarly affected total cholesterol levels. We have confirmed this identity for LDL levels.100,101 Several metabolic studies, however, have shown that in contrast to high-oleic-acid diets, high-carbohydrate diets lower HDL cholesterol concentrations and raise triglyceride levels. These effects of high carbohydrate intakes have been confirmed in epidemiological studies,102 suggesting that the theoretically adverse actions of carbohydrates on HDL and triglycerides are long lived. Still higher intakes of total fat, even if they consist mainly

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**TABLE 3. Potential Disadvantages of High Intakes of Polyunsaturated Fatty Acids (Linoleic Acid)**

- No epidemiological support
- Cancer promotion (animals) (implicated in human cancer)
- Suppression of immune system (animals)
- Lowering of HDL cholesterol (higher intakes)
- Increased risk for cholesterol gallstones
- Promotion of LDL oxidation

HDL, high density lipoprotein; LDL, low density lipoprotein.
of oleic acid, may have drawbacks. Higher-fat diets may promote weight gain and have been implicated in the development of cancer, although a recent review of epidemiological studies seems to exonerate oleic acid from this latter effect. More studies thus will be required to determine whether carbohydrate or oleic acid is the preferable replacement for saturated fatty acids. In truth, both probably are acceptable, and their use together should allow for more variety in cholesterol-lowering diets.

The possibility of greater dietary variety is enhanced further by our recent confirmation that dietary stearic acid does not increase cholesterol levels. Previous studies had raised this possibility, and our confirmation means that saturated fatty acids do not invariably increase cholesterol levels. Indeed, prior investigations have also shown that medium-chain saturated fatty acids are not hypercholesterolemic. The cholesterol-raising saturates thus appear to be limited to three: lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0). The failure of several saturated acids to raise cholesterol levels expands the potential for more variety to the diet.

**Causes of Primary Moderate Hypercholesterolemia**

Primary moderate hypercholesterolemia is here defined as a total cholesterol level in the range of 240–289 mg/dl (LDL cholesterol of 160–209 mg/dl). Most patients with moderate hypercholesterolemia have all of the acquired causes of borderline-high cholesterol described above. To have still higher cholesterol levels, genetic abnormalities probably are required. In most cases, 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 are responsible for raising the LDL cholesterol level from the borderline range to the moderately elevated range. These categories are based largely on a recent study by Vega et al, in which LDL turnover measurements were performed in 108 middle-aged men with primary moderate hypercholesterolemia, in 16 men with borderline-high cholesterol levels, and in 14 men with heterozygous familial hypercholesterolemia (FH). Patients with concomitant hypertriglyceridemia were excluded from this study. The abnormalities responsible for moderate hypercholesterolemia in these patients will be examined.

**Low Clearance Rates for Low Density Lipoprotein**

These LDL turnover studies revealed that some patients with primary moderate hypercholesterolemia had unusually low clearance rates (low FCRs) for LDL compared with those subjects with borderline LDL cholesterol levels (Figure 9). Two possible mechanisms might explain these unusually low FCRs (Figure 10). First, a portion of the patients may have an excessive depression in LDL receptor activity, beyond that typically occurring with borderline-high levels, and second, other patients may possess LDL particles that bind poorly to LDL receptors. To distinguish between these two mechanisms, we performed a study in which the patients' own LDL particles (autologous LDL) were labeled and reinfected with labeled, normal (homologous) LDL. Two different isotopes of iodine—125I and 131I—were used to label the two forms of LDL. We hypothesized that if both isotopes disappeared slowly but at the same rates, then the patient's own LDL was normal and LDL receptor activity was reduced. However, if the patient's own LDL disappeared at a slow rate but the normal LDL disappeared at a normal rate, then the patient's LDL was defective and was bound poorly to LDL receptors.

Figure 11 shows the results of one such study. In this patient, both forms of LDL decayed at the same rate; hence, this patient was considered to have a low LDL receptor activity and normal LDL particles. The former might have had several origins. For example, the patient could have a mild form of heterozygous FH manifested by only moderately high LDL levels. Several instances of heterozygous FH with only moderately high LDL levels have been reported recently; such patients presumably have mitigating factors that prevent severe hypercholesterolemia. Alternatively, a low LDL receptor activity might reflect defective regulation of LDL receptor synthesis. Such could result from excessive sensitivity to dietary cholesterol or saturated fatty acids, beyond the typical response. An abnormality of this type might reside in the gut (hyperabsorption of cholesterol) or in the liver (excessive sensitivity to suppressive nutrients). Indeed, some individuals appear to be unusually sensitive to the cholesterol-raising action of saturated fatty acids, which leads to hypercholesterolemia.

Figure 12 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; the patient's LDL thus appeared to be abnormal. To explore this possibility, we sought the collaboration of Robert Mahley and coworkers in San Francisco. As a result, Thomas Innerarity and associates\textsuperscript{114} found that this patient's LDL bound poorly to LDL receptors in tissue culture, and Karl Weisgraber and coworkers\textsuperscript{115} noted that it reacted abnormally with a single antibody targeted to the receptor-binding domain of the LDL receptor. These findings confirmed that the patient's LDL was abnormal. Further studies in Bryan McCarthy's laboratory\textsuperscript{116} identified the abnormality as a glutamine-to-arginine transformation at position 3,500 of apo B-100. This same abnormality was identified in other relatives of the patient. Subsequently, the "3,500 defect" has been found in several other families, suggesting that familial defective apo B-100 (3,500 mutation) is responsible for many cases of hypercholesterolemia.\textsuperscript{117}

In the course of our turnover studies,\textsuperscript{107} other patients were identified in whom autologous LDL decayed more slowly than homologous LDL. These patients do not have the 3,500 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, abnormalities that remain to be elucidated.

Increased Input of Low Density Lipoprotein

Another potential cause of moderate hypercholesterolemia is an increased input of LDL, that is, an

\begin{figure}
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\includegraphics[width=\textwidth]{figure10}
\caption{Diagrams of two potential mechanisms for the low fractional catabolic rates for low density lipoprotein (LDL) in patients with primary moderate hypercholesterolemia (see Figure 9). The possibilities are a moderate (~25%) reduction in LDL receptor activity (panel A) and a defective apolipoprotein (apo) B-100, resulting in a failure of LDL to interact normally with LDL receptors (panel B). See the legend to Figure 1 for additional details and explanation of abbreviations.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure11}
\caption{Simultaneous isotopic-decay curves of two labeled low density lipoprotein (LDL) particles in a patient (L.S.) with primary moderate hypercholesterolemia. The two decay curves, which die away at similar rates, consist of the patient's own LDL (O) and that of a normal subject (\textbullet). FCR, fractional catabolic rate.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure12}
\caption{Simultaneous isotopic-decay curves of two labeled low density lipoprotein (LDL) particles in a patient (G.R.) with primary moderate hypercholesterolemia. The two decay curves consist of the patient's own LDL (O) and that of a normal subject (\textbullet). The patient's own labeled LDL decays at a much slower rate than that of the normal subject. FCR, fractional catabolic rate.}
\end{figure}
increased conversion of VLDL to LDL. Three possible mechanisms are shown in Figure 13. One cause of high LDL input is a reduced activity of LDL receptors, as observed most dramatically in patients with homozygous FH9118; in this case, fewer VLDL particles are removed by hepatic LDL receptors, and hence more are converted to LDL (Figure 13A). Overproduction of LDL should be accompanied by a low FCR for LDL, as indeed occurs in patients with FH118 (Figure 14). Second, there could be an overproduction of apo B-containing lipoproteins by the liver (Figure 13B); with this defect, 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, that is, direct hepatic removal of VLDL (and VLDL remnants) is reduced, allowing for a greater conversion of VLDL to LDL (Figure 13C). In this case, availability of LDL receptors for clearance of LDL should be increased because receptors would be less loaded with VLDL particles; hence, the FCR for LDL should be relatively high. Even so, high levels of LDL should be present because VLDL particles have greater affinity for LDL receptors than does LDL itself, and the relatively slow uptake of LDL by receptors should lead to an increase in LDL concentrations.107 These latter two patterns of LDL kinetics have been observed in our patients with moderate hypercholesterolemia (Figures 15 and 16), and we might speculate about their causes.

We postulate that increased input of LDL associated with low-normal FCRs for LDL is most consistent with an overproduction of VLDL apo B, with increased conversion of VLDL to LDL on that basis (Figure 13B). This abnormality may be one mechanism whereby obesity contributes to borderline-high cholesterol levels (Figure 7). A still greater increment in VLDL apo B input may be the result of genetic hypersensitivity to obesity. Whether primary hypersecretion of VLDL apo B, independent of obesity, truly exists has not been determined, but it has been postulated to be the underlying defect of familial combined hyperlipidemia.119,120 Even if the

**FIGURE 13.** Diagram of possible mechanisms for an increased input of low density lipoprotein (LDL) apolipoprotein (apo) B. These include (A) reduced activity of LDL receptors (with enhanced conversion of very low density lipoprotein [VLDL] to LDL), (B) hepatic overproduction of VLDL apo B, and (C) decreased hepatic clearance of VLDL, with increased conversion of VLDL to LDL.

**FIGURE 14.** Bar graphs of kinetic parameters for low density lipoprotein (LDL) in 22 adults with heterozygous familial hypercholesterolemia (FH) compared with 14 normal middle-aged men. The high levels (mg/dl) of LDL cholesterol and LDL apolipoprotein (apo) B in FH patients are due to both increased input rate (mg/kg·d) and decreased fractional catabolic rate (FCR) for LDL (pools/day) (see Figure 13A).
The input of LDL apo B has been reported to be abnormally high in familial combined hyperlipidemia and the related condition called hyperapobetalipoproteinemia (hyperapo B). These disorders are often characterized by LDL particles having an unusually low cholesterol to apo B ratio (1.25 or less). We observed that eight of 52 patients (14%) with a high input of LDL (with or without high FCRs) had an LDL cholesterol to apo B ratio of less than 1.25 and hence could be classified as having hyperapo B. None of the 52 patients had hypertriglyceridemia, but nonetheless, we postulate that their hypercholesterolemia was largely secondary to defects in metabolism of VLDL, either to increased input or to decreased removal (Figures 13B and 13C). Thus, if these defects were inherited, other family members could have elevated VLDL levels, which is consistent with the diagnosis of familial combined hyperlipidemia. Thus, it is likely that at least some of the patients with high LDL input would have been classified as having familial combined hyperlipidemia had full family studies been done.

Enrichment of Low Density Lipoprotein With Cholesterol Ester

A final mechanism whereby LDL cholesterol levels may be raised from the borderline zone to moderately elevated is by enrichment of LDL particles with cholesterol ester (Figure 17). This abnormality is revealed by an increase in the LDL cholesterol to apo B ratio, and it was observed commonly in our patients with moderate hypercholesterolemia (Figure 18). The LDL cholesterol to apo B ratio in 37 patients of this type averaged 1.62±0.17 (mean±SD) compared with a ratio of 1.42±0.12 for 16 subjects with borderline-high cholesterol. In none of the other groups with moderate hypercholesterolemia was the average ratio high. The mechanism for an increase in the LDL cholesterol to apo B ratio is unknown, but it may be related to one of the factors influencing the cholesterol ester content of LDL, for example, LCAT, CETP, residence time of LDL, or the cholesterol content of newly secreted lipoproteins. Most
but for patients who have established CHD, a greater line zone by dietary therapy alone may be sufficient, if the desirable range is the goal of therapy, genetic defects in LDL metabolism, a reduction of LDL levels to the desirable range will not be possible. In the others who have moderate or polygenic hypercholesterolemia, a decrease of cholesterol levels to the borderline zone 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 moderate hypercholesterolemia on the basis of excessive sensitivity to diet. A decrease of cholesterol levels to the desirable range will not have the power to 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 borderline or desirable cholesterol levels. This is because multiple metabolic defects must be overcome. The combination of a bile acid sequestrant with either nicotinic acid or a hydroxymethylglutaryl coenzyme A reductase inhibitor appears to be adequate therapy in most patients with severe hypercholesterolemia.

Treatment of Moderate Hypercholesterolemia

The NCEP recommended that the first line of treatment for moderate hypercholesterolemia be dietary therapy, that is, reduction in the intake of saturated fatty acids, cholesterol, and total energy consumption. Based on our previous discussion, these dietary changes should decrease total cholesterol levels by approximately 50 mg/dl; such a change would 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 moderate hypercholesterolemia on the basis of excessive sensitivity to diet; in these patients, dietary therapy, that is, reduction in the intake of saturated fatty acids, cholesterol, and total energy consumption, appears to be adequate therapy in most patients with moderate hypercholesterolemia. Although dietary therapy is indicated as an adjunct for management of severe hypercholesterolemia, it generally will not have the power to 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 borderline or desirable cholesterol levels. This is because multiple metabolic defects must be overcome. The combination of a bile acid sequestrant with either nicotinic acid or a hydroxymethylglutaryl coenzyme A reductase inhibitor appears to be adequate therapy in most patients with severe hypercholesterolemia.
Summary
This review underlines the concept that multiple factors are responsible for hypercholesterolemia in the American public. Dietary factors (cholesterol, saturated fatty acids, and obesity) clearly raise the cholesterol level, and they 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 synthesis after menopause is an important contributor to elevated cholesterol in postmenopausal 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 heterozygous FH, most cases of severe hypercholesterolemia appear to be the result of the coexistence of at least two defects in LDL metabolism, and as a rule, they can be treated successfully only by using cholesterol-lowering drugs in combination.

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