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ABSORPTION AND METABOLISM OF DIETARY CHOLESTEROL

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For many years the metabolism of cholesterol has been a subject of great interest. Cholesterol is required for normal cell function. It is the precursor of steroid hormones and bile acids. It is also a major constituent of atherosclerotic plaques and most gallstones. Much has been learned about cholesterol metabolism in both animals and man during the last 20 years. Research nevertheless has shown that the metabolism of cholesterol varies from one species to another. Results from animal studies consequently cannot always be extrapolated to man. For this reason, many important questions remain to be answered for both the metabolism of cholesterol and the role of cholesterol in the pathogenesis of atherosclerosis and cholelithiasis. Some of the questions specifically related to the role of dietary cholesterol are examined in this review.

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CHOLESTEROL ABSORPTION

Cholesterol enters the intestinal tract from two sources—the diet and bile. The intestinal mucosa also may contribute a small amount. Intakes of cholesterol in American adults average from 400 to 500 mg/day; biliary outputs of cholesterol range from 800 to 1200 mg/day in normal adults (35). Biliary cholesterol is entirely unesterified, but a portion of dietary cholesterol may be esterified with fatty acids. Any cholesterol ester entering the intestine is deesterified rapidly by pancreatic cholesterol esterase (81). Before absorption, cholesterol must be solubilized by micelles containing conjugated bile acids and hydrolytic products of triglycerides and lecithin—fatty acids, monoglycerides, and lysolecithin (43, 44). Unesterified cholesterol is solubilized in the hydrophobic center of micelles. Bile acids are crucial for cholesterol absorption (73). Micelles also promote cholesterol absorption by facilitating transport across the unstirred layer of water adjacent to the surface of the luminal cell (84). This transport occurs by simple diffusion. Movement of cholesterol through the unstirred layer, rather than the penetration of the microvillus membrane, appears to be the rate-limiting step for its absorption. The micelle as a whole does not penetrate the cell membrane. Passage of cholesterol through the structured lipid of the membrane occurs by monomolecular passive diffusion. Although the above generalizations reflect a greatly increased knowledge of cholesterol absorption, several important questions remain unanswered about quantitative aspects of absorption in man.

How Much Dietary Cholesterol is Absorbed in Man?

The proportion of dietary cholesterol actually absorbed in humans has been a subject of considerable dispute (38). This uncertainty is the result of several factors, not the least of which is methodology. Most methods for estimating cholesterol absorption use isotopic tracers. One technique is to estimate the amount of radioactivity entering plasma following oral administration of labelled cholesterol (89). Another determines the amount of unabsorbed label excreted in feces (5, 14, 64, 74). Early estimates using tracer methods suggested that only about 30% of dietary cholesterol is absorbed (34, 37). More recent estimates generally are higher (14). The use of tracers unfortunately presents several problems. For example, the low values obtained in earlier studies may have been due to failure to get the tracer into the proper physical state for absorption.

Another approach to measurement of cholesterol absorption is to use an intestinal perfusion technique (37). Absorption estimated by these methods often is in the range of 60–80% (37, 61). Absorption of dietary cholesterol thus may be greater than previously thought. Nonetheless, we are uncertain because an entirely satisfactory method for determining the absorption of cholesterol in the usual American diet has not been developed.

Are Endogenous (Biliary) and Exogenous Cholesterol Absorbed to the Same Extent?

Dietary cholesterol has received particular attention because it is thought to increase plasma concentrations of cholesterol, which in turn may accelerate atherosclerosis. In this regard, however, biliary cholesterol should not be overlooked. Inputs of biliary cholesterol are about twice those of dietary cholesterol (35). Once biliary cholesterol passes into the gut it should have the same potential for raising plasma cholesterol as does dietary cholesterol. Biliary cholesterol actually may be absorbed better than dietary cholesterol. Intestinal perfusion studies have shown that absorption of endogenous cholesterol is frequently in the range of 60–80% (37, 61). Absorption of dietary cholesterol is usually reported to be lower (33, 34). If endogenous and exogenous cholesterol are absorbed differently, the difference might be explained by their physical states. Biliary cholesterol enters the intestine in micellar solution (43, 44). Ingested cholesterol is mostly dissolved in dietary triglycerides. To be absorbed, dietary cholesterol must be transformed into a micellar state. This extra step may not be 100% efficient; if it is not, absorption of exogenous cholesterol would be less than that of endogenous cholesterol.

What Causes Variability of Cholesterol Absorption in Normal People?

Estimates of percentage absorption of dietary cholesterol vary from person to person. Isotopic tracer measurements reveal rates varying from 20–80%. Reasons for this variability are unclear. Several factors could decrease real or apparent absorption of cholesterol. Since bile acids are required for cholesterol absorption (73), a relatively low pool size of bile acids could decrease absorption. Any increase in rate of intestinal transit should decrease time available for absorption. A reduction in secretion of pancreatic lipase would leave a larger oil phase to take up cholesterol from the micellar phase. The type of diet ingested could be a factor. A low-fat diet may reduce the quantity of fatty acids available for formation of mixed micelles. Certain vegetable products can interfere with absorption of cholesterol. Perhaps of greatest importance is the intestinal mucosa's capacity to esterify cholesterol; any deficiency in the esterifying enzyme in the mucosa should reduce the amount of cholesterol absorbed.

Finally, biliary cholesterol undoubtedly competes with exogenous cholesterol for absorption (37). Input rates of biliary cholesterol are variable. Higher inputs should reduce absorption of exogenous cholesterol. If so, only absorption of exogenous cholesterol, not absolute absorption—i.e. endogenous + exogenous cholesterol—would be reduced. None of the above possibilities has been studied systematically.

How Does Cholesterol Absorptive Capacity Relate to Plasma Cholesterol Concentrations?

One factor determining the plasma cholesterol level may be the intestinal absorptive capacity for cholesterol. By increasing dietary cholesterol, plasma cholesterol can be made to rise in most people. An increase of about 10% is typical (3, 6, 10, 12, 42, 47, 58). These changes are more likely to be seen in metabolic ward studies than in people on ad libitum diets. In contrast, blocking cholesterol absorption by a variety of means lowers plasma cholesterol. Reductions of 10–15% are common (32). These findings imply that changing absorption of both exogenous and endogenous cholesterol, from minimum to maximum amounts, can alter the plasma cholesterol by as much as 25%.

Since changes in absorption can affect cholesterol concentrations, we might ask whether individual variability in absorption of cholesterol affects plasma cholesterol. Previous studies have shown that patients with familial hypercholesterolemia do not overabsorb cholesterol (12). These patients, however, have a genetic disorder of lipoprotein metabolism unrelated to dietary cholesterol. Other types of patients with high plasma cholesterol could have increased absorptive capacity. A low cholesterol concentration likewise might be due to reduced absorption. An example of the latter could be American Indians, who have both a low plasma cholesterol and apparently a low percentage absorption (61).

Do Plasma Cholesterol Concentrations Depend Only on Dietary Cholesterol Intakes in the Range of 0–500 mg Per Day?

Several reports suggest that the maxmum rise in plasma cholesterol in response to dietary cholesterol is reached at cholesterol intakes of 400–500 mg/day (3, 6, 10, 11, 42, 47, 58). These reports imply that the plasma cholesterol is exquisitely sensitive to changes in dietary cholesterol at low cholesterol intakes but not at higher intakes. A mechanism for this effect is difficult to visualize. At low levels of dietary cholesterol the ratio of biliary-to-dietary cholesterol is high. Relatively small changes in dietary cholesterol should not alter this ratio markedly. More studies are needed to determine the response in plasma cholesterol over a wide range of cholesterol intakes.

Are Some Patients Responders and Others Nonresponders to Dietary Cholesterol?

Studies on cholesterol feeding in primates have indicated that within the same species some animals respond to dietary cholesterol with an increase in plasma cholesterol; others do not (19, 20, 21, 54). This finding has fostered the concept that some humans are responders and others are nonresponders to dietary cholesterol. This concept has some basis from human studies (1, 24a, 59a, 61a). The idea has clinical implications: Testing might distinguish those who

should from those who need not decrease their intake of cholesterol. Not all investigations in man, however, support the concept of a widely variable response. Mattson (58) examined effects of increasing intakes of dietary cholesterol on plasma concentrations in a group of young male volunteers. Essentially all the subjects responded with increases in plasma cholesterol, and the degree of increase was remarkably uniform. This study thus casts some doubt on the hypothesis that some normal humans are responders while others are not. Whether a similar uniformity in response to dietary cholesterol is present in patients with different types of hyperlipidemia remains to be determined.

Does a Limited Absorption of Dietary Cholesterol Protect Against Severe Hypercholesterolemia in Humans?

In many animal species, the feeding of large amounts of cholesterol results in severe hypercholesterolemia. In chickens, rabbits, and even some primates, concentrations of cholesterol can exceed 1000 mg/dl. This does not occur in man. Only small rises at most can be induced in human subjects. Can the lack of a great rise in cholesterol concentrations be explained by a limited absorptive capacity, as some have suggested? This intriguing concept remains to be proven. Comparative studies on the maximum absorptive capacities of humans and other animal species have not been carried out. Although species differences in absorptive capacity could be a factor, other factors, particularly those related to clearance of lipoproteins, may be more important. Rats, for example, appear to have the ability to absorb large quantities of cholesterol, and yet they are resistant to hypercholesterolemia (82). They seem to be protected by other mechanisms. It is interesting to speculate on the response in plasma cholesterol concentrations that would occur in man if cholesterol absorption could be increased to several grams per day.

One possible explanation for the apparent upper limit in response of plasma cholesterol to dietary cholesterol is that absorptive capacity is "saturated" at relatively low intakes. Although this is an attractive idea, the question of maximal absorptive capacity has not been studied adequately. One study showed that up to 1 g of dietary cholesterol can be absorbed per day (65). Another indicated that percentage absorption is not reduced markedly by raising dietary intake (5). Thus, the concept of saturation as it pertains to absorption of dietary cholesterol cannot be accepted without further investigation.

Why Are Plant Sterols so Poorly Absorbed?

The differential in absorption rates of cholesterol and plant sterols is remarkable. Only a few milligrams of beta sitosterol are absorbed daily (68). This sterol differs from cholesterol only by the presence of an ethyl group on the side

chain. Campesterol, which has a side-chain methyl group, also is absorbed poorly. The differences in absorption between cholesterol and plant sterols almost certainly cannot be explained by micellar solubility. Passive diffusion into the mucosal cell probably is similar for the two classes of sterols. The most likely difference lies in rates of esterification before incorporation into chylomicrons. Plant sterols may be poorly esterified by mucosal acylCoA:cholesterol acyl transferase (ACAT). Whether intestinal ACAT is specific for cholesterol is unknown.

The probability that enzyme specificity is responsible for differences in absorption of cholesterol and plant sterols is enhanced by recent discovery of a disorder called hyperbetasitosterolemia (4). This disease is characterized by a marked increase in absorption of sitosterol, elevated plasma sitosterol, tendon xanthomas, and possible premature atherosclerosis. A likely mechanism for this response involves loss of ACAT specificity, but no data are available at present to prove this hypothesis.

Do High-Cholesterol Diets Promote Development of Cancer of the Colon?

One major hypothesis suggests that colon cancer can result from excess cholesterol in the diet (52, 53, 88). Certain types of steroids are known to be carcinogenic. Dietary cholesterol might theoretically be responsible for cancer in two ways: *(a)* Bacteria in the large intestine convert unabsorbed dietary cholesterol into two other neutral steroids—coprostanol and coprostanone (59). *(b)* Cholesterol that is absorbed may enhance formation of bile acids in the liver; the bile acids are transformed into a host of acidic steroids in the colon (31). It has been postulated that the apparent relationship between a low plasma cholesterol and cancer may be related to increased excretion of endogenous cholesterol or enhanced conversion of cholesterol into bile acids (63a). Whether newly absorbed dietary cholesterol actually enhances the synthesis of bile acids is discussed below. The carcinogenicity of fecal neutral and acidic steroids has not been proven. The hypothesis is attractive, but it remains nothing more than an hypothesis. Little progress has been made in the last decade towards resolving this issue.

CHYLOMICRONS

Most dietary cholesterol is absorbed with chylomicrons. These lipoproteins contain mainly triglycerides that have been produced by the intestinal mucosa from newly absorbed fat. They are secreted into intestinal lymph and enter the blood stream through the thoracic duct. As chylomicrons pass into the peripheral circulation they come in contact with an enzyme, lipoprotein lipase, located on the surface of capillary endothelial cells. The triglycerides undergo hydrolysis with release of free fatty acids at this site. After lipolysis is almost

complete, chylomicron "remnants" are released back into the circulation and are cleared rapidly by the liver.

What is the Fate of Chylomicron Cholesterol?

It has been generally accepted that during interaction of chylomicrons with peripheral lipoprotein lipase, triglycerides are hydrolyzed to fatty acids and glycerol, and cholesterol remains with the particle throughout lipolysis. If this is the case, cholesterol stays with the chylomicron remnant and is removed from the circulation by the liver. Remnants are known to be rich in cholesterol, which would support this mechanism. There are, however, other possibilities.

Chylomicrons contain both unesterified and esterified cholesterol. It has been postulated that unesterified cholesterol, located in the surface coat of these particles, may be released during lipolysis. If so, the surface cholesterol may be taken up by high-density lipoproteins (HDL) (79). The fate of the cholesterol ester is another question. Lipoprotein lipase does not contain cholesterol esterase activity, nor has this latter enzyme been found in association with lipoprotein lipase. This suggests that removal of cholesterol ester from chylomicrons does not occur in peripheral tissues. Proof on this point, however, is lacking. The potential importance of this issue is revealed in the following question.

Is Chylomicron Cholesterol Atherogenic?

This question really has two parts. First, we might inquire whether chylomicrons can enter the arterial wall directly, as can low–density lipoproteins, and deposit their cholesterol in the subintimal region. Second, during lipolysis of chylomicrons at the arterial endothelial surface, is cholesterol released that enters the arterial wall? Neither of these questions has been resolved, but both stimulate interesting speculation.

There is little evidence that chylomicrons are directly atherogenic. These lipoproteins are large and probably penetrate the arterial endothelium poorly. The best evidence that chylomicrons per se are not atherogenic comes from patients who have a congenital deficiency of lipoprotein lipase. These patients have extreme elevations of chylomicrons and yet are relatively immune from atherosclerotic disease (25). If chylomicrons are directly atherogenic, these patients should have marked atherosclerosis.

Under certain circumstances, however, chylomicrons might become atherogenic. If loss of arterial endothelium occurs, chylomicrons might penetrate the artery wall. An example could be patients with diabetes mellitus. These patients have increased atherosclerosis and frequently have chylomicronemia (7). If diabetes causes endothelial damage, chylomicrons might penetrate the arterial wall and thereby contribute to atherosclerosis.

Zilversmit (90) has proposed another mechanism whereby chylomicron cholesterol could be atherogenic. He has suggested that chylomicrons may

attach to the endothelial surface of the arterial wall; during lipolysis of triglycerides by lipoprotein lipase, some of the cholesterol contained within the chylomicron may be released and make its way into the subintimal region. The release of cholesterol during lipolysis has been documented by Fielding (22). This is an intriguing hypothesis, but evidence to support its importance for human atherosclerosis is virtually nonexistent.

Are Chylomicron Remnants Atherogenic?

Even if chylomicrons themselves do not contribute to atherosclerosis, chylomicron remnants could be atherogenic. For instance, Ross & Zilversmit (66) have shown that a major fraction of cholesterol in plasma of cholesterol-fed rabbits is associated with chylomicron remnants. Uptake of chylomicron remnants by the liver in rabbits seems to be delayed when they contain large amounts of dietary cholesterol. Since atherosclerosis can be induced by cholesterol feeding in rabbits, the chylomicron remnant could be an important atherogenic lipoprotein. Whether chylomicron remnants are atherogenic in man is another question. It has not been shown that feeding large amounts of cholesterol in man is associated with delayed clearance of chylomicron remnants. Concentrations of chylomicron remnants in man are normally low because of the great efficiency of the liver in their removal (36). This rapid clearance of remnants could be an important protective mechanism for the prevention of atherosclerosis in man. The possibility nevertheless must be kept open that exposure of the arterial wall to small amounts of chylomicron remnants for prolonged periods could cause deposition of cholesterol in atherosclerotic plaques.

HEPATIC CHOLESTEROL METABOLISM

As cholesterol ester enters the liver with chylomicron remnants, its fatty acid apparently is released by an esterase. The resulting unesterified cholesterol can have four fates: It can be *(a)* reesterified and stored as cholesterol ester, *(b)* secreted into plasma with lipoproteins, *(c)* converted into bile acids, and *(d)* secreted into bile as cholesterol itself. The relative magnitudes of these pathways appear to be a function of the species and the metabolic state. Another important action of unesterified cholesterol is to inhibit the synthesis of cholesterol by the liver cell. The following questions pertain to intrahepatic events of cholesterol metabolism in man.

To What Extent Does Dietary Cholesterol Inhibit the Hepatic Synthesis of Cholesterol?

Synthesis of cholesterol is inhibited by dietary cholesterol in many animal species (82). The extent to which this occurs in man remains to be shown.

Circumstantial evidence supports the concept of feedback inhibition in the human liver. When the absorption of cholesterol in inhibited, a compensatory increase in synthesis of cholesterol occurs (32). Presumably this increase occurs in the liver. Other evidence comes from cholesterol balance studies, from which total body synthesis of cholesterol can be estimated (65). When dietary cholesterol is increased, total-body synthesis of cholesterol is reduced in many, but not all, subjects.

These studies strongly imply that feedback is active in human beings. Nonetheless, even if dietary cholesterol does inhibit cholesterol synthesis in the liver the effect may not be entirely elastic—i.e. there may not be a one-for-one decrease in hepatic synthesis associated with an increase in cholesterol absorption.

Does Dietary Cholesterol Stimulate the Synthesis of Bile Acids?

In rats (86) and dogs (63), increasing dietary cholesterol increases synthesis of bile acids. Newly absorbed cholesterol thus is transformed rapidly into bile acid. This mechansim may protect against the development of hypercholesterolemia in these species. In cholesterol balance studies carried out at the Rockefeller University (32, 65), increasing dietary cholesterol did not promote bile acid production. Most of these patients had hypercholesterolemia. Lin & Conner (51), on the other hand, have claimed that prolonged feeding of dietary cholesterol to normal subjects results in an increased synthesis of bile acids.

Compared to many animal species, humans in general have a small fractional conversion of hepatic cholesterol into bile acids (82). A low conversion rate in humans may contribute to our relatively high levels of plasma cholesterol. This species "abnormality" could be stressed further when the diet is rich in cholesterol. Further research is needed to determine whether and to what extent dietary cholesterol stimulates bile acid formation in normal man.

Does Cholesterol in Chylomicron Remnants Have a Unique Metabolism in the Liver?

Perhaps it is reasonable to assume that cholesterol entering in the liver cell with chylomicron remnants becomes part of a single "metabolic pool" of cholesterol. It may mix completely with cholesterol from other sources (e.g. lipoprotein cholesterol and newly synthesized cholesterol). Another possibility is that it has a unique fate. For example, it could be secreted directly into bile, or it could be incorporated specifically into lipoproteins or converted rapidly into bile acids. Such a metabolic channeling of cholesterol has been postulated, but little concrete evidence supports it. Metabolic channeling of cholesterol in the liver cell may exist, but it has not been proven. If there is metabolic channeling, the following question seems particularly important.

Is Dietary Cholesterol Uniquely Destined for the Plasma Compartment?

Upon first consideration it might be concluded that there is nothing unique about newly absorbed dietary cholesterol. Once it enters the liver with chylomicron remnants it could become mixed with endogenous cholesterol and thereby lose its identity. If this is true, however, it is difficult to explain why relatively small amounts of dietary cholesterol sometimes cause an inordinate rise in plasma cholesterol levels in some persons. This fact is especially perplexing when one considers that a marked increase in the body's production of cholesterol in the obese state usually causes little or no rise in plasma cholesterol concentrations (87). In other words, it is possible that newly absorbed cholesterol has a different effect on the plasma cholesterol concentration than newly synthesized cholesterol. If so, perhaps the difference relates to sites of entry into body pools. Newly absorbed cholesterol finds its way to the liver with chylomicron remnants. If the liver contributes a relatively small fraction of total body synthesis of cholesterol, most newly synthesized cholesterol would originate in extrahepatic tissues. Eventually, of course, any cholesterol produced in extrahepatic tissues must be returned to the liver for excretion. If all cholesterol entering the liver, regardless of its source, is mixed equally, then newly absorbed cholesterol cannot be unique. On the other hand, if there is metabolic channeling of cholesterol in the liver cell, the mechanism of entry could be significant. Newly synthesized cholesterol from peripheral tissues could be channeled into bile (70). Newly absorbed cholesterol in contrast might be shunted into lipoprotein synthesis. These considerations draw our attention to the potential significance of metabolic channeling, examined in more detail below.

How Much of Total Body Synthesis of Cholesterol Occurs in the Liver?

It has long been thought that the liver is the major site of cholesterol synthesis in the body (15–17). More recent studies suggest that this may not be so in all species. In some animals, especially in the rat, squirrel, monkey, and hamster, the liver appears to make a major contribution to total body synthesis (82). The fraction of the total appears to be less in the rabbit and guinea pig (82). The amount of cholesterol produced in the human liver is unknown.

If the human liver synthesizes relatively small amounts of cholesterol, this could have important implications for the body's economy of cholesterol. One protection against an excess of newly-absorbed cholesterol in some animals seems to be feedback inhibition of hepatic cholesterol synthesis. A reduction in synthesis thus theoretically can "neutralize" an increment of newly absorbed cholesterol; but this would not occur if hepatic synthesis of cholesterol is low. A decrease in synthesis could not compensate for an increased influx of new

cholesterol. Feedback inhibition consequently would lose its importance as a protective mechanism against absorption of excess cholesterol. It thus seems crucial to know how much of the total body synthesis of cholesterol occurs in the liver of human subjects.

BILIARY CHOLESTEROL

By What Mechanism is Cholesterol Secreted into Bile?

Little is known about how cholesterol is secreted into bile. Cholesterol in bile is solubilized by bile acids and lecithin, but the molecular events whereby this occurs are unknown. The best evidence suggests that cholesterol is present in canalicular membranes complexed with lecithin, and as bile acids are secreted into the lumen of the canaliculus both cholesterol and lecithin are entrained or leached out of the membrane (40, 60, 85). This mechanism can explain entrance of cholesterol into bile over a wide range of secretion. Still, entirely different mechanisms are possible—e.g. a specific secretory process. If the latter exists, however, it has never been identified.

How Much Dietary Cholesterol Reenters the Bile as Cholesterol Itself?

Our previous studies showed that increasing dietary cholesterol enhances biliary lipid outputs (65). Newly absorbed cholesterol presumably is removed in part by secretion into bile. The amount eliminated by this mechanism, however, is unknown. Two factors could reduce the magnitude of the increment: *(a)* feedback inhibition of cholesterol synthesis, and *(b)* enhanced conversion of cholesterol into bile acids. The maximum increment for most people should be about 250 mg/dl, since this is approximately the amount of dietary cholesterol absorbed. Real increments probably are lower than the maximum, but their range has not been determined.

Does Dietary Cholesterol Contribute to Formation of Cholesterol Gallstones?

If the bile is the major route of removal of absorbed dietary cholesterol, high-cholesterol diets could contribute to development of cholesterol gallstones. The excess synthesis associated with obesity is clearly a major factor responsible for supersaturated bile in overweight patients (2). Besides making too much cholesterol, many obese patients may ingest excessive dietary cholesterol along with an increased caloric intake. An excess of newly absorbed cholesterol combined with an overproduction of cholesterol may raise the biliary cholesterol to a level high enough to initiate nucleation of cholesterol in bile. The specific role of dietary cholesterol in cholelithiasis has not been

explored adequately. A crucial factor may be how much dietary cholesterol reenters bile as unchanged cholesterol.

Why is Human Bile So Highly Saturated with Cholesterol?

Of all species, humans have the most highly saturated bile (8, 82). For given quantities of bile acids and lecithin, human bile is exceedingly enriched with cholesterol, compared with other species. This fact probably accounts for the relatively high incidence of cholesterol gallstones in humans. Several factors might contribute to the high saturation of human bile. One is a low fractional conversion of hepatic cholesterol into bile acids. The result is that more cholesterol is excreted in bile where there is less bile acid to dissolve it. Another reason could be a greater metabolic channeling in man. If cholesterol from lipoproteins is shunted directly into bile, the result could be a high output of cholesterol into bile, without a compensatory increase in synthesis of bile acids; this could account for the high saturation of bile. The fact that cholesterol absorption in the rat causes a striking increment in synthesis of bile acids (86) suggest that metabolic channeling might occur less in such species than in man. Biliary cholesterol is not increased by high-cholesterol diets in the rat, and bile remains highly unsaturated (82). Whether differences in hepatic response to cholesterol input from various lipoproteins between certain animals (e.g. rats and dogs) and humans are qualitative or merely quantitative remains to be worked out.

VERY-LOW-DENSITY LIPOPROTEINS

The major lipoprotein secreted by the liver is very-low-density lipoprotein (VLDL). VLDL includes a group of lipoproteins rich in triglycerides. They range in size from 300–500 Å (30). Indirect evidence suggests that the lipid core of newly secreted VLDL is mainly triglyceride, with little if any cholesterol ester (27, 28). The surface coats of these particles contain unesterified cholesterol, phospholipids, and apolipoproteins including the C and E apoproteins, as well as apo B-100 (41, 57).

As VLDL circulate in plasma they undergo several changes. They first begin to acquire cholesterol ester in their neutral lipid core; this in derived mainly from HDL by way of exchange with triglyceride from VLDL (27, 28). At the same time VLDL come in contact with lipoprotein lipase on the surface of endothelial cells. This lipase degrades triglycerides with release of free fatty acids and glycerol. The residual particle is a VLDL remnant. In many animal species, such as rat and dog, VLDL remnants are removed rapidly by the liver, as are chylomicron remnants. In normal man, however, most VLDL remnants are transformed into low-density lipoproteins (LDL). Although the primary function of VLDL is thought to be transport of triglycerides, these

lipoproteins probably play a definite role in metabolism of cholesterol. Several important questions regarding VLDL-cholesterol have not been resolved.

Does the Amount of Cholesterol Absorbed Affect Quantities of Cholesterol Secreted in VLDL?

Causes of the rise in plasma cholesterol associated with an increase in cholesterol intake are not well understood. One possibility is that the liver secretes more cholesterol into the plasma. If so, the excess could be associated with VLDL. If high-cholesterol diets increase secretion of VLDL-cholesterol we must ask whether this excess secretion occurs as esterified or unesterified cholesterol. Available evidence indicates that most cholesterol ester is formed in the plasma compartment by action of the enzyme, lecithin cholesterol acyltransferase, on unesterified cholesterol (27, 28). Still, some cholesterol ester may well be secreted with triglycerides in VLDL. Secretion of cholesterol esters with VLDL occurs in rats (50, 62, 78), and this mechanism also may exist for man. If so, it should be activated maximally by high-cholesterol diets, as occurs in rats. The high concentrations of cholesterol in lipoproteins of certain cholesterol-fed animals is probably the result of increased output of cholesterol ester by the liver (50, 62, 78). The extent to which the pathway occurs in humans has never been examined.

What is the Fate of VLDL-Cholesterol?

A major portion of cholesterol in VLDL is transferred to LDL in the normal conversion of VLDL to LDL. The cholesterol content of VLDL per lipoprotein particle, however, is higher than that of LDL particles. Some cholesterol therefore must be lost from VLDL in its conversion to LDL. A possible site of loss could be at lipolysis by lipoprotein lipase. The mechanisms could be similar to those described above for chylomicrons. If chylomicrons can contribute cholesterol to the arterial wall during lipolysis, the same process should occur with VLDL. Indeed, the Zilversmit hypothesis may apply more to VLDL than to chylomicrons (90).

The loss of cholesterol from VLDL may not occur entirely during lipolysis. It could take place at the final step in conversion of VLDL remnants to LDL. Here the liver may play a role. In several species VLDL remnants are removed almost completely by the liver. This process seems to be receptor-mediated, and the receptors apparently recognize apo E on remnants (56). Conversion of VLDL remnants to LDL also may require apo E receptors (41). The first step may be attachment of VLDL remnants to hepatic apo E receptors but without internalization of the particle (41). Further modification of remnants may occur at this site. Hepatic triglyceride lipase could be one mediator of this transformation, removing most of the remaining core triglyceride before release of the particle back into the circulation as LDL. If this mechanism pertains, partial

removal of cholesterol ester could also occur at the same site. This would require a cholesterol esterase to work in concert with triglyceride lipase. Whether such a mechanism actually exists is unknown. Since it could explain the apparent loss of cholesterol ester in transformation of VLDL to LDL, it is worthy of further investigation.

Does Excess Dietary Cholesterol Increase the Atherogenicity of VLDL?

If high-cholesterol diets increase secretion of cholesterol into VLDL we might inquire whether the resulting VLDL are more atherogenic than normal VLDL. In experimental animals, such as the rabbit and dog, feeding of cholesterol stimulates formation of a cholesterol-rich, β-VLDL (56). These particles appear atherogenic in these species. Preliminary reports suggest that cholesterol feeding in man results in production of a similar abnormal lipoprotein (56). More studies are needed to determine the extent to which β-VLDL are produced after ingestion of cholesterol.

LOW-DENSITY LIPOPROTEINS

LDL includes a spectrum of lipoproteins ranging in size from 175–250 Å. These lipoproteins contain mostly cholesterol ester in their lipid core. Apo B-100 constitutes more than 95% of the protein of this particle (41, 57). Little triglyceride is present. Although most LDL is derived normally from VLDL, recent work suggests that a portion of LDL can be secreted directly by the liver—i.e. independent of VLDL (45, 75, 76, 80). The mechanism for this latter phenomenon is unknown. Presumably a newly secreted "pre-LDL" is transformed into LDL in plasma. Whether this pre-LDL enters the plasma in the heavier portion of VLDL, or directly into the LDL density range, remains to be determined.

In recent years the fate of LDL has received much attention. The major portion of plasma LDL is removed through specific cell-surface receptors of LDL (29). About 70–80% of LDL seems to be removed by the receptor pathway (29). Sites of removal include both liver and extrahepatic tissues. Up to 50% of LDL may be by the liver, although this fraction has not been determined for humans. The remaining LDL is removed by nonreceptor pathways. In tissue culture, modification of apo B structure is required to achieve nonreceptor uptake of LDL, but modification may not be required in vivo.

Since the feeding of cholesterol raises LDL in several animal species and in man, several questions can be raised about the mechanisms involved.

Does Dietary Cholesterol Cause Direct Secretion of LDL?

If secretion of cholesterol esters in lipoproteins is increased by high-cholesterol diets, the result might be an enhanced direct secretion of LDL. This is to say,

cholesterol-ester rich particles might bypass the usual VLDL pathway. Such a mechanism in fact might lead to direct secretion of "mature" LDL by the liver. These particles might theoretically be differentiated from LDL derived from VLDL by the composition of cholesterol ester fatty acids. Most cholesterol esters produced by the LCAT reaction contain linoleic acid, while those formed in the liver mostly have oleic acid. Thus, if feeding of high-cholesterol diets were to raise the proportion of oleic acid in cholesterol esters of LDL, increased direct secretion would be implied. Unfortunately, there is exchange of cholesterol esters between lipoproteins, and it cannot be assumed that the composition of cholesterol esters found in LDL necessarily reflects the composition secreted by the liver. A rise in the ratio of oleic acid to linoleic acid in cholesterol esters of total plasma nontheless would be consistent with this mechanism.

Does a High-Cholesterol Diet Stimulate Synthesis of Apolipoprotein B (apo B)?

Apo B is the major structural apoprotein of VLDL and LDL. Recent evidence from our laboratory indicates that LDL concentrations in most people are regulated mainly by synthetic rates of LDL-apo B (46). Those with high concentrations of LDL have the greatest production rates of LDL-apo B. In people with high LDL, an increased input of apo B into LDL could be derived either from VLDL or by direct secretion of LDL (45, 75, 76, 80). Factors regulating the synthesis of apo B are poorly understood. Apo B production could be controlled in part by hepatic concentrations of cholesterol. Ingestion of dietary cholesterol could stimulate the synthesis of LDL-apo B. Ginsberg et al (26) recently reported that feeding of cholesterol did not stimulate synthesis of LDL-apo B. In this study, however, two factors may have hidden a possible effect of dietary cholesterol. First, in the control period of this study the subjects were given 150 mg/1000 cal of cholesterol, and this amount was increased to 500 mg/1000 cal in the second period. At an intake of 150 mg/1000 cal the maximum effect of dietary cholesterol on apo B synthesis may already have been realized. In accord with this possibility was the finding that a further increment of dietary cholesterol in the second period did not raise in total LDL levels. The effects of dietary cholesterol thus may have been masked by the moderate intakes in the control period. The potential effect of newly absorbed cholesterol on LDL-apo B synthesis is revealed by a study carried out recently in our laboratory. We observed that blockage of cholesterol absorption reduces synthesis of LDL-apo B in human subjects. The amount of cholesterol absorbed thus appears to affect apo B synthesis. Nevertheless, as suggested by the study of Ginsberg et al (26), there may be a ceiling on the effect. Perhaps newly absorbed dietary cholesterol influences synthesis of apo B only to a certain point, and further input may have no additional effect on production of this apoprotein.

Does Dietary Cholesterol Regulate the Uptake of LDL by the Liver or Other Tissue?

As shown in tissue culture studies, a major factor regulating the uptake of LDL by the receptor pathway is the concentration of cholesterol within the cell (29). When cellular concentrations rise, synthesis of receptors is reduced and uptake of LDL is decreased. If this mechanism also occurs in vivo in the liver, it could explain in part the rise of LDL levels during feeding of cholesterol. As more dietary cholesterol enters the liver cell, the production of LDL receptors on the surface of liver cells may decrease. Plasma concentrations of LDL therefore should rise. Studies in experimental animals indicate that reduction in hepatic concentrations of cholesterol by administration of either bile-acid sequestrants or cholesterol-synthesis inhibitors increases the clearance of LDL (48, 72). By the same token, a rise in hepatic concentrations of cholesterol could decrease the number of receptors on the liver cell and thus reduce hepatic uptake of cholesterol. Whether this mechanism is responsible for the rise in LDL of a high-cholesterol diet remains to be determined.

Does Dietary Cholesterol Redirect LDL Clearance to Extrahepatic Tissue?

If cholesterol in the diet expands hepatic concentrations of cholesterol and thereby reduces uptake of LDL by the liver, LDL could be directed elsewhere for removal. Under such circumstances, increased amounts of cholesterol might be distributed to extrahepatic tissues. This raises the possibility of a gradual but progressive accumulation of cholesterol in extrahepatic tissues. One site of accumulation might be the arterial wall. Dietary cholesterol thus could promote atherogenesis by means other than raising LDL levels—i.e. by promoting peripheral uptake of LDL.

Do High-Cholesterol Diets Lead to Formation of Highly Atherogenic LDL?

In subhuman primates the feeding of cholesterol results in the formation of unusually large LDL (67). These particles appear to have increased atherogenic potential. It is important to know whether the same occurs in humans. The question remains open, but available evidence suggests that cholesterol feeding does not enlarge LDL in man (69). This does not rule out the possibility that excess cholesterol in the diet might produce transitory increases in LDL-cholesterol that are not seen in the fasting state. Even if enrichment occurs only during part of the day, the resulting particles could contribute to atherosclerosis. More studies thus are needed to determine the full impact of dietary cholesterol on the composition of LDL.

How Do Dietary Cholesterol and Saturated Fats Interact to Raise LDL Levels?

A recent report claimed that dietary cholesterol causes a rise in LDL only when given concurrently with a diet rich in saturated fatty acids (69). When dietary cholesterol was fed with large amounts of polyunsaturated fatty acids, LDL levels did not increase. The reason for this difference is not apparent, but it could be linked to the basic mechanisms whereby the two types of fatty acids affect LDL concentrations. There are conflicting reports on the exact mechanisms of LDL lowering by polyunsaturates. Several factors may interact to produce the changes in LDL levels caused by these two types of fatty acid.

One hypothesis is that polyunsaturated fatty acids lower LDL-cholesterol by altering the composition of LDL (77). For example, these fatty acids might occupy more space within the lipoprotein and thereby sterically exclude cholesterol from the particle. This hypothesis implies that polyunsaturated fats would not reduce the concentrations of LDL particles but only the amount of cholesterol that can be carried by these lipoproteins. This mechanism could explain the lack of rise in LDL-cholesterol during feeding of dietary cholesterol with large amounts of polyunsaturated fatty acids. LDL particles enriched in polyunsaturates might not have the capability of incorporating more cholesterol.

Although this hypothesis is attractive, a recent study from our laboratory did not support it (83). We carried out a detailed analysis of LDL composition of diets rich in saturated fatty acids and in polyunsaturates. LDL-cholesterol concentrations fell during feeding of polyunsaturates. So did all other constituents of LDL. The composition did not change significantly. The concentrations of LDL particles therefore appeared to be reduced. This finding implies that the LDL lowering action of polyunsaturates is not obtained by changing LDL composition but through altering its metabolism.

A decrease in LDL concentrations could occur either by reducing the synthesis of LDL or by increasing its clearance. Although polyunsaturated fatty acids may decrease the synthesis of LDL in some patients, the predominant action seems to be to promote clearance (71). The most likely mechanism for the rise in LDL with cholesterol feeding is to reduce LDL receptors and hence its clearance (although this is by no means the only mechanism). Presumably the actions of polyunsaturates in lowering LDL override those of dietary cholesterol, but no explanation for this paradox is readily available.

HIGH-DENSITY LIPOPROTEINS AND REVERSE CHOLESTEROL TRANSPORT

Concentrations of cholesterol in extrahepatic tissues can be increased by at least two mechanisms: de novo synthesis or uptake of LDL. Since cholesterol cannot

be degraded in peripheral tissues, it must be returned to the liver for excretion or degradation. This process has been called "reverse cholesterol transport". Its mechanisms have not been worked out fully. They are thought to involve HDL. In order to understand current concepts of reverse cholesterol transports, we must keep in mind the basic pathways of HDL metabolism.

HDL represents a group of small lipoproteins with diameters ranging from 70–100 Å. These particles contain mainly cholesterol ester in their neutral lipid core. Their lipoprotein coats consist of unesterified cholesterol, phospholipids, and apolipoproteins. The major apoproteins are apo A-I and apo A-II. Both are water-soluble apoproteins and can move from one lipoprotein to another. Small amounts of C and E apoproteins are also present.

The liver and gut probably secrete particles called nascent HDL (39). These are disc-shaped lipoproteins containing the apo As, phospholipids, and possibly apo E. They have a high affinity for unesterified cholesterol, which can be acquired from other lipoproteins, red blood cells, or other extraheptic cells. Through reaction with LCAT, which is activated by apo A-I, unesterified cholesterol in the outer coat is esterified; the resulting cholesterol ester is incorporated into the core. The result is a small spherical particle called HDL_3. This particle has a molecular weight of about 175,000.

As HDL_3 circulate they begin to take on additional constituents. Cellular cholesterol may be a continuing source of HDL-cholesterol. Cholesterol also may be acquired during the degradation of triglyceride-rich lipoproteins (VLDL and chylomicrons). Unesterified cholesterol, phospholipids, and other soluble apoproteins (Cs and Es) released during lipolysis seem able to enter the surface coat of HDL (79). These changes along with the continuing action of LCAT can expand HDL_3, producing a larger particle, HDL_2. The latter has an aggregate molecular weight of about 340,000.

HDL can transfer some of its cholesterol ester to VLDL in exchange for triglyceride (9). This cholesterol ester undergoes various fates as discussed above. It may enter the liver either during catabolism of VLDL or by hepatic uptake of LDL. The disposition of the remaining HDL-cholesterol is poorly understood. HDL seems not to be removed by specific cell-surface receptors. Clearance of whole HDL particles is likely, but the mechanism is unknown.

Whether HDL is the immediate receptor of extrahepatic cholesterol has not been resolved. Fielding & Fielding (23, 24) suggest that a smaller complex consisting of apoprotein D and LCAT may be the active receptor for unesterified cholesterol. The apo D-LCAT complex may esterify cholesterol and then transfer the ester to other lipoproteins, particularly HDL. Another intriguing concept is that apo E may play an important role in removal of cholesterol from extrahepatic cells. Nonhepatic cells have been shown to produce apo E (1a), and when cells in tissue culture are loaded with cholesterol they secrete

cholesterol complexed with apo E into the surrounding media. This cholesterol–apo E complex in vivo may acquire apo A to produce HDL_C (56). In animals HDL_C is removed rapidly by the liver (56). The secretion of apo E from cells thus could play a vital role in reverse cholesterol transport.

Much has yet to be learned about cholesterol metabolism in peripheral (extrahepatic) tissue and about how extrahepatic cholesterol is returned to the liver. Several questions appear particularly important for man.

Do High-Cholesterol Diets Inhibit Extrahepatic Synthesis of Cholesterol?

Most investigators agree that increasing the cholesterol content of diet inhibits hepatic synthesis of cholesterol. This has been proven amply in animals, and it may occur in man. Whether dietary cholesterol directly or indirectly blocks the synthesis of extrahepatic cholesterol is another issue. Previous studies in humans show that for most patients synthesis of cholesterol in the whole body cannot be inhibited completely by high-cholesterol diets (65). On the other hand, some degree of negative feedback may occur. This could be the result of several mechanisms. Both VLDL and LDL could deliver more cholesterol to peripheral tissues, which could increase intracellular concentrations and thereby inhibit synthesis. Alternatively, diets high in cholesterol may interfere with removal of cholesterol from tissues. A possible mechanism is overloading of HDL with cholesterol during lipolysis, which could reduce its capacity to accept cholesterol from the cell membranes of extrahepatic tissues. The accumulation of cholesterol in peripheral cells could secondarily inhibit their synthesis of cholesterol.

Does Dietary Cholesterol Raise Concentrations of HDL-Cholesterol?

Studies in experimemtal animals have shown that high-cholesterol diets can increase concentrations of HDL-cholesterol. Since HDL seems not to be in the major pathway of input and clearance of dietary cholesterol, the way in which concentrations of HDL-cholesterol could be raised is not entirely clear. Two possibilities exist. One is transfer of chylomicron cholesterol to HDL. A current hypothesis is that lipolysis of triglyceride-rich lipoproteins causes release of unesterified cholesterol from the surface coat with transfer to HDL (79). Through the action of LCAT, this cholesterol is esterified and moves into the neutral-lipid core of the particle; accumulation of cholesterol in this way may promote conversion of HDL_3 to HDL_2. The second possibility is that cholesterol accumulates in peripheral tissues during lipolysis of chylomicrons or VLDL. This would require an enhanced reverse cholesterol transport that could raise HDL-cholesterol.

If Dietary Cholesterol Raises HDL-Cholesterol, Could this Rise Protect Against Atherosclerosis?

It is generally believed that a high concentration of HDL, as relected by HDL-cholesterol, may protect against atherosclerosis (18, 49). The particular mechanism responsible for elevating HDL-cholesterol, however, may determine the relationship to protection. If a rise in HDL is the result of overloading of this lipoprotein with exogenous cholesterol, the mobilization of endogenous cholesterol from peripheral tissues could be impaired. If so, a high HDL-cholesterol could signify increased atherogenesis. Under other circumstances, a high HDL-cholesterol could reflect a primary increase in uptake of endogenous cholesterol from extrahepatic tissues; if so, this could indicate a protective action.

Whether increasing cholesterol in the diet will raise HDL cholesterol in man has not been demonstrated conclusively, although at least one study supports such a result (55). It is highly unlikely that this change would protect against atherosclerosis. It should be pointed out, nevertheless, that the nature of the link between increased HDL and decreased risk for atherosclerotic disease is poorly understood; obviously a beneficial effect of the rise in HDL-cholesterol with cholestrol feeding cannot be ruled out. At least, an increase in HDL during cholesterol feeding may reflect a compensatory and protective mechanism. If a high-cholesterol diet delivers more cholesterol to peripheral tissues, a high HDL could signify an active reverse cholesterol. The rise in HDL-cholesterol with cholesterol feeding thus might indicate an enhanced protective capacity.

Does Dietary Cholesterol Inhibit Reverse Cholesterol Transport?

The final site of clearance of cholesterol released from extrahepatic tissues is the liver. Current evidence suggests that a significant portion of cholesterol entering the liver is carried in LDL and perhaps VLDL remnants (29). The former, and perhaps the latter to some extent, attach to receptors for apo B before hepatic uptake (29). As indicated above, high-cholesterol diets may elevate hepatic concentrations of cholesterol and thereby reduce apo·B receptors. If so, diets rich in cholesterol could retard one of the final steps in reverse cholesterol transport. This could lead not only to an elevation in plasma LDL but also to a redistribution of sites of LDL removal to extrahepatic tissues. The effects of dietary cholesterol therefore could be greater than suggested by the small rise in LDL concentrations resulting from cholesterol feeding. Thus exogenous cholesterol could be dangerous if it retards mobilization of endogenous cholesterol from peripheral tissues (including from the arterial wall). The following question therefore must be considered.

Does Dietary Cholesterol Expand Extrahepatic Pools of Cholesterol?

If high-cholesterol diets interfere with reverse cholesterol transport they might expand extrahepatic pools of cholesterol. Release of cholesterol from triglyceride-rich lipoproteins during lipolysis also might increase peripheral pools by ways discussed above. Finally, any rise in LDL produced by high-cholesterol diets could promote tissue uptake of LDL and expansion of body pools. Studies in dogs have failed to show that cholesterol feeding increases concentrations of cholesterol in tissues outside the liver (63). The same, however, may not be true for humans. LDL levels are much higher in man than in dogs. More plasma cholesterol thus may be diverted to peripheral tissues in man. Therefore, specific studies on pool sizes of cholesterol in humans after prolonged feeding of cholesterol will be required before this question can be answered.

MAJOR UNRESOLVED ISSUES

In recent years there has been increasing acceptance of the concept that raising dietary cholesterol increases plasma cholesterol in many if not most people. This increase, which occurs largely in LDL, should promote atherogenesis and increase the risk for coronary heart disease. The approximate increase in risk can be calculated from standard risk-factor tables. The degree of rise in plasma cholesterol, however, does not necessarily provide a complete picture of the role of dietary cholesterol in the pathogenesis of atherosclerosis. The possibility must be considered that excess cholesterol in the diet could act through other mechanisms to promote atherogenesis. Some of the questions outlined above reveal the complexity of cholesterol metabolism in man and point out several possible relationships between dietary cholesterol and atherosclerosis that have not been explored adequately.

Particularly important is whether dietary cholesterol carried on lipoproteins other than LDL (e.g. chylomicrons and VLDL) is atherogenic. The concept that dietary cholesterol could specifically induce a highly atherogenic lipoprotein (e.g. β-VLDL or large LDL) is particularly intriguing. Furthermore, the possibility that hepatic cholesterol derived from the diet could interfere with LDL uptake and thereby redirect LDL clearance to extrahepatic tissues needs further exploration. Finally, excess hepatic cholesterol may retard other pathways of reverse cholesterol transport and thus could promote atherosclerosis.

The notion of metabolic channeling of cholesterol in the liver cell needs further exploration. Whether newly absorbed cholesterol is destined primarily for secretion into bile or transport into lipoproteins has not been determined.

Whether cholesterol carried on VLDL-remnants, LDL, or HDL is channeled directly into bile, or is the preferred precursor for bile acids, is also unknown. Finally, if channeling of cholesterol in the liver cell occurs, this could have important implications for mechanisms of formation of supersaturated bile and cholesterol gallstones.

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