REGULATION OF PLASMA LDL-CHOLESTEROL LEVELS

Remojadas, Veracruz –
Late Classical Period

MEDICAL GRAND ROUNDS

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INTRODUCTION

Cholesterol, an essential constituent of living tissues, plays critical roles as a structural component of most biological membranes and is the immediate precursor for a number of essential vitamins, steroid hormones, and bile acids. It is of critical importance, therefore, that the cells of the major tissues of the body be assured a continuous supply of this compound. To meet this need, a complex series of transport, biosynthetic and regulatory mechanisms has evolved. Generally, cholesterol can be acquired from the environment through the absorption of dietary cholesterol or synthesized de novo from acetyl CoA within the body. More cholesterol usually enters the body through these two mechanisms than is used during normal metabolic turnover so that the excess must be metabolized and/or excreted to prevent a potentially hazardous accumulation of sterol. Unfortunately, mammalian tissues do not possess enzymes capable of extensive degradation of the sterol nucleus. The best that can be done is to modify certain of the substituent groups on the hydrocarbon tail or on the ring structure of the sterol molecule. Hence, cholesterol is excreted from the body either as the unaltered molecule or after biochemical modification to other sterol products, such as bile acids and steroid hormones.

The availability of cholesterol in the diets of different animal species varies enormously and even in the same species, including man, may change markedly from day to day. Thus, it is apparent that there must be regulatory mechanisms operative that adjust the rate of cholesterol synthesis within the body and/or the rate of cholesterol excretion from the body to accommodate the varying amounts of sterol that are being absorbed from moment to moment from the diet. Generally these regulatory mechanisms function very well, so that there is little net accumulation of cholesterol over the lifetime of many animals, yet sufficient sterol is always available to meet the metabolic needs of the various tissues. In a few species, and in particular in man, subtle imbalances do develop that can lead to elevation in circulating levels of plasma cholesterol or to excessive secretion of cholesterol into bile. In the first instance, this metabolic abnormality may lead to cholesteryl ester accumulation in cells within the walls of arteries and produce clinically apparent atherosclerotic disease. In the second instance, the bile may become supersaturated with sterol, leading to the precipitation of cholesterol and, ultimately, to clinically apparent cholelithiasis.

This review covers the newer data on the major events that regulate circulating plasma LDL-choleterol levels.

GENERAL FEATURES OF CHOLESTEROL BALANCE IN MAN AND VARIOUS EXPERIMENTAL ANIMALS

The general features of cholesterol balance that must be taken into consideration in man and in various experimental animals are shown in Fig. 1. The body pool of cholesterol in the adult remains essentially constant. The content of sterols in various tissues varies markedly, from about 0.5 g per kg of muscle to 15 g per kg of brain, but averages approximately 1.4 g per kg of tissue for the body as a whole. Thus, a 70-kg man contains about 100 g of cholesterol while a 0.2-kg rat has only 0.3 g of total sterol. Not all compartments of this body pool are equally accessible to metabolic interactions or to exchange with cholesterol carried in the blood. Based upon an analysis of die-away curves, for example, the body pool of cholesterol has been divided into three functionally distinct areas. These include a rapidly miscible pool (pool A), a more slowly exchangeable pool (pool B) and a pool of cholesterol that is either only very slowly miscible or nonexchangeable (pool C). In the baboon the size of pool A equals about 0.3 g per kg of body weight and includes nearly all of the sterol present in tissues like the blood, small intestine, lung, liver and spleen, and a lesser proportion of the cholesterol in a variety of other organs including skeletal muscle. Pool B is larger, equaling about 0.6 g per kg of body weight, and includes a portion of the cholesterol present principally in tissues such as adipose tissue, skeletal muscle and skin. Pool C, the nonexchangeable cholesterol pool, contains about 0.5 g of sterol per kg of body weight and is made up largely of cholesterol present in brain, bone, skin, skeletal muscle and adipose tissue.

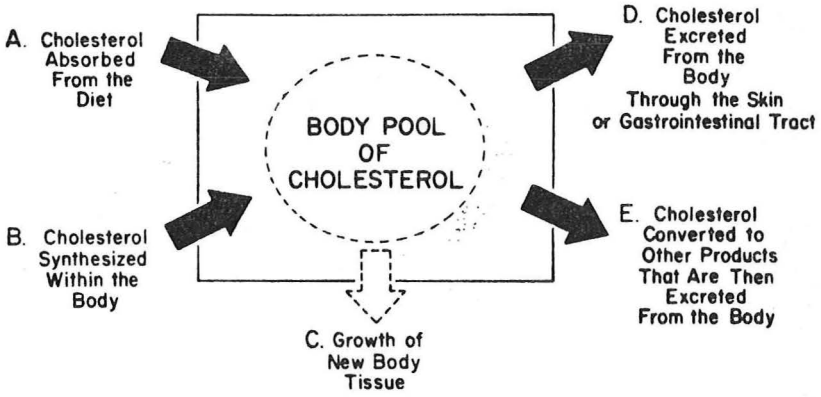


Fig. 1

New cholesterol can be added to the body pool from only two sources. Either preformed sterol is absorbed from dietary sources across the gastrointestinal mucosa or, alternatively, the cholesterol molecule is synthesized de

novo from acetyl CoA in a variety of different tissues within the body. The sum of these two processes constitutes the total input of cholesterol into the body pool each day. Similarly, there are only two major pathways for the removal of cholesterol from the body. The unmodified cholesterol molecule may be lost directly from the body pool. This takes place through the sloughing of oily secretions and cells from the skin, through the desquamation of cells from the stomach, small intestine, and colon and through the movement of cholesterol into pancreatic, gastric, intestinal and canalicular secretions. Of these various routes, secretion of cholesterol through the canalicular membrane of the hepatocyte is usually of greatest quantitative importance. Alternatively, the cholesterol molecule may first be metabolized to another product such as bile acids, adrenocorticosteroids or testosterone which, in turn, is excreted from the body through the urine or gastrointestinal tract.

In the growing animal there is necessarily a greater input of cholesterol into the body than output since, on the average, there is a net accumulation of about 1.4 g of sterol for each kg of body weight gained. Once adulthood is reached and body weight becomes constant, however, the input of cholesterol into the system must equal output. Thus, even though there may be net accumulation of a very small quantity of cholesterol in critical locations, e.g., in the walls of the coronary arteries, the content of cholesterol in the blood and in the other organs remains essentially constant over many years.

CHOLESTEROL ABSORPTION RATES IN DIFFERENT SPECIES

The first of the two major sources for sterol in the body pool is dietary cholesterol absorbed through the gastrointestinal tract. Apparently every animal is capable of absorbing dietary cholesterol to at least some degree, although there are remarkable differences among the various species in the rate of such intestinal transport. Most data indicate that cholesterol movement into the intestinal epithelial cell is a passive process that does not depend upon the expenditure of metabolic energy or upon the intervention of membrane receptors. Thus, the magnitude of the unidirectional flux of cholesterol across the brush border is a linear function of the concentration of this molecule in the luminal fluid, is independent of the presence of structurally related sterol molecules and manifests a relatively low temperature dependency and, hence, a low activation energy. However, recently it has been shown that it is not the rate of membrane translocation that limits the overall velocity of cholesterol

uptake into the intestinal epithelial cell but, rather, it is the rate at which the cholesterol molecule can diffuse from the bulk phase of the intestinal contents through the unstirred water layers overlying the intestinal microvilli. Thus, as with most nonpolar, poorly soluble substances, the rate of intestinal uptake of cholesterol is limited by the rate of diffusion up to the brush border and not by the rate at which this solute penetrates this limiting membrane. It is at the level of these unstirred water layers that bile acid micelles appear to exert their critical function in facilitating cholesterol absorption. Mixed micelles, composed of bile acids and the products of lipolysis of dietary lipids such as fatty acids and β-monoglycerides, solubilize dietary cholesterol. These micellar structures can carry large amounts of sterol up to the aqueous-microvillar interface overcoming, in effect, the resistance engendered by the unstirred water layers to cholesterol absorption and greatly increasing the velocity of sterol uptake per unit length of intestine. Hence, in the intact animal or in man, adequate concentrations of bile acids must be present in the intestinal contents to have relatively rapid sterol absorption: in the absence of such surface active agents the rate of cholesterol absorption is markedly reduced.

The overall process of net cholesterol absorption from the intestinal lumen to the blood is still more complex than these considerations of brush border translocation might imply since there are a number of enzymatic reactions in the intestinal contents and within the intestinal epithelial cell that also could influence the rate of net uptake. Following ingestion, for example, complex foods must be digested by the peptidases and lipases secreted into the intestinal lumen by the pancreas in order to release the largely unesterified, dietary sterols. The small amount of dietary cholesteryl esters is hydrolyzed by another pancreatic enzyme, cholesteryl esterase. This unesterified cholesterol from the diet, along with the unesterified cholesterol reaching the intestinal lumen from the bile, is then solubilized in the complex structure of the mixed micelle. Following the movement of this carrier up to the brush border, the dietary sterol diffuses into the cytosolic compartment of the intestinal absorptive cell where it presumably mixes with a pool of newly synthesized cholesterol. A large proportion of this intracellular cholesterol pool is esterified to long chain fatty acids and incorporated into the structure of the nascent chylomicron. This lipoprotein particle is then secreted from the epithelial cell by an exocytotic process, enters the intestinal lymphatic system

and, hence, eventually reaches the circulating blood. It is apparent that under different physiological circumstances or in different animal species there could be significant variation in the velocity of any one of these steps that might ultimately limit the rate of net cholesterol uptake into the body pool from the diet.

While little data are available delineating the velocity of each of these steps in different animals, measurements have been made of the overall rates at which dietary cholesterol is absorbed in several species. The methods used to make these measurements are relatively insensitive and are the subject of continuing discussion and controversy. Nevertheless, these data do suggest that there are remarkable differences in the amounts of cholesterol that can be absorbed by man and by different experimental animals. A 70 kg man, for example, can absorb several hundred mg of cholesterol per day. A much smaller animal such as a 0.2 kg rat or a 1.5 kg rabbit may absorb, in absolute terms, nearly as much. These differences are made more apparent when the rate of net cholesterol absorption is expressed per kg of body weight. On a relatively high cholesterol intake, man absorbs only about 2-4 mg of cholesterol per day per kg body weight. In contrast, other species such as the rat, rabbit and dog can absorb from 35 to 50 times this amount. On the basis of findings such as these, it has been postulated that this limited capacity to absorb cholesterol may be one of the major mechanisms that protects man against the detrimental effects of excessive dietary cholesterol intake.

CHOLESTEROL SYNTHESIS RATES IN THE WHOLE ANIMAL

The second of the major sources for cholesterol in the body pool is de novo synthesis of sterol by the major organ systems. The rate at which cholesterol is synthesized within the body of man or the experimental animal has been measured by two different types of procedures. One method involves measuring sterol balance across the body. With this technique the amount of cholesterol excreted from the body in the feces as neutral (cholesterol and its bacterial degradation products) and acidic (bile acids) sterols is quantitated in the steady state. After taking into account the amounts of cholesterol that are eaten in the diet and lost from the skin or converted to steroid hormones, and after correcting for any sterol that may be completely degraded by intestinal bacteria, it is possible to calculate the rate of total cholesterol synthesis per day in the experimental subject.

The rate of whole body sterol synthesis has been determined more directly by measuring the rate at which an animal incorporates [^{3}H]water into sterols under in vivo conditions. By assuming that 1.45 μg atoms of carbon are incorporated into cholesterol for every mg atom of ^{3}H, it is possible to calculate the absolute amount of cholesterol that is synthesized under a given circumstance from the amount of ^{3}H that appears in the body sterol pool over a relatively short period of time. The external sterol balance technique and the [^{3}H]water incorporation procedure appear to yield comparable results. For example, the rate of sterol synthesis in the squirrel monkey equals about 30-34 mg of cholesterol per day per kg of body weight whether measured by sterol balance or by isotope incorporation. Similarly, about 20-24 mg of cholesterol are synthesized each day in a 0.2 kg rat when quantitated by either technique.

Using such methods, rates of whole body cholesterol synthesis have been measured in man and in a variety of animals under conditions where dietary cholesterol intake was low. The absolute amount of cholesterol synthesized varies markedly from animal to animal and is even very different between animals of similar weight, e.g., rat and hamster or the guinea pig and squirrel monkey. As was the case with species differences in cholesterol absorption, these variations in rates of sterol synthesis are emphasized by expressing the data as the amount of cholesterol synthesized per kg of body weight. Thus, man can synthesize about 9 mg of cholesterol/day/kg of body weight while the rat is capable of making over 13 times more sterol, or about 118 mg/day/kg body weight. In general, there is an inverse, although imperfect, relationship between the rate of whole animal sterol synthesis and body weight. The larger animals and man generally synthesize much less sterol per unit weight than the small animals, particularly the rat.

RATES OF HEPATIC CHOLESTEROL SYNTHESIS IN VITRO AND IN VIVO

[^{3}H]water should be the ideal substrate with which to quantitate rates of cholesterol synthesis in the liver since this method is not subject to the serious errors inherent in the use of various [^{14}C]substrates for this purpose. Furthermore, recent experimental work has demonstrated that the C/^{3}H incorporation ratio is approximately the same in liver tissue derived from a variety of animal species. Hence, the rates of [^{3}H]water incorporation into cholesterol should provide an accurate appraisal of both the relative and absolute rates of hepatic sterol synthesis as measured under in vitro conditions.

Rates of cholesterol synthesis have been measured in liver specimens obtained from a variety of animal species that had been maintained on a low intake of dietary cholesterol. As summarized in Fig. 2, very large variations are seen among the different species when these rates of synthesis are expressed per g of liver tissue. The rat again manifests an extremely high rate of cholesterol synthesis reflecting the high rate of whole body sterol synthesis found in the same species. The rates of sterol synthesis are all much lower in the other species, including man. Another point to be emphasized, and that will be discussed in detail later, is that there is no general correlation between the rates of hepatic cholesterol synthesis and the relative amounts of cholesterol secreted into the bile of each of these species or of their respective propensities to develop cholesterol gallstones.

While these comparisons are of considerable interest, they still do not answer the fundamentally important question as to the quantitative importance of the liver to whole body sterol synthesis in each of these species, particularly under in vivo conditions. Fortunately, [^{3}H]water also can be utilized to make such measurements. When administered intravenously, this substrate rapidly equilibrates with the total pool of body water: hence, the SA of the intracellular water that is being incorporated into cholesterol presumably equals the SA of water in the circulating plasma and this can be easily sampled. Furthermore, provided that the experimental subject is not given any exogenous water, the SA of the body pool remains essentially constant for long periods of

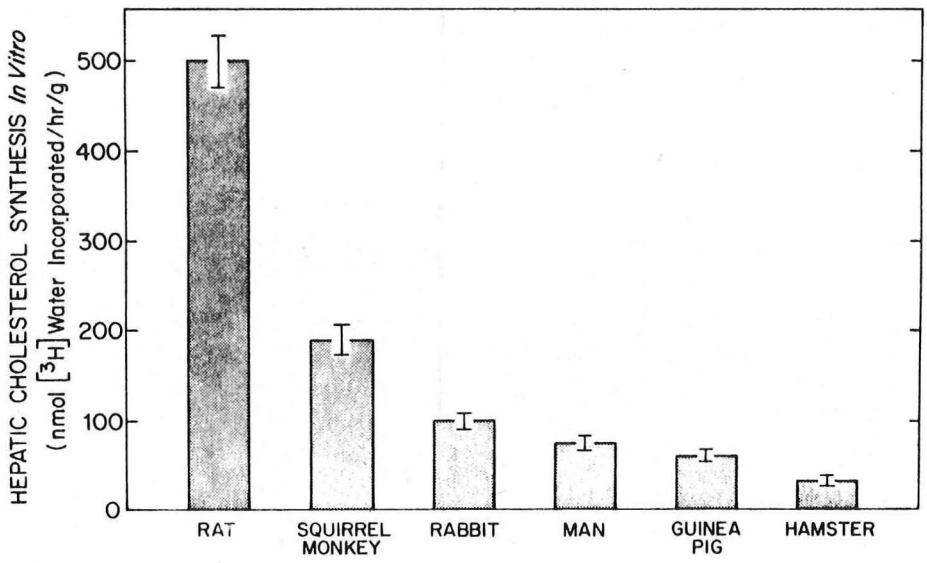


Fig. 2

time. Hence, the rates of incorporation of [^{3}H]water into cholesterol under these conditions also provide an accurate measure of rates of sterol synthesis in vivo.

Fig. 3 shows the amount of newly synthesized sterol that is found in one g of the major organs of the rat (panel A) and squirrel monkey (panel B) 1 hr after the intravenous administration of [^{3}H]water. The highest content of [^{3}H]cholesterol is found in the liver, endocrine glands and various parts of the gastrointestinal tract. All other tissues contain some newly synthesized sterol, but the amounts are low when expressed per g of tissue.

To relate these values to whole body synthesis rates, the weight of each organ must be taken into consideration. This has been done in the data shown in Fig. 4. The whole rat incorporates 31.6 μmol of [^{3}H]water into sterol per hr while in the squirrel monkey this value equals 28.9 μmol/hr. These incorporation rates correspond to the synthesis of 24 mg and 22 mg of cholesterol per day in these two species, respectively. In the rat, 51% of the newly synthesized sterol is found in the liver, 12% in the small intestine, 12% in the skin and 13% in the carcass (mainly muscle and bone). This same group of organs also contains the majority of the newly synthesized sterol found in the monkey. Thus, while the rates of synthesis per g of skin and muscle are very low, these two organs make up such a large fraction of the body weight of the animals that they also are major sites for the synthesis of sterols.

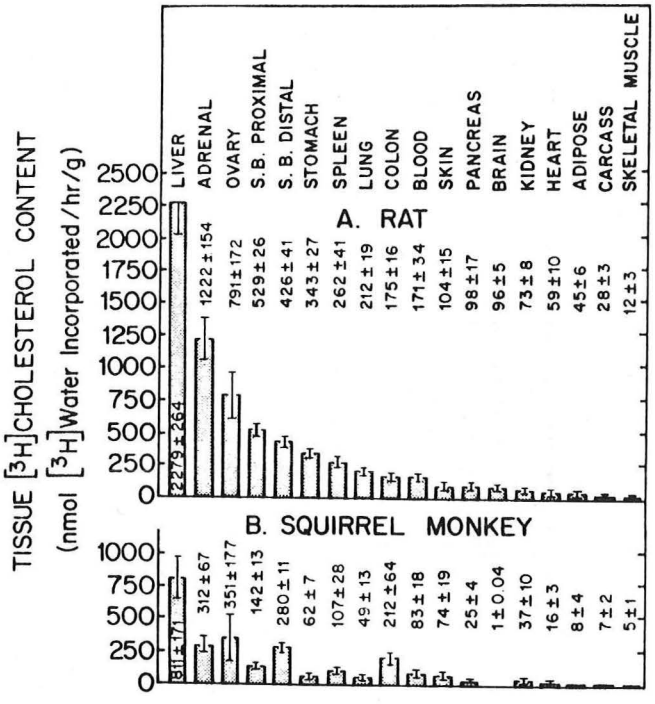


Fig. 3

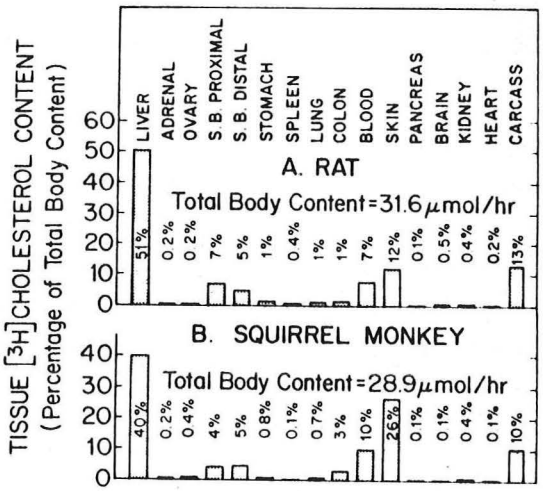


Fig. 4

Under these in vivo conditions there have been small shifts of newly synthesized cholesterol from the sites of origin to other organs. Quantitatively, the most important shifts occur out of the liver and small intestine. In the rat, for example, within 1 hr nearly half of the cholesterol that has been synthesized in the intestine is transferred to the liver and approximately 30% of that which is synthesized in the liver has moved out into the plasma and extrahepatic tissues. Taking these shifts into consideration, it has been reported that in this species only about 50% of total body sterol synthesis occurs in the liver, 24% in the small intestine, 8% in the skin and 18% in the remaining tissues in the body.

Based upon this type of analysis, the quantitative importance of the liver to total body sterol synthesis in different animal species under the condition of low dietary cholesterol intake has been measured in a number of different species as shown in Fig. 5. In the rat and squirrel monkey approximately half of whole body sterol synthesis takes place in the liver. In the other species in which such measurements have been made, however, hepatic cholesterol synthesis contributes less than one-third of the sterol synthesized in the body each day. These results clearly support the prediction that previous work utilizing a variety of [^{14}C]substrates to quantitate the importance of the major organ systems for endogenous sterol synthesis has grossly underestimated the role of extrahepatic tissues. In a number of species the contribution of gastrointestinal tract, muscle and skin equals or exceeds that of the liver.

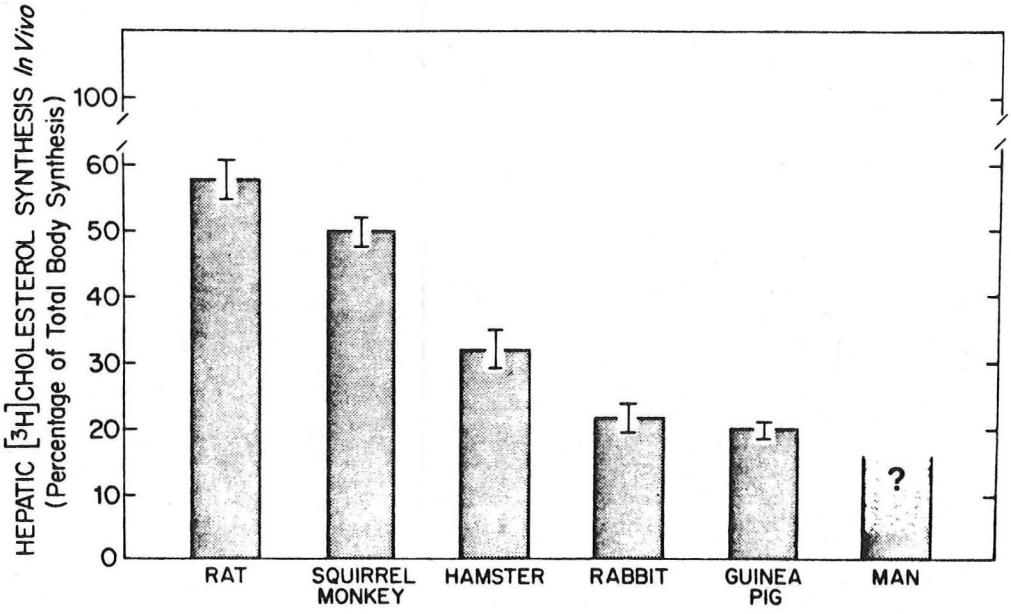


Fig. 5

CHOLESTEROL TRANSPORT THROUGH THE PLASMA

While the liver may be quantitatively less important as a site for sterol synthesis than was formerly believed, it nevertheless remains the key organ for the regulation of cholesterol balance within the intact animal. It is the liver that largely compensates for changes in cholesterol input into the body from the diet, that synthesizes various lipoprotein particles which deliver sterol to certain peripheral tissues, that takes up other lipoprotein particles carrying cholesterol from the extrahepatic tissues back to the liver, and that secretes cholesterol and bile acids from the body. The movement of cholesterol through plasma and its targeted uptake by specific tissues is largely articulated by special classes of lipoproteins interacting with specific cell-surface receptors present on the parenchymal cells of many organs. The major pathways for the transport of cholesterol among the various tissue compartments of the body are outlined in Fig. 6. The amounts of cholesterol shown entering and leaving the body pool through the various input and output pathways are representative for normal man.

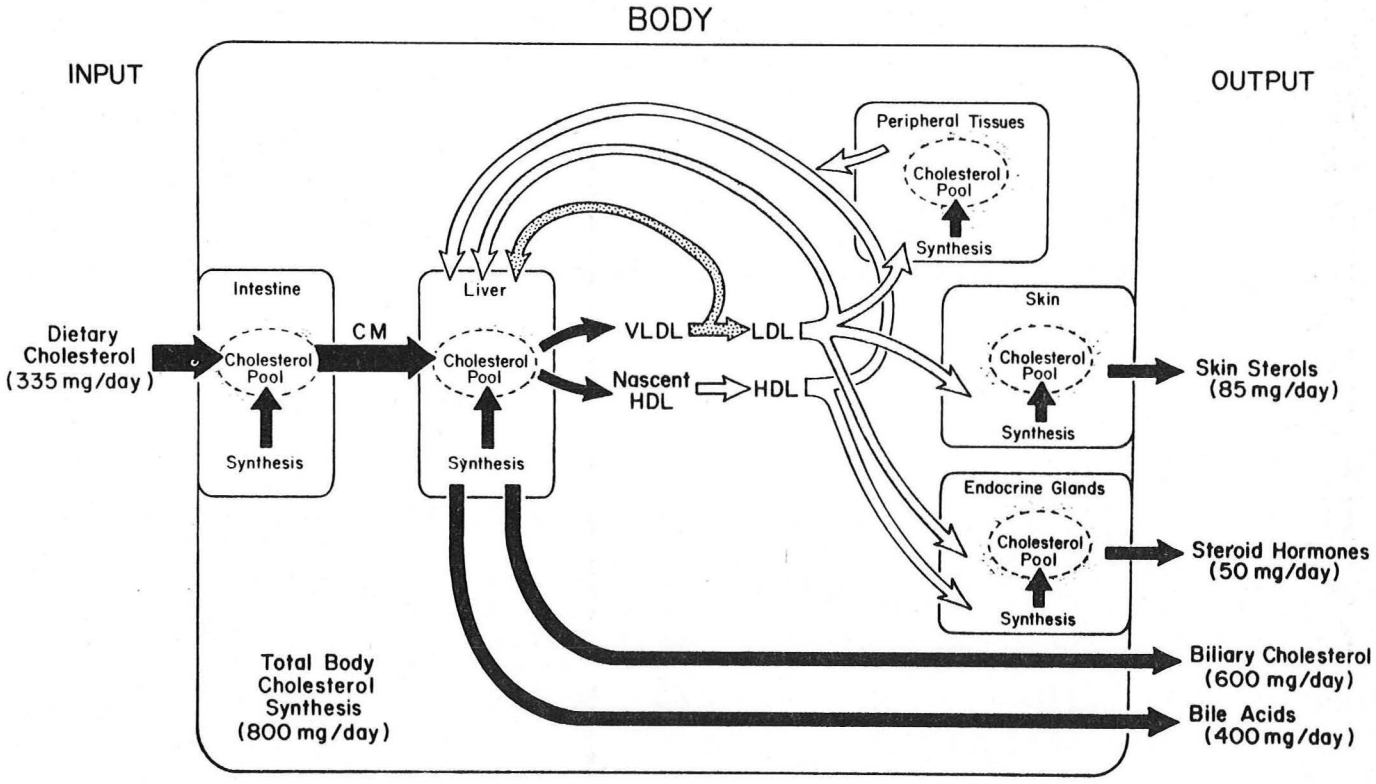


Fig. 6

Dietary cholesterol, along with endogenous cholesterol that has been secreted into the intestinal lumen in bile and other secretions, is taken up by absorptive cells located predominantly in the proximal portion of the small intestine. There it mixes with an additional pool of cholesterol that has been synthesized locally and, after being largely esterified, is incorporated into the nascent chylomicron (CM) particle. Thus, while most exogenous or dietary cholesterol enters the body carried in the CM, a significant proportion of the cholesterol present in this particle may be of endogenous origin since the amount of biliary cholesterol entering the bowel lumen or synthesized within the intestinal wall may be large compared to the amount of cholesterol available for absorption from the diet.

The nascent particle contains predominantly apoproteins A-I (apoA-I) and B (apoB). Once it enters the lymph, however, the CM acquires apoproteins E (apoE) and C (apoC) through interaction with other lipoproteins such as high density lipoproteins (HDL). This family of C apoproteins serves two important functions: first, the presence of large amounts of apoC, relative to apoE, appears to prevent uptake of the particle by the liver and, second, one component of this family, apoC-II, activates the enzyme lipoprotein lipase (LPL). This enzyme is situated on the luminal surface of capillaries found predominantly in muscle and adipose tissue and rapidly hydrolyzes much of the triglyceride present in the core of the CM. This liberates large amounts of free fatty acid that are then taken up and stored or metabolized in the adjacent tissues. As the triglyceride in the core of the CM is removed, the particle becomes smaller in size and loses some of its surface components including unesterified cholesterol, phospholipid and the apoproteins A-I and C. Presumably because of the decrease in the ratio of apoC to apoE, the partially metabolized CM, or CM remnant, is recognized by the hepatocyte and is rapidly and essentially quantitatively cleared by the liver. This uptake occurs by way of a high velocity, saturable transport system that probably depends upon the presence of receptors on the liver parenchymal cells that interact with the apoE of the remnant. Since the effective K_m for this transport process is so low, the plasma is cleared essentially completely of the particle within minutes. Thus, by this mechanism much of the cholesterol from the diet or bile that is absorbed across the intestine or that is synthesized within the bowel wall is delivered directly to the liver.

Just as the CM serves to transport triglyceride and cholesterol out of the intestine, the very low density lipoproteins (VLDL) serve a similar function in

transporting triglyceride and cholesterol out of the liver. These particles also contain apoB (although of higher molecular weight than the apoB of the CM), apoC and apoE. The triglyceride carried in VLDL is largely disposed of in peripheral tissues as this nonpolar lipid in the core of the lipoprotein is hydrolyzed by LPL and a remnant particle is formed. This remnant, like that formed by the action of LPL on the CM, is rapidly and quantitatively taken up by the liver. An alternative pathway exists for VLDL in that this particle may be metabolized through an intermediate density lipoprotein fraction to low density lipoproteins (LDL) which contain essentially only the B apoprotein. It is unclear where this transformation takes place, although the liver may be involved. In some species such as the rat, the majority of VLDL produced by the liver is metabolized through the remnant pathway while in man, a much larger proportion of the VLDL is metabolized to LDL. Hence, depending upon the species, a variable proportion of the cholesterol derived from the hepatic sterol pool and incorporated into VLDL ends up circulating in the plasma LDL fraction.

Many tissues of the body, including the liver, possess specific cell-surface receptors that recognize and bind lipoproteins containing the B and/or E apoproteins and these binding sites are referred to as LDL receptors. Tissues that contain these receptor sites bind and internalize the LDL particle and so acquire cholesterol to partially meet their metabolic needs. All tissues of the body also contain a second transport mechanism for removing LDL from the plasma that does not involved the LDL receptor and, therefore, is referred to as receptor-independent LDL transport. New techniques are now available that allow the quantitation of the importance of each of these processes in the transport of LDL out of the plasma compartment and into each organ.

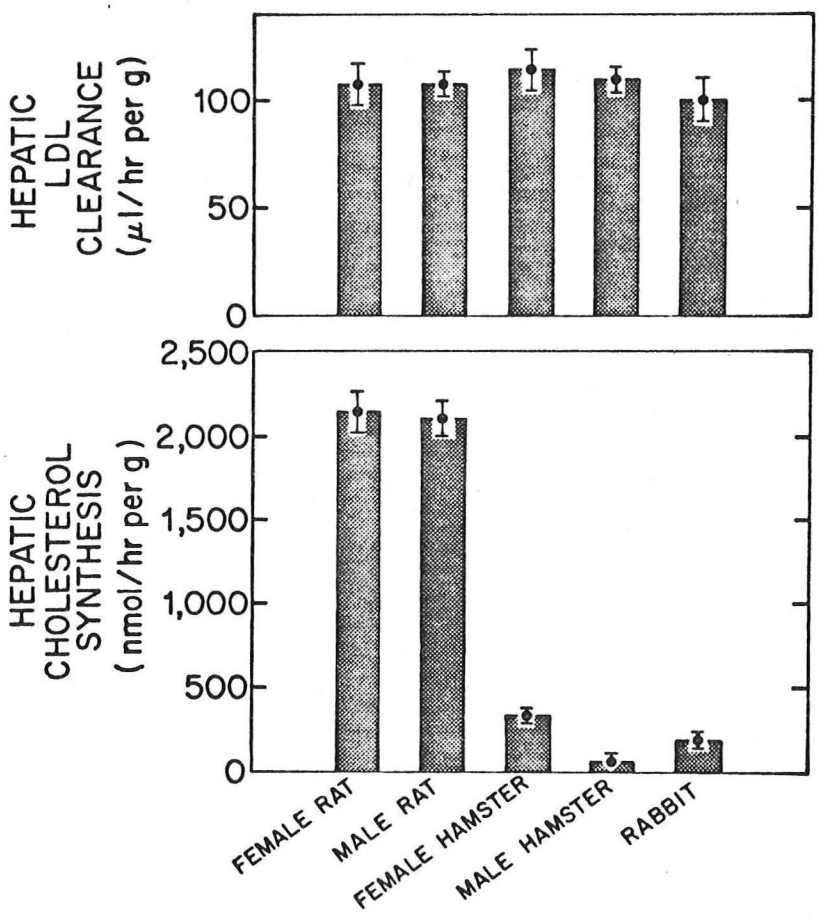
RATES OF LDL UPTAKE IN VARIOUS ORGANS

With the development of radiolabeled markers for LDL that are retained by tissues after uptake and the primed-continuous infusion technique for quantitating LDL transport into different tissues, it has become possible to measure the rates of LDL uptake in all major organs of the experimental animal in vivo. These transport rates can be measured under circumstances where the rate of uptake is linear with respect to the time of infusion, where differential losses of the radioactive label are minimized and where the circulating levels of LDL can be experimentally altered to any desired value.

Such measurements have now been made in a number of species, both male and female, including the rat, hamster, rabbit and dog, and essentially three findings have been seen in all species. First, the liver manifests a very high rate of LDL uptake with clearance rates that equal approximately 100 µl/h per g in most animals. This organ accounts for the uptake of 55-75% of all LDL cleared from the plasma in the species that have been studied thus far. The gastrointestinal tract is the only other tissue that clears significant amounts of LDL. Second, LDL uptake by most other extrahepatic organs is very low and is quantitatively of little importance. Third, unlike the other organs LDL uptake by the endocrine glands is highly variable from species to species. For example, some species such as the hamster rely primarily on cholesterol that is newly synthesized for hormone production while other species such as the rat acquire most of the cholesterol needed by this endocrine gland from the uptake of HDL-cholesterol.

Thus, unlike rates of cholesterol synthesis, there is relatively little variation among different species in the importance of the liver for the clearance of plasma LDL. This important observation is summarized by the data presented in Fig. 7. As shown in the lower panel, the rate of hepatic sterol synthesis in animals fed a low-cholesterol diet may vary as much as 50-fold (the rat compared to the male hamster, for example) yet the liver is the primary site for LDL uptake and degradation in all of these species. Thus, there is no relationship between the rate of cholesterol synthesis and the rate of

Fig. 7



LDL transport in the basal state. Indirect evidence also suggests that the liver is the primary site for LDL removal from the circulation in man.

MECHANISMS OF LDL UPTAKE IN VARIOUS ORGANS

It is now clear that there are two basic mechanisms for the removal of LDL from the plasma. These include the uptake process mediated by the LDL receptor and that transport mechanism that is independent of the LDL receptor. Since the LDL receptors in a given species do not interact well with LDL from a second species and since methylation of the LDL molecule further blocks this binding reaction, methylated human LDL (methyl-hLDL) has been used to quantitate the receptor independent component of LDL transport in the tissues of various animal species. When labeled with [^{14}C]sucrose and administered to animals as a primed-continuous infusion, the receptor independent clearance of LDL takes place in every organ at rates that vary from 1-10 μl/h per g. Only the spleen manifests a significantly higher rate of receptor independent clearance. Thus, as summarized in Fig. 8 in the case of the rat, and as has been found in all other species that have been examined, receptor independent LDL transport occurs in all organs in the body. In contrast, a large component of receptor dependent LDL transport is seen only in those organs that manifest particularly high rates of LDL uptake. These tissues include the liver, the various endocrine glands and several abdominal organs such as the kidney and small intestine. Thus, in all species that have been examined thus far, the following generalizations can be made. In tissues with high rates of LDL transport such as the liver and endocrine glands, > 90% of the LDL uptake is receptor dependent. LDL uptake in the intestinal epithelial cell, in contrast, is approximately half mediated by the receptor dependent process and half mediated by the receptor independent process. Finally, in most of the other tissues in the body, the observed low rates of LDL uptake are largely mediated by the receptor independent process.

When the rates of tissue uptake shown in Fig. 8 are multiplied by whole-organ weights, then the rates of LDL uptake by each whole organ in the body are obtained. These data are summarized in the case of the rat in Fig. 9. In this species, as well as in other animal species including man, approximately one-third of the LDL cleared from the plasma space is removed by receptor independent processes while the remainder is mediated by the receptor dependent transport system. As summarized in Fig. 8, this receptor independent LDL transport activity is distributed in many organs in the body. In contrast, the

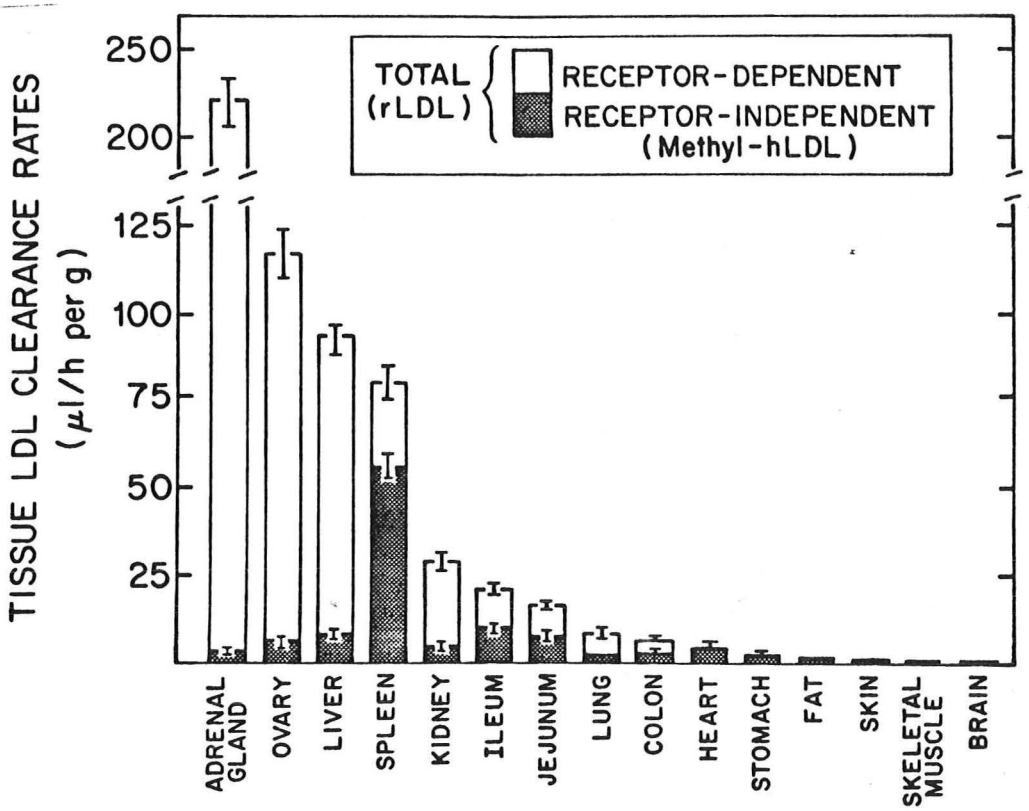

TISSUE LDL CLEARANCE RATES
(μl/h per g)
250
200
125
100
75
50
25
TOTAL (rLDL)
RECEPTOR-DEPENDENT
RECEPTOR-INDEPENDENT
(Methyl-hLDL)
ADRENAL GLAND
OVARY
LIVER
SPLEEN
KIDNEY
ILEUM
JEJUNUM
LUNG
COLON
HEART
STOMACH
FAT
SKIN
SKELETAL MUSCLE
BRAIN

Fig. 8

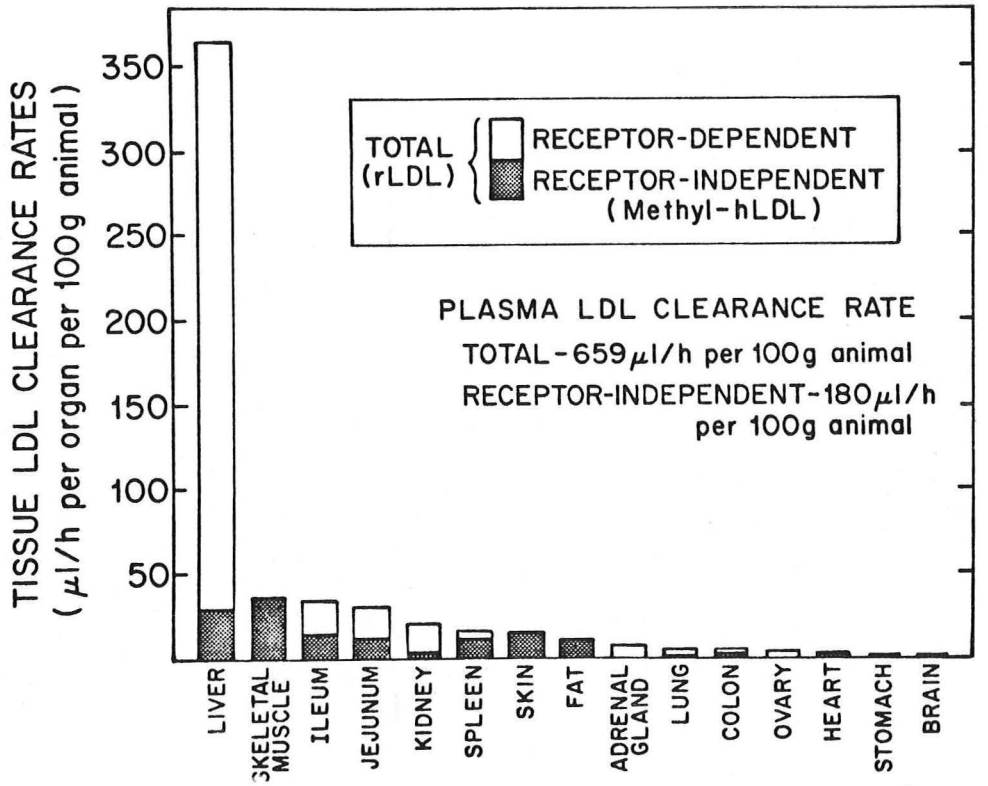

TISSUE LDL CLEARANCE RATES
(μl/h per organ per 100g animal)
350
300
250
200
150
100
50
TOTAL (rLDL)
RECEPTOR-DEPENDENT
RECEPTOR-INDEPENDENT
(Methyl-hLDL)
PLASMA LDL CLEARANCE RATE
TOTAL-659μl/h per 100g animal
RECEPTOR-INDEPENDENT-180μl/h
per 100g animal
LIVER
SKELETAL MUSCLE
ILEUM
JEJUNUM
KIDNEY
SPLEEN
SKIN
FAT
ADRENAL GLAND
LUNG
COLON
OVARY
HEART
STOMACH
BRAIN

Fig. 9

receptor dependent transport activity is localized essentially to only two tissues: these include the liver and, to a lesser extent, the small intestinal epithelium. Hence, not only does the liver and intestine in most species account for the clearance of 70-80% of the circulating LDL pool, but these same two organs contain nearly 90% of the receptor dependent LDL transport activity that can be identified in these species.

Thus, in the rat, hamster, rabbit and man, about two-thirds of the circulating LDL pool is cleared by receptor mediated means. Probably 90% of this is accounted for by transport into the liver and intestine. In contrast, about one-third of the LDL in the plasma pool is cleared by receptor independent mechanisms and this transport activity is distributed in a number of different organs other than the liver and intestine. It should also be emphasized that these two transport processes are probably localized on the same parenchymal cells in each organ although such information is only available in the case of the liver and intestine.

KINETICS OF RECEPTOR DEPENDENT AND RECEPTOR INDEPENDENT LDL TRANSPORT

These quantitative relationships, however, are true only in normal animals and man with normal circulating levels of LDL-cholesterol. Under circumstances where the plasma LDL concentration is either lowered or elevated, the relative importance of receptor dependent and receptor independent uptake in each organ changes depending upon the kinetic characteristics of each of the transport processes in that organ. By adding mass quantities of LDL to the primed-continuous infusion, it is possible to abruptly elevate the circulating plasma concentration of either the homologous LDL or the methyl-hLDL in a given species. In this manner the rate of clearance of LDL-cholesterol uptake can be measured in every organ as a function of the plasma LDL-cholesterol concentration. It should be emphasized that during the 4-6 h interval over which such measurements are made, infusion of mass quantities of LDL does not down-regulate LDL receptor activity in any tissue.

Such detailed kinetic curves are now available for a number of species and an example is shown in Fig. 10 for the liver of the hamster. As is apparent in panel A, the clearance of LDL by the receptor independent mechanism is constant at about 10 μl/h per g. Thus, the rate of uptake of LDL-cholesterol by this process is a linear function of the plasma LDL concentration, as shown in panel B. In contrast, total LDL clearance, which contains a large receptor

dependent component, decreases as the plasma LDL level is elevated and the LDL receptors become relatively more saturated. At very high plasma LDL-cholesterol levels, the rate of receptor dependent LDL transport becomes quantitatively much less important, even though there is no suppression of LDL-receptor activity. This is also illustrated in Fig. 10. The point labeled X represents the normal plasma LDL-cholesterol level in the hamster. At this concentration about 90% of hepatic LDL uptake is receptor dependent. However, if there is overproduction of LDL and the plasma LDL-cholesterol rises, the receptor independent component of total hepatic LDL uptake becomes progressively more important as shown at points Y and Z.

Another important point illustrated by this curve is that similar amounts of cholesterol can be taken up into the liver by the receptor independent process as by the receptor dependent transport system, although the plasma cholesterol level must be increased to accomplish this. For example, at a plasma LDL-cholesterol concentration of 25 mg/dl, about 30 µg/h per g of LDL-cholesterol is taken up into the normal liver by predominantly receptor-dependent transport. Essentially the same amount of cholesterol can be taken up by the receptor independent system if the plasma LDL-cholesterol concentration is raised to about 300 mg/dl (panel B, Fig. 10).

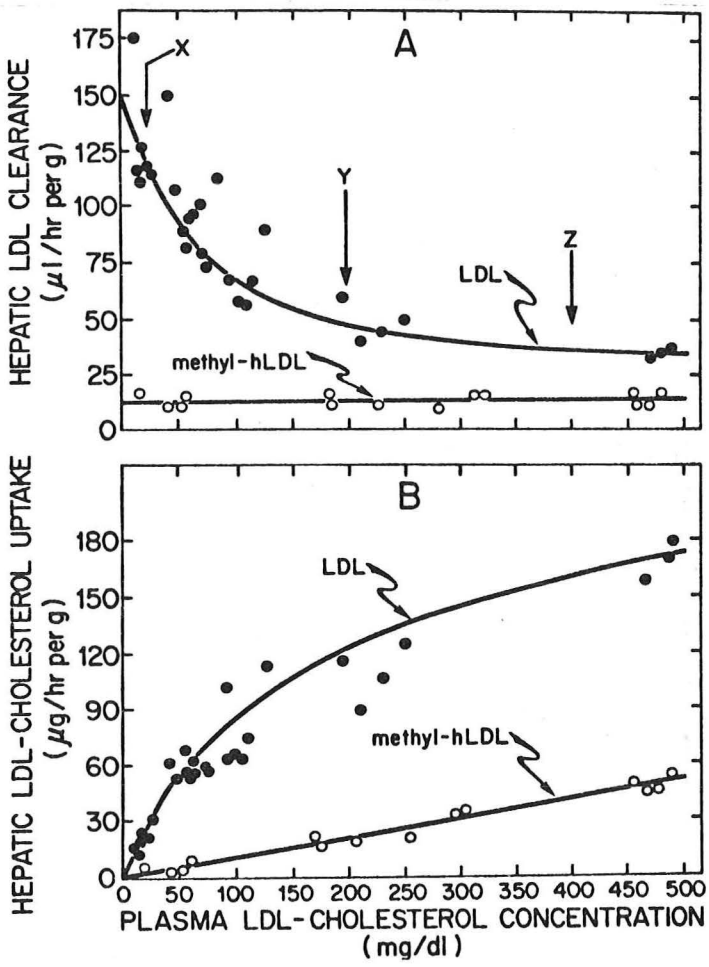


Fig. 10

That LDL-cholesterol taken up by either the receptor dependent or receptor independent process exerts similar metabolic regulation of cholesterol synthesis is illustrated by the data shown in Fig. 11. In this particular study in the rat, the liver was allowed to take up approximately 300 µg of LDL cholesterol over a 14 h period by either the receptor dependent pathway (homologous LDL, rLDL) or

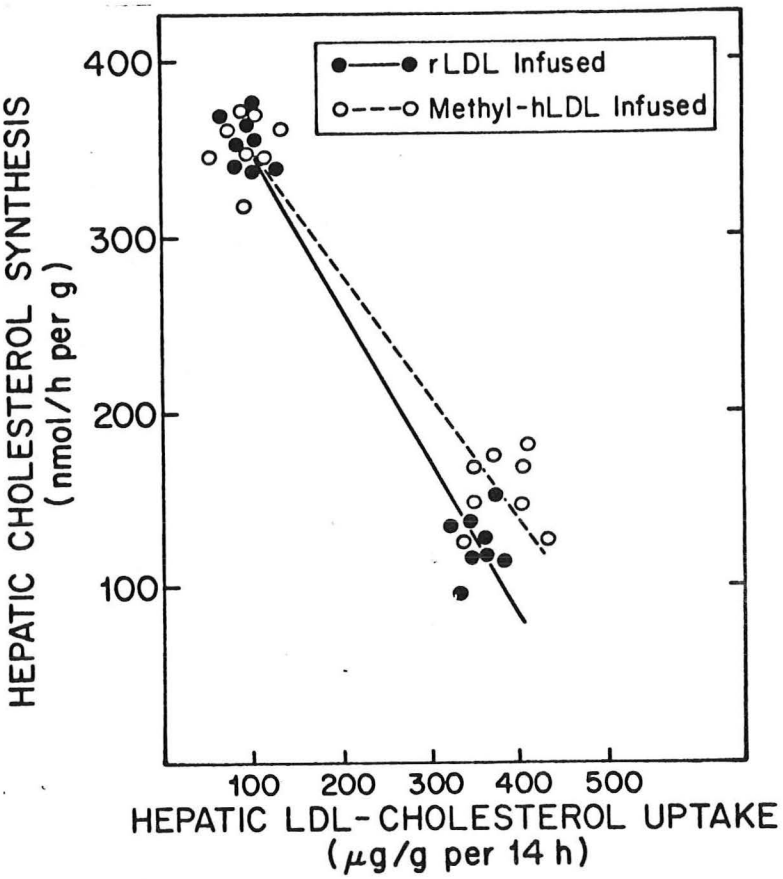

rLDL Infused
Methyl-hLDL Infused
HEPATIC CHOLESTEROL SYNTHESIS
(nmol/h per g)
400
300
200
100
100 200 300 400 500
HEPATIC LDL-CHOLESTEROL UPTAKE
(μg/g per 14 h)

Fig. 11

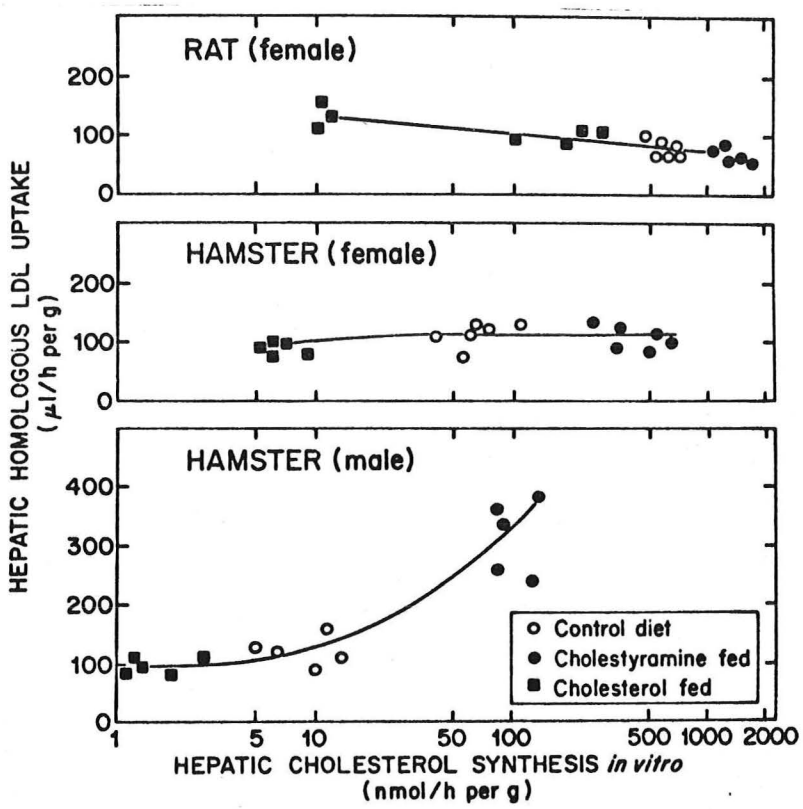

RAT (female)
HAMSTER (female)
HAMSTER (male)
HEPATIC HOMOLOGOUS LDL UPTAKE
(μl/h per g)
200
100
0
400
300
200
100
0
Control diet
Cholestyramine fed
Cholesterol fed
1 5 10 50 100 500 1000 2000
HEPATIC CHOLESTEROL SYNTHESIS in vitro
(nmol/h per g)

Fig. 12

receptor independent pathway (methyl-hLDL). As is apparent in this figure the uptake of similar amounts of LDL cholesterol by these two transport systems resulted in essentially identical amounts of suppression of hepatic cholesterol synthesis.

Thus, because of these kinetic differences in the receptor dependent and receptor independent pathways, the receptor independent transport system becomes the predominant mechanism for LDL removal from the plasma under any circumstance where there is marked overproduction of LDL or where LDL receptor activity is reduced below normal levels by genetic or environmental factors. Nevertheless, under these conditions cholesterol balance across the individual tissues and across the whole-animal is maintained, but this balance is achieved by a marked elevation in the circulating levels of LDL-cholesterol.

INTERRELATIONSHIP BETWEEN HEPATIC CHOLESTEROL SYNTHESIS AND RECEPTOR DEPENDENT LDL-UPTAKE

Receptor-independent LDL clearance is not only constant at all plasma LDL-cholesterol concentrations (Fig. 10) but, in addition, it is constant under different nutritional states and under circumstances where rates of sterol synthesis in the various organs have been changed. In contrast, the rate of receptor dependent LDL transport can, in a few experimental situations, be altered. Since the great majority of receptor dependent LDL transport demonstrated in the whole animal resides in the liver, it follows that regulation of hepatic receptor dependent LDL transport should be of greatest importance in the regulation of plasma LDL-cholesterol levels.

Unlike isolated cells, however, there is no relationship between the rate of sterol synthesis in the liver and LDL uptake. As illustrated in Fig. 12, for example, in both the rat and female hamster the rate of cholesterol synthesis in the liver can be increased or decreased over a very large range without altering the rate of LDL transport. As long as the change in sterol synthesis fully compensates for the induced change in cholesterol flux across the liver, the rate of LDL transport (and the circulating level of LDL-cholesterol) remains constant. This compensatory capacity is much lower in an animal like the male hamster that, in the basal state, synthesizes cholesterol at a rate that equals only 1-2% of the rate seen in the rat. Thus, feeding cholestyramine to this animal induces a loss of sterol from the liver that cannot be compensated for by an increase in sterol synthesis and, hence, receptor dependent (but not receptor

independent) LDL clearance increases. Receptor activity may also be suppressed by feeding this animal relatively large amounts of cholesterol (not shown in Fig. 12). Thus, whether or not a given manipulation alters hepatic LDL clearance (and circulating LDL-cholesterol levels) critically depends upon the capacity of that particular animal (or man) to compensate for the maneuver by a change in hepatic cholesterol synthesis.

REGULATION OF HEPATIC RECEPTOR DEPENDENT LDL UPTAKE

With these interrelations defined and with complete kinetic curves available for both the receptor dependent and receptor independent transport systems, it is now possible to explore quantitatively some of the major factors that regulate circulating LDL-cholesterol levels. First, however, there are several important considerations concerning whole-animal turnover data that must be understood. Fig. 13 represents the theoretical curves for LDL turnover in the whole animal or man. The two curves in the lower panel show the rate of total LDL transport out of the plasma and the receptor independent component as a function of the concentration of plasma LDL-cholesterol. In the upper panel, these same data are presented as LDL clearance rates or as the fractional catabolic rate of the LDL molecule. These curves represent the rates of whole-body LDL transport (expressed as uptake,

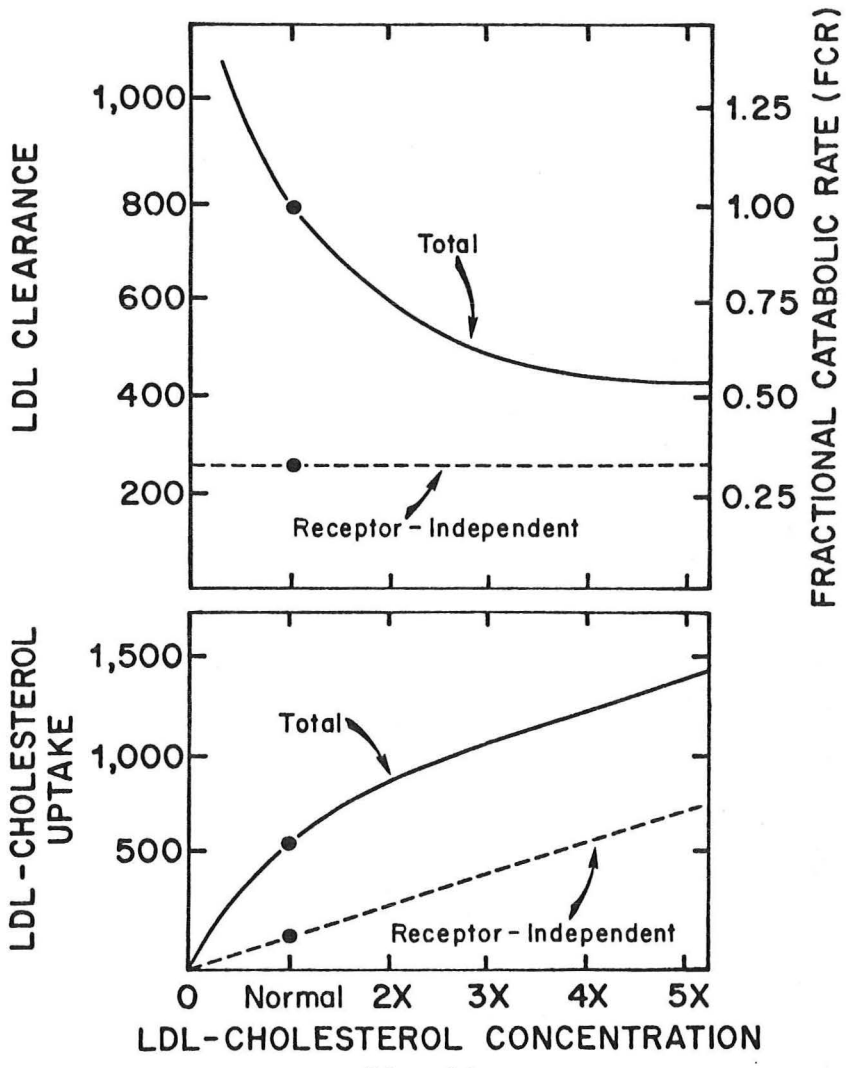


Fig. 13

clearance or FCR) as the plasma LDL-cholesterol level is varied from 0 to 5 times the normal level and under circumstances where there has been no change in LDL-receptor activity in any organ. The solid points show the situation in the normal animal or man where about two-thirds of LDL transport is receptor dependent and one-third is receptor independent. If the animal or man is subjected to a maneuver that raises the plasma LDL-cholesterol level, it necessarily follows that the clearance or FCR must decrease. Conversely, if the manipulation lowers the plasma LDL-cholesterol level, the clearance or FCR must increase. These changes take place even though there has been no change in receptor dependent LDL transport in the body. The point to be emphasized is that a change in LDL clearance or FCR alone cannot be interpreted as a change in receptor dependent LDL transport. This would also occur, for example, if the LDL production rate was altered. Data on LDL clearance or FCR can only be interpreted if superimposed upon the kinetic curves constructed for each experimental animal. If the experimental data fall above or below this curve at a particular LDL-cholesterol concentration, then there has been an increase or decrease, respectively, in the receptor dependent component of LDL transport.

LDL METABOLISM IN MAN

The values for the four parameters that define LDL transport in normal man have been determined and can be used to construct the appropriate curves that define the relationship between plasma LDL-cholesterol concentrations and variations in the transport parameters. These parameters are P^* - 0.0030 mg/h per kg per mg/dl; J_{max} - 0.78 mg/h per kg; and K_m - 90 mg/dl.

Once the normal curves describing both total and receptor independent LDL-cholesterol uptake have been defined, the relative contribution of receptor dependent uptake to total LDL-cholesterol degradation can be calculated at any given plasma LDL-cholesterol concentration. These data are shown in panel C of Fig. 14. As is apparent, at a normal plasma LDL concentration receptor dependent LDL-cholesterol uptake accounts for approximately 62% of total LDL-cholesterol degradation, a value that is somewhat lower than those reported in other species. However, this percentage figure in man was measured at plasma LDL-cholesterol concentrations of approximately 70-80 mg/dl whereas in the animal studies the plasma LDL-cholesterol level was commonly 20-25 mg/dl. As is evident in panel C, however, at a plasma LDL-cholesterol concentration in man of

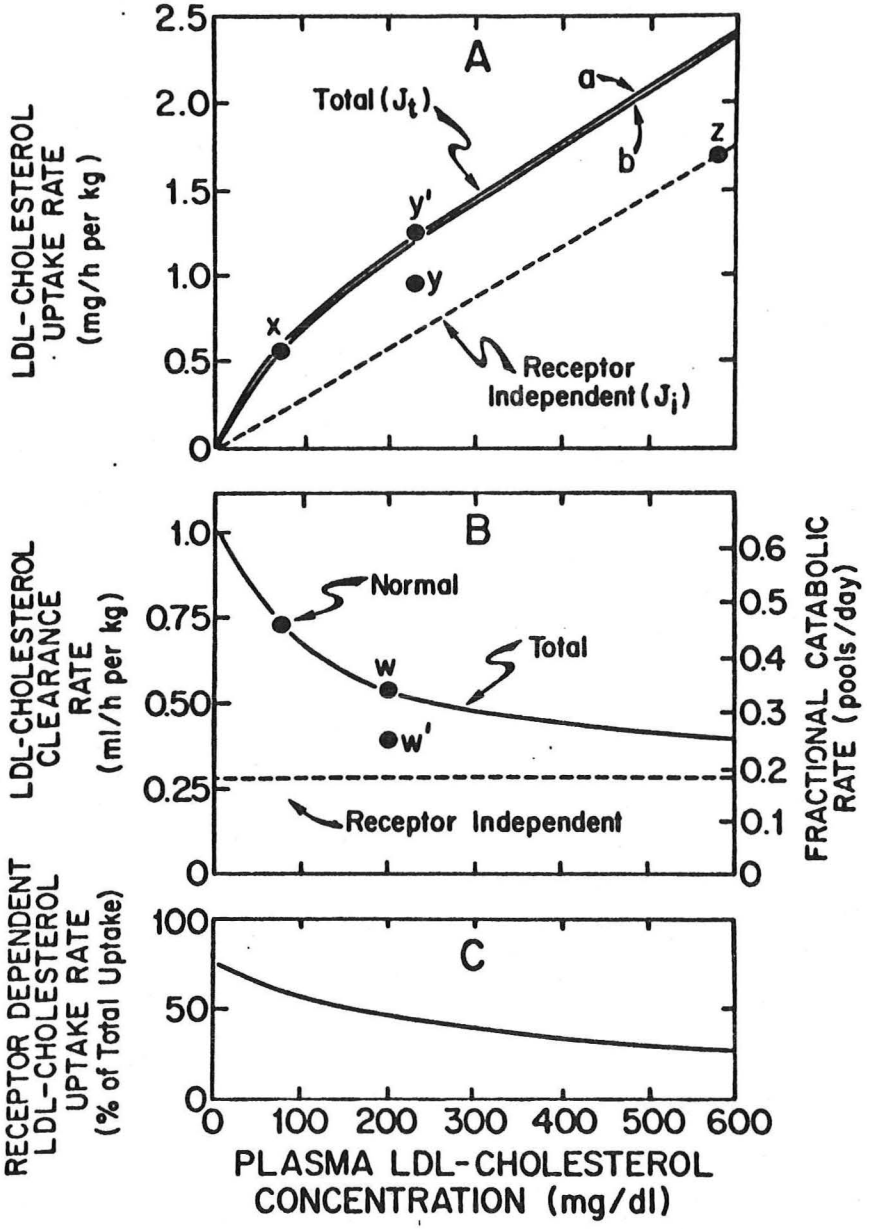

A
B
C
Total (J_t)
Receptor Independent (J_i)
a
b
x
y
y'
z
Normal
Total
w
w'
Receptor Independent
LDL-CHOLESTEROL UPTAKE RATE (mg/h per kg)
LDL-CHOLESTEROL CLEARANCE RATE (ml/h per kg)
FRACTIONAL CATABOLIC RATE (pools/day)
RECEPTOR DEPENDENT LDL-CHOLESTEROL UPTAKE RATE (% of Total Uptake)
PLASMA LDL-CHOLESTEROL CONCENTRATION (mg/dl)
0
0.5
1.0
1.5
2.0
2.5
0.25
0.50
0.75
0.1
0.2
0.3
0.4
0.5
0.6
50
100
200
300
400
500
600

Fig. 14

20-25 mg/dl, receptor dependent LDL transport would account for approximately 70% of total LDL clearance. Thus, receptor dependent LDL transport is of equal importance to overall LDL degradation in adult man as it is in the rat, hamster and rabbit. This relatively low figure of 62%, therefore, provides no evidence for down-regulation of receptor activity in the healthy, young human subjects used to construct these standard transport curves.

Having derived the kinetic parameters for LDL transport in normal man, it was next possible to use these values to construct a new set of curves that predict how the plasma LDL-cholesterol concentration will change under circumstances where there have been systematic variations in either the receptor number (J_{max}) or the rate of LDL production and degradation (J_t). Although not shown in Fig. 15, it is also possible to construct similar curves showing the relationship between the plasma LDL-cholesterol level and changes in the K_m value. Panel A of Fig. 15, for example, shows the predicted relationships between the rate at which LDL-cholesterol is produced and degraded and the plasma LDL-cholesterol concentration under circumstances where the amount of LDL receptor activity (J_{max}) has been varied from 0 to 4 times the normal value (0.78 mg/h per kg). These same

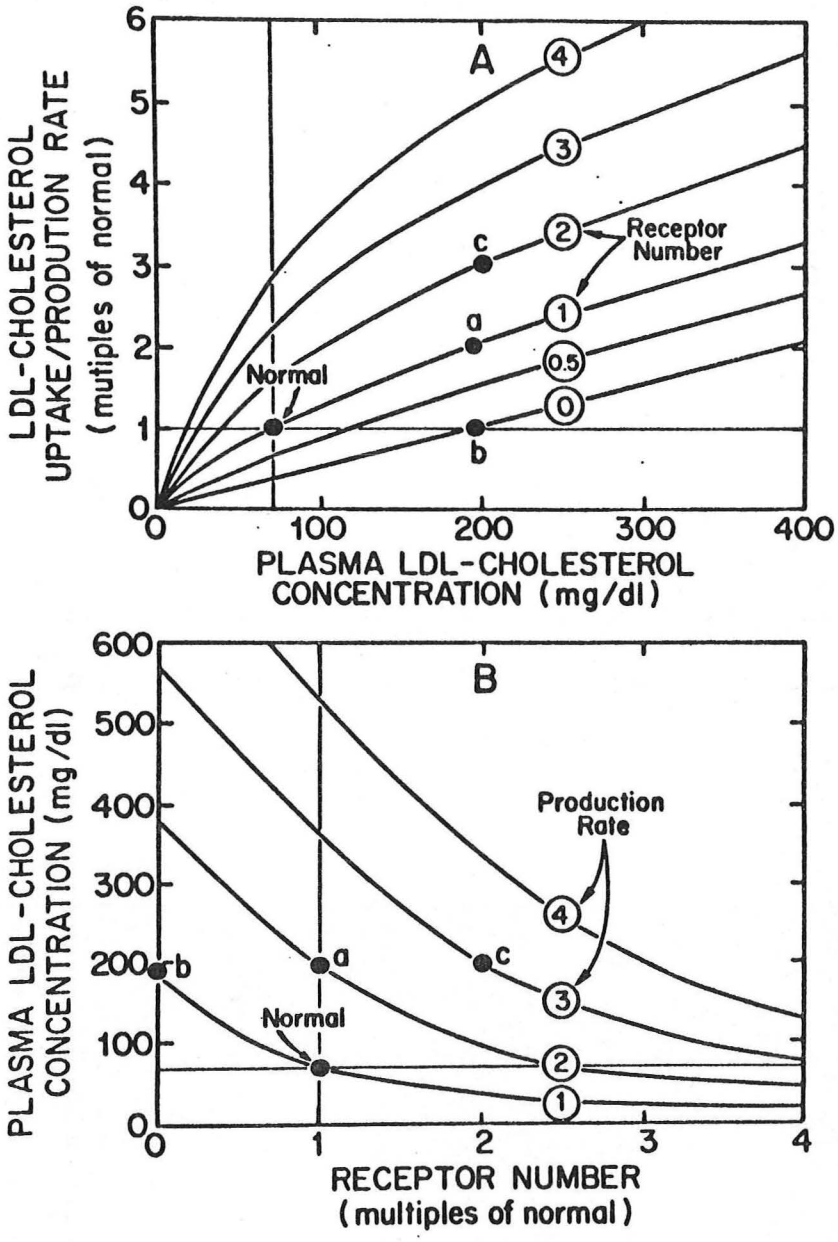


Fig. 15

data are shown in panel B except that the plasma LDL-cholesterol concentration has been plotted against receptor number (J_{max}) under circumstances where the LDL production rate has been varied from 1 to 4 times the normal value (0.55 mg/h per kg).

The values found in young subjects are also indicated in Fig. 15 along with several important examples, labeled a, b and c, that might occur in man under pathological conditions. First, point a represents the situation in an individual with a 2-fold increase in the rate of LDL-cholesterol production but a constant receptor number. As a consequence of this change, the plasma LDL-cholesterol concentration would increase from approximately 70 to 200 mg/dl. Importantly, the FCR in this patient would not remain constant but would drop from approximately 0.47 to 0.36 pools/day. In panel B this same situation is shown by vertical movement along a line of constant receptor number to the curve representing twice the normal production rate. In this format it is somewhat easier to appreciate that the receptor number remained constant. A second example is represented by point b where the production rate has been kept constant but the patient has lost all LDL receptor activity. In panel A this would be perceived as movement from the normal situation, horizonally to the curve representing 0 receptors. Alternatively, in panel B this movement would occur along the isobar of normal production, leftwards from normal to 0 receptors. An important point illustrated by this example is that total loss of all LDL receptor activity would increase the plasma LDL-cholesterol concentration only modestly to approximately 183 mg/dl. Thus, a quantitatively similar rise in plasma LDL-cholesterol levels results from either a 2-fold increase in the LDL production rate or from total loss of LDL receptor activity. A final example is illustrated by point c. In this situation the LDL-cholesterol production rate has been increased 3-fold while the amount of LDL receptor activity has been doubled. In this case, the increased receptor dependent LDL transport just compensates for the increased production rate so that the plasma LDL-cholesterol concentration in the steady-state also equals approximately 200 mg/dl.

Obviously, these curves are very useful in that they predict what any change in receptor number or production rate will do to the steady-state plasma LDL-cholesterol concentration. In the experimental animal, where each of these variables can be measured directly, such curves can be used to predict the effects of any manipulation of the system on the plasma LDL-cholesterol level.

However, in the case of human turnover studies, these curves have one additional, very important use. In such studies essentially only two pieces of experimental information are obtained, the plasma LDL-cholesterol level and the LDL-cholesterol production rate. When these two variables are plotted on the standard curves shown in Fig. 15, it is possible to read off the third variable, the receptor number in that particular subject. Thus, these curves not only provide the means for understanding the effects of various pharmacological and physiological manipulations on plasma LDL-cholesterol levels, but they also provide the basis for actually quantitating LDL receptor activity in intact man.

The utility of these kinetic curves for the interpretation of human LDL turnover data can best be illustrated by the analysis of three sets of data from the literature. The most useful graphic representations of the normal relationships that exits between the plasma LDL-cholesterol concentration, LDL-cholesterol production rate and LDL receptor number are the two formats shown in Fig. 15. Hence, these two formats are utilized for the analysis of these three sets of data, as shown in Fig. 16.

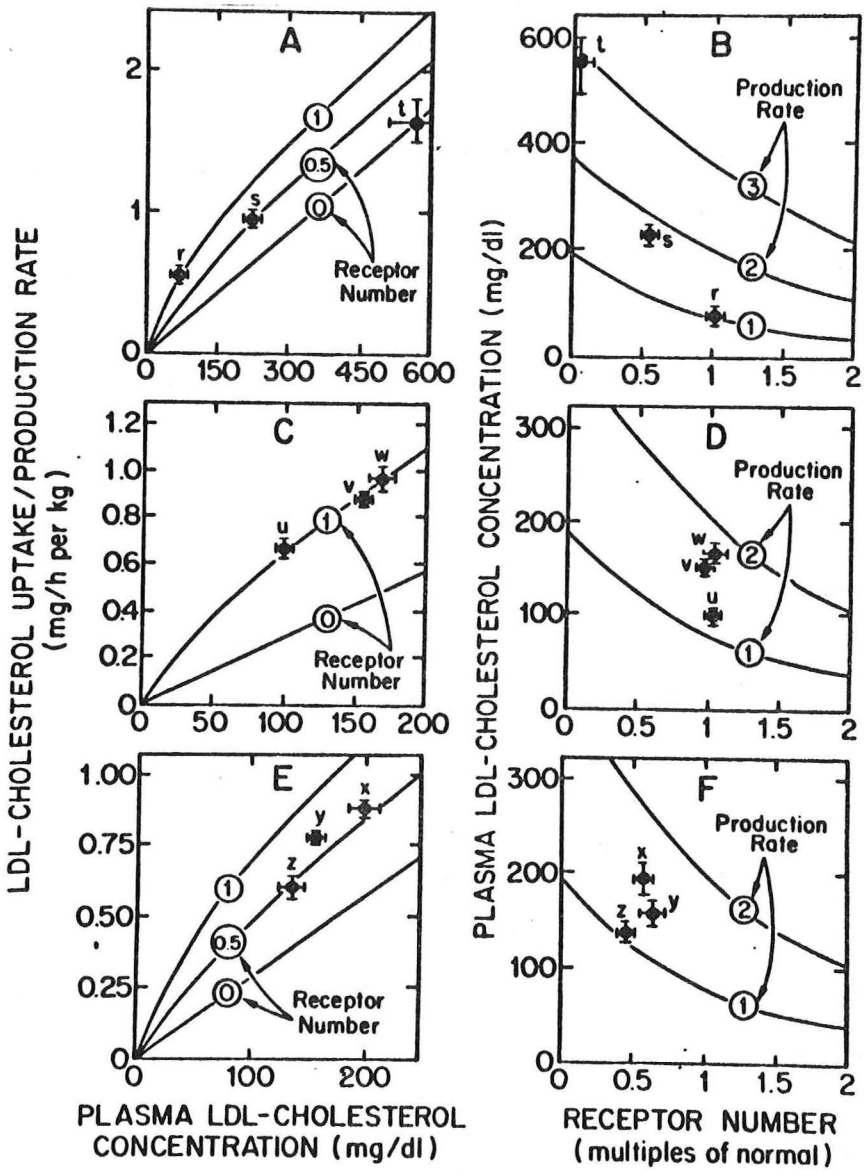


Fig. 16

Panels A and B illustrate the results obtained from turnover studies in patients who genetically lack LDL receptor activity as reported by Bilheimer,

Stone and Grundy (3). The normal, control population is represented by the point labeled r (n=6) while those patients with heterozygous familial hypercholesterolemia are shown by point s (n=6) and those subjects with homozygous disease are shown as point t (n=7). As illustrated by these plots, with loss of LDL receptor activity there is an associated increase in LDL production rates so that the patients with homozygous disease produced almost three times as much LDL-cholesterol per h per kg as the control group. It should be noted that when the two experimentally determined values in these studies, i.e., the plasma LDL-cholesterol concentration and the LDL production rate, are plotted on the standardized curves (panel B) the derived receptor number in these subjects equaled 48% of normal in the heterozygous group and 4% in the homozygous patients. These results fit closely with those that would be expected from the gene-dose effect. This analysis also provides the basis for quantitating the importance of receptor loss versus increased LDL production as the cause of the hypercholesterolemia seen in these patients. As illustrated by the standard curves, complete loss of LDL receptor activity would result in only a modest increase in the plasma LDL-cholesterol concentration, from the control value of about 70 mg/dl to 180 mg/dl (panel B). It is the 3-fold increase in production rate that further increases the plasma cholesterol concentration from 180 mg/dl to the value of 568 mg/dl observed in the patients with homozygous disease.

The second set of data analyzed in Fig. 16 involves the effect of aging on plasma LDL-cholesterol levels. While it has been recognized for some time that plasma LDL-cholesterol concentrations increase with age, there has been controversy as to whether this increase is primarily due to down-regulation of LDL receptor activity or, alternatively, is the result of overproduction of LDL-cholesterol. By pooling the results of several turnover studies performed in ostensibly normal individuals (3,12,13) and stratifying the patients on the basis of age, we have obtained three groups with which to carry out this analysis. In panels C and D the points labeled u represent a group of young individuals ranging in age from 20 to 30 y.o. with a mean age of 25 y.o. (n=25). The points labeled v represent a group of subjects ranging in age from 40 to 60 y.o. with a mean age 56 y.o. (n=22) and point w represents patients over the age 60 y.o. (n=7). As shown in panel C, with aging (u to w), there is a progressive increase in the LDL-cholesterol production rate and plasma LDL-cholesterol concentration. However, these points move along the isobar for the situation

where receptor number remains constant at the normal value. This is also shown graphically in panel D, where it can be appreciated that the only change that appears to occur with aging is an increase in LDL-cholesterol production while there is no observable alteration in LDL receptor number. Thus, at least in this series of patients ranging in age from 22 to 68 y.o., the observed increase in plasma LDL-cholesterol concentration (as well as the observed decrease in FCR) associated with aging is due entirely to an increase in LDL-cholesterol production rates.

Finally, the data in panels E and F illustrate the changes that occur in one group of patients treated to lower circulating plasma cholesterol levels. This group of subjects include 12 patients recently described by Grundy and Vega (14) all of whom had plasma cholesterol concentrations in excess of 250 mg/dl and evidence of coronary artery disease. The mean age of these patients was 59 y.o. and none had a family history suggestive of familial hypercholesterolemia. The findings in this group upon entry into the study are plotted as point x in panels E and F. It is apparent that despite the absence of a history of familial hypercholesterolemia, this group of patients was remarkably similar to the previously described group of patients with heterozygous familial hypercholesterolemia. They had similar plasma LDL-cholesterol concentrations, appeared to express approximately 50% of the normal level of LDL receptor activity and had LDL-cholesterol production rates that were elevated 1.6-fold above normal levels. It is possible that environmental factors such as diet were responsible for the changes observed in these patients. Regardless of the nature of the defect seen in these subjects, however, when this group was treated with a low dose (10 mg twice each day) (point y) or high dose (20 mg twice each day) (point z) of the drug mevinolin, the plasma LDL-cholesterol concentration declined along the isobar representing a constant receptor number. Thus, at least in this group of patients, the effect of mevinolin therapy was to reduce the LDL-cholesterol production rate to near normal values under circumstances where there was no consistent change in LDL receptor activity. In contrast to this result, in another group of patients with heterozygous familial hypercholesterolemia, mevinolin therapy lowered plasma LDL-cholesterol levels by essentially doubling the calculated receptor number under circumstances where the LDL production rate remained unchanged (15). While the physiological reason for this different response is not apparent, the use of these standard curves did allow quantitation of receptor number in these two groups of patients.

SUMMARY

The plasma LDL-cholesterol concentration in the steady-state is determined by the rate of LDL-cholesterol production relative to the rate of LDL-cholesterol removal from the plasma. This latter process, in turn, is dictated in a complex manner by the rates of receptor independent (defined by P^*) and receptor dependent LDL uptake (defined by K_m and J_{max}). In animal experiments, each of these variables can be measured directly so that the exact mechanism of an alteration in plasma LDL-cholesterol levels can be ascertained with considerable accuracy. Such measurements are not possible in man since LDL turnover studies yield data on only two of these variables, i.e., the plasma LDL-cholesterol concentration and the LDL-cholesterol production rate. However, reasonable values for P^*, K_m and J_{max} can be derived for man. Furthermore, using these values kinetic curves can be constructed that describe the relationship in normal, young human subjects between the plasma LDL-cholesterol concentration, the LDL-cholesterol production rate and the LDL receptor number. Thus, by measuring the LDL-cholesterol concentration and LDL-cholesterol production rate in any group of patients or in the same patients before and after some dietary or pharmacological manipulation, it is possible to determine indirectly LDL receptor number. Such an analysis should prove extremely valuable in more accurately assessing the mechanisms of change observed in plasma cholesterol levels in any clinical situation.

Finally, two caveats should be reemphasized. First, in constructing these curves for analysis of turnover data it would be prudent for investigators to define P^* directly in the patient population under study. While animal studies have shown the value of this parameter to be constant under a variety of conditions, it is conceivable that P^* may vary in certain patient groups. Second, while the plasma LDL-cholesterol concentration is primarily dictated by the LDL production rate and receptor number, changes in receptor affinity (K_m) may also have an effect under certain circumstances. Such K_m effects will clearly have to be defined in animal experiments or by the use of binding studies under in vitro conditions.

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