Regulation of plasma levels of low-density lipoprotein cholesterol: interpretation of data on low-density lipoprotein turnover in man

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ABSTRACT At the present time the most useful technique with which to examine the kinetics of low-density lipoprotein (LDL) cholesterol in vivo is the labeled LDL turnover study. However, a major limitation of this method is that, despite its ability to accurately measure both the plasma LDL concentration and LDL production rate, it cannot directly quantify LDL receptor activity. The present study defines the equations that describe the relationship between LDL cholesterol production rate, LDL receptor number, and plasma LDL cholesterol concentration. These equations provide a method that allows calculation of total LDL receptor activity based on the results of an LDL turnover study. With the use of this technique and data from previously published series, the effects of the genetic absence of receptors, aging, and the treatment of hypercholesterolemia with mevinolin on LDL cholesterol kinetics were analyzed.


THERE ARE NOW many excellent studies in man that examine the kinetics of low-density lipoprotein (LDL) metabolism using the labeled LDL turnover technique.1,2 However, with this method there are essentially only two experimental measurements that can be made: (1) the plasma LDL cholesterol or LDL protein concentration during the course of the study and (2) the rate at which the labeled LDL disappears from the plasma. This latter value is normally expressed in terms of a fractional catabolic rate (FCR) or the turnover rate of the LDL molecule and is usually expressed as the pools of LDL degraded per day (pools/day). When multiplied by the LDL pool size, this value gives the mass of LDL degraded each day. At steady state, when plasma LDL concentration is constant, this rate of LDL degradation must necessarily equal the rate of LDL production.

However, to understand the regulation of plasma LDL cholesterol levels in the blood, it is not only necessary to have values for the rates of LDL production but, in addition, it is essential that some measure of LDL receptor number also be available. While some investigators have attempted to equate changes in the FCR with changes in receptor number, this clearly is not valid. Since approximately two-thirds of the LDL pool is degraded by a saturable, receptor-dependent system,3,4 any event that increases the plasma LDL concentration must necessarily decrease the measured FCR. Similarly, if a manipulation lowers the plasma LDL concentration, the measured FCR values must increase. It should be emphasized that both of these changes occur even though the receptor number in the whole animal or in man in a particular situation may have remained constant. These changes in FCR merely reflect changes in the degree of saturation of the receptor-dependent component of LDL degradation.

Thus, to make any inferences concerning changes in receptor number from a turnover study, it is first necessary to quantitate the changes that occur in the FCR with alterations in plasma LDL cholesterol concentrations under conditions in which the receptor number remains constant. Any alteration in the observed FCR that is greater than predicted from these data can then be used to make valid inferences about receptor number. Complete kinetic analysis of these relationships has now been carried out in the rat, hamster, and rabbit.5-8. While such detailed studies cannot be undertaken in man, the purpose of this article is to report the

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basic transport parameters that describe LDL degradation in normal human subjects and thus the two essential experimental measurements, i.e., the plasma LDL cholesterol concentration and metabolic production rate, can then be used to define changes in receptor number in intact man under a variety of physiologic and pharmacologic manipulations.

Methods

All data used in this study came from two sources. The first group of experimental data was obtained from animal studies performed in this laboratory and previously published. These are presented in this report in abbreviated form to illustrate important concepts. The second group of data from human LDL turnover studies was obtained from previously published series and, in each case, the source of these data is appropriately referenced.

In intact man, as well as in a variety of experimental animals, approximately two-thirds of the removal of LDL from the plasma space takes place by receptor-dependent transport. The rates of uptake by this process can be described by classic Michaelis-Menten kinetics, and therefore the rate of receptor-dependent uptake ($J_d$) is defined by the following relationship:

$$J_d = \frac{(J_{max})(C)}{K_m + C}$$

(1)

where $J_{max}$ represents the maximal achievable transport rate, $K_m$ is the concentration required to achieve half of this maximal transport rate, and $C$ equals the concentration of LDL in the plasma. $J_{max}$ reflects receptor number and in this study is expressed as the micrograms or milligrams of LDL cholesterol transported by the receptor-dependent process per hour per gram of tissue. In the case of the whole hamster or man this rate is normalized to 100 g (hamster) or 1.0 kg (man) body weight. $K_m$ presumably reflects the affinity of the LDL receptor for the LDL particle and is expressed as the milligrams of LDL cholesterol per deciliter of plasma. The same units are used for $C$, the concentration of LDL cholesterol in the plasma.

Receptor-independent uptake ($J_i$) is a linear function of the concentration of the particle in plasma so that this process is described by the equation:

$$J_i = (P^*) (C)$$

(2)

where $P^*$ is the proportionality constant of this relationship and is analogous to a membrane permeability coefficient. In this study $P^*$ is expressed as the micrograms or milligrams of LDL cholesterol that are taken up per hour per gram of tissue when $C$ equals 1.0 mg/dl. Alternatively, in the whole animal or man the data are again normalized to a body weight of either 100 g (hamster) or 1.0 kg (man).

In the case of LDL cholesterol uptake and degradation, both receptor-dependent and receptor-independent transport occur simultaneously so that equations 1 and 2 must be combined and this yields the relationship that describes the rate of total LDL cholesterol uptake and degradation ($J_t$) as a function of the plasma LDL cholesterol concentration:

$$J_t = \frac{(J_{max})(C) + (P^*) (C)(K_m) + (P^*) (C^2)}{K_m + C}$$

(3)

Two features of this equation should be emphasized. First, equation 3 is correct only under the special circumstance in which the concentration of LDL cholesterol at the site of the LDL receptors is essentially identical to the measured concentration in the plasma. If a significant diffusion barrier were to exist between the plasma and the parenchymal cell a more general form of the equation would have to be used. The general form of this equation has been previously described. In using it to analyze the kinetics of LDL transport in both the rat and hamster, however, we found that the resistance of the diffusion barrier was essentially equal to 0, and therefore equation 3 is applicable. Second, at steady state ($J_t$) must equal the metabolic production rate of LDL cholesterol. Thus, equation 3 can be rewritten to give the steady-state concentration of plasma LDL cholesterol under circumstances in which there has been a change in $J_t$, a change in LDL receptor number ($J_{max}$), or a change in the receptor affinity for the LDL particle ($K_m$). In this report, however, only changes in $J_t$ and $J_{max}$ will be considered, although it is very possible that changes in LDL structure may have important effects on $K_m$ values under certain circumstances.

In practice, data from studies in man were fitted to equation 3 by true nonlinear regression analysis with the use of a computerized least squares fitting algorithm based on a previously published method. Initial values for parameters in the transport equations were first estimated from the shape of the experimental curves. By an iterative technique, the computer program then converged toward values for each of the parameters that minimized the sum of errors squared. The iterative procedure was stopped when the change made in all parameters was less than 1% of their previous value. An adjusted $r^2$ value was calculated to define the "goodness of fit" of the final curve to the experimental data. The variance in all parameters is expressed as $\pm 1$ SEM.

Results

To construct the kinetic curves that describe the relationship between the plasma LDL cholesterol concentration, LDL production, and receptor-dependent and independent LDL degradation, it is necessary to have experimental values for $J_{max}$, $K_m$, and $P^*$ for each organ in the body. After adjustment for organ weight, such values can then be summed to give the appropriate kinetic curves that describe the characteristics of LDL turnover in the whole animal. Such curves are now available for the hamster, rat, and rabbit and one such set of data is presented here to illustrate the methods used to construct such "whole animal" kinetic curves for man.

In the experimental animal, the plasma LDL cholesterol concentration can be immediately elevated and maintained at any value with the use of a primed/continuous infusion of homologous LDL. After this manipulation, $J_t$ into each organ can be assessed by measurement of the accumulation of radiolabeled homologous LDL over time. The receptor-independent component of this uptake process can also be assessed with use of methylated heterologous LDL, which is not recognized by the LDL receptor.

When the appropriate regression curves are fitted to such data for determination of either total LDL uptake

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*Spady DK: Unpublished observations.*
(equation 3) or receptor-independent LDL transport (equation 2), the best-fit kinetic curves are obtained for each organ. Examples of such curves are shown in figure 1 for four organs of the hamster. The curves in panel A show the relationship between the plasma LDL cholesterol concentration and total LDL cholesterol uptake per gram of liver, adrenal gland, intestine, and kidney. Figure 1, B, shows the receptor-independent component of this uptake process in these same organs. In addition to fitting the curves to the experimental data, the computer program used for this fitting process also yields values for each of the variables in the equations. Thus, for example, there were marked differences in the rate of receptor-dependent LDL transport (reflecting differences in receptor number) in these tissues, as revealed by the \( J_{\text{max}} \) values of 126, 55, 17, and 6 \( \mu g/hr/g \), respectively, in the liver, adrenal gland, intestine, and kidney. The variation in receptor-independent LDL transport was much less, as indicated by the fact that the values of \( P^* \) in these same organs varied less than twofold.

After these rates of total and receptor-independent LDL transport in all organs were multiplied by the appropriate organ weights, the sum of the values yielded the rate of LDL transport into all of the organs in the whole animal at any plasma LDL cholesterol concentration. Such curves are shown for the hamster weighing 100 g in figure 2, A. These curves represent the rates of both total and receptor-independent LDL removal from the plasma under circumstances in which the plasma LDL cholesterol concentration was varied from 0 to 500 mg/dl and in which \( J_{\text{max}} \) was kept constant. As is apparent, the rate of removal of plasma LDL cholesterol by the receptor-independent process increased linearly with the plasma LDL cholesterol concentration. This relationship, however, was curvilinear for total LDL cholesterol uptake, reflecting the saturable nature of the receptor-dependent transport process that is included in the total transport figures. It should be noted that the rate of \( J_t \) equals the rate of LDL cholesterol degradation, which at steady state equals the rate of LDL cholesterol production.

These same data can also be plotted as clearance values (ml of plasma cleared/hr/100 g animal) or as FCRs (pools/day) as a function of the plasma LDL cholesterol level. Such curves are shown in figure 2, B, and graphically illustrate the decline in the clearance rate or FCR that occurs with elevation of the plasma LDL cholesterol concentration even when the \( J_{\text{max}} \) is kept constant. For example, a normal hamster with a plasma LDL cholesterol concentration of 22 mg/dl had an LDL cholesterol uptake (degradation/production) rate of 128 \( \mu g/hr/100 g \) animal and a FCR of 3.41 pools/day (point x). Increasing LDL cholesterol production to 587 \( \mu g/hr/100 g \) resulted in a fall in the FCR to 1.71 pools/day (point y). This occurred because of progressive saturation of the receptor-dependent transport process, even though \( J_{\text{max}} \) was constant. As shown in figure 2, C, this in turn is accompanied by a decline in the fraction of total LDL cholesterol uptake that is due to the receptor-dependent pathway from 75% to 57%, since the receptor-independent

**FIGURE 1.** Kinetic curves for LDL transport in the hamster. A. The relationship between total \( (J_t) \) LDL transport into various tissues of the hamster and the plasma LDL cholesterol concentration. B. The same relationship for the receptor-independent component \( (J_r) \) of total LDL uptake. These data were obtained from a study of 28 animals, as described in Spady et al., and the lines represent the curves fitted to the individual data points. In these examples both \( J_t \) and \( J_r \) are expressed in terms of the micrograms of LDL cholesterol taken up and degraded per hour per gram of tissue.
pathway, unlike the receptor-dependent route, is not saturable.

Thus, such curves illustrate the complex relationship that exists in this experimental animal between the plasma LDL cholesterol concentration and the rate of LDL uptake (degradation), even under circumstances in which \( J_{ma} \) is kept constant at 485 \( \mu g/hr/100 \ g \), the mean value of \( K_m \) is fixed at 91 mg/dl, and \( P^* \) equals 1.23 \( \mu g/hr/100 \ g \) per mg/dl. This relationship becomes even more complex once changes are introduced into the receptor number, as can be done, for example, by altering the intake of dietary cholesterol, triglyceride, or bile acids.\(^{10, 11}\)

Obviously, experimental curves such as these in which the kinetic relationships in the whole animal between plasma LDL cholesterol levels, LDL production rates, and LDL receptor activity were derived from transport curves quantitated in individual organs cannot be obtained in man. However, because of the nature of several parameters in the equations, it has become apparent that these curves can be constructed for man with the use of values from published turnover data and several assumptions that have proven to be correct in animal experiments. The steps involved in the construction of such LDL transport curves for man are illustrated in figure 3.

The first parameter of the equations that can be fixed is the proportionality constant for the receptor-independent component (\( P^* \)). Since receptor-independent transport in the whole animal is linear with respect to

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**FIGURE 2.** Kinetic curves for LDL transport (degradation) in the whole hamster. The two curves shown in A represent the summation of the rates of total and receptor-independent LDL cholesterol uptake by 14 major organs of the hamster. These curves represent the micrograms of LDL cholesterol taken up and degraded each hour in the whole animal and are normalized to a body weight of 100 g. B. These same curves expressed as either LDL cholesterol clearance rates or as FCRs. C. The percentage of total LDL degradation in the hamster that is receptor dependent as a function of the plasma LDL cholesterol concentration. The significance of the points labeled \( x \) and \( y \) is described in the text.

**FIGURE 3.** Derivation of the kinetic curves for LDL transport (degradation) in normal man. A. The results of deriving the kinetic curves for LDL transport in man by two separate methods (curves a and b). B. The average of these same curves is expressed in terms of clearance rates or FCRs at different plasma LDL cholesterol concentrations. C. The percentage of total LDL transport that is receptor dependent at different concentrations of plasma LDL cholesterol. The significance of the specific points labeled \( w \), \( x \), \( y \), and \( z \) is discussed in the text.
the plasma LDL cholesterol concentration (see figure 2 and Spady and colleagues\textsuperscript{5, 10}), the magnitude of this variable can be determined in man with a derivative of LDL that does not interact with the LDL receptor or, alternatively, it can be measured directly with native LDL in patients who lack LDL receptor activity. In such patients, the rate of LDL cholesterol production and removal from the plasma averages approximately 1.7 mg/hr/kg body weight and the mean plasma LDL cholesterol concentration equals 568 mg/dl.\textsuperscript{3} These data are plotted as point z in figure 3, A. From these data the value of P* in man can be calculated to equal 0.0030 mg/hr/kg per mg/dl. Furthermore, the average FCR in such patients equals about 0.18 pool/day, which corresponds to a clearance rate of LDL cholesterol equal to 0.30 ml/hr/kg. Since all three of these variables are independent of the plasma LDL cholesterol level, the kinetic curves defining receptor-independent transport in man can be firmly fixed and are shown in figure 3, A and B.

There are two methods available with which to construct the curves defining total LDL transport in man. With the first method it is possible to fix three points along the curve that relates total LDL cholesterol uptake to plasma LDL cholesterol concentration. The first of these points is the origin and the second reflects the values found in normal man. For this second point, data from a group of young men in whom the mean LDL cholesterol uptake (degradation) rate was reported to equal 0.55 mg/hr/kg and the average plasma LDL cholesterol concentration equaled 76 mg/dl (shown as point x, figure 3, A) were used.\textsuperscript{3} The third point was obtained from published data in patients with heterozygous familial hypercholesterolemia.\textsuperscript{3} In such patients an average LDL cholesterol uptake (degradation) of 0.95 mg/hr/kg was reported at a mean plasma LDL cholesterol concentration of 230 mg/dl (point y, figure 3, A). If the heterozygotic patient expresses only 50\% of the normal complement of LDL receptors, then point y should be midway between the point defined by the receptor-independent uptake curve at a plasma LDL concentration of 230 mg/dl and the point on the normal human curve constructed with 100\% of the receptor complement. This third point can therefore be fixed and is labeled y'. The shape of the LDL uptake curve is dictated by equation 3 and must pass through these three points. The value of P* in the equation has been firmly fixed and equals 0.0030 mg/hr/kg per mg/dl so that there are only two remaining parameters, K_m and J_{max}, to be derived. In two other mammalian species, it has previously been established that the K_m value for the LDL receptor in most organs falls within a narrow range, with an average value of approximately 90 mg/dl.\textsuperscript{6} Thus, assuming that this value is also correct for man, one can then calculate that the value of J_{max} is equal to approximately 0.80 mg/hr/kg. From these parameters, then, it is possible to draw the complete curve that describes the relationship between the plasma LDL cholesterol concentration and the rate of LDL cholesterol uptake (degradation) in normal man. This curve is labeled a in figure 3, A.

The second method available to derive this curve obviates the necessity of assuming a value for K_m. To fit a curve whose shape is dictated by equation 3 to a set of experimental data by nonlinear regression methods, the data set must contain measured LDL production rates over a relatively wide range of plasma LDL cholesterol concentrations. Three groups of patients were selected from the literature for inclusion in this analysis. These included six young, healthy subjects originally reported by Bilheimer et al.\textsuperscript{3} and an additional group of 19 young men that were considered by Grundy et al.\textsuperscript{12} to also represent healthy young subjects. In these two groups of patients, LDL cholesterol concentrations ranged from 50 to 154 mg/dl and the LDL cholesterol production rates varied from 0.44 to 0.91 mg/hr/kg. A third group of subjects also was included that had heterozygous familial hypercholesterolemia.\textsuperscript{3} The uptake rates observed in these patients were corrected to those values that would be anticipated if such individuals expressed 100\% of their receptor number (as described above). Nonlinear regression analysis of the data from these 31 patients was then performed with the established value of 0.0030 mg/hr/kg per mg/dl for P*. The curve that best fitted these data is labeled b in figure 3, A. The best-fit value derived from this analysis for K_m equaled 88.2 ± 16.0 mg/dl and that for J_{max} equaled 0.76 ± 0.06 mg/hr/kg.

It is apparent that these two methods yielded essentially identical kinetic curves (a and b) and essentially identical absolute values of K_m and J_{max} for receptor-dependent LDL transport in vivo in man. Thus, in summary, this analysis suggests that the kinetic curves describing both receptor-dependent and receptor-independent LDL transport (degradation) in man can be described with the following set of average parameters: P* = 0.0030 mg/hr/kg per mg/dl; J_{max} = 0.78 mg/hr/kg; and K_m = 90 mg/dl.

These same curves are also shown in figure 3, B, expressed either in terms of LDL cholesterol clearance or as FCRs. It is important to note that the curve describing total LDL cholesterol turnover again represents the situation in which J_{max} has been kept constant so that the decline seen in the FCR with increasing
concentrations of plasma LDL cholesterol represents progressive saturation of the receptor-dependent transport system. For example, an increase in the plasma LDL cholesterol level from the normal value of about 70 to 200 mg/dl (point w) must be associated with a fall in the FCR from about 0.47 to 0.36 pool/day.

Finally, once these 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 figure 3, C. 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 to 80 mg/dl, whereas in the animal studies the plasma LDL cholesterol level was commonly 20 to 25 mg/dl. As is evident in figure 3, C, however, at a plasma LDL cholesterol concentration in man of 20 to 25 mg/dl, receptor-dependent LDL transport would account for approximately 70% of total LDL clearance. Thus, the importance of receptor-dependent LDL transport to overall LDL degradation is equal in adult man and in the rat, hamster, and rabbit. This relatively low figure of 62%, therefore, provides no evidence of downregulation of receptor activity in the healthy young human subjects from whom data were obtained 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, based on equation 3, that predict how the plasma LDL cholesterol concentration will change under circumstances in which there have been systematic variations in either $J_{\text{max}}$ or $J_t$. Although not shown in figure 5, it is also possible to construct similar curves showing the relationship between the plasma LDL cholesterol level and changes in the $K_m$ value. Figure 4, A, 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 in which the $J_{\text{max}}$ has been varied from 0 to 4 times the normal value (0.78 mg/hr/kg). These same data are shown in figure 4, B, except that the plasma LDL cholesterol concentration has been plotted against $J_{\text{max}}$ under circumstances in which the LDL production rate has been varied from 1 to 4 times the normal value (0.55 mg/hr/kg).

![Figure 4: Relationship between plasma LDL cholesterol concentration and the production rate and receptor number. A, The relationship between the LDL cholesterol production rate and the plasma LDL cholesterol concentration under circumstances in which the receptor number in the body is varied from 0 to 4 times the normal value. In this case, the normal value for receptor number was defined as that number that would account for the maximal transport ($J_{\text{max}}$) of 0.78 mg of LDL cholesterol per hour per kilogram. B, The relationship between the plasma LDL cholesterol concentration and LDL receptor number under circumstances in which the LDL production rate was varied from 1 to 4 times the normal value. In this case the normal production rate equaled 0.55 mg of LDL cholesterol per hour per kilogram. The results in normal young subjects are shown as a single point. The significance of the points labeled a, b, and c is discussed in the text.](http://circ.ahajournals.org/)

The values found in young subjects are also indicated in figure 4, along with several important examples (labeled a, b, and c) that might occur in man under pathologic conditions. First, point a represents the situation in an individual with a twofold increase in the rate of LDL cholesterol production but a constant receptor number. As a consequence of this change, the plasma LDL concentration would increase from 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 pool/day. In figure 4, 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: here the production rate has been kept constant but the patient has lost all LDL receptor activity. In figure 4, A, this would be perceived as horizontal movement from the normal situation to the curve representing 0 receptors. Alternatively, in figure 4, B, this movement would occur along the isobar of normal production leftward 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 level results from either a twofold 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 threefold 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 at 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 at the steady state to the plasma LDL cholesterol concentration. In the experimental animal, in which 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 figure 4, 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 pharmacologic and physiologic manipulations on plasma LDL cholesterol levels, but they also provide the basis for actually quantitating LDL receptor activity in intact man.

Discussion

The kinetics of LDL degradation, as with any particle transported out of the plasma by both receptor-dependent and independent pathways, is defined by three parameters: $K_m$, $J_{max}$, and $P^*$. In the experimental animal it is relatively straightforward to define these parameters in each individual organ, as well as in the whole animal. In man, however, the problem is somewhat more difficult. In this study, using methods defined in experimental animal preparations and data from human LDL turnover studies, we have derived reasonable estimates for the value of each of these parameters in the normal human subject. While we believe that these values are accurate, two important points regarding their derivation should be recognized.

First, the parameter most easily calculated is that of $P^*$, the proportionality constant for receptor-independent LDL transport, since this value is derived directly from the LDL production rate and plasma LDL cholesterol concentration found in patients with homozygous familial hypercholesterolemia. This calculation, however, assumes that all LDL degradation occurs by the receptor-independent pathway in such patients and that this identical pathway exists in the normal population. There is now considerable evidence in both man and experimental animals that this assumption is correct. Furthermore, in animals in which receptor-independent LDL degradation can be measured directly with methylated heterologous LDL preparations, there is also good evidence that the rate of LDL degradation is directly proportional to the plasma LDL cholesterol concentration over a wide range, and therefore it is valid to use a single well-defined point on this line to calculate $P^*$. Second, the value of $K_m$ for the receptor-dependent LDL transport process was derived from turnover studies in man, but the fact that this derived value of about 90 mg/dl is nearly identical to the values determined directly in two other mammalian species in vivo provides support for its validity.

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 exist between the plasma LDL cholesterol concentration, LDL cholesterol production rate, and LDL receptor number are the two formats shown in figure 4. Hence, these two formats are used for the analysis of these three sets of data, as shown in figure 5.

Figure 5, A and B, illustrates the results obtained from turnover studies in patients who genetically lack LDL receptor activity, as reported by Bilheimer et al. The normal control population is represented by the point labeled $r$ ($n = 6$), while those patients with homozygous familial hypercholesterolemia are repre-
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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 to 180 mg/dl (figure 5, B). It is the threefold 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 figure 5 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 downregulation 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 and stratifying the patients on the basis of age, we have obtained three groups with which to carry out this analysis. In figure 5, C and D, the points labeled u represent a group of young individuals ranging in age from 20 to 30 years with a mean age of 25 years (n = 25). The points labeled v represent a group of subjects ranging in age from 40 to 60 years with a mean age 56 years (n = 22), and point w represents patients over the age 60 years (n = 7). As shown in figure 5, 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 in which receptor number remains constant at the normal value. This is also shown graphically in figure 5, 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 years, 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 figure 5, E and F, illustrate the changes that occurred in one group of patients who received treatment to lower circulating plasma cholesterol levels. This group of subjects included 12 patients recently described by Grundy and Vega, 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 years, and none had a family history suggestive of familial hypercholesterolemia. The findings in this group on entry into the

FIGURE 5. Representative data for LDL production rates and LDL receptor number in three different clinical situations. A and B. The experimental data obtained in control subjects (r) and in patients with heterozygous (s) and homozygous (t) familial hypercholesterolemia. C and D. Published data for different groups of normal subjects whose ages were 20 to 30 (u), 40 to 60 (v), and >60 years (w). E and F. Data obtained in a group of hypercholesteremic patients (x) treated with either low (y) or high (z) doses of mevinolin.
study are plotted as point x in figure 5, 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 iso-
bar representing a constant receptor number. Thus, at least in this group of patients, the effect of mevinolin was to reduce the LDL cholesterol production rate to near-normal values under circumstances in which there was no consistent change in LDL receptor activity. In contrast to this result, in another group of patients with heterozygous familial hypercholesterolemia, mevinolin lowered plasma LDL cholesterol levels by essentially doubling the calculated receptor number under circumstances in which the LDL production rate remained unchanged. While the physiologic 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.

In summary, the plasma LDL cholesterol concentra-
tion at steady state is determined by the rate of LDL cholesterol production relative to the rate of removal of LDL cholesterol 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-depen-
dent LDL uptake (defined by K_m and J_m). In animal experiments, each of these variables can be mea-
sured directly so that the exact mechanism of an alteration in plasma LDL cholesterol levels can be ascertained with considerable accuracy. Such measure-
ments 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, as reviewed in this report, reasonable values for P*, K_m, and J_m can be derived for man. Furthermore, with these values kinetic curves can be constructed (figure 4) that describe the relationship in normal young hu-
man 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 pharmacologic manipulation, it is possible to indirectly determine 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 K_m may also have an effect under certain circumstances. Such K_m effects will have to be defined in animal experiments or by the use of binding studies under conditions in vitro.

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