De novo production of low density lipoproteins: fact or fancy

David M. Shames and Richard J. Havel

Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143-0130

Abstract Many investigators, observing an apparent dilution in the plasma specific activity (SA) of apolipoprotein B-100 (apoB) in low density lipoprotein (LDL) as compared with that in very low density lipoprotein (VLDL) after injection of radiolabeled VLDL, have formulated kinetic hypotheses incorporating the concept of de novo production of LDL to explain their data in humans and other mammals. These hypotheses, with rare exception, do not account for the kinetic heterogeneity known to exist in the apoB of human VLDL on the basis of size and in the apoB of rabbit VLDL on the basis of size and presence of apolipoprotein E. When a logical analysis of such kinetic heterogeneity of apoB in plasma VLDL is performed, it becomes clear that the apparent dilution of the SA of apoB in LDL relative to that in VLDL can be explained without the requirement for de novo production of LDL. Although this alternative hypothesis, incorporating the concept of kinetic heterogeneity of apoB in VLDL, does not exclude the process of de novo production of LDL, which so many investigators have invoked to explain their data, it does raise a question as to the existence of such a process since an alternative hypothesis can explain such data just as well. Clearly, more experimental data on the kinetic heterogeneity of human and other mammalian VLDL are needed before a reasonable choice can be made between these two hypotheses. —**Shames, D. M., and R. J. Havel.** De novo production of low density lipoproteins: fact or fancy. *J. Lipid Res.* 1991. **32:** 1099–1112.

Supplementary key words very low density lipoprotein • intermediate density lipoprotein • lipoprotein kinetics • multicompartmental analysis • integral equation analysis • precursor product relationships

Isolated hepatic perfusion experiments with livers from fed New Zealand white (NZW) and Watanabe heritable hyperlipidemic (WHHL) rabbits have shown that very little, if any, apolipoprotein B-100 (apoB) is secreted with a density greater than 1.006 g/ml (1). We have incorporated this information into our analysis of in vivo kinetic data on apoB metabolism in very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low density lipoproteins (LDL) obtained from experiments in NZW and WHHL rabbits. The hypothesis of no direct de novo production of either IDL or LDL apoB was found to be consistent with all of our tracer and mass data (2–5). Nevertheless, other investigators have interpreted their in vivo kinetic data on apoB metabolism in the rabbit (6), miniature pig (7–9), monkey (10, 11), many patients with hyperlipoproteinemia (12–25), and some normal subjects (19, 22) to show significant de novo production of IDL or LDL. These differences between our conclusions and those of many other investigators may reflect species variation in the metabolism of apoB; however, an alternative explanation for this discrepancy can also be considered. The finding of de novo production of LDL could be an implication of the particular type of kinetic hypothesis various investigators have chosen to fit their data, and consequently, other kinetic hypotheses that do not require de novo production of LDL but which are equally consistent with their data may be found.

We have recently evaluated this possibility by taking the alternative approach with respect to our own in vivo kinetic data in normal and WHHL rabbits. We tested the hypothesis that significant de novo production of LDL (30% and 40%, respectively) exists in these rabbits (26). The compartmental model we formulated, which incorporates the assumption of de novo production of LDL and which we know to be erroneous for these animals, fitted our kinetic and mass data nearly as well as the model in which no de novo production of LDL was assumed. This insight with respect to our own data raised the question as to whether the many other kinetic studies in the literature purporting to show the existence of de novo production of LDL could be reinterpreted to be consistent with no de novo production of LDL. Some investigators using compartmental modeling as a means of data analysis have suggested that their requirement for de novo production of LDL may be the result of a small, rapidly turning over, hepatogenous pool of apoB in VLDL with enough

Abbreviations: SA, specific activity; apoB, apolipoprotein B-100; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; NZW, New Zealand white; WHHL, Watanabe heritable hyperlipidemic; FH, familial hypercholesterolemia; FCR, fractional catabolic rate.

conversion to LDL to explain the LDL mass (11, 21, 25). Because of its small size, this pool would not be easily detected with the tracer kinetic techniques commonly used, even though significant transport of apoB could occur along this pathway. To evaluate the requirment for de novo production of LDL, we have submitted this hypothesis as well as several others to a more formal analysis of precursor-product relationships in the setting of the conversion of apoB from VLDL to LDL. Our analysis shows that the process of de novo production of LDL is not required to explain any of the above referenced data.

METHODS AND RESULTS

The types of kinetic approaches that purport to "show" de novo production of LDL can be grouped for our analysis into six categories. These are: *1*) LDL production greater than VLDL production; *2*) apparent dilution of LDL specific activity (SA) relative to VLDL SA after radiolabeled VLDL injection; *3*) LDL production greater than apoB transport from VLDL to LDL; *4*) compartmental modeling requiring an input into LDL to explain the mass of apoB in LDL after the apoB tracer data in VLDL and LDL have been accommodated; *5*) LDL SA less than IDL SA at time of peak LDL SA; and *6*) early radioactivity in IDL and LDL relative to VLDL after injection of radiolabeled amino acid precursors of apoB. Each of these hypotheses suggesting de novo production of LDL has been reevaluated in the context of alternative explanations that do not require such a process.

1) LDL turnover greater than VLDL turnover

Soutar, Myant, and Thompson (12) estimated the transport of LDL in three homozygous and two heterozygous patients with familial hypercholesterolemia (FH) after administration of radiolabeled LDL as the amount of radioactivity excreted per 24-h period divided by the LDL SA at the beginning of that period. VLDL transport was estimated as the product of the VLDL apoB mass in plasma multiplied by its fractional catabolic rate (FCR), calculated as the best single slope through the apoB tracer data in VLDL during the first 16 h after radiolabeled VLDL injection. The apoB transport in LDL was calculated to be 72% greater than that in VLDL, on average, in the three homozygous patients. Given the assumption that all of the apoB in VLDL was converted to LDL, de novo production of LDL was 42% (0.72/1.72) of LDL transport. If some of the apoB were lost irreversibly as VLDL or as IDL, then de novo production of LDL would be greater than 42% of LDL transport.

In this analysis, kinetic homogeneity of VLDL and LDL is assumed. If a second metabolic component is present in VLDL with a rapid fractional turnover rate, its steady state mass would be small and therefore missed in the kinetic analysis, provided that its apoB were radiolabeled in proportion to apoB mass. Thus, even though a significant transport of apoB from VLDL to LDL could occur along this pathway, it would likely be missed. Consequently, the production rate of apoB in VLDL could be underestimated to an extent that it appeared to be less than LDL transport. In fact, our initial estimate of apoB production rate in the WHHL rabbit (3) was only 59% of a later estimate obtained after we became aware of a rapidly turning over component of apoB in VLDL representing only 3.3% of the apoB mass (4).

2) Apparent dilution of LDL specific activity relative to VLDL specific activity after radiolabeled VLDL injection

At least three groups of investigators (6–10) have purported to show de novo production of LDL by finding apparent dilution in the SA of apoB in plasma LDL relative to apoB SA in VLDL after radiolabeled VLDL injection (27). Mathematically this analysis takes the form of showing that

$$\frac{\int_0^\infty B_{LV}\,dt}{\int_0^\infty B_{VV}\,dt} < 1.0 \qquad \textit{Eq. 1)}$$

where B_{LV} and B_{VV} represent the SA responses of apoB in plasma LDL and VLDL, respectively (units of fraction of administered dose per unit mass), after radiolabeled VLDL injection. According to this analysis, if there is no de novo production of LDL, Eq. 1 is equal to unity. Goldberg et al. (10) have defined a dilution fraction, DF, which is given by

$$DF = 1 - \frac{\int_0^\infty B_{LV}\,dt}{\int_0^\infty B_{VV}\,dt} \qquad \textit{Eq. 2)}$$

The value of this expression is the fraction of the LDL production rate, which is derived from a direct or de novo route.

There are two major problems with this analysis. The first is the requirement for $t = \infty$ extrapolation along the slope of the line through the terminal VLDL and LDL SA data to evaluate the definite integrals shown in Eqs. 1 and 2. It is relatively easy to increase significantly the value of $\int_0^\infty B_{LV}\,dt$ by simply extrapolating along a somewhat smaller or more shallow final slope. This is especially easy to do when the slope of the line through the terminal points of the LDL SA data is small, as is often the case with data from patients with FH (12–15, 19, 23), WHHL rabbits (3–5), and miniature pigs (7–9).

To test the hypothesis that Eqs. 1 and 2 can assume a wide variety of values under the above set of conditions, a theoretical experiment was performed using data taken

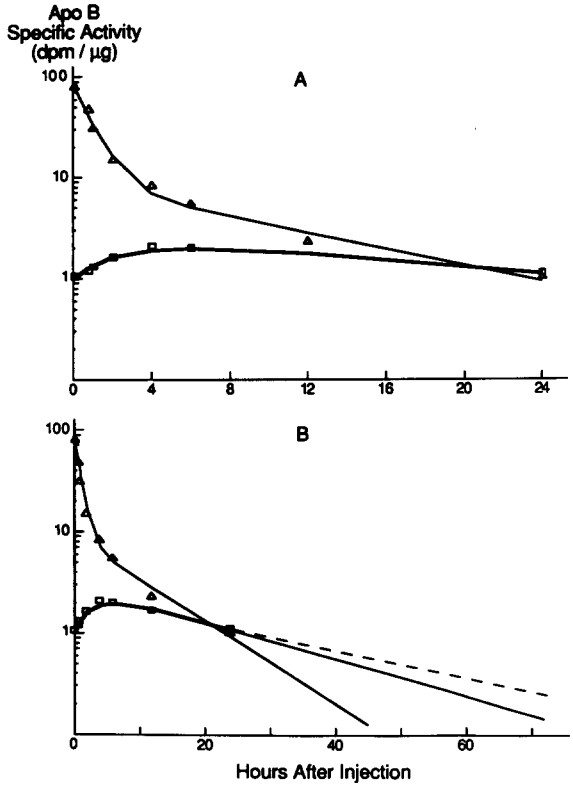

Fig. 1. ApoB SA data in plasma VLDL (△) and LDL (□) after injection of homologous radiolabeled VLDL into miniature pigs; from Fig. 1 of Huff et al. (7). Thin solid lines through data points represent best biexponential fits to data. Heavy solid lines through LDL SA data represent near superimposition of biexponential and triexponential fits to these data, the former without constraints and the latter subjects to the constraint that de novo production of LDL is 0. Dashed line represents the extrapolative implications of the triexponential function beyond the time of the last datum. Evaluation of integrals of SA responses with Eq. 2 suggests that de novo production rates of LDL are 62% and 0% of total LDL transport when LDL data are fitted by the biexponential and triexponential functions, respectively. Model fits to data over the 24 h of data collection (A) are compared to the extrapolative implications of each model beyond the interval of data collection (B).

from Fig. 1A of Huff and Telford (7). The data points portrayed in our **Fig. 1A and B** were taken from their figure as accurately as possible. Using the CONSAM computer program (28–30), we fitted biexponential functions to both the VLDL and LDL SA data, as suggested by Huff and Telford, corresponding to the solid lines though the data. These functions were then integrated analytically from t = 0 to ∞ and their ratios were calculated as in Eqs. 1 and 2. In fact, when this was done, the dilution fraction was calculated to be 62%, a value satisfyingly close to that reported by Huff and Telford (7), given the slight errors that might occur when numerical data are extracted from a published graph of these data. Using the CONSAM program, it is relatively easy to refit the LDL SA data of Fig. 1 subject to the constraint that the value of DF be 0, (Eq. 2) corresponding to no de novo production of LDL. We performed this test assuming a triexponential equation for LDL, an assumption we feel is reasonable given the fact that the IDL and LDL subsystems would add at least one eigenvalue of complexity beyond that found in the VLDL subsystem. The results of this test are also shown in Fig. 1A where the heavy solid line through the LDL SA data represents the near superimposition of the fits of the biexponential and triexponential functions. It is clear that there is no significant difference between the fits to the VLDL and LDL SA data generated by the hypotheses of 62% and 0% de novo production of LDL ($P > 0.5$ by Fisher F statistic of residual variance ratio). Nevertheless, there is a significant difference between these two hypotheses beyond the data base as shown in Fig. 1B where the LDL responses of each kinetic hypothesis are extrapolated to 72 h as compared to 24 h in Fig. 1A. Consequently, without additional information on this system (such as additional SA data considerably beyond the final 24-h time point or direct measurements of de novo production of LDL from isolated hepatic perfusion experiments), a wide range of estimates of the degree of de novo production of LDL is possible, all of which are equally consistent with the data.

A further problem with Eqs. 1 and 2 is the fact that kinetic homogeneity of apoB in VLDL and LDL must be assumed. This is clearly not the case in the rabbit, where kinetic heterogeneity related to varying particle size in VLDL (4) and the presence or absence of apoE (2–4) is seen in VLDL, IDL, and even LDL. Kinetic heterogeneity is also seen in humans (31, 32), where particle size is a strong determinant of the rate of metabolism and fate of apoB in VLDL. It will be shown below that even if the data were known to perfect precision and extended out in time manyfold greater than is normally the case in experiments such as those performed by Huff et al. (7–9) and Goldberg et al. (10), Eqs. 1 and 2 can lead to significant errors in determining the presence and magnitude of de novo production of LDL because of the failure to take account of the metabolic heterogeneity of apoB in plasma (see Compartmental modeling).

3) LDL production greater than apoB transport from VLDL to LDL

Two groups of investigators have postulated the existence of de novo production of LDL in the cynomolgus monkey (10) and in patients with heterozygous FH (15) by attempting to show that the transport of apoB from VLDL to LDL is less than LDL production. These investigators argued that if apoB transport in plasma VLDL (R_{VV}) in units of mass per unit time is given by

$$R_{VV} = \frac{1}{\int_0^\infty B_{VV}\,dt} \qquad \textit{Eq. 3)}$$

where B_{VV} is the SA of apoB in plasma VLDL after in-

jection of radiolabeled VLDL in units of fraction of administered dose per unit mass, and the fraction of apoB in VLDL converted to LDL ($F_{V\rightarrow L}$) is given by

$$F_{V\rightarrow L} = \frac{\int_0^\infty B_{LV}\,dt}{\int_0^\infty B_{LL}\,dt} \qquad Eq.\ 4)$$

where B_{LV} and B_{LL} represent the plasma LDL SA responses (fraction of administered dose per unit mass) after injection of radiolabeled VLDL and LDL, respectively, then the rate of transport of apoB in VLDL to LDL is given by

$$R_{VV}F_{V\rightarrow L} = \frac{1}{\int_0^\infty B_{VV}\,dt}\,\frac{\int_0^\infty B_{LV}\,dt}{\int_0^\infty B_{LL}\,dt} \qquad Eq.\ 5)$$

Furthermore, if the rate of LDL transport (R_{LL}) in units of mass per unit time is given by

$$R_{LL} = \frac{1}{\int_0^\infty B_{LL}\,dt} \qquad Eq.\ 6)$$

where B_{LL} is the apoB SA in plasma LDL after injection of radiolabeled LDL in units of fraction of dose of radiolabeled LDL per unit of mass, then the fraction of LDL derived from VLDL is given by

$$\frac{R_{VV}F_{V\rightarrow L}}{R_{LL}} = \frac{\int_0^\infty B_{LV}\,dt}{\int_0^\infty B_{VV}\,dt} \qquad Eq.\ 7)$$

This expression, however, is the same as that of Eq. 1. Thus, this analysis, using the simple algebra of integral equations, shows that the independent data on LDL production, as provided through Eq. 6, are redundant to the information provided by VLDL and LDL SA data, obtained after radiolabeled VLDL injection, for estimating the fraction of LDL derived from VLDL. These data are clearly necessary, however, for the estimation of the fraction of apoB in VLDL that is converted to LDL. It is thus seen that this technique must have the same limitations with respect to calculation of de novo production of LDL as that discussed in section 2. These limitations are those of the extrapolative implications of the kinetic hypothesis beyond the last datum and the assumption of kinetic homogeneity.

4) Compartmental modeling

Perhaps the greatest body of information purporting to show the existence of de novo production of LDL has been developed using compartmental analysis of apoB tracer data in VLDL and LDL after injection of radiolabeled VLDL and LDL (11, 17, 20–25, 31). These studies have taken the general form of developing a compartmental model to fit the apoB tracer data in VLDL, IDL, and LDL after injection of VLDL and LDL radiolabeled with ^{125}I and ^{131}I. Once this has been accomplished, the steady state solution is performed, and the calculated LDL mass is often found to be too low to explain the measured LDL mass, thus requiring an additional input into LDL. This additional input is throught to be de novo ("direct") production of LDL. The percentage of de novo production of LDL as compared to total LDL production, reported by investigators using compartmental analysis in different groups of patients, has varied widely, sometimes being zero but often is greater than 50% (11, 17, 20–25, 31).

We have recently shown (26) that up to 30% de novo production of LDL can easily be accommodated by a compartmental model developed by us to describe apoB tracer data in the NZW rabbit, where we have independent information that no de novo production is present. This percentage of de novo production of LDL can go up to 40% in a model for apoB kinetics in the WHHL rabbit, where again independent information is available that little or no de novo production of LDL exists. Ths wide compatibility of a compartmental model to the tracer and steady state data under such different assumptions (either 0 or 30–40% de novo production of LDL) can be explained on the basis of different $t = \infty$ extrapolations of the model beyond the last datum according to the different steady state hypotheses mentioned above.

Some of the investigators whose compartmental modeling has led them to suggest de novo production of LDL have preferred to hypothesize instead a small but rapidly turning over pool of VLDL particles with high conversion to LDL. Using exogenous labeling techniques, this pool is nearly "invisible" compared with the considerably larger, more slowly turning over apoB pools in VLDL. Consequently, the transport of apoB through this pool in VLDL and its conversion to LDL are not detected with conventional tracer techniques. Such a process of apoB metabolism in VLDL and conversion to LDL implies significant kinetic heterogeneity, a concept already well established in the literature of apoB metabolism. As mentioned above, Packard et al. (31) and Stalenhoef et al. (32) clearly documented kinetic heterogeneity of apoB in humans on the basis of the particle size. We have also shown such a process in VLDL on the basis of particle size in NZW and WHHL rabbits (4) and have shown the existence of both B,E and B particles, each with very different kinetics and transition probabilities to LDL (2–4).

In attempting to understand the nature of such a small, nearly invisible pool of apoB in VLDL, hypothesized above, within the framework of known kinetic heterogeneity of apoB in VLDL, we were led to perform a logical analysis of kinetic heterogeneity in the apoB system. This

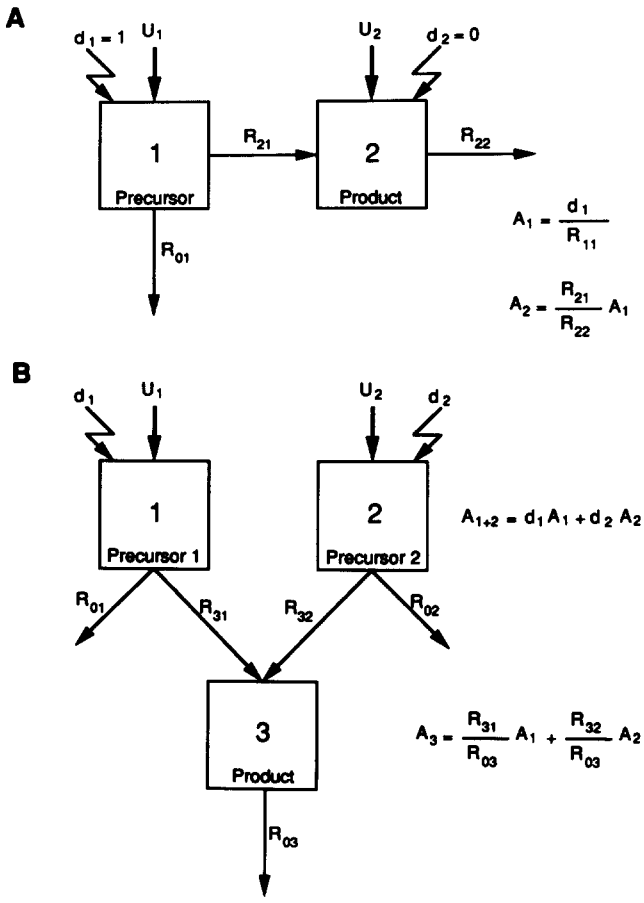

Fig. 2. Compartmental models describing relationship between precursor and product SA in metabolic systems exhibiting kinetic homogeneity (A) and heterogeneity (B) of precursor. U_i, independent production rate of material into compartment i from outside of system; d_i, fraction of injected dose of tracer bound to material in compartment i; R_{ij}, transport rate of material to compartment i from compartment j; R_{ii}, total transport rate of material out of compartment i and A_i, $t = \infty$ integral of SA response in compartment i.

analysis showed that kinetic heterogeneity in any precursor-product system could result in the SA of the product being less than that of the precursor without independent production of the product. This is the same as saying that the apparent dilution of the LDL SA response relative to the VLDL SA response after radiolabeled VLDL injection does not necessarily imply de novo production of LDL. The same conclusion follows for compartmental modeling where the counterpart of the apparent dilution of apoB SA in LDL relative to that in VLDL is an LDL mass too large to be explained on the basis of the conversion of apoB tracer in VLDL to LDL.

A relatively simple way of formalizing the above argument takes advantage of the algebra of integral equations and those relationships among these equations nicely demonstrated by Goldberg et al. (10) and Le et al. (33). In a general precursor product system where compartment 1 is converted to compartment 2 but not necessarily completely (**Fig. 2A**), and where the areas under the SA responses (fraction of administered dose of radioactivity per unit mass) for compartments 1 and 2 evaluated from $t = 0$ to ∞ are given by A_1 and A_2, respectively, then

$$A_1 = \frac{d_1}{R_{11}} \qquad \textit{Eq. 8)}$$

where d_i is a unit dose of radioactivity injected into compartment i and R_{ii} is the transport through this compartment in mass per unit time. Also

$$A_2 = \frac{R_{21}}{R_{22}} A_1 \qquad \textit{Eq. 9)}$$

where R_{ij} is the transport to compartment i from compartment j. When there is no independent input into compartment 2 other than transport from compartment 1, $R_{22} = R_{21}$, and $A_2 = A_1$.

Now let us hypothesize a simple precursor-product system exhibiting kinetically different processes of conversion of precursor to product where no independent production of product exists, as shown in Fig. 2B. It is assumed that compartments 1 and 2, both of which are precursors of the product in compartment 3, are labeled in proportion to mass. Consequently, the ratio, d_1/d_2 is equal to that of M_1/M_2 where M_i is the steady state mass in compartment i. To create kinetic heterogeneity in this system, we will assume that $A_1 < A_2$, which is the same thing as assuming that the residence time of the precursor in compartment 1 is shorter than the residence time of the precursor in compartment 2. The relationship of the area under the SA response of any compartment i, A_i, to the residence time of compartment i, t_i, is obvious from a generalization of Eq. 8, where L_{ii} is the fractional turnover rate of compartment i.

$$A_i = \frac{d_i}{R_{ii}} = \frac{d_i}{M_i L_{ii}} = \frac{d_i}{M_i} t_i \qquad \textit{Eq. 10)}$$

Since d_i/M_i is the same for any compartment in the system that is exogenously labeled in proportion to mass, it is clear that A_i is proportional to t_i.

Let us now assume that the kinetic heterogeneity of the precursor system is not known to the investigator, who instead assumes a kinetically homogeneous system. Thus, a mixture of individual SAs is measured as the precursor SA. The area under the SA curve of this mixture of precursor SAs, A_{1+2}, is given by the average of the SAs of the individual precursors weighted for relative mass (33). Thus

$$A_{1+2} = \frac{M_1 A_1 + M_2 A_2}{M_1 + M_2} = d_1 A_1 + d_2 A_2 \qquad \textit{Eq. 11)}$$

Using Eqs. 9 and 11, the area under the SA response of the product, A_3, shown in compartment 3, is given by

$$A_3 = \frac{R_{31}}{R_{03}} A_1 + \frac{R_{32}}{R_{03}} A_2 \qquad Eq.\ 12)$$

since R_{31}/R_{03} and R_{32}/R_{03} are proportional to the relative masses generated in compartment 3 as result of R_{31} and R_{32}, respectively.

If we define the fraction of the total transport of product coming from precursors in compartments 1 and 2 as r_{31} and r_{32}, respectively, where $r_{31} = R_{31}/R_{03}$ and $r_{32} = R_{32}/R_{03}$, then the ratio of the areas under the product and precursor SA responses can be simply expressed as

$$\frac{A_3}{A_{1+2}} = \frac{r_{31}A_1 + r_{32}A_2}{d_1A_1 + d_2A_2} = \frac{r_{31}A_1 + (1 - r_{31})A_2}{d_1A_1 + (1 - d_1)A_2} \qquad Eq.\ 13)$$

It is clear from this expression that when the fraction of the total transport to product from a particular precursor is equal to the fraction of the label or mass associated with that precursor, that is, when $r_{31} = d_1 = M_1/(M_1 + M_2)$ then

$$\frac{A_3}{A_{1+2}} = 1 \qquad Eq.\ 14)$$

Under these conditions, the area under the SA response of the product is equal to that of precursor. However, when the fraction of the total transport to product for a given precursor is greater than its relative amount of label or mass and when that particular precursor has a shorter residence time than a second precursor, that is, when $r_{31} > d_1$ [i.e., $r_{31} > M_1/(M_1 + M_2)$] and $A_1 < A_2$, then

$$\frac{A_3}{A_{1+2}} < 1 \qquad Eq.\ 15)$$

and when $r_{31} < d_1$ [i.e., $r_{31} < M_1/(M_1 + M_2)$] and $A_1 < A_2$, then

$$\frac{A_3}{A_{1+2}} > 1 \qquad Eq.\ 16)$$

Thus, in this type of system, which exhibits kinetic heterogeneity of precursor and no independent production of product, the area under the product SA response after injection of exogenously labeled precursor can be equal to, less than, or greater than the area under the precursor SA response. Consequently, when kinetic heterogeneity of the precursor is a possibility, one cannot safely infer de novo production of the product when the area under the product SA response is less than that of the precursor. An apparent dilution of product SA relative to precursor SA seen in integral equation analysis corresponds to the requirement for independent production of product using compartmental analysis, because the mass of the product is too large to be explained on the basis of the conversion of precursor to product defined by the tracer. It is clear from this analysis that a small, rapidly turning over precursor pool in VLDL with a conversion rate to LDL relatively greater than its relative mass, hypothesized by a number of investigator to explain a large LDL mass, is simply a particular case of kinetic heterogeneity in VLDL, where the residence time of one of the VLDL precursors is very short, resulting in an area under the SA response that is vanishingly small and therefore not even detected in the measurement of the plasma disappearance of the precursor.

Fig. 3 uses the simple model of Fig. 2B to show numerical examples describing situations where the SA of the product is equal to (Fig. 3A), greater than (Fig. 3B), and less than the SA of the precursor (Fig. 3C, D). Fig. 3D gives a numerical example of this last situation occurring when a fraction of the precursor turns over so rapidly that it is not even detected in the precursor data, in this case being only 1% of the apoB mass in VLDL.

Some investigators have hypothesized kinetic heterogeneity of the LDL system to explain their data in patients with hyperlipoproteinemia (23, 34–36). We have observed this phenomenon in NZW and WHHL rabbits and have attempted to explain it on the basis of the presence or absence of apoE (2, 3). Such a process can magnify or reduce any differences in apoB SA of LDL relative to VLDL when kinetic heterogeneity in VLDL also exists. An example of this effect is shown in **Fig. 4A** where the weighted average of the SAs in the product is calculated using Eqs. 9 and 11. Here the apparent 21% enhancement in product SA relative to precursor where kinetic homogeneity of the precursor is assumed to exist (Fig. 3B) can be converted into an apparent 9% dilution effect when kinetic heterogeneity of the product is also assumed. Alternatively, an apparent dilution in the SA of the product of 34% (Fig. 3C) can disappear when kinetic heterogeneity of product is also assumed, as shown in Fig. 4B. However, apparent dilution or enhancement of SA in the product cannot occur as a result of kinetic heterogeneity in the product unless kinetic heterogeneity is also present in the precursor system, as shown in Eq. 9.

A final point to be made about the use of compartmental modeling to estimate the magnitude of apparent direct production of LDL has to do with the concept of hypothesis testing. Using a software package such as the SAAM-CONSAM computer program, it is relatively easy to test hypotheses different from the first one found to fit the data. As mentioned above, a typical strategy one often uses in compartmental modelling is to hypothesize a simple model that is unique and consistent with all the apoB

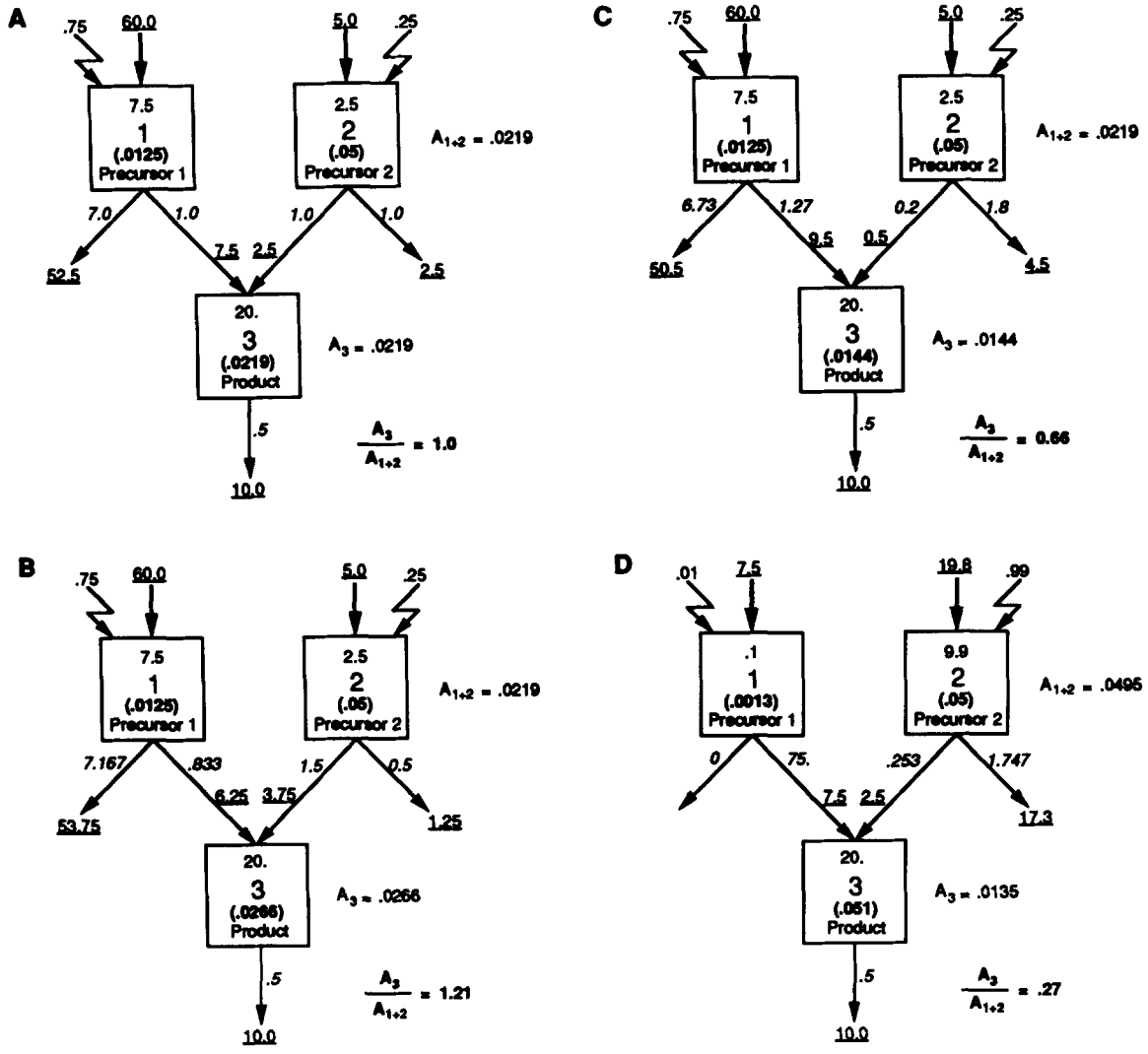

Fig. 3. Numerical examples of compartmental model describing a metabolic system exhibiting kinetic heterogeneity of precursor and no independent production of product where the ratio of the $t = \infty$ integrals of the product SA to the mixture of precursor SAs is unity (A), greater than unity (B), less than unity (C), and considerably less than unity (D). The latter case is that of a rapidly turning over precursor pool with relatively small mass but relatively large transport to product. Steady state amounts of material in each compartment shown at top of squares; compartment number in center in bold type; and $t = \infty$ integrals of SA responses of compartments in parentheses at bottom of squares. Transport rates are underlined, rate constants are italicized, and fractions of administered dose are at beginning of jagged arrows. A_i is the $t = \infty$ integral of the SA response in compartment i and A_{i+j} is the $t = \infty$ integral of the SA responses in compartments i and j taken as a mixture of SAs.

tracer data. One then performs a steady state solution, perhaps assuming that the VLDL mass is known. If the calculated LDL mass is less than the measured mass of LDL, then de novo production of LDL is assumed to exist and is easily calculable. However, one can proceed further in this analysis. One can actually test the hypothesis that de novo production does not exist by attempting to force the rate constants of the model to a set of values whose steady state solution is consistent with the measured LDL mass without invoking de novo production of LDL. The fit of the model to the tracer data will probably become worse as compared with the situation where this steady state constraint is not imposed. Nevertheless, the fit to the tracer data subject to the steady state constraint that de novo production is zero may be so close to the unconstrained fit to the data that a clear choice between the two hypotheses is not possible on the basis of model consistency with all available data. We have attempted to make this point clear with out own data (26). However, when the assumption that de novo production of LDL is zero yields kinetic responses by the model that are systematically different from the tracer and mass data, a further possibility is to increase the complexity of the kinetic heterogeneity of the precursor subsystem in the model to obtain a better fit.

We have applied this strategy of alternative hypothesis testing to a number of models already in the literature. The most interesting of these is that of James et al. (23)

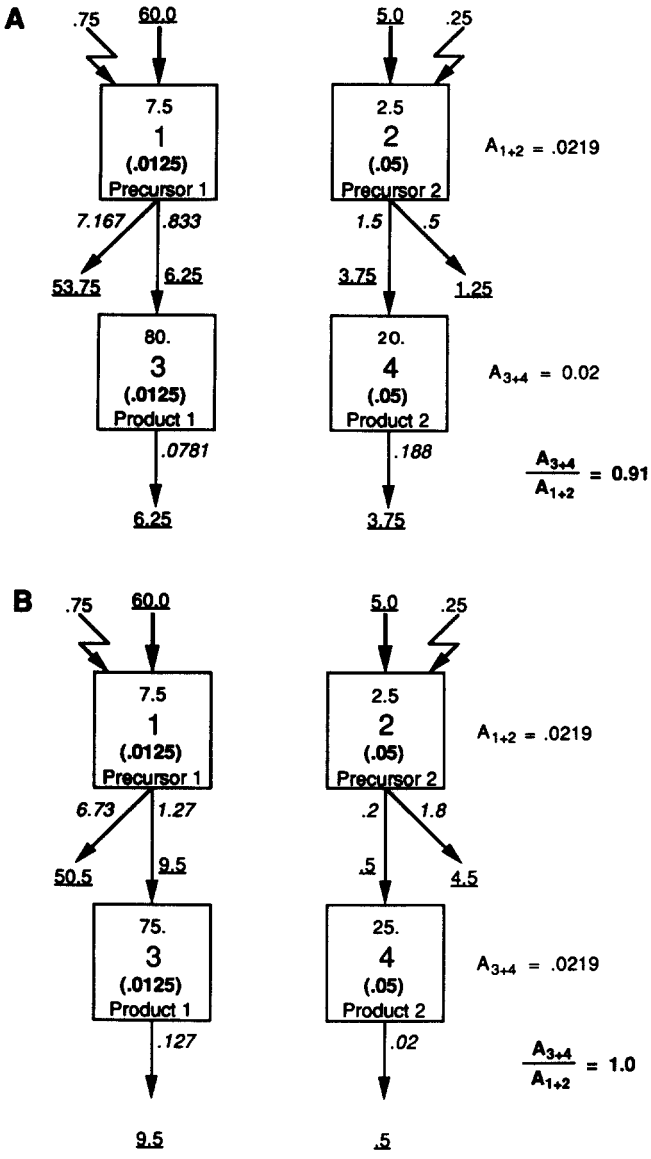

Fig. 4. Numerical examples of compartmental model describing a metabolic system exhibiting kinetic heterogeneity of both precursor and product. The latter can result in the ratio of the t = ∞ integrals of the product to precursor SA responses becoming less (A) or greater (B) than that when kinetic homogeneity of the product is present as in Figs. 3B and 3C, respectively. Same notation as in **Fig. 3.**

who have developed an elaborate model containing kinetic heterogeneity of apoB in VLDL, IDL, and LDL to describe the conversion of apoB from VLDL to LDL and compared their results in patients with FH with those in normal subjects. Using their model, the authors estimated that considerable de novo production of LDL occurred in five FH patients without portocaval shunting. The largest de novo production of LDL was calculated for their patient FH5, whose de novo production directly into IDL and LDL accounted for 66% of his total LDL transport.

We reasoned that if we could test the hypothesis that essentially no de novo production existed in this patient and found it to be consistent with their data, then the hypothesis of no de novo production would also fit the data from their other patients, where the percent of LDL transport arising from de novo production was considerably less. Consequently, we used the authors' model to generate a set of perfect simulated data assuming initial conditions corresponding to simultaneous injection of ^{131}I-labeled $VLDL_1$ (S_f 60–400) and ^{125}I-labeled $VLDL_2$ (S_f 20–60). To provide expected random fluctuations in these simultations, we randomized the data using a normally distributed table of random numbers ($\mu = 0$ and $\sigma = 1$) assuming a fractional standard deviation of 0.1. We then assumed that no de novo production of LDL was present and attempted to adjust the constants of their model to fit this assumption as well as the set of tracer data simulated from their model and the mass data obtained from patient FH5. We also sought to perform this test without changing the kinetics of the LDL subsystem.

Slight systematic deviations of the model responses with the randomized simulated data set were produced by this maneuver. However, by increasing the complexity of the kinetic heterogeneity beyond that already present in small VLDL (S_f 20–60), we were able to obtain a set of kinetic responses that were not significantly different from those generated from their model which assumed a de novo production rate of LDL of 66% ($P > 0.1$ by Fisher F statistic of residual variance ratio).

The results of this analysis are shown in Figs. 5 and 6. Their model, assuming a large de novo LDL and IDL production, is shown in **Fig. 5A** and ours, assuming no de novo production of LDL or IDL, is shown in Fig. 5B. Compartment 13 (bold circle) in our model represents the only difference in structure between these two models. The responses of these two models compared with the randomized data simulated from their model are shown in **Figs. 6A** and **6B,** respectively. It is clear from Fig. 6 that no rational choice is possible between their hypothesis of 66% and ours of 0% de novo production of LDL based on the consistency of each hypothesis with the data. When integral equation analysis is applied to the responses of the model with no de novo production of LDL (Fig. 6B), there is an apparent dilution of the LDL SA relative to VLDL SA (as defined by Eq. 2) of 80% and 84%, corresponding to the S_f 60–400 and S_f 20–60 VLDL tracer injections, respectively. These integral equation calculations serve as another example of the fact that apparent dilution of LDL SA relative to VLDL SA even of large magnitude does not necessarily imply de novo production of LDL when a particular type of kinetic heterogeneity of VLDL is present, as is clearly the case in our modification of the model proposed by James et al. (23).

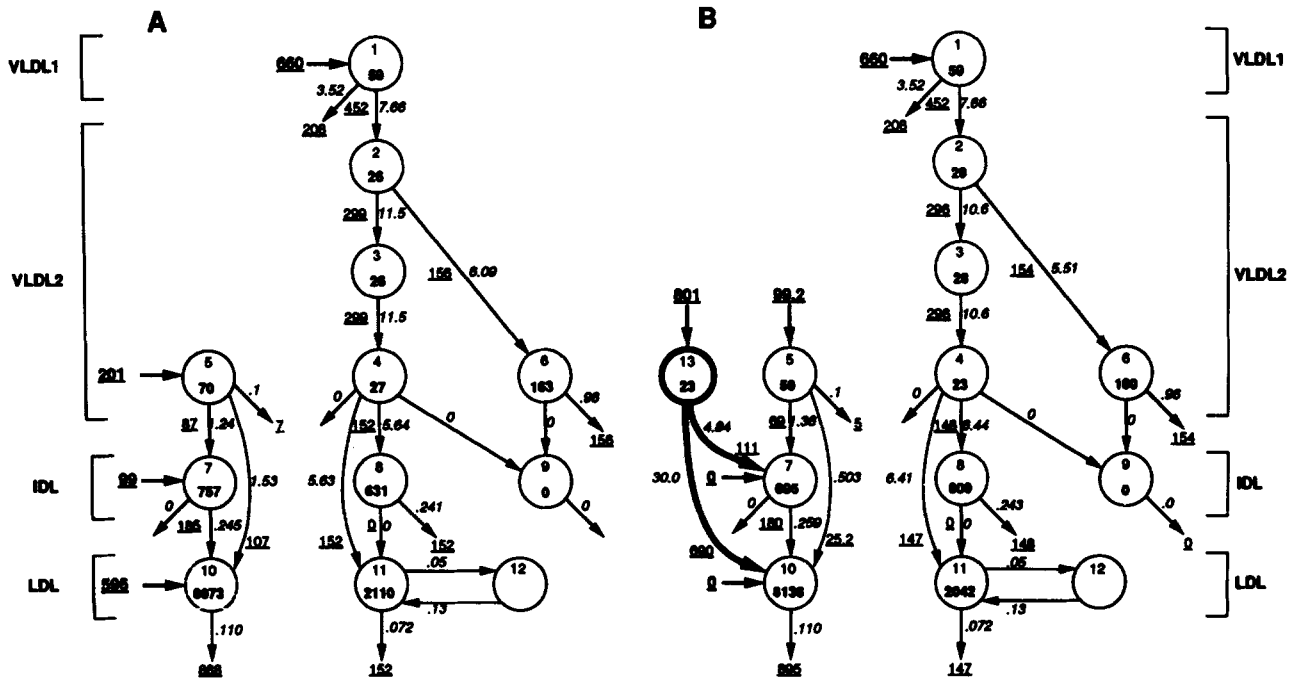

Fig. 5. Compartmental model formulated by James et al. (23) to describe the plasma kinetics of apoB in large (S_f 60–400) and small (S_f 20–60) VLDL and its conversion to IDL and LDL. Model constants and steady state solution proposed by James et al. (A) to explain tracer and mass data from patient FH5 assume considerable de novo production of IDL and LDL. Additions to model (compartment 13 and rate constants L(7,13) and L(10,13) in bold lines), alternative set of values for rate constants and steady state solution proposed by us (B) to explain the same data set assume no de novo production of IDL or LDL. Amounts (mg) of plasma apoB in each compartment shown at bottom of circles with compartment number shown at top. Transport rates (mg/day) are underlined, and rate constants (day^{-1}) are in italics. Compartment 12 represents extravascular compartment for LDL.

5) LDL specific activity less than IDL specific activity at time of peak LDL specific activity

A number of investigators (7–9, 13, 14, 37, 38) have used a type of kinetic analysis first described by Zilversmit (39) to determine the presence and estimate the fraction of LDL transport derived from a de novo pathway after injection of radiolabeled VLDL. This hypothesis, which assumes kinetic homogeneity of the apoB in VLDL, suggests that the ratio of the peak LDL SA to the IDL SA at the same time provides a measure of the fraction of LDL derived from IDL. Soutar, Myant, and Thompson (13, 14) found that this ratio was less than unity in most FH homozygotes but not in FH heterozygotes or normal subjects. Sigurdsson, Nicoll, and Lewis (37) studied seven normal and seven hyperlipidemic subjects and found in all that the LDL SA response crossed the IDL SA response at or near the peak value for LDL SA, an observation they interpreted as the absence of de novo production of LDL. Reardon, Fidge, and Nestel (38) studied four normal and eleven hyperlipidemic subjects with the same technique and obtained results similar to those of Sigurdsson et al. (37). However, Huff and Telford (7) came to very different conclusions when they applied this technique in the miniature pig. They estimated that as much as 80% of LDL was not derived from VLDL in these animals and that this percentage was reduced significantly after treatment with lovastatin and cholestyramine (8, 9).

As pointed out by Zilversmit (39), the use of this analytic technique assumes kinetic homogeneity in the precursor system. However, if kinetic heterogeneity is present in IDL, the peak value for LDL SA may occur at, after, or before the time at which the LDL SA response crosses the IDL SA response in the absence of any de novo production of LDL. The same can be said for the IDL SA response relative to that for VLDL.

These relationships are clearly delineated in **Fig. 7** where precursor and product SA responses A, B, C and D are generated from the four models without de novo production of LDL presented in Fig. 3A, B, C, and D, respectively. Vertical lines are drawn through the product SA responses at their peaks to demonstrate the relationship of the peak SA of the product to the SA of the precursor at this time. Only model A generates a set of precursor and product SA responses that are correctly interpreted with the Zilversmit technique. Using this technique, one would conclude significant de novo production of product from the responses in Fig. 7C and 7D when, in fact, such responses are due solely to kinetic heterogeneity in the precursor system. Finally, the paradoxical effect of the peak SA in the product occurring after the product SA response crosses that of the precursor as seen in Fig. 7B is

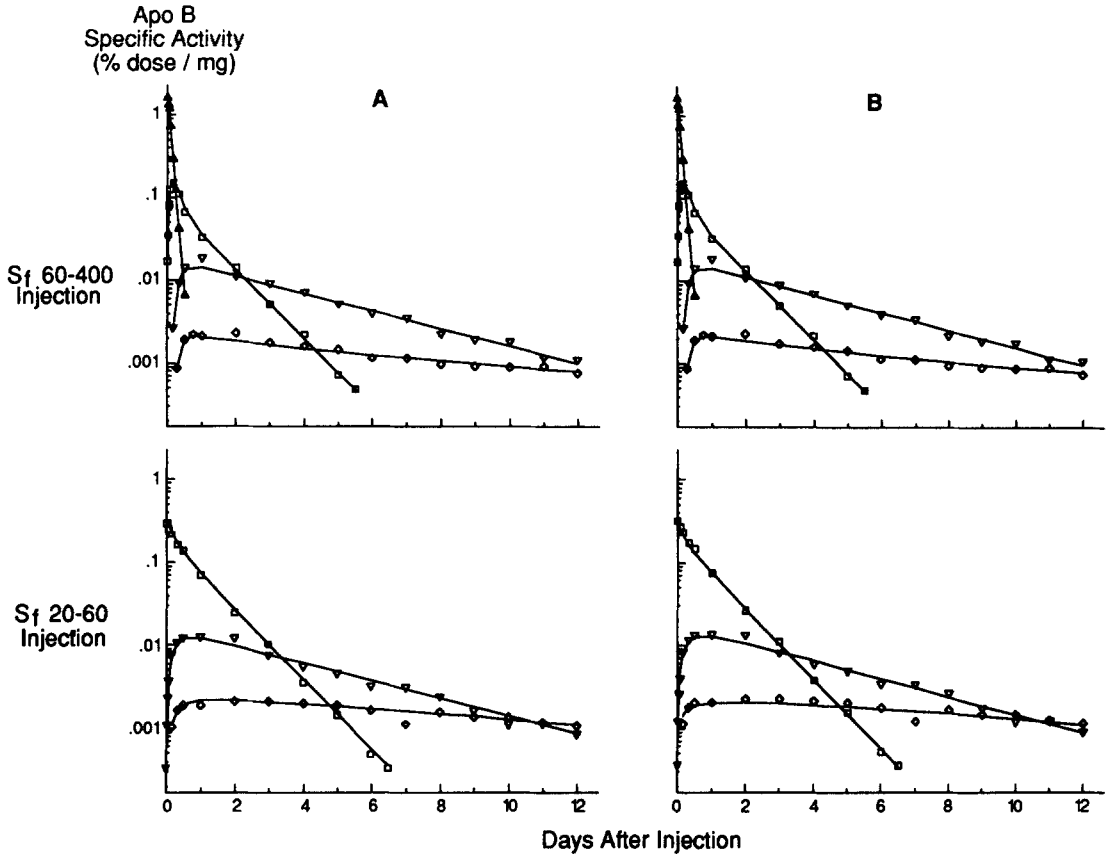

Fig. 6. Theoretical set of randomized, normally distributed (μ = 0, σ = 1, FSD = 0.1) apoB data generated from compartmental model proposed by James et al. (23) to fit data obtained from their patient FH5 with homozygous, familial hypercholesterolemia. Top graphs represent apoB SA in S_f 60-400 VLDL (△), S_f 20-60 VLDL (□), IDL (▽), and LDL (◇) after injection of radiolabeled S_f 60-400 VLDL. Bottom graphs use same notation for data representing apoB SA in S_f 20-60 VLDL, IDL, and LDL following injection of radiolabeled S_f 20-60 VLDL. Lines through data represent responses obtained from model in Fig. 5 using rate constants proposed by James et al. (23) which incorporate the concept of de novo production of LDL (A) and those proposed by us which do not incorporate de novo production of LDL (B).

easily explainable by the kinetic heterogeneity in model B. It is clear that the Zilversmit technique cannot be used reliably to determine the presence and estimate the magnitude of de novo production of LDL when kinetic heterogeneity exists in VLDL or IDL. In fact, if the Zilversmit technique is used to calculate the percentage of de novo production of LDL from the IDL and LDL SA data shown in Fig. 6B, a data set generated from a model without de novo production of LDL, one estimates a value of either 86% or 81% of LDL production by the de novo route depending on whether data from the S_f 60-400 or S_f 20-60 VLDL tracer injections are analyzed.

6) Early tracer activity in IDL and LDL relative to that in VLDL after injection of radiolabeled amino acid precursors of apoB

Several groups of investigators have hypothesized significant degrees of de novo production of IDL and LDL to explain the early rise of radioactivity in apoB in IDL and LDL relative to that in VLDL after injection of [^{3}H]leucine (16, 18, 25) and [^{75}Se]selenomethionine (19). The analysis presented above demonstrates that a rapidly turning over precursor pool in VLDL with relative transport to IDL and LDL greater than its relative mass in VLDL could explain the early activity in and masses of IDL and LDL without the assumption of de novo production of IDL and LDL. Furthermore, even without invoking the possibility of kinetic heterogeneity in VLDL, the early rise of IDL and LDL radioactivity is also explainable on the basis of artifacts in the data resulting from incomplete ultracentrifugal separation of kinetically distinct components of apoB in VLDL, IDL, and LDL, as previously discussed by us (5).

DISCUSSION

We have shown that the hypothesis of de novo production of LDL in animals and man which has been used to explain the in vivo plasma kinetics of apoB SA in VLDL,

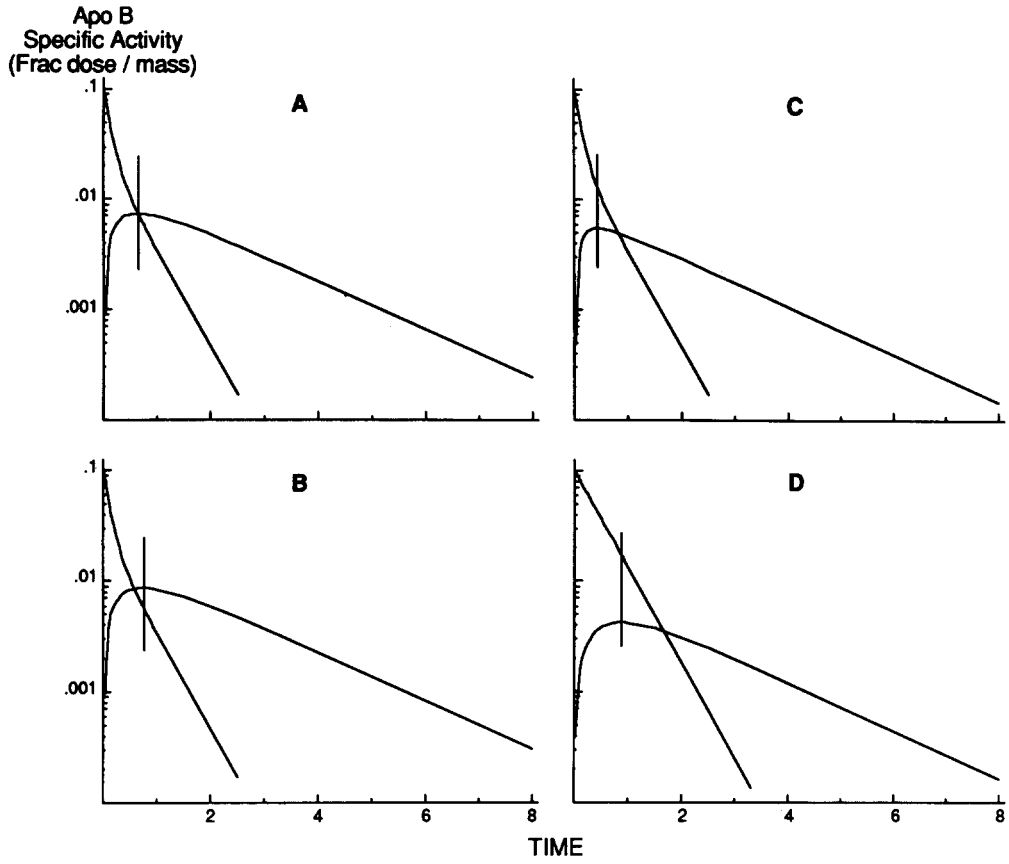

Fig. 7. Specific activity responses of precursor and product corresponding to models A, B, C, and D in Fig. 3. Vertical lines through peak SA response of product from each model define relationship to precursor SA at the same time. Peak product SA equal to (A), greater than (B), and less than (C, D) precursor SA at same time is explainable by kinetic heterogeneity in precursor system where relative transport rate of precursor to product is proportional to (A), less than (B), and greater than (C, D) relative mass of precursor component with shorter residence time.

IDL, and LDL after injection of either endogenously or exogenously labeled tracers is not required to explain these data. The hypothesis of de novo production of LDL, whether taking the form of a compartmental model or some simplified measure of a compartmental model (e.g., integral equations, Zilversmit technique), explains an apparent dilution in the SA of apoB in LDL relative to that in VLDL or IDL by direct production of LDL, IDL, or both. However, by making the assumption of kinetic heterogeneity in VLDL, known to exist on the basis of particle size in rabbit and man (4, 31, 32) and content of apoE in the rabbit (2–4), the apparent dilution of SA of LDL can be explained without the need for de novo production of LDL. Even when no kinetic heterogeneity exists in VLDL, de novo production of LDL may not be needed to explain the data if the extrapolative implications of the kinetic hypothesis beyond the last datum are changed such that an apparent dilution of LDL SA over the time course of the experimental data disappears when the integral of the LDL SA response is evaluated to $t = \infty$. This possibility of increasing the calculated area under the LDL SA response by changing the extrapolative consequences of the model is especially easy to accomplish when the slope of the line through the terminal points of the LDL data is relatively small, a finding seen in many patients with hyperlipoproteinemia where the fractional catabolic rate of LDL may be reduced.

The decrease in product SA relative to precursor SA as a result of kinetic heterogeneity in the precursor system rather than due to de novo production of product at first seems paradoxical. However, this concept becomes obvious when one goes through the integral equation logic discussed in Eqs. 8–16 and Figs. 2–4. Expressed in the simplest terms, when there is kinetic heterogeneity in the precursor system and when the relative transport of precursors to product is not identical to the relative masses of the precursors, the area under the SA of the product will be either less or greater than that of the mixture of precursors when exogenous labeling of precursor(s) is employed. In the former case this occurs when a precursor with a short residence time has a rate of steady state transport to product relatively greater than its relative mass as

compared with the other precursors. The latter possibility will occur when the same precursor has a transport to product relatively less than its relative mass.

Direct tests of the hypothesis of de novo production of LDL have mainly taken the form of isolated hepatic perfusion experiments in animals. These have yielded inconsistent results. In perfusates from normally fed animals, VLDL are the predominant lipoprotein containing apoB that accumulate, either during recirculating perfusions or in the single pass mode. In normal rabbits (1), as noted above, and guinea pigs (40), little or no newly synthesized apoB accumulates in the usual density range of LDL. In normally fed rats and nonhuman primates, some investigators have found newly synthesized apoB in perfusate LDL during single pass perfusions (41, 42).[1] In liver perfusates of cholesterol-fed rats and guinea pigs, accumulation of LDL tends to be more pronounced, reflecting the cholesterol enrichment of newly synthesized lipoproteins from cholesterol-loaded livers (40, 44), although, as discussed elsewhere (40), gradual release of plasma LDL sequestered in the space of Disse can complicate interpretation of the data obtained. Greater accumulation of LDL in liver perfusates has not been observed in cholesterol-fed African green or Japanese monkeys (45, 46), in which some newly synthesized apoB accumulates in perfusate LDL whether or not the animals have been maintained on cholesterol-rich diets. A compelling inconsistency of the hypothesis of de novo LDL production derived from in vivo kinetic data has been found in cynomolgus monkeys by Goldberg et al. (47), who demonstrated the absence of radioactivity in LDL after intravenous injection of [^{3}H]leucine when conversion of VLDL to LDL was halted by antibodies to lipoprotein lipase. It is worth remembering that this was the same animal system employed to "demonstrate" de novo production of LDL (10) using technique 2) discussed above.

The paradoxical observation of an increase in the integral of the apoB SA data in IDL relative to that in VLDL after injection of exogenously labeled VLDL was reported some time ago by Reardon et al. (38). This observation has been confirmed and explained by Le et al. (33) using the delipidation cascade where sequential pools in the cascade, because they have also been labeled exogenously, are enriched in SA relative to their immediate precursor pool. However, even when Beltz et al. (25) used a delipidation cascade of up to eight compartments, they were unable to explain the relatively high apoB SA in IDL without hypothesizing a sequestered pool of apoB with IDL kinetics along the conversion pathway of VLDL to LDL. It is worth noting that the kinetic heterogeneity in the precursor system exhibited in Fig. 3B is another mechanism that will result in enrichment of the product SA (in this case IDL). In fact, such a process, in combination with the delipidation cascade, is an alternative hypothesis to that of a sequestered pool of IDL offered by Beltz et al. (21, 25) to explain the unexpectedly high apoB SA in IDL after injection of radiolabeled VLDL.

Huff et al. (8, 9) have considered the increase in apoB SA in LDL relative to that in VLDL following injection of radioiodinated VLDL in miniature pigs treated with lovastatin and cholestyramine to be the result of a marked decrease in the de novo production rate of LDL as compared to that occurring in untreated animals. An alternative hypothesis that fits their data just as well is that lovastatin and cholestyramine either reduce the kinetic heterogeneity normally present in the VLDL of these animals or change the relative disposition of these VLDL particles such that their relative transport to IDL and LDL is more proportional to their relative mass. Neither of these two possible mechanisms requires the concept of de novo production of LDL to explain their data.

As shown in Fig. 4, the increase or decrease in the integral of product SA relative to that of precursor SA when kinetic heterogeneity of the precursor is present can be magnified or reduced when kinetic heterogeneity of the product is also present. Such confounding effects make it imperative to delineate clearly any kinetic heterogeneity in both precursor and product systems before an accurate description of transport of precursor to product can be obtained.

Finally, it is worth mentioning the confusing results that can be produced by incomplete ultracentrifugal separation of kinetically distinct components of apoB (5). Such distortion of the tracer data can account for early activity in IDL and LDL relative to VLDL after injection of radiolabeled amino acid precursors of apoB which could be interpreted to suggest de novo production of LDL. Conversely, when such artifacts occur in data obtained after injection of VLDL, radiolabeled exogenously, the apparent dilution of LDL SA caused by kinetic heterogeneity of VLDL or by de novo production of LDL could be masked if LDL radioactivity were spuriously increased by contamination with IDL or VLDL.

The logical analysis presented above cannot be used to refute kinetic hypotheses which invoke the assumption of de novo production of LDL to explain the data in any of the animal or human studies discussed above. It does, however, point out that other hypotheses based on kinetic heterogeneity in VLDL can explain the data in these studies just as well as the hypothesis of de novo production of LDL. It is also important to note that the type of kinetic heterogeneity in VLDL that can result in apparent dilution of SA in LDL as compared to VLDL has been documented in humans and rabbits (2–5, 31, 32). Clearly,

[1]Lipoproteins isolated from Golgi fractions obtained from livers of normally fed rats, which have been shown to be uncontaminated with multivesicular bodies containing endocytosed lipoproteins, include very few particles in the size range of plasma LDL or smaller (43).

better analytical techniques for assessing the degree of metabolic heterogeneity that exists in apoB metabolism in VLDL are needed before this question can be answered definitively. Consequently, it seems to us that the wisest approach at this time to the question of de novo production of LDL is to proceed with caution, being always alert to the fact that any conclusions drawn from the analysis of kinetic data, no matter how intuitively obvious, are hypothetical and therefore may require revision when new data become available. We hope that our alternative hypothesis which explains the apparent dilution of the SA of apoB in LDL after injection of labeled VLDL on the basis of kinetic heterogeneity of apoB in VLDL rather than de novo production of LDL will encourage new types of experiments, which will allow a definitive rejection of one or the other of these hypotheses.

This research was supported by a grant from the National Institutes of Health (HL-14237, Arteriosclerosis Specialized Center of Research).

Manuscript received 16 July 1990, in revised form 3 January 1991, and in re-revised form 15 March 1991.

REFERENCES

1. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* **80:** 6096–6100.
2. Yamada, N., D. M. Shames, J. B. Stoudemire, and R. J. Havel. 1986. Metabolism of lipoproteins containing apolipoprotein B-100 in blood plasma of rabbits: heterogeneity related to the presence of apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* **83:** 3479–3483.
3. Yamada, N., D. M. Shames, and R. J. Havel. 1987. Effect of LDL receptor deficiency on the metabolism of apoB-100 in blood plasma: kinetic studies in normal and Watanabe heritable hyperlipidemic (WHHL) rabbits. *J. Clin. Invest.* **80:** 507–515.
4. Yamada, N., D. M. Shames, K. Takahashi, and R. J. Havel. 1988. Metabolism of apolipoprotein B-100 in large very low density lipoproteins of blood plasma. *J. Clin. Invest.* **82:** 2106–2113.
5. Shames, D. M., N. Yamada, and R. J. Havel. 1990. Metabolism of apoB-100 in lipoproteins separated by density gradient ultracentrifugation in normal and Watanabe heritable hyperlipidemic rabbits. *J. Lipid Res.* **31:** 753–762.
6. Ghiselli, G. 1982. Evidence that two synthetic pathways contribute to the apolipoprotien B pool of the low density lipoprotein fraction of rabbit plasma. *Biochim. Biophys. Acta.* **711:** 311–315.
7. Huff, M. W., and D. E. Telford. 1985. Direct synthesis of low density lipoprotein apoprotein B in the miniature pig. *Metabolism.* **34:** 36–42.
8. Huff, M. W., D. E. Telford, K. Woodcroft, and W. L. P. Strong. 1985. Mevinolin and cholestyramine inhibit the direct synthesis of low density lipoprotein apolipoprotein B in miniature pigs. *J. Lipid Res.* **26:** 1175–1186.
9. Huff, M. W., and D. E. Telford. 1989. Regulation of low density lipoprotein apoprotein B metabolism by lovastatin and cholestyramine in miniature pigs: effects on LDL composition and synthesis of LDL subfractions. *Metabolism.* **38:** 256–264.
10. Goldberg, I. J., N. A. Le, H. N. Ginsberg, J. J. R. Paterniti, and W. V. Brown. 1983. Metabolism of apoprotein B in the cynomolgus monkey: evidence for independent production of low-density lipoprotein apolipoprotein B. *Am. J. Physiol.* **244:** E196–E201.
11. Marzetta, C. A., F. L. Johnson, L. A. Zech, D. M. Foster, and L. L. Rudel. 1989. Metabolic behavior of hepatic VLDL and plasma LDL apoB-100 in African green monkeys. *J. Lipid Res.* **30:** 357–370.
12. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very low and low density lipoproteins in familial hypercholesterolemia. *Atherosclerosis.* **28:** 247–256.
13. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1979. Metabolism of apolipoprotein B-containing lipoproteins in familial hypercholesterolemia. *Atherosclerosis.* **32:** 315–325.
14. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1982. The metabolism of very low density and intermediate density lipoproteins in patients with familial hypercholesterolemia. *Atherosclerosis.* **43:** 217–231.
15. Janus, E. D., A. Nicoll, R. Wootton, P. R. Turner, P. J. Magill, and B. Lewis. 1980. Quantitative studies of very low density lipoprotein: conversion to low density lipoprotein in normal controls and primary hyperlipidaemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolaemia. *Eur. J. Clin. Invest.* **10:** 149–159.
16. Phair, R. D., M. G. Hammond, J. A. Bowden, M. Fried, W. R. Fisher, and M. Berman. 1975. A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. *Fed. Proc.* **34:** 2263–2270.
17. Berman, M., M. Hall, III, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
18. Fisher, W. R., L. A. Zech, P. Bardalaye, G. Warmke, and M. Berman. 1980. The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL. *J. Lipid Res.* **21:** 760–774.
19. Eaton, R. P., R. C. Allen, and D. S. Schade. 1982. Metabolic heterogeneity of low density lipoprotein-apoB production in familial hypercholesterolemia: an analytical model solution of tracer data. *J. Lipid Res.* **23:** 738–746.
20. Ginsberg, H. N., N. A. Le, and J. C. Gibson. 1985. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. *J Clin. Invest.* **75:** 614–623.
21. Beltz, W. F., Y. A. Kesäniemi, B. V. Howard, and S. M. Grundy. 1985. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, intermediate density lipoproteins and low density lipoproteins. *J. Clin. Invest.* **76:** 575–585.
22. Kesäniemi, Y. A., W. F. Beltz, and S. M. Grundy. 1985. Comparisons of metabolism of apolipoprotein B in normal subjects, obese subjects and patients with coronary heart disease. *J. Clin. Invest.* **76:** 586–595.
23. James, R. W., B. Martin, D. Pometta, J. C. Fruchart, P. Duriez, P. Puchois, J. P. Farriaux, A. Tacquet, T. Demant, R. J. Clegg, A. Munro, M. F. Oliver, C. J. Packard, and J. Shepherd. 1989. Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. *J. Lipid Res.* **30:** 159–169.

24. Arad, Y., R. Ramakrishnan, and H. N. Ginsberg. 1990. Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J. Lipid Res.* **31:** 567–582.
25. Beltz, W. F., Y. A. Kesäniemi, N. H. Miller, W. R. Fisher, S. M. Grundy, and L. A. Zech. 1990. Studies on the metabolism of apolipoprotein B in hypertriglyceridemic subjects using simultaneous administration of tritiated leucine and radioiodinated very low density lipoprotein. *J. Lipid Res.* **31:** 361–374.
26. Havel, R. J., and D. M. Shames. 1990. Kinetic studies of the origin of apolipoprotein B-100 in low density lipoproteins of normal and Watanabe heritable hyperlipidemic rabbits. *In* Hypertriglyceridemia, Hypercholesterolemia, Hypocholesterolemia and In Vivo Kinetics. C. L. Malmendier, editor. Plenum Publishing Corp., New York. In press.
27. Shipley, R. A., and R. E. Clark. 1972. Tracer Methods for In Vivo Kinetics. New York, Academic Press. 93–98.
28. Berman, M., and M. F. Weiss. 1978. SAAM Manual. DHEW Publication No. (NIH) 78-180, National Institutes of Health, Bethesda, MD.
29. Berman, M., W. F. Beltz, P. C. Greif, R. Chabay, and R. C. Boston. 1983. CONSAM Users Guide. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.
30. Foster, D. M., and R. C. Boston. 1983. The use of computers in compartmental analysis: the SAAM and CONSAM program. *In* Compartmental Distribution of Radiotracers. J. S. Robertson, editor. CRC Press, Boca Raton. 73–142.
31. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
32. Stalenhoef, A. F. H., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.
33. Le, N. A., R. Ramakrishnan, R. B. Dell, H. N. Ginsberg, and W. V. Brown. 1986. Kinetic analysis using specific radioactivity data. *Methods Enzymol.* **129:** 384–394.
34. Berman, M. 1979. Kinetic analysis of turnover data. *Prog. Biochem. Pharmacol.* **15:** 67–108.
35. Foster, D. M., A. Chait, J. J. Albers, R. A. Failor, C. Harris, and J. D. Brunzell. 1986. Evidence for kinetic heterogeneity among human low density lipoproteins. *Metabolism.* **35:** 685–696.
36. Chait, A., D. M. Foster, J. J. Albers, R. A. Failor, and J. D. Brunzell. 1986. Low density lipoprotein metabolism in familial combined hyperlipidemia and familial hypercholesterolemia: kinetic analysis using an integrated model. *Metabolism.* **35:** 697–704.
37. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. *J. Clin. Invest.* **56:** 1481–1490.
38. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein B apoprotein in man. *J. Clin. Invest.* **61:** 850–886.
39. Zilversmit, D. B. 1960. The design and analysis of isotope experiments. *Am. J. Med.* **29:** 832–848.
40. Guo, L. S. S., R. L. Hamilton, R. Ostwald, and R. J. Havel. 1982. Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* **23:** 543–555.
41. Marsh, J. B., and C. E. Sparks. 1982. The effect of fasting on the secretion of lipoproteins and two forms of apoB by perfused rat liver. *Proc. Soc. Exp. Biol. Med.* **170:** 178–181.
42. Teramoto, T., T. Watanabe, T. Matsushima, Y. Horie, and H. Oka. 1990. Characterization of nascent lipoproteins produced by perfused rat liver: evidence of hepatic secretion and post-secretory modification of nascent lipoproteins. *J. Biochem.* **107:** 519–525.
43. Hamilton, R. L., A. Moorehouse, and R. J. Havel. 1991. Isolation and properties of nascent lipoproteins from highly purified rat hepatocytic Golgi fractions. *J. Lipid Res.* **32:** 529–543.
44. Noel, S. P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubinstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
45. Johnson, F. L., L. L. Swift, and L. L. Rudel. 1987. Nascent lipoproteins from recirculating and nonrecirculating liver perfusions and from the hepatic Golgi apparatus of African green monkeys. *J. Lipid Res.* **28:** 549–564.
46. Teramoto, T., H. Kato, Y. Hashimoto, M. Kinoshita, T. Watanabe, H. Oka, and C. Naito. 1987. Effect of dietary cholesterol on production of lipoproteins and apolipoproteins by perfused livers from Japanese monkeys (*Macaca fuscata*). *Eur. J. Clin. Invest.* **17:** 522–529.
47. Goldberg, I. R., N. A. Le, H. N. Ginsberg, R. M. Krauss, and F. T. Lindgren. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J. Clin. Invest.* **81:** 561–568.