Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men[1]

John S. Millar,[2,*] Alice H. Lichtenstein,[*] Marina Cuchel,[*] Gregory G. Dolnikowski,[†] David L. Hachey,[**] Jeffrey S. Cohn,[*] and Ernst J. Schaefer[3,*]

Lipid Metabolism Laboratory[*] and Mass Spectrometry Laboratory,[†] Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111; and Children's Nutrition Research Center,[**] Baylor College of Medicine, Houston, TX 77030

Abstract Levels of plasma very low density lipoprotein (VLDL) and low density lipoprotein (LDL) constituents increase with age. In an attempt to further define the mechanisms responsible for these changes, kinetic studies of VLDL and LDL apolipoprotein (apo) B-100 were carried out in 19 normolipidemic male subjects with plasma total cholesterol and triglyceride levels below the 90th percentile whose ages ranged from 24 to 73 years. Subjects were maintained on standardized diets consisting of 47–49% of calories as carbohydrate, 15% protein, and 36–40% fat (15–17% saturated, 15–17% monounsaturated, 6% polyunsaturated) with 150 mg cholesterol/1000 kcal. At the end of the diet period, the metabolism of apoB-100 within VLDL, intermediate density lipoprotein (IDL), and LDL was studied in the fed state using a primed-constant infusion of [$^2H_3$]leucine. Data were fit to a multicompartmental model to determine residence times and production rates of apoB-100 in each fraction. There were significant positive correlations between age and VLDL, IDL, and LDL apoB-100 concentrations (r = 0.50, 0.62, and 0.69; P = 0.03, 0.004, and 0.001, respectively). There was a positive correlation between age and the production rate of VLDL apoB-100 (r = 0.50, P = 0.03), but there was no significant relationship between age and either IDL or LDL apoB-100 production rates. Age was also positively correlated with the residence time of LDL apoB-100 (r = 0.68 P = 0.001). Our data suggest that the age-associated increase in VLDL apoB-100 is due to an increased production rate of this constituent, whereas the age-associated increase in LDL apoB-100 is due to an increased residence time of these particles in plasma.—**Millar, J. S., A. H. Lichtenstein, M. Cuchel, G. G. Dolinikowski, D. L. Hachey, J. S. Cohn, and E. J. Schaefer.** Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men. *J. Lipid Res.* 1995. **36:** 1155–1167.

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The incidence of coronary heart disease (CHD) in the United States increases with age and is associated with an age-related increase in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol levels (1). Elevated levels of VLDL cholesterol are associated with decreased levels of high density lipoprotein (HDL) cholesterol, the latter being an independent risk factor for the development of CHD (2). Elevated levels of LDL cholesterol are also a risk factor for the development of CHD (3). Current National Cholesterol Education Program (NCEP) guidelines include age as an independent risk factor for the development of CHD (males ≥ 45 years, females ≥ 55 years) (4). Projections indicate that the percentage of older individuals in the United States population will increase well into the next century (5). Therefore, the percentage of the population with this additional risk factor will also be increasing. From a public health perspective, it is important to determine mechanisms responsible for these age-related changes in VLDL and LDL cholesterol levels so that measures can be taken to prevent or minimize their occurrence.

Previous investigations into the mechanism behind the age-related increase in LDL cholesterol have studied the metabolism of apolipoprotein (apo) B-100, the major protein constituent of the LDL particle. LDL apoB-100 levels, like LDL cholesterol levels, have been observed to increase with advancing age (6). Kinetic studies of LDL apoB-100 have demonstrated that this increase is partially attributable to an increase in the residence time[4] of the LDL particle (7–9). However, findings regarding the relationship between age and LDL apoB-100 production rate have not been as consistent. LDL apoB-100 production rates have been reported to increase with advancing age

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; CHD, coronary heart disease; apo, apolipoprotein; FCR, fractional catabolic rate; FSR, fractional synthetic rate; FSD, fractional standard deviation; BMI, body mass index.

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[2]Current address: Department of Pathological Biochemistry, Glasgow Royal Infirmary, Glasgow G4 OSF, Scotland.

[3]To whom reprint requests should be addressed.

[4]Residence time, the average lifetime of a particle in plasma, is calculated as (1/fractional catabolic rate) or, under steady state conditions, as (1/fractional synthetic rate) where synthesis is equal to catabolism.

(7) while others have found no relationship between LDL apoB-100 production rate and age (8, 9).

Although an age-related increase in plasma triglyceride concentrations may be due to an increased residence time of chylomicron remnants (10, 11), the mechanism behind the age-related increase in VLDL cholesterol concentrations is unknown. Using a stable isotope-labeled leucine tracer, we have designed studies to characterize changes in the metabolism of VLDL, IDL, and LDL apoB-100 that are responsible for increases in these particles with advancing age.

METHODS

Subjects

Nineteen male volunteers between the ages of 24 and 73 years with total plasma cholesterol and triglyceride levels below the 90th percentile for age and sex norms (3) underwent a complete medical history and physical examination. The subjects were in good health and had normal hepatic, renal, and thyroid function. They did not smoke, and were not taking medications known to affect plasma lipid levels. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. Subjects consumed a diet containing 45–49% carbohydrate, 15% protein, 36–40% fat (15–17% saturated, 15–17% monounsaturated, and 6% polyunsaturated fatty acids) and 180 mg cholesterol/1000 kcal/day for 5 days (subjects 1, 2, 3, 5, 6, and 10) or 4 to 6 weeks (all other subjects). All food and drink were provided to the subjects and caloric intake was adjusted to maintain body weight.

Experimental protocol

At the end of the diet period the subjects underwent a primed-constant infusion with [$^2H_3$]leucine (99.8% atom % excess) (12). After a 12-h overnight fast, subjects consumed hourly meals similar in composition to the previously provided study diet, the first representing 4/23rds (subjects 1, 2, 3, 5, 6, and 10) (12) or 1/20th (all other subjects) (13) of the daily caloric intake and subsequent meals were equally divided among the remaining daily caloric intake. Five hours after the first meal, at zero hour of the infusion protocol, subjects were given a bolus injection (10 μmol/kg) immediately followed by a constant infusion of [$^2H_3$]leucine (10 μmol/kg/h). The duration of the infusion period was 15 h. Blood samples were drawn at the following timepoints: 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15 h.

Lipoprotein fraction preparation and lipid quantitation

Blood was collected in tubes containing EDTA (0.1% final concentration). Fasting blood samples were obtained after a 14-h overnight fast. Plasma was separated from red cells in a refrigerated centrifuge at 3,000 rpm for 20 min at 4°C. Lipoprotein fractions representing triglyceride-rich lipoprotein (d < 1.006 g/ml), which contains chylomicrons and VLDL, intermediate density lipoprotein (IDL, d 1.006–1.019 g/ml), and LDL (d 1.019–1.063 g/ml) were isolated by sequential ultracentrifugation. As it was assumed that all of the apoB-100 in the triglyceride-rich lipoprotein fraction was contained in VLDL, this fraction will henceforth be referred to as VLDL. Plasma was assayed for total cholesterol and triglyceride using enzymatic reagents as previously described (14). HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with dextran sulfate-MgCl from plasma as previously described (15).

Isolation and quantitation of apoB

ApoB was assayed in plasma, VLDL, and IDL with a noncompetitive, enzyme-linked immunosorbent assay using immunopurified polyclonal antibodies as previously described (6). In the fed state, approximately 97% of the VLDL apoB mass is apoB-100, therefore, it was assumed that the VLDL apoB-100 mass was equal to the VLDL apoB mass (6). LDL apoB-100 concentrations were calculated as the difference between the plasma apoB and the sum of the VLDL and IDL apoB concentrations. ApoB-100 was isolated from VLDL and LDL by preparative SDS polyacrylamide gel electrophoresis (4–22.5%) using a Tris-glycine buffer system as previously described (16).

Determination of isotopic enrichment

Polyacrylamide gel bands containing apoB-100 were hydrolyzed in 12 N hydrochloric acid at 100°C for 24 h. The hydrolysates were dried under nitrogen, resuspended in 1 N acetic acid, and the free amino acids were isolated using a Dowex AG-50W-X8 100–200 mesh cation exchange resin. Amino acids were eluted from the resin with 3 M ammonium hydroxide and dried. The sample was then propylated, converted to the N-heptafluorobutyramide derivatives, and extracted into ethyl acetate prior to analysis on a Hewlett-Packard 5890/5988A gas chromatograph/mass spectrometer (17).

Kinetic analysis

Fractional synthetic and production rates were calculated using a multicompartmental analysis of isotopic enrichment data from VLDL, IDL, and LDL apoB-100 samples drawn during the infusion period. Data were expressed as % (labeled/unlabeled) leucine (also known as tracer/tracee) as recommended by Cobelli et al. (18). Data were corrected for naturally occurring leucine in the M + 3 (labeled) peak by subtracting the zero hour (background) ratio at this mass without further manipulation because a high-purity tracer of 99.8% will result in ratios identical, when rounding, to those obtained using the equations from reference 18 (**Table 1**). Corrected ratios

TABLE 1. Comparison of methods of calculating % (labeled/unlabeled) leucine ratios at different tracer purities

Sample Enrichment	M + 0 Peak Area	M + 3 Peak Area	M + 3 / M + 0	Enrichment[a] (99.8% Tracer Purity)	Enrichment[a] (80.0% Tracer purity)	Labeled / Unlabeled[a,b] (99.8% Tracer Purity)	Labeled / Unlabeled[a,b] (80.0% Tracer Purity)	(M + 3 / M + 0) Sample − (M + 3 / M + 0) Background
			%	%	%	%	%	%
Background	1×10^6	2×10^3	0.20					
Low	1×10^6	3×10^3	0.30	0.10	0.10	0.10[c]	0.13	0.10
Medium	1×10^6	5.2×10^3	5.20	4.74	4.74	5.0	6.3	5.0
High	1×10^6	10.2×10^3	10.20	9.06	9.06	10.0	12.8	10.0

[a] Calculated as in reference 18.
[b] Rounded value.
[c] As calculated in the current study.

were then analyzed with the CONSAM 30 software program (19). Two dummy data points, representing the theoretical maximal enrichment, also referred to as the plateau value, of apoB-100, calculated as previously described (13), were inserted into each data set at 350 and 400 h to ensure that the VLDL, IDL, and LDL data sets all reached an identical plateau value and to improve the reproducibility of the analysis. The mass of each lipoprotein fraction (pool size) was calculated as the product of the plasma concentration of that fraction and the estimated plasma volume (4.5 percent of body weight). The mass of each lipoprotein fraction was accounted for in the model by dividing the sum of the function(s) describing the kinetic behavior of each lipoprotein fraction by the corresponding apoB-100 pool size (equation 1). This is identical to fitting data expressed as tracer units to these same function(s) (equation 2).

$$[\%\ (\text{labeled/unlabeled})] = f(x)/(\text{pool size}) \qquad \textit{Eq. 1)}$$

$$[\%\ (\text{labeled/unlabeled})] * (\text{pool size}) = f(x) \qquad \textit{Eq. 2)}$$

The model used in analyzing the kinetic data is depicted in **Fig. 1.** Because the metabolic studies were carried out to 15 h, the model was simplified to one in which the parameters could be identified with reasonable certainty based on fractional standard deviations of the parameter estimates. The precursor pool (compartment 11) is followed by a delay chain, which represents the time required for the synthesis and secretion of apoB-100 from the liver. This parameter was fixed to a value of 30 min (20) to decrease the number of adjustable parameters in the model. There are two inputs into the VLDL fraction, one representing particles that turn over rapidly and the other with a slower turnover, as might be expected from a remnant particle. The rapid VLDL enters a compartment (compartment 21), that represents a compartment containing large VLDL. Compartments 21 to 24 represent a classical VLDL delipidation chain, a concept originally proposed by Phair et al. (21) and later supported by others (22–24) which, in the current case, was necessary to fit the delay in appearance of label in the IDL and LDL data. The rate constants between compartments 21 and 31 are set as being equal, again, to limit the number of adjustable parameters. There are sites of direct catabolism from each compartment along the delipidation chain. From VLDL, apoB-100 enters a rapid IDL compartment (compartment 31), and eventually the LDL

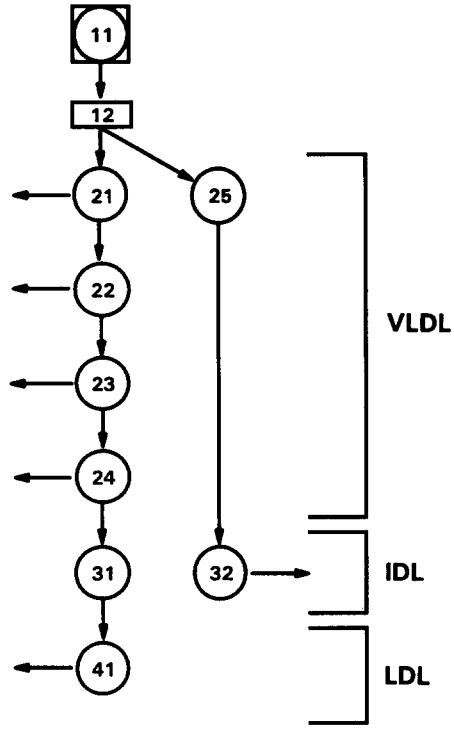

Fig. 1. The compartmental model used in the analysis of the data from this study. Compartment 11: precursor amino acid pool; 12 represents the synthetic and secretory delay of VLDL apoB-100; compartments 21 to 25: VLDL; compartments 31 and 32: IDL; compartment 41: LDL. Horizontal arrows leaving any compartment represent catabolism of apoB-100 from that compartment.

compartment (compartment 41). The slow VLDL pathway consists of a single compartment (compartment 25) that has a relatively increased residence time when compared to material in compartments 21 to 24. VLDL from this compartment is the precursor for IDL remnants (compartment 32) which also have a relatively increased residence time as compared to material in compartment 31. Although the possibility exists for apoB-100 from the IDL remnant to continue to LDL, this was unnecessary to fit the LDL and was not included in the model. Therefore, all IDL remnants in compartment 32 are assumed to be catabolized directly from plasma. The mean parameter estimates and fractional standard deviations are listed in the appendix for younger (<45 years), and older (<45 years) subjects from the study.

The fractional catabolic rate (FCR) of each fraction represents the weighted sum, based on the mass of each compartment within that fraction, of the individual rate constants leaving that fraction. The equations used for the FCR calculations for VLDL, IDL, and LDL apoB-100 are shown as equations 3–5, respectively. M(x) is the mass of compartment x and L(y,x) is the rate constant for the fraction of apoB-100 in compartment x entering compartment y. L(0,x) is the rate constant for the fraction of apoB-100 in compartment x being catabolized directly from plasma. Residence times were calculated by taking the reciprocal of the FCR (1/FCR). Production rates were obtained by multiplying the FCR by the fraction mass and dividing the result by the body weight.

$$\text{VLDL FCR} = \frac{[M(21)*L(0,21)]+[M(22)*L(0,22)]+[M(23)*L(0,23)]+[M(24)]+[L(0,24)]+[M(24)*L(31,24)]+[M(25)*L(32,25)]}{[M(21)+M(22)+M(23)+M(24)+M(25)]} \quad \textit{Eq. 3)}$$

$$\text{IDL FCR} = \frac{[M(31)*L(41,31)]+[M(32)*L(0,32)]}{M(31)+M(32)} \quad \textit{Eq. 4)}$$

$$\text{LDL FCR} = \frac{[M(41)*L(0,41)]}{M(41)} \quad \textit{Eq. 5)}$$

We have shown that plasma leucine enrichments remain constant during the course of the infusion (13, 17). We therefore assumed a constant enrichment of the precursor pool and used a constant precursor enrichment in calculating the kinetic parameters. Due to our study extending to 15 h we were unable to estimate kinetic parameters associated with multiple LDL compartments (25) and must assume that the rate constant for the removal from the LDL compartment represents the average kinetic behavior of an LDL particle in plasma.

Model selection

Previously, we chose simplified approaches in the analysis of kinetic data obtained during the course of a 15-h infusion of stable isotope-labeled amino acid tracer (12, 13, 17). These analyses yielded valuable insights into the metabolism of apolipoproteins under a variety of circumstances in our hands and by others (26–29). There are, however, limitations to performing these types of analyses (30). Although the results from these previous studies may be qualitatively correct, the quantitative results may be significantly different from the actual result, based on the heterogenous kinetic behavior of the particle under study (23, 31). We therefore analyzed the current data using multicompartmental modeling. There are two basic types of multicompartmental models of VLDL, IDL, and LDL apoB-100 metabolism in the literature. The first type uses a classical VLDL delipidation chain from which IDL and LDL are derived, as well as remnant compartments in VLDL (21–24). The second type has collapsed the VLDL delipidation chain into one compartment in an attempt to limit the number of adjustable parameters but is otherwise similar to the first (32). Although the second type of model may satisfy mathematical constraints required to obtain unique parameter estimates for the model, it has sacrificed a physiological component that most previous investigators have felt necessary to include for the past 20 years. We sought to work with a model that satisfies the physiological structure of the VLDL, IDL, and LDL system as it is thought to be today and, at the same time, satisfies mathematical criteria that determine how well a model fits a set of kinetic data. The objective of these experiments was to estimate the residence time and production rates of VLDL, IDL, and LDL apoB-100 in this set of subjects. By examining the results of previous studies we have found a model that is able to embody the features that are in the VLDL, IDL, and LDL apoB-100 metabolic system as it is known today while being able to provide reproducible parameter estimates.

Model assumptions

As it is not possible to measure all variables required to perform multicompartmental modeling of the kinetic data, the analysis, as with any kinetic analysis, is conducted using a number of assumptions. We assume that plasma leucine is in equilibrium with the hepatic leucine tRNA pool and that the hepatic tRNA pool maintains a constant level of enrichment during the course of the infusion as reflected by a constant plasma enrichment (12, 13). The apoB-100 from VLDL, IDL, and LDL is derived from the same precursor pool that results in newly formed apoB-100 in VLDL, IDL, and LDL having an identical enrichment of stable isotope-labeled leucine. This would result in these proteins achieving an identical maximal level of enrichment, or plateau value, in plasma if ex-

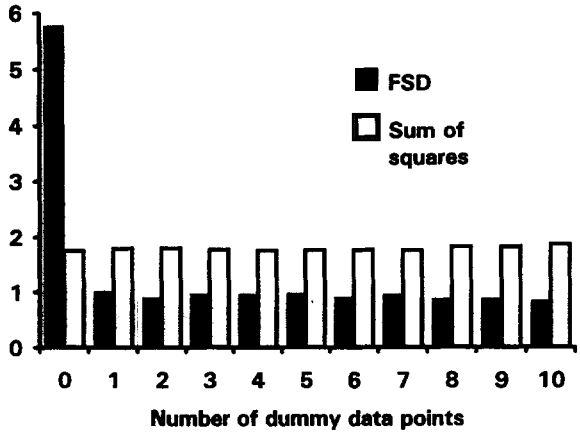

Fig. 2. Graph illustrating the effect of including 0 to 10 dummy data points in each set of VLDL, IDL, and LDL kinetic data on the total fractional standard deviation (FSD) of the parameter estimates and on the sum of aquares of error.

perimental conditions had been maintained for more than the 15-h duration of the study to a point where the enrichment in each fraction failed to increase further. We assume that we are able to measure the maximal level of enrichment for each subject using the VLDL apoB-100 kinetic data. The assumption is made that these studies were conducted under steady state conditions, where the FCR is equal to the fractional synthetic rate (FSR), by inducing a constant production of lipoproteins with frequent feedings (12, 13). We assume that the time required for each subject to synthesize and secrete VLDL apoB-100 is approximately 30 min. We also make the assumption that all VLDL remnants are synthesized as such de novo and are converted to IDL remnants that are removed directly from plasma.

Statistical analyses

Statistical analyses were performed with Microsoft Excel version 4.0 (Microsoft Corporation, Seattle, WA). Pearson correlation coefficients were calculated to test for correlations between parameters. The Student's *t*-test was used to assess mean differences between groups.

RESULTS

The effect of including dummy data points, representing the theoretical level of leucine enrichment of the precursor from which these particles were derived, on the fractional standard deviation (FSD) of the parameter estimates and the sum of squared errors are shown in **Fig. 2.** There was a substantial improvement in the sum of the FSDs by including one dummy data point in each of the VLDL, IDL, and LDL data sets. Additional dummy data points resulted in a sum of FSDs that was similar to the VLDL, IDL, and LDL data sets with only one dummy data point. There was no difference in the sum of squares of data sets analyzed with and without additional dummy data points. There were differences in the level at which the VLDL, IDL, and LDL data sets arrived at a plateau enrichment value in data sets that were extrapolated to 200 h when no dummy data points were included in the analysis (**Fig. 3A**). When dummy data points were included there were no differences in the plateau enrichment value for the VLDL, IDL, and LDL data sets (Fig. 3B).

The characteristics of the subjects are presented in **Table 2.** The age range of the subjects was from 24 to 73 years. Age was significantly correlated with total cholesterol and triglyceride concentrations (Table 2). In contrast, age was not correlated with HDL cholesterol concentrations and body mass index (BMI). There was

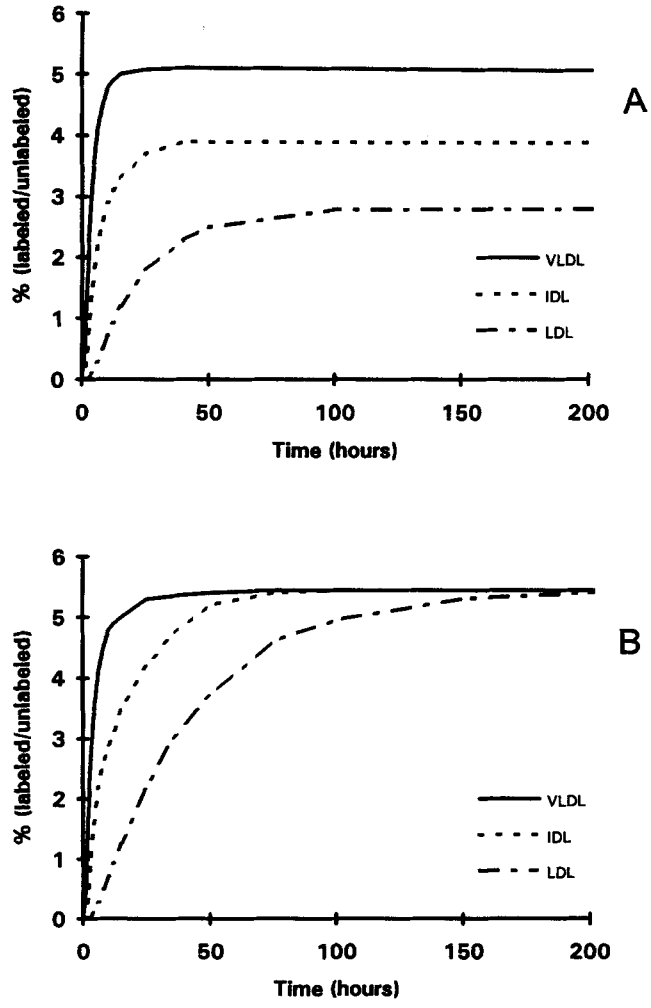

Fig. 3. Kinetic curves, extrapolated to 200 h, for the solutions to VLDL, IDL, and LDL data sets in one subject without (A) and with (B) dummy data points included in each data set.

TABLE 2. Subject characteristics

Subject	Age	BMI	Total Cholesterol	Total Triglyceride	HDL Cholesterol
	yr	*kg/m²*	*mg/dl*	*mg/dl*	*mg/dl*
1	24	20.0	143	33	56
2	25	23.2	144	61	37
3	25	34.5	151	76	47
4	27	26.7	157	70	37
5	28	22.7	149	63	59
6	29	24.7	135	115	36
7	33	26.1	140	81	42
8	36	23.4	172	118	32
9	37	22.7	174	81	45
10	41	23.5	196	76	49
11	46	26.1	189	114	37
12	47	21.5	181	98	45
13	53	26.8	159	174	29
14	60	25.8	211	79	48
15	62	28.3	174	85	34
16	65	25.0	195	128	39
17	69	24.6	233	81	42
18	71	25.0	225	182	46
19	73	27.1	204	125	49
Mean ± SD	45 ± 17	25.1 ± 3.1	175 ± 30	97 ± 38	43 ± 8
r^a		0.12	0.85[b]	0.59[c]	−0.11

[a] Pearson correlation coefficient versus age.
[b] $P < 0.001$.
[c] $P < 0.01$.

also no significant correlation between age and the calculated VLDL apoB-100 plateau value for the subjects (data not shown).

TABLE 3. Residence times and production rates for VLDL apoB-100

Subject	Fed VLDL ApoB-100	Residence Time	Production Rate
	mg/dl	*days*	*mg/kg per day*
1	3.0	0.25	5.3
2	2.8	0.08	15.1
3	2.0	0.06	12.2
4	10.1	0.11	40.0
5	5.1	0.18	12.7
6	3.3	0.13	10.3
7	2.6	0.11	10.6
8	4.6	0.04	48.2
9	5.4	0.05	46.1
10	3.7	0.06	27.6
11	3.9	0.08	15.3
12	6.3	0.03	31.5
13	15.9	0.30	23.6
14	24.7	0.31	35.1
15	4.1	0.17	10.4
16	12.3	0.10	55.0
17	2.2	0.04	22.7
18	11.1	0.13	37.8
19	11.6	0.03	64.5
Mean ± SD	7.1 ± 5.9	0.12 ± 0.09	27.6 ± 17.3
r^a	0.50[b]	0.01	0.50[b]

[a] Pearson correlation coefficient versus age.
[b] $P < 0.05$.

The results of the analysis of VLDL apoB-100 kinetic data are shown in **Table 3.** Age was not significantly correlated with VLDL apoB-100 residence time ($r = 0.01$, $P = 0.97$) but was positively correlated with production rate of VLDL apoB-100 ($r = 0.50$, $P = 0.03$) (**Figs. 4A and 4B**).

The results of the analysis of the IDL apoB-100 kinetic data are shown in **Table 4.** Age was not correlated with IDL apoB-100 residence time ($r = 0.20$, $P = 0.41$) or production rate ($r = 0.33$, $P = 0.17$).

The results of the LDL apoB-100 kinetic analyses are shown in **Table 5.** There was a significant positive correlation of age with LDL apoB-100 residence time ($r = 0.68$, $P = 0.001$) (**Fig. 5A**). Age was not correlated with the production rate of LDL apoB-100 ($r = 0.15$, P 0.54) (Fig. 5B).

Subjects were divided on the basis of age into an older group (>45 years) and a younger group (<45 years), according to the current NCEP guidelines at which age, in males, becomes an additional risk factor for the development of CHD (4). Representative kinetic curves for a younger subject and older subject, selected for having a relatively decreased and increased LDL apoB-100 residence time, respectively, are shown in **Fig. 6A and 6B.** The mean ages of the younger and older subjects were 31 and 61 years, respectively (**Table 6**). There was no difference in the mean VLDL apoB-100 residence time (0.13 ± 0.11 vs. 0.11 ± 0.07 day) or production rate (32.8 ± 17.8 vs. 22.8 ± 16.3 mg/kg per day) between older and younger subjects. There were no significant

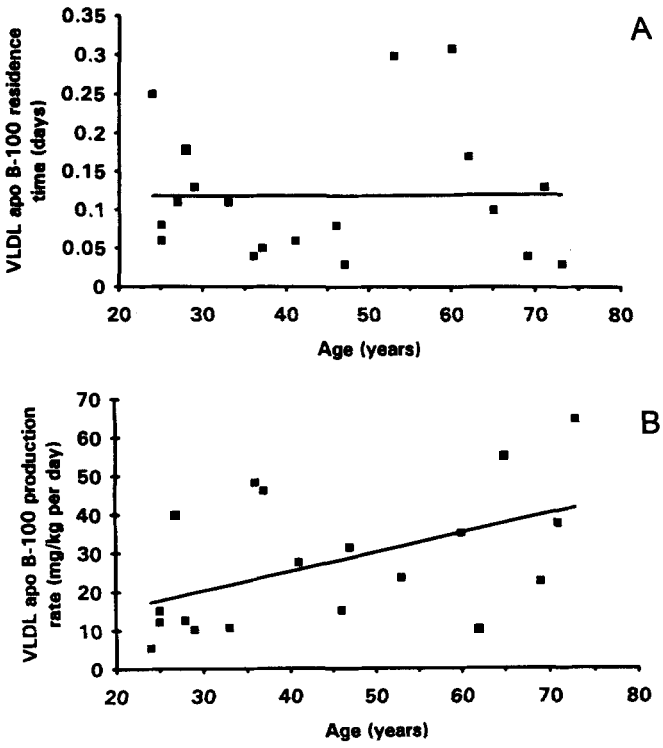

Fig. 4. Relationship between age and VLDL apoB-100 residence time (A) and production rate (B).

differences in the IDL apoB-100 residence time (0.04 ± 0.02 vs. 0.03 ± 0.01 day) or production rates (14.4 ± 2.7 vs. 13.2 ± 4.8 mg/kg per day) between older and younger subjects, respectively. The LDL apoB-100 residence time of the older subjects was significantly higher than that of the younger subjects (3.46 ± 0.25 vs. 2.42 ± 0.55 days), with no significant differences in the production rates (12.2 ± 2.6 vs. 12.3 ± 4.0 mg/kg per day).

TABLE 4. Residence times and production rates for IDL apoB-100

Subject	Fed IDL ApoB	Residence Time	Production Rate
	mg/dl	*days*	*mg/kg per day*
1	0.6	0.05	5.4
2	0.4	0.02	13.6
3	0.6	0.02	12.1
4	1.0	0.04	13.2
5	0.7	0.03	12.5
6	1.2	0.02	10.1
7	0.4	0.02	11.3
8	1.0	0.04	13.4
9	0.7	0.02	16.5
10	1.4	0.05	24.2
11	1.7	0.02	12.7
12	0.6	0.02	14.8
13	2.4	0.08	14.6
14	2.3	0.06	17.0
15	0.7	0.03	10.4
16	1.8	0.06	13.6
17	0.9	0.03	16.7
18	1.5	0.04	18.7
19	2.4	0.02	11.3
Mean ± SD	1.2 ± 0.7	0.04 ± 0.02	13.8 ± 3.9
r^a	0.62[b]	0.20	0.33

[a] Pearson correlation coefficient versus age.
[b] $P < 0.01$.

TABLE 5. Residence times and production rates for LDL apoB-100

Subject	Fed LDL ApoB	Residence Time	Production Rate
	mg/dl	*days*	*mg/kg per day*
1	34	3.21	4.7
2	51	1.54	14.9
3	60	2.31	11.6
4	86	2.98	13.0
5	56	2.45	10.3
6	56	2.60	9.6
7	51	2.19	10.6
8	82	2.98	12.4
9	62	1.67	16.7
10	99	2.31	19.3
11	106	3.79	12.6
12	105	3.47	13.7
13	69	2.98	10.4
14	69	3.21	9.6
15	73	3.47	9.5
16	89	3.47	11.5
17	136	3.47	17.6
18	115	3.79	13.7
19	96	3.47	11.2
Mean ± SD	79 ± 26	2.91 ± 0.68	12.3 ± 3.3
r^a	0.69[b]	0.68[b]	0.15

[a] Pearson correlation coefficient versus age.
[b] $P < 0.001$.

DISCUSSION

The results of this study indicate that the residence time of LDL apoB-100 decreases with advancing age in men. In addition it was observed that VLDL apoB-100 production rate increases with advancing age. There was no evidence for a relationship between age and the production rate of LDL apoB-100 even though subjects over a broad age range were studied. These findings, in part, account for the positive correlations between the plasma VLDL, IDL, and LDL apoB-100 concentrations with age.

A significant positive correlation of age and the production rate of VLDL apoB-100 was observed in the current study. In a recent review on the topic of hepatic apoB-100 secretion it was concluded that a major determinant of hepatic apoB-100 secretion is the amount of lipid substrate present in the liver (33). The differences observed in the production rates of VLDL apoB-100 between older and younger individuals in the current study may relate to differences in the hepatic supply of endogenous or

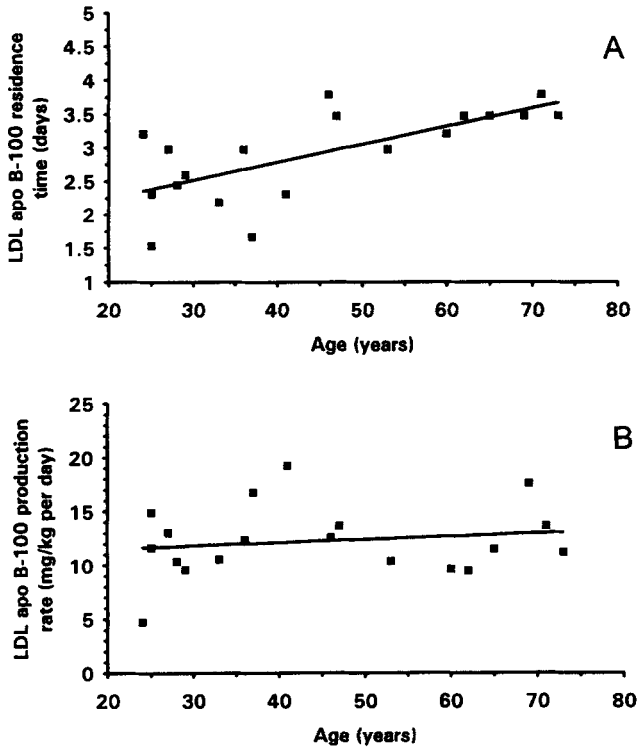

Fig. 5. Relationship between age and LDL apoB-100 residence time (A) and production rate (B).

dietary lipid. In the current study, subjects were provided with diets of similar composition to control dietary lipid content. Thus, an explanation for the current findings would be that there are either differences between older and younger individuals in the processing of dietary and/or endogenous lipid by the liver or that there are differences in endogenous lipid content between the livers of younger and older subjects. Although it is not known whether there are differences in the processing of dietary lipid between younger and older individuals, it is known that the percentage of lean body mass decreases with age (34). Thus, even though there were no significant differences in BMI between older and younger subjects in this study, it is likely that the older subjects had a greater percentage of body fat than the younger subjects. If this were the case, then the additional body fat could result in an increased flux of free fatty acids to the liver and an increased rate of hepatic triglyceride synthesis. This, in turn may lead to increased production rates of VLDL apoB-100 in the older subjects, similar to what has been reported in obesity (35).

A significant correlation between age and IDL apoB-100 concentration in plasma was also observed in this study. There was no significant relationship between the IDL apoB-100 residence time or production rate and age. However, there were positive correlations between age and both the IDL apoB-100 residence time and production rate which, combined, could account for the age associated increase in IDL apoB-100 concentrations.

The lack of association of age with LDL apoB-100 production rate in this study is similar to the observations reported by Ericsson et al. (9) and Miller (8). This is in contrast to observations reported by Grundy, Vega, and Bilheimer (7) who found that older subjects had higher LDL apoB-100 production rates than younger subjects. It might be expected that a similar percentage of IDL apoB-100 would be converted to LDL apoB-100 regardless of age as was seen in the VLDL to IDL conversion. However, results from the current analysis indicate that, although there is an increased production of VLDL apoB-100 with advancing age, the percentage of these particles that are cleared from the plasma without being converted to LDL increases with advancing age as well, the net result being no increase in the production of LDL apoB-100 with advancing age. The specific pathways that

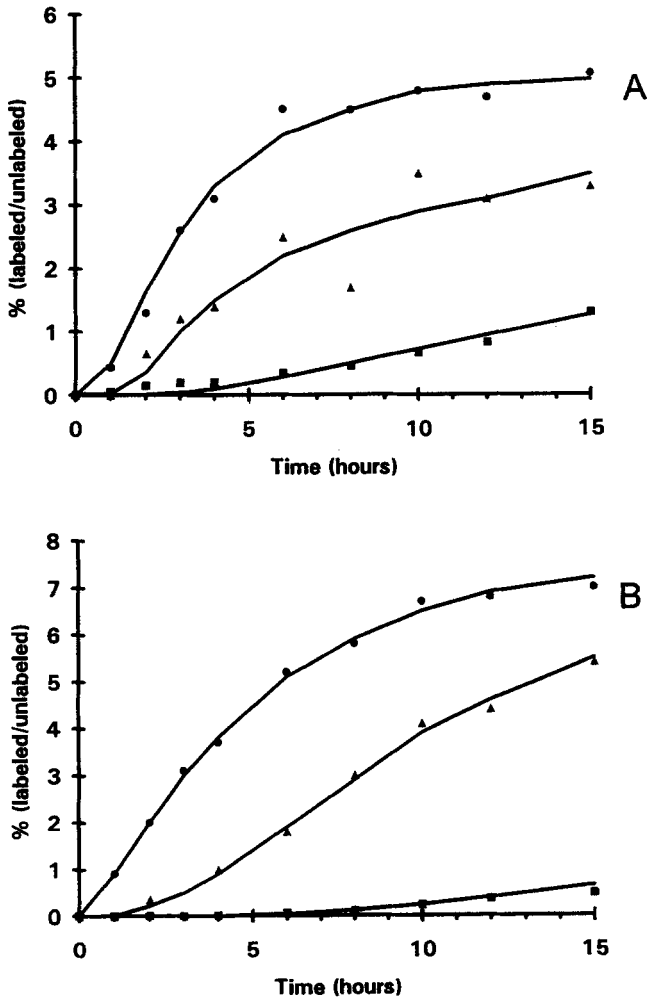

Fig. 6. Kinetic curves for the solutions to the VLDL (circles), IDL (triangles), and LDL (squares) data sets for a representative younger (A) and older (B) subject.

TABLE 6. Mean values for younger and older subject groups

Variable	Younger (n = 10)	Older (n = 9)	*P* Value
Age (years)	31 ± 6	61 ± 10	
BMI (kg/m^2)	24.7 ± 3.9	25.6 ± 1.9	0.57
Total cholesterol (mg/dl)	156 ± 19	197 ± 24	<0.001
Triglyceride (mg/dl)	77 ± 25	118 ± 38	0.01
HDL cholesterol (mg/dl)	44 ± 9	41 ± 7	0.42
VLDL apoB-100 (mg/dl)	4.3 ± 2.3	10.2 ± 7.1	0.02
VLDL apoB-100 residence time (days)	0.11 ± 0.07	0.13 ± 0.11	0.54
VLDL apoB-100 production rate (mg/kg per day)	22.8 ± 16.3	32.8 ± 17.8	0.22
IDL apoB (mg/dl)	0.8 ± 0.3	1.6 ± 0.7	0.006
IDL apoB-100 residence time (days)	0.03 ± 0.01	0.04 ± 0.02	0.40
IDL apoB-100 production rate (mg/kg per day)	13.2 ± 4.8	14.4 ± 2.7	0.52
LDL apoB-100 (mg/dl)	64 ± 19	95 ± 23	0.005
LDL apoB-100 residence time (days)	2.42 ± 0.55	3.46 ± 0.25	<0.0001
LDL apoB-100 production rate (mg/kg per day)	12.3 ± 4.0	12.2 ± 2.6	0.94

are involved in the clearance of the additional newly secreted particles observed with increasing age are one or more of the "remnant" pathways involved in VLDL metabolism (compartments 25, 31, and 32).

The results observed in this study regarding LDL apoB-100 residence time are consistent with what has been observed previously (7–9). Miller (8) conducted a meta-analysis that included data from 57 male subjects (age range approximately 20 to 70 years) who participated in comparably conducted studies of LDL apoB-100 metabolism. It was reported that there was a significant negative correlation of age with LDL apoB-100 FCR. Ericsson et al. (9) studied male subjects between the ages of 21 and 80 years and reported that there was a significant negative correlation of LDL apoB-100 FCR with age. Grundy et al. (7) studied LDL apoB-100 metabolism in younger subjects (mean age 24.9 years) and older subjects (mean age 55.6 years) and reported that there was a significantly lower FCR in the older subjects when compared to the younger subjects.

Spady and Dietschy (36) hypothesized that the age-related increase in LDL cholesterol levels is related to diet as there are no differences in the relative receptor activity and production rates of LDL in hamsters of different ages fed a relatively low fat diet. However, the general finding of total and/or LDL cholesterol levels increasing with advancing age seen in the United States has been observed in some subpopulations consuming a relatively low-fat diet (37–39) but not others (40–42). This would suggest that, in free-living humans, other non-dietary mechanisms may be responsible for an age-related increase in total and LDL cholesterol levels. Ericsson et al. (9) were able to increase the LDL apoB-100 FCR in older subjects to levels comparable to those of younger subjects by administration of cholestyramine. This led to their hypothesis that the mechanism responsible for a decreased LDL apoB-100 FCR with increasing age is a decrease in 7α-hydroxylase activity as they had previously demonstrated an age-related decrease in bile acid production (43). Recent data have demonstrated that 7α-hydroxylase activity decreases with advancing age in humans which may be due to an age-related decrease in circulating growth hormone levels (44, 45). This situation, hypothetically, would result in an accumulation of cholesterol and 25-hydroxycholesterol precursors within the hepatocyte, which could down-regulate LDL receptor activity (46). Additional support for this hypothesis comes from clinical studies that have demonstrated that growth hormone administration results in increased hepatic LDL receptor expression (47). An alternative hypothesis is that with advancing age there is down-regulation of hepatic LDL receptors in response to an increased hepatic uptake of cholesterol-rich VLDL and IDL particles. This hypothesis is similar to that proposed by Davignon, Gregg, and Sing (48) to explain the increase in LDL cholesterol associated with apoE4 homozygosity. Increased uptake of cholesterol-rich VLDL and IDL in vivo would presumably increase the hepatic cholesterol content and result in a down-regulation of LDL receptors, the result being a decreased LDL apoB-100 FCR.

While most studies that have used radioiodinated apoB-100 to study normal subjects and analyzed the data using a multicompartmental model have been conducted in the fasting state, we find that results obtained for the current kinetic analysis are similar to results for subjects studied using radioiodinated VLDL under similar conditions (i.e., in the constantly fed state) (49). We found that the residence time for VLDL apoB-100 ranged from 0.03 to 0.31 days as compared to 0.02 to 0.19 days, and the production rate of VLDL apoB-100 ranged from 5.3 to 64.5 mg/kg per day as compared to 16.8 to 117.6 mg/kg per day. We found that the residence time for LDL apoB-100 ranged from 1.54 to 3.79 days as compared to 1.22 to 2.17 days, and the production rate of LDL apoB-100 ranged from 4.7 to 19.3 mg/kg per day as compared to 10.9 to 18.7 mg/kg per day.

Limitations of the study

A number of interesting new findings have emerged from the present study. It must be stressed that the conclusions drawn in this study, as with any apolipoprotein kinetic study, are based on a number of assumptions. If one or more of these assumptions are incorrect, then conclusions drawn in this study may differ from those we have arrived at.

There are practical limitations on the number of blood samples that can be drawn during the course of a lipoprotein kinetic study that utilizes stable isotope-labeled tracers. If subjects, particularly older subjects, are participating in a long term study where their blood chemistries are monitored on a regular basis and are to participate in a kinetic study, which is the case for some of the subjects in the current study, then there are strict limitations as to the total amount of blood, hence, number of blood samples, that can be taken during the infusion period itself. Ideally one would like to collect a larger number of timepoints to improve the confidence in the parameter estimates derived from fitting a model to the kinetic data. This would also have the benefit of allowing the investigator to use a more complex model with fewer constraints, the analysis of which would not be as dependent on assumptions as was the current model.

New questions

We have observed that the production rate of VLDL apoB-100 increases with advancing age. If we are correct in hypothesizing that increased hepatic triglyceride is responsible for the increased apoB-100 secretion, then a logical question is why does hepatic triglyceride content increase with advancing age?

We have also found that there is no difference in the residence time of VLDL apoB-100 between older and younger subjects. Is the same also true for chylomicron apoB-48? Or are there differences that have been suggested in studies comparing retinyl ester and postprandial triglyceride clearance in young and old?

Another interesting question is, if VLDL apoB-100 production increases with age, why is there not a concomitant increase in LDL production? Do older subjects produce larger VLDL particles that are less likely to be converted to LDL, or are the particles similar in composition and content to those secreted by younger subjects, and are there alterations in the metabolism in plasma of these particles which result in their clearance before reaching LDL?

In summary, we have found that increases in VLDL apoB-100 concentrations seen with age are associated with an increased production rate. It was also found that age-associated increases in LDL apoB-100 concentrations are associated with a increased LDL apoB-100 residence time with no change in the production rate. These findings may be due, in part, to changes in body composition with age. We speculate that an increased fat body mass may result in elevated plasma free fatty acid concentration. As a consequence of this there is overproduction of triglyceride by hepatocytes of older individuals resulting in excess VLDL apoB-100 production, a decreased conversion of IDL to LDL, and delayed fractional catabolism of LDL due to down-regulation of hepatic LDL receptor activity.

APPENDIX

Mean compartment masses, rate constants, and transport rates as determined by multicompartmental modeling of kinetic data from younger subjects

	M(21)	M(22)	M(23)	M(24)	M(25)	M(31)	M(32)	M(41)
Mean ± SD	33 ± 30	30 ± 19	26 ± 13	23 ± 9	30 ± 28	13 ± 7	19 ± 22	2220 ± 742
	L(22,21)	L(23,22)	L(24,23)	L(0,21)	L(0,22)			
Mean ± SD	47 ± 27	47 ± 27	47 ± 27	5 ± 7	5 ± 7			
% FSD	12	12	12	6	6			
	L(0,23)	L(0,24)	L(31,24)	L(32,25)	L(0,32)			
Mean ± SD	5 ± 7	5 ± 7	47 ± 27	3 ± 1	4 ± 2			
% FSD	6	6	12	12	16			
	L(41,31)	L(0,41)						
Mean ± SD	83 ± 54	0.434 ± 0.111						
% FSD	10	6						

M(x) is the mass of compartment x in milligrams; L(y,x) is the rate constant for the fractional rate of transfer of apoB-100 from compartment x to compartment y per day.

APPENDIX

Mean compartment masses, rate constants, and transport rates as determined by multicompartmental modeling of kinetic data from older subjects

	M(21)	M(22)	M(23)	M(24)	M(25)	M(31)	M(32)	M(41)
Mean ± SD	89 ± 64	63 ± 61	48 ± 42	37 ± 29	103 ± 84	14 ± 14	48 ± 30	3338 ± 649

	L(22,21)	L(23,22)	L(24,23)	L(0,21)	L(0,22)
Mean ± SD	47 ± 27	47 ± 27	47 ± 27	5 ± 7	5 ± 7
% FSD	12	12	12	8	6

	L(0,23)	L(0,24)	L(31,24)	L(32,25)	L(0,32)
Mean ± SD	5 ± 7	5 ± 7	47 ± 27	3 ± 1	4 ± 2
% FSD	6	6	12	12	16

	L(41,31)	L(0,41)
Mean ± SD	83 ± 64	0.434 ± 0.111
% FSD	10	6

M(x) is the mass of compartment x in milligrams; L(y,x) is the rate constant for the fractional rate of transfer of apoB-100 from compartment x to compartment y per day.

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