Positive linear correlation between the length of truncated apolipoprotein B and its secretion rate: in vivo studies in human apoB-89, apoB-75, apoB-54.8, and apoB-31 heterozygotes

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Abstract Apolipoprotein B (apoB), the major protein component of triglyceride-rich lipoproteins secreted from the liver, plays crucial roles in the secretion, transport, and receptor-mediated clearance of lipoproteins. A minority of cases of familial hypobetalipoproteinemia is due to genetically determined truncations of apoB-100 that range in size from apoB-9 to apoB-89, but truncated apoBs smaller than apoB-27.6 were not detected in plasma. To ascertain the physiologic bases of the hypobetalipoproteinemia, we studied in vivo metabolic parameters of the products of both the normal and mutant apoB alleles in human apoB truncation/apoB-100 heterozygotes (apoB-89/apoB-100, n = 2, apoB-75/apoB-100, n = 2; apoB-54.8/apoB-100, n = 6; apoB-31/apoB-100, n = 1) using endogenous labeling with [^{13}C]leucine, mass spectrometry, and multicompartmental modeling. All truncated forms of apoB were secreted at reduced rates. The secretion rates of apoB-89, apoB-75, apoB-54.8, and apoB-31 were 92%, 64%, 37%, and 12%, respectively, of the respective apoB-100s on a molar basis. Additionally, particles containing apoB-89, apoB-75, and apoB-54.8 had increased fractional catabolic rates (FCR), while apoB-31-containing particles had a decreased FCR. On regression analysis, the secretion rate was linearly linked to the length of the truncated apoB (r^2 = 0.86, P < 0.0001), with secretion being reduced by 1.4% for each 1% of apoB truncated. The linear regression line of apoB size versus apoB secretion rate has a zero intercept for apoB secretion at apoB-28, which is consonant with the apparent absence in plasma of truncations smaller than apoB-25. We conclude that secretion of apoB in vivo is dependent on the length of the truncation of apoB, possibly because the smaller the truncated apoB, the less it is protected from intracellular degradation.—**Parhofer, K. G., P. H. R. Barrett, C. A. Aguilar-Salinas, and G. Schonfeld.** Positive linear correlation between the length of truncated apolipoprotein B and its secretion rate: in vivo studies in human apoB-89, apoB-75, apoB-54.8, and apoB-31 heterozygotes. *J. Lipid Res.* **37:** 844–852.

Supplementary key words hypobetalipoproteinemia • lipoprotein metabolism • apoB-100 • apoB truncations • kinetic models

Apolipoprotein B-100 (apoB-100), the major structural protein component of triglyceride-rich lipoproteins synthesized in human liver, consists of 4536 amino acids. ApoB-100 plays crucial roles in the intravascular transport of lipids in apoB-100-containing lipoproteins and in the LDL-receptor mediated clearance of the same lipoproteins. It is also crucial in the hepatic assembly and secretion of nascent lipoproteins. Another form of apoB is apoB-48 which is secreted by enterocytes as an integral protein of chylomicrons. Cell-culture studies indicate that the rate of secretion and degradation of apoB-100 is closely linked to the lipid supply (1, 2). The availability of lipids most likely regulates secretion leaving the unsecreted apoB for degradation.

Truncations of apoB-100 in humans are associated with hypobetalipoproteinemia, and truncations as short as 9% (apoB-9) and as long as 89% (apoB-89) of the full length apoB-100 have been described (3), with the numbers denoting the size of the truncation in relation to apoB-100. However, only truncated forms larger than apoB-27 have been found in plasma (4–6). ApoB-truncation-containing LDLs isolated from plasmas of affected individuals are smaller in size and more dense than LDL particles containing apoB-100 (4, 7, 8). Studies

Abbreviations: VLDL, IDL, LDL, HDL, very low, intermediate, low, and high density lipoproteins; apo, apolipoprotein; FCR, fractional catabolic rate; PR, production rate; LRP, LDL receptor-related protein (α^2-macroglobulin receptor); mRNA, messenger ribonucleic acid; SDS, sodium dodecyl sulfate.

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on secretion of truncated forms of apoB from stably transfected hepatoma cells also indicate that the amounts of lipid per particle and the sizes of the particles are directly related to the length of the truncated apoB (9, 10). The relatively few metabolic studies of apoB-truncations that have been carried out in humans in vivo show that both apoB-100 and apoB truncations are produced at reduced rates (e.g., 8, 11–15). In the present report we examine the quantitative relationship between the lengths of apoB truncations and their rates of secretion in vivo in humans. We present results on metabolic studies in heterozygotes for apoB-31/apoB-100, apoB-54.8/apoB-100, apoB-75/apoB-100, and apoB-89/apoB-100, using endogenous labeling of apoB with [^{13}C]leucine as a tracer and a multicompartmental model for data analysis (16). In order to describe the relation of apoB length to apoB secretion over the whole range of truncated apoB, we have used data on some subjects parts of which have been published previously (8, 12, 13, 15), as indicated in the tables.

METHODS

Study protocol

The following heterozygotes were studied: two heterozygotes for apoB-89 (apoB-89/apoB-100), two heterozygotes for apoB-75 (apoB-75/apoB-100), six heterozygotes for apoB 54.8 (apoB-54.8/apoB-100) and one heterozygote for apoB-31 (apoB-31/apoB-100). The characteristics of these patients are shown in **Table 1.** All patients were healthy and taking no medications. Details of the procedure for the kinetic studies have been published previously (16). In brief, the subjects did not change their regular diet 10 days prior to or throughout the 110-h study period. After subjects had fasted for 10 h, a primed constant infusion of [1-^{13}C]leucine (MSD Isotopes, Montreal, Canada, isotopic purity 99%) was started, consisting of a bolus (0.85 mg • kg^{-1}), immediately followed by an 8-h constant infusion of the tracer at 0.85 mg • kg^{-1} • h^{-1}. After the initial bolus, the subjects remained fasting for another 16 h. The subjects were then allowed to continue their regular diet, and subsequent samples were drawn after fasting for 10 to 12 h. In total, 42 plasma samples were drawn for assays of plasma amino acid enrichment. Thirty-two samples were drawn for apoB leucine enrichment. VLDL (d 1.006 g/ml), IDL (1.006–1.019 g/ml), and LDL (1.019–1.063 g/ml) were isolated by sequential ultracentrifugation. In the apoB-31 heterozygote the HDL fraction (1.063–1.25 g/ml) was also isolated. Aliquots for determination of VLDL-, IDL-, and LDL-apoB pool sizes were drawn on five occasions during the course of the study.

Analytical methods

Truncated apoB and apoB-100 were separated by 3–6% gradient polyacrylamide gel electrophoresis at each time point from each lipoprotein fraction (17). Coomassie blue-stained bands of the variant truncated apoBs, apoB-48, and apoB-100, were clearly separated from each other. Bands were excised and hydrolyzed in 12 N HCl for 16 h at 110°C. Amino acids were isolated from the hydrolyzed gel pieces or from 0.3 ml plasma by cation exchange chromatography (AG50W-X8, Bio-Rad, Richmond, CA) and derivatized to n-acetyl-n-propanol esters (18). Enrichments were determined by GC–MS (19) and subsequently converted to tracer/tracee ratios as previously described (20). This conversion takes the non-negligible mass of stable isotope tracers into account.

Total apoB levels in VLDL, IDL, and LDL fractions were measured by radioimmunoassay (21). The protein bands corresponding to apoB-100 and the truncated apoB were scanned by laser densitometry on an LKB Model 2202 Ultrascan (Bromma, Sweden). Areas under the peaks corresponding to apoB-100 and truncated apoB were quantitated using the program SigmaScan (Jandel Scientific, Corte Madera, CA). Based on the

TABLE 1. Characteristics of studied heterozygotes

Truncation	Cholesterol	Triglycerides	Plasma ApoB	VLDL-ApoB-100	VLDL-ApoB Truncation
			mg/dl		
ApoB-89 (2)[a]	105 ± 12	51 ± 8	27.2 ± 1.9	1.25 ± 0.45	0.6 ± 0.05
ApoB-75 (2)[b]	109 ± 18	59 ± 9	21.6 ± 0.02	2.78 ± 0.51	0.56 ± 0.14
ApoB-54.8 (6)[c]	104 ± 9	51 ± 15	30.4 ± 12.9	1.95 ± 0.93	0.22 ± 0.09
ApoB-31 (1)[d]	119	28	25	1.24	1.0[e]

[a]Ref. 12 for kinetic analysis.
[b]Ref. 16 for kinetic analysis.
[c]Ref. 16 for kinetic analysis of two heterozygotes.
[d]Ref. 22 for original description of molecular defect.
[e]HDL-ApoE truncation.

assumption that both forms of apoB have the same chromogenicity (that means that a band of 1 mg of apoB-100 has the same chromogenicity as a band of 1 mg of truncated apoB), the pool sizes of the truncated apoB and apoB-100 were estimated by scanning three to five samples from different time points in each lipoprotein fraction and averaging the ratios. The pool size of apoB-31 was based on a plasma concentration of 1.0 mg • dl^{-1} (22).

Kinetic analysis

VLDL, IDL, and LDL tracer data of apoB-100, apoB-89, apoB-75, and apoB-54.8 were analyzed by a simple multicompartmental model (**Fig. 1A**). The data on apoB-89 and apoB-75 have been published in detail elsewhere (8, 12). The model consists of a precursor compartment (compartment 1) and an intracellular delay compartment accounting for the synthesis of apoB and the assembly of lipoproteins (compartment 2). Compartments 10 and 11 are used to account for the kinetics of the VLDL-apoB fraction and represent a minimal delipidation chain. From either VLDL compartment apoB can leave the plasma or be converted to IDL-apoB (compartment 20). LDL-apoB (compartment 30) is derived from the IDL fraction or directly through a pathway from compartment 10. ApoB can leave the plasma from any compartment. It is assumed that plasma leucine (compartment 1) is the source of the leucine that is incorporated into apoB. Plasma leucine tracer/tracee ratio curves were similar to those described earlier (16) and similar in all subjects. A tri-exponential function (16) was used to fit the plasma leucine tracer/tracee ratios and was used as a forcing function (20) in the model. This model is the simplest model that is consistent with our tracer data and the current understanding of lipoprotein metabolism. In addition, because we are comparing the kinetics of truncated apoB with that of apoB in the same individual at the same time, our results are to some degree model independent. In this study the CONSAM/SAAM (23, 24) programs were used to fit the model to the observed tracer data. Metabolic parameters for apoB-100 and the truncated apoB are subsequently derived from the best fit. The fractional catabolic rate (FCR) of VLDL-apoB is the weighted average (related to mass distribution) of the FCR of pools 10 and 11. The FCR of each VLDL pool is the sum of individual rate constants, thus including conversion to IDL or LDL as well as removal from plasma. Because of the low mass of IDL apoB in some subjects with apoB-75 and apoB-89, tracer/tracee ratios could not be determined. To fit the model to these subjects' data it was assumed that all IDL was converted to LDL. In these subjects the FCR of IDL was free to adjust to provide the delay necessary to fit the LDL data.

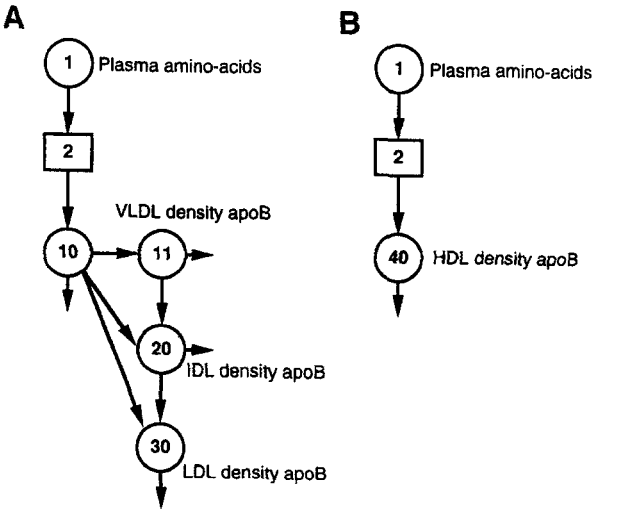

Fig. 1. Panel A shows the multicompartmental model for apoB-100, apoB-89, apoB-75, and apoB-54.8. Panel B shows the model for apoB-31. Compartment 1: plasma leucine tracer/tracee ratio (forcing function); compartment 2: delay compartment (synthesis of apoB and secretion); compartments 10 and 11: VLDL-apoB; compartment 20: IDL-apoB; compartment 30: LDL-apoB; compartment 40: HDL-apoB (for details see Methods).

ApoB-54.8 is found predominantly in the VLDL fraction (25). Some apoB-54.8 also floats in the IDL and LDL density ranges, this, however, was not enough to determine accurate metabolic parameters. ApoB-54.8 tracer data were therefore analyzed without differentiating between irreversible loss from plasma and conversion to IDL and LDL. The fact that only little apoB-54.8 is detectable in lipoprotein fractions other than VLDL indicates that the conversion rate to IDL and/or LDL is minimal.

ApoB-31 is only detectable in the HDL density range (22) therefore a different approach was used to determine metabolic parameters for this truncation. ApoB-31 was isolated from the HDL fraction as described above for apoB in the other lipoprotein fractions. ApoB-31 tracer data were analyzed by a one compartment model (Fig. 1B), which represents an extremely simplified version of the model shown in Fig. 1A. This assumes that there is a kinetically homogenous group of lipoprotein particles containing apoB-31, which float in the HDL density range. The more complex model shown in Fig. 1A could have been used, however, most of the pathways required to describe apoB-100 and the other truncations would not be required to describe the apoB-31 tracer data. Parameters for apoB-100 were determined as described above for the other heterozygotes.

ApoB secretion rates were determined for all truncations. In addition, FCRs were determined for apoB-89 VLDL, apoB-75 VLDL, apoB-54.8 VLDL, and apoB-31 HDL. LDL parameters and VLDL to IDL/LDL conversion rates could only be determined in apoB-89 and

apoB-75 heterozygotes. A more complete description of these parameters has been published previously (8, 12). The modeling process was performed independently for the truncated apoB and apoB-100 in each patient and then compared to each other. Thus, apoB-100 served as an internal control representing the metabolism of "normal" apoB-containing particles. As the secreted lipoprotein particles contain only one molecule of apoB, the ratio of the secretion rate between apoB-100 and the truncated apoB was calculated on a molar basis.

Statistical analysis

All data are expressed as mean ± standard deviation, except where stated differently. Linear regression analysis was performed using InStat software, Graph PAD, San Diego, CA.

RESULTS

Concentrations of plasma apoB and VLDL-apoB are represented in Table 1. These values represent means of 5 samples obtained during the study period. ApoB concentrations remained stable (coefficients of variation were < 5%) throughout the study indicating steady-state conditions. In all 11 patients the concentrations of truncated apoB were lower than respective concentrations of apoB-100. However, the ratios of truncated apoB to apoB-100 varied considerably.

Plasma leucine tracer/tracee ratios were very similar in all subjects (not shown) and did not differ from previously published data in hyperlipidemic and normolipidemic subjects (16, 26). The plasma leucine tracer/tracee ratio curves were fitted with tri-exponential functions that were used to define the forcing functions in the modeling process.

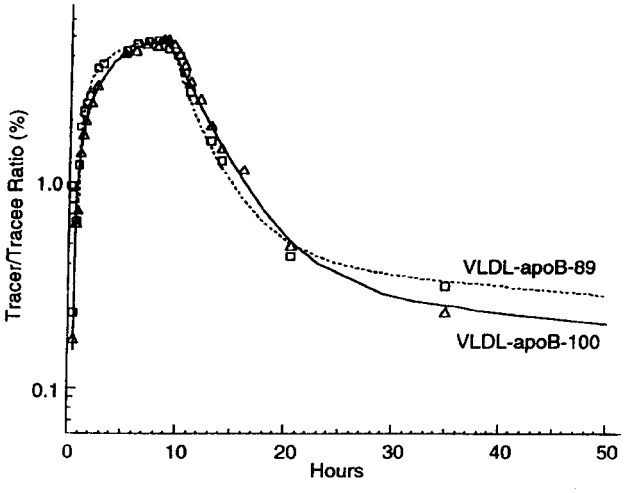

Fig. 2. VLDL-apoB-89 and apoB-100 leucine tracer/tracee ratios in an apoB-89/apoB-100 heterozygote. Observed values (symbols) and model predicted values (lines) for apoB-89 (dotted line) and apoB-100 (solid line) are shown.

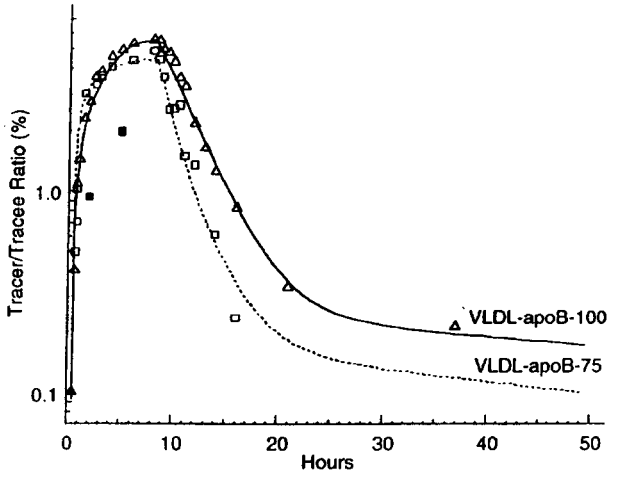

Fig. 3. VLDL-apoB-75 and apoB-100 leucine tracer/tracee ratios in an apoB-75/apoB-100 heterozygote. Observed values (symbols) and model predicted values (lines) for apoB-75 (dotted line) and apoB-100 (solid line) are shown. Symbols with X represent unweighted observations, these points, as well as the 21-h value for apoB-75 (tracer/tracee ratio < 0.1) were ignored for fitting the model to the data. The 37-h point for apoB-75 was not available for determining the level of enrichment.

Figures 2–5 show apoB leucine tracer/tracee ratios in representative subjects. The symbols represent observed values, while the lines represent the best fit of the multicompartmental model to the tracer data. There is good agreement between the model derived fits and the observed tracer data. VLDL-apoB leucine tracer/tracee ratios for an apoB-89/apoB-100 heterozygote are shown (**Fig. 2**). The rate of appearance of label in the truncated apoB tracer/tracee curve is faster than that for apoB-100, thus the VLDL-apoB-89 curve reached a plateau earlier. The downward slope of the apoB-89 curve at the end of the infusion period is also faster, i.e., the rate of disappearance of labeled apoB-89 was faster than apoB-100. Kinetically, this indicates that the FCR of the truncated apoB is faster than that of the apoB-100. VLDL tracer data for an apoB-75 heterozygote are shown in **Fig. 3.** Features similar to those described for the apoB-89 were present in this truncation, indicating that VLDL-apoB-75 also had an increased FCR compared with apoB-100. The FCR of VLDL-apoB-54.8 also is higher than the FCR of VLDL-apoB-100 (**Fig. 4**). Five of the six apoB-54.8 heterozygotes had similar findings. One of the apoB-54.8 heterozygotes had a lower FCR for VLDL apoB-54.8 than for VLDL-apoB-100. Note that in addition to differences in slopes, the plateau values for apoB-54.8 were low compared with apoB-100. This was present in all six subjects. **Figure 5** shows the leucine tracer/tracee ratios of VLDL-apoB-100 and HDL-apoB-

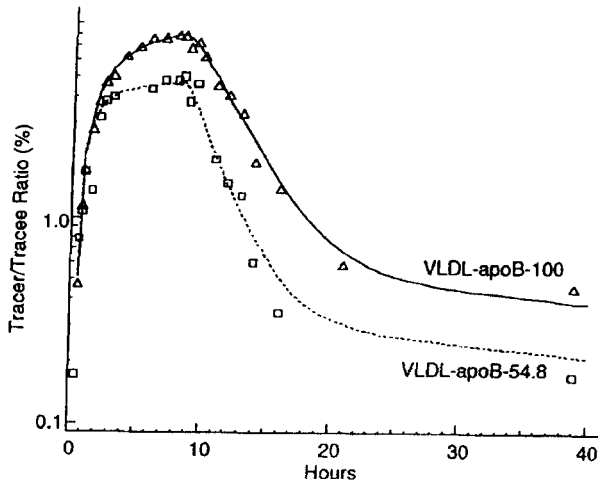

Fig. 4. VLDL-apoB-54.8 and apoB-100 leucine tracer/tracee ratios in an apoB-54.8/apoB-100 heterozygote. Observed values (symbols) and model predicted values (lines) for apoB-54.8 (dotted line) and apoB-100 (solid line) are shown. The 21-h point was unweighted for fitting because the measured tracer/tracee ratio was below 0.1

31 for the apoB-31 heterozygote. The rate of appearance and disappearance of labeled apoB-31 is considerably slower than that of apoB-100 in the VLDL fraction. This indicates that the FCR of apoB-31 is very low compared with apoB-100 in the same subject.

While the qualitative differences between the kinetics of the truncated apoB and apoB-100 can be appreciated from the figures directly, multicompartmental modeling is necessary to quantify the kinetic parameters. **Table 2** shows the FCRs of apoB-100 and the truncated forms of apoB. Although the absolute values varied considerably between subjects (columns 2 and 3), the ratios were remarkably similar in all subjects with a given truncation, as indicated by the low standard deviation in column 4. The FCRs of VLDLs containing apoB-89 or apoB-75 were increased uniformly compared to apoB-100. While the mean FCR of apoB-54.8-containing VLDLs also was increased relative to apoB-100-containing VLDLs, reflecting the values in five subjects, the FCR of VLDL-apoB-54.8 was lower in one subject. The FCR of apoB-31-containing lipoproteins was decreased compared to VLDL-apoB-100 and was comparable to that of LDL-apoB-100.

The modeling process also revealed metabolic parameters for LDL apoB-89 and apoB-75 and conversion rates of VLDL to LDL for these two truncations. While these data are published in detail elsewhere (8, 12), it is worth mentioning here that LDL containing apoB-89 or apoB-75 have an FCR that is significantly increased compared to the FCR of LDL apoB-100 in the same subjects (0.61 d^{-1} vs. 0.33 d^{-1} and 0.61 d^{-1} vs. 0.46 d^{-1}, respectively). The increased FCR of apoB-89-VLDL and apoB-75-VLDL reflects an enhanced irreversible loss of particles from each lipoprotein fraction rather than an increased conversion of VLDL to LDL. Thus, less apoB-89 or apoB-75 is ultimately converted to the LDL fraction (18% vs. 80% and 5% vs. 23%).

Table 3 shows secretion rates for VLDL apoBs. Compared to apoB-100, the secretion rates of truncated apoBs were uniformly decreased. Although the absolute values again varied considerably among subjects (columns 2 and 3), the ratios were very similar in all subjects with a given truncation, as indicated by the low standard deviation in column 4. On a molar basis the secretion rate of apoB-89 was 92%, apoB-75 was 64%, apoB-54.8 was 37%, and apoB-31 was only 13% of the respective apoB-100 secretion rates in the same subjects. When plotted as a function of apoB size relative to apoB-100, apoB secretion rates formed a straight line, with a zero intercept for apoB secretion at apoB-28 (**Fig. 6**). The conversion from mg to moles was performed to account for the fact that the lipoproteins under discussion contain only one molecule of apoB per particle. However, it is worth mentioning that the presentation of the data on a mg-based format also reveals a linear relationship between the length of apoB truncation and the secretion rate. Such an analysis would define a different slope (reduction in secretion by 1.5% for each 1% of apoB truncated) and predict a different zero intercept for apoB secretion (apoB-34).

DISCUSSION

In previous studies of heterozygous subjects we sought explanations at the metabolic level for the low levels of apoB-100 and truncated apoBs in plasma, and found that both apoB-100 and the truncations to be

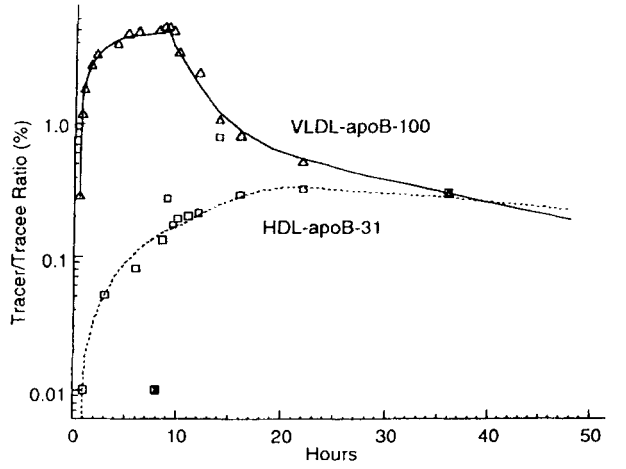

Fig. 5. VLDL-apoB-100 and HDL-apoB-31 leucine tracer/tracee ratios in the apoB-31/apoB-100 heterozygote. Observed values (symbols) and model predicted values (lines) for apoB-100 (solid line) and HDL-apoB-31 (dotted line) are shown. The symbol with an X represents an unweighted observation, which was ignored for fitting the model to the data.

TABLE 2. Fractional catabolic rate (FCR)

Truncation	FCR		Ratio of FCRs (ApoB-Truncation to ApoB-100)
	VLDL ApoB-100	VLDL ApoB-Truncation	
	pools/day		
ApoB-89[a,d]	8.9 ± 4.1	12.9 ± 6.4	1.44 ± 0.06
ApoB-75[b,d]	9.4 ± 0.4	20.2 ± 2.4	2.15 ± 0.16
ApoB-54.8[c,d]	7.2 ± 4.7	11.4 ± 5.6	1.58 ± 0.58
ApoB-31	12.0	0.55[e]	0.045

[a]Ref. 12 for kinetic analysis.
[b]Ref. 8 for kinetic analysis.
[c]Ref. 16 for kinetic analysis of two heterozygotes.
[d]Ref. 15 for kinetic analysis of ApoB-100 in subject with truncated ApoB.
[e]HDL - ApoB truncation.

produced at low rates relative to appropriate controls (8, 12, 13, 15). In the present studies we explored the relationship between the lengths of the various apoB truncations and their metabolism. Using an endogenous tracer methodology, we found strong evidence for a decreased secretion of truncated apoB compared to apoB-100 in human heterozygote subjects. An essentially linear relationship between the length of truncated apoB and the secretion rate was observed. Our results indicate that apoB secretion is reduced by approximately 1.4% for each 1% of apoB truncated and that truncated forms of apoB smaller than apoB-28 will not be secreted into plasma.

A compartmental model was used to fit the tracer data and calculate apoB metabolic parameters. Although compartmental models are widely used and accepted, the comparison of apoB-100 and apoB-truncations within subjects as in this study is, in fact, largely independent of the model used because the parameters for the truncated apoB are compared to the parameters for apoB-100 in the same subject, at the same time, and under identical conditions. The fact that apoB metabolism was studied in simple heterozygotes permitted each subject's apoB-100 to serve as an internal control for his/her own truncated apoB. Although our model allows for apoB to be secreted only into the VLDL (apoB-89, apoB-75, apoB-54.8) or HDL (apoB-31) fraction, an integrated model of apoB metabolism (VLDL, IDL, LDL) to determine secretion rates is used to exclude the possibility that significant amounts of apoB are secreted directly into other compartments. Theoretically, secretion into the IDL or LDL fraction should be detectable independently of the method of tracer application. In practical terms, however, the most sensitive method to exclude or confirm direct apoB secretion into IDL or LDL compartments is the use of multiple endogenous and exogenous tracers introduced at different levels of the lipoprotein cascade. Nevertheless, using a single endogenous tracer, there was no evidence of direct secretion of apoB-89 or apoB-75 into lipoprotein fractions other than VLDL. While the secretion of minimal amounts of apoB-54.8 into lipoprotein fractions other than VLDL and of apoB-31 into lipoprotein fractions other than HDL cannot be excluded completely, there was no evidence of such a pathway. Thus, based on our data, we can exclude that significant amounts of apoB are secreted into lipoprotein fractions other than VLDL (in the cases of apoB-89, apoB-75, apoB-54.8) or HDL (in the case of apoB-31).

In comparison to apoB-100, all truncations of apoB were secreted at reduced rates (Table 3), and size was directly and linearly correlated with secretion rate (Fig. 6). The regression line relating apoB size with apoB secretion rate intersected the abscissa at apoB-27.6. This is compatible with the absence of detectable truncated apoBs shorter than apoB-27.6 in plasma (4–6). However, the absence of apoBs smaller than apoB-27.6 may be more apparent than real. Very small amounts of truncated apoB in plasma may be present but not be detected due to the relative insensitivity of the methods used. Trace amounts of apoB are detected by sensitive immunoassays even in the more severe low apoB condition, abetalipoproteinemia (27).

Our metabolic studies also could be interpreted as showing that the secretion of truncated apoB from the hepatocyte is normal, but that significant immediate reuptake of truncated apoB occurs, perhaps mediated by apoE. This concept is supported by cell studies demonstrating that cultured hepatocytes can efficiently secrete truncated apoB (28, 29). But if the secretion rate from hepatocytes is normal, then the rate of immediate reuptake must be linked to the length of the truncated apoB. Previous studies from our own laboratory, however, indicate that the receptor binding of apoB-89 and apoB-75 is not altered sufficiently to account for such a rapid reuptake (8, 12, 14, 25). Thus, truncated apoB are most likely secreted at a decreased rate from hepatocytes.

TABLE 3. Secretion rate

Truncation	Secretion Rate		Ratio of Secretion Rates[a]
	ApoB-100	ApoB-Truncation	(ApoB-Truncation to ApoB-100)
	mg/kg/day		
ApoB-89[b,e]	5.4 ± 4.0	4.4 ± 32.	0.92 ± 0.03
ApoB-75[c,e]	10.6 ± 1.5	5.1 ± 0.6	0.64 ± 0.01
ApoB-54.8[d,e]	6.2 ± 2.9	1.3 ± 0.7	0.37 ± 0.15
ApoB-31	6.7	0.25	0.12

[a]Ratio calculated on a molar basis.
[b]Ref. 12 for kinetic analysis.
[c]Ref. 8 for kinetic analysis.
[d]Ref. 16 for kinetic analysis of two heterozygotes.
[e]Ref. 15 for kinetic analysis of apoB-100 in subjects with truncated apoB.

Yao et al. (9) speculated that the association with lipid is a prerequisite for secretion and thus the inefficient secretion of short truncated apoBs (apoB-18 to apoB-23) may be due to their inability to acquire a sufficient amount of lipid. This speculation is supported by the finding that in all studied cell systems some apoB is degraded intracellularly (1, 2, 9, 30–32). The rate of secretion and degradation is influenced by the availability of fatty acids, i.e., the addition of oleate results in a decreased degradation of apoB-100 and an enhanced secretion of triglyceride enriched apoB-containing particles (2, 28, 32). It could be that truncated apoB are secreted less efficiently as they are associated with less lipid. However, in a recently published study using rat hepatoma cells stably transfected with truncated apoB (33), it was sh wn that the reduction in apoB secretion induced by monomethylethanolamine, a specific inhibitor of apoB secretion (34), is not a function of assembly of the apoB into a buoyant lipoprotein particle, indicating a more complex regulation of apoB secretion. It seems questionable however, whether the situation in humans can be deducted from cell studies. As mentioned above, not all truncations are secreted by stably transfected hepatoma cells at low efficiency in vitro. For example McLeod et al. (29) reported that despite an impaired lipid recruitment of truncated apoB, there was no reduction in the secretion rate of lipoproteins containing the truncated apoB in such a cell model. The discrepancies between the results presented here and those found in hepatomas could be related to differences between actions of viral gene promoters used in vitro and the natural promoters of our subjects, or in processing of human apoB-containing VLDL precursors in human hepatocytes and rat hepatomas.

The enhanced FCRs of particles containing apoB-89 or apoB-75 are most likely mediated by LDL-receptor mechanisms (8, 12, 14) as these truncations possess the putative LDL-receptor recognition sites and, in fact, interact with LDL-receptors with increased affinities (7, 8, 14). However, other factors, such as a slightly different apolipoprotein composition (e.g., increased apoE content or altered apoE/apoC ratio) or conformational

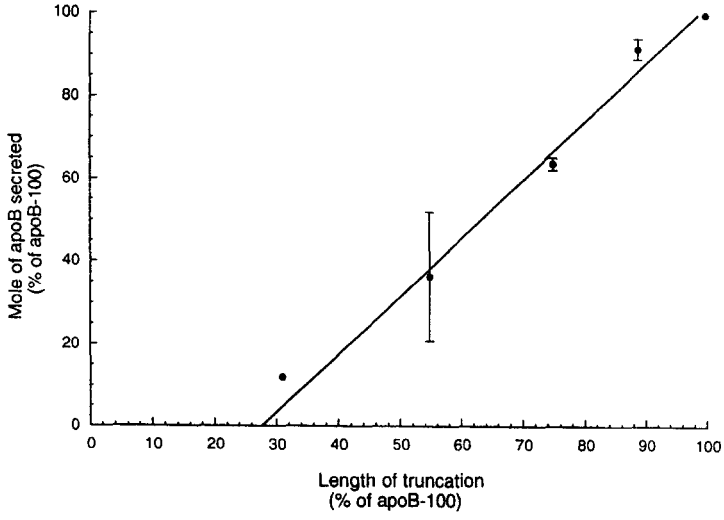

Fig. 6. Secretion of truncated apoB as a function of the size of the apoB truncation. ApoB secretion is expressed on a molar basis as a percentage of apoB-100 secretion determined under identical conditions in the same subjects (see Table 3). The linear regression formula is as follows: $y = -38.9 + 1.4x$, $r^2 = 0.86$, $P < 0.0001$. Brackets indicate standard deviations.

changes leading to an increased uptake by LRP or VLDL receptors may be further mechanisms. The increased FCR of apoB-89 and apoB-75 reflects an enhanced irreversible loss of particles from each lipoprotein fraction rather than an increased conversion of VLDL to LDL. Thus, less apoB-89 or apoB-75 is ultimately converted to the LDL fraction. This is probably also true for apoB-54.8 because of the low concentrations of apoB-54.8 in the IDL and LDL density ranges. The increased FCRs of the VLDL containing apoB-54.8 remains unexplained. Although this truncation does not possess the LDL-receptor recognition region, an increased apoE content or altered apoE/apoC ratio could mediate an increased uptake through the LDL receptor. However, it may be that VLDL-containing apoB-54.8 truncations are removed from plasma by other efficient mechanisms. The low relative FCR in one subject out of six with apoB-54.8 may be due to the significantly higher FCR of VLDL-apoB-100 of the one subject compared with the five others, i.e., the observed lower relative catabolism of apoB-54.8 may be the consequence of a high apoB-100 metabolism rather than a low apoB-54.8 metabolism. In any case, the tissue loci of catabolism of apoB-54.8 and the quantitative contributions of each tissue to the catabolism remain to be determined.

Plateau tracer/tracee curve values of VLDL-apoB-89 and VLDL-apoB-75 were nearly identical with their respective VLDL-apoB-100 values, suggesting that the truncations and apoB-100 were derived from the same precursor pools. By contrast, the values for VLDL-apoB-54.8 were consistently lower than the plateau values for VLDL-apoB-100 at the end of the primed constant infusions in all six subjects (Fig. 4). This consistent but unexpected finding for apoB-54.8 could indicate that the wild-type and mutant proteins originated from two different leucine precursor pools and/or different tissues, i.e., the liver and intestine. However, the intestine is unlikely as a source of apoB-54.8 secretion because in the intestine the mutant mRNA would be edited to form apoB-48 the same way as the mRNA for apoB-100 (35). Furthermore, the low plateau values for apoB-54.8 were not due to poor separation of apoB-54.8 from apoB-48. These proteins are clearly separable by SDS-gel electrophoresis (25). Therefore, it is likely that both apoB-54.8 and apoB-100 are produced in the livers of these heterozygotes as other tissues do not seem to express apoB in adults (36). The different plateau values could also be a consequence of a constant dilution of the sample from unknown sources related perhaps to the different masses of apoB-100 and apoB-54.8 and therefore be without physiologic correlate. The calculated FCRs and hence secretion rates, however, would not be affected by such a constant dilution, because the time when the plateau is reached would not change. Therefore, it is unlikely that the plateau values in any way influenced the observed correlation of length of truncation and secretion rate.

A single pool model was used to fit the apoB-31 tracer data (Fig. 5). This model assumes that the plasma apoB-31 pool was kinetically homogeneous. Although we did not investigate the hypothesis that apoB-31 could be derived from two tissues, intestine and liver (35), the model-generated curve was consistent with the tracer data. ApoB-31 particles appear to have grossly perturbed kinetic parameters, indicating that they do not participate normally in lipoprotein metabolism. The site or method of catabolism of apoB-31 particles has not been identified.

In summary, although much remains to be learned about the intracellular metabolism of apoB truncations and about their tissue sites of removal from plasma, the present data indicate that secretion rates of truncations are lower than secretion rates of apoB-100 in the same subjects. Furthermore, there is a direct relationship between secretion rates and size: the shorter the truncated form, the lower the secretion rate. These metabolic findings account, in part, for the hypobetalipoproteinemia seen in our subjects.

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