Apolipoprotein B metabolism in hypertriglyceridemic diabetic patients administered either a fish oil- or vegetable oil-enriched diet

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Abstract The effect on apolipoprotein B kinetics of a diet enriched in either fish oil or safflower oil was investigated in five hypertriglyceridemic (HTG), non-insulin-dependent diabetic subjects. The fish oil diet decreased plasma triglycerides and VLDL-apoB but increased LDL-apoB and LDL-cholesterol. Total plasma apoB concentration did not change, nor did the increased VLDL-apoB secretion present in these HTG subjects, which, accompanied by impaired lipolysis, accounted for their elevated VLDL. The fish oil-induced fall in VLDL resulted from a decrease in secretion without a change in residence time. The IDL fraction, which also contained small VLDL, was the primary site for the secretion of apoB particles in the HTG subjects. On the fish oil diet there was a further, compensatory increase in the secretion of these lipoproteins such that the transport of apoB in IDL remained the same, as did its mass. In the HTG subjects the major portion of IDL lipoproteins was catabolized, with LDL-apoB production comprising the lesser quantity. On the fish oil diet, a shift in the channeling of the lipoprotein output from IDL resulted in a decrease in the catabolic pathway and an increase in conversion to LDL. As the residence time of LDL did not change, this increased input gave rise to the larger mass of LDL-apoB seen in these hypertriglyceridemic subjects when receiving a fish oil diet.—**Fisher, W. R., L. A. Zech, and P. W. Stacpoole.** Apolipoprotein B metabolism in hypertriglyceridemic diabetic patients administered either a fish oil- or vegetable oil-enriched diet. *J. Lipid Res.* 1998. **39:** 388–401.

Supplementary key words apolipoprotein B (apoB) • fish oil • SAAM • compartmental modeling • [^{3}H]-leucine • tracer kinetics • hypertriglyceridemia • diabetes

The dietary intake of marine fish oil, a source of long chain, n–3 fatty acids, has long been recognized to decrease plasma triglycerides (1–3). This effect is associated with a fall in plasma VLDL-apoB concentration and with decreased secretion of these lipoproteins (4, 5). In humans, severe hypertriglyceridemia is most commonly associated with impaired glucose tolerance or overt non-insulin-dependent diabetes (3); however, the seeming appropriateness of the use of fish oil in treating these patients has been challenged by reports of resultant increases in LDL cholesterol concentration and worsening diabetic control (3). This tracer kinetic study was undertaken to examine, in greater detail, the effect of dietary fish oil on apoB metabolism in hypertriglyceridemic, non-insulin-dependent patients. Subjects were studied twice, on isocaloric, isofat diets in which the predominant dietary fat was either safflower oil or fish oil. The pronounced changes observed in apoB metabolism that affect VLDL, IDL, and LDL are generally consistent with observations that have been reported in animal and in vitro studies.

METHODS

Clinical protocol

This research was approved by the Institutional Review Board of the University of Florida and informed consent was obtained from each subject. Five adults, one female and four males, with non-insulin-dependent diabetes mellitus were investigated. **Table 1** summarizes

Abbreviations: VLDL, very low density lipoprotein ($S_f > 100$ lipoproteins); IDL, intermediate density lipoprotein (S_f 20–100 lipoproteins); LDL, low density lipoprotein (S_f 4–10 lipoproteins); apoB, apolipoprotein B; C(i), compartment i; L(i, j), fractional transfer coefficient (or the rate constant) for the transfer of substance from C(j) to C(i); R(i, j), transport or flux of apoB from C(j) to C(i); R(IDL) etc., transport of apoB through the IDL fraction; M(IDL) etc., mass of apoB within the IDL fraction; RT(IDL) etc., residence time of apoB within the IDL fraction; VO, vegetable oil; FO, fish oil.

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pertinent clinical data. None was receiving medication known to alter glucose or lipid metabolism during their participation. Each subject was studied twice during 21-day admissions to the Clinical Research Center, Shands Hospital, University of Florida, once while receiving a supplement of n–3 enriched oils and again with a supplement of n–6 oils. These oils were provided by the Fish Oils Test Materials Program, NIH, and were dispensed as 1 g soft gelatin capsules containing either purified menhaden oil or safflower oil. The former contained 249 mg/g of n–3 oils (EPA and DHA), the latter <1 mg/g of n–3 oils. The two branches of the study were separated by a least 1 month, and during each session, for 1 month prior to and throughout their admission on the CRC, subjects received a weight maintenance diet composed of 15% of calories as protein, 60% carbohydrate, and 25% fat, of which 15% was either fish oil or safflower oil and 10% dietary fat. After 1 week of dietary stabilization on the CRC ward, a nasogastric feeding tube was passed. For the next 48 h the subjects received a constant liquid feeding containing the same protein and carbohydrate content, but omitting the supplemental oil and reducing the fat content to 20 g/day to minimize chylomicron formation. Thereafter, the previous diet was reinstituted. Twelve h after initiating this liquid feeding, the subjects received 5 μCi/kg [4, 5-^{3}H]l-leucine intravenously. Frequent blood samples were drawn during the subsequent 36 h and, thereafter, in the fasting state, over 2 weeks, at the times shown in the figures. On the day prior to initiating the liquid feeding and on the last day of the study, blood was also drawn to quantitate plasma apoB levels of VLDL, IDL, and LDL.

Quantitation of plasma leucine

Five ml of blood was drawn at each sampling timepoint to measure plasma free leucine concentration and specific radioactivity, as previously described (6).

TABLE 1. Data for subjects receiving n-6- and n-3-enriched diets

			Triglycerides		Plasma Cholesterol		LDL-Chol		HDL-Chol		VLDL-Chol		IDL-Chol	
Subject	Age/Sex	Wt	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
		kg						*mg/dl*						
J	67/F	59	308	149	161	149	82	96	19	30	71	22	212	86
L	64/M	106	297	145	254	258	148	194	30	35	103	24	157	94
K	59/M	63	853	280	226	199	25	97	19	21	485	56	338	191
Z	67/M	84	1208	275	320	223	77	75	20	59	1302	196	81	64
P	34/M	94	287	155	144	164	54	74	22	25	86	14	181	119
Average			591	201	221	199	77	107	22	34	410	62	194	111
P (2-tail)[a]			0.01		ns		0.08		ns		0.01		0.01	

			Plasma ApoB		VLDL-ApoB		IDL-ApoB		LDL-ApoB	
			n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
						mg/dl				
J	67/F	59	77	68	4.3	0.8	21	12	52	55
L	64/M	106	97	101	6.9	4.4	21	14	69	82
K	59/M	63	76	102	12.0	5.0	31	24	33	73
Z	67/M	84	100	112	41.0	14.0	6	27	53	71
P	34/M	94	45	72	4.5	4.3	15	18	25	50
Average			79	91	13.7	5.7	19	19	46.4	66.2
P (2-tail)[a]			ns		0.04		ns		0.03	

			Plasma Lipid to ApoB Ratios					
			VLDL-TG/VLDL-ApoB		IDL-TG/IDL-ApoB		LDL-Chol/LDL-ApoB	
			n–6	n–3	n–6	n–3	n–6	n–3
J	67/F	59	16.5	27.5	10.1	7.2	1.6	1.7
L	64/M	106	14.9	5.5	7.5	6.7	2.1	2.4
K	59/M	63	40.4	11.2	10.9	8.0	0.8	1.3
Z	67/M	84	31.8	14.0	14.7	2.4	1.5	1.1
P	34/M	94	19.1	3.3	12.1	6.6	2.2	1.5
Average			24.5	12.3	11.1	6.2	1.6	1.6
P (2-tail)[a]			ns		0.07		ns	

[a]Student's *t*-test run on log-transformed values.

Fractionation of plasma lipoproteins and measurement of apoB specific radioactivity

At each sampling, 45 ml of blood was mixed with inhibitors of proteolysis and bacterial growth and the samples were placed on ice (6). The plasma was separated and lipoproteins were fractionated by sequential ultracentrifugational flotation, in a Beckman ultracentrifuge with a Ti 50.2 rotor at 5°C, by underlayering the plasma beneath the solvent and recovering the lipoproteins at the meniscus. The fractionation scheme utilized was that of Gustafson, Alaupovic, and Furman (7). Trace amounts of chylomicrons were recovered at density 1.006 g/ml, after flotation at 17,500 rpm for 20 min, and were discarded. Large VLDL, $S_f > 100$, was isolated at the same density but at 35,000 rpm for 1 h. A fraction containing IDL and small VLDL S_f 20–100, was subsequently recovered by centrifugation at density 1.02 g/ml for 20 h, and is referred to as IDL hereafter. The rationale for collecting this fraction is based on two considerations. First, the buoyant density of IDL is 1.004 mg/ml (8). Thus, when attempting the separation of IDL from VLDL at density 1.006 g/ml, much of the IDL distributes in the 1.006 g/ml supernatant and is recovered along with small VLDL. Second, the traditional IDL fraction is present in plasma in too small an amount for measurement of its specific radioactivity when drawing repeated samples from subjects. Accordingly, we separated large VLDL and then recovered small VLDL and the entire IDL within a second fraction. Finally, LDL was isolated at density 1.06 g/ml for 20 h. Recovered lipoproteins were frozen in 20% sucrose pending isolation of apoB by preparative, acrylamide gel electrophoresis in an SDS buffer. The recovered apoB was quantitated by a fluorescamine protein assay, and specific radioactivity was determined by scintillation counting, as described previously (9).

The experimental kinetic data were analyzed by mathematical compartmental modeling using the SAAM computer program (10). Initially, each data set was analyzed as total plasma radioactivity using our previously published triglyceride model (11). Inconsistencies with the model that could be generalized for either the fish oil or vegetable oil perturbations were then incorporated into a revised model, and all the studies were reanalyzed. The data were then analyzed as specific radioactivity and inconsistencies between the total and specific activity analyses were resolved. Finally, the fish oil and vegetable oil data for each subject were analyzed in the same equation so that we could determine the statistical significance of parameters that differed between the two studies. Those parameters that differed but did not reach statistical significance were set to a common value and the equations were solved again. The parameters reported in **Tables 2** and **3** are from this final analysis. Model-derived residence times, masses and transports were analyzed by correlations and by *t*-tests for the means of paired samples (from the two diets) for individual subjects .

ApoB concentration in total plasma and in lipoprotein fractions was measured by an immunoturbidimetric assay (12). The average difference between the laboratory-determined and model-derived masses was 11 ± 12% (mean ± SD).

ApoB model

The apoB model (**Fig. 1**) used in analyzing these data originated with Phair et al. (13), and has been refined by numerous studies using both radioiodinated apoB and [^{3}H]-leucine tracers (11, 14–16). As in our other studies with the latter tracer, plasma leucine was used as a forcing function to drive apoB synthesis, C(1), and the tail of the forcing function was adjusted to enable the model-derived IDL tail to fit the radioactivity of the tracer (6). A recycling loop in the apoB synthesis pathway was required to fit the hump on the descending limb of VLDL and IDL (6).

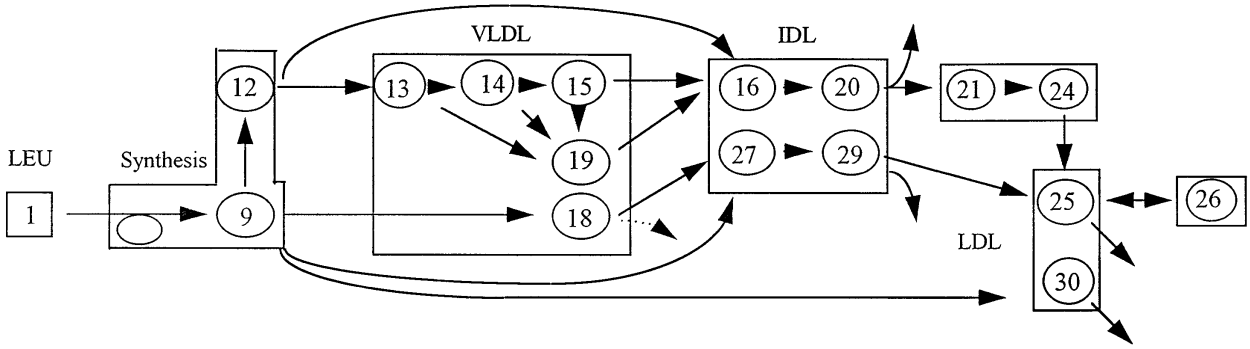

Fig. 1. The apoB model used in the mathematical analysis of the kinetic data. The model may be considered a mathematical analog of the physiological system. See the text for a description of the model.

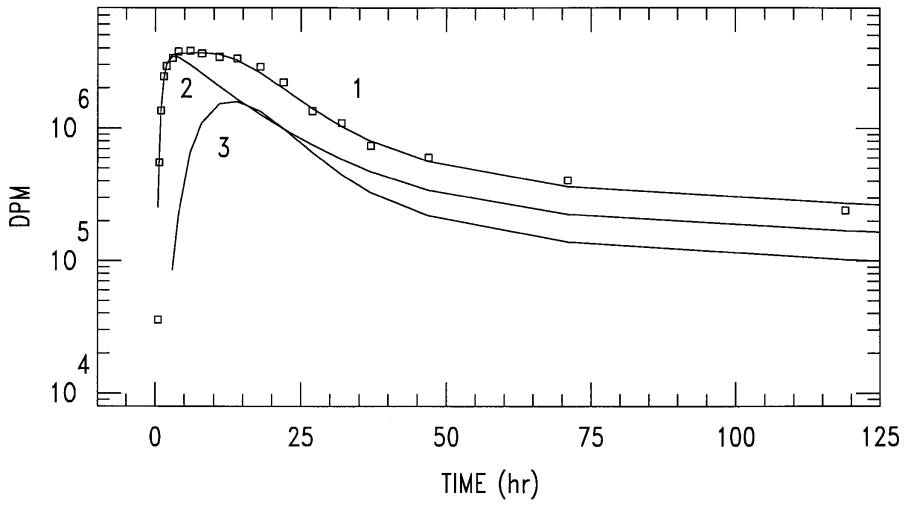

Fig. 2. VLDL kinetics. The experimental VLDL data from study Z:VO analyzed using the ApoB model. Curve 1: The computer-generated fit to the experimental data. Curve 2: The computer simulation when L(13, 12) is set = 0 demonstrates the need for a delayed input of presumably triglyceride-enriched apoB that enters a delipidation chain which sustains the broad peak of VLDL. Curve 3: The simulation when L(18, 9) is set = 0 demonstrates the requirement for an early input that establishes the rapid rise in tracer activity and then decays. The determinants of the kinetic tail, here truncated at 125 h but that extends over several weeks and beyond, have been analyzed (6).

The 10 studies in these five subjects revealed that newly synthesized apoB initially appeared in plasma at several points in the metabolic cascade that characterizes the metabolism of this protein. Table 2 reports the transport of apoB along the metabolic pathways shown in Fig. 1 for each of the 10 studies. In addition, the average flux along each pathway expressed as a fraction of total apoB secretion (average fractional transport) is also recorded, and this value will be referred to subsequently when describing the model.

Metabolic channeling is an important feature of apoB physiology (16, 17). Approximately one-quarter of apoB is secreted as large VLDL, L(18, 9), one-third as IDL, L(27, 9), and 5% as LDL, L(30, 9), in what constitutes a fast path for the channeling of apoB to LDL. An additional 40% appears in plasma after a delay, L(12, 9), which is thought to result from the time required in the hepatocyte to package additional lipid in an enriched fraction of VLDL particles (17). Lipoproteins formed by this pathway initially appear in plasma

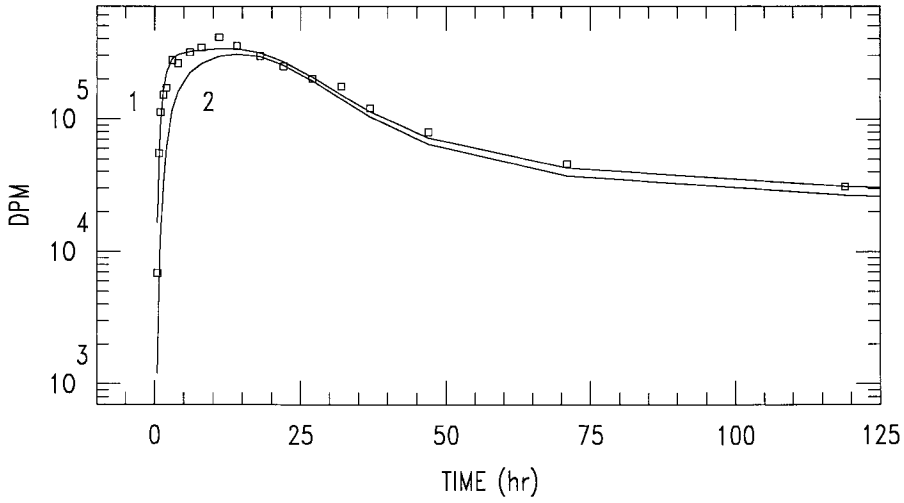

Fig. 3. IDL kinetics, study Z:VO. The requirement of an early input pathway of nascent IDL-apoB. Curve 1: The model-generated fit of IDL. Curve 2: The computer simulation when L(27, 9) is set = 0. Though the input by L(27, 18) is substantial, it arrives too late to fit the rapid rise in the data.

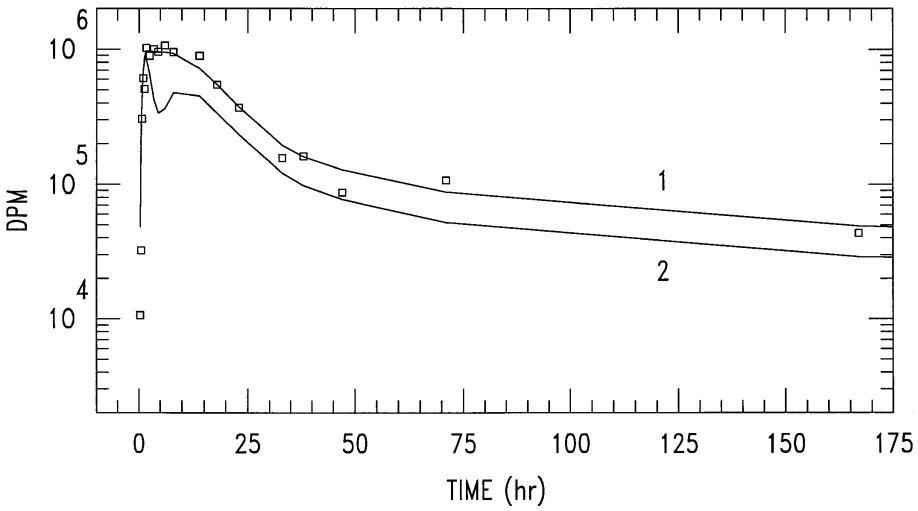

Fig. 4. IDL kinetics, study K:VO. The requirement of a delayed input of nascent IDL-apoB. Curve 1: The model-generated fit. Curve 2: The computer simulation when L(16, 12) is set = 0. ApoB transport through the delipidation chain is inadequate to fit the IDL.

as either large VLDL, L(13, 12) or as smaller IDL, L(16, 12). **Figure 2** shows the model-generated fit to VLDL and the simulations resulting when alternately L(13, 12) and L(18, 9) have been set to zero, thus demonstrating the requirement for both pathways in fitting these data. While large VLDL-apoB that enters C(18) is rapidly converted to IDL, that entering C(13) is metabolized along a three-compartment delipidation chain, as previously described, enroute to IDL (12). The residence time of apoB in large VLDL is from 2 to 3 h, Table 3.

Except in one study [L(0, 18) for Z:VO, shown as a dotted arrow in the model], all of apoB that is secreted as large VLDL is converted to the smaller sized particles that comprise the IDL fraction. However, the 57% of total apoB that is secreted as nascent IDL particles, [R(27, 9) + R(16, 12)], constitutes the major route of entry into plasma for apoB in these hypertriglyceridemic subjects (Table 2). The requirements for the nascent input of apoB into IDL arise because apoB transport through IDL, R(IDL), is appreciably greater than R(VLDL), and the kinetic curves reflect this fact (**Fig. 3** and **Fig. 4**). Thus, apoB may enter the IDL fraction through pathways which provide either a rapid or a delayed input (Fig. 1). The former, [C(9)→C(27) and C(9)→C(18)→C(27)], model the early rise in the IDL kinetic curve. The latter, [C(12)→C(16) and C(15)→C(16)], provide a delayed input of tracer required to broaden the peak. IDL is also modeled with an internal delay that may be viewed as an extension of the delipidation chain. This delay serves to round and broaden the peak of tracer activity. The need to maintain separate fast and slow pathways through IDL becomes evident from the kinetics of LDL (see below). The time required to process a particle in its transit through IDL is about 4 h (Table 3) and as the data do not permit a distinction to be made in the transit time for the two pathways through IDL, their rates have been set equal.

A striking feature of large VLDL metabolism, observed in five of 10 studies, was the occurrence of a very slowly turning-over apoB pool, C(19), derived from large VLDL, C(13)→C(15), and fed into IDL.[3] Though <1% of apoB is transported by this route, the mass of apoB in C(19) is about 100 mg, as a consequence of its prolonged residence time of some 3 days, which, however, is very poorly determined. The requirement for this pool is demonstrated in **Fig. 5**, where it is shown to sustain the tail of the VLDL-apoB curve that otherwise declines at far too rapid a rate. Berman et al. (14), using a radioiodinated VLDL-apoB tracer, reported kinetic evidence for a pool of presumably remnant β-VLDL particles with a slow turnover time that was readily apparent in subjects with Type 3 hyperlipoproteinemia, i.e., subjects homogeneous for an apoE2 phenotype, but was also observed in endogenous hypertriglyceridemia. This pool was designated compartment 21, and it has been observed in a number of kinetic studies using radioiodinated VLDL-apoB tracers (16), but it has not previously been demonstrated with a leucine tracer.

[3]The magnitude of the apoB transport along this pathway, R(16, 19), is so low that its destination cannot be determined, and the decision to direct the output from C(19) to C(16) is arbitrary.

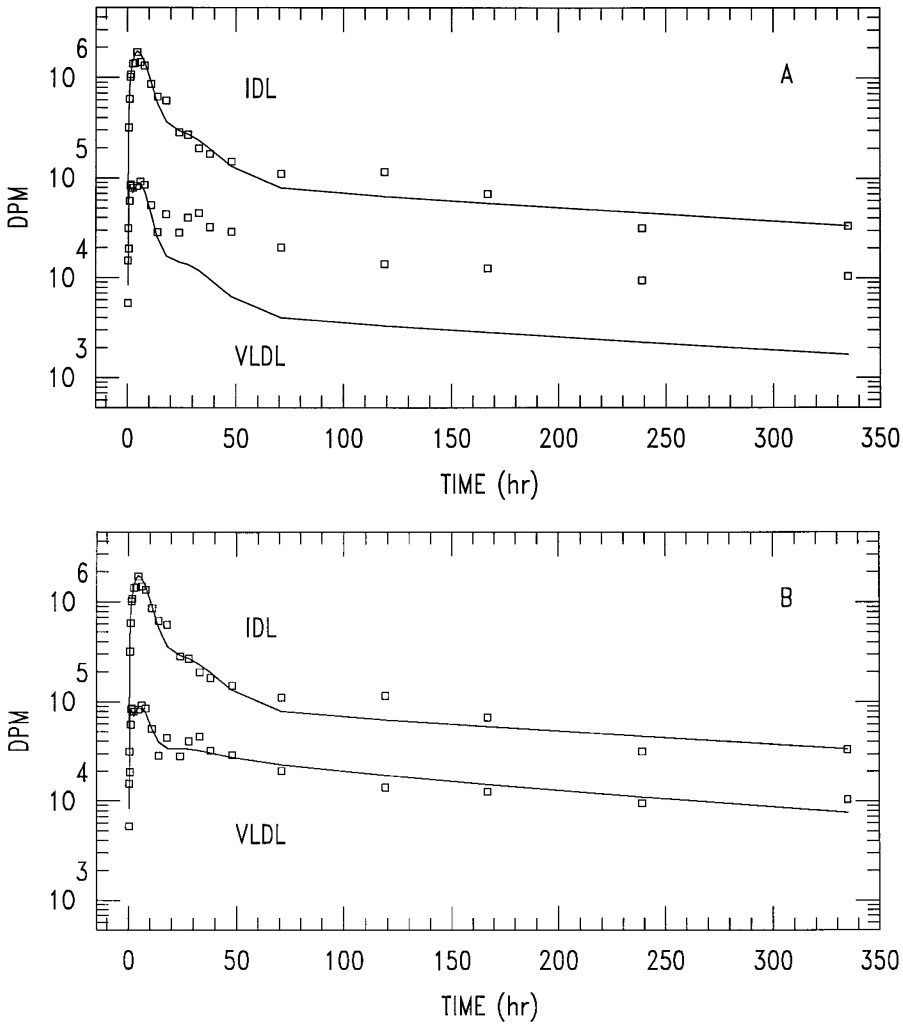

Fig. 5. The modeling of β-VLDL from study K:FO. A: When IDL-apoB, the main secretory product of apoB, has been fit, the kinetic tail on VLDL decays too rapidly. B: Introduction of C(19), which is analogous to C(21) of Berman et al. (13), enables the tail to be elevated and sustained over 2 weeks of observation.

Three-quarters of apoB secreted into plasma is lost from IDL in these hypertriglyceridemic individuals [R(IDL Loss), Table 2]. About 29% is lost from the slow pathway, L(0, 20), and 43% is lost from the rapidly metabolized IDL, L(0, 29). Thus, only about one-quarter of plasma apoB eventually appears in LDL. That apoB which has traversed the delipidation chain in VLDL and constitutes the slow pathway from IDL to LDL, C(21)→C(24), is of particular interest. This delay is required to fit the peak and declining activity in LDL, as discussed below. In seven of 10 studies, the 21 mg/h of IDL-apoB that traverses this pathway, R(21, 20), disappears from plasma for a period of about 4h and then reappears as LDL. By contrast, apoB that is metabolized along the fast path to LDL is promptly converted from C(29) to C(25) and thus to LDL. To our knowledge, this delay has not been reported by other investigators. The intriguing questions, of course, are where these lipoproteins reside for several hours during their conversion to LDL and why lipoproteins following the fast path do not require this delay.

LDL production can be resolved into three pathways (Fig. 1). As recorded in Table 2, delayed input of tracer to LDL can be channeled through the delipidation chain and the slow pathway through IDL to LDL. This apoB input to LDL accounts for about 11% of total apoB flux, [R(21, 20)]. A second input, channeled through C(18), acquires additional apoB in C(27) and delivers 8% of total apoB to LDL through this faster route, [R(25, 29)]. About 5% of apoB appears to enter directly into LDL, where it is required to account for the very rapid appearance of tracer, [R(30, 9)]. The flux through these pathways differs among subjects (Table 2). **Figure 6A**, in which L(21, 20) has been set

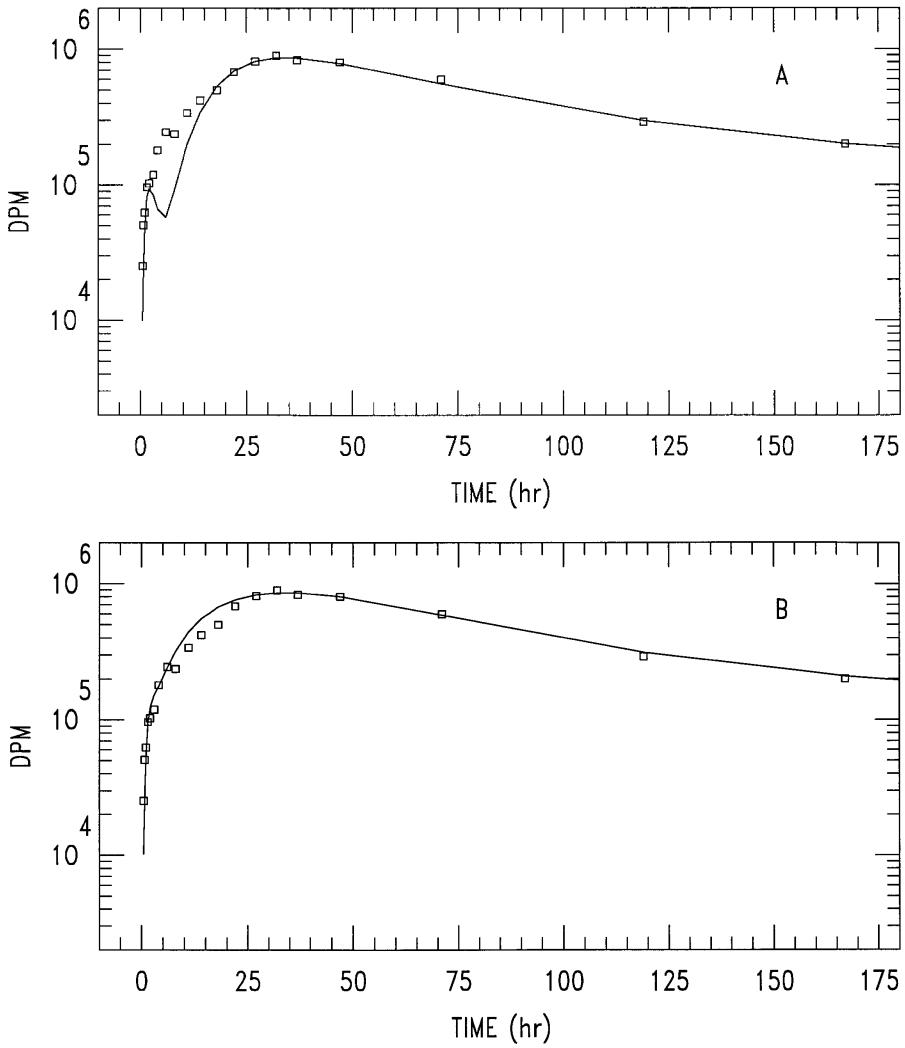

Fig. 6. The channeling of apoB into LDL by three pathways. Model-generated plots of the LDL data from study Z:VO demonstrating channeling of apoB through the three input pathways to LDL. A: The input through L(25, 29) is set = 0, while the input through the delipidation pathway, L(21, 20), is adjusted so as to fit the peak and the tail. One clearly sees the small input of nascent apoB, L(30, 9), that fits the initial 4 data; however, lacking the input from the faster IDL pathway the remaining early portion of the curve cannot be fit. B: The input through the delipidation pathway is blocked, L(21, 20) = 0. Again, the flux through L(25, 29) is increased so as to fit the peak and tail, but the early points on the curve are then overshot. These LDL data were satisfactorily fit by a combination of all three pathways (result not shown).

equal 0, demonstrates the requirement for the slow pathway in order to fit the peak and tail of the LDL curve. Figure 6B, in which L(25, 29) = 0, shows the consequence of the loss of the rapid pathway through IDL, with the resultant inability to fit the ascent to the peak of LDL. However, as demonstrated in both these figures, this input through IDL arrives too late to provide an optimal fit of the four early time points, which are better fit by a small direct input of nascent LDL R(30, 9). C(30) is a small compartment whose output, whether into C(25) or directly out of the system as shown in Fig. 1A, is not well resolved, though in this subject the latter appears to be the case. The residence time of LDL-apoB is about 2 days, but there is considerable variation among the subjects (Table 3).

Thus, in all 10 studies the kinetic analysis resolves apoB input pathways that enter both VLDL and IDL but also with a small direct input to LDL. A portion of apoB is rapidly metabolized, while another portion progresses through a series of delays that constitute the delipidation chain. IDL is the metabolically most dynamic lipoprotein fraction. Ninety-five percent of apoB

TABLE 2. ApoB transport on various paths in subjects receiving n–6- and n–3-enriched diets

	R(APB)[a] 1[b]		R(VLDL) 0.38[b]		R(13,12) 0.15[b]		R(18,9) 0.23[b]		R(16,19) 0.01[b]		R(IDL) 0.91[b]	
Subjects	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
						mg/h						
J	183	136	55	29	22	16	33	13	0.4	0	173	109
L	161	173	107	22	28	10	78	11	1.2	0.9	151	167
K	248	114	109	7	49	2	60	5	0	0.3	248	112
Z[c]	243	252	165	101	61	37	104	64	0	0	154	228
P	131	249	67	56	17	34	49	21	0.5	1	131	247
Average	193	185	101	43	35	20	65	23	0.42	0.44	171	173
P (2-tail)[d]	ns		0.01		0.01		0.01		ns		ns	

	R(27,9) 0.32[b]		R(16,12) 0.25[b]		R(27,9)+R(16,12) 0.57[b]		R(IDL Loss) 0.76[b]		R(0, 20) 0.29[b]		R(0,29) 0.43[b]	
	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
						mg/h						
J	0	80	117	0	117	80	148	86	121	0	27	86
L	44	45	0	99	44	144	122	132	12	110	110	22
K	70	41	69	64	139	105	205	75	83	41	122	34
Z[c]	22	43	48	84	70	127	198	171	93	69	24	102
P	64	191	0	0	64	191	107	190	10	5	97	185
Average	40	80	47	49	87	129	156	131	64	45	76	86
P (2-tail)[d]	ns		ns		0.05		0.03		ns		ns	

	R(IDL→LDL) 0.19[b]		R(21,20) 0.11[b]		R(25,29) 0.08[b]		R(LDL) 0.24[b]		R(0,25) 0.19[b]		R(30,9) 0.05[b]	
	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
						mg/h						
J	25	23	19	16	5.7	7	35	50	25	23	10	27
L	30	35	17	0	13	35	40	42	30	35	10	7
K	43	37	35	25	8	12	44	39	43	37	0.5	2
Z[c]	37	57	16	52	21	5	45	81	38	57	7	24
P	22	56	7	29	15	27	24	60	23	56	1	4
Average	31	42	18.8	24.4	13.0	17	38	54	32	42	6	13
P (2-tail)[d]	0.05		ns		ns		0.04		0.05		ns	

[a] Average apoB production for all 10 studies, expressed as mg/kg/day, is 58 ± 20 (mean ± SD).
[b] Average transport on this path/average total apoB transport.
[c] R(0, 18) = 0 for all studies except Z:VO where the value is 81 mg/h.
[d] Data converted to fraction of total apoB transport on each path for each subject and *t*-test performed on these fractional transports.

traverses this fraction, which is the major site of secretory input and from which three-quarters of apoB is catabolized, as only about one-quarter of secreted apoB eventually is converted to LDL in these hypertriglyceridemic subjects.

RESULTS

Response of plasma lipids to fish oil diet

The switch from a safflower to a fish oil diet resulted in some profound changes in plasma lipids (Table 1). A highly significant decrease in total plasma triglycerides was accompanied by a decrease in VLDL-apoB but not in IDL or total plasma apoB. The fall in the triglyceride/apoB ratios of the VLDL and IDL fractions, though not reaching statistical significance, provides evidence of a decrease in triglyceride content, and thus the size, of these particles.

Plasma cholesterol did not change with diet, but the increase in the observed values of the LDL-cholesterol is in agreement with the rise in LDL-apoB concentration in response to fish oil. The LDL-cholesterol/LDL-apoB ratio remained unchanged, implying that no change occurred in the size distribution of these lipoproteins.

Relationships among pool masses, transports, and residence times

As is evident from Table 1, the five subjects varied greatly in their plasma lipid levels, and, for some, strik-

TABLE 3. ApoB masses and residence times for subjects receiving n–6- and n–3-enriched diets

	Masses (mg)						Residence Times (h)					
	VLDL		IDL		LDL		VLDL		IDL		LDL	
Subjects	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3	n–6	n–3
J	95	34	568	298	1356	1510	0.55	1.1	3.3	2.7	39	30
L	268	163	926	735	3520	3760	2.5	1.3	6.1	4.4	89	90
K	380	120	804	603	975	2120	3.5	1.7	3.2	5.4	22	54
Z	1500	354	717	1220	1590	2670	7.0	1.9	4.6	5.3	36	33
P	187	140	632	838	1025	2065	2.8	2.6	4.9	3.4	43	35
Average	486	162	729	739	1693	2425	3.27	1.72	4.42	4.24	45.8	48.4
P (2-tail)[a]	0.01		ns		0.04		ns		ns		ns	

[a]Student's *t*-test performed on log-transformed data.

ing changes occurred upon substituting the fish oil for the vegetable oil diet. In order to investigate changes produced by the dietary perturbation, it was first necessary to obtain an overview of the metabolism of apoB in this small population of subjects. Table 2 and Table 3 provide the results of measurement of residence times and apoB transports in VLDL, IDL, and LDL.

As would be expected, the mass of VLDL-apoB is strongly correlated with its residence time, in agreement with the generally recognized view of the importance of lipolysis in the metabolism of these lipoproteins. However, the mass is also strongly correlated with the secretory rates of VLDL-apoB by each of its input pathways. By contrast, total apoB production does not correlate significantly with VLDL mass, which is not surprising as only 38% of apoB is secreted as VLDL, Table 2, R(VLDL). The correlation of VLDL-TG with both mass and secretion is consistent.

IDL is a large lipoprotein fraction, and as recorded in Table 2, it provides the major transport route for apoB (Table 2, Average Fractional Transport). Again, the correlations are revealing, **Table 4**. The mass of IDL-apoB, M(IDL), does not correlate with the residence time, RT(IDL), but it does correlate with the quantity of apoB transported, R(IDL), and also with that amount converted to LDL, R(IDL→LDL). M(IDL) also fails to correlate with the catabolic loss of apoB from IDL, R(IDL Loss), and thus the mechanism controlling this catabolic process appears to be independent of the mass of IDL.

As modeled, Fig. 1, apoB is channeled through IDL to LDL as two nonintersecting pathways, as previously discussed. Fifty-one percent of apoB is transported through the fast pathway [the sum of R(0, 29) + R(25, 29)] and 40% by the delayed path [the sum of R(21, 20) + R(0, 20)]. Because some 90% of apoB transport is channelled through IDL, it was to be anticipated that apoB transport, R(IDL), would correlate highly with total apoB secretion, R(apoB) and this was the case. The greatest proportion of nascent apoB secretion is directed into IDL; R(27, 9) + R(16, 12) = 57% of total, secreted apoB. Because the output from this lipoprotein fraction is the sum of the pathways for the conversion to LDL, R(IDL→LDL), and for direct loss from plasma, R(IDL Loss), their observed correlations with R(IDL) are as expected. As residence time seems not to be a determinant of IDL mass, entry of apoB into this fraction and its output appear to be independently reg-

TABLE 4. Correlations (*r*) of masses (M), transports (R), and residence times (RT)

Parameter 1	vs.	Parameter 2	*r*	*P*
M(VLDL)	vs.	R(ApoB)	0.484	ns
		R(13, 12)	0.771	<0.01
		R(18, 9)	0.781	<0.01
		RT(VLDL)	0.934	<0.01
M(IDL)	vs.	R(ApoB)	0.622	<0.1
		R(IDL)	0.663	<0.05
		R(27, 9) + (16, 12)	0.246	ns
		R(IDL>LDL)	0.742	<0.02
		R(IDL Loss)	0.592	<0.01
		RT(IDL)	0.531	ns
		M(VLDL)	0.185	ns
R(IDL)	vs.	R(ApoB)	0.873	<0.01
		R(27, 18) + (27, 9)	0.585	<0.1
		R(IDL Loss)	0.841	<0.01
		R(IDL>LDL)	0.787	<0.01
		RT(IDL)	−0.243	ns
M(LDL)	vs.	R(ApoB)	−0.062	ns
		R(LDL)	0.271	ns
		R(IDL>LDL)	0.215	ns
		RT(LDL)	0.844	<0.01
R(LDL)	vs.	R(ApoB)	0.623	<0.1
		R(IDL>LDL)	0.807	<0.01
		R(30, 9)	0.596	<0.1
Triglycerides (TG)	vs.	R(ApoB)	0.485	ns
		R(VLDL)	0.794	<0.01
		M(VLDL)	0.889	<0.01
		CHOL	0.658	<0.05
		LDL-CHOL	−0.280	ns
VLDL-TG	vs.	M(VLDL)	0.978	<0.01
		R(VLDL)	0.808	<0.01
Plasma Chol	vs.	M(LDL)	0.436	ns
LDL-Chol	vs.	M(LDL)	0.819	<0.01

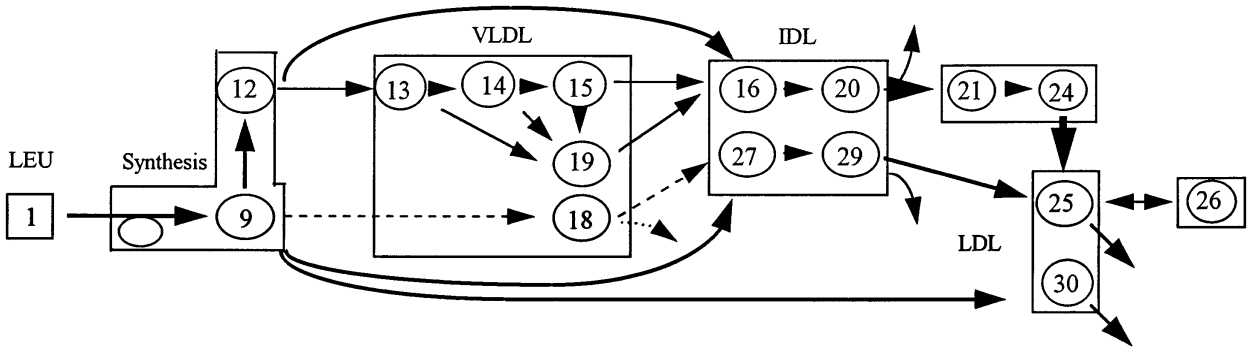

Fig. 7. The apoB model, as shown in Fig. 1, modified to demonstrate the changes in apoB metabolism produced by the fish oil diet. The intensity of the lines depicting metabolic pathways are adjusted to reflect the fish oil-induced changes in metabolism in accordance with the data tabulated in Table 2. The principal changes are the decrease in the production of VLDL, with a shift in apoB secretion to the smaller particles of the IDL fraction, and a shift in the ratio of IDL catabolism to conversion in favor of conversion to LDL, resulting in an increase in the concentration of LDL.

ulated processes which between them determine the mass of IDL. In these hypertriglyceridemic subjects, 76% of total secreted apoB is lost from plasma when it exits this fraction, with only 19% being converted to LDL [the remaining 5% of apoB flux is in the form of nascent LDL secretion, R(30, 9)]. The effect of a change from the vegetable to fish oil diet on these processes will be explored subsequently.

The mass of LDL is correlated solely with the residence time. Only 24% of secreted apoB becomes LDL (Table 2) and, accordingly, R(LDL) correlates poorly with R(apoB) but is highly correlated with the conversion of IDL to LDL, R(IDL→LDL). Nascent secretion of LDL, R(30, 9), is measured at about 5%, and this correlates, though poorly, with LDL-apoB transport. The strong correlation of LDL-cholesterol with the mass of LDL-apoB is as anticipated.

Effect of change from a vegetable oil to a fish oil diet on apoB metabolism

Table 3 reports the effects of a switch from a vegetable oil to a fish oil diet on the masses and residence times of apoB in the three lipoprotein fractions under study. There was a highly significant decrease in the mass of VLDL-apoB, no change in that of IDL, and a significant increase in the mass of LDL. The decrease in the mass of VLDL induced by fish oil, with the associated fall in plasma triglycerides, occurred in the absence of a significant decrease in residence time. A similar situation pertains with LDL. Despite these changes in the lipoprotein pools, the total plasma content of apoB did not change significantly (Table 1).

The transport of apoB along each segment of the pathways shown in the model in Fig. 1 has been measured for each subject on both diets, and Table 2 reports those values that either change with diet or are major transport pathways that are unresponsive to diet.[4] **Figure 7** provides a visual image of the changes in the metabolic pathways of apoB that result from the fish oil diet, as tabulated in Table 2. Because of the large differences in apoB production that occurred among the subjects, in analyzing these data, they were converted to fractional transports, i.e., the transport along a specific pathway divided by the total apoB flux, R(apoB), for that study, and differences in the means were evaluated by paired, two-sample *t*-tests.

Significant differences are clearly evident in apoB transport as a result of a change in diet, even in this small sample of five subjects. Total apoB production remained unchanged. However, on the fish oil diet VLDL-apoB secretion was strikingly reduced, and this reduction affected both input pathways, but primarily R(18, 9). As the residence time of VLDL-apoB decreased insignificantly, the fall in production rate was largely responsible for the decrease in the size of the VLDL pool.

The metabolism of IDL is a different situation. The apoB-mass, residence time, and flux remain unchanged, though the size of these lipoprotein particles appears to decrease. The input of apoB from VLDL was reduced, mostly because of the reduction in the flux through C(18), R(18, 9). However, this reduction was compensated by an increased secretion of nascent apoB, [R(27, 9) + R(16, 12)], with the major input going to compartment 27. Thus, the flux of apoB along the fast pathway remains about the same.

As noted previously, apoB output from IDL was di-

[4]The decision to limit the data presented in this report was made in the interest of space; however, the author will be glad to provide a full tabulation of the data to those who request it.

vided between a catabolic pathway and conversion to LDL; however, the ratio of degradation to conversion was altered by diet. Thus, fish oil caused a decrease in catabolism, R(IDL Loss), that was compensated by an increase in the channeling of IDL-apoB to LDL, R(IDL→LDL). This increase in the production of LDL-apoB on the fish oil diet, in the absence of a change in the residence time of LDL, accounted for the enlargement of the LDL pool, and was predominantly the result of a shift in the channeling of the output from IDL to favor LDL production.

DISCUSSION

The proper interpretation of human kinetic studies requires a knowledge of the biological system that is generally obtained by animal and in vitro experiments. However, determining the relevance of such observations to human physiology is only possible through the investigation of human subjects. Here we report an examination of the response of the apoB-containing lipoproteins to a dietary perturbation, accomplished by interchanging diets enriched in vegetable oil or fish oil, in hypertriglyceridemic, non-insulin-dependent diabetic humans. In order to understand the changes in apoB metabolism produced by fish oil, it was first necessary to determine the kinetics of apoB within this group of subjects.

Several laboratories have examined the metabolism of apoB in hypertriglyceridemic diabetic subjects using radioiodinated apoB tracers and these have been reviewed recently (18, 19). Packard, et al. (20, 21) and Kissebah (22) have used radioiodinated VLDL tracers to measure apoB secretory input and loss occurring along the dual pathways by which apoB is channeled from VLDL to LDL in hypertriglyceridemic and hypercholesterolemic subjects. In our laboratory, we have used [^{3}H]-leucine as an endogenous tracer in examining apoB kinetics in similar subjects (11), and the findings from these distinctly different methodologies are strongly reinforcing (23). Total apoB production is increased in hypertriglyceridemia. The average total apoB production, when expressed as mg/kg per day, in the 10 studies reported here, was 58 (sd 20) which agrees closely with the value of 57 (sd 20) mg/kg per d previously reported by us in an earlier study of hypertriglyceridemic subjects (24). These values contrast with that of 34 (sd 21) mg/kg per day for normolipidemic subjects (24). It is, of course, well recognized that impaired lipolysis is of major importance in the induction of hypertriglyceridemia associated with diabetes (18). The strong correlation between VLDL mass and residence time is thus consistent with the importance of lipolysis in determining the magnitude of the hypertriglyceridemia in our subjects, but VLDL-apoB secretion is also a strong determinant of mass (Table 4).

The lipoprotein fractionation protocol we have used in these studies, which combines small VLDL, $S_f < 100$, with IDL, was adopted for practical reasons as enumerated in Methods; however, it also has a physiologic rationale. Large VLDL particles are substrates for the VLDL and triglyceride-rich lipoprotein receptors that are expressed on endothelial cells and macrophages, respectively, and may contribute to the atherogenicity attributed to VLDL (25, 26). Small VLDL and IDL, however, are readily cleared by receptors of the LDL receptor family that are present in the liver (27). It is presumably within this lipoprotein fraction that kinetic studies from our laboratory, and by others, have demonstrated a metabolic fork by which apoB may either be channeled into LDL or irreversibly removed from plasma (11, 21, 22, 28, 29). Apparently the catabolism of these remnants is an apoE-mediated process (30–32). In the present study, we demonstrated very little loss of apoB from large VLDL but about three-quarters of apoB irreversibly disappeared from plasma as small VLDL or IDL, while the remainder was converted to LDL, Table 2. This finding is in general agreement with an earlier report from our laboratory (24). The fork in the output from IDL is of crucial importance in understanding LDL metabolism and its response to fish oil.

Many studies have examined the effects of fish oil on lipid metabolism in humans and animals, and they have been well reviewed (1–3). The changes in plasma lipid concentration are dependent on whether fish oil is an add-on to a control diet or whether the study is designed as a comparison between fish oil- and vegetable oil-enriched diets, as here, where the comparison was with safflower oil. In general, a fall in plasma triglycerides is seen with fish oil that is dose-dependent and reflects a decrease in VLDL levels. Total plasma apoB concentration tends not to change or may decrease. The response of LDL-cholesterol and LDL-apoB is variable, not only depending on diet but on patient selection. In familial combined and hypertriglyceridemic individuals, when changing from a vegetable to a fish oil diet, most, but not all, reports show a rise in LDL-cholesterol (1–3). Schectman, Kaul, and Kissebah (33) have provided an interesting analysis of the response of LDL-cholesterol as a function of LDL heterogeneity. As in animals, in our subjects total apoB production did not change in response to fish oil. Thus, the same number of apoB particles was secreted on both diets, but with fish oils these were lipid-depleted, smaller lipoproteins species (1, 34).

The delivery of fatty acids to the liver is a major determinant of both triglyceride synthesis and VLDL secretion (35). Fish oils inhibit both processes while stimulating fatty acid oxidation and phospholipid synthesis. The suppression of triglyceride synthesis, with preferential incorporation of n–3 fatty acids into phospholipid has been explained by the inhibition of phosphotidate phosphohydrolase activity by n–3 fatty acids (36–39). N–3 fatty acids also affect the intracellular degradation of apoB and thus its secretion (reviewed in 35).

Experiments in African green monkeys are particularly relevant to understanding the response of primates to fish oil and in relating these to observations in rodents (34). Perfused monkey livers have decreased secretion of cholesteryl ester and triglyceride but maintain the same apoB output. Thus the same number of particles is produced, but their size is smaller. However, these plasma lipoproteins had a higher phospholipid to core (triglyceride and cholesterol ester) lipid ratio. Liver perfusion and autopsy analyses showed that, while n–3 fatty acids were well incorporated into glyceride synthesis, they were diverted away from secretory triglyceride and into hepatic phospholipids, consistent with preferential synthesis of phospholipids.

Decreased availability of hepatic triglyceride for VLDL assembly provides an explanation for the reduction in VLDL-apoB secretion. Such an observation was initially reported in humans using a ^{125}I-labeled VLDL tracer, where a decrease in VLDL-apoB production rate was found in the absence of a change in the FCR of VLDL (4). VLDL-triglyceride metabolism was also studied in five hypertriglyceridemic humans using a [^{3}H] glycerol tracer (5). Here, also, a decrease in production was found to explain the fall in triglyceride concentration in the absence of a change in FCR. In concordance, an examination of post-heparin lipoprotein lipase and hepatic lipase activity in 12 hypertriglyceridemic patients failed to demonstrate any change induced by a fish oil diet (40). Our findings with respect to VLDL metabolism are in agreement.

Studies on the effects of fish oils on LDL metabolism have been complicated by the variability in the LDL response. Furthermore, investigators differ in their findings concerning fish oil-induced changes in lipid composition of human LDL from normal subjects (41, 42), but these differences may result from the structural heterogeneity that occurs in human LDL (33). Conflicting data have been reported concerning the effect of fish oils on hepatic LDL-receptor activity when examined in rats and in HepG2 cells (41, 43, 44); however, such discrepancy may well be the result of differences in the association of apoE with LDL (44). The single reported kinetic study on LDL metabolism in seven normal human subjects was performed with ^{125}I-labeled LDL (45). Subjects were studied on a control diet prepared with vegetable oils to provide n–6 fatty acids and these were substituted by salmon oil or Maxepa. LDL-cholesterol and LDL-apoB concentrations fell, due to a decrease in LDL production rate, but the LDL fractional catabolic rate did not change. Thus, this study provided no evidence for a change in LDL-receptor activity in humans. These findings are consistent with ours in that we also failed to observe a change in the fractional catabolic rate of LDL. Rather, the increase in LDL concentration observed in our hypertriglyceridemic subjects arose from an increase in the production rate of LDL-apoB. In the absence of a change in residence time, the increased flux of apoB from IDL to LDL became the determinant of the increase in LDL mass in response to fish oil.

Increased flux into LDL can be understood in the light of the fish oil-induced changes in the metabolism of small VLDL and IDL. Accompanying the production of a lesser number of VLDL, there is a compensatory increase in the production of IDL. The output from IDL is split by a metabolic fork, and kinetic studies in miniature pigs have demonstrated that fish oil redirects this channeling towards increased conversion of VLDL and IDL to LDL production (46). In our subjects, fish oil produces a similar effect with a decrease in the R(IDL Loss) pathway and an increase in R(IDL $>$ LDL). The resultant rise in LDL production accounts for the increase in LDL mass. In an interesting study, Linga et al. (44) have demonstrated that in cynomolgus monkeys, fish oils reduce apoE association with LDL, resulting in a reduced affinity of the LDL for the LDL-receptor. They review evidence that apoE binding to the receptor is lipid dependent and speculate that an increased phospholipid composition of lipids in fish oil lipoproteins may reduce apoE binding and receptor affinity. One is tempted to extend this reasoning to postulate that an altered lipid composition of IDL from fish oil reduces apoE-mediated binding to cellular receptors and results in enhanced conversion of LDL. There are a number of metabolic conditions in which the flux along the arms of this fork is shifted; however, fish oil is of particular interest, because it is a dietary perturbation and, hence, is reversible.

In conclusion, we have investigated the response in apoB kinetics to a substitution of dietary fish oil for safflower oil in hypertriglyceridemic, non-insulin-dependent diabetic subjects. While these subjects demonstrated the anticipated fall in VLDL-triglyceride concentration and VLDL-apoB production, the interesting finding is the manner in which apoB responds to this dietary perturbation, in keeping with the hypothesis that apoB metabolism adjusts in response to changing states of

lipid metabolism. Thus, decreased availability of triglyceride for VLDL secretion is compensated by production of the same number of smaller, triglyceride-depleted apoB particles. The reduction in flux through the catabolic pathway for IDL, resulting in an increased production of LDL, must arise from a decrease in the hepatic clearance of IDL in the presence of fish oil. Such dietary-induced shifts in apoB metabolism have been previously demonstrated with a high carbohydrate diet that increases secretion of large VLDL and the catabolic rate of LDL (47). While it is important to understand these changes in apoB kinetics, it is risky to extrapolate from a knowledge of the altered kinetics of the carrier apoproteins, induced by whatever cause, to conclusions regarding their medical significance. Such is the case with the use of fish oil as a potential therapy to lower triglycerides in the hypertriglyceridemic diabetic. However, knowing the cause of the increase in LDL concentration that occurs in these subjects provides a rationale for combining the administration of fish oils with that of a statin.

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