Abnormal clearance of postprandial S_f 100–400 plasma lipoproteins in insulin-dependent diabetes mellitus

Angeliki Georgopoulos[1] and Robert D. Phair

Division of Endocrinology and Metabolism, Department of Medicine, and Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Abstract Studies were carried out in three normolipidemic non-obese men with insulin-dependent diabetes mellitus (IDDM) and three normal men, to assess whether the clearance of postprandial S_f 100–400 lipoproteins is decreased in IDDM. $S_f > 100$ lipoproteins isolated from plasma 4.5 h after fat ingestion were labeled with ^{125}I and injected into the same subject intravenously. ApoB radioactivity was measured over time in $S_f > 400$, S_f 100–400, and S_f 20–100 lipoproteins isolated from plasma and analyzed using a kinetic model that included both fast and slow delipidation cascades, where lipolysis and uptake of particles by the liver and other tissues were represented. Fractional catabolic rates of S_f 100–400 lipoproteins (min^{-1}) were decreased in diabetic versus control subjects: fast = 0.170 ± 0.126 versus 0.680 ± 0.242 (mean ± SD) ($P < 0.05$, two-tailed) and slow = 0.011 ± 0.006 versus 0.031 ± 0.015 ($P < 0.05$, one-tailed). Kinetic analysis showed that the data were consistent with decreased uptake by the tissues for the fast cascade (diabetic, 0.084 ± 0.082, vs. control, 0.617 ± 0.328, $P < 0.05$, one-tailed). A similar trend was observed for the slow cascade. There were no significant differences between the two groups in the intraplasma lipolysis rates of S_f 100–400 particles. Analysis of the composition of the injected particles showed that they were total cholesterol (TC)-versus triglyceride (TG)-enriched ($P < 0.001$, log-ratio analysis of composition) in IDDM subjects. In conclusion, the decreased clearance of postprandial S_f 100–400 lipoproteins in IDDM men appears to result from decreased tissue uptake (fast cascade) rather than impaired lipolysis, and may be related to cholesterol enrichment. —**Georgopoulos, A., and R. D. Phair.** Abnormal clearance of postprandial S_f 100–400 plasma lipoproteins in insulin-dependent diabetes mellitus. *J. Lipid Res.* 1991. **32:** 1133–1141.

Supplementary key words postprandial lipoproteins • kinetic analysis • SAAM

Cardiovascular disease is the major cause of death in diabetic patients above the age of 20 (1). The mechanism is unknown (2–4). Plasma triglycerides (TG) are elevated in diabetes (5, 6) and have been shown to be an independent risk factor for atherosclerosis (7). They are carried by very low density lipoproteins (VLDL), chylomicrons (CM) and their remnants. Remnant particles have been implicated in the development of atherosclerosis in type III hyperlipoproteinemia (8). Studies in diabetic animals have shown decreased clearance of chylomicron remnants (9, 10). It is therefore possible that the clearance of postprandial TG-rich remnants is decreased in diabetic human subjects and that such a decrease could lead to deposition of lipid in cells of the blood vessel wall, especially if these lipoproteins are cholesterol-enriched (11), as in type III hyperlipoproteinemia. The present study was undertaken to investigate whether the clearance of postprandial TG-rich lipoproteins of S_f 100–400 is decreased in insulin-dependent diabetes mellitus (IDDM).

MATERIAL AND METHODS

All participating study subjects were non-obese, normolipidemic E_3/E_3 males. Two groups were studied: control men and patients with IDDM, as defined by the criteria of the American Diabetes Association. The study subject characteristics are shown in **Table 1**. The groups were matched for age, percentage of ideal body weight (107 ± 9.5% in controls vs. 105 ± 11.5% in diabetics; mean ± SD), plasma TG (114 ± 51.5 mg/dl in controls vs. 89 ± 21.5 in diabetics), total cholesterol (TC) (188 ± 19.2 mg/dl in controls vs. 164 ± 20.2 in diabetics), and high density lipoprotein (HDL) cholesterol (49 ± 10.2 mg/dl in controls vs. 36 ± 9 mg/dl in diabetics). The diabetic subjects were in poor control as shown by a plasma glucose level of 247 ± 77 mg/dl

Abbreviations: TG, triglyceride; CM, chylomicrons; VLDL, very low density lipoproteins; HDL. high density lipoproteins; LDL, low density lipoproteins; IDDM, insulin-dependent diabetes mellitus; TC, total cholesterol; CHO, carbohydrate; PL, phospholipid; LDL, lipoprotein lipase.

[1]To whom correspondence should be addressed at: Department of Medicine, VA Medical Center, One Veterans Drive, Minneapolis, MN 55417.

TABLE 1. Clinical characteristics of control and insulin-dependent diabetic (IDDM) men

Subjects	Age	IBW	PG	TC	TG	HDL	Hba_1	Daily Insulin Dose
	yr	*%*		*mg/dl*			*%*	*U/24 h*
Control (n = 3)								
Mean	27	107	85	188	114	49		
± SD	6.6	9.5	9.3	19.2	51.5	10.2		
IDDM (n = 3)								
Mean	33	105	247	164	89	36	12.8	53
± SD	4.9	11.7	77	20.2	21.5	9	0.6	6.4

IBW, ideal body weight; PG, plasma glucose; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; Hba_1, hemoglobin a_1.

(mean ± SD) (vs. 85 ± 9.3 mg/dl in the controls) and an Hba_1 of 12.8 ± 0.6% (normal range: 3.9–7.7%). The daily mean insulin dose of the diabetic patients (53 ± 6.4 units) was not excessive, suggesting the absence of significant insulin resistance in our study patients. Dietary evaluations, as assessed by 3-day recalls, revealed no differences in the percent calories from fat, carbohydrate (CHO), and protein between the study groups. Fat intake was 30–35% and CHO intake 50–55%. Study subjects exercised 1–2 h per week. None of the study subjects had hypertension, anemia, abnormalities in thyroid, renal or liver function, consumed alcohol on a regular basis, or was taking any medication except for insulin in the diabetic subjects. The duration of diabetes ranged from 7 to 15 years. Background retinopathy and mild peripheral neuropathy but not nephropathy, coronary, carotid, or peripherial vascular disease were present in the diabetic subjects, as assessed by history, physical examination, and resting electrocardiogram.

Study protocol

All participating subjects signed the consent form. They were asked to remain on their usual diet and exercise patterns for 2 weeks preceding the study, abstain from any alcohol intake for at least 3 days, and fast for 13–14 h preceding the study. A baseline blood sample was obtained at 8:30 AM after which the study subject ingested a fat formula containing corn oil mixed with skim milk and fruit flavoring, 70 g/m^2 body surface. No other food or drink (except water) was allowed for 5 h. Diabetic subjects were given 60% of their daily insulin dose to avoid hypoglycemia. At 4.5–5 h plasmapheresis was performed. The subject was then given his lunch and the remaining insulin dose, as needed. He was also placed on saturated potassium iodide administration to protect his thyroid from radioactive iodine uptake that could occur after injection of ^{125}I-labeled lipoprotein 3 days later. The harvested plasma was used to isolate postprandial TG-rich lipoproteins which were iodinated, characterized, filtered, tested for pyrogens, and re-injected into the same subject (autologous experiment) 72 h later. The subjects were admitted to the Johns Hopkins Clinical Research Center the evening before the injection. After a 13–14 h fast, an intravenous bolus of 55 μCi ^{125}I-labeled TG-rich lipoproteins ($S_f > 100$) was administered at 8:30 AM. Blood samples were drawn on ice before and 7–8 times (2, 5, 10, 15 or 20, 30 or 40, 50 or 60, 70 or 80, and 90 or 100 min) after the IV bolus injection. Study subjects ate breakfast and diabetic subjects received their daily insulin dose 120 min after the injection. Using density gradient ultracentrifugation, TG-rich lipoprotein subfractions were isolated from each blood sample ($S_f > 400$, 100–400, and 20–100), and apoB radioactivity was counted as described below.

Biochemical methods

Cholesterol and TG measurements in plasma and lipoprotein subfractions were performed using colorimetric enzymatic methods (kits BMD #704121 and Seradyn #47161, respectively). Lipoprotein protein was measured by the Bradford method (12) and phospholipid by the Bartlett method (13). HDL cholesterol was measured by heparin–manganese precipitation, according to the Lipid Research Clinic procedure (14). Plasma glucose was determined by the glucose oxidase method (15) and Hba_1 by agarose gel electrophoresis (16).

Lipoprotein preparation and isolation after plasmapheresis. TG-rich lipoproteins ($S_f > 100$) were isolated after overlayering plasma with saline-EDTA (d 1.006 g/ml) using an SW_{28} rotor and spinning at 31.2 × 10^6 *g*-min at 10°C. The isolated lipoproteins were further concentrated and purified by raising their density to 1.063 g/ml with KBr and overlayering them with a solution containing 1.1% NaCl, 10 mM HEPES, 0.01% EDTA (pH 7.4), and respinning them at 31.2 × 10^6 *g*-min (17). The re-isolated $S_f > 100$ lipoproteins were dialyzed against Tris-EDTA buffer (pH 7.4) and iodinated by the method of MacFarlane, as modified by Fidge and Poulis (18). After iodination, the lipoproteins were dialyzed against 24 l of buffer, mixed with autologous fasting plasma to induce transfer of apoC radioactivity to HDL. They were then re-isolated using KBr and HEPES as described above, dialyzed and characterized. The characteristics of the injectate are shown in **Table 2**. The injected lipoproteins had

TABLE 2. Characteristics of injected S_f 100–400 lipoproteins in control and IDDM men

Subjects	Specific Activity	Dose	% TCA Precipitable	ApoB	
				S_f 100–400	S_f > 400
	cpm/ng	*μCi*		*% of protein*	
Control					
Mean	27.5	55.0	92.7	38.0	7.1
± SD	4.3	4.6	1.1	10.0	2.0
IDDM					
Mean	24.4	55.0	94.9	31.6	8.7
± SD	10.3	4.4	2.5	3.0	2.2

similar specific activities (27.5 ± 4.3 cpm/ng of protein, mean ± SD, in controls vs. 24.4 ± 10.3 in diabetics), and the distribution of the radioactivity in trichloroacetic acid (TCA)-soluble (92.1% ± 1.1 in controls vs. 94.9% ± 2.5 in diabetics) and apoB counts (Table 2) were not different in the two groups. Before injection to the study subjects, the lipoproteins were diluted, filtered through a 0.45 μm Millipore filter, and tested for pyrogens by the limulus test (19).

Lipoprotein isolation from plasma after injection of ^{125}I-labeled S_f > 100 lipoprotein. Three postprandial TG-rich lipoprotein fractions (S_f > 400, 100–400 and 20–100) were isolated by ultracentrifugation using the method of Lindgren, Jensen, and Hatch (20) as modified by Redgrave and Carlson (21); recoveries were 88–94%. This method requires the creation of a salt density gradient and the isolation of subfractions by successive centrifugations in an SW41.Ti rotor. Four ml of plasma was adjusted to d 1.1 g/ml by adding KBr. NaCl-KBr solutions d 1.065 g/ml (3 ml) followed by d 1.02 g/ml (3 ml) and d 1.006 g/ml (2 ml) containing 0.01% EDTA were layered successively over the plasma. The conditions of isolation were 4.5×10^6 *g*-min for S_f > 400, 31.2×10^6 *g*-min for S_f 100–400 and 152×10^6 *g*-min for S_f 20–100.

ApoB radioactivity measurements in the TG-rich lipoprotein subfractions were carried out after isolation of apoB by polyacrylamide gel electrophoresis according to the method of Kane, Hardman, and Paulus (22) using 4–30% Pharmacia gradient gels. The top part of the gel containing both apoB-100 and apoB-48, as identified by high molecular weight standards (Pharmacia) as well as LDL apoB and VLDL apoB-100 and B-48 from a subject with type III hyperlipoproteinemia, was cut out and counted in a gamma counter with a 60% efficiency. All apoB measurements presented represent total apoB counts. We made that decision based on the reasoning that postprandial S_f 100–400 particles consist of a mixture of apoB-100- and apoB-48-containing particles. In our experimental protocol we measured the clearance of this mixture of particles, harvested 4.5 h after fat ingestion, labeled with ^{125}I and re-injected into the same subjects 72 h later.

Kinetic analysis. The multicompartment model described below was developed under the standard assumptions of tracer kinetics. During development, alternate models were formulated and tested using the SAAM/CONSAM (version 30) (23) software (RFKA, University of Washington, Seattle) running on a VAX 8650 computer. Parameter estimates and uncertainties were obtained by a generalized nonlinear least squares procedure (24).

Statistical methods. Comparisons of the intercompartmental rate constants L(i, j) for control and diabetic subjects were performed by the Student's *t*-test (25).

Compositional analysis. For analysis of the lipoprotein composition, the log-ratio analysis of compositions (26) was used as previously described (27). More specifically, at every time point the lipoprotein mass concentration (mg/ml) was calculated by adding the measurements of the concentration of TG, TC, protein, and phospholipid (PL). Since cholesteryl esters were not measured, the mass of fatty acids esterified with cholesterol was not included in the calculated values. Study groups were compared with regard to the composition of the lipoprotein mass concentration using a statistical analysis appropriate for compositional data (26). Compositional data cannot be analyzed by 'standard' statistical techniques because they are subject to a unit-sum constraint and inappropriate analysis can lead to erroneous conclusions. Instead, a multivariate analysis has been developed (26) which provides proper testing of hypotheses and models of compositional data. In the present work the null hypothesis to be tested is that the lipoprotein composition of the diabetic subjects does not differ from that of normal subjects. More specifically, we tested the statistical significance of the relative TC versus TG enrichment of the diabetic lipoproteins as compared to normal. This was tested using the multivariate version of the Behrens-Fisher statistic, as described in page 157 of ref. 26, and the significance probability is obtained from the chi-square distribution. The significance probability level reported in Results refers to the test statistic above. The calculations for this statistical analysis were carried out using the Microcomputer Package for the Statistical Analysis of

Compositional data (CODA) by J. Aitchison (Chapman and Hall, distributors).

RESULTS

Kinetic data

Figs. 1 and 2 show the apoB radioactivity in S_f 100–400 and S_f 20–100 lipoproteins in the control (**Fig. 1**) and the diabetic (**Fig. 2**) subjects. Data are expressed as fraction of the injected ^{125}I dose per ml of plasma in each apoB compartment. ApoB radioactivity in $S_f > 400$ was too low to be accurately measured and is therefore not included in the figures. Inspection of the curves shows that despite heterogeneity present within the

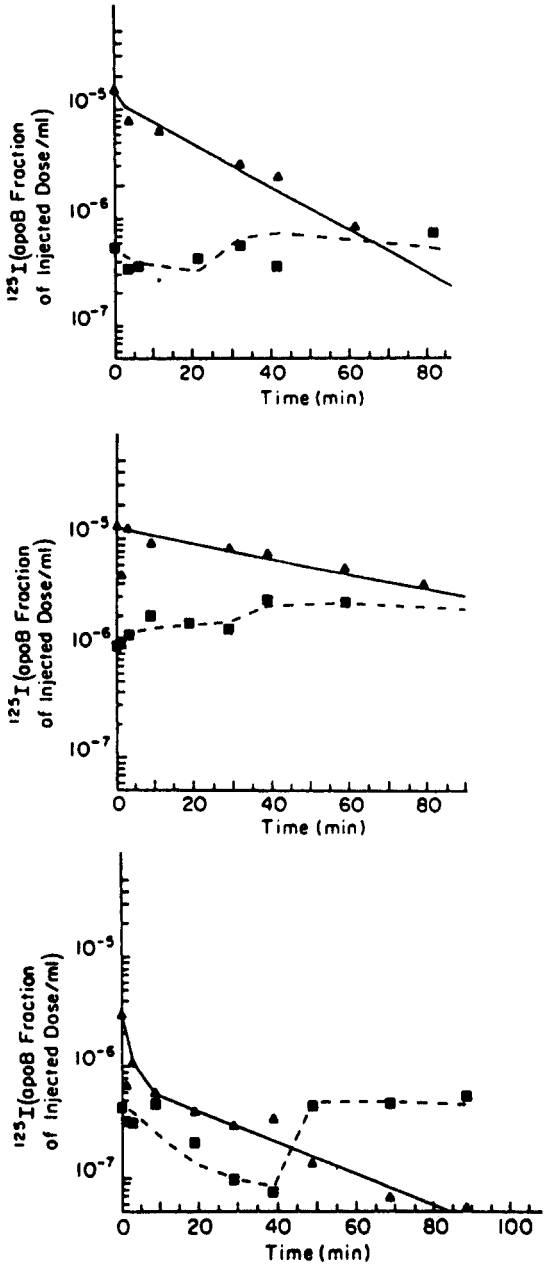

Fig. 1. ^{125}I-labeled apoB in plasma S_f 100–400 (▲) and S_f 20–100 (■) in three normal men. The lines represent solutions of the model that were obtained after least-squares fitting; solid line, S_f 100–400; interrupted line, S_f 20–100. The actual fraction of the injected ^{125}I in each apoB compartment is much less than 1.0 because many of the injected cpm were associated with apoE and apoCs.

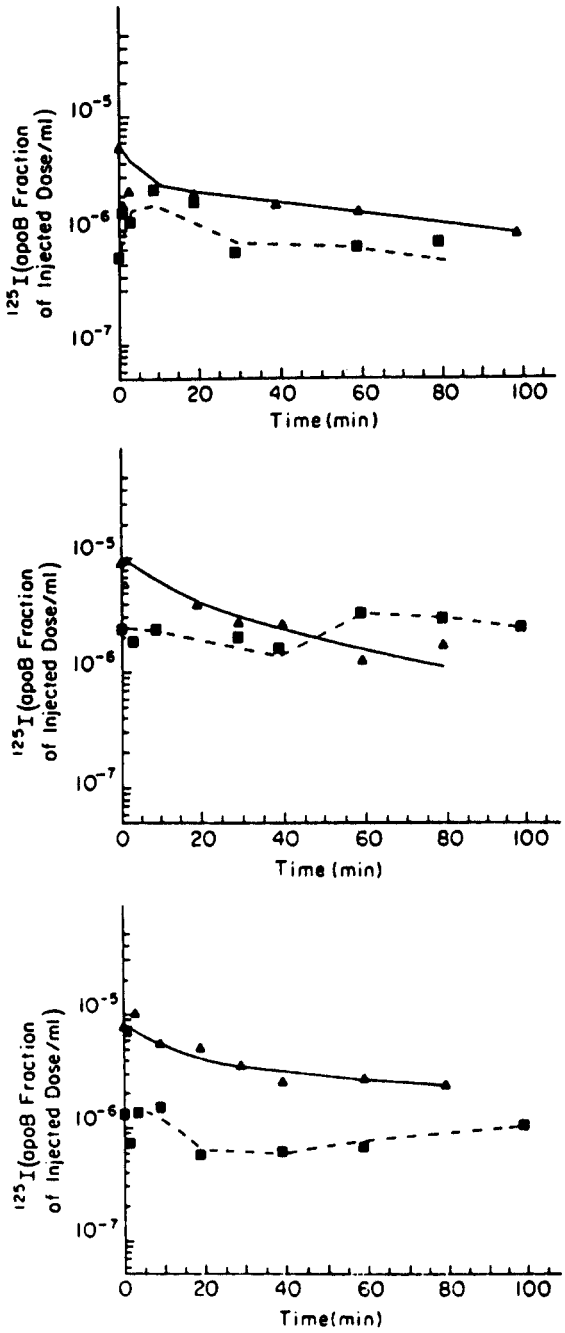

Fig. 2. ^{125}I-labeled apoB in plasma S_f 100–400 (▲) and S_f 20–100 (■) in three IDDM men. The lines represent solutions of the model that were obtained after least-squares fitting; solid line, S_f 100–400; interupted line, S_f 20–100. The actual fraction of the injected ^{125}I in each apoB compartment is much less than 1.0 because many of the injected cpm were associated with apoE and apoCs.

S_f 100–400 particles, as evidenced by the shape of the decay curves, the curves of the control group as a whole are more variable than those of the diabetic group. Overall, the turnover rate of apoB in S_f 100–400 is slower in the diabetic subjects.

Development of kinetic model

A model was developed based on current concepts of lipoprotein metabolism and on postprandial S_f 100–400 and S_f 20–100 apoB kinetics from control subjects. The proposed model assumes that particles of $S_f > 20$ (probable diameter > 35 nm) are too large to exchange significantly with extracellular fluid, so that biphasic kinetics must reflect kinetic heterogeneity of particles within a single S_f class. It therefore (**Fig. 3**) includes two parallel lipolytic cascades characterized by different turnover rates. The fast cascade represents lipolysis from $S_f > 400$ (compartment #7) to S_f 100–400 (#4) to S_f 20–100 (#5) to $S_f < 20$ (#6); and the slow cascade represents lipolysis from S_f 100–400 (#1) to S_f 20–100 (#2) to $S_f < 20$ (#3). Since $S_f < 20$ particles were not isolated in our studies, compartments 6 and 3 represent clearance of the particles by both lipolysis and tissue uptake, as indicated in Fig. 3. Direct removal of both fast and slow S_f 100–400 lipoprotein particles appears to be required by the data; if all the activity injected as S_f 100–400 was forced to appear in S_f 20–100, the predicted activity in S_f 20–100 either had the right shape and the wrong magnitude, or the right magnitude and the wrong shape; in other words it did not fit the data. Direct uptake of particles with density S_f 20–100 was not resolvable from the data because there were too few counts in particles $S_f < 20$ for accurate analysis. Vascular interconversion of fast and slow particles could not be resolved in these experiments. This should not be taken to rule out the possibility of such interconversion. To account for the delayed appearance of plasma apoB radioactivity in S_f 20–100, many minutes after the injection, recycling of particles from the extravascular compartment (#8) into the plasma in the S_f 20–100 range was added. Recycling was more evident in the data from control subjects than in diabetics, but all the data sets required some delayed reappearance of radioactivity that had previously escaped from plasma. Since the ^{125}I would be lost if apoB were hydrolyzed, this process must represent prolonged extravascular processing (perhaps LPL/hepatic TG-lipase or cellular endocytosis and resecretion). Recycled particles had to enter the slow cascade; recycling to fast S_f 20–100 particles could not be resolved. The clearance of apoB in $S_f > 400$ lipoproteins could not be measured with precision since the apoB content of these particles was no more than 7% of the protein content (28) and the particle clearance in normolipidemic subjects was rapid on the time scale of these experiments. Therefore, all apoB values in this compartment (#7) were calculated from *a*) the distribution of activity in the injectate, *b*) the appearance of this activity only in the fast cascade of S_f 100–400, and *c*) the assumption that one-half of these particles were cleared directly (mostly by the liver). Control subjects were analyzed first. Rate constants obtained from the analysis of control data served as the starting point in fitting the diabetic data. When such a fit was not possible, rate constants were adjusted using the minimum change postulate (29).

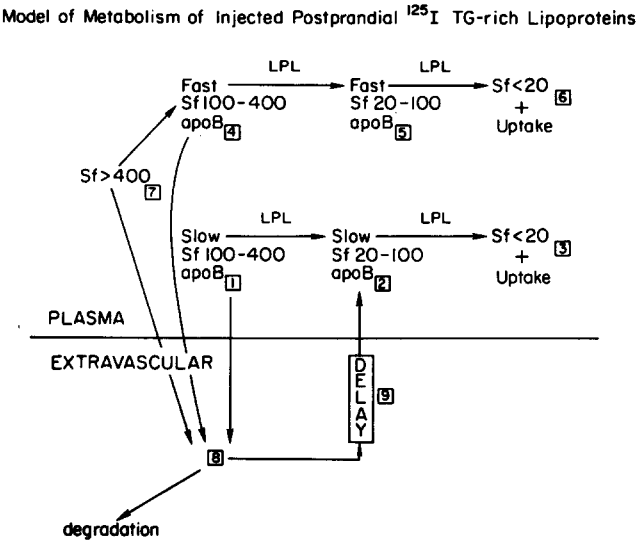

Fig. 3. Current kinetic model of human postprandial S_f 100–400 lipoproteins. Two parallel cascades of fast and slow particles are included. Lipolysis and tissue uptake of the particles are represented. Numbers identify compartments.

During least squares fitting only a subset of the model parameters could be identified (i.e., estimated with coefficients of variation less than 0.5). These were L(8,4), L(5,4), L(8,1), L(2,1), L(6,5), L(3,2), IC(4), IC(1), IC(5), IC(2), $F_{recycling}$ [i.e., L(9,8)/L(8,8)], and DT(9) (**Table 3**, A and B). The remaining parameter values remained fixed during the least-squares fitting procedure; their values were L(4,7) = 0.5 min^{-1}, L(8,7) = 0.5 min^{-1}, L(8,8) = 5 min^{-1}.

Findings

As shown in Table 3A, the turnover rate of diabetic S_f 100–400 particles (as measured by total apoB radioactivity) was significantly slower than that of controls for both the fast [L(4,4), $P < 0.05$, two-tailed *t*-test] and the slow [L(1,1), $P < 0.05$, one-tailed] cascades. Moreover, the decreased turnover rate of the diabetic S_f 100–400 particles was not due to decreased lipolysis rates [L(5,4)], as one might have expected to occur in diabetes, but rather to decreased uptake of the particles by the extravascular tissues [L(8,4), $P < 0.05$, one-tailed]. A similar trend was seen for the slow cascade (L(8,1) in Table 3A) but did not reach statistical significance because of the small sample

TABLE 3. Model parameters (mean ± SD) in control and diabetic subjects

Parameters	Tissue Uptake	Lipolysis	S_f 100–400 Total Turnover	S_f 100–400 Distribution of Label	S_f 20–100 Lipolysis and Uptake	S_f 20–100 Distribution of Label
				fraction of apoB dose		*fraction of apoB dose*
A. Cascade						
Fast cascade	L(8,4)	L(5,4)	L(4,4)	IC(4)	L(6,5)	IC(5)
Control	0.617 ± 0.328	0.064 ± 0.087	0.680 ± 0.242	0.043 ± 0.033	0.140 ± 0.034	0.008 ± 0.006
Diabetic	0.084 ± 0.082	0.087 ± 0.069	0.170 ± 0.126	0.077 ± 0.017	0.146 ± 0.098	0.013 ± 0.009
	$P < 0.05$	NS	$P < 0.05$	NS	NS	NS
Slow cascade	L(8,1)	L(2,1)	L(1,1)	IC(1)	L(3,2)	IC(2)
Control	0.028 ± 0.017	0.003 ± 0.002	0.031 ± 0.015	0.148 ± 0.119	0.015 ± 0.009	0.005 ± 0.007
Diabetic	0.009 ± 0.005	0.002 ± 0.001	0.011 ± 0.006	0.069 ± 0.014	0.011 ± 0.006	0.014 ± 0.020
	NS	NS	$P < 0.05$	NS	NS	NS
B. Recycling	Recycling Fraction	Recycling Delay[a]				
Control	0.152 ± 0.129	32.7 ± 9.3				
Diabetic	0.392 ± 0.257	36.0 ± 3.6				
	NS	NS				

L, rate constant (min^{-1}); IC, initial conditions, one-tailed test. Numbers inside parentheses correspond to model compartment as indicated in Fig. 3. (Note: The sum of ICs does not add up to unity because it represents only apoB counts that are a fraction of the total counts present in the isolated lipoprotein fractions; apoC and apoE counts account for the rest.)

[a]The data do not permit iterative adjustment of this delay time. The numbers in this column were estimated based on manual minimization of residuals.

size and the intersubject variability. There were no resolvable differences between the two groups in the turnover rates of S_f 20–100 lipoproteins [L(5,5) and L(2,2)]. There were also no differences in the recycled fraction, the delay time [DT(9)] for recycling of apoB to S_f 20–100, or the initial conditions. The complete set of kinetic parameters is included as an Appendix.

Compositional analysis

To investigate whether the observed difference in the clearance of the postprandial lipoproteins between normal and diabetic subjects was due to differences in the injected lipoproteins, the composition of S_f 100–400 lipoproteins was compared in the normal and diabetic subjects. As shown in **Fig. 4**, the composition of the diabetic lipoproteins differed significantly from that of the normal subjects. More specifically, the diabetic lipoproteins were TC- versus TG-enriched ($P < 0.001$, log-ratio analysis of (TG, TC) subcomposition, see Methods).

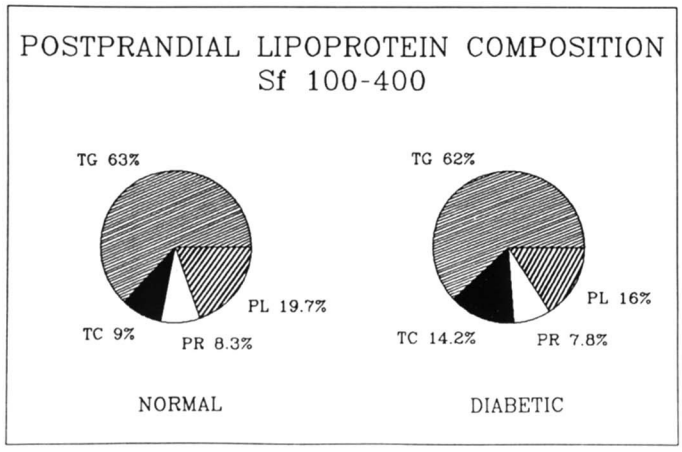

Fig. 4. Mean lipoprotein mass composition in postprandial S_f 100–400 lipoproteins in normal and IDDM men studied; TG, triglycerides; TC, total cholesterol; PR, protein; PL, phospholipids.

DISCUSSION

The purpose of this study was to investigate whether the clearance of postprandial TG-rich S_f 100–400 lipoproteins was decreased in insulin-dependent diabetic men. The range of S_f 100–400 was selected since in the postprandial state it should be enriched in chylomicron remnants which have been shown to be atherogenic in animals (30) and humans (8). Total apoB radioactivity was used as a marker of particle clearance, since it stays with the particle until the particle is removed from the circulation. Although until recently apoB-100 particles were considered to be of hepatic origin and B-48 of intestinal origin, there is recent evidence that the human intestine also produces B-100 particles (31). Therefore, intestinal origin of some B-100-containing particles in the postprandial state cannot be ruled out. Indeed, studies have shown that the apoB-100 mass in the postprandial state increases (32) and represents the greater portion of apoB mass in d 1.006 g/ml lipoproteins. We, therefore, decided to count

total apoB in our studies. To analyze the results, we used a model (Fig. 3) that included the processes involved in the clearance of postprandial TG-rich lipoproteins, i.e., lipolysis and tissue uptake. Moreover, the model included both fast and slow lipolytic cascades. These parallel cascades were consistent with the kinetic data; they are similar to those described by Yamada et al. (33) for endogenous rabbit lipoprotein metabolism and might therefore correspond to apoE-rich (fast) and apoE-poor (slow) lipoproteins. Alternatively, the two cascades might represent the metabolism of apoB-48 and apoB-100 particles. In any case, it is probable that all of our labeled particles were postprandial since even the slowest of them had a turnover time (100 min) that was 2.6-fold faster than the fastest diabetic (NIDDM) VLDL turnover time reported by Taskinen, Packard, and Shepherd (34) for endogenous particles.

Our data are consistent with the hypothesis that in IDDM men the clearance of postprandial S_f 100–400 lipoproteins is decreased, since a significantly decreased fractional catabolic rate was indeed observed in diabetic men (Table 3A). This was found to be the case for both the slow and fast cascades, suggesting that whatever the nature of the defect in diabetes, it is not limited to a specific, kinetically distinct subfraction of the S_f 100–400 lipoproteins.

Decreased chylomicron remnant clearance has been reported previously in diabetic animal studies by some (9, 10) but not by other (35) investigators. To our knowledge, this is the first report of decreased postprandial lipoprotein clearance in IDDM human subjects. We believe that our findings reflect the changes in lipoprotein metabolism caused by diabetes since our study subjects were selected to be non-obese, normolipidemic, and were matched with control subjects for factors other than IDDM, i.e., body weight, race, age, and sex. Moreover, since all subjects had the E_3/E_3 phenotype, differences in the apoE phenotype could not account for the decreased clearance of S_f 100–400 lipoproteins in our diabetic subjects. Previous studies in hypertriglyceridemic NIDDM subjects have shown decreased apoB clearance of $S_f > 400$ lipoproteins (36) which was thought to be secondary to decreased LPL activity. In this report the presence of hypertriglyceridemia (269–2,854 mg/dl) in all diabetic subjects made it difficult to assess the isolated effect of diabetes.

Analysis of our data to assess which process could account for the decreased fractional catabolic rate of the diabetic S_f 100–400 particles showed that tissue clearance of the particles (fast cascade) rather than lipolysis rates was decreased (Table 3A). Similar conclusions were reached by Redgrave and Callow (37) using lipid emulsions as a model for the metabolism of TG-rich lipoproteins in rats. These investigators found decreased uptake of the particles by both the liver and extrahepatic tissues. Recent studies in NIDDM subjects (34) showed a lack of effect of insulin treatment on the delipidation of $VLDL_1$ (S_f 60–400) to $VLDL_2$ (S_f 20–60).

What could be the mechanisms accounting for the decreased tissue clearance of postprandial lipoproteins in diabetes? Since in our studies each subject received his own particles (autologous experiment), diabetic particles were injected into diabetic subjects and normal particles into normal subjects. It is thus possible that the decreased clearance of postprandial S_f 100–400 lipoproteins in diabetic subjects could be due to either abnormal particle composition or abnormal clearing mechanisms, or both. Compositional analysis of the lipoproteins showed that the diabetic particles were cholesterol-enriched. This is in agreement with previous findings by us (27) and other investigators (38) that showed similar changes in fasting TG-rich lipoproteins in diabetic subjects. It is, however, unclear whether these compositional abnormalities can explain the decreased uptake of the particles by the extravascular tissues. Recent studies have shown that compositional changes induced by incubating LDL with hepatic TG-lipase affected the apoB configuration and its binding to the LDL receptor (39). It is therefore conceivable that compositional changes in TG-rich particles would also cause changes in the binding of the particles to their receptors.

Our estimates of apoE content in S_f 100–400 lipoproteins by gel electrophoresis did not show any differences between normal and diabetic subjects (data not shown), but we cannot rule out changes in the apoE configuration or extent of glucosylation. It is also possible that we were not able to detect any differences in apoE content between the two groups because our S_f 100–400 particles were isolated by ultracentrifugation, which might have accounted for differential loss of apoE in the two study groups. Since apoE exchanges with HDL, it is possible that after re-injection of the particles to our study subjects there were differences in the extent of apoE transfer in the diabetic and normal S_f 100–400 lipoproteins. It is also possible that greater glucosylation of the diabetic apoE could account for differential binding to cellular receptors. Alternatively, differences in the content of other apolipoproteins, i.e., apoC-II versus apoC-III or apoA-I and apoA-IV between normal and diabetic subjects could have also interfered with the uptake of chylomicron remnants by the liver (40, 41).

Another new finding of this study is the delayed input into the circulation of particles in the S_f 20–100 range in both the control and diabetic subjects. In light of the 37 min (average) delay involved in the appearance of these particles, this process may represent release into the circulation of intracellularly modified smaller apoB-containing particles originating from the injected $S_f > 100$ particles. Alternatively, prolonged processing of the particles by endothelial or extravascular lipases may occur since the recycling apoB was found in S_f 20–100

particles which are denser than the injected S_f 100–400 particles. We believe that intracellular processing is more likely because vascular lipases are unlikely to contribute a pure delay to the observed kinetics, but further studies are needed to elucidate the mechanisms involved.

In conclusion, our hypothesis is consistent with the results of our studies that the clearance of postprandial S_f 100–400 lipoproteins is decreased in normolipidemic nonobese IDDM men. According to our model, this appears to be mainly due to decreased uptake of the particles by the liver and other tissues rather than decreased intravascular lipolysis and could be related to the increased atherosclerotic risk present in diabetes.

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APPENDIX. Kinetic parameters for three normal (Nor-1,..., Nor-3) and three IDDM (Dia-1,..., Dia-3) subjects. Compartments are numbered as in Fig. 3. L(i,j) are expressed as (min^{-1}), DT(9) as (min).

	Subjects					
Parameters	Nor-1	Nor-2	Nor-3	Dia-1	Dia-2	Dia-3
IC(7)	0.0752	0.0241	0.0103	0.0372	0.0206	0.0532
L(4,7)	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
L(8,7)	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
IC(4)	0.0512	0.0070	0.0717	0.0572	0.0858	0.0881
L(5,4)	0.0282	0.1630	0.0000	0.1530	0.0915	0.0155
L(8,4)	0.8120	0.2380	0.8000	0.1630	0.0000	0.0879
L(4,4)	0.8402	0.4010	0.8000	0.3160	0.0915	0.1034
F to liver[a]	0.9664	0.5935	1.0000	0.5158	0.0000	0.8501
IC(1)	0.0184	0.1730	0.2520	0.0539	0.0713	0.0814
L(2,1)	0.0000	0.0058	0.0027	0.0016	0.0008	0.0026
L(8,1)	0.0320	0.0096	0.0428	0.0089	0.0045	0.0147
L(1,1)	0.0320	0.0154	0.0455	0.0105	0.0053	0.0173
F to liver	1.0000	0.6257	0.9398	0.8502	0.8492	0.8497
IC(5)	0.0077	0.0010	0.0140	0.0080	0.0239	0.0080
L(6,5)	0.1200	0.1800	0.1200	0.0511	0.2470	0.1400
IC(2)	0.0024	0.0134	0.0003	0.0041	0.0023	0.0361
L(3,2)	0.0050	0.0200	0.0200	0.0150	0.0043	0.0150
L(0,8)	4.5700	3.5000	4.6500	4.2500	1.6900	3.1800
L(9,8)	0.4310	1.5000	0.3500	0.7500	3.3100	1.8200
L(8,8)	5.0010	5.0000	5.0000	5.0000	5.0000	5.0000
Recyc fract[b]	0.0862	0.3000	0.0700	0.1500	0.6620	0.3640
DT(9)	43.0000	30.0000	25.000	33.000	35.000	40.000

[a]F to liver, fraction of apoB in a given compartment that is destined for extravascular tissue (presumably, liver). This is calculated as L(8,i)/L(i,i).
[b]Recycling fraction, L(9,8)/L(8,8) as described in the text.

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