Plasma Low Density Lipoprotein Transport Kinetics in Noninsulin-dependent Diabetes Mellitus

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ABSTRACT Plasma low density lipoprotein (LDL) transport kinetics were determined from the disappearance of ^{125}I-LDL injected into age- and weight-matched groups of 13 normal subjects, 20 mild diabetics, and 8 moderately severe diabetic patients (fasting plasma glucose <150 and >150 mg/100 ml, respectively). In mild diabetics, LDL apo-lipoprotein-B (apo-B) synthetic rate (SR) was significantly greater than normal. The fractional catabolic rate (FCR), however, was also increased so that plasma LDL concentration remained normal. In moderately severe diabetics, LDL SR was normal but FCR was reduced resulting in increased plasma LDL cholesterol and apo-B concentrations. In normal subjects, moderate obesity was associated with increased LDL secretion. In diabetic subjects, however, changes in LDL turnover were of equal magnitude in obese and nonobese patients. In normolipemic and hyperlipemic mild diabetic subjects with equal degrees of glucose intolerance, both LDL apo-B SR and FCR were greater than normal. The magnitude of these increases, however, was lower in the hyperlipemic individuals. Stepwise regression analysis revealed that both LDL SR and FCR correlated positively and linearly with insulin response to glucose loading, but negatively and curvilinearly with fasting plasma glucose and glucose response. We propose that in noninsulin-dependent diabetes, mild hyperglycemia is accompanied by increased LDL turnover, despite normal plasma LDL levels, whereas moderately severe hyperglycemia is associated with decreased LDL catabolism, resulting in increased plasma LDL levels. These changes cannot be attributed to the presence of obesity or hypertriglyceridemia, and may relate to varying degrees of insulin resistance and decreased insulin secretion affecting plasma very low density lipoprotein (VLDL) secretion, VLDL conversion to LDL, and LDL catabolism. Both increased LDL turnover in mild diabetes and delayed removal of LDL in moderately severe diabetes could increase cholesterol ester availability to peripheral tissues, and may result in an increased risk of atherosclerosis.

INTRODUCTION

In man, plasma low density lipoprotein (LDL)[1] plays a major role in the transport of cholesterol ester (CHE) to peripheral tissues. Abnormalities in the metabolic behavior of this lipoprotein could lead to accelerated delivery of CHE to vascular structures, resulting in an increased risk of atherosclerosis. As recently reviewed (1, 2), ~90% of CHE formed is initially transferred to very low density lipoprotein (VLDL). During catabolism of VLDL, a major fraction of its apolipoprotein-B (apo-B) together with CHE becomes associated with LDL. The LDL particle can acquire additional CHE in the circulation (1) and under certain conditions LDL may be secreted directly into the plasma (3, 4).

No detailed information is currently available concerning the effects of diabetes mellitus upon the in vivo metabolism of plasma LDL in man. There are several reasons, however, to suspect that the abnormal milieu in this disease might adversely affect the met-

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[1] *Abbreviations used in this paper*: apo-B, apolipoprotein-B; CHE, cholesterol ester; FCR, fractional catabolic rate; FPG, fasting plasma glucose; LDL, low density lipoprotein; SR, synthetic rate; TG, triglycerides; TMU, tetramethylurea; VLDL, very low density lipoprotein.

abolic behavior of LDL. First, the increased cholesterol synthesis (5) and cholesterol esterifying capacity (lecithin:cholesterol acyltransferase activity) (6) and the relative enrichment of LDL with triglycerides (7) suggest an abnormality in the formation of VLDL, its conversion to LDL, or both. Indeed, we have recently shown that enhanced VLDL apo-B secretion is a fundamental defect in diabetes and that in some diabetic individuals, conversion of VLDL to LDL is greater than normal (8). Second, the lack of insulin action in diabetes, whether due to insulin resistance, decreased insulin secretion, or both, might impair receptor-mediated uptake and catabolism of LDL, as it does in cultured cells in vitro (9, 10). Third, the nonenzymatic glycosylation of LDL apo-B, if it occurs to a significant extent in vivo, could affect the catabolic rate and the metabolic fate of LDL (11). Finally, diabetes is frequently associated with obesity and hyperlipidemia and these disorders may independently affect plasma LDL transport.

The present study was therefore undertaken to examine the in vivo kinetics of plasma LDL transport in well-defined groups of noninsulin-dependent diabetic subjects, separating the effects of diabetes from those due to associated obesity and hyperlipidemia. The relationships of prevailing plasma insulin levels and severity of hyperglycemia to the disorder in LDL transport were also explored.

METHODS

Subjects. Kinetic studies were completed in 13 normolipemic, nondiabetic healthy volunteers and 28 adult patients with newly diagnosed noninsulin-dependent diabetes (type II diabetes). All subjects were Caucasian and their clinical characteristics are shown in Table I. Diabetic patients were enrolled in their order of appearance at the diabetic clinic and their willingness to participate in the study. Patients with a history of familial hyperlipoproteinemia were excluded. The patient's history included knowledge of any family member with documented hyperlipidemia, xanthomatosis, or premature coronary heart disease. First-degree relatives were also requested to obtain plasma lipid measurements via their own physician and to report their results to us. The patient's history was confirmed in the families of seven patients (subjects 42, 43, 45, 48, 51, 54, and 58) in whom a sufficient number of first-degree relatives were available for lipid determinations in our laboratory. Routine laboratory tests were also performed to confirm normal thy-

TABLE I
Clinical Characteristics and Plasma Lipid Levels

Subject group	No.	Sex (M/F)	Age	Body wt		Plasma concentration	
				kg	% Ideal	TG	CH
			yr			*mg/100 ml*	
Normal subjects							
All subjects	13	8/5	43±1.9	71±3.2	115±4.0	109±7	176±9
Nonobese	8	5/3	45±2.6	64±2.5	106±3.6	99±6	164±9
Obese	5	3/2	40±2.5	82±3.0*	130±3.2*	125±14	195±20
Mild diabetic subjects							
All subjects	20	10/10	45±1.5	76±2.1	119±3.5	250±30‡	200±5
Normolipemic	9	5/4	44±2.6	74±3.3	120±5.3	121±8	185±4
Nonobese	5	3/2	43±2.9	68±2.2	108±4.0	121±12	186±7
Obese	4	2/2	44±5.2	82±4.1*	135±2.9*	120±12	184±2
Hyperlipemic	11	5/6	45±1.8	78±2.7	118±4.9	356±33‡§	211±8‡§
Nonobese	6	3/3	47±2.4	72±2.9	106±3.0	329±16	211±12
Obese	5	2/3	43±2.8	87±1.2*	134±2.8*	388±30	212±9
Moderately severe diabetic subjects							
All subjects	8	4/4	43±2.8	75±3.1	122±6.3	366±30‡§	214±4‡§
Nonobese	4	2/2	44±3.0	68±3.1	107±4.2	370±43	220±5
Obese	4	2/2	42±5.1	82±1.7*	139±1.3*	361±36	207±4

M, males; *F*, females; *TG*, triglycerides; *CH*, cholesterol. *Obese*, 120–150% of ideal body weight.
Values are means±SEM; individual data are shown in Table I of the Appendix.
* Significantly different from nonobese subjects in the same group, $P < 0.001$.
‡ Significantly different from normals, $P < 0.005$.
§ Significantly different from normolipemic mild diabetics, $P < 0.001$.

roid, liver, and kidney function. Patients with morbid obesity, extreme lipemia, gross proteinuria, history of excessive alcohol consumption, or intake of drugs known to affect lipid or carbohydrate metabolism were excluded. The presence of either acute illness or severe diabetes (plasma glucose concentrations at 7 and 11 a.m. and at 4 and 10 p.m. >300 mg/100 ml) requiring immediate management were contraindications for participation in the study.

The diagnosis of diabetes was based upon the criteria of the National Diabetes Data Group (12). Diabetes was considered to be present when plasma glucose concentration in both the 2-h sample and in another sample exceeded 200 mg/100 ml. Diabetic subjects were separated according to their fasting plasma glucose (FPG) concentration into two main groups: mild diabetics (FPG <150 mg/100 ml) and moderately severe diabetics (FPG >150 mg/100 ml). As shown in Table I, the three groups (normal, mild diabetics, and moderately severe diabetics) were comparable in composition with respect to age, sex, body weight, and percentage of ideal body weight. Ideal body weight for the midpoint of the range for medium body frame was determined from the Metropolitan Life Insurance Company tables. Each group included both nonobese and moderately obese subjects (<120% and 120–150% of ideal body weight, respectively). The ratio of nonobese to obese individuals in each group was approximately equal.

Patients in the mild diabetic group included those with normal fasting plasma triglyceride levels of <150 mg/100 ml (within the 90th percentile of the similarly aged normal population) (13), and those with fasting hypertriglyceridemia of >250 mg/100 ml (well above the 95th percentile for the normal population) (13). The normolipemic and hyperlipemic subgroups were of similar age and weight and their fasting and postglucose challenge plasma concentrations were comparable. All of the moderately severe diabetic patients were hyperlipemic but their mean plasma lipid levels were not significantly different from those of the hyperlipemic mild diabetic subjects (Table I). Quantitative analysis of plasma lipoproteins isolated by ultracentrifugation revealed type IV hyperlipoproteinemia, the increase in plasma triglycerides being due to an increase in VLDL, the cholesterol to triglyceride ratio in this fraction being <0.30.

Studies were initiated during the period of tenure of Dr. Kissebah at St. Mary's Hospital Medical School, London, and continued at the Medical College of Wisconsin, Milwaukee, Wisconsin. Approximately one-third of the subjects in each study group was recruited from the former institution. All investigations were performed under the direct supervision of Dr. Kissebah.

Procedures and methods. Subjects were hospitalized in the Metabolic Ward or the Clinical Research Center and fed a weight maintenance diet providing 40% of the calories as carbohydrates, 20% as protein, and 40% as fat with a polyunsaturated to saturated fat ratio of 0.4 and cholesterol intake of ~600 mg/d. This diet was started 1 wk before and maintained throughout the study period. The nature of the investigation was explained to the patient and informed consent obtained. The study was approved by the Human Research Review Committees of St. Mary's Hospital and the Medical College of Wisconsin.

Preparation of ^{125}I-LDL. Approximately 100 ml of venous blood was withdrawn from the patient after an overnight fast using EDTA (1 mg/ml) as an anticoagulant. Plasma was separated by low speed centrifugation at 4°C. The patient's own LDL fraction (d = 1.019–1.063 g/ml) was separated using an ultracentrifugation procedure (14). The lipoprotein fraction was then washed and concentrated by flotation through a density solution of 1.063 g/ml at 105,000 g for 20 h. The concentrated material (12–15 mg of protein) was dialyzed against several changes of 0.9% saline containing 0.10% EDTA and an aliquot was labeled with ^{125}I using the iodine monochloride technique of MacFarlane (15) as modified for lipoproteins (16). Carrier-free Na ^{125}I was obtained from the Amersham Corp., Arlington Heights, IL. The iodination procedure resulted in the introduction of ~0.5 atom of iodine per molecule, assuming a molecular weight for LDL of 2×10^6 and a protein composition of 25% by weight. Unbound ^{125}I was removed by gel chromatography followed by repeated dialysis against six to eight changes of 0.9% saline. Samples of the lipoprotein fraction used for iodination gave single precipitation lines when tested by immunoelectrophoresis (17). In the iodinated preparation, <3% of the total radioactivity was extractable in chloroform:methanol (2:1, vol/vol) and <1% was detected as free iodine, the remaining radioactivity being bound to the protein moiety. The labeling efficiency ranged between 16 and 42%. An aliquot of the ^{125}I-LDL was subjected to gel chromatography on Sephadex G-50 superfine 2 × 50-cm column and a single homogenous peak was eluted at the same site as native unlabeled LDL. Also, when an aliquot of ^{125}I-LDL was mixed with the patient's plasma and the LDL fraction (d 1.019–1.063) was reisolated by ultracentrifugation, >98% of the added radioactivity was recovered in this fraction, suggesting that the physical properties of iodinated LDL were not significantly altered by the iodination procedure. Furthermore, when radioactivity was measured in urine specimens collected after injection of the ^{125}I-LDL into the patient, no indication of early rapid degradation of the labeled material was found. The iodinated material was sterilized by Millipore filtration (0.22 μm) and mixed with sterile human albumin to protect against radiodestruction of LDL. All procedures for preparation of tracers were performed under strict sterile conditions. Aliquots were also tested for pyrogenic, bacterial, and fungal contamination.

Administration of *^{125}I-LDL and collection of samples.* Subjects were given Lugol's iodine (5 drops/d) before starting the study and for 2–3 wk after to block thyroidal uptake of radioactive iodine. The radioactive material (10–15 mg of protein and 60–100 μCi of ^{125}I) was diluted in sterile saline and given intravenously as a pulse injection. Blood samples were obtained 10 min after the injection at 1-h intervals for the first 12 h, every 2 h for a further 8 h, and every 4–8 h for the next 24 h. Fasting blood samples were also obtained daily for a total period of 2–3 wk. Urine specimens were collected at similar time intervals in glass bottles containing sodium iodate and potassium iodide crystals.

Analysis of samples. Plasma samples were centrifuged quantitatively to isolate the LDL fraction (d 1.019–1.063). This fraction was subsequently washed by flotation through 2 vol of a density solution 1.063 g/ml. The respun lipoprotein was mixed with an equal volume of 4.2 M tetramethylurea (TMU) and the mixture kept at room temperature for 2 h to allow selective precipitation of the apo-B (18). The mixture was filtered through a Whatman Gf/C glass fiber disk (Whatman, Inc., Clifton, NJ) placed in a Millipore Pyrex microanalysis fret support. The precipitate was washed with a mixture of equal volumes of physiological saline and TMU followed by distilled water and then by chloroform methanol (1:1, vol:vol). The glass fiber disk containing the delipidated TMU-insoluble precipitate (apo-B) was transferred to a counting vial for radioassay. Samples of the injected tracer were treated simultaneously and the total dose of injected material was checked by weighing the syringe contents. Radioactivity was corrected for physical decay using ^{125}I

standard counted simultaneously. After radioassay, the TMU-insoluble proteins of the lipoprotein fraction were determined using a modification of the method of Lowry et al. (19). Sodium hydroxide (0.2 ml of 2 mol/liter solution) was added to the radioactivity counting vial and the mixture allowed to stand overnight at room temperature. To this mixture 2.0 ml of Na_2CO_3 (40 g/liter) was added, followed by 0.04 ml of an aqueous solution of $CuSO_4$ (10 g/liter) and NaK tartrate (20 g/liter). After 10 min at room temperature, 0.2 ml of H_2SO_4 (1.0 mol/liter) was added, followed by 0.2 ml Folin reagent (0.5 mol/liter). The sample was then centrifuged for 10,000 *g* min and the clear supernatant aspirated for colorimetry. For the preparation of standards, samples of LDL (*d* 1.020–1.050) containing 10–100 μg of protein, assayed by the standard procedure of Lowry et al. (19) using an albumin standard, were mixed with TMU and processed by the procedures described above. Nonpolar lipids were extracted from the reaction mixture in chloroform before colorimetry. The water content of hydrated bovine albumin used for preparation of the working standards was determined by gravimetry. No chromogenicity factor was used since purified apo-B, determined gravimetrically and corrected for the mass of carbohydrate moiety, was found to produce identical colorimetric readings as anhydrous bovine albumin (18). Using ^{125}I-LDL samples from normal and diabetic individuals, recovery of ^{125}I-apo-B during the TMU precipitation procedures was >98% and recovery of the apo-B protein ranged between 97 and 102%. Recoveries were assessed against calculated LDL apo-B content determined according to the standard procedure of Kane et al. (18) (LDL apo-B = total LDL protein − TMU-soluble protein). Lipid-bound radioactivity was also accounted for in determining ^{125}I-apo-B recovery. Correction for incomplete recovery (82–87%) occurring during the LDL washing procedures was based upon measurements of cholesterol in the initial LDL density spin and in the final preparation processed for TMU precipitation.

Calculation and interpretation of results. The curve peeling technique of Matthews (20) was used to derive the exponential functions best describing the radioactivity decay data. In most subjects, two exponentials were observed but in two normal subjects and three diabetic patients, a third exponential was also found. The mean percentage of the total LDL pool removed during this third decay phase, however, was <8% of the total removal rate. Data analysis, therefore, was performed using the two-compartment model of Matthews. From the slopes of the two exponentials, b_1 and b_2, and their intercepts with zero time axis, c_1 and c_2, the fractional catabolic rate (*FCR*) was calculated as follows:

$$FCR\ (d^{-1}) = \frac{1}{\frac{c_1}{b_1} + \frac{c_2}{b_2}}.$$

The synthetic rate (*SR*) of plasma LDL was then calculated as:

$$SR\ (mg/kg/d) = \frac{FCR\ (d^{-1}) \times steady\ state\ LDL\ pool\ (mg)}{body\ wt\ (kg)}.$$

The steady-state LDL pool was determined from mean plasma LDL concentration and the initial distribution space of labeled LDL. The latter was derived from the radioactive dose and plasma radioactivity 10 min after injection of the tracer.

In this calculation, it is assumed that the intravascular LDL pool equilibrates with, and is exchanging with, an extravascular pool and that catabolism of LDL protein is occurring at a site in close proximity to the intravascular compartment. It is also assumed that the metabolism of LDL during the experimental period is proceeding at a steady state and, hence, the absolute synthetic rate is equated to its rate of catabolism. In the present study, steady state was considered to be present when plasma LDL concentrations during the course of the investigation and the ratio of radioactivity in urine to that in plasma (U/P) varied by <10%.

Other methods. Plasma triglycerides and cholesterol were determined in venous blood obtained after an overnight fast. Plasma LDL (*d* 1.019–1.063) obtained quantitatively by ultracentrifugation was also subjected to cholesterol estimation. Analyses were performed using standard Lipid Research Clinic autoanalyzer II semiautomated techniques (21). Oral glucose tolerance tests using 40 g of glucose/m^2 body surface area were performed after an overnight fast. Plasma samples were collected between zero time and 120 min after oral glucose administration and analyzed for glucose (22) and immunoreactive insulin (23). Glucose or insulin area in units (24) represents the sum of one-half the value at 0 time, one-half the value at 120 min, plus values at 30, 60, and 90 min.

Statistical analysis. Analysis of variance was used to test the matching of the subject groups. Comparison between the groups was assessed using Student's *t* test for unpaired data. Correlations of plasma insulin and glucose and their interactive effects on LDL kinetic parameters were evaluated by stepwise multiple regression analyses using the Statistical Package for Social Sciences manual (25). Results are presented as mean±SEM.

RESULTS

Fig. 1 shows representative radioactivity curves from each of the study groups. As shown in Table II, the rate of LDL apo-B synthesis in normal healthy individuals averaged 13.2±1.0 mg/kg per d. The LDL

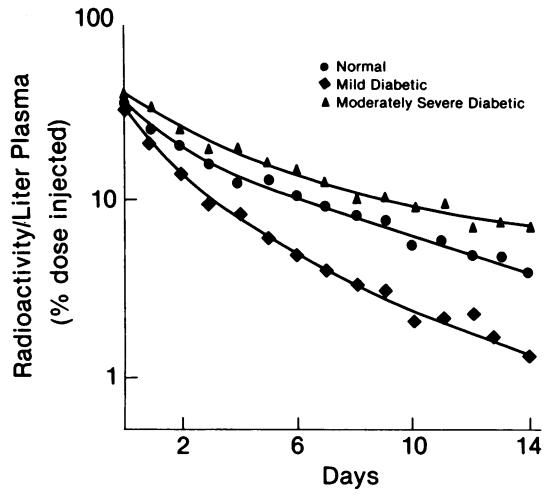

FIGURE 1 Typical ^{125}I-LDL apo-B radioactivity data obtained in a normal subject, a mild diabetic, and a moderately severe diabetic patient.

TABLE II
Plasma LDL Metabolism

Subject group	LDL concentration		LDL apo-B kinetics	
	CH	Apo-B	FCR	SR
	mg/100 ml		d^{-1}	*mg/kg/d*
Normal subjects				
All subjects	109±7	63±5	0.42±0.02	13.2±1.0
Nonobese	98±9	54±4	0.43±0.03	11.5±1.1
Obese	128±8*	78±7*	0.42±0.02	15.9±0.8*
Mild diabetic subjects				
All subjects	109±6	64±2.4	0.67±0.04‡	21.1±1.5‡
Normolipemic	115±5	65±3.7	0.84±0.03‡	27.0±1.5‡
Nonobese	116±8	62±4.6	0.88±0.02	27.4±2.4
Obese	114±2.5	68±6.2	0.80±0.07	26.8±1.9
Hyperlipemic	104±9	63±3.3	0.53±0.04‡§	16.2±0.8‡§
Nonobese	107±16	61±4.9	0.53±0.06	15.7±1.2
Obese	100±8	66±4.8	0.53±0.05	16.8±0.8
Moderately severe diabetic subjects				
All subjects	136±6‡‖	77±1.4‡‖	0.31±0.02‡‖	12.1±0.7‖
Nonobese	144±8	76±2.0	0.29±0.03	10.9±0.9
Obese	129±4	78±2.0	0.33±0.02	13.3±0.8

Obese, 120–150% of ideal body weight.
Values are means±SEM; individual data are shown in Table II of the Appendix.
‡ Significantly different from normals, $P < 0.05$ or less.
§ Significantly different from normolipemic mild diabetics, $P < 0.001$.
‖ Significantly different from hyperlipemic mild diabetics, $P < 0.005$.
* Significantly different from nonobese subjects in the same group, $P < 0.025$.

apo-B FCR was 0.42±0.02 d^{-1}. LDL apo-B SR in the mild diabetic group as a whole (21.1±1.5 mg/kg per d) was significantly higher than normal. The FCR in this group (0.67±0.04 d^{-1}) was also increased; hence, the plasma concentrations of LDL apo-B and cholesterol were not significantly different from normal. Patients with moderately severe diabetes, on the other hand, had significant increases in plasma LDL apo-B and cholesterol levels. This increase was associated with a significant reduction in LDL apo-B FCR (0.31±0.02 d^{-1}) whereas the SR (12.1±0.7 mg/kg per d) was not significantly different from normal.

Relationship to obesity level. In obese normolipemic nondiabetic subjects, LDL apo-B synthesis was significantly higher than that of nonobese individuals (Table II), LDL apo-B SR being positively correlated with the percentage of ideal body weight ($r = 0.55$, $P < 0.05$). LDL apo-B FCR, however, was not significantly different between obese and nonobese individuals. Both plasma LDL apo-B and cholesterol concentrations were higher in the obese subjects but remained within the normal range.

Among normolipemic and hyperlipemic mild diabetic patients, the rate of LDL apo-B synthesis increased in both the nonobese and obese individuals to the same extent (Table II). Furthermore, no significant differences were found between obese and nonobese subjects with respect to their LDL apo-B FCR or LDL apo-B and cholesterol concentrations. Similarly, no significant differences in LDL apo-B turnover measurements were found between obese and nonobese individuals with moderately severe diabetes.

Relationship to hyperlipidemia. Table II shows that in both normolipemic and hyperlipemic mild diabetic patients with similar degrees of glucose intolerance, LDL apo-B SR and FCR were significantly higher than normal. The rate of LDL apo-B synthesis, however, was much higher in normolipemic than in hyperlipemic individuals (27±1.5 vs. 16.2±0.8 mg/kg per d, $P < 0.001$). LDL apo-B FCR in the normoli-

TABLE III
Glucose Insulin Relationship

Subject group	Glucose		Insulin	
	Fasting	Response	Fasting	Response
	mg/100 ml	*U*	*μU/ml*	*U*
Normal subjects	83±2.0	527±7	12±2	221±15
Mild diabetic subjects				
Normolipemic	127±2.9*	847±17*	22±5*	299±22*
Hyperlipemic	130±3.0*	827±16*	24±4*	222±26‡
Moderately severe diabetic subjects	181±14.2*‡§	1,038±31*‡§	21±5	103±4*‡§

Glucose or insulin response represents the integral area in arbitrary units calculated from plasma glucose or insulin levels achieved during an oral glucose tolerance test. Values are means±SEM; individual data are shown in Table III of the Appendix.

* Significantly different from normals, $P < 0.05$.

‡ Significantly different from normolipemic mild diabetics, $P < 0.05$.

§ Significantly different from hyperlipemic mild diabetics, $P < 0.01$.

pemic subjects (0.84±0.03 d^{-1}) was also greater than in the hyperlipemic patients (0.53±0.04 d^{-1}). Hypertriglyceridemic, moderately severe diabetic subjects had much lower LDL apo-B SR (12.1±0.7 mg/kg per d) and FCR (0.31±0.02 d^{-1}) than mild diabetic subjects with comparable plasma lipid levels.

Relationship to plasma insulin and glucose. Table III shows that in mild diabetic patients with or without hyperlipemia, the mean fasting plasma insulin concentration was significantly higher than in normal subjects. Moderately severe diabetic subjects also tended to have increased fasting plasma insulin. This increase, however, did not attain statistical significance, a wide range of fasting plasma insulin (9 to 40 μU/ml) being observed in this group. During oral glucose challenge, normolipemic mild diabetic patients achieved a significantly higher plasma insulin response than normal subjects. Compared to normolipemic mild diabetics, patients with hyperlipemia and a similar degree of glucose intolerance had a lower insulin response that was not significantly different from the normal subjects. The insulin response of moderately severe diabetic patients, on the other hand, was significantly lower than normal.

Fig. 2 shows that among all of the diabetic subjects studied, the rate of LDL apo-B synthesis was linearly correlated with the insulin response to oral glucose administration. With decreasing insulin response, LDL apo-B SR was proportionately reduced ($r = 0.80$, $P < 0.001$). As shown in Fig. 3, decreases in plasma insulin response were also associated with declining LDL FCR ($r = 0.89$, $P < 0.001$). No significant correlations were observed between fasting plasma insulin levels and any of the LDL kinetic indices.

A negative curvilinear relationship was found between fasting plasma glucose concentration and both LDL apo-B SR (Fig. 4) and LDL apo-B FCR (Fig. 5). Similar relationships were observed with the post-glucose challenge plasma glucose response. Within the range of glycemia of the mild diabetics, LDL apo-B SR and FCR were minimally affected by the level of plasma glucose. With progression of glycemia toward

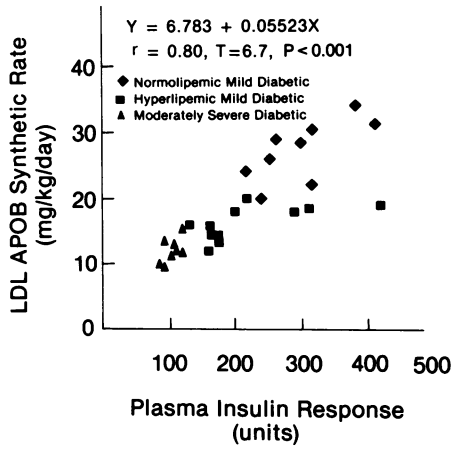

FIGURE 2 Relationship between the plasma insulin response to glucose loading and LDL apo-B synthetic rate.

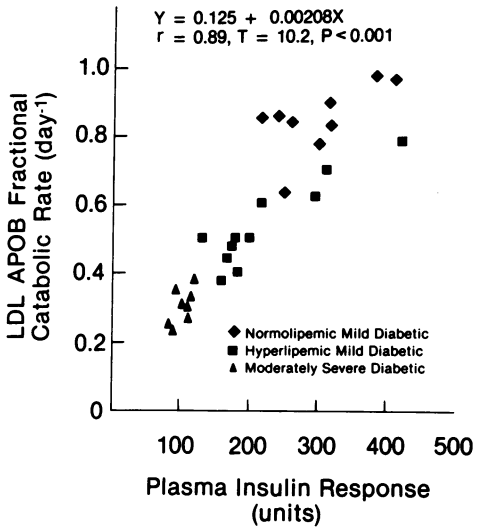

FIGURE 3 Relationship between the plasma insulin response and LDL apo-B FCR.

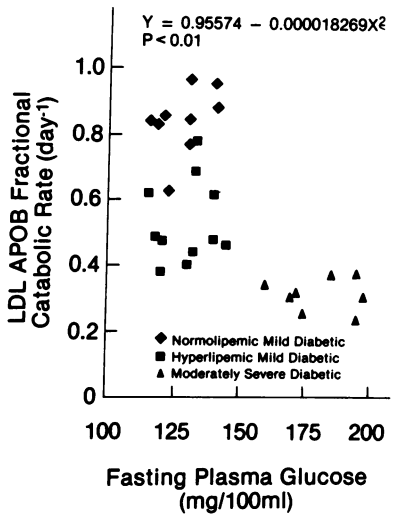

FIGURE 5 Correlation between fasting plasma glucose and LDL apo-B FCR.

the moderately severe diabetic range, however, increases in plasma glucose were associated with reductions in both LDL apo-B synthetic and fractional catabolic rates. Hence, in moderately severe diabetic patients, mean LDL apo-B SR and FCR were significantly lower than those of mild diabetics with a similar degree of elevation in plasma triglyceride levels (Table II). Stepwise multiple regression analysis, however, revealed that in diabetic patients, plasma LDL apo-B SR and FCR were most strongly related to the insulin response ($P < 0.0001$ and < 0.0001, respectively), whereas their association with plasma glucose levels did not contribute a significant, independent effect.

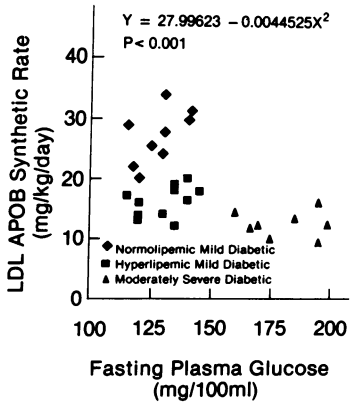

FIGURE 4 Correlation between fasting plasma glucose and LDL apo-B synthetic rate.

DISCUSSION

Elevated plasma levels of cholesterol and LDL cholesterol are among the factors predisposing to an increased incidence of coronary heart disease in diabetes (26, 27). Many diabetic patients, however, have normal plasma LDL cholesterol concentrations, and yet their risk of developing ischemic heart disease remains greater than that of the nondiabetic population (28). The present study reveals that abnormalities in LDL metabolism, which may not be reflected by abnormal plasma LDL concentrations, are present in diabetes. Thus, in noninsulin-dependent diabetic patients with mild fasting hyperglycemia and normal plasma LDL levels, the rate of LDL synthesis was increased but compensated for by enhanced catabolism so that the plasma level remained within the normal range. This kinetic pattern suggests enhanced delivery of LDL CHE to peripheral tissues and is similar to that observed in normolipemic subjects with premature coronary heart disease (29), and in patients with familial combined hyperlipidemia who have elevated plasma VLDL but normal LDL levels (30), and are similarly prone to accelerated atherogenesis (31). With more severe fasting hyperglycemia, LDL synthesis was not increased but catabolic rate was reduced, resulting in a higher plasma LDL concentration. The prolonged residence of LDL in plasma of these patients may also result in an increased risk of atherosclerosis.

Obesity is a frequent companion of noninsulin-dependent diabetes and is itself associated with increased cholesterol transport (32), increased secretion of VLDL

triglyceride (33) and apo-B (34), and enhanced VLDL apo-B conversion to LDL (34). In the present study, moderate obesity in normolipemic nondiabetic subjects was associated with increased LDL apo-B synthesis, this increase being proportionate to the level of obesity. The effects of diabetes upon plasma LDL transport kinetics, however, could not be accounted for by the presence of obesity since the changes in LDL turnover were equally manifest in nonobese and obese individuals. Furthermore, obesity did not exert an additive effect to that of diabetes.

Hyperlipidemia is another common feature of noninsulin-dependent diabetes, ~40% of diabetic patients having increased plasma VLDL levels (35). Endogenous hypertriglyceridemia is associated with enhanced production of cholesterol (36), VLDL triglycerides (37, 38), and apo-B (39). The increased LDL turnover in the mild diabetics, however, cannot be attributed to the presence of hypertriglyceridemia since (*a*) nondiabetic individuals with familial hypertriglyceridemia do not have increased LDL synthesis (4, 30), a major fraction of VLDL being removed from the plasma as remnant VLDL particles (40); (*b*) none of our patients had a history of familial lipoprotein disorder; and (*c*) the increase in LDL turnover in mild diabetics was present in normolipemic as well as hyperlipemic patients. Furthermore, the coexistence of hypertriglyceridemia with mild diabetes resulted in a decrease rather than an increase in LDL turnover; hypertriglyceridemic mild diabetic subjects had significantly lower LDL SR and FCR than normolipemic subjects with an equal degree of glucose intolerance, and hypertriglyceridemic moderately severe diabetics had still lower LDL turnover rates.

In normal subjects, LDL particles are thought to be derived solely from VLDL catabolism (4). In patients with familial hypercholesterolemia (4) and some hypertriglyceridemic individuals (41), however, a fraction of the LDL is secreted directly into the plasma without prior formation of VLDL. As shown in Fig. 6, LDL apo-B synthetic rate in our diabetic patients correlated closely with the rate of LDL formation from VLDL reported previously in the same subjects (8). The intercept of the line representing this correlation with the vertical axis was not significantly different from zero, indicating that in diabetic patients, as in normal subjects, LDL synthesis could be largely accounted for by its formation from VLDL. With currently available techniques, however, estimates of VLDL apo-B secretion and its conversion to LDL are approximations, and hence, formation of LDL from other sources cannot be entirely excluded.

The effects of diabetes on plasma LDL metabolism may result from complex interactions between varying

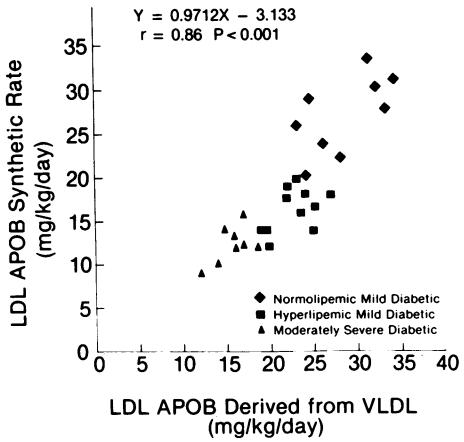

FIGURE 6 Correlation between LDL apo-B synthetic rate and LDL apo-B derived from VLDL. Individual values for LDL apo-B formed from VLDL, determined simultaneously in the same subject, have been reported previously (8).

degrees of insulin resistance, decreased insulin secretion, hyperglycemia, and increased FFA flux, which characterize the abnormal metabolic milieu in this syndrome (42–44). These interactions could affect plasma LDL transport at three major sites: VLDL secretion, LDL formation from VLDL, and LDL catabolism. Based upon the present findings and previous research, our overall interpretation of the effects of diabetes on these events is depicted in Fig. 7.

In noninsulin-dependent diabetic subjects with or without hypertriglyceridemia, VLDL secretion is increased (8, 44–46). This increase may result from insulin resistance in peripheral tissues leading to an enhanced supply to the liver of glucose and FFA, both of which stimulate VLDL production. Insulin action in the liver, however, occurs at a much lower K_m (half-maximal effective insulin concentration (43), and may remain unimpaired even in moderately severe diabetics so that insulin continues to support VLDL synthesis. Thus, despite the progressive decline in insulin response from normolipemic to hyperlipemic mild to hyperlipemic moderately severe diabetes, VLDL secretion rate is increased in all three groups as a result of the accompanying progressive increase in hepatic supply of glucose and FFA. This contrasts both with the situation in nondiabetic subjects, in whom the availability of these substrates is limited and the VLDL secretion rate is proportional to the degree of insulinemia (47, 48), and with more severe forms of diabetes, in which extreme insulin deficiency may result in impaired VLDL secretion (46).

Insulin regulation of the enzyme lipoprotein lipase may also provide a control step for the formation of

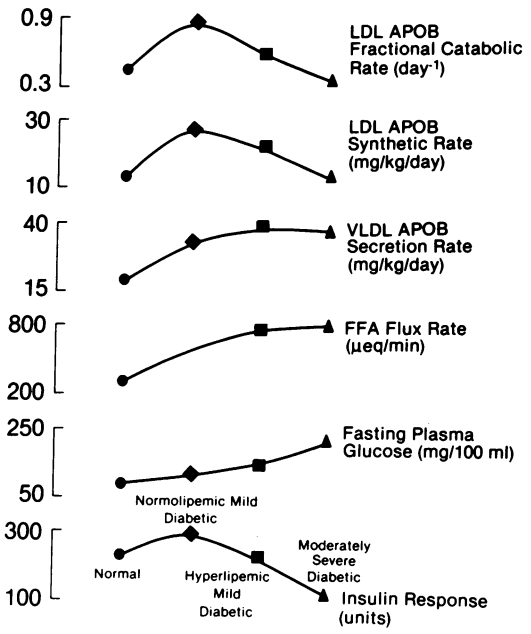

FIGURE 7 Relationship of LDL apo-B transport kinetics to other metabolic indices in normal and noninsulin-dependent diabetic subjects. VLDL apo-B secretion rate, determined simultaneously in the same subject, has been reported previously (8). Individual subjects in the present study are identified by the same subject number as in the previous publication. Values for plasma FFA flux are derived from previously reported data of normal and diabetic subjects similar in age and body weight (39, 44).

LDL from VLDL. Circulating VLDL enters a lipolytic cascade, initiated at the capillary endothelium via the activity of this enzyme, through which the particle is gradually depleted of triglycerides. Whereas complete delipidation of VLDL results in its transformation to LDL, partial delipidation leads to its conversion to remnant VLDL particles. Both adipose tissue and heparin-releasable lipoprotein lipase are diminished in diabetes (49, 50). Insulin stimulation of lipoprotein lipase activity and VLDL delipidation, however, occurs at a much lower insulin level than that required for glucose disposal since (*a*) moderate degrees of insulin resistance and fasting hyperglycemia are associated with normal adipose tissue lipoprotein lipase and postheparin plasma lipolytic activity (51), (*b*) increased VLDL secretion in normolipemic mild diabetic subjects is accompanied by a rise in VLDL removal (8), and (*c*) mild elevations in plasma glucose have a minimal effect upon LDL synthesis (Fig. 4). We therefore suggest that in normolipemic mild diabetic subjects, the plasma insulin response was sufficient to activate the VLDL lipolytic cascade, hence the increase in VLDL secretion was associated with complete delipidation of VLDL to LDL and enhanced LDL synthesis. The lower insulin response in the hyperlipemic mild diabetics, on the other hand, was accompanied by partial impairment of VLDL delipidation resulting in a lower LDL synthetic rate. With the even greater reduction in insulin response in the moderately severe diabetic patients, the VLDL delipidation cascade was severely compromised so that LDL synthesis was not increased despite enhanced VLDL formation, most of the VLDL presumably being removed from the plasma as remnant particles.

The significant correlation between plasma-insulin response and LDL fractional catabolic rate supports the thesis that insulin may regulate LDL catabolic rate in vivo. Addition of physiological concentrations of insulin to fibroblast cultures in vitro enhances receptor-mediated uptake and degradation of LDL apo-B (9, 10), the major pathway for removal of LDL from plasma (52). Induction of hyperinsulinemia by total parenteral nutrition, insulin infusion, or high sucrose feeding is associated with increased in vivo catabolism of plasma LDL (53–55). The level of insulinemia in our normolipemic mild diabetic subjects may thus be sufficient to allow an increase in LDL catabolic rate in response to the increase in LDL synthesis. With the progressive decrease in insulin response, however, there was a progressive decline in the LDL catabolic rate. Indeed, LDL FCR in moderately severe diabetics in whom the insulin response was markedly impaired was lower than normal, resulting in a raised plasma LDL concentration.

Finally, diabetes could affect LDL metabolism via the nonenzymatic glycosylation of LDL apo-B. LDL apo-B can be glycosylated by exposure to glucose in vitro (11, 56) and this process may also occur in vivo (11, 57). Evidence derived from animal studies or cell culture lines suggest that this glycosylation is accompanied by a decrease in LDL FCR (11). The relationship between plasma glucose and LDL FCR shown in the present study, however, implies that, if present, the effect of nonenzymatic glycosylation on LDL catabolism could only be of significance in moderately severe forms of diabetes. This effect might, indeed, be mediated by the marked reduction in circulating insulin that usually accompanies severe degrees of hyperglycemia. Alternatively, nonenzymatic glycosylation could induce the chemical modification of a subpopulation of LDL that is either too small or is removed from the plasma at too slow a rate to be revealed by our procedures. Further studies are required in which the decay rates of radiolabeled glycosylated and nonglycosylated LDL simultaneously injected into the patient are compared.

APPENDIX

TABLE I
Clinical Characteristics and Plasma Lipid Levels

Subject number	Sex	Age	Body wt		Plasma concentration	
			kg	% Ideal	TG	CH
		yr			*mg/100 ml*	
Normal subjects						
1	M	46	55	96	90	162
2	M	45	53	90	109	181
3	M	55	66	110	100	164
4	M	35	68	115	69	127
5	M	35	64	116	108	168
6	F	42	64	98	84	172
7	F	51	76	116	126	190
8	F	48	66	105	104	146
9*	M	37	82	125	128	188
10*	M	43	91	135	150	220
11*	M	33	72	130	152	198
12*	F	48	84	136	104	186
13*	F	38	82	126	86	184
Mild diabetic subjects						
Normolipemic						
31	M	34	76	107	122	179
32	M	46	64	96	150	172
33	M	52	66	118	100	198
34	F	43	64	104	146	206
35	F	42	68	116	88	174
36*	M	38	72	139	129	188
37*	M	51	92	140	150	179
38*	F	55	82	132	106	186
39*	F	33	84	128	96	184
Hyperlipemic						
40	M	38	66	105	425	269
41	M	52	78	116	340	198
42	M	51	64	100	330	218
43	F	47	68	96	305	202
44	F	42	75	106	276	182
45	F	52	82	112	298	198
46*	M	46	87	126	420	238
47*	M	36	89	142	380	216
48*	F	48	86	134	460	220
49*	F	37	84	129	396	196
50*	F	49	82	136	286	189
Moderately severe diabetic subjects						
51	M	39	72	118	486	222
52	M	52	68	98	294	216
53	F	46	64	104	384	209
54	F	40	67	108	316	234
55*	M	56	86	139	278	211
56*	M	38	72	138	494	214
57*	F	32	82	136	362	206
58*	F	44	86	142	310	198

M, male; *F*, female.
* Moderately obese (120–150% ideal body wt).

TABLE II
Plasma LDL Metabolism

Subject number	LDL concentration		LDL apo-B kinetics	
	CH	Apo-B	FCR	SR
	mg/100 ml		d^{-1}	*mg/kg/d*
Normal subjects				
1	106	46.2	0.34	7.8
2	124	54.2	0.48	13.0
3	100	40.0	0.49	9.8
4	76	48.1	0.28	6.1
5	53	74.8	0.40	14.9
6	113	58.5	0.45	13.2
7	125	69.5	0.43	14.8
8	87	42.6	0.56	12.0
9*	114	62.4	0.46	14.3
10*	152	97.8	0.35	17.1
11*	139	82.0	0.38	15.6
12*	123	59.6	0.48	14.3
13*	110	86.1	0.42	18.2
Mild diabetic subjects				
Normolipemic				
31	109	68.8	0.84	28.8
32	97	70.2	0.97	34.1
33	131	56.4	0.85	24.0
34	140	68.4	0.88	30.1
35	101	46.5	0.86	20.0
36*	116	52.8	0.83	21.9
37*	107	64.8	0.96	31.1
38*	118	72.6	0.77	28.0
39*	116	82.4	0.63	26.0
Hyperlipemic				
40	184	82.6	0.38	15.7
41	94	64.8	0.61	19.8
42	104	58.9	0.48	14.1
43	96	54.2	0.44	11.9
44	73	48.6	0.78	18.9
45	89	56.2	0.49	13.7
46*	124	78.6	0.46	18.1
47*	101	68.4	0.48	16.4
48*	111	70.2	0.39	14.0
49*	86	52.0	0.69	17.9
50*	79	56.6	0.62	17.5
Moderately severe diabetic patients				
51	142	76.8	0.31	11.9
52	136	79.2	0.23	9.1
53	131	69.6	0.37	12.9
54	165	78.4	0.25	9.8
55*	135	79.2	0.30	11.9
56*	130	74.0	0.32	11.8
57*	125	82.8	0.38	15.7
58*	124	78.2	0.35	13.7

* Moderately obese (120–150% ideal body wt).

TABLE III
Glucose Insulin Relationship

Subject number	Glucose		Insulin	
	Fasting	Response	Fasting	Response
	mg/100 ml	*U*	*μU/ml*	*U*
Normal subjects				
1	72	484	8	170
2	80	546	10	207
3	88	540	7	173
4	92	523	6	159
5	86	535	10	201
6	76	476	12	171
7	80	554	11	201
8	89	554	8	220
9*	86	496	12	249
10*	82	547	24	290
11*	84	536	17	269
12*	79	539	16	223
13*	87	518	21	341
Mild diabetic subjects				
Normolipemic				
31	115	798	26	260
32	130	825	32	380
33	129	880	8	215
34	140	876	18	314
35	120	902	7	242
36*	117	760	11	316
37*	138	816	38	410
38*	128	869	46	303
39*	123	899	9	247
Hyperlipemic				
40	118	816	16	160
41	138	783	29	221
42	120	808	46	174
43	136	896	13	168
44	134	894	11	420
45	117	762	18	182
46*	144	865	31	198
47*	138	803	48	128
48*	129	777	24	186
49*	134	901	17	311
50*	116	795	8	296
Moderately severe diabetic subjects				
51	198	1,044	40	108
52	195	1,139	11	89
53	186	1,023	8	107
54	174	1,006	42	86
55*	168	995	30	105
56*	172	1,026	12	111
57*	194	1,175	9	122
58*	160	897	14	96

* Moderately obese (120–150% ideal body wt).

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