Association Between Low-Density Lipoprotein Composition and Its Metabolism in Non–Insulin-Dependent Diabetes Mellitus

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Atheroma is related to low-density lipoprotein (LDL) composition. LDL in diabetic patients—a group with increased risk of severe atheroma—has been shown by our group and others to have various compositional alterations that are potentially atherogenic. Little is known about the relationship between LDL turnover and composition. This study examined the relationship between LDL composition and turnover in non–insulin-dependent diabetes mellitus (NIDDM) patients. Twenty-two NIDDM patients with a mean plasma cholesterol of 6.6 ± 1.5 mmol/L were studied. Twelve subjects were hypercholesterolemic (mean cholesterol, 7.7 ± 0.8 mmol/L), and eight of these agreed to be studied a second time after 4 weeks of treatment with simvastatin. LDL was isolated by density gradient ultracentrifugation, iodinated, and reinjected into the patient. LDL turnover was determined by measuring the clearance of [^{125}I]-LDL from plasma over a 10-day period. The LDL residence time, determined using a biexponential model, correlated negatively with the body mass index (BMI) ($r = -.73$, $P < .001$) and serum triglycerides ($r - .57$, $P < .01$). There was a significant inverse correlation between LDL residence time and the LDL esterified to free cholesterol ratio in hypercholesterolemic subjects ($r = -.94$, $P < .001$). There was a significant inverse relationship between LDL residence time and both hemoglobin A_{1c} (HbA_{1c}) and fasting blood glucose in these subjects before treatment ($P < .005$). After simvastatin therapy, the relationships were no longer significant. Simvastatin treatment was associated with a shorter LDL residence time ($P < .01$) and a decrease in LDL glycation ($P < .001$) with virtually no change in diabetic control (HbA_{1c}, 6.0% ± 3.1% *v* 6.3% ± 3.3%, NS). This study suggests that a decrease in residence time by upregulation of the LDL receptor with simvastatin alters LDL composition in a way that is likely to render the particle less atherogenic.

THE INCREASED INCIDENCE of atherosclerosis in diabetes is well known. Many studies have convincingly demonstrated that low-density lipoprotein (LDL) cholesterol is the most important lipoprotein predictor of cardiovascular disease in diabetes.[1-3] The most common lipid abnormality in diabetes is increased serum triglyceride. The relationship between hypertriglyceridemia and abnormalities in LDL particle size and composition has been extensively investigated in diabetes.[4-6] In nondiabetic subjects, Caslake et al[7] have shown that elevations of serum triglyceride were associated with slower clearance by the LDL receptor route but had no effect on LDL clearance by nonspecific uptake. Reducing plasma triglycerides was found to alter LDL composition and increase LDL catabolism.[8] In diabetes, LDL turnover studies show conflicting results. Kissebah et al[9] showed that LDL turnover was retarded in poorly controlled non–insulin-dependent diabetes mellitus (NIDDM) patients, whereas it was accelerated in well-controlled NIDDM patients. Howard et al[10] have shown reduced LDL turnover in obese diabetic Pima Indians compared with body mass index (BMI)-matched nondiabetic subjects. In NIDDM, improvement of diabetic control with insulin increased lipoprotein turnover and decreased plasma triglyceride levels.[11]

Compositional abnormalities that occur in LDL in NIDDM may account for some of the increased risk of atherosclerosis in this population. These compositional abnormalities include increased LDL glycation,[12,13] increased LDL linoleic acid,[14,15] and a higher esterified to free cholesterol ratio. We have shown that LDL glycation, elevated LDL linoleic acid/oleic acid, and an elevated esterified to free cholesterol ratio. We have shown that LDL glycation, elevated LDL linoleic acid/oleic acid, and an elevated esterified to free cholesterol ratio were associated with impaired uptake of the LDL particle by the LDL receptor, failure to downregulate cellular cholesterol synthesis,[16,17] and macrophage cholesterol accumulation.[18] Others have shown increased uptake of glycated LDL by pathways other than the classic LDL receptor pathway.[19] LDL with increased linoleic acid and increased glycation is more easily oxidized in vitro.[14,15] and may therefore be more atherogenic.

Little information exists on the relationship between compositional abnormalities and LDL catabolism in vivo. The reduced uptake of altered LDL by the LDL receptor in vitro suggests that in vivo it may be cleared more slowly. Alternately, a faster turnover may occur due to non–receptor-mediated uptake, particularly in peripheral tissues. The relationship between LDL composition and catabolism may have implications for our understanding of the atherogenicity of diabetic LDL. We therefore investigated the relationship between LDL turnover and composition in NIDDM subjects. The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors decrease LDL cholesterol by blocking cellular cholesterol synthesis. They also inhibit apolipoprotein B production[20] and may upregulate cellular LDL receptor activity.[21] This increases LDL clearance and reduces the time LDL circulates in the plasma, which may influence the composition of LDL. Simvastatin has been shown in the Scandinavian Simvastatin Survival Study[22] (a secondary prevention trial) to reduce the risk of major coronary heart disease events by 55% in diabetic patients. We therefore used this drug as a tool to explore the relationship between the composition and turnover of LDL.

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SUBJECTS AND METHODS

Subjects

Twenty-two randomly selected NIDDM patients (14 men and eight postmenopausal women) agreed to take part in the study. They were divided into two groups, subjects with serum cholesterol less than 6.5 mmol/L (n = 10) and greater than 6.5 mmol/L (n = 12), to define a group of patients suitable for treatment with a cholesterol-lowering drug. Patients with renal, thyroid, or liver disease were excluded, as were patients on medication that might alter lipoprotein metabolism. Patient characteristics are shown in Table 1. The BMI was 21 to 37 kg/m^2, with a mean BMI of 29.9 ± 4.1 kg/m^2, and most patients were well controlled, with a mean hemoglobin A_{1c} (HbA_{1c}) of 5.8% ± 2.4% (normal value, <4.9%). Three of the patients were on dietary therapy alone, 18 were treated with diet and oral hypoglycemic agents, and one was treated with diet and insulin. This patient (A.M.) was included in the analysis and is indicated in the figures. The patients were in a steady state as regards diet, weight, and glycemic control before and during the study period. The patients were compliant with a diet in which fat comprised 40% of total calories: 20% as polyunsaturated fat, 10% as monounsaturated fat, and 10% as saturated fat. All subjects provided informed consent, and the study was approved by the Hospital Ethics Committee.

Study Protocol

Blood was drawn at the beginning of the study for determination of lipoproteins, indices of glycemic control, and LDL isolation. Patients received potassium iodide (200 mg/d) for 3 days prior to injection of [^{125}I]-LDL and for 14 days following LDL turnover. Baseline LDL turnover was determined for all subjects. Ten subjects with a cholesterol level greater than 6.8 mmol/L were treated with simvastatin (40 mg/d). Eight patients (three men and five postmenopausal women) completed 1 month of treatment. One patient withdrew because of intercurrent illness, and the other for social reasons. In the remaining eight patients, LDL turnover and LDL composition were reexamined after the 4-week treatment.

Table 1. Characteristics of Normocholesterolemic and Hypercholesterolemic NIDDM Patients Before and After Statin Treatment

Characteristic	Normocholesterolemic (n = 10)	Hypercholesterolemic (n = 12)	Hypercholesterolemic Pre-Statin (n = 8)	Hypercholesterolemic Post-Statin (n = 8)
Age (yr)	57.0 ± 4.1	67.5 ± 3.0*	67.4 ± 4.0	
BMI (kg/m^2)	30.7 ± 2.7	28.5 ± 1.4	28.5 ± 1.7	28.5 ± 1.6
HbA_{1c} (%)	5.6 ± 1.5	6.0 ± 3.1	6.0 ± 3.1	6.3 ± 3.3
Fasting plasma glucose (mmol/L)	7.1 ± 0.9	7.8 ± 3.3	8.2 ± 2.9	8.0 ± 3.3
Fasting plasma insulin (mIU/L)	18.4 ± 18.0	10.4 ± 7.9	11.4 ± 8.6	19.8 ± 16.9
Insulin/glucose ratio	2.5 ± 1.3	1.4 ± 1.4	1.5 ± 1.1	2.5 ± 2.4
Plasma cholesterol (mmol/L)	5.4 ± 0.3	7.7 ± 0.8†	7.7 ± 0.6	4.8 ± 0.9‡
Plasma LDL cholesterol				
mmol/L	3.3 ± 0.3	4.9 ± 0.3†	5.0 ± 0.3	2.6 ± 0.9‡
mg/mL	1.27 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.0 ± 0.3‡
Plasma triglycerides (mmol/L)	2.1 ± 0.4	2.8 ± 0.4	3.0 ± 1.4	3.35 ± 1.3

*$P < .05$ *v* normocholesterolemic.
†$P < .01$ *v* normocholesterolemic.
‡$P < .01$ *v* pretreatment (paired *t* test).

Methods

Blood for the kinetic study was drawn in the fasting state into EDTA-containing evacuated tubes. Plasma was obtained by centrifugation within 1 hour. LDL was isolated by a modification of the method of Lindgren et al.[23] For each patient, 6.5 mL plasma was brought to density 1.118 g/mL by addition of 1.108 g NaCl. Beckman ultraclear centrifuge tubes (Beckman, High Wycombe, UK) were precoated with polyvinyl alcohol.[24] A discontinuous NaCl/KBr density gradient was formed by slowly running the following solutions down the inside of each centrifuge tube: 0.5 mL plasma at 1.345 g/mL, 1.8 mL at 1.118 g/mL, 0.9 mL at 1.099 g/mL, 0.9 mL at 1.086 g/mL, 1.8 mL at 1.079 g/mL, 1.8 mL at 1.072 g/mL, 1.8 mL at 1.064 g/mL, and 1.8 mL at 1.059 g/mL. The density was checked using a DMA 46 densitometer (Anton Parr, Graz, Austria), and the solutions were autoclaved. The centrifuge tubes were placed in a Beckman SW 41 Ti rotor in a Beckman L7-55 ultracentrifuge and run at 40,000 rpm for 6 hours and 40 minutes. The top 1 mL containing very–low-density lipoprotein (VLDL) and intermediate-density lipoprotein was removed by aspiration. Following a second run at 34,000 rpm for 15 hours, the LDL (Svedberg units, 0 to 12) was aspirated in the top 0.5 mL. Apoprotein electrophoresis confirmed that the LDL preparation contained no apolipoprotein E, apolipoprotein A, or apolipoprotein (a). The salt density solution was removed on a Sephadex PD10 column (Pharmacia, Uppsala, Sweden).

LDL was iodinated with [^{125}I] using a modification[25] of the iodine monochloride method,[26] and free iodine was removed on a Sephadex PD10 column (Pharmacia, Umea, Sweden). Labeling efficiency was approximately 50%. Greater than 99% of the [^{125}I] was trichloroacetic acid–precipitable, and less than 0.02% could be extracted into chloroform. Thirty microcuries of autologous [^{125}I]-LDL was reinjected intravenously. Blood samples were taken 20 minutes after injection and daily thereafter for 10 days. The plasma was separated and stored at −70°C. At the end of each patient study, [^{125}I] activity was measured using a LKB Wallac 1275 Mini-gamma counter (Turku, Finland).

The extinction curves of plasma [^{125}I] counts were analyzed by a curve-peeling technique and resolved into two exponential decay curves. The LDL fractional catabolic rate (FCR) was calculated using the Matthews equation,[27] FCR = 1/[(C1/b1) + (c2/b2)], where the extinction curve is described by the function, $X1 = C_1e^{-b1t} + C2\exp(-b2t)$. The residence time (1/FCR) represents the mean circulating time of a LDL molecule and was used as an expression of LDL turnover. Compositional analysis was performed on LDL isolated by sequential ultracentrifugation (density, 1.021 to 1.063 g/mL).[28] Plasma and LDL total and free cholesterol were determined using enzymatic colorimetric assays (intraassay and interassay variation, 4.6% and 4.8%, respectively; Boehringer, Mannheim, Germany). Plasma triglyceride was determined by a similar method. The LDL protein level was measured by a modification of the Lowry technique,[29] and plasma LDL cholesterol was determined using the Friedewald equation. LDL glycation was estimated by affinity-gel chromatography using an aminophenylborate gel.[30] Intraassay and interassay variations for the method were 7.7% and 8.1%, respectively. Blood HbA_{1c} was determined using an enzyme immunoassay kit (Novo-Nordisk, Cambridge, UK; normal value, <4.9%). Plasma insulin was determined by a microparticle enzyme immunoassay (Abbot Diagnostic Laboratories, IL).

LDL Fatty Acids

Heptadecanoic acid (100 μg) was added as an internal standard to LDL (1 mg/mL protein) and the lipids were extracted by a modification of the method of Folch et al.[31] The organic fraction was dried with

anhydrous sodium sulfate. The samples were dried under nitrogen and transmethylated.[32] Following transmethylation, the fatty acid methyl esters were extracted into hexane, dried under nitrogen, and reconstituted in iso-octane immediately prior to determination by gas-liquid chromatography. The fatty acids were analyzed in a Shimadzu GC-14A gas chromatograph (Kyoto, Japan) equipped with a capillary fused silica Permabond FFAP-DF-0.1 (25 m × 0.25 mm ID) column (Durren, Germany). Fatty acids were expressed as a percentage of total fatty acids. The intraassay and interassay variations were 1.8% and 2.6%, respectively.

Statistical Analysis

Results are expressed as the mean ± SD. Statistical analysis was performed using the Student *t* test or paired Student *t* test and ANOVA for comparisons that were normally distributed. Nonparametric results are shown as the median and range and were logarithmically transformed prior to analysis. Correlation coefficients were determined by linear and multiple regression analysis using Statworks software (Islandier, NY) on an Apple Macintosh computer (Apple Computer, Cupertino, CA). *P* values less than .05 were considered statistically significant.

RESULTS

Subject characteristics are shown in Table 1. The normocholesterolemic patients were younger than the hypercholesterolemic patients ($P < .05$). Diabetic control was similar in normocholesterolemic and hypercholesterolemic patients, and did not change following statin treatment in the eight statin-treated hypercholesterolemic subjects. Fasting plasma insulin was similar in all groups, as was the insulin to glucose ratio. The eight hypercholesterolemic patients had a mean plasma cholesterol of 7.7 ± 0.6 mmol/L, and this decreased significantly to 4.8 ± 0.9 mmol/L following treatment ($P < .01$), a level similar to the value in normocholesterolemic subjects. The decrease in plasma cholesterol following treatment reflected the reduction in plasma LDL cholesterol ($P < .01$). The posttreatment level in hypercholesterolemic patients was not significantly different from the normocholesterolemic value (2.6 ± 0.9 *v* 3.3 ± 0.3 mmol/L).

The mean LDL esterified to free cholesterol ratio was 2.9 ± 0.1 for normocholesterolemic and 3.1 ± 0.4 for hypercholesterolemic NIDDM patients (Table 2). Treatment with the statin decreased the LDL esterified to free cholesterol ratio in six of eight hypercholesterolemic patients treated. Two subjects, the insulin-treated patient (A.M.) and one other, showed an increase in the esterified to free cholesterol ratio. The percent linoleic acid was similar in LDL from normocholesterolemic and hypercholesterolemic patients. Linoleic acid decreased significantly from 36.5% ± 7.0% to 32.0% ± 3.7% of the fatty acid content ($P < .05$) in hypercholesterolemic patients following statin treatment. LDL glycation was slightly but not significantly higher in hypercholesterolemic patients and decreased significantly from 3.2% ± 2.2% to 1.3% ± 1.0% ($P < .01$) after statin treatment, without a change in glycemic control. The mean LDL residence time was significantly longer in hypercholesterolemic patients compared with normocholesterolemic patients (3.4 ± 0.8 *v* 2.7 ± 0.2 days, $P < .05$). For the eight hypercholesterolemic patients treated with statin, the pretreatment residence time was 3.4 ± 0.02 days, and this decreased significantly to 2.8 ± 0.2 days after simvastatin treatment ($P < .01$) (Fig 1). Subject A.M. had a very short residence time, and this increased post-statin.

Table 2. LDL Composition at Baseline and in Hypercholesterolemic Patients Before and After Statin Treatment

Parameter	Normocholesterolemic (n = 10)	Hypercholesterolemic (n = 12)	Hypercholesterolemic Pre-Statin (n = 8)	Hypercholesterolemic Post-Statin (n = 8)
LDL esterified/free cholesterol ratio	2.9 ± 0.1	3.1 ± 0.4	3.1 ± 0.4	3.2 ± 1.1
LDL linoleic acid (% of total fatty acids)	37.6 ± 2.8	36.6 ± 8.1	36.5 ± 7.0	32.0 ± 3.7†
LDL oleic acid (% of total fatty acids)	19.6 ± 4.0	21.8 ± 3.3	20.2 ± 3.4	22.3 ± 2.9
LDL glycation (%)	2.2 ± 1.2	2.8 ± 2.0	3.2 ± 2.2	1.3 ± 1.0*
LDL residence time (days)	2.7 ± 0.2	3.4 ± 0.8‡	3.4 ± 0.2	2.8 ± 0.2*

*$P < .01$ *v* pretreatment (paired *t* test).
†$P < .05$ *v* pretreatment (paired *t* test).
‡$P < .05$ *v* normocholesterolemic.

Significant correlations with LDL glycation are shown in Table 3 for 22 patients at baseline and eight hypercholesterolemic patients pretreatment. Examination of the eight hypercholesterolemic patients pretreatment showed greater *r* values for the correlation with glycemic control, plasma triglyceride, and esterified to free cholesterol ratio versus the 22 patients (Fig 2).

LDL residence time correlated inversely with plasma triglycerides ($r = -.57$, $P < .01$) and the BMI ($r = -.73$, $P < .001$) (Fig 3). There was a positive curvilinear relationship between the BMI and fasting plasma insulin to glucose ratio ($r = .61$, $P < .05$). However, there was no correlation between the BMI and fasting triglycerides or HbA_{1c}. Multiple regression analysis of these 22 subjects showed that the BMI was independently related to the residence time ($P < .001$) when plasma triglycerides were included in the model.

There was an inverse relationship between LDL residence time and both HbA_{1c} ($r = -.89$, $P < .005$) and fasting blood glucose ($r = -.90$, $P < .005$) (Fig 4) in hypercholesterolemic patients pretreatment. These relationships were lost following

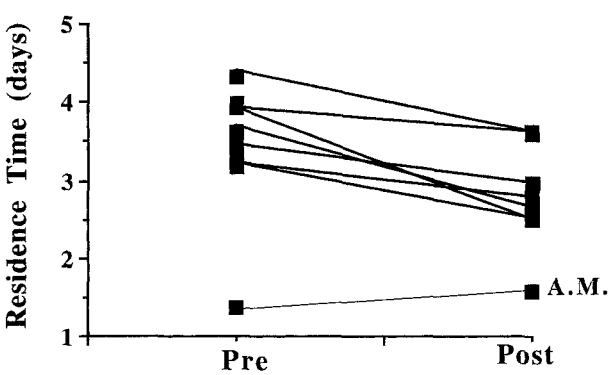

Fig 1. LDL residence time (1/FCR) determined by curve-peeling analysis of plasma [^{125}I]-LDL disappearance curves before (Pre) and after (Post) 4 weeks of simvastatin treatment (40 mg/d). The mean residence time of LDL from 8 hypercholesterolemic NIDDM patients decreased significantly from 3.4 ± 0.2 to 2.8 ± 0.2 days ($P < .01$) following statin treatment.

Table 3. Correlations in 22 Patients at Baseline and in Eight Hypercholesterolemic Patients Pretreatment

Correlation	r	P
22 patients		
LDL glycation correlated with		
HbA_{1c}	.45	.05
Fasting blood glucose	.48	.05
Plasma triglyceride	.62	.005
Esterified/free cholesterol	.52	.02
LDL esterified/free cholesterol correlated with		
Plasma triglyceride	.72	.001
Plasma cholesterol	.64	.001
LDL residence time correlated inversely with		
Plasma triglyceride	−.57	.01
BMI	−.73	.001
8 hypercholesterolemic patients		
LDL residence time correlated inversely with		
HbA_{1c}	−.89	<.005
Fasting blood glucose	−.90	<.005
LDL esterified/free cholesterol	−.94	<.001
Plasma triglyceride	−.90	<.005

upregulation of the LDL receptor by simvastatin. LDL residence time correlated inversely with the LDL esterified to free cholesterol ratio in these patients ($r = -.94$, $P < .001$) (Fig 5). This correlation was lost after treatment. LDL residence time also correlated inversely with serum triglyceride ($r = -.90$, $P < .005$) before but not after treatment.

Multiple regression analysis of the hypercholesterolemic subjects prior to simvastatin treatment showed that the LDL esterified to free cholesterol ratio has an independent association with the residence time ($P < .05$) when plasma triglycerides and either HbA_{1c} or fasting glucose are included in the model.

DISCUSSION

This study examined the relationship between LDL composition in NIDDM and LDL residence time. Our main findings

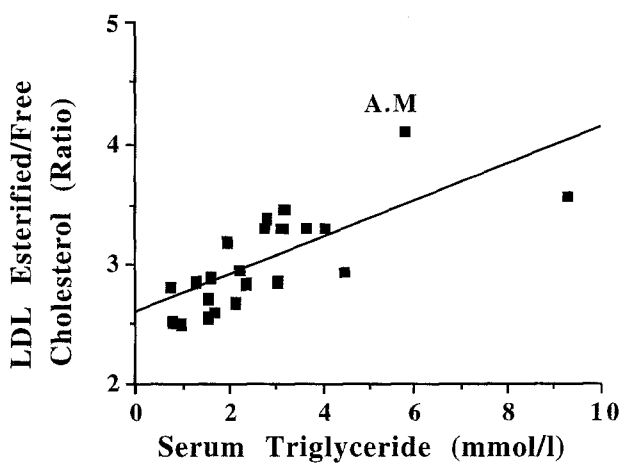

Fig 2. Correlation between the LDL esterified/free cholesterol ratio and fasting serum triglyceride in the total group of patients at baseline (N = 22, $r = .72$, $P < .001$).

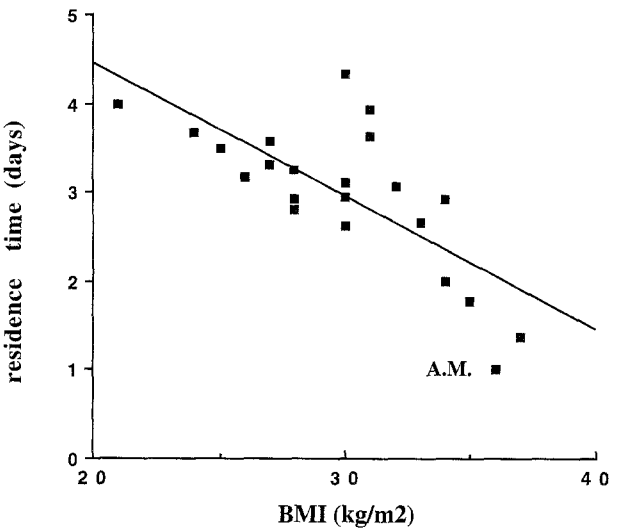

Fig 3. Correlation between LDL residence time (1/FCR) determined by curve-peeling analysis of plasma [^{125}I]-LDL disappearance curves and BMI in the total group of patients at baseline (N = 22, $r = .73$, $P < .001$).

were that LDL residence time was significantly increased in NIDDM patients with hypercholesterolemia (Table 2) and that the reduction in LDL using simvastatin normalized LDL residence time and significantly reduced LDL glycation. This was associated with a significant reduction in LDL linoleic acid.

In a previous study, we have shown that LDL from NIDDM patients has an increased esterified to free cholesterol ratio that is associated with an increased linoleic acid content.[12,14,15] Since linoleic acid has two double bonds, it is easily oxidizable, and in those studies, we demonstrated a correlation between percent linoleic acid and LDL oxidizability in vitro.[14,15,17,33] Oxidized LDL is taken up by the scavenger receptor in an unregulated manner, which leads to cholesterol accumulation and foam cell formation. We have previously shown decreased regulation of cellular cholesterol synthesis by diabetic LDL in vitro, and we have related LDL fatty acid composition to cellular cholesterol synthesis in mononuclear cells,[12,34] suggesting impaired uptake of the altered LDL by the receptor pathway.

LDL clearance was analyzed according to the method of Matthews,[27] which is based on the assumption that LDL behaves kinetically as a single homologous pool. It is possible to show, using the urinary excretion rate of radioactive iodine from LDL that has been catabolized, that LDL usually behaves as at least two pools.[8,35] Matthews' analysis is therefore an approximation that yields less kinetic data than the newer more sophisticated methods that have been developed to study VLDL subfraction metabolism.[36] The fact that LDL behaves kinetically as two or more mathematical pools does not establish that in vivo LDL exists as two distinct forms in terms of composition, or that any distinct LDL phenotype corresponds to a particular kinetic pool. Thus, it is not surprising that Caslake et al[8] found very similar results with regard to LDL turnover with fenofibrate using their multicompartmental model in comparison to a previous study with the drug bezafibrate using the Matthews method. Hence, we and others[37] feel confident using the Matthews method to measure the FCR.

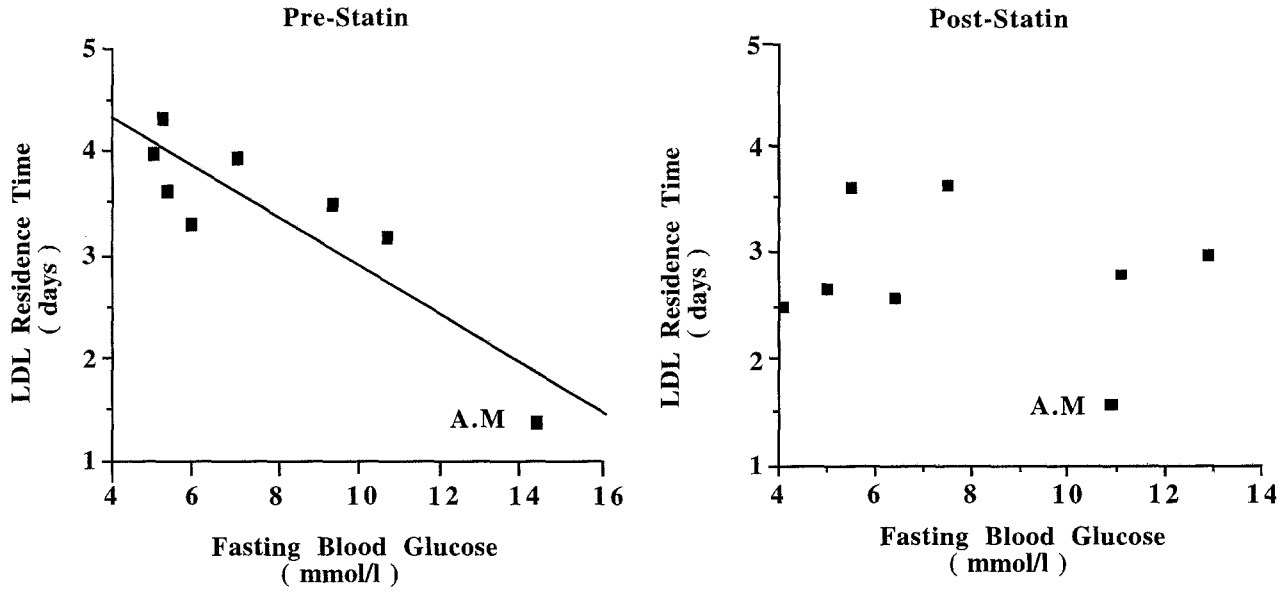

Fig 4. Correlation between LDL residence time (1/FCR) determined by curve-peeling analysis of plasma [^{125}I]-LDL disappearance curves and fasting blood glucose in 8 hypercholesterolemic patients before and after statin treatment. Pretreatment, there was a significant inverse correlation (n = 8, $r = -.88$, $P < .005$), which was lost after treatment.

We have previously shown that simvastatin decreased the esterified to free cholesterol ratio in LDL of NIDDM patients.[34] In the present study, there was an inverse correlation between the esterified to free cholesterol ratio and the residence time in hypercholesterolemic patients (Fig 5). This was an unexpected finding, as we have previously shown that LDL from diabetic and hypercholesterolemic patients, which had a high esterified to free cholesterol ratio, was a poor regulator of cellular cholesterol synthesis, suggesting defective LDL receptor uptake. Simvastatin reduced the linoleic acid content of LDL, suggesting that the higher linoleic acid initially may have been associated with greater oxidation of LDL, and thus the relationship of the LDL esterified to free cholesterol ratio to the residence time may have been mediated through increased oxidation of a more easily oxidized LDL and a shift to the scavenger receptor. Six of the patients on simvastatin had a decrease in the LDL residence time. All of these patients became normocholesterolemic and had a reduced esterified to free cholesterol ratio after simvastatin treatment, whereas the two patients in whom the residence time either did not change

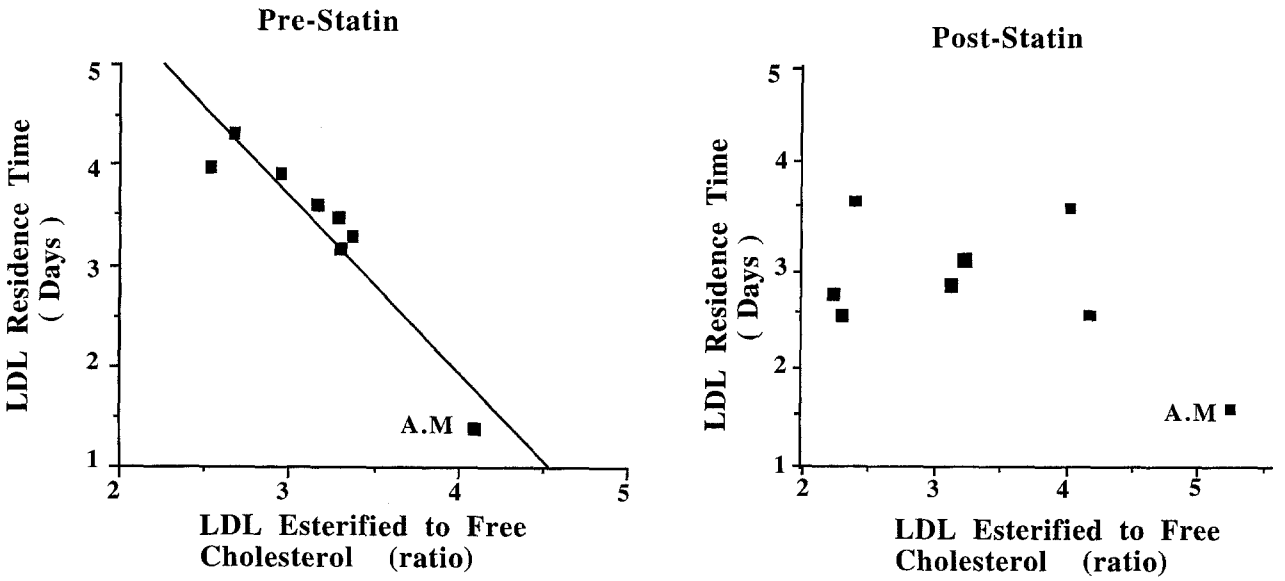

Fig 5. Correlation between LDL residence time (1/FCR) determined by curve-peeling analysis of plasma [^{125}I]-LDL disappearance curves and the LDL esterified to free cholesterol ratio before and after statin therapy. Pretreatment, there was a significant inverse correlation between residence time and LDL esterified to free cholesterol ratio ($r = -.94$, $P < .001$). After treatment, there was no correlation.

or slightly increased (Fig 1) had no change in the esterified to free cholesterol ratio. This suggests that the degree of LDL esterification and LDL residence time may be dependently regulated.

The relationship we found between the BMI and LDL clearance rate has not been previously described. Insulin resistance has been related to hypertriglyceridemia and LDL size.[6] In abdominal obesity, particularly in NIDDM, there is excess portal delivery of free fatty acids to the liver, resulting in overproduction of VLDL, rich in triglyceride.[8,38,39] This subfraction of VLDL is thought to give rise to small dense LDL.[4] Gylling and Miettinen[40] have demonstrated that in NIDDM, the turnover rate is faster for dense LDL versus light LDL, although no data on LDL composition were reported in their study. Taskinen et al[11] demonstrated an increase in LDL catabolism on insulin therapy in seven NIDDM patients. Mazzone et al[41] have shown in an in vivo euglycemic clamp study in nondiabetic subjects that insulin infusion accelerates LDL catabolism. The one patient on insulin treatment in our study (A.M.) had a very short residence time, and this patient had the highest fasting plasma insulin.

Mazzone et al[41] also demonstrated, in human mononuclear leukocytes that insulin stimulated LDL degradation. Their experiment lasted only 4 hours, suggesting an effect on the nonreceptor pathway, since upregulation of LDL receptors would not have occurred in this short time. Thus, hyperinsulinemia appears to play a major role in the correlation between obesity and LDL turnover. There was a significant correlation between diabetic control and LDL residence time (Fig 4). Diabetic control influences the esterified to free cholesterol ratio; however, there was no change in diabetic control in our study. Therefore, our study suggests that LDL cholesterol esterification, not LDL glycation, affects the residence time. Upregulation of the LDL receptor by simvastatin was associated with a decrease in LDL glycation due to the shorter residence time of LDL. Kortlandt et al[42] showed an acceleration in the catabolism of native, and particularly of glycated, LDL after the onset of diabetes in a non–insulin-requiring alloxan-diabetic rabbit model. We have found an association between LDL glycation and LDL receptor-mediated uptake of LDL by human macrophages in vitro.[43] The evidence favors the increased catabolism of LDL in diabetes being due to nonreceptor clearance. Thus, studies on LDL residence time should be considered in relation to LDL composition. In nondiabetic subjects, simvastatin has been shown to reduce LDL oxidizability.[44,45] The decrease in LDL glycation and in LDL linoleic acid content following upregulation of the LDL receptor suggests that HMGCoA reductase inhibitors lead to the production of a less oxidizable LDL in NIDDM.

In conclusion, this study, which was performed in a small number of patients due to the complexity of the investigations, supports the hypothesis that LDL clearance is related to obesity and LDL composition, including glycation. Upregulation of the LDL receptor decreases LDL residence time and thereby decreases LDL glycation without an alteration in diabetic control, and also decreases LDL linoleic acid. These changes are compatible with less oxidizable and hence less atherogenic LDL particles.

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