Effect of Treatment With a Hydroxymethylglutaryl Coenzyme A Reductase Inhibitor on Fasting and Postprandial Plasma Lipoproteins and Cholesteryl Ester Transfer Activity in Patients With NIDDM

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Patients with non-insulin-dependent diabetes mellitus (NIDDM) have a greater risk of developing coronary heart disease than would be expected from a similar degree of hyperlipidemia in nondiabetic populations. Accelerated transfer of cholesteryl esters (CET) from high-density lipoprotein (HDL) to low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), a process that is associated with atherosclerosis, may be a possible explanation for this. CET, plasma lipoprotein concentration, and mass in the fasting and postprandial state have been examined in 31 hyperlipidemic patients with NIDDM before and after 8 weeks of treatment with the hydroxymethylglutaryl (HMG)–coenzyme A (CoA) reductase inhibitor pravastatin in a double-blind, placebo-controlled, parallel group study. Body mass index, glycemic control, and blood pressure remained unaltered during the study period. Compared with placebo, pravastatin decreased fasting serum cholesterol ($P < 0.001$) and LDL cholesterol ($P < 0.002$) levels. The high basal CET (34.4 ± 13.1 nmol · ml^{-1} · h^{-1}) was decreased significantly by pravastatin treatment (27.5 ± 13.7 nmol · ml^{-1} · h^{-1}, $P = 0.013$). There was a fall in the total cholesterol, free cholesterol, and phospholipid content of the S_f 0–12, 20–60, and 60–400 lipoproteins (all $P = 0.001$). Lecithin: cholesterol acyl transferase activity was not altered. The postprandial increase in VLDL cholesterol 5 h after a standardized mixed meal was attenuated after pravastatin treatment ($P = 0.011$). Inhibition of hepatic cholesterol synthesis with an HMG-CoA reductase inhibitor in hyperlipidemic patients with NIDDM decreased serum cholesterol and the free cholesterol content of triglyceride-rich lipoprotein, thereby decreasing the transfer of cholesteryl ester from HDL to LDL and VLDL. *Diabetes* 44:460–465, 1995

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BMI, body mass index; CAD, coronary artery disease; CET, cholesteryl ester transfer; CETP, cholesteryl ester transfer protein; CoA, coenzyme A; HDL, high-density lipoprotein; HMG, hydroxymethylglutaryl; LCAT, lecithin:cholesterol acyl transferase; LDL, low-density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; VLDL, very-low-density lipoprotein.

Patients with diabetes, particularly those with non-insulin-dependent diabetes mellitus (NIDDM), have an increased prevalence of coronary artery disease (CAD) (1). Some of this increased coronary risk can be attributed to the presence of conventional coronary risk factors, which occur with greater frequency in NIDDM, but many diabetic patients have premature CAD despite the absence of clinically obvious coronary risk factors (2). Evidence suggests that the disordered lipoprotein metabolism in NIDDM involves not only an increase in lipoprotein concentrations, but also an alteration in their composition, particularly an increase in the lipid content of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) and a decrease in high-density lipoprotein (HDL) (3). There is evidence in nondiabetic subjects that such alterations are associated with the increased transfer of cholesteryl esters (CET) from HDL to LDL and VLDL (4). CET is part of the process of reverse cholesterol transport, a mechanism by which excess cholesterol from the tissues is transported back to the liver (5), but accelerated plasma CET, which results in enrichment of LDL and VLDL with cholesterol, has been shown to be associated with premature atherosclerosis (6). In transgenic mice, the expression of human or simian cholesteryl ester transfer protein (CETP), a protein that also influences plasma CET, leads to premature atherosclerosis (7).

We and others have recently reported that plasma from patients with NIDDM has accelerated CET (4,8), and this may be related to the free cholesterol content in VLDL and LDL (8). We have further investigated plasma CET in NIDDM by examining the effect of an hydroxymethylglutaryl (HMG)–coenzyme A (CoA) reductase inhibitor, pravastatin, on plasma CET and lipoproteins in the fasting and postprandial state.

RESEARCH DESIGN AND METHODS

Patients with NIDDM receiving treatment with diet and sulfonylureas (glibenclamide or gliclazide) for at least 6 months and aged between 18 and 60 years were recruited from outpatient clinics in the Diabetes Centre and the Lipid Clinic at Manchester Royal Infirmary. The study, which had the approval of the hospital's ethical committee, was restricted to patients with a serum total cholesterol >6.5 mmol/l on two separate occasions and who had adequate glycemic control (HbA_1 <11.5%) within 4 weeks of entering the study. All patients were

TABLE 1
Glycemic control and fasting serum lipid and plasma lipoprotein concentrations in placebo and pravastatin groups before and after treatment

	Placebo			Pravastatin		
	Before treatment	After treatment	*P*	Before treatment	After treatment	*P*
BMI (kg/m^2)	27.8 ± 4.3	28.0 ± 4.2	NS	27.5 ± 3.5	27.6 ± 3.6	NS
Blood glucose (mmol/l)	9.7 ± 2.8	11.0 ± 3.6	NS	10.5 ± 2.9	11.1 ± 3.6	NS
HbA_1 (%)	9.3 ± 2.6	9.2 ± 2.3	NS	9.4 ± 2.2	8.8 ± 1.8	NS
Serum cholesterol (mmol/l)	7.5 ± 0.8	7.6 ± 0.8	NS	7.5 ± 1.0	6.0 ± 1.0	0.001
Serum triglycerides (mmol/l)	2.33 (1.09–6.88)	2.73 (1.37–6.58)	NS	3.29 (1.1–4.93)	2.86 (1.7–8.14)	NS
LDL cholesterol (mmol/l)	4.4 ± 1.0	4.4 ± 0.6	NS	4.2 ± 1.1	2.9 ± 0.5	0.002
HDL cholesterol (mmol/l)	1.27 ± 0.38	1.28 ± 0.41	NS	1.16 ± 0.23	1.18 ± 0.21	NS
HDL_2 cholesterol (mmol/l)	0.70 ± 0.35	0.72 ± 0.33	NS	0.60 ± 0.21	0.59 ± 0.18	NS
HDL_3 cholesterol (mmol/l)	0.58 ± 0.09	0.56 ± 0.12	NS	0.56 ± 0.10	0.58 ± 0.14	NS
VLDL cholesterol (mmol/l)	0.51 (0.27–1.48)	0.63 (0.21–1.84)	NS	0.87 (0.20–1.78)	0.75 (0.38–1.74)	NS
LCAT activity ($nmol \cdot ml^{-1} \cdot h^{-1}$)	68.2 ± 21.3	68.1 ± 25.6	NS	54.5 ± 14.4	53.5 ± 14.1	NS
CET activity ($nmol \cdot ml^{-1} \cdot h^{-1}$)	34.7 ± 18.9	38.1 ± 18.3	NS	34.4 ± 13.1	27.5 ± 13.7	0.013

Data are means ± SD or median (range). *P* values are for differences in fasting values before and after treatment in each group.

interviewed by a dietitian before entry to the study to ensure that they had been on a stable diet for at least 6 months. Other criteria for inclusion were normal serum creatine kinase activity and a stable body mass index (BMI). Patients with proteinuria and levels of serum creatinine >120 μmol/l and serum triglyceride >10 mmol/l were excluded, as were patients taking lipid-lowering medication, those with a history of myocardial infarction, or those who had cardiac surgery within the last 3 months.

All patients entered a single-blind placebo–run-in period of 4 weeks, followed by a double-blind parallel group comparison of pravastatin with placebo for 8 weeks. Blood samples were taken after a 12-h overnight fast for serum lipids, and HDL cholesterol was measured by precipitation at the start of the 4-week placebo–run-in period. Patients then entered the single 8-week treatment period with either placebo or 40 mg pravastatin in a single dose at night, and blood samples were taken at the start and end of the treatment period for serum lipid analyses, isolation of lipoproteins, and determination of CET in the fasting state and 5 h after a standard mixed meal. Serum triglycerides were also measured 3 h after the meal. The test meal, given at 10 A.M., consisted of a McDonald's Quarterpounder With Cheese, french fries, and milk. This provided 51 g of fat, 40 g protein, 84 g carbohydrate, and 908 kcal (9). Serum was stored at −20°C for the measurement of serum lipids in batches. EDTA plasma was used for the separation of lipoproteins and to determine lecithin:cholesterol acyl transferase (LCAT) activity and CET.

Total cholesterol was measured by the CHOD-PAP method (Biostat, Stockport, U.K.). Free cholesterol was measured by a cholesterol oxidase method (BCL, Lewes), and triglycerides were measured by a GPO-PAP method (BCL, Lewes). Phospholipids were measured by an enzymic method that uses phospholipase D-choline oxidase and peroxidase-antiperoxidase (BCL, Lewes). Lipids in the lipoprotein subfractions were measured by adding 2,4,6-tribromo-3-hydroxybenzoic acid to the reagent (v:v 1:11) to enhance the sensitivity of the colorimetric reaction (10). Total protein in the lipoprotein fractions was determined by the bicinichoninic acid method (11) (Pierce & Warriner, Chester, U.K.).

Lipoproteins were isolated by both sequential and density-gradient ultracentrifugation using the Beckman L8–55 M ultracentrifuge (Beckman, Palo Alto, CA) (12) to examine the role of large LDL and small VLDL particles. VLDL were isolated as the supernatant after ultracentrifugation of 5 ml EDTA plasma overlayered with 1 ml 1.006 g/ml saline in a Beckman 50.3 Ti rotor for 24 h at 40,000 rpm. Total HDL and HDL_3 were obtained by ultracentrifugation of EDTA plasma at 40,000 rpm for 48 h in a Beckman 50.3 Ti rotor, in two separate tubes where the density was adjusted to 1.063 and 1.125 g/ml, respectively. The cholesterol content of the infranatant and supernatant from the 1.063 g/ml spin and the infranatant from the 1.125 g/ml spin were determined. HDL_2 cholesterol was determined by subtracting HDL_3 cholesterol from total HDL cholesterol concentration. LDL cholesterol was calculated by subtraction. Similar results were obtained using the Friedwald formula (data not presented).

Discontinuous density-gradient ultracentrifugation was used to isolate lipoproteins in the range S_f 0–12 (LDL), S_f 12–20 (intermediate-density lipoprotein), S_f 20–60 (small VLDL), and S_f 60–400 (large VLDL) (12). The sample was prepared by adding 0.341 g solid NaCl or 0.55 g NaCl for hyperlipidemic specimens to 2 ml plasma, to obtain a final density of 1.118 g/ml. Solutions of density varying from 1.0988 to 1.0588 g/ml were layered on top of the plasma sample. Ultracentrifugation was carried out at 23°C in a Beckman 40.1 Ti rotor on a Beckman model L8 55 M preparative ultracentrifuge. The supernatants were removed after four sequential runs to obtain the S_f 60–400 fraction (39,000*g* for 98 min), S_f 20–60 fraction (18,500*g* for 15 h 41 min), S_f 12–20 fraction (39,000*g* for 2 h 35 min), and S_f 0–12 fraction (30,000*g* for 2 h 10 min). All fractions were frozen soon after isolation and analyzed in batches for total protein and lipids.

LCAT activity was measured by a modification of the Stokke and Norum method (13), and an estimate of CET was obtained by the rate of accumulation of cholesteryl esters on LDL and VLDL determined by an isotopic assay that uses endogenous lipoproteins (14). In brief, plasma was incubated at 37°C with a [^{3}H]cholesterol:albumin emulsion for 3 h, and the appearance of radiolabelled cholesteryl esters on VLDL and LDL was measured by precipitation of these lipoproteins from incubated serum by sodium phosphotungstate/magnesium chloride. The rate of CET was estimated from the radioactivity in the HDL-containing supernatant and the accumulation of radiolabelled cholesteryl esters in the precipitate containing VLDL and LDL. The coefficients of variation for the LCAT and CET assays were 4 and 12%, respectively.

Statistical analysis. Statistical analyses were carried out using Minitab (Minitab, State College, PA). Variables were compared using paired Student's *t* test and one-way analysis of variance. The Mann-Whitney *U* test was used to determine differences in variables that had a nongaussian distribution.

RESULTS

Patients. A total of 35 patients entered the study, but 4 patients were withdrawn from the study; 1 had an acute myocardial infarction during the placebo–run-in phase, 1 had elective coronary artery bypass surgery, and 2 moved from the area during the treatment phase. Of those that completed the study, there were 15 patients (mean age 57.6 [39–69] years) in the placebo group and 16 patients (mean age 56.9 [39–67] years) in the group randomly assigned to treatment with pravastatin. Median duration of diabetes was 84 (6–180) months in the placebo group and 78 (10–204) months in the pravastatin group. The two prerandomization baseline serum

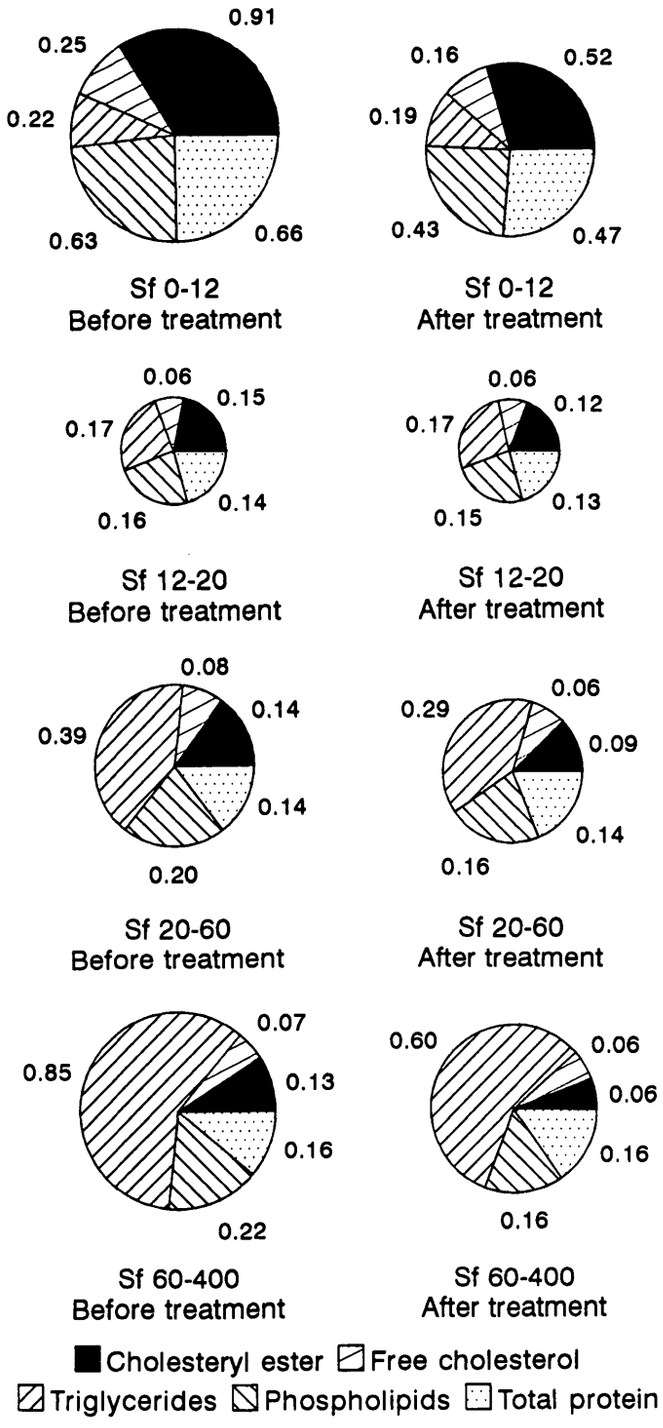

FIG. 1. Composition of S_f 0–12, S_f 12–20, S_f 20–60, and S_f 60–400 lipoproteins before and after treatment with pravastatin. The angular fraction of each segment of the circle indicates the percentage contribution of each component to the total lipoprotein mass. The area occupied by each segment indicates the plasma mass concentration of each component (indicated in g/l by the number adjacent to the segment), and the overall area of the circles indicates the total lipoprotein concentration. See RESULTS for significance of decreases in lipoprotein mass and components with pravastatin treatment.

lipid values (before or after entry to the 4-week placebo–run-in phase) were not significantly different in either treatment group.

Fasting responses to treatment. Throughout the study there were no significant changes in BMI, blood glucose level, or HbA_1 in either the placebo or pravastatin group (Table 1). Serum and LDL cholesterol levels remained unchanged in the placebo group, but they were decreased significantly (P = 0.001) in the pravastatin-treated group. Neither HDL cholesterol nor its subfractions and VLDL cholesterol levels changed in either the placebo or pravastatin-treated patients. CET decreased in the pravastatin group from 34.4 ± 13.1 to 27.5 ± 13.7 nmol · $ml^{-1} \cdot h^{-1}$ (P = 0.013), but CET remained unchanged in the placebo group (reference range for nondiabetic subjects [n = 67]: 17.8 ± 6.5 nmol · $ml^{-1} \cdot h^{-1}$). LCAT activity did not change in either group.

In the pravastatin group, there was a decrease in the total cholesterol (P = 0.014), free cholesterol (P = 0.004), and phospholipid (P = 0.004) concentrations of the S_f 0–12 fraction (Fig. 1). A similar decrease in total and free cholesterol and phospholipids was seen in the S_f 20–60 (P = 0.026, P = 0.022, and P = 0.044) and S_f 60–400 (P = 0.014, P = 0.004, and P = 0.004) fractions. Thus, there was a significant decrease in the mass of S_f 0–12, 20–60, and 60–400 fractions associated with pravastatin therapy. No changes were observed in any of the fractions in the placebo group.

Postprandial responses to treatment

Serum lipids. Postprandial serum triglycerides rose significantly 5 h after the test meal in both placebo (P = 0.013) and pravastatin groups (P = 0.001) (Table 2).

Postprandial changes in plasma CET and LCAT activities. Compared with fasting values, 5-h postprandial CET and LCAT activities were higher in both the placebo and pravastatin groups, both before and after treatment, but the rises were not statistically significant. After treatment, the pravastatin group had the smallest area under the curve (calculated by the trapezium rule) (157.5 ± 79.8) in comparison with either the untreated phase (190.8 ± 65.8) or to patients in the placebo-treated group (before treatment: 210.5 ± 95.8 or after treatment: 204.5 ± 89.5) (Fig. 2).

Plasma lipoproteins. LDL, VLDL, HDL, and its subfractions and S_f 0–12, 12–20, and 20–60 fractions did not change significantly after the meal in the placebo and pravastatin groups, either before or after treatment. However, before treatment, in both the placebo and pravastatin groups, there were significant postprandial increases in the cholesterol, triglyceride, and total protein content in the S_f 60–400 lipoproteins (Table 2). After the treatment phase, these postprandial increases remained in the placebo group, but were abolished in the pravastatin group (Table 2).

Comparison of pre- and post-treatment 5-h postprandial concentrations. In the pravastatin group, 5-h postprandial serum and LDL cholesterol values were significantly lower compared with the 5-h pretreatment values (P = 0.001 for both) (Table 3). Pre- and post-treatment 5-h postprandial serum lipid and plasma lipoprotein concentrations were similar in the placebo group (Table 3).

DISCUSSION

Our results confirm the previous findings of increased CET reported in patients with NIDDM (4). Studies of hyperlipidemic patients, in whom CET is increased (15,16), indicate that the rate of CET is determined mainly by the composition of fasting and postprandial lipoproteins rather than by the mass of CETP (17,18). A previous study found that there was no increase in CETP mass in NIDDM, but that accelerated CET was related to the increased free cholesterol content of

TABLE 2
Postprandial changes in the concentration of components of S_f 60–400 lipoproteins before and after treatment in placebo and pravastatin groups

	Placebo group			Pravastatin group		
	Fasting values	5-h postprandial values	*P*	Fasting values	5-h postprandial values	*P*
Before treatment						
Total cholesterol (mmol/l)	0.26 (0.1–1.07)	0.51 (0.08–0.96)	0.03	0.52 (0.05–1.08)	0.54 (0.13–0.79)	NS
Free cholesterol (mmol/l)	0.14 (0.03–0.57)	0.24 (0.05–0.43)	0.035	0.19 (0.05–0.36)	0.27 (0.07–0.40)	0.033
Triglycerides (mmol/l)	0.57 (0.2–1.88)	0.82 (0.11–2.74)	0.011	0.97 (0.10–2.08)	1.12 (0.24–2.09)	0.05
Phospholipids (mmol/l)	0.20 (0.07–0.60)	—*	—*	0.28 (0.02–0.45)	—*	—*
Total protein (g/l)	0.13 (0.02–0.5)	0.35 (0.06–0.65)	0.004	0.16 (0.09–0.55)	0.34 (0.08–0.05)	0.05
After treatment						
Total cholesterol (mmol/l)	0.28 (0.05–1.21)	0.48 (0.02–1.00)	NS	0.32 (0.8–0.95)	0.39 (0.1–0.9)	NS
Free cholesterol (mmol/l)	0.13 (0.03–0.57)	0.24 (0.04–0.50)	0.012	0.16 (0.05–0.52)	0.21 (0.04–0.43)	NS
Triglycerides (mmol/l)	0.56 (0.11–2.80)	1.04 (0.15–2.32)	0.025	0.68 (0.24–1.74)	0.91 (0.28–1.72)	NS
Phospholipids (mmol/l)	0.19 (0.04–0.73)	—*	—*	0.21 (0.07–0.69)	—*	—*
Total protein (g/l)	0.2 (0.05–0.04)	0.44 (0.05–0.65)	0.035	0.16 (0.07–0.6)	0.29 (0.09–0.7)	0.04

Data are median (range). *P* values are for differences between fasting and 5-h postprandial values. *Not done.

the lipoproteins (4). In vitro experiments also suggest that the increased free cholesterol content of LDL and VLDL leads to an increase in the rate of CET (15), and there is evidence that the increased free cholesterol content of lipoproteins is related to increased coronary heart disease risk (19). A possible explanation for the increased CET in NIDDM may be the increased secretion of VLDL, which can lead to hyperlipidemia (20) and also results in increased free cholesterol content of lipoproteins (18).

Lipoprotein abnormalities exist even in normolipidemic diabetic patients (21,22) and remain uncorrected despite intensive insulin therapy (20,23). We have previously observed in NIDDM patients that the increased free cholesterol content of LDL and accelerated CET were independent of serum lipid concentrations (8). This raises the possibility that diabetes per se may lead to dyslipidemia. The overproduction of VLDL, seen also in patients with familial combined hyperlipidemia (24), which is clinically associated with NIDDM (25), may be a possible feature of diabetic dyslipidemia. However, catabolic defects resulting from partial lipoprotein lipase deficiency may also lead to hyperlipidemia (26). In addition, the degree of dyslipidemia may be related to glycemic control (27), the hypoglycemic agent used (20,28), and the mean serum lipid levels in the population from which the patients studied have been selected (29).

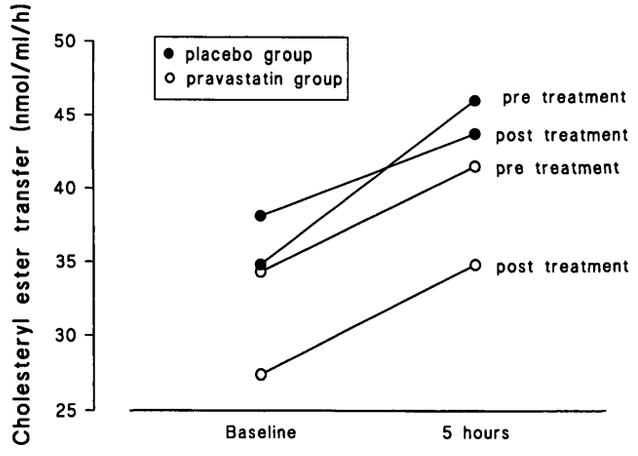

FIG. 2. Mean fasting and 5-h postprandial CET activity in the placebo and pravastatin groups before and after treatment with pravastatin or placebo.

Our findings indicate that suppressing hepatic cholesterol production is effective in decreasing CET activity in NIDDM. The most likely explanation for this decrease is an alteration in lipoprotein composition induced by HMG-CoA reductase inhibition. There were significant changes in the concentrations and composition of LDL and both small and large VLDL after treatment with pravastatin, particularly a reduction in the free cholesterol content of the lipoproteins. It is likely that this effect of pravastatin, in reducing CET, is also shared by other HMG-CoA reductase inhibitors. Yoshino et al. (30) found that pravastatin decreased VLDL and LDL concentrations in diabetic patients. Simvastatin also decreased VLDL cholesterol and VLDL apolipoprotein B in NIDDM (31) and lovastatin decreased LDL and VLDL cholesterol in one study (32), but only LDL in another (33). Thus, the HMG-CoA reductase inhibitors, as a class of drugs, are effective in the treatment of diabetic dyslipidemia. The variation in response, particularly in lipoprotein concentrations, is likely to reflect the heterogeneity of patients in different studies.

In the present study, pravastatin produced a small, statistically insignificant decrease in fasting serum triglycerides. Two previous studies of pravastatin in NIDDM have reported a significant lowering of serum triglycerides in patients taking just 10 mg pravastatin/day (30,34), but variable triglyc-

TABLE 3
Differences in 5-h postprandial serum lipids, plasma lipoprotein concentrations, and LCAT and CET activities before and after treatment in placebo and pravastatin groups

	Placebo group			Pravastatin group		
	Before treatment	After treatment	*P*	Before treatment	After treatment	*P*
Total cholesterol (mmol/l)	7.4 ± 1.0	7.5 ± 0.8	NS	7.5 ± 0.9	6.0 ± 0.8	NS
Serum triglycerides (mmol/l)	3.89 (1.82–11.62)	4.41 (1.62–15.99)	NS	5.43 (1.44–10.15)	3.69 (1.57–9.49)	NS
LDL cholesterol (mmol/l)	4.2 ± 0.9	4.2 ± 0.9	NS	4.1 ± 0.8	2.8 ± 0.5	0.001
VLDL cholesterol (mmol/l)	0.88 (0.29–2.06)	0.91 (0.37–2.38)	NS	0.94 (0.37–1.79)	0.76 (0.35–1.77)	NS
HDL cholesterol (mmol/l)	1.24 ± 0.31	1.21 ± 0.45	NS	1.11 ± 0.26	1.18 ± 0.28	NS
LCAT activity ($nmol \cdot ml^{-1} \cdot h^{-1}$)	70.5 ± 20.2	70.2 ± 19.0	NS	60.9 ± 14.8	66.9 ± 22.3	0.02
CET activity ($nmol \cdot ml^{-1} \cdot h^{-1}$)	45.5 ± 19.4	43.7 ± 17.5	NS	41.9 ± 13.2	34.9 ± 18.2	NS

Data are means ± SD or median (range). *P* values are for differences between 5-h postprandial values before and after treatment in each group.

eride responses to simvastatin and lovastatin have been observed in NIDDM patients (31–33,35). We also observed that pravastatin prevented the postprandial cholesterol enrichment of lipoproteins, most significantly in the S_f 60–400 (VLDL) fraction. CET is increased during the postprandial phase, and prolonged postprandial lipemia, often observed in NIDDM (36), is likely to lead to a more atherogenic profile due to a prolonged increase in CET. Treatment with pravastatin resulted in small decreases in postprandial triglyceride-rich lipoproteins and in CET. The mechanism by which pravastatin prevents postprandial rises in CET is likely to be different from that induced by fibrates (37). Turnover studies suggest that HMG-CoA reductase inhibitors selectively remove the cholesteryl ester–enriched remnants of the triglyceride-rich lipoproteins (38). Thus, a decrease in lipoproteins that accept cholesteryl ester from HDL is the most likely cause for a decrease in CET.

The nature of hyperlipoproteinemia will influence the choice and dose of the lipid-lowering medications, but in initiating therapy in diabetic patients, one needs to be particularly cognizant of the effects of drugs that may help to ameliorate some of the underlying abnormalities which are predominant in diabetic patients (39). Our study shows that pravastatin effectively lowered serum cholesterol levels and caused qualitative and quantitative improvements in lipoprotein composition without affecting glycemic control (40). Moreover, the decrease in atherogenic CET suggests that HMG-CoA reductase inhibitors are a valuable addition to the therapy of diabetic dyslipidemia.

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