Atorvastatin Treatment Beneficially Alters the Lipoprotein Profile and Increases Low-Density Lipoprotein Particle Diameter in Patients With Combined Dyslipidemia and Impaired Fasting Glucose/Type 2 Diabetes

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Diabetic dyslipidemia is featured by hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol levels, and elevated low-density lipoprotein (LDL) cholesterol commonly in the form of small, dense LDL particles. First-line treatment, fibrates versus statins or both, of dyslipidemia in diabetic patients has been the focus of debate. We investigated the potential hypolipidemic effects of atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor with good triglyceride lowering properties, in patients with combined dyslipidemia and evidence of impaired fasting glucose or type 2 diabetes. Twenty patients were recruited for the study, and after a 60-day wash out period, baseline measurements of lipoprotein parameters, LDL particle diameter, and apolipoprotein B (apoB) degradation fragments were obtained. The group was then randomized, in a double-blinded manner, into 2 subgroups. Group A received atorvastatin (80 mg) and group B received placebo daily for 60 days. After the first treatment period, all patients were reanalyzed for the above parameters. The treatment regime then crossed over for the second treatment period in which group A received placebo and group B received atorvastatin (80 mg) daily for 60 days. All parameters were remeasured at the end of the study. Treatment with atorvastatin resulted in a statistically significant reduction in total cholesterol (41%), LDL cholesterol (55%), triglycerides (TG) (32%), and apoB (40%). Mean LDL particle diameter significantly increased from 25.29 ± 0.24 nm (small, dense LDL subclass) to 26.51 ± 0.18 nm (intermediate LDL subclass) after treatment with atorvastatin (n = 20, $P < .005$). At baseline, LDL particles were predominantly found in the small, dense subclass; atorvastatin treatment resulted in a shift in the profile to the larger and more buoyant LDL subclass. Atorvastatin treatment did not produce consistent changes in the appearance of apoB degradation fragments in plasma. Our results suggest that atorvastatin beneficially alters the atherogenic lipid profile in these patients and significantly decreases the density of LDL particles produced resulting in a shift from small, dense LDL to more buoyant and less atherogenic particles.

THE HIGHLY ATHEROGENIC dyslipidemia associated with insulin resistance, impaired fasting glucose, and type 2 diabetes mellitus is characterized by elevated plasma concentrations of triglycerides (TG), low concentrations of high-density lipoprotein (HDL) cholesterol, and the predominance of small, dense low-density lipoprotein (LDL) particles. First-line treatment of diabetic dyslipidemia has been the subject of debate in recent literature.[1,2] Studies have assessed the efficacy of fibric acid derivatives,[3] statins,[4-6] and even compared statins with fibrates.[7-9] Fibrates effectively lower TG levels, increase HDL cholesterol, increase LDL particle diameter, and cause a shift in the distribution of LDL subtypes; however, fibrates do not lower LDL cholesterol levels. Statins effectively lower LDL cholesterol, total cholesterol, apolipoprotein B (apoB), and reduce all LDL subtypes, but they do not induce a shift in the LDL subtype distribution.[10] Hypertriglyceridemia alters lipoproteins and leads to the formation of small, dense LDL particles.[11] The TG lowering effect of fenofibrate has been suggested to contribute to the shift from the small, dense LDL particle subtype to the more buoyant particle subtype.[9]

LDL cholesterol lowering in diabetic patients has been suggested to be a priority in improvement of the dyslipidemia observed in these patients.[12] 3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, such as statins, effectively lower plasma levels of LDL cholesterol, reduce coronary events and overall mortality, and are currently the drug of choice in treating patients with hyperlipidemia.[8,13] Statins have also been shown to exert positive effects in patients with type 2 diabetes. A subgroup of type 2 diabetic patients in the Scandinavian Survival Study and in the Cholesterol and Recurrent Events (CARE) trial exhibited significant reductions in the incidence of major coronary events as a result of drug therapy.[14,15] Studies comparing different statins determined that atorvastatin effectively decreased TG, LDL cholesterol, apoB, and total cholesterol in patients with mixed dyslipidemia with and without type 2 diabetes[4] and in type 2 diabetics with hypercholesterolemia.[5] We, therefore, examined the effect of atorvastatin, a statin with good TG-lowering properties, on LDL particle size and plasma levels of apoB degradation products, in patients with the typical dyslipidemia associated with impaired fasting glucose and type 2 diabetes.

Previous investigation into the intracellular biogenesis and degradation of apoB in human hepatocytes has suggested that a 70- to 85-kd apoB fragment may be generated upon intracellular degradation of apoB in the liver.[16,17] The generation of the 70- to 85-kd apoB fragment may thus be a direct indication of the degree to which full-length apoB (apoB-100) is degraded by the hepatocytes. It may also be an indicator of the formation of a translocation arrested apoB,[17] which is a form of intracellular apoB that is secretion-incompetent and, therefore, des-

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tined for degradation in the cell. We hypothesized that in patients treated with atorvastatin, intrahepatic apoB degradation may be enhanced, resulting in an increased formation of the 70- to 85-kd apoB fragment and thus an elevation in the plasma concentration of this apoB degradation intermediate.

Our primary objective in this study was to determine the effects of atorvastatin on the density profile of the plasma apoB-containing lipoproteins and the plasma levels of small, dense LDL particles in patients with combined dyslipidemia and impaired fasting glucose or type 2 diabetes. Our secondary objective was to determine the effects of atorvastatin on the appearance of the 70- to 85-kd apoB fragment in plasma before and after therapy.

MATERIALS AND METHODS

Study Design

Twenty patients with either impaired fasting glucose (IFG) or type 2 diabetes as per American Diabetes Association (ADA) criteria were enrolled in the study. Criteria defining the condition were as follows: plasma TG greater than 2.5 mmol/L, HDL cholesterol less than 0.9 mmol/L for men and less than 1.1 mmol/L for women, total cholesterol/HDL cholesterol ratio greater than 5, IFG or type 2 diabetes mellitus,[18] and/or hypertension. Patients signed informed consent, and a full medical history and physical examination was obtained from each patient. The protocol of this study was approved by the Windsor Human Research and Ethics Committee. The exclusion criteria were as follows: (1) hypersensitivity to HMG-CoA reductase inhibitors, (2) concomitant use of erythromycin or macrolide antibiotics, cyclosporin, azole antifungals, trazodone, systemic steroids other than oral contraceptives, or hormone replacement therapy, (3) pregnancy or breastfeeding, (4) type 1 diabetes, (5) thyrotropin (TSH) above 5.5 mU/mL, (6) aspartate transaminase (AST) greater than twice the upper limits of normal, (7) creatine kinase (CK) greater than 3 times the upper limits of normal, and (8) alcohol consumption of greater than 14 drinks/week. Patients followed a low-fat and low-cholesterol Canadian Diabetes Association (CDA)-approved diet and were all monitored by a dietician. All lipid-lowering agents were discontinued for 8 weeks for patients who were eligible to participate in the study. After this period, baseline measurements of fasting plasma glucose, glycohemoglobin, CK, AST, total cholesterol, apoB, TG, nonesterified fatty acids (NEFA), HDL cholesterol, LDL cholesterol, insulin, C-peptide, LDL particle size, and apoB fragments were obtained. Baseline measurements were evaluated to determine whether each patient fulfilled the inclusion/exclusion criteria to continue in the treatment phase of the study. Twenty patients matching the criteria were then randomized in a double-blinded manner into 2 treatment groups. Group A received atorvastatin (80 mg) daily and group B received placebo for 60 days. At the end of this period, blood samples were drawn from each patient, and repeat measurements were performed. After this initial treatment period, the treatment regimes then crossed over with group A receiving placebo and group B receiving atorvastatin (80 mg) daily for 60 days. At the end of this second treatment period, all parameters described above were remeasured. At each visit, patients were evaluated for adverse events. All patients completed the study except for 1 patient in Group A who moved out of town after the second treatment period.

Measurement of Biochemical Analytes

Patient blood samples were drawn after a 12-hour fast into tubes containing EDTA. Blood was centrifuged, and plasma/serum was separated immediately and analyzed. The remaining samples were refrigerated or frozen for long-term storage. Glucose, glycohemoglobin, CK, AST, total cholesterol, HDL cholesterol, and TG levels were determined on an automatic analyzer (Hitachi 705, San Jose, CA). LDL cholesterol was calculated from the Friedewald formula. LDL cholesterol was not calculated for patients with TG levels greater than 4.5 mmol/L, as the formula is invalid for TG levels in this range.

ApoB, C-Peptide, Insulin, and Free Fatty Acid Measurements

ApoB, C-peptide, insulin, and free fatty acid levels were measured from serum samples. ApoB was measured by immunonephelometric analysis performed on a Dade Behring (Mississauga, Canada) BNII system using antisera to apoB. C-peptide levels were determined on an Immunlite (DPC; Intermedical Ltd, West Malling, UK) instrument using a chemiluminescent competitive immunoassay. Insulin was determined using a solid phase, 2-site chemiluminescent enzyme-labeled immunometric assay on an Immunlite instrument (no cross-reactivity with proinsulin, C-peptide, or glucagon in this insulin assay). Free fatty acid levels were measured using an enzymatic assay kit (Wako Chemicals, Richmond, VA) on a Cobas Mira analyzer (Roche, Laval, Canada).

Homeostasis Model Assessment of Insulin Resistance

We assessed insulin resistance in these patients before and after atorvastatin treatment using the homeostasis model assessment (HOMA) originally described by Matthews et al.[19] This method calculates insulin resistance/sensitivity based on mathematical modeling of fasting insulin and glucose levels using the following formula: fasting serum insulin (μU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5.

LDL Particle Diameter

Diameter of LDL particles in whole plasma was assessed by nondenaturing polyacrylamide gradient gel electrophoresis (2.5% to 15%) using previously described methods[20,21] with some minor modifications. Ten microliters of plasma was subjected to electrophoresis after adjustment to 25% sucrose with a stock solution of 40% sucrose. Gradient gels were prerun for 15 minutes at 125 V. Samples and protein standards were loaded and gels were then run for 15 minutes at 20 V, 15 minutes at 70 V, and finally at 150 V for an additional 30 hours at 4°C. Two and a half microliters of a suspension of carboxylated latex beads (diluted 10-fold to 0.1 to 0.105 g/cc) was added to the protein standard lane 2 hours after the initiation of electrophoresis to prevent the binding of the marker proteins to the beads. Alternatively, latex beads were loaded in a separate lane. After electrophoresis, gels were stained overnight in 0.04% Oil Red O in 60% ethanol and were destained in 10% acetic acid. Protein markers were selectively stained with 0.05% Coomassie R-250 in 50% methanol, 10% acetic acid by soaking a narrow piece of filter paper and placing it over the appropriate lane. The distribution of LDL isoforms for each patient was determined by measuring the migration distance of each LDL band observed. Diameters were assigned to each LDL band by extrapolation on a standard curve of migration distances measured for proteins of known particle diameter and from carboxylated latex beads (38 nm, Duke Scientific, Palo Alto, CA). The protein standards used included thyroglobulin (17 nm), ferritin (12.2 nm), and catalase (10.2 nm) (High Molecular Weight Calibration Kit, Pharmacia Biotech, Baie d'Urfé, Canada). Intensities of the LDL subclasses were determined by densitometric analysis of each LDL band. Gels were scanned with the Kodak (Rochester, NY) Digital Science 1D Gel Documentation System using a 530 nm filter, which detects the Oil Red O stain. Net band intensity was determined for each LDL isoform observed.

Determination of Plasma ApoB Degradation Fragments

Plasma samples were subjected to chemiluminescent immunoblotting for determination of apoB degradation fragments. Plasma was

diluted 10-fold and was resolved on a 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) mini-gel. Resolved apoB degradation fragments were transferred electrophoretically onto a nitrocellulose membrane (Amersham Pharmacia) overnight using a Bio-Rad (Mississauga, Canada) Wet Transfer System. The primary antibody used to detect the apoB fragments was a goat antihuman apoB polyclonal antibody (Genzyme, Mississauga, Canada). The secondary antibody was an antigoat immunoglobulin G (IgG) peroxidase conjugate (Sigma, St Louis, MO). Membranes were treated with ECL detection reagents and were exposed to Hyperfilm (Amersham Pharmacia). Densitometric scans of the immunoblots were performed to quantify the fragments present. Fragment sizes were estimated from the position of molecular weight markers (Rainbow marker from Amersham Life Science).

Statistical Analysis

Statistical significance was calculated by performing 2-tailed paired Student's *t* test analysis. Pearson's correlation test was used for assessment of correlation between continuous variables. Differences were considered significant if *P* values were $\leq .05$. All *P* values are 2-tailed.

RESULTS

Eight women and 12 men were enrolled in the study. The mean age of the study participants was 59.4 years. The mean body mass index (BMI) at the start of the study was 35.6 and was unchanged at the end of the study. Fifteen patients were hypertensive, dyslipidemic, and diabetic, 4 patients had diabetes and dyslipidemia, and 1 patient had impaired fasting glucose and dyslipidemia. Among the patients with diabetes mellitus, 7 were on diet and oral hypoglycemic agents, 3 were on diet and insulin therapy, and 9 were on diet, insulin therapy, and oral hypoglycemic agents. Baseline measurements of fasting plasma glucose, cholesterol, apoB, TG, NEFA, HDL cholesterol, LDL cholesterol, insulin, and C-peptide levels were obtained after a 60-day wash out period. These parameters were remeasured at the end of the first treatment period and again at the end of the study.

Plasma levels of various lipid and metabolic parameters measured over the course of the study for group A (patients treated with atorvastatin after baseline) are summarized in Table 1. Each number represents the mean value for each metabolite calculated for each part of the study, namely, baseline, atorvastatin treatment, and placebo. Treatment with atorvastatin resulted in a statistically significant 40% reduction in apoB ($n = 10$, $P < .005$), a 38.9% reduction in total cholesterol ($n = 10$, $P < .005$), a 35% reduction in TG ($n = 10$, $P = .01$), and a 50% reduction in LDL cholesterol levels ($n = 9$ for baseline and atorvastatin treatment, $P < .005$; note: LDL cholesterol could not be calculated for patients with TG levels greater than 4.5 mmol/L, thus n values vary for this group). HDL cholesterol levels significantly decreased after placebo treatment ($n = 10$, $P = .007$), suggesting that atorvastatin treatment may have maintained or slightly increased HDL levels. Plasma C-peptide levels decreased, although not significantly, following atorvastatin treatment, suggesting a trend towards a reduction in the insulin production rate; this decrease was maintained after the second treatment or placebo period. Glucose/insulin and the C-peptide/insulin ratios were also calculated. The glucose/insulin ratio decreased after atorvastatin treatment, but did not reach statistical significance. The C-peptide/insulin ratio decreased significantly after atorvastatin treatment ($n = 10$, $P < .05$).

The trends observed over the course of the study for group B (placebo treatment after baseline) are presented in Table 1. The mean values for each parameter displayed describe the measurements obtained at baseline, after placebo treatment, and after atorvastatin treatment. As expected, the parameters measured did not significantly change following placebo treatment. Following treatment with atorvastatin, patients in group B exhibited a statistically significant 40% decrease in apoB ($n = 10$, $P < .005$), a 43% decrease in total cholesterol ($n = 10$, $P < .005$), a 29% decrease in TG ($n = 10$, $P < .005$), and a 61% decrease in LDL cholesterol levels ($n = 8$ for placebo measurement and $n = 9$ for atorvastatin treatment, $P < .005$; note: LDL cholesterol could not be calculated for patients with TG levels greater than 4.5 mmol/L, thus n values vary for this group).

To generate a larger sample size, the 2 groups were amalgamated according to treatment regime. Thus, baseline mea-

Table 1. Mean Values of Metabolites Measured

Metabolite	Group A			Group B		
	Baseline	Atorvastatin	Placebo	Baseline	Placebo	Atorvastatin
Total cholesterol (mmol/L)	6.60 ± 0.51	4.03 ± 0.29*	6.64 ± 0.54	5.97 ± 0.37	6.08 ± 0.27	3.48 ± 0.18*
TG (mmol/L)	3.44 ± 0.68	2.23 ± 0.36†	4.48 ± 1.11	4.95 ± 1.29	4.17 ± 0.71	2.97 ± 0.79*
LDL cholesterol (mmol/L)	4.08 ± 0.43	2.04 ± 0.26*	4.13 ± 0.52	3.74 ± 0.32	3.94 ± 0.21	1.55 ± 0.21*
HDL cholesterol (mmol/L)	0.89 ± 0.04	0.94 ± 0.03	0.83 ± 0.03	0.81 ± 0.07	0.84 ± 0.07	0.82 ± 0.08
Total cholesterol/HDL cholesterol ratio	7.48 ± 0.65	4.29 ± 0.30*	8.17 ± 0.75	7.92 ± 0.88	7.47 ± 0.48	4.58 ± 0.52*
ApoB (g/L)	1.24 ± 0.07	0.74 ± 0.05*	1.36 ± 0.10	1.13 ± 0.08	1.19 ± 0.07	0.72 ± 0.06*
Free fatty acids (mmol/L)	0.68 ± 0.07	0.61 ± 0.08	0.59 ± 0.07	0.67 ± 0.07	0.67 ± 0.08	0.58 ± 0.06
Glucose (mmol/L)	7.99 ± 0.49	7.76 ± 0.66	9.29 ± 0.79	9.31 ± 1.03	8.99 ± 0.79	9.33 ± 1.11
Insulin (pmol/L)	202.7 ± 52.5	232.9 ± 50.2	197.7 ± 35.8	332.5 ± 143.5	336.3 ± 114.6	316.3 ± 99.7
C-peptide (ng/mL)	3.45 ± 0.45	2.69 ± 0.40	2.33 ± 0.29	3.88 ± 0.51	3.75 ± 0.75	3.39 ± 0.69

NOTE. Baseline levels were taken after an 8-week wash out period. Plasma and serum samples were drawn after a 12-hour fast. For group A, samples were drawn at baseline, after 60 days of atorvastatin treatment (80 mg daily), and after 60 days of placebo treatment. For group B, samples were drawn at baseline, after 60 days of placebo treatment, and after 60 days of atorvastatin treatment (80 mg daily). Data are means ± SEM.

*$P < .005$.

†$P < .05$.

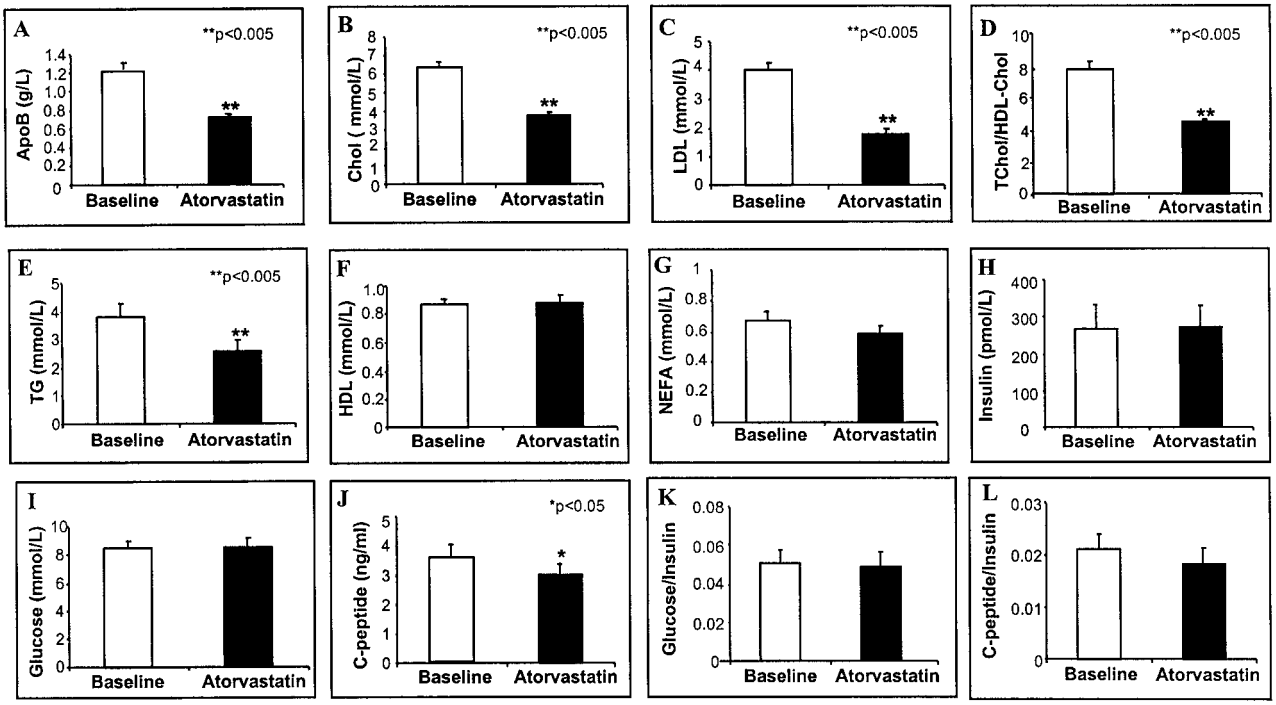

Fig 1. Lipid and metabolic parameters in the amalgamated patient group. Data from group A and group B was amalgamated according to treatment regime to allow for comparison of the effect of atorvastatin on the group of all 20 patients. Baseline levels were derived by combining baseline levels from group A with placebo levels from group B (because these levels directly preceded drug treatment). Atorvastatin-treated levels were derived by combining the levels obtained at the end of the 60-day drug treatment period from both groups A and B. (A-L) Represent plasma/serum levels of apoB, total cholesterol, LDL cholesterol TG, total cholesterol/HDL cholesterol ratio, TG, HDL cholesterol, NEFA, insulin, glucose, C-peptide levels (n = 20, mean ± SEM). As measures of insulin resistance and insulin secretion, we also calculated glucose/insulin and C-peptide/insulin ratios. ApoB, apolipoprotein B; chol, cholesterol; HDL-chol, high-density lipoprotein-cholesterol; LDL-chol, low-density lipoprotein-cholesterol; NEFA, nonesterified fatty acids; SEM, standard error of the mean; TG, triglyceride.

surements for group A were combined with placebo measurements for group B (because these were the measurements that directly preceded the initiation of atorvastatin). It is noteworthy to mention that the limitation of such a practice is that both groups had different drug-free intervals prior to drug therapy, which may potentially result in unanticipated effects. However, in many clinical studies, an 8-week wash out period is sufficient to eliminate any residual drug effects. Amalgamating baseline levels (group A) with placebo levels from group B is based on this assumption, because both groups had at least an 8-week wash out period; thus, it is assumed that levels from both groups would represent baseline lipid levels. Furthermore, statistical analysis showed that baseline levels from group A were not significantly different from placebo levels (group B). Accordingly, levels measured after 60 days of treatment with atorvastatin from group A were combined with drug treatment levels measured from group B. Graphic representation of the grouped results for each metabolite is summarized in Fig 1. Atorvastatin treatment significantly reduced apoB, total cholesterol, TG, and LDL cholesterol by 40%, 41%, 32%, and 55%, respectively (for apoB, TG, and total cholesterol: n = 20, $P <$ .005; for LDL cholesterol: n = 17 for baseline, n = 18 for atorvastatin group; note: LDL cholesterol could not be calculated for patients with TG levels greater than 4.5 mmol/L, thus n values vary for this group). There was a 42% statistically significant reduction in the ratio of total cholesterol/HDL cholesterol in response to atorvastatin treatment (n = 20, $P <$ .005). HDL cholesterol was not significantly affected by atorvastatin treatment; this may be due to the small sample size of our study. Grouped C-peptide levels were significantly reduced after atorvastatin treatment (n = 20, $P <$ .05).

HOMA of Insulin Resistance

At baseline, the HOMA score calculated was 13.25 ± 9.26. Atorvastatin treatment did not significantly change the HOMA score (13.59 ± 9.93). Thus, it appears that statin therapy does not affect insulin resistance in our study (note: insulin values were obtained using an assay that does not cross-react with proinsulin, C-peptide, or glucagon).

Effect of Atorvastatin on LDL Particle Diameter

LDL particle diameter was classified by size into 3 different subgroups using previously described intervals.[21-23] LDL-I represents the large LDL particle group having a diameter greater than 26.8 nm, LDL-II represents the intermediate group having a diameter ≥ 26.0 nm and ≤ 26.8 nm, and LDL-III represents the small, dense LDL subclass, which describes LDL particles less than 26.0 nm in diameter.

Atorvastatin treatment resulted in a statistically significant

increase in mean particle diameter calculated for subjects in group A. Figure 2A depicts the change in mean LDL particle diameter over the course of the study. At baseline, the mean particle diameter was determined to be 24.98 ± 0.26 nm, which falls within the small, dense LDL subclass. This mean diameter increased significantly ($n = 10$, $P < .005$) to 26.64 ± 0.21 nm, which falls within the intermediate LDL size range, in response to atorvastatin treatment. After placebo treatment, the mean LDL particle diameter was reduced to a diameter of 24.85 ± 0.31 nm and thus resumed small, dense LDL classification. Figure 2B represents the change in mean LDL particle diameter for patients in group B. After atorvastatin treatment, the mean particle diameter increased from 25.60 ± 0.42 nm (LDL-III subclass) to 26.40 ± 0.31 nm (LDL-II subclass); although this increase did not reach statistical significance, a shift from LDL-III to LDL-II was observed. However, amalgamating both treatment groups (to generate a larger treatment group as described above) results in an overall statistically significant increase in mean LDL particle diameter from 25.29 ± 0.24 nm to 26.51 ± 0.18 nm ($n = 20$, $P < .005$) (Fig 2C); again, this represents a shift from the small, dense LDL particle size to the intermediate class. Each patient possessed a distinct number of LDL isoforms; on average, each patient had 2 or 3 different isoforms with 1 isoform being predominant. The predominant LDL band was defined as the isoform that was most intensely stained with Oil Red O. Figure 2D represents the mean diameter of the predominant LDL isoform determined from the amalgamated group prior to and after atorvastatin treatment. The mean diameter of the predominant LDL subclass at baseline was 24.21 ± 0.19 nm; this diameter increased significantly after atorvastatin treatment to a diameter of 25.36 ± 0.21 nm ($n = 20$, $P < .005$). Although both diameters fall within the range for small, dense LDL, atorvastatin appeared to decrease the density of the predominant LDL isoform in addition to giving rise to other isoforms of the intermediate and large classification.

The distribution of mean LDL particle diameters calculated for each patient was determined and is displayed in Fig 3. At baseline, all patients in group A (Fig 3A) had mean LDL particle diameters in the small, dense subclass, while patients in group B (Fig 3B) had 6 patients in the LDL-III subclass and 4 patients in the LDL-II subclass. In group A, atorvastatin treatment resulted in the shift of mean LDL particle diameter to the more buoyant subclass, with 4 patients having a mean diameter in LDL-II, 4 patients in the LDL-I subclass, and only 2 patients in the LDL-III subclass. Subsequent treatment with placebo reverted the distribution to baseline observations in which a majority of the patients possessed a mean particle diameter within the small, dense subclass. The initial placebo treatment in group B resulted in a distribution consistent with baseline observations. Atorvastatin treatment resulted in a shift of the mean LDL particle distribution in which 4 of the patients corresponded to the LDL-I subgroup, 3 patients possessed the LDL-II subclass, and 3 patients exhibited the LDL-III subclass.

Effect of Atorvastatin Treatment on the Appearance of ApoB Fragments in Plasma

Plasma levels of apoB fragments were examined by Western blotting to determine the effect of atorvastatin treatment. The 70- to 85-kd fragment was detected in the plasma of most of the 20 patients examined. We observed another fragment, stronger in intensity with a molecular weight of approximately 155 kd. There was considerable variability, however, in the pattern and abundance of apoB degradation intermediates detected in patient plasma. Examples of some representative immunoblots are depicted in Fig 4. The data from 3 of the 20 patients appeared to be consistent with our hypothesis. Two of the 3 patients produced strong fragments after atorvastatin treatment, while there were no fragments detected initially (baseline). The third patient had faint fragments at baseline that increased in intensity after drug treatment (Fig 4A). However, in most of the patients examined, the appearance and/or abundance of apoB fragments was found to be independent of atorvastatin treatment. Six patients had strong bands present at baseline that disappeared after atorvastatin treatment (Fig 4B). Consistent with this pattern, an additional patient had weak fragments present at baseline that disappeared after treatment. Conversely, 3 patients possessing strong fragments at baseline presented with weaker fragments after atorvastatin treatment (Fig 4C). The remaining 6 patients did not exhibit any changes in fragmentation pattern. Four of these patients did not have any fragments at baseline, 1 had strong fragments present, and 2 others had weak fragments present (Fig 4D).

DISCUSSION

Therapeutic treatment of diabetic dyslipidemia, namely by fibrates, statins, or both, has been the focus of debate.[1,2] A study in patients with diabetic dyslipidemia comparing lovastatin with gemfibrozil indicated that lovastatin was significantly more effective in lowering total cholesterol, LDL, LDL:HDL ratio, and directly measured LDL:HDL than gemfibrozil.[7] A study by Paolisso et al[24] in dyslipidemic, non–insulin-dependent diabetic patients, indicated that atorvastatin was more potent than simvastatin in lowering TG levels. Thus, extrapolating these data to our current study, atorvastatin may be expected to be very effective in the primary and secondary preventions of coronary heart disease (CHD) in patients with combined dyslipidemia and impaired fasting glucose or type 2 diabetes.

Atorvastatin decreased plasma cholesterol, LDL cholesterol, apoB, TG, and the total cholesterol to HDL cholesterol ratio by 41%, 55%, 40%, 32%, and 42%, respectively, in our group of patients, consistent with published reports of the efficacy of this drug in hyperlipidemic patients without evidence of diabetes.[25,26] C-peptide levels decreased significantly in response to atorvastatin treatment ($n = 20$, $P < .05$). This might indicate a decrease in insulin production potentially due to an increase in insulin sensitivity. This was unexpected, as statins are not thought to be insulin sensitizers. A recent report indicates that pravastatin therapy reduced the risk of developing diabetes in post hoc analysis of patients in the West of Scotland Coronary Prevention Study (WOSCOPS).[27] A study comparing atorvastatin to simvastatin found that both drugs improved insulin resistance, as determined by the HOMA index and that changes in TG were positively correlated with changes in the HOMA index.[24] We assessed insulin resistance in our patients before and after atorvastatin treatment using HOMA. In contrast to

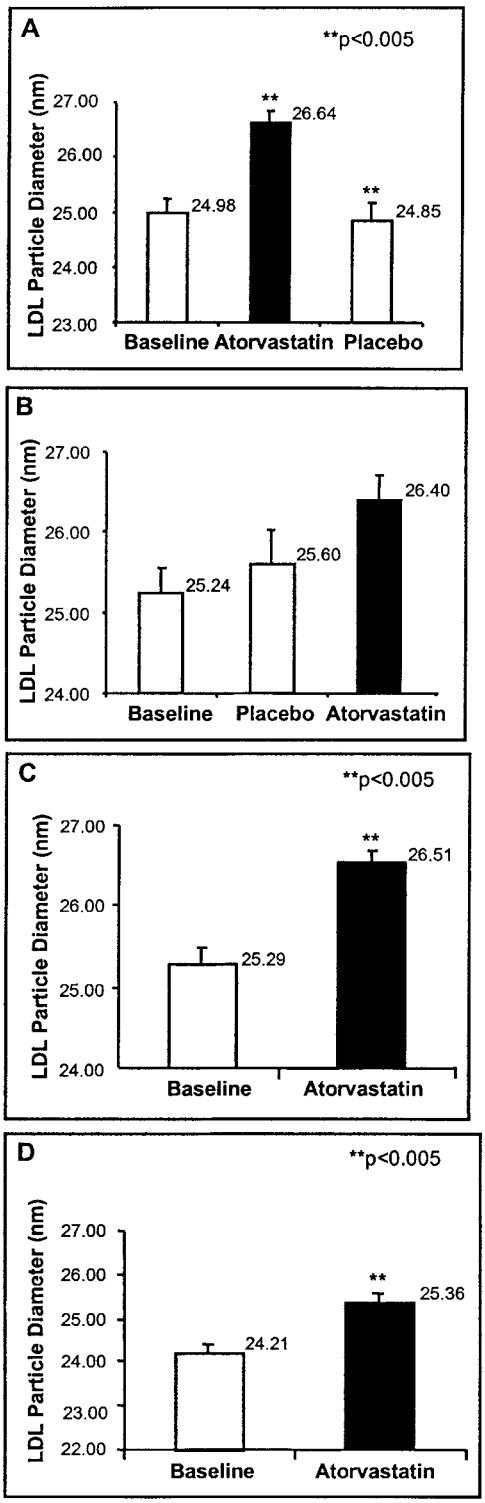

Fig 2. Determination of mean LDL particle diameter. LDL particle species were determined after electrophoresis of whole plasma samples on 2.5% to 15% nondenaturing polyacrylamide gradient gels that were subsequently stained with Oil Red O. LDL particle diameter for each isoform was estimated from a calibration curve of latex beads and proteins of known particle diameter. A diameter was assigned to each isoform detected for each patient. A mean LDL particle diameter was then calculated for each patient. (A) The mean particle diameter was determined for patients in treatment group A at baseline, after 60 days of treatment with atorvastatin, and after placebo treatment. (B) The mean LDL particle diameter was determined for patients in treatment group B at baseline, after 60 days of placebo treatment, and after atorvastatin treatment. (C) Effect of atorvastatin on mean LDL particle diameter in the amalgamated patient group (n = 20, mean ± SEM). (D) Effect of atorvastatin on the predominant LDL isoform detected in the amalgamated patient group (n = 20, mean ± SEM).

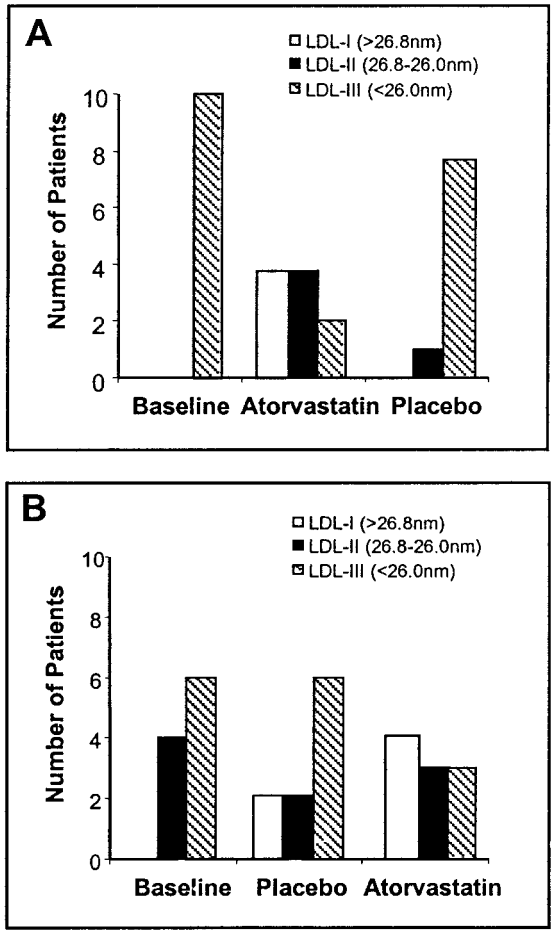

Fig 3. Distribution of within-patient mean particle diameter in different LDL subclasses. LDL particle diameters were classified into 3 subgroups using previously described intervals used to define LDL subclasses. Large LDL (LDL-I) particles had a diameter greater than 26.8 nm, intermediate LDL particles (LDL-II) had a diameter of ≥ 26.0 nm and ≥ 26.8 nm, and small, dense LDL (LDL-III) was less than 26.0 nm. Mean LDL particle diameter was calculated for LDL isoforms detected in each patient, and each patient was then classified into the 3 subgroups. (A) Distribution of mean LDL particle diameters detected in patients from treatment group A at baseline, after atorvastatin treatment, and after placebo treatment. (B) Distribution of mean LDL particle diameters detected in patients from treatment group B at baseline, after placebo, and after treatment with atorvastatin.

Paolisso et al,[24] we did not observe a significant difference in the HOMA score in response to atorvastatin treatment. However, we did confirm that these patients were, in fact, insulin

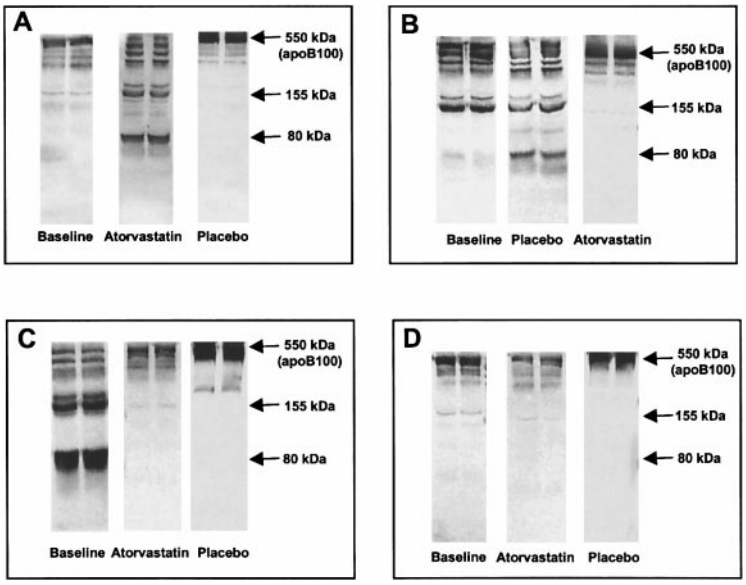

Fig 4. Immunoblot analysis of apoB degradation fragments in plasma of patients. Plasma levels of apoB degradation fragments were visualized by chemiluminescent immunoblotting with antihuman apoB antibody. Shown are blots of 4 representative patients from both treatment groups A (atorvastatin after baseline) and B (placebo after baseline) exhibiting diverse fragmentation patterns in response to statin therapy. (A) Shows a blot of 1 patient (of 3) in treatment group A. (B) Shows a representative blot of 1 patient (of 6) in treatment group B. (C) Shows a blot of 1 patient (of 3) in treatment group A. (D) Shows a blot of 1 patient (of 2) in treatment group A.

resistant, as calculated HOMA scores were within the range of those determined in diabetic patients.[28] Thus, atorvastatin did not appear to impact insulin sensitivity in our group of patients. Further studies with longer treatment periods and more patients may provide more insight into this possibility.

Previous studies have investigated the role of statins and/or fibrates on small, dense LDL in patients with familial combined hyperlipidemia (FCHL).[29-33] A study in FCHL patients determined that atorvastatin treatment significantly reduced all major LDL subspecies including light, intermediate, and dense LDL levels, and that the reduction of absolute lipoprotein mass was preponderant in the dense LDL subspecies.[34] A recent study comparing the efficacy of atorvastatin versus fenofibrate on LDL subfraction distribution in type 2 diabetic patients determined that atorvastatin treatment decreased all LDL subfractions, but did not change LDL subtype distribution.[9] The study also determined that fenofibrate treatment resulted in a significant decrease in TG and induced a shift in the LDL distribution from small, dense LDL to intermediate dense LDL.

With respect to LDL particle size in our study, atorvastatin treatment was accompanied by an increase in the mean LDL particle diameter from 25.29 ± 0.24 nm to 26.51 ± 0.18 nm, which suggests that all circulating LDL subclasses were reduced. Similar results of the effect of atorvastatin treatment on LDL subclasses were reported in FCHL patients[34] and more recently in type 2 diabetic patients.[9] However, our study is the first to associate atorvastatin treatment with the improvement of abnormal LDL particle distribution, as we observed an effective shift in LDL subclass from predominantly LDL-III classification to LDL-I and LDL-II. This may be due to the significant (32%) decrease in TG levels observed in our patients after atorvastatin treatment. The combination of these changes, coupled with a 40% decrease in plasma apoB, the latter representing a reduction in the number of circulating LDL particles, may contribute substantially to a decrease in the risk of cardiovascular complications in patients with combined dyslipidemia and impaired fasting glucose or type 2 diabetes. However, the contribution of increasing LDL particle diameter in reducing coronary incidence or coronary events has not yet been established. Based on results from fibrate trials, the reduction of coronary events with fibrate treatment far exceeds what one would have predicted from a decrease in LDL cholesterol alone; the difference was partially attributed to the effects of lowering serum TG levels.[35,36] We postulate that the decrease in circulating LDL particles may be due to decreased assembly and secretion of apoB-containing particles, such as very–low-density lipoprotein (VLDL) from the liver in addition to increased removal of apoB-containing lipoproteins via LDL receptor-mediated uptake. In vitro studies in the human hepatoma cell line, HepG2, indicated that atorvastatin decreased secretion of apoB-containing lipoproteins by inhibiting cholesterol synthesis and increasing intracellular degradation of apoB.[37]

We did not find any significant correlation between the changes in the mean LDL particle diameter with the changes in plasma TG, LDL cholesterol, apoB, or total cholesterol-to-HDL cholesterol ratio. The absence of correlation between the decrease in plasma TG and the increase in mean LDL particle diameter may be due to the small sample size. Conversely, it is conceivable that atorvastatin may also exert effects beyond TG lowering that have direct effects on LDL particle size.

Evaluation of the plasma levels of the 70- to 85-kd fragment did not correlate with the effects of atorvastatin treatment in our patient group. Previous studies have detected a 70-kd fragment that originates from the N-terminal domain of apoB.[16,38] The 70- to 85-kd fragment appears to accumulate in the plasma of patients with homozygous abetalipoproteinemia, a disease caused by genetic mutations in the gene for microsomal TG transfer protein.[39] In HepG2 cells, atorvastatin treatment results in decreased translocation of apoB across the endoplasmic reticulum and results in increased intracellular degradation of apoB.[37] Thus, we initially hypothesized that atorvastatin would induce intrahepatic deg-

radation and result in the presence of specific apoB degradation fragments in plasma. However, it was rather unexpected that the fragment was present in a significant proportion of patients at baseline, prior to the initiation of atorvastatin therapy. Thus, the appearance and/or abundance of specific apoB fragments cannot be used reliably as a marker for the extent of intrahepatic degradation of apoB in patients undergoing hypolipidemic therapy.

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