Effect of Improving Glycemic Control on Low-Density Lipoprotein Particle Size in Type 2 Diabetes

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The current study sought to assess the effect of improving glycemic control in type 2 diabetes on the components of diabetic dyslipidemia, especially low-denisty lipoprotein (LDL) size. A total of 33 type 2 diabetic patients (48.5% women, age 59.6 ± 11.1 years, body mass index [BMI] 28.9 ± 4.9, diabetes duration 6 [0 to 40] years, 40.7% on insulin) were seen at the hospital because of poor glycemic control (hemoglobin A_{1c} [HbA_{1c}] 10.33% ± 1.89%). Triglyceride, LDL-cholesterol (LDLc, Friedewald/ultracentrifugation), high-density lipoprotein HDL-cholesterol (HDLc, direct method), apolipoproteins AI (apoAI) and B (apoB) (immunoturbidimetry), and LDL size (gradient gel electrophoresis) were measured at baseline and after improvement in glycemic control (decrease ≥ 1 percentage point in HbA_{1c} and final $HbA_{1c} \leq 8\%$). Improvement in glycemic control (HbA_{1c} 7.01% ± 0.63%, $P < .0005$ *v* baseline) after a follow-up of 3.5 (range, 1 to 13) months resulted in a significant reduction in LDLc (3.34 ± 1.02 *v* 3.62 ± 1.15 mmol/L, $P < .05$) and apoB (1.07 ± 0.25 *v* 1.17 ± 0.29 g/L, $P < .01$) and an increase in HDLc (1.21 ± 0.32 *v* 1.13 ± 0.34 mmol/L, $P < .05$) and apoAI (1.36 ± 0.24 *v* 1.27 ± 0.24 mmol/L, $P < 0.01$) in the whole group, and an increase in LDL particle size (25.61 ± 0.53 *v* 25.10 ± 0.31 nm, $P < .005$) in the 14 patients showing LDL phenotype B at baseline. No significant changes were seen in body weight or BMI. We conclude that improvement of glycemic control in type 2 diabetes improves most of the components of diabetic dyslipidemia, including a shift towards larger LDL particles in subjects with phenotype B.

DIABETIC DYSLIPIDEMIA, seen in type 2 diabetes and the metabolic syndrome, comprises moderate hypertriglyceridemia, low high-density lipoprotein cholesterol (HDLc), normal or slightly increased low-density lipoprotein cholesterol (LDLc), increased number of atherogenic particles, reflected by high apolipoprotein B (apoB) concentrations, and a predominance of small dense LDL particles (phenotype B).[1,2] LDL phenotype B increases the risk of coronary events[3] and is displayed by as many as 50% of patients with type 2 diabetes.[4] In addition, the increase in LDL size is associated with a slower progression of coronary atherosclerosis.[5] Optimization of glycemic control improves most components of diabetic dyslipidemia,[6,7] although very few studies have assessed its effect on LDL particle size. In addition, in these studies, glycemic optimization was achieved after rapid weight loss in obese patients[8] or with insulin therapy in lean patients,[4] and differences have been described between sulfonylureas and insulin.[9] The aim of this study was to assess the effect of improving glycemic control with different therapeutic interventions, in a stepwise manner, on the components of diabetic dyslipidemia, especially LDL particle size.

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MATERIALS AND METHODS

Study Design

A group of poorly controlled type 2 diabetic subjects was followed and assessed before and after improvement of glycemic control (a reduction of at least 1% point in HbA_{1c} and a final $HbA_{1c} \leq 8\%$) in a longitudinal intervention study. Different therapeutic interventions were used in a stepwise manner. Life-style intervention was intensified in all patients: they were encouraged to reduce saturated fat intake, increase physical exercise and quit smoking, and given oral agents when needed. Metformin was considered the oral agent of choice, in the absence of contraindications, when the patient was overweight or obese. Otherwise, sulfonylureas were used, but glitazones were not. If monotherapy was insufficient, both agent groups were combined, and/or insulin was used, with a progressively more complex program.

Patients

Thirty-three consecutive type 2 diabetic subjects, admitted to the hospital or seen in the outpatient diabetes clinic because of poor glycemic control, were included in the study, after excluding those receiving lipid-lowering treatment. A history and physical examination were performed, after the patients gave their informed consent.

Laboratory Measurements

Total cholesterol and triglyceride were measured by enzymatic methods, and HDLc by a direct method (Roche Diagnostics, Basel, Switzerland). LDLc was calculated using Friedewald's formula[10] when triglyceride did not exceed 3.45 mmol/L (300 mg/dL). Otherwise, ultracentrifugation was performed, and LDLc was estimated in the infranatant after separating the $d < 1.006$ kg/L fraction. ApoB and apoAI were measured by an immunoturbidimetric method (Roche Diagnostics) calibrated against the World Health Organization (WHO)/International Federation of Clinical Chemistry (IFCC) reference standard SP3-07 for apoB and SP1-01 for apoAI.[11] LDL size was determined by electrophoresis on gradient (2% to 16%) polyacrylamide gel, cast in the laboratory, according to the method described by Nichols et al, with modifications.[12] The gels were scanned (Gel-DOC 2000, Bio-Rad, Hercules, CA) and migration distances (from the top of the gel to the most prominent band) were measured. A pool containing sera with 4 LDL fractions whose diameter (22.9 ± 0.7, 24.5 ± 0.6, 26.2 ± 0.5, and 28.4 ± 0.9 nm) had been previously assessed by electron micros-

copy was used as control. LDL particle subclasses were classified as predominantly small LDL or phenotype B (diameter $\leq$ 25.5 nm) and non-small LDL (phenotype A, diameter $>$ 25.5 nm).[3] Both intragel and intergel imprecisions were below 1%. HbA_{1c} was measured by ion-exchange high-performance liquid chromatography (HPLC; Variant, Bio-Rad), with normal values ranging from 4.6% to 5.8% (mean, 5.1% $\pm$ 0.3%).

Statistical Analysis

Analysis was performed using SPSS 8.0 statistical package for Windows (SPSS, Chicago, IL). Continuous variables are expressed as the mean $\pm$ SD (gaussian distribution) or as median and range, and qualitative data, as percentages. Comparison of patient features before and after intervention was performed using Student's *t* test for paired data (gaussian distribution), Wilcoxon's test (non-gaussian distribution) for quantitative data, and McNemar's test for qualitative variables. Bivariate correlations were perfomed between changes in HbA_{1c} and in the different lipidic variables, and logistic regression analysis was performed to find predictors of increase in LDL size. Tests were 2-tailed, and $P < .05$ was considered significant.

RESULTS

Improvement of glycemic control was achieved with lifestyle intervention alone in 6.5% of the patients, with oral agents in 22.6% (6 metformin alone and 1 combined with sulfonylureas), with diet, metformin plus insulin in 9.7% and with diet plus insulin in 71%, after a follow-up of 3.5 (range, 1 to 13) months. Weight and BMI did not change with improved glycemic control (median decrease in HbA_{1c} 2.9 [1.1 to 10.2] percentage points) (Table 1). A significant reduction in LDLc and apoB and an increase in HDLc and apo AI were seen. Triglyceride concentrations, LDL size, and the frequency of phenotype B did not change significantly after glycemic optimization, and only the increase in HDLc was significantly correlated with the change in HbA_{1c} ($r = -0.396$).

However, 12 of 14 patients with LDL phenotype B at baseline experienced an increase in LDL size (*v* 6 of 19 with phenotype A, $P = .005$). There were no differences in initial glycemic control among patients with phenotype A and B after glycemic optimization. Patients with phenotype B at baseline had shorter diabetes duration, were more likely to be treated with diet only (Table 2), and showed other components of diabetic dyslipidemia (triglyceride, 1.35 [0.50 to 4.05] mmol/L *v* 1.10 [0.52 to 6.69] mmol/L, $P = 0.051$; HDLc, 0.95 $\pm$ 0.20 mmol/L *v* 1.27 $\div$ 0.36 mmol/L, $P = .005$). Although no differences in the new treatment were found between the patients showing an increase in LDL size and the remaining patients, those treated with diet only at baseline were more likely to show improvement in LDL size. However, these patients showed features of the metabolic syndrome. They had, on average, central obesity (BMI 30.4 $\pm$ 4.0 kg/m^2, waist girth 103 $\pm$ 2.5 cm), hypertriglyceridemia (2.21 [1.2 to 4.65] mmol/L), low HDLc (0.91 $\pm$ 0.16 mmol/L), normal LDLc (3.33 $\pm$ 1.23 mmol/L), and small LDL (25.05 $\pm$ 0.43 nm).

Baseline LDL size was the only lipidic predictor of change in LDL size ($r = -0.654$, $P < .0005$), and no other potential marker of LDL size (triglyceride, LDLc/apoB ratio)[12] predicted this change. The correlation between LDLc/apoB ratio and LDL size was significant during poor ($r = 0.385$, $P < .05$), but not during good control ($r = 0.315$, $P = .074$). Logistic regression analysis showed that baseline LDL size predicted increase in LDL size correctly in 84.9% of cases, and the addition of baseline triglyceride or triglyceride and HbA_{1c} increased this percentage to 87.8% and 93.9%, respectively ($P < .0001$ for all models).

Table 1. Main Anthropometric and Laboratory Features Before and After Improvement of Glycemic Control

	Poor Control	Good Control
BMI (kg/m^2)	28.92 $\pm$ 4.88	29.18 $\pm$ 4.87
HbA_{1c} (%)	10.54 $\pm$ 2.05	7.01 $\pm$ 0.63*
Total cholesterol (mmol/L)	5.60 $\pm$ 1.22	5.39 $\pm$ 1.21
Triglyceride (mmol/L)	1.52 (0.73-5.07)	1.26 (0.50-6.69)
LDLc (mmol/L)	3.62 $\pm$ 1.15	3.34 $\pm$ 1.02†
HDLc (mmol/L)	1.13 $\pm$ 0.34	1.21 $\pm$ 0.32†
apoAI (mmol/L)	1.27 $\pm$ 0.24	1.36 $\pm$ 0.24‡
apoB (g/L)	1.17 $\pm$ 0.29	1.07 $\pm$ 0.25‡
Lipoprotein(a) (mg/L)	258 (<80-2,619)	319 (<80-2,619)
LDL size (nm)		
Total	25.62 $\pm$ 0.54	25.70 $\pm$ 0.51
Phenotype A	25.99 $\pm$ 0.29	25.77 $\pm$ 0.49
Phenotype B	25.10 $\pm$ 0.31	25.61 $\pm$ 0.53§
Phenotype B (%)	42.4	30.3
LDLc/apoB ratio (mmol/g)	3.04 $\pm$ 0.46	3.12 $\pm$ 0.48

*$P < .0005$, †$P < 0.05$, ‡$P < .01$, §$P < .005$ *v* poor control.

Table 2. Main Clinical Features of the Patients Included in the Study

	All Patients	Phenotype A	Phenotype B
N	33	19	14
Male/female	51.5/48.5	42.1/57.9	64.3/35.7
Age (yr)	59.6 $\pm$ 11.15	61.9 $\pm$ 11.9	56.6 $\pm$ 9.6
BMI (kg/m^2)	28.9 $\pm$ 4.9	28.7 $\pm$ 5.2	29.6 $\pm$ 4.2
Menopause	93.4	100	90.9
Hypertension	56.3	63.2	46.2
Smoking	28.1	21.1	38.5
Diabetes duration (yr)	6 (0-40)	12 (0-40)	3 (0-24)*
Treatment			
No treatment	21.9	21.1	23.1
Diet only	18.8	5.3	38.5*
Oral agents†	18.8	21.1	15.4
Insulin plus metformin	9.4	10.6	7.7
Insulin	31.3	42.1	15.4
Retinopathy	28.6	47.1	0*
Nephropathy			
Microalbuminuria	30.4	31.6	28.6
Proteinuria	9.1	10.5	7.1
Stroke	6.3	10.5	0
Coronary heart disease	9.4	15.8	0
Peripheral vascular disease	18.8	26.3	7.7

NOTE. Qualitative variables presented as percentages, quantitative variables as mean $\pm$ SD or median (range).

*$P < .05$ *v* phenotype A.

†Three metformin, 2 sulphonylureas, and 1 both.

DISCUSSION

Our results support previous work showing a favorable effect of improving glycemic control on lipid and lipoprotein concen-

trations both in type 1[4,14] and type 2 diabetic patients.[4,15] However, our major findings are related to its effect on LDL particle size, extending a previous study of ours[4] in 2 main aspects. First, the patients included in this study are more representative of the type 2 diabetic population: the previous study included mostly lean (mean BMI 25.9 kg/m^2) type 2 diabetic patients whose glycemic control was improved with insulin treatment alone. In addition, the complete normalization of dyslipidemia with glycemic optimisation in that study suggests that they were insulinopenic, more similar to subjects with type 1 diabetes.[14] Second, the favorable change in LDL size induced by glycemic improvement is only observed in those patients with LDL phenotype B at baseline.

Although studies assessing the effect of glycemic optimization on LDL size are scarce, most show an improvement of this component.[4,15,16] Greater increases in HbA_{1c}, changes in BMI, the type of treatment used to improve glycemic control, and more severe lipidic disorders at baseline may account for different responses in the different studies,[4,16] baseline triglyceride concentrations being higher in those studies showing both a decrease in triglyceride concentrations and an increase in LDL size.[4,15,16] No significant changes in BMI were seen in the present study, and neither baseline triglyceride nor their increments were correlated with changes in LDL size. Nevertheless, small numbers and high variability in triglyceride concentrations may account for this lack of correlation. A small study published by Rivellese et al showed a more positive effect on LDL size when glycemic optimization was achieved with insulin than with glibenclamide, despite similar HbA_{1c} at the end of both treatments.[9] Although no differences were found in our study in treatment distribution between patients in whom LDL size increased and the rest, reduced patient number limits the conclusions that can be drawn from comparison between groups. However, in previous studies involving oral agents, only glitazones have proved to increase LDL size.[17]

The fact that only the patients with phenotype B showed a significant increase in LDL size in the present study, and that the increase in LDL size was inversely and strongly correlated with baseline LDL size, supports the hypothesis that the more severe the disorder, the greater the response to glycemic improvement. The fact that patients treated with diet only at baseline, who showed features of the metabolic syndrome, also showed an increase in LDL size, points in the same direction. Unfortunately, no easily measured predictors of change in LDL size were found.[13]

In conclusion, glycemic optimization is a good tool to improve the components of diabetic dyslipidemia; although individually modest, these changes seem globally significant since they involve an increase in HDLc and ApoAI, a reduction in LDLc and apoB, and, in the patients with LDL phenotype B at baseline, a shift towards larger LDL particles. However, probably not only glycemic optimization, but also life-style intervention plays a role in the improvement of the different components of diabetic dyslipidemia.

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