

Effect of intensive lipid-lowering strategy on low-density lipoprotein particle size in patients with type 2 diabetes mellitus

S.D.J.M. Niemeijer-Kanters^a, G.M. Dallinga-Thie^a, F.C. de Ruijter-Heijstek^a,
A. Algra^{b,c}, D.W. Erkelens^a, J.D. Banga^{a,*}, H. Jansen^d

^a Department of Internal Medicine, G02.228, University Medical Center Utrecht, Heidelberglaan 100, PO Box 85500, 3508 GA Utrecht, The Netherlands

^b Julius Center for Patient Oriented Research, University Medical Center Utrecht, Heidelberglaan 100, PO Box 85500, 3508 GA Utrecht, The Netherlands

^c Department of Neurology, University Medical Center Utrecht, Heidelberglaan 100, PO Box 85500, 3508 GA Utrecht, The Netherlands

^d Department of Internal Medicine and Department of Biochemistry, Erasmus University, Rotterdam, The Netherlands

Received 29 December 1999; received in revised form 14 June 2000; accepted 22 August 2000

Abstract

A preponderance of small dense LDL particles is strongly associated with the occurrence of atherosclerotic disease. Although several studies have documented an increased prevalence of small dense LDL particles in diabetes mellitus no data are available to show the effect of lipid-lowering treatment upon the improvement of LDL particle size. In the present study we examined the effect of lipid-lowering treatment, following an intensive lipid-lowering strategy for 30 weeks pursuing ADA recommended target lipid levels, on LDL particle size in 50 type 2 diabetic patients with moderate hyperlipidemia. At week 0, 24 patients (48%) were characterized by small dense LDL phenotype pattern B. After the treatment period a shift towards normal LDL particle size was observed in 17 patients but seven patients (29%) showed the more atherogenic LDL subclass pattern B. After treatment, plasma HDL-cholesterol was significantly lower ($P < 0.05$) in these patients compared to those who had LDL subclass pattern A. Multivariate regression analysis revealed VLDL-cholesterol or triglycerides and HDL₃-cholesterol as independent determinants for LDL particle size. Change in HDL₂-cholesterol was an independent determinant for change in LDL particle size. In conclusion, a strategy of intensive lipid-lowering, with the intention to reduce triglyceride levels below 1.7 mmol/l, may be insufficient to ensure improvement in LDL size in all patients. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: LDL particle size; Type 2 diabetes mellitus; Lipid-lowering therapy; HDL-cholesterol; Triglycerides

1. Introduction

The worldwide incidence and prevalence of diabetes, especially type 2, are increasing. Diabetes accounts for 6–12% of the total health care expenditure in industrialized countries [1]. Cardiovascular complications are the major cause of morbidity and mortality in diabetes [2]. Therefore, recognition of both quantitative and qualitative abnormalities that increase the risk of atherosclerotic complications is essential. Hypercholesterolemia and hypertriglyceridemia are proven predic-

tors of coronary heart disease mortality in diabetes mellitus [3,4]. The lipoprotein profile in type 2 diabetes is abnormal, with hypertriglyceridemia and reduced HDL-cholesterol plasma concentrations as the dominant features [5].

Plasma triglyceride concentration is a main determinant of LDL particle size, apart from genetic factors [6–8]. LDL comprises a heterogeneous spectrum of particles that differ in size, density, chemical composition and atherogenicity [9]. Analysis of LDL subclass distributions by density and particle size has revealed marked variability among individual subjects. On the basis of the predominant particle size, patterns of LDL on gradient gel electrophoresis are commonly divided in either pattern A, if the LDL fraction contains mainly

* Corresponding author. Tel.: +31-30-2507399; fax: +31-30-2518328.

E-mail address: j.d.banga@digd.azu.nl (J.D. Banga).

large LDL, or pattern B, if small dense LDL particles are present. Patients with diabetes frequently have an abnormal composition of LDL particles and a predominance of small dense LDL [10,11]. Reaven et al. [12] found that subjects with LDL pattern B are more often insulin resistant, have higher glucose, insulin, and triglyceride levels, lower HDL-cholesterol levels, and higher blood pressure than those with pattern A. Available data suggest a plasma triglyceride concentration threshold of 1.3–1.7 mmol/l for the presence of small dense LDL [13]. The preponderance of small dense LDL increases at concentrations of plasma triglycerides in the upper normal range [7].

Various post-hoc subgroup analyses of larger, randomized trials indicate that patients with small dense LDL respond more favourably to lipid-lowering treatment with regard to arteriographic change and that changes in lipoprotein subclasses may be better predictors of arteriographic outcome than the LDL-cholesterol level [14–16]. The guidelines of the American Diabetes Association and the National Cholesterol Education Program (NCEP) suggest target values for plasma lipid levels in subjects with diabetes [17,18]. To our knowledge, there are no published studies that have assessed whether a strategy of intensive lipid-lowering with the intention to reach these target values can improve LDL subclass distribution in diabetic patients. The aim of our study was to investigate the effect of an intensive lipid-lowering strategy on LDL particle size in patients with type 2 diabetes mellitus. Furthermore, we examined determinants of LDL particle size in these patients.

2. Methods

2.1. Patients

Patients with type 2 diabetes mellitus, classified according to the World Health Organization criteria [19] were recruited from the outpatient clinic for diabetes of the University Medical Center Utrecht [20]. Inclusion criteria were: LDL-cholesterol > 2.6 mmol/l or triglycerides > 1.7 mmol/l or HDL-cholesterol < 0.9 mmol/l for men and < 1.1 mmol/l for women. The three target lipid levels to be reached were: LDL-cholesterol < 2.6 mmol/l and triglycerides < 1.7 mmol/l and HDL-cholesterol > 0.9 mmol/l for men and > 1.1 mmol/l for women. These values were derived from recommendations by the American Diabetes Association and the National Cholesterol Education Program at the time of the study [18,21]. Exclusion criteria were: premenopausal state without adequate contraception; liver or renal function impairment (creatinine > 200 mmol/l); other contra-indication to the study medication; hypothyroidism or excessive alcohol consumption

(> 5 consumptions/day). The study protocol was approved by the Ethics Committee of the University Medical Center Utrecht and all patients gave written informed consent.

2.2. Design

The study started with a run-in period of 6 weeks if HbA_{1c} at baseline was $\leq 8.5\%$, or 12 weeks if HbA_{1c} at baseline was $\leq 8.5\%$. In these 12 weeks, glycemic control was improved as much as practically feasible, scheduling extra visits. A dietician recommended a NCEP Step I diet to all participants on the basis of a 4-day dietary recall: total fat intake $< 30\%$ of Kcal, saturated fat intake $< 10\%$ of total Kcal, and < 300 mg of cholesterol per day [18].

We used medication steps of incremental dosages and combinations of drugs to reach target lipid values. The purpose was explicitly to investigate the effect of this lipid-lowering strategy on LDL particle size, and not to test one drug in particular. After the run-in period, patients started with lipid-lowering medication at week 0, which was extended every six weeks until all three target values for plasma lipids were reached. If all target values were achieved, the lipid-lowering medication was stabilized and kept constant for the remaining study period. The medication-steps were:

Step 1: simvastatin 20 mg nocte if only LDL-cholesterol > 2.6 mmol/l, or gemfibrozil 600 mg b.d. if triglycerides > 1.7 mmol/l and/or HDL-cholesterol < 0.9 mmol/l for men and < 1.1 mmol/l for women, irrespective the level of LDL-cholesterol.

Step 2: simvastatin 20 mg nocte and gemfibrozil 600 mg b.d. in combination.

Step 3: step 2 plus acipimox 250 mg b.d.

Step 4: step 2 plus acipimox 250 mg t.d.s.

Step 5: step 2 plus acipimox 500 mg t.d.s.

When patients experienced adverse events, simvastatin could be replaced by pravastatin (40 mg nocte) and gemfibrozil by bezafibrate (200 mg t.d.s.). The total follow-up was 36 or 42 weeks for all patients, depending on the duration of the run-in period (6 or 12 weeks).

2.3. Laboratory investigations

Venous blood was collected after an overnight fast of at least 10 h. Plasma was prepared by immediate centrifugation for analytical analysis. Plasma triglycerides and cholesterol were measured in triplicate by commercial colorimetric assays (triglycerides GPO-PAP and Monotest Cholesterol kit, respectively, Boehringer Mannheim). Plasma HDL-cholesterol was determined by precipitation procedures with heparin-manganese chloride [22]. Plasma lipoprotein fractions were isolated by means of density gradient ultracentrifugation [23].

Plasma apo B and apo AI were determined using immunonephelometric assays, using polyclonal rabbit anti-human apo B or apo AI antiserum, and standards with the assigned values according to the International Federation of Clinical Chemistry (Behringwerke AG, Marburg, Germany) [24].

The LDL subclass patterns were identified by electrophoresis on 2–16% PAGE gels as described by Jansen et al. [25]. The gels were prepared with an LKB 11300 Ultrograd gradient mixer [26]. In each gel, reference sera with known LDL size were applied to lane 1 and 6 of a total of 12 lanes. The LDL size in these sera had been determined using a set of LDL standards (ranging from 246 to 287 Å), kindly provided by Dr R.M. Krauss (San Francisco, CA). The gels were stained with Oil Red O. The center of the most prominent LDL band was marked on the gel. The migration distance of the bands from the top of the gel was measured. The average LDL particle diameter was estimated from a quadratic extrapolation of a plot of the logarithm of the diameter of the standards versus the migration distance of the standards. LDL particles with a peak diameter ≤ 257 Å were classified as small dense LDL or LDL subclass pattern B and LDL particles with a peak diameter > 257 Å as large buoyant LDL particles or LDL subclass pattern A. The variation coefficient of the LDL particle diameter assay was 1.9%.

A two-antibody sandwich immunoassay of Cholesterol Ester Transfer Protein (CETP) was set up according to Mezdoor et al. [27] with major modifications as described below. The coating was performed with a combination of monoclonal antibodies TP1 (5 mg/ml in PBS) and TP2 (2.5 mg/ml) during an overnight incubation at 70°C. To prevent non-specific binding, plates were blocked with 1% BSA at room temperature for 2 h. Samples were tested in 20, 40 and 80-fold dilution whereas standard plasma was diluted from 10- to 160-fold with 0.1% Triton X-100 and 1% BSA in PBS. As a secondary antibody TP20, labeled with digoxigenine, was used. Antidigoxigenine Fab fragments coupled to peroxidase were added. Absorbance, after addition of TMB, was read after 30 min incubation and termination of the reaction with H₂O₂ at 450 nm. The interassay and intraassay coefficient of variance were 7.8 and 6.0%, respectively.

2.4. Statistical analysis

Data are presented as means \pm standard deviation. Changes in variables were described by differences between values at the end of the study and those at the start (including 95% confidence intervals based on the paired *t*-test for dependent samples). Differences between groups were evaluated for significance by the *t*-test for independent samples.

Linear regression analysis was performed to evaluate the determinants of LDL particle size. The continuous variables (cholesterol, VLDL-cholesterol, IDL-cholesterol, LDL-cholesterol, HDL-cholesterol, HDL₂-cholesterol, HDL₃-cholesterol, triglycerides, CETP, apo AI, apo B) were divided into tertiles. If LDL particle diameter was essentially the same in adjacent tertiles, these were taken together for later analyses. Associations between different parameters were determined by Pearson correlation coefficients. Variables selected from univariate analysis (selected by significance level of 0.10 or less) were entered into a stepwise multivariate model.

The statistical package SPSS[®] for Windows 8.0 (SPSS, Chicago, IL) was used for data analysis. *P* < 0.05 was considered statistically significant.

3. Results

LDL particle size before and after lipid-lowering treatment was determined in 50 type 2 diabetic patients. Their main characteristics at the start of the run-in period are shown in Table 1.

At week 0, the start of the lipid-lowering medication, 24 patients (48%), 19 men and five women, were characterized by LDL subclass pattern B. After the treatment period of 30 weeks a shift towards normal LDL particle size was observed in 17 patients. Seven patients (29%), four men and three women, retained the more atherogenic LDL subclass pattern B.

At the end of the study period, four patients (three with pattern A at week 0, one with pattern B at week 0) were treated with a statin alone, 17 (10 with pattern A, seven with pattern B) with a statin and a fibrate, 15 (eight with pattern A, seven with pattern B) with a statin and a fibrate and acipimox 250 mg b.d., one (pattern B) with a statin and a fibrate and acipimox 250 mg t.d.s. and 13 (five with pattern A and eight with pattern B) with a statin and a fibrate and acipimox 500 mg t.d.s.. The patient treated with a statin and a fibrate and acipimox 250 mg t.d.s. and 11 of the 13 patients

Table 1
Baseline characteristics of the type 2 diabetic patients^a

<i>N</i>	50
Age (years)	58.6 \pm 8.7
Men (%)	64
Duration of diabetes (years)	9.4 \pm 8.6
Diet only/oral polyglycemic agent only/insulin only/oral hypoglycemic agent and insulin (%)	10/36/44/10
HbA _{1c} (%)	8.3 \pm 1.5
Body mass index (kg/m ²)	27.9 \pm 4.1
Systolic blood pressure (mmHg)	146 \pm 22
Diastolic blood pressure (mmHg)	86 \pm 9
Ever smoker (%)	68

^a Data are presented as mean \pm standard deviation or percentage.

Table 2
Plasma lipids, apolipoproteins and CETP according to LDL subclass phenotype at week 0^a

	Pattern A	Pattern B	Difference (95% CI)
<i>N</i>	26	24	
Cholesterol (mmol/l)	5.5 ± 0.9	5.4 ± 0.9	-0.2 (-0.7-0.3)
VLDL-cholesterol (mmol/l)	0.43 ± 0.30	0.90 ± 0.43	0.48 (0.27-0.68) ^b
IDL-cholesterol (mmol/l)	0.31 ± 0.15	0.39 ± 0.15	0.07 (-0.01-0.16)
LDL-cholesterol (mmol/l)	3.1 ± 0.7	2.8 ± 0.6	-0.3 (-0.7-0.1)
HDL-cholesterol (mmol/l)	1.0 ± 0.2	0.8 ± 0.2	-0.2 (-0.3-0.1) ^b
HDL ₂ -cholesterol (mmol/l)	0.30 ± 0.11	0.25 ± 0.07	-0.05 (-0.10-0.003)
HDL ₃ -cholesterol (mmol/l)	0.73 ± 0.15	0.58 ± 0.12	-0.15 (-0.22-0.07) ^b
Triglycerides (mmol/l)	1.6 ± 0.8	2.7 ± 1.0	1.1 (0.6-1.6) ^b
CETP (mg/ml)	3.1 ± 0.5	3.3 ± 1.1	0.2 (-0.3-0.7)
Apo AI (g/l)	1.4 ± 0.2	1.3 ± 0.2	-0.1 (-0.2-0.1) ^c
Apo B (g/l)	1.2 ± 0.2	1.2 ± 0.2	0.1 (-0.1-0.2)
LDL diameter (Å)	268 ± 3	252 ± 5	

^a Data are presented as mean ± standard deviation.

^b *P* < 0.001.

^c *P* < 0.01.

treated with a statin and a fibrate and acipimox 500 mg t.d.s. did not reach the predetermined lipid goals. However, during the 30-week treatment period their LDL-cholesterol decreased on average by 0.8 ± 1.0 mmol/l, their triglycerides by 1.2 ± 1.3 mmol/l and their HDL-cholesterol increased by 0.1 ± 0.2 mmol/l. All other patients did reach the target levels. Four of the seven patients with pattern B after treatment were treated with a statin and a fibrate and three were treated with a statin and a fibrate and acipimox t.d.s.

Age, duration of diabetes, antidiabetic treatment, and glycemic control were similar in patients exhibiting LDL subclass phenotype A and B at week 0. Baseline body mass index (BMI) was higher in the patients with LDL phenotype B at week 0 compared to those with phenotype A: 29.2 ± 3.7 and 26.6 ± 4.1 kg/m², respectively. A total of 79% of the patients with pattern B at week 0 were male compared to 50% of the patients with pattern A. There were no differences in baseline BMI, glycemic control, age or duration of diabetes between the patients who maintained or changed their LDL phenotype B. A total of 57% of the patients with pattern B after treatment were male.

Table 2 shows levels of plasma lipids, apolipoproteins and CETP mass according to LDL subclass phenotype

at week 0. Plasma lipid profiles of patients with LDL subclass phenotype B at week 0 were characterized by significantly elevated plasma triglycerides and VLDL-cholesterol levels and significantly decreased plasma HDL-cholesterol levels, mainly HDL₃-cholesterol and plasma apo AI, compared to patients with phenotype A. Table 3 shows levels of plasma lipids, apolipoproteins and CETP mass before and after lipid-lowering, in the patients characterized by LDL subclass phenotype A before and after treatment (*n* = 26). Total cholesterol, VLDL-cholesterol, IDL-cholesterol, LDL-cholesterol, triglycerides, CETP and apo B decreased significantly in these subjects. HDL-cholesterol, HDL₂-cholesterol, HDL₃-cholesterol and apo AI increased significantly. Table 4 depicts levels of plasma lipids, apolipoproteins and CETP mass before and after lipid-lowering in the patients characterized by LDL subclass phenotype B before and A after treatment (*n* = 17). LDL particle size increased significantly from 253 ± 5 to 266 ± 6 Å, respectively. A significant decrease in total cholesterol, VLDL-cholesterol, IDL-cholesterol, LDL-cholesterol, triglycerides, CETP and apo B was found. HDL-cholesterol, HDL₂-cholesterol and HDL₃-cholesterol increased significantly. Table 5 shows levels before and after treatment in patients with pattern B

Table 3

Levels of plasma lipids, apolipoproteins and CETP mass at week 0 and week 30 in 26 patients characterized by LDL subclass phenotype A before and after treatment^a

	Week 0	Week 30	Difference (95% CI)
Cholesterol (mmol/l)	5.5 ± 0.9	4.2 ± 0.6	-1.4 (-1.8-0.9) ^b
VLDL-cholesterol (mmol/l)	0.43 ± 0.30	0.19 ± 0.14	-0.24 (-0.34-0.13) ^b
IDL-cholesterol (mmol/l)	0.31 ± 0.15	0.18 ± 0.11	-0.13 (-0.19-0.07) ^b
LDL-cholesterol (mmol/l)	3.1 ± 0.7	2.2 ± 0.5	-0.8 (-1.1-0.6) ^b
HDL-cholesterol (mmol/l)	1.0 ± 0.2	1.4 ± 0.3	0.4 (0.3-0.5) ^b
HDL ₂ -cholesterol (mmol/l)	0.30 ± 0.11	0.47 ± 0.13	0.17 (0.12-0.22) ^b
HDL ₃ -cholesterol (mmol/l)	0.73 ± 0.15	0.98 ± 0.20	0.25 (0.16-0.34) ^b
Triglycerides (mmol/l)	1.6 ± 0.8	1.0 ± 0.3	-0.6 (-0.9-0.3) ^b
CETP (mg/ml)	3.1 ± 0.5	2.8 ± 0.7	-0.3 (-0.6-0.0) ^d
Apo AI (g/l)	1.4 ± 0.2	1.5 ± 0.2	0.1 (0.1-0.2) ^c
Apo B (g/l)	1.2 ± 0.2	0.8 ± 0.2	-0.3 (-0.4-0.2) ^b
LDL diameter (Å)	268 ± 3	269 ± 7	2 (-1-4)

^a Data are presented as mean ± standard deviation.

^b *P* < 0.001.

^c *P* < 0.01.

^d *P* < 0.05.

Table 4

Levels of plasma lipids, apolipoproteins and CETP mass at week 0 and week 30 in 17 patients characterized by LDL subclass phenotype B before and A after treatment^a

	Week 0	Week 30	Difference (95% CI)
Cholesterol (mmol/l)	5.4 ± 0.9	3.9 ± 0.6	-1.4 (-1.9–-1.0) ^b
VLDL-cholesterol (mmol/l)	0.85 ± 0.44	0.29 ± 0.14	-0.56 (-0.76–-0.37) ^b
IDL-cholesterol (mmol/l)	0.36 ± 0.15	0.21 ± 0.11	-0.14 (-0.22–-0.07) ^c
LDL-cholesterol (mmol/l)	2.8 ± 0.7	2.2 ± 0.5	-0.6 (-0.9–-0.3) ^c
HDL-cholesterol (mmol/l)	0.8 ± 0.2	1.2 ± 0.2	0.3 (0.3–0.4) ^b
HDL ₂ -cholesterol (mmol/l)	0.24 ± 0.07	0.35 ± 0.12	0.10 (0.05–0.16) ^c
HDL ₃ -cholesterol (mmol/l)	0.59 ± 0.12	0.83 ± 0.15	0.24 (0.19–0.30) ^b
Triglycerides (mmol/l)	2.7 ± 1.2	1.1 ± 0.3	-1.6 (-2.1–-1.0) ^b
CETP (mg/ml)	3.2 ± 1.1	2.7 ± 0.7	-0.5 (-0.9–-0.2) ^b
Apo AI (g/l)	1.3 ± 0.2	1.3 ± 0.2	0.1 (0.0–0.1)
Apo B (g/l)	1.2 ± 0.2	0.9 ± 0.2	-0.3 (-0.4–-0.2) ^b
LDL diameter (Å)	253 ± 5	266 ± 6	13 (10–16) ^b

^a Data are presented as mean ± standard deviation.

^b $P < 0.001$.

^c $P < 0.01$.

before and after treatment ($n = 7$). Total cholesterol, VLDL-cholesterol, IDL-cholesterol, LDL-cholesterol, triglycerides and apo B decreased significantly. HDL-cholesterol and HDL₃-cholesterol increased significantly. HDL₂-cholesterol and apo AI did not change in these patients. There was a slight tendency towards a larger LDL particle diameter after treatment ($P = 0.10$). Table 6 depicts levels of plasma lipids, apolipoproteins and CETP according to LDL subclass phenotype at week 30. Only HDL-cholesterol was significantly lower in patients with pattern B at the end of the treatment period. All patients who changed from phenotype B to phenotype A attained the treatment goal for plasma triglyceride level (< 1.7 mmol/l), two of seven patients who maintained phenotype B did not reach this target level.

A total of 17 subjects already had lipid-lowering medication prescribed at baseline. At week 0, 11 of these 17 patients (65%) were characterized by LDL subclass pattern B, despite their medication. This medication was replaced by the study medication at week 0. Nine of the 11 patients changed to pattern A after treatment by intensive lipid-lowering according to the study protocol. All analyses were also performed after exclusion of these 17 patients and yielded comparable

results (data on file).

VLDL-cholesterol, IDL-cholesterol, HDL-cholesterol, HDL₂-cholesterol, HDL₃-cholesterol, triglycerides and apo AI were selected in univariate regression analysis as determinants of LDL particle size. HDL-cholesterol and apo AI were not entered into the multivariate model because of strong associations with HDL₂-cholesterol and HDL₃-cholesterol. Stepwise multivariate regression analysis of the other selected variables showed that VLDL-cholesterol and HDL₃-cholesterol were independent determinants of LDL particle diameter (adjusted $R^2 = 0.51$, $P < 0.001$). The regression coefficients were -8 (95% CI -13 – -3) and 24 (95% CI 10 – 38) for VLDL-cholesterol and HDL₃-cholesterol, respectively. Because VLDL-cholesterol and triglycerides were strongly correlated ($r = 0.88$) a similar model containing triglycerides and HDL₃-cholesterol could be developed with regression coefficients of -3 (95% CI -5.3 – -1.0 , $P < 0.01$) and 25 (95% CI 10 – 39 , $P < 0.01$), respectively.

Regression analysis was also performed to investigate whether changes in plasma lipids, apolipoproteins and CETP were determinants of changes in LDL diameter in the patients with LDL subclass pattern B at week 0 ($n = 24$). The change in HDL₂-cholesterol was the only

Table 5

Levels of plasma lipids, apolipoproteins and CETP mass at week 0 and week 30 in 7 patients characterized by LDL subclass phenotype B before and after treatment^a

	Week 0	Week 30	Difference (95% CI)
Cholesterol (mmol/l)	5.4 ± 0.9	3.8 ± 0.7	-1.5 (-2.5–-0.6) ^b
VLDL-cholesterol (mmol/l)	1.03 ± 0.42	0.36 ± 0.32	-0.67 (-0.96–-0.39) ^b
IDL-cholesterol (mmol/l)	0.46 ± 0.14	0.25 ± 0.07	-0.20 (-0.36–-0.05) ^c
LDL-cholesterol (mmol/l)	2.7 ± 0.6	2.0 ± 0.4	-0.7 (-1.3–-0.1) ^c
HDL-cholesterol (mmol/l)	0.8 ± 0.2	1.1 ± 0.2	0.3 (0.1–0.4) ^b
HDL ₂ -cholesterol (mmol/l)	0.25 ± 0.08	0.31 ± 0.08	0.06 (-0.01–0.14)
HDL ₃ -cholesterol (mmol/l)	0.55 ± 0.11	0.78 ± 0.17	0.23 (0.14–0.32) ^b
Triglycerides (mmol/l)	2.8 ± 0.7	1.4 ± 0.7	-1.4 (-2.1–-.6) ^c
CETP (mg/ml)	3.4 ± 1.2	3.1 ± 1.0	-0.3 (-1.2–0.5)
Apo AI (g/l)	1.3 ± 0.1	1.3 ± 0.2	0.0 (-0.1–0.2)
Apo B (g/l)	1.2 ± 0.2	0.9 ± 0.2	-0.3 (-0.5–-0.1) ^c
LDL diameter (Å)	249 ± 5	252 ± 5	3 (-1–7)

^a Data are presented as mean ± standard deviation.

^b $P < 0.001$.

^c $P < 0.01$.

Table 6
Plasma lipids, apolipoproteins and CETP according to LDL subclass phenotype at week 30^{a,b}

	Pattern A	Pattern B	Difference (95% CI)
<i>n</i>	43	7	
Cholesterol (mmol/l)	4.1 ± 0.6	3.8 ± 0.7	-0.2 (-0.7-0.3)
VLDL-cholesterol (mmol/l)	0.23 ± 0.14	0.36 ± 0.32	0.13 (-0.17-0.43)
IDL-cholesterol (mmol/l)	0.19 ± 0.11	0.25 ± 0.07	0.06 (-0.03-0.15)
LDL-cholesterol (mmol/l)	2.2 ± 0.5	2.0 ± 0.4	-0.2 (-0.6-0.1)
HDL-cholesterol (mmol/l)	1.3 ± 0.3	1.1 ± 0.2	-0.2 (-0.5-0.0) ^c
HDL ₂ -cholesterol (mmol/l)	0.42 ± 0.14	0.31 ± 0.08	-0.11 (-0.22-0.00)
HDL ₃ -cholesterol (mmol/l)	0.92 ± 0.19	0.78 ± 0.17	-0.14 (-0.29-0.02)
Triglycerides (mmol/l)	1.0 ± 0.3	1.4 ± 0.7	0.3 (-0.3-0.9)
CETP (mg/ml)	2.8 ± 0.7	3.1 ± 1.0	0.3 (-0.3-0.9)
Apo AI (g/l)	1.5 ± 0.2	1.3 ± 0.2	-0.1 (-0.3-0.0)
Apo B (g/l)	0.9 ± 0.2	0.9 ± 0.2	0.0 (-0.08-0.19)
LDL diameter (Å)	268 ± 7	252 ± 5	

^a Data are presented as mean ± standard deviation.

^b $P < 0.001$.

^c $P < 0.05$.

variable selected by univariate analysis (adjusted $R_2 = 0.22$, $P < 0.05$). The regression coefficient was 38 (95% CI 11–64).

4. Discussion

We evaluated the effect of intensive lipid-lowering treatment on LDL particle size in 50 patients with type 2 diabetes mellitus. Plasma lipid profiles of subjects with LDL subclass phenotype B were characterized by elevated triglycerides and VLDL-cholesterol levels and decreased HDL-cholesterol levels. LDL subclass pattern B was found in 24 patients (48%) before treatment. After the treatment period a shift towards pattern A was observed in 17 patients, but seven patients still showed the more atherogenic LDL phenotype pattern B. After treatment, plasma HDL-cholesterol was significantly lower in these patients compared to those with LDL subclass pattern A. VLDL-cholesterol and triglycerides were strongly correlated. Multivariate regression analysis showed that VLDL-cholesterol or triglycerides and HDL₃-cholesterol were independent determinants of LDL particle size, and change in HDL₂-cholesterol was an independent determinant of change in LDL particle size.

Hypertriglyceridemia in diabetes can be explained by an increased hepatic VLDL overproduction and impaired catabolism of triglyceride-rich particles. It has been postulated that high plasma triglyceride influences LDL size and density through a cycle of lipid exchange [13]. Higher levels of triglyceride-rich particles in diabetes allow enhanced CETP-mediated exchange of cholesteryl ester from LDL and HDL for triglycerides from VLDL and chylomicrons, leading to enrichment of LDL and HDL with triglycerides. Hydrolysis of core triglycerides by hepatic lipase, which is often increased in type 2 diabetes, results in the formation of small dense LDL and HDL. This causes small dense HDL₃ to predominate at the expense of the larger and cholesteryl-ester-rich HDL₂.

McNamara et al. [6] found significant associations between change in LDL size and change in triglyceride and HDL-cholesterol concentrations in 227 untreated non-diabetic participants of the Framingham Offspring Study. Lahdenperä et al. [7] described that serum triglyceride was the major determinant of LDL size in both patients with type 2 diabetes and non-diabetic subjects with or without coronary artery disease. They found a potential threshold effect for triglycerides in determining LDL size, with increasing prevalence of small dense LDL particles at triglycerides above the level of 1.7 mmol/l. Also HDL-cholesterol and the activity of hepatic lipase appeared to be independently associated with LDL particle size in this study. Multivariate analysis performed by Tan et al. [28] revealed that plasma triglycerides levels, CETP and hepatic lipase activity accounted for ~20% of the variability in LDL particle density in patients with type 2 diabetes. CETP mass was not independently associated with LDL size in our study. Hokanson et al. [29] found that small dense LDL was associated with low lipoprotein lipase and high hepatic lipase activities in normolipidemic subjects. In men with familial hypercholesterolemia treated with atorvastatin, changes in LDL size correlated to changes in triglycerides concentration, but not to changes in hepatic lipase activity [30]. In women, atorvastatin did not affect LDL size or pattern. Zambon et al. [16] reported the results of intensive lipid-lowering in non-diabetic subjects. Changes in LDL density on therapy were strongly associated with changes in hepatic lipase activity.

LDL size may contribute to an increased risk of atherosclerosis in a number of ways. Small dense LDL has been reported to bind to the arterial wall with greater affinity than native LDL [31]. Small dense LDL may also be more susceptible to glycation and oxidation [32]. The role of small dense LDL as a risk factor for coronary heart disease is becoming well established. LDL size was significantly smaller in men and women with coronary artery disease than in controls in a prospective, population-based study [33]. In another

nested case-control study, men with a myocardial infarction diagnosed during follow-up had significantly smaller LDL diameter than controls [34]. However, after adjustment for triglyceride level, LDL particle size was no longer associated with risk of myocardial infarction. The results from a third prospective study showed that the presence of small dense LDL particles was associated with an increased risk of subsequent development of ischemic heart disease in men [35]. Gaziano et al. [36] found that small dense LDL was not independent of plasma triglyceride level as a predictor of myocardial infarction in a case-control study.

To our knowledge, this study is the first to report the effect of lipid-lowering treatment upon the improvement of LDL particle size in diabetes. Despite a decrease of mean plasma triglycerides from 2.8 to 1.4 mmol/l, seven patients retained LDL subclass pattern B after treatment. Plasma HDL-cholesterol was lower in these patients than in the subjects with pattern A. In multivariate analysis HDL₃-cholesterol was an important determinant of LDL particle size. These findings mean that lowering plasma triglycerides levels below the ADA recommended target level is not enough to revert LDL size from dense towards more buoyant particles in all patients with type 2 diabetes. The triglyceride target level of 1.7 mmol/l may be insufficient to ensure improvement in LDL size. Furthermore, low plasma HDL-cholesterol levels may prevent a change from pattern B to pattern A. Behavioral interventions such as weight loss, increased physical activity and smoking cessation may be useful to raise HDL-cholesterol [17]. However, genetic predisposition may also contribute to the persistence of LDL subclass pattern B in these seven patients [8].

We included 17 subjects who already had lipid-lowering medication prescribed at baseline. A total of 65% of these patients showed LDL phenotype pattern B, despite their medication. This finding stresses the importance of pursuing target lipid levels instead of describing a standard dosage of a lipid-lowering agent.

Recent studies indicate that LDL size may also be influenced by treatment with thiazolidinediones. Troglitazone treatment increased LDL-cholesterol, but improved the ratio of large buoyant to small dense LDL in 15 obese subjects with type 2 diabetes [37]. The changes in lipoprotein profiles were unrelated to changes in insulin sensitivity.

In conclusion, a strategy of intensive lipid-lowering, with the intention to reduce triglyceride levels below 1.7 mmol/l, may be insufficient to ensure improvement in LDL size in all patients.

Acknowledgements

The authors wish to thank Dr R.M. Krauss for kindly providing them with a set of LDL standards. This work

was financially supported by the Dutch Prevention Fund (grant no. 28-2437). S.D.J.M. Niemeijer-Kanters is recipient of a grant from the Dutch Organization for Scientific Research (NWO).

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