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LDL-cholesterol/apolipoprotein B ratio is a good predictor of LDL phenotype B in type 2 diabetes

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Abstract LDL phenotype B is a component of diabetic dyslipidaemia, but its diagnosis is cumbersome. Our aim was to find easily available markers of phenotype B in a group of type 2 diabetic subjects. We studied 123 type 2 diabetic patients (67.5% male, aged 59.3±10.1 years, mean HbA1c 7.4%). Clinical features and fasting total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol (LDLc, using Friedewald's equation and an alternative formula), apolipoprotein B (apoB), lipoprotein (a) and LDL particle size (on gradient polyacrylamide gel electrophoresis) were assessed. Patients with phenotypes A (predominant LDL size ≥25.5 nm) and B (<25.5 nm) were compared, and regression analysis was performed to find the best markers of LDL particle. Cut-off points were obtained and evaluated as predictors of phenotype B (kappa index). Patients with phenotype B (36%) showed higher total cholesterol, triglyceride and apolipoprotein B, and lower HDL cholesterol and LDLc/apoB ratio. Triglyceride was the best predictor of LDL particle size (r=-0.632, p<0.01), but an LDLc/apoB ratio below 1.297 mmol/g detected phenotype B best (sensitivity 65.9%, specificity 92.4%, kappa=0.611). Although triglyceride concentration is the best predictor of LDL size in type 2 diabetes, LDLcholesterol/apolipoproteinB ratio is the best tool to detect phenotype B.

Key words LDL size • LDL phenotype B • LDL cholesterol/apolipoprotein B ratio • Type 2 diabetes

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Introduction

LDL cholesterol (LDLc) is a strong predictor of coronary heart disease, and lowering LDLc has proved to reduce mortality and cardiovascular events [1] in diabetic subjects. However, most diabetic patients do not have increased LDLc, but display other characteristics of diabetic dyslipidaemia, which comprise moderate hypertriglyceridaemia, low HDL cholesterol (HLDc), increased apolipoprotein B (apoB) and an increased proportion of small, dense LDL particles (phenotype B) [2, 3]. The latter has been associated with coronary heart disease in several cross-sectional [4, 5] and longitudinal [6, 7] studies, and is present in as many as 30%–50% of type 2 diabetic patients [2, 7, 8]. Thus, the measurement of LDL size can provide important information for cardiovascular risk assessment in these patients. Nevertheless, the determination of LDL size is not easy: both density gradient ultracentrifugation and electrophoresis on gradient polyacrylamide gels, which are the most frequently used methods [9], are cumbersome and time-consuming. Therefore, in addition to the determination of triglyceride, HDLc and LDLc, as is currently recommended [10], and even apoB, it would be useful to be able to predict the presence of LDL phenotype B from clinical or analytical markers. The aim of the present study was to search for the markers that best predict LDL particle phenotype from among easily available clinical and analytical variables in a group of type 2 diabetic subjects.

Material and methods

Patients

A total of 123 type 2 diabetic patients from a university hospital were included in the study, after excluding those receiving treatment or in situations known to affect lipid metabolism, unrelated to their diabetes. Patients with hypertension were not being treated with non-selective betablockers or high-dose diuretics. History and physical examination, including anthropometric parameters, were performed. Diabetes, smoking, hypertension, peripheral vascular disease, coronary heart disease, ischaemic cerebrovascular disease, retinopathy and nephropathy were defined and evaluated as described elsewhere [3].

Laboratory determinations

Creatinine, total cholesterol and triglyceride were measured by enzymatic methods; HDLc was measured by a direct method using specific polyethylene glycol-pretreated enzymes (Roche Diagnostics, Basel, Switzerland).

We calculated LDLc by Friedewald et al.'s formula [11] when triglyceride did not exceed 3.45 mmol/l (300 mg/dl), by dividing total triglyceride (in mmol/l) by 2.17 ($VLDL_c$). When triglyceride was ≥3.45 mmol/l (n=11), we measured LDLc by ultracentrifugation in fresh or frozen serum stored at -80º C for no more than 96 hours, as is the usual procedure in our laboratory. LDLc was also estimated by an equation ("alternative formula"), previously developed by us [12, 13]. The equation (LDLc=0.385 x total cholesterol + 2010 x apoB - 0.342 x triglyceride) includes apoB, triglyceride and total cholesterol concentrations (all in mmol/l, except apoB, in g/l) and has proved to be more accurate than Friedewald et al.'s equation in type 2 diabetic patients [12, 13].

ApoB was measured by an immunoturbidimetric method (Tina-quant, Roche Diagnostics) calibrated against the WHO/IFCC reference standard SP3-07. Lipoprotein (a) was measured by immunoturbidimetry (Roche Diagnostics), with a detection limit of 80 mg/l.

LDL size was detemined by electrophoresis on gradient (2%–16%) polyacrylamide gel, cast in the laboratory, according to the method described by Nichols et al., with modifications [14]. Plasma samples (10 µl) were applied to the gel in a final concentration of 10% sucrose, stained with Sudan black (prepared in the laboratory using ethylene-glycol and 0.1% (w/v) Sudan black, from Sigma). Electrophoresis was performed in a refrigerated cell for a prerun of 60 minutes at 120 V, followed by 30 minutes at 20 V, 30 minutes at 70 V and 16 hours at 100 V. Pooled sera containing 4 LDL fractions whose diameters (22.9±0.7, 24.5±0.6, 26.2±0.5 and 28.4±0.9 nm) had been previously assessed by electron microscopy was used as control. The gels were scanned, and migration distances (from the top of the gel to the most prominent band) were measured. The predominant LDL particle diameter of each sample was calculated from a calibration line using the 4 standards of known diameter. LDL particle subclasses were classified as predominantly small LDL or phenotype B (diameter <25.5 nm) and non-small LDL (phenotype A, diameter ≥25.5 nm) [4]. Both intra- and inter-gel imprecisions were below 1%. $HbA1_c$ was measured by ion-exchange HPLC (Variant, Bio-Rad, Hercules, CA, USA), normal values ranging from 4.6% and 5.8%.

Statistical analyses

Analysis was performed using SPSS 8.0 statistical package for Windows (SPSS, Illinois, USA). Continuous variables were expressed as mean and standard deviation (gaussian distribution) or as median and range; qualitative data were expressed as percentages. Comparison between groups was performed using Student's *t* (gaussian distribution) and Mann-Whitney's U (non-gaussian distribution) tests for quantitative data and chi-squared test for qualitative variables. Tests were two-tailed, and a *p* value below 0.05 was considered significant.

Bivariate correlations were analysed between LDL size and other continuous data. Multivariate analysis, which consecutively included all continuous variables, was performed to ascertain the best independent markers of LDL particle size. Using the regression equations, cut-off points for the diagnosis of phenotype B were calculated for the best markers. Sensitivity, specificity and concordance with the true diagnosis were then assessed using kappa indexes (K). Values between 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.0, showed fair, moderate, good and very good concordance, respectively [15].

Results

We searched for clinical or analytical markers that best predict LDL particle phenotype in a group of 123 type 2 diabetic patients attending a university hospital (Table 1). According to gradient polyacrylamide gel electrophoresis, 79 (64%) of patients had LDL phenotype A while 44 (36%) had phenotype B (Table 1). The two groups defined by LDL phenotype did not differ significantly in clinical or anthropometrical characteristics.

Table 2 shows the lipoproteic parameters of the patients involved in the study, together and according to LDL phenotype. The 44 patients with phenotype B had significantly higher total cholesterol, triglyceride and apoB, but lower HDL_c and LDL_c/apoB ratio than those with phenotype A.

LDL particle size was strongly correlated (r=-0.632, $p<0.0005$) with triglyceride (Fig. 1a). LDL size was more weakly correlated with HDLc (r=0.332, $p<0.0005$), non-HDLc (r=-0.301, $p=0.001$) and apoB (r=-0.202, $p=0.025$). Although LDL size was not correlated with LDLc concentrations, it was correlated with LDLc/apoB ratio (Fig. 1b). The correlation was stronger when the alternative formula was used to calculate LDL_c (r=0.561, $p<0.0005$) than when Friedewald et al.'s equation or ultracentrifugation (as appropriate) was used to estimate LDLc (r=0.436, $p<0.0005$). No significant correlation was found with HbA1c, diabetes duration, albuminuria or serum creatinine, neither was there any correlation with BMI, waist circumference or waist/hip ratio in men or women.

Table 1 Clinical features of the 123 type 2 diabetic patients included in the study

	All patients (n=123)	LDL phenotype	
		A (n=79)	B (n=44)
Women, n (%)	40 (32.5)	24 (30.4)	16 (36.4)
Menopausal women, n (%)[a]	35 (87.5)	20 (83.3)	15 (93.8)
Age, mean (SD), years	59.3 (10.1)	59.9 (10.9)	58.4 (8.4)
BMI, mean (SD), kg/m^2	28.1 (3.7)	27.7 (3.8)	28.7 (3.4)
Hypertension, n (%)	61 (51.3)	37 (48.7)	24 (55.8)
Smoking, n (%)	29 (23.5)	20 (25.3)	9 (20.4)
Diabetes duration, median (range), years	8 (0–37)	10 (0–35)	6 (0–37)
Treatment, n (%)			
Diet only	31 (26.5)	17 (22.1)	14 (38.3)
Oral agents	35 (30.6)	23 (29.9)	13 (31.0)
Insulin	50 (42.9)	36 (46.8)	14 (33.2)
Insulin plus oral agents[b]	8 (6.8)	6 (7.8)	2 (4.8)
Retinopathy, n (%)	43 (34.9)		
Nephropathy, n (%)	60 (49.1)	45 (57.0)	21 (47.7)
Microalbuminuria	48 (42.9)	31 (44.3)	17 (40.5)
Proteinuria	5 (4.5)		
Renal failure	2 (1.7)	3 (4.3)	2 (4.8)
Cardiovascular disease, n (%)	49 (41.5)		
Stroke, n (%)	7 (5.9)	2 (2.9)	0 (0)
Coronary heart disease, n (%)	25 (20.3)	36 (48.0)	
Peripheral vascular disease, n (%)	35 (28.4)	18 (24.0)	6 (13.9)
Hyperuricemia, n (%)	33 (26.8)	22 (27.8)	11 (25.0)

[a] Percent of 40 women

[b] Included in the previous two groups

Table 2 Lipoproteic profiles of the 123 patients included in the study, together and by LDL phenotype. Values are mean (SD) unless otherwise noted

	All patients (n=123)	LDL phenotype	
		A (n=79)	B (n=44)
HbA1c, %[a]	7.4 (5.2–16)	7.4 (5.2–13)	7.5 (5.7–16)
Total cholesterol, mmol/l	5.62 (1.17)	5.39 (1.10)	6.05 (1.19)
Triglyceride, mmol/l[a]	1.41 (0.56–10.5)	1.05 (0.61–3.30)	2.3 (1.0–10.5)*
HDL cholesterol, mmol/l	1.19 (0.29)	1.27 (0.29)	1.05 (0.23)*
VLDL cholesterol, mmol/l[a]	0.65 (0.26–4.48)	0.49 (0.26–1.29)	1.08 (0.46–4.48)*
LDLc, mmol/l			
Friedewald or ultracentrifugation[b]	3.61 (0.93)	3.57 (0.94)	3.68 (0.92)
Alternative formula	3.97 (0.90)	3.98 (0.86)	3.96 (0.98)
Apolipoprotein B, g/l	1.15 (0.25)	1.11 (0.24)	1.24 (0.23)*
Lipoprotein (a), mg/l[a]	274 (<80–1532)	251 (<80–1532)	290 (<80–1505)
LDLc/apoB ratio, mmol/g			
Friedewald or ultracentrifugation[b]	1.20 (0.14)	1.24 (0.12)	1.14 (0.16)*
Alternative formula	1.30 (0.12)	1.39 (0.08)	1.23 (0.20)*
LDL size, nm	25.8 (24.4–27.0)	26.0 (25.5–27.0)	25.2 (24.4–25.5)*

* $p<0.005$ vs. patients with phenotype A

[a] Values are median (range)

[b] Depending on triglyceride concentration

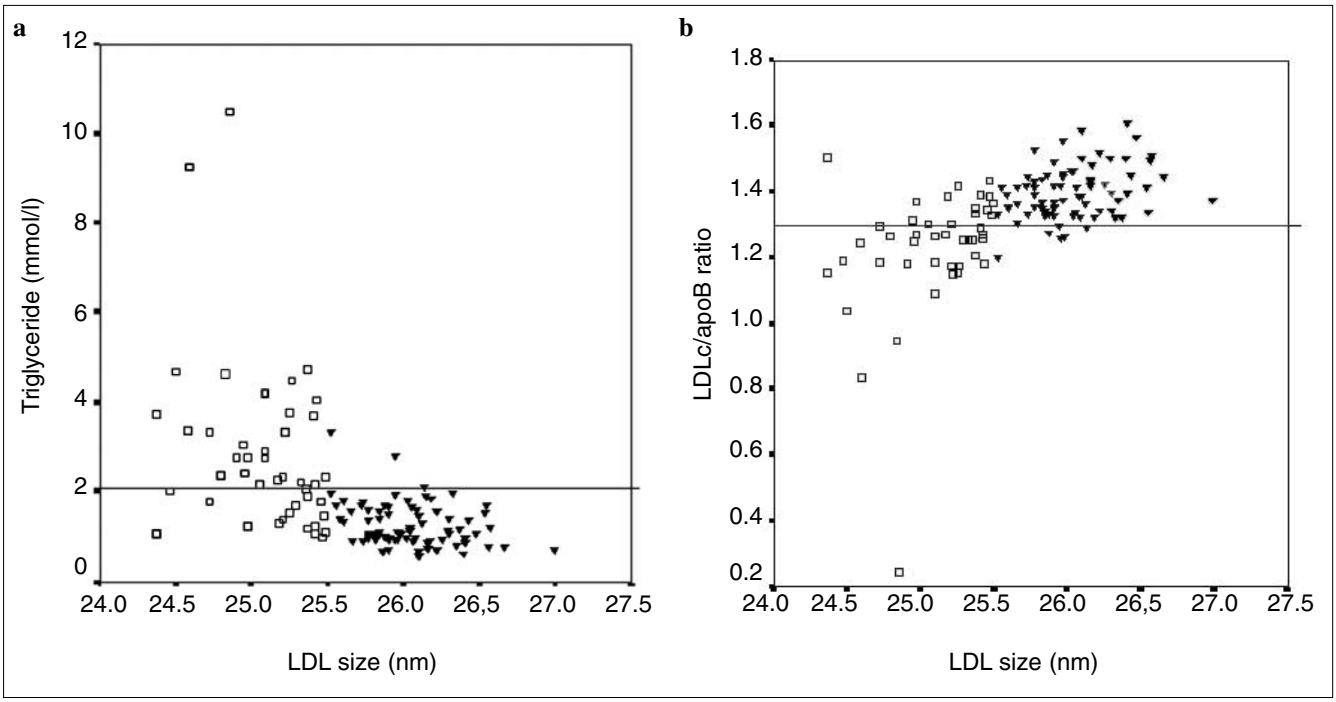

Fig 1a,b Correlations between serum lipids and LD particle size (LDL phenotype) for 123 diabetic patients. **a** Triglyceride concentration vs. LDL size (r=–0.632, $p<0.0005$). **b** LDLc/apoB ratio vs. LDL size, when LDLc was determined using the alternative formula (r=0.561, $p<0.0005$). *Horizontal lines* represent cut-off values to predict LDL phenotype B (LDL size <25.5 nm). ▼ Subjects with phenotype A, □ Subjects with phenotype B

In multivariate analysis, both triglyceride and LDLc/apoB ratio (when LDLc was determined by the alternative formula) were good predictors of LDL particle size. When controlled for triglyceride, LDLc/apoB ratio was no longer significantly correlated with LDL size, whereas triglyceride remained significantly correlated with LDL particle size after controlling for LDLc/apoB ratio. In all patients, triglyceride alone was the best predictor of LDL size, and the addition of other variables did not significantly improve its predictive value.

The LDLc/apoB ratio (when LDLc was determined by the alternative formula), calculated by the regression equation to match an LDL size of 25.5 nm (ratio=0.159 x LDL size - 2.758; $p<0.0005$), produced a cut-off point of 1.297 mmol/g (0.5 in mg/mg), which had a sensitivity of 65.9% and a specificity of 92.4% for the diagnosis of LDL phenotype B (K=0.611). In contrast, the cut-off point obtained for triglyceride, 2.1 mmol/l (triglyceride=40.736-1.515 x LDL size; $p<0.0005$), showed a sensitivity and specificity of 61.4% and 97.5% (K=0.470), respectively. A triglyceride cut-off point of 1.7 mmol/l (recommended by the European Policy Group) [16] showed a sensitivity and specificity of 72.7% and 86.1%, respectively, and a moderate concordance (K=0.591) with LDL phenotype defined by electrophoresis (see Fig. 1a). No significant difference was found in the results after excluding two outliers with triglyceride concentrations above 9 mmol/l (data not shown). Neither the combination of triglyceride and LDLc/apoB ratio (kappa index 0.602), nor the introduction of HDLc, improved the diagnostic value of the ratio.

When classified according to gender, triglyceride remained the best predictor of LDL particle size for males (r=-0.602 vs. r=0.502 for the LDLc/apoB ratio), whereas both parameters were similarly predictive for women (r=-0.684 vs. r=0.689, respectively). Among men, a ratio below 1.288 (ratio = 0.169 x LDL size - 3.022) showed a sensitivity and specificity of 50.0% and 94.5%, respectively (K=0.493). Among women, an LDLc/apoB ratio below 1.308 (ratio=0.150 x LDL size - 2.517) showed a sensitivity of 81.25% and specificity of 91.67% (K=0.737) for the diagnosis of LDL phenotype B. The triglyceride value obtained from the regression equation was similar in men (2.13 mmol/l) and women (2.15 mmol/l), and their diagnostic values did not reach that of the LDLc/apoB ratio in neither gender (K=0.415 and K=0.574, for men and women, respectively).

Discussion

The present study demonstrates that type 2 diabetic patients with good average glycaemic control display a high proportion of LDL phenotype B, which is in agreement with previous studies [2, 7]. Not unexpectedly, LDL size was correlated with triglyceride, HDLc and apoB, although not with

glycaemic control or anthropometric parameters. However, our main finding is that LDLc/apoB ratio is a good predictor of LDL particle size, and the best tool to identify patients with LDL phenotype B. Although LDLc/apoB ratio is just an estimation, and its concordance with LDL particle size is only "good", it may serve as a surrogate marker of LDL size and, thus, be potentially useful in risk assessment and evaluation of response to therapy in type 2 diabetic subjects.

When comparing patients with phenotypes A and B, age and sex distributions were similar, which could be explained by the narrow age-range of the patients in this study, with an absence of very young people, and the fact that most women were post-menopausal. In addition, non-diabetic men display smaller LDL particles than women [2, 17], but in diabetic subjects, no difference is found between genders in the prevalence of small, dense LDL particles [2], in agreement with the present study. Diabetes-dependent parameters were similar, regardless of LDL phenotype, but other components of diabetic dyslipidaemia were evident in the group with phenotype B. Indeed, lipoproteic alterations, rather than diabetic status (duration, glycaemic control, treatment modality) seem to be related to LDL particle size. Some [18, 19], but not all [20] previous studies have shown an association of insulin resistance and glycaemic control [2] with particle size. When found, this association is not independent of the dyslipidaemia that typically coexists with insulin resistance [19], and improvement in glycaemic control is associated with a decrease in triglyceride and an increase in HDLc [2], which are strongly correlated with LDL particle size. Unlike the present finding, microalbuminuria has previously been described to be associated with decreased LDL size. However, this association has also been attributed to coexisting dyslipidaemia, including fasting and post-prandial hypertriglyceridaemia [21].

In multivariate analysis, triglyceride proved to be the best independent predictor of LDL size in this study, on its own explaining 40% of LDL size variance ($r=0.632$, $r^2=0.40$). This is in agreement with previous studies, which have shown triglyceride to explain 20%–50% of LDL particle size in non-diabetic and diabetic subjects [18–20, 22]. The cut-off point obtained for triglyceride to distiguish between phenotypes A and B is close to the goal recommended by the National Cholesterol Education Program and the American Diabetes Association [10, 23]. Nevertheless, the triglyceride cut-off point which best separated both phenotypes was that recommended by the European Policy Group [16], similar to that obtained in previous studies [2, 24], though some authors showed a shift in LDL phenotype when triglyceride exceed concentrations as low as 1.1–1.5 mmol/l (95–130 mg/dl), both in non-diabetic [25] and diabetic subjects [26]. Despite being the best predictor of LDL particle size, an overlap was found in triglyceride concentrations between both LDL phenotypes, making them only a moderate marker of phenotype B. Thus, alternative predictors should be searched for.

LDLc, the main therapeutic goal in the management of dyslipidaemia, is often normal or only slightly increased in type 2 diabetic subjects, and does not give information on LDL particle size, as is also shown in the present study. On the other hand, diabetic dyslipidaemia comprises not only increased triglyceride and low HDLc, but also a high prevalence of hyper-apoB-dependent dyslipidaemic phenotypes, regardless of triglyceride concentrations [3]. Because more than 90% of apoB is on LDL particles, patients with small dense LDL (relatively poor in cholesterol) should be expected to have low LDLc/apoB ratios, as has been described previously [5, 18]. To our knowledge, the LDLc/apoB ratio has not been evaluated as a predictor of LDL particle size in diabetic patients before. In the present study, LDLc/apoB ratio was not as good a marker of LDL size as triglyceride, but proved to be a better diagnostic tool to identify patients with LDL phenotype B. This could be influenced by the biological variability of triglyceride concentration, which is much higher than that of the LDLc/apoB ratio [27]. Men had lower LDLc/apoB ratios for each LDL subfraction in a previous study [5] and in the present study. In women, LDLc/apoB ratio was as good a predictor of LDL particle size as triglyceride, and showed a good concordance with electrophoresis, being the best tool to diagnose phenotype B. Although the availability of a more accurate, maybe direct, method for the determination of LDLc will probably improve the value of LDLc/apoB ratio as a predictor of LDL particle size, the use of an alternative formula for the estimation of LDLc improves the well-known bias of Friedewald et al.'s equation in diabetic subjects [13]. This is supported by the lack of prediction of LDLc/apoB ratio when the method to determine LDLc is affected by high triglyceride concentrations [28, 29].

Finally, the fact that the addition of other lipidic parameters to triglyceride or LDLc/apoB ratio did not improve LDL size estimation is probably because all these disorders are different manifestations of a common lipoproteic derangement in type 2 diabetes.

The results of this study should be borne in mind when deciding upon the indication and choice of optimal therapy for dyslipidaemia in type 2 diabetes, because LDL phenotype provides information for risk stratification, especially useful in patients with borderline LDLc concentrations (100–130 mg/dl) [23]. We conclude that, although triglyceride concentration is the best sole predictor of LDL size in type 2 diabetic patients, an LDLc/apoB ratio below 1.3 mmol/g is the best tool to diagnose phenotype B. This fact is even more evident in women, where 47.5% of LDL particle size variation is explained by this ratio. Thus, the measurement of apoB in type 2 diabetic subjects allows us not only to estimate LDLc more accurately [13] and identify hyper-apoB-dependent dyslipidaemic phenotypes [3], but also to reliably predict the presence of small dense LDL particles.

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