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

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 (HDLc), 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 beta-blockers 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 (VLDLc). 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 determined 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%. HbA1c 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 HDLc and LDLc/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 LDLc (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.