Technical brief

DIRECT LDL-CHOLESTEROL MEASUREMENT IN TYPE 2 DIABETIC PATIENTS

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Direct LDLc in type 2 diabetes

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1 table

1 figure

22 references

LDL-cholesterol (LDLc) is the main therapeutic goal in the treatment of diabetic dyslipidemia (1). Betaquantification, the designated comparison method for the determination of LDLc is cumbersome and time-consuming, and needs an ultracentrifuge and qualified staff (2). Friedewald's equation (3) is the most frequently used method for the calculation of LDLc, but shows unacceptable bias in diabetic patients (4,5,6). We recently described an alternative formula, including apolipoprotein B (apoB), which proved to be more accurate than Friedewald's equation in the estimation of LDLc in diabetic (7) and hypertriglyceridemic patients (8). Direct methods have also been proposed, but none has been widely accepted yet (5,9,10).

We recently assessed LDLc-Plus (Roche Diagnostics, Basel, Switzerland), a newly-described direct homogeneous method (11), and showed that it measures cholesterol associated with IDL and LDL particles, and, after reassignation of the value of the calibrator, produced similar values for LDLc as betaquantification (LDLc-BQ) (12).

The principal aim of the present study was to assess the accuracy of the direct homogeneous assay LDLc-Plus in the evaluation of LDLc in type 2 diabetic patients, and to compare it and the calculations made with Friedewald's and our alternative formula, which includes apoB, with LDLc-BQ.

A total of 86 type 2 diabetic patients with fair average glycemic control (HbA1c 7.76 ± 1.72 %) were studied, after they had given their informed consent. After an overnight fast, blood samples were drawn into tubes containing EDTA. Plasma was separated by centrifugation, and total cholesterol and triglyceride were immediately measured in fresh samples, by enzymatic methods, HDLc, by a direct, homogeneous method, and apolipoprotein B, by immunoturbidimetry (all from Roche Diagnostics, Basel, Switzerland). These concentrations were used for the calculations mentioned. Plasma was kept at -20ºC, for a median of 3 months, until betaquantification and the

measurement of LDLc by the direct method were performed. To estimate LDLc, several methods were used: betaquantification (estimation of LDLc, after separation and measurement of cholesterol in the d< 1.006 Kg/L fraction by ultracentrifugation, and measurement of HDLc by a direct method in the infranatant), Friedewald's formula (3), our alternative formula [LDLc-apoB = (0.385 x total cholesterol) + (2.010 x apoB) - (0.342 x triglyceride)](7) and the direct method LDLc-Plus (cat. no. 1985604; Roche Diagnostics), following the manufacturers instructions, after adding 0.31 mmol/L to the stated value of the calibrator, following the results we previously found (12). With commercial controls containing two different LDL-c concentrations (Precinorm® L and Precipath® H, Roche Diagnostics), LDLc-Plus showed a run-to-run imprecision <2.0%. Freezing is known to affect physical and biological properties of LDL particles (13). Thus, it might interfere with the detergent-based LDLc method. In addition, variations in LDLc-BQ after storage have also been described (14). Since frozen samples were used for LDLc-Plus and LDLc-BQ determination, to correct for this possible effect, LDLc was measured in a set of 15 different samples (triglyceride 0.46-5.31 mmol/L - two samples above 4,51 mmol/l-, LDLc 2.12-4.63 mmol/L) before and after 3 months at -20ºC. A 4% and 2.5% decrease in LDLc-BQ and LDLc-Plus, respectively, was attributable to freezing. These corrections were applied to the measurements obtained in the frozen samples studied.

Analysis was performed using SPSS 8.0 statistical package for Windows (SPSS, Illinois, USA). Quantitative data are expressed as mean and standard deviation (gaussian distribution) or as median and range (non-gaussian distribution). Student's t for paired data was used to compare LDLc by the different methods with LDLc-BQ. Bivariate analysis was performed to find predictors of LDLc-Plus and each method's bias. A regression equation was obtained for each LDLc method, and was used to

calculate the cut-off points equivalent to those recommended for risk stratification by the National Cholesterol Education Program according to LDLc-BQ (15). All tests were two-tailed, and p was considered significant when < 0.05.

The patients' mean concentrations were as follows: apolipoprotein B 1.19±0.23 g/l, total cholesterol 5.62±1.07 mmol/l, triglyceride 1.61 (0.51-10.05; five patients showing triglyceride> 4.51 mmol/l), HDLc 1.17±0.32 mmol/l, and LDLc-BQ 3.69±0.88 mmol/l. All the methods used to measure LDLc produced means which were significantly different from LDLc-BQ ($p<0.0005$ for all). Friedewald's formula (LDLc-Fd) underestimated (3.53±0.95 mmol/l), whereas LDLc-Plus and LDLc-apoB overestimated LDLc (3.85±0.99 and 3.87±0.87 mmol/l, respectively). All LDLc concentrations (LDLc-BQ, LDLc-Fd, LDLc-Plus and LDLc-apoB) were correlated with apoB (r between 0.835 and 0.876), and LDLc-Plus also correlated with VLDLc/triglyceride ratio (R = 0.328). The correlation between each method and LDLc-BQ produced the following regression equations. LDLc-Plus = LDLc-BQx1.087 - 0.163, r = 0.963; LDLc-Fd = LDLc-BQx1.036 - 0.296, r = 0.951; LDLc-apoB = LDLc-BQx0.942 + 0.391, r = 0.950. The confidence interval of the slope included 1 for LDLc-apoB, whereas the confidence interval of the "y"-axis intersection included 0 for LDLc-Fd and LDLc-Plus, which shows that LDLc-apoB bias is constant to LDLc-BQ concentrations, whereas LDLc-Fd and LDLc-Plus show a proportional bias. The bias of each method against LDLc-BQ is shown in figure 1. The LDLc-apoB bias was only correlated with triglyceride (R = -0.452), whereas the bias for LDLc-Plus correlated with triglyceride ($R=0.286$, $p= 0.008$) and apoB ($R= 0.420$, $p< 0.0005$) concentrations, and for LDLc-Fd, with apoB (R = 0.232, p = 0.031). VLDLc/triglyceride ratio was directly and more strongly correlated with bias than any other variable for LDLc-Plus and for LDLc-Fd ($R=0.493$ and 0.665, respectively, $p<0.0005$ for both). As mentioned, LDLc-BQ

concentrations showed significant association with apoB values, but not with VLDLc/triglyceride, these results reflecting the relatively small contribution of IDL mass to LDLc concentrations obtained by this method.

When cut-off points for LDLc recommended by NCEP (15) were calculated with the equations shown above, we obtained the results displayed in table 1. LDL-c Plus produced the best agreement with LDLc-BQ at 2.59 and 3.36 mmol/L, whereas LDLc-Fd was the most accurate measure at 4.13 mmol/L, and LDLc-apoB at 4.92 mmol/L. Although the reference value was included in the confidence interval for all three methods, uncertainty was greater for LDLc-Fd, as is revealed by the dispersion of its values.

Our study analyzed the usefulness of a direct homogeneous method when compared with calculations used for the LDLc estimation. Previous data on direct methods in diabetic patients are disparate: immunofiltration shows advantages over Friedewald's equation in one study (5), but displays a significant overestimation when compared with betaquantification according to another study (9), whereas a detergent-based method has given encouraging results (10). The present study assessed a new detergent-based, direct, homogeneous method known to measure the cholesterol contained in LDL and IDL particles (12,16,17). VLDLc/triglyceride ratio, a known marker of IDL, was the variable most strongly correlated with bias, both for LDLc-Fd and for LDLc-Plus. IDL particles have previously proved to cause an underestimation of LDLc by Friedewald's equation (18), and to cause an overestimation of HDL-cholesterol measured by a direct method which uses a common reagent with the LDLc-Plus assay (19,20). We previously suggested that LDLc-Plus was understandardized, at least in its earlier formulations, and that it only measured 80% of LDLc and 65% of IDLc in non-diabetic subjects (12). The strong correlations found between LDLc-Plus and both apoB and

VLDLc/triglyceride ratio suggest that the cholesterol content of these particles is also measured in diabetic patients, and the correlation of these parameters with LDLc-Plus bias also supports the fact that particle proportion may have a principal effect on this bias. Re-assignement of a new value to the calibrator used in the assay corrected the constant bias found for LDLc-Plus in non-diabetic patients (12); however, in the present study, LDLc-Plus showed a proportional bias. Both facts suggest that the bias of LDLc-Plus in our present samples might be influenced by differences in their relative proportion of IDL and LDL particles, more than to a calibration underassignment. This fact may be amplified in diabetic dyslipidemia, which is known to include an increase in IDL particles. Nevertheless, at low LDLc concentrations (cut-off points of 2.59 and 3.36 mmol/l), at which drug intervention is recommended in diabetic subjects, only LDLc-Plus showed a bias below 4% when compared with LDLc-BQ, whereas LDLc-Fd underestimates LDLc, in agreement with previous data (21,22). At higher LDLc concentrations, LDLc estimated by Friedewald's or our alternative formula seem more accurate than LDLc-Plus. However, at these concentrations, way above the therapeutic goals, this fact becomes clinically irrelevant.

In summary, in type 2 diabetic subjects, LDLc-plus provides estimations of LDLc which are no better than those provided by easily-performed calculations for high LDLc concentrations, but seems to be more accurate at low LDLc concentrations, which are those at which drug intervention is assessed in these high-risk patients. Nevertheless, more studies are needed to confirm these results.

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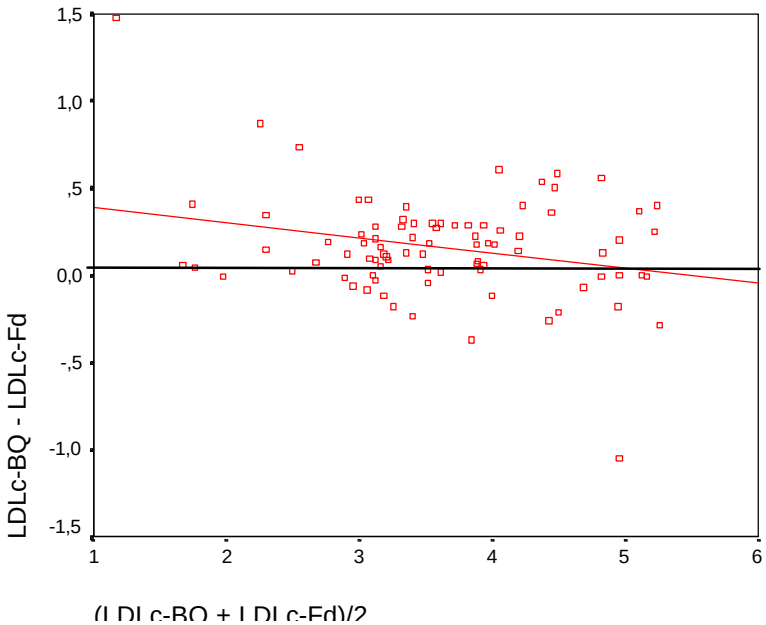
Table 1: Concentrations estimated for the different methods (with their confidence intervals), equivalent to the cut-off points recommended by the National Cholesterol Education Program, as compared with betaquantification.

LDLc-BQ	**2.59**	**3.36**	**4.13**	**4.92**
LDLc-Fd	2.38 (-8.1%)	3.18 (-5.4%)	4.00 (-3.2%)	4.80 (-2.4%)
	(1.92-2.85)	(2.66-3.70)	(3.40-4.56)	(4.16-5.43)
LDLc-apoB	2.83 (+9.3%)	3.55 (+5.6%)	4.27 (+3.9%)	5.02 (+2.1%)
	(2.39-3.25)	(3.07-4.03)	(3.75-4.80)	(4.44-5.60)
LDLc-Plus	2.65 (+2.3%)	3.49 (+3.9%)	4.33 (+4.8%)	5.19 (+5.5%)
	(2.23-3.07)	(3.01-3.96)	(3.80-4.84)	(4.61-5.76)

For abbreviations, see text. Concentrations are all in mmol/L

Fig 1: Comparison of all three methods with LDLc-BQ.

1a



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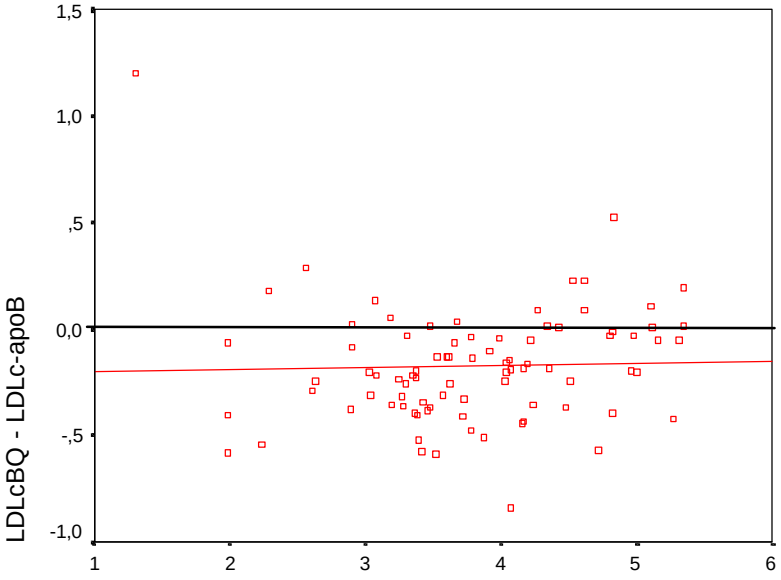

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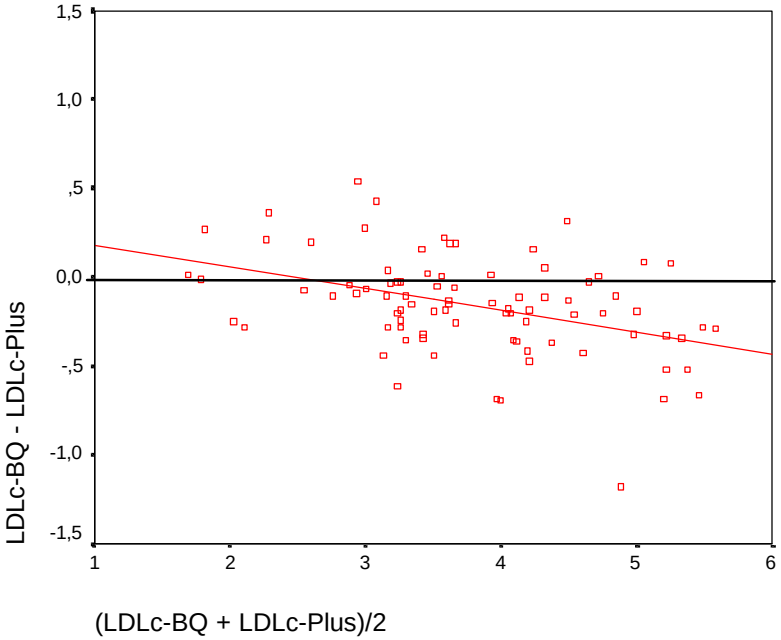

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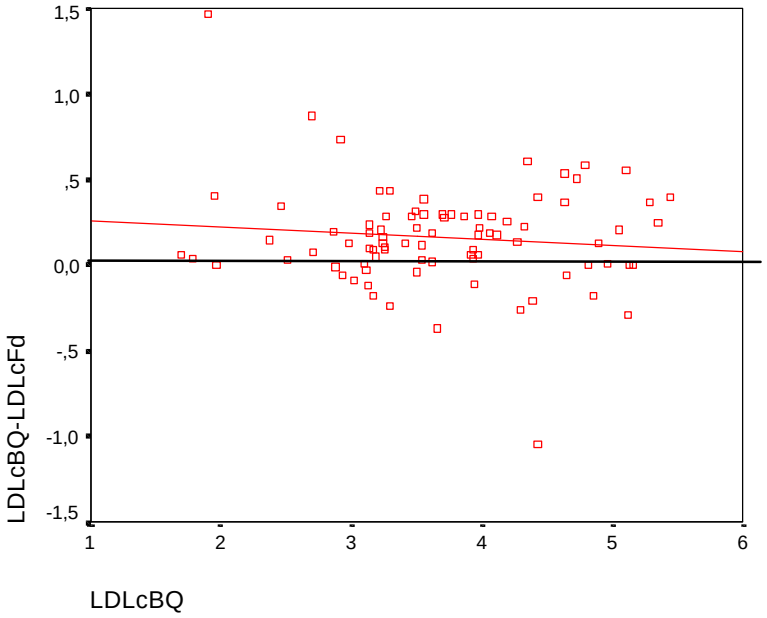

r = -0.106
p < 0.05

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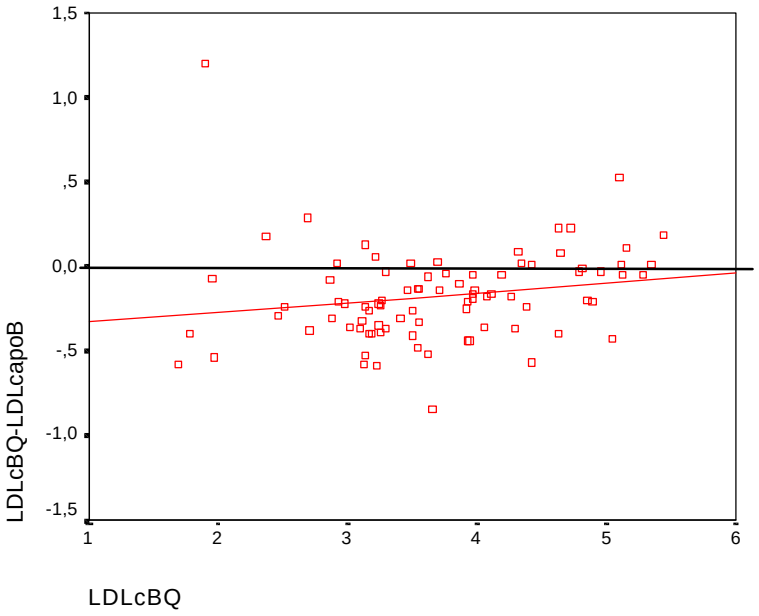

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p > 0.05

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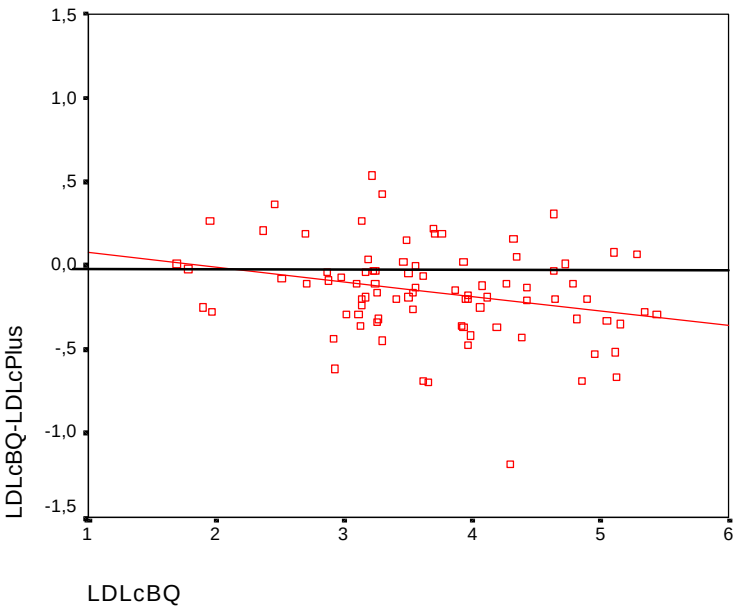

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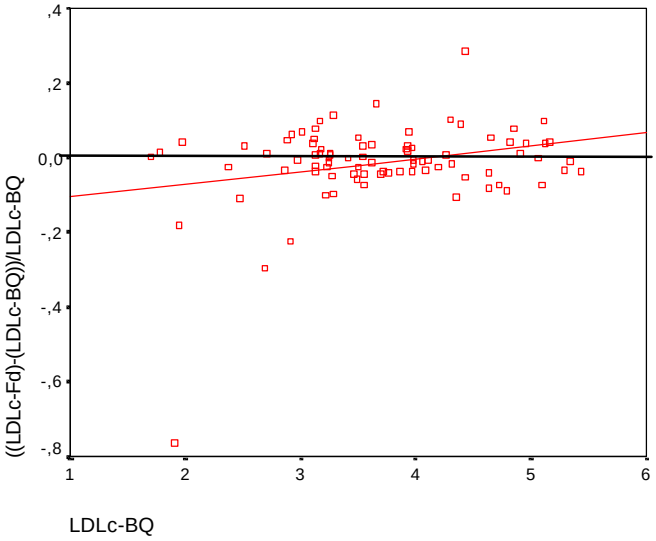
1a



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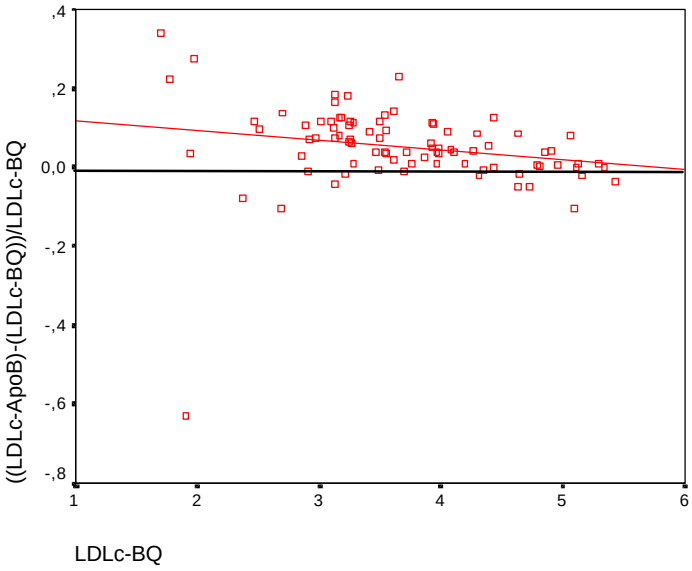
1b



r = -0.202
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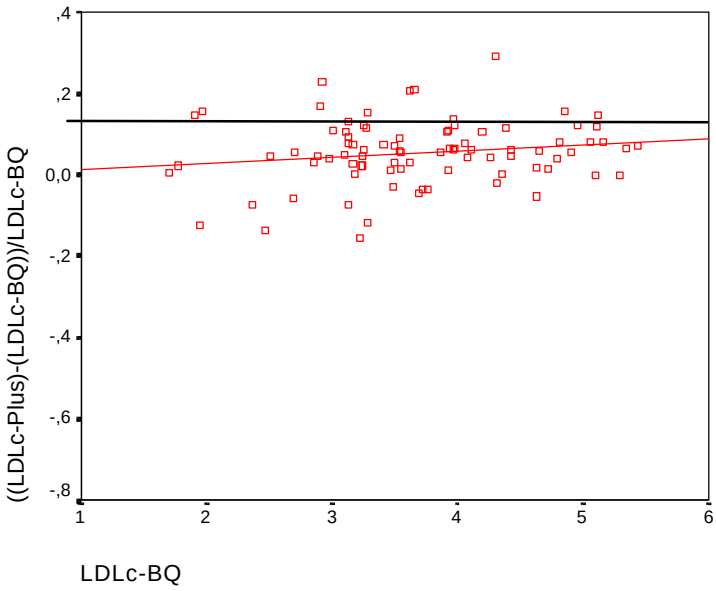

r = 0.161
p = 0.13

LDL-CHOLESTEROL/APOLIPOPROTEIN B RATIO IS A GOOD PREDICTOR OF LDL PHENOTYPE B IN TYPE 2 DIABETES.

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Predictors of LDL size in type 2 diabetes

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2 tables

1 figure

29 references

Abstract

Aim. LDL phenotype B is a component of diabetic dyslipidaemia, but its diagnosis is cumbersome. Our aim is to find easily available markers of phenotype B in a group of type 2 diabetic subjects.

Methods. We studied 123 type 2 diabetic patients (67.5% male, age 59.3±10.1 years, HbA1c 7.4% (5.2-16)). Clinical features and fasting total cholesterol and triglyceride, HDL cholesterol, LDL cholesterol (Friedewald's equation and an alternative formula), apolipoprotein B, lipoprotein (a) and LDL particle size (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).

Results. Patients with phenotype B (36%) showed higher total cholesterol, triglyceride and apolipoprotein B, and lower HDL cholesterol and LDLcholesterol/apolipoproteinB ratio. Triglyceride was the best predictor of LDL particle size (r -0.632, p<0.01), but an LDLcholesterol/apolipoproteinB ratio below 1.297 mmol/g detected phenotype B best (sensitivity 66%, specificity 92%, kappa: 0.611).

Conclusions. 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.

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 much 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 gel, 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], or 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 is to search for the markers their combination, which best predict LDL particle phenotype among easily available clinical and analytical variables in a group of type 2 diabetic subjects.

RESEARCH DESIGN 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 being in situations known to affect lipid metabolism, unrelated to their diabetes. Patients with hypertension were not treated with non-selective betablockers or high-dose diuretics. Anamnesis 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]. Their main clinical features are displayed in table 1.

Laboratory determinations:

Total cholesterol and triglyceride were measured by enzymatic methods, and HDLc, by a direct method, using polyethylene-glycol pretreated enzymes to specifically measure HDLc (Roche Diagnostics, Basel, Switzerland).

We calculated LDLc by Friedewald's formula [11] when triglyceride did not exceed 3.45 mmol/L (300 mg/dl), dividing total triglyceride (in mmol/L) by 2.17. When triglyceride were ≥3.45 mmol/l (N = 11), we measured LDLc by ultracentrifugation in fresh or frozen serum stored at -80ºC for no more than 96 hours, as is the usual procedure in our laboratory. LDLc was also estimated by an equation, previously developed by us [12,13], which includes apoB, triglyceride and total cholesterol (all in mmol/l, except apoB, in g/l) (LDLc = 0.385 x total cholesterol + 2,010 x apoB - 0.342 x triglyceride) and which has proved to be more accurate than Friedewald'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 80mg/l.

LDL size was detemined by electrophoresis on gradient (2-16%) polyacrylamide gel, cast in the laboratory, according to the method described by Nichols, with modifications [14]. A volume of 10µl of plasma samples was applied on lanes 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 120V, followed by 30 minutes at 20V, 30 minutes at 70V and 16 hours at 100V. 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 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%.

Statistical analysis

Analysis was performed using SPSS 8.0 statistical package for Windows (SPSS, Illinois, USA). Continuous variables are expressed as mean and standard deviation (gaussian distribution) or as median and range, and qualitative data, as percentages. Comparison between groups was performed using Student's t (gaussian distribution) and Mann-Whitney's U test (non-gaussian distribution) for quantitative data and chi-squared 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. Then, their sensibility, specificity and concordance with the true diagnosis were assessed using kappa indexes (K). Values between 0.21-0.40, 0.41-0.60, 0.61-0.80 and 0.81-1.0, show fair, moderate, good and very good concordance, respectively [15].

RESULTS

Table 2 shows the lipoproteic parameters of the patients involved in the study, and the differences in clinical and analytical features between subjects with and without phenotype B. The 36% of patients (N = 44) with phenotype B differed from the rest in no clinical or anthropometrical, but only lipidic features. On the other hand, LDL particle size was found to be strongly correlated (r -0.632, p<0.0005) with triglyceride, and more weakly 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 with LDLc/apoB ratio (r 0.436, p<0.0005 using Friedewald's equation/ultracentrifugation to estimate LDLc), especially when the alternative formula was used to calculate LDLc (r 0.561, p<0.0005). No significant correlation was found

with HbA1c, nor with diabetes duration, albuminuria or serum creatinine. Neither was there any correlation with BMI, waist circumference or waist/hip ratio in men or women.

In multivariate analysis, both triglyceride and LDLc/apoB ratio (LDLc by the newly-proposed 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, 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 (by the newly-proposed 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), whereas 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 figure 1). 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 and 0.689, respectively). In the male group, a ratio below 1.288 (ratio = 0.169 x LDLsize -3.022) showed a sensitivity and specificity of 50 and 94.5%, respectively (K 0.493). In the female group, an LDLc/apoB ratio below 1.308 (ratio = 0.150 x LDL size -2.517) showed 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 value did not reach that of the LDLc/apoB ratio in neither gender (K 0.415 and 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 distribution 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 postmenopausal. 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 it is 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 what the present study shows, 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 postprandial 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 show 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 is 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, 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 are known to show lower LDLc/apoB ratios for each LDL subfraction in previous [5] as 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's equation in diabetic subjects [13]. The lack of prediction of LDLc/apoB ratio in other studies which use methods to determine LDLc which are interfered by high triglyceride concentrations support this fact [28,29].

Finally, the fact that the addition of other lipidic parameters to triglyceride or LDLc/apoB ratio does 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 born 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 may 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 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 hyperapoB-dependent dyslipidaemic phenotypes [3], but also to reliably predict the presence of small dense LDL particles.

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Table 1: Main clinical features of the 123 type 2 diabetic patients included in the study.

Male/female (%)	67.5/32.5
Age (years)	59.3±10.1
BMI (Kg/m^2)	28.1±3.7
Menopause (%, only women included)	87.2
Hypertension (%)	51.3
Smoking (%)	23.5
Diabetes duration (years)	8 (0-37)
Treatment (%):	
Diet only	26.5
Oral agents	30.6
Insulin	42.9
Insulin plus oral agents	6.8
Retinopathy (%)	34.9
Nephropathy (%)	49.1
Microalbuminuria	42.9
Proteinuria	4.5
Renal failure	1.7
Cardiovascular disease (%)	37.6
Stroke (%)	5.9
Coronary heart disease (%)	20.3
Peripheral vascular disease (%)	28.4

Table 2: Main laboratory results of all the patients included in the study and differences in clinical and metabolic features according to LDL phenotype.

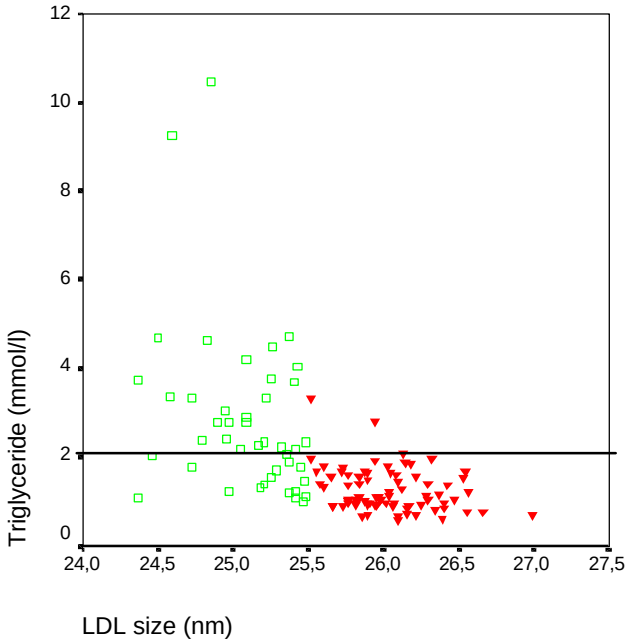
	All patients N = 123	Phenotype A N = 79	Phenotype B N = 44
Age (years)		59.9±10.9	58.4±8.4
Male/female (%)		70/30	64/36
Menopause (%)		83	94
BMI (Kg/m^2)		27.7±3.8	28.7±3.4
Diabetes duration (years)		10 (0-35)	6 (0-37)
Insulin treatment (%)		55	39
Smoking (%)		25	21
Hypertension (%)		49	56
Hyperuricemia (%)		28	25
Nephropathy (%)		57	48
HbA1c (%)	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)	1.41 (0.56-10.5)	1.05 (0.61-3.30)	2.3 (1.0-10.5)$^{+}$
LDLc (mmol/l) -Friedewald/ultracentrifugation -Alternative formula	 3.61±0.93 3.97±0.90	 3.57±0.94 3.98±0.86	 3.68±0.92 3.96±0.98
HDL cholesterol (mmol/l)	1.19±0.29	1.27±0.29	1.05±0.23^{+}
VLDL cholesterol (mmol/l)	0.65(0.26-4.48)	0.49(0.26-1.29)	1.08(0.46-4.48)$^{+}$
Apolipoprotein B (g/l)	1.15±0.25	1.11±0.24	1.24±0.23^{+}
Lipoprotein (a) (mg/l)	274 (<80-1532)	251 (<80-1532)	290 (<80-1505)
LDLc/apoB ratio (mmol/g)			

-Friedewald/ultracentrifugation -Alternative formula	1.20±0.14 1.30±0.12	1.24±0.12 1.39±0.08	1.14±0.16 [+] 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) [+]

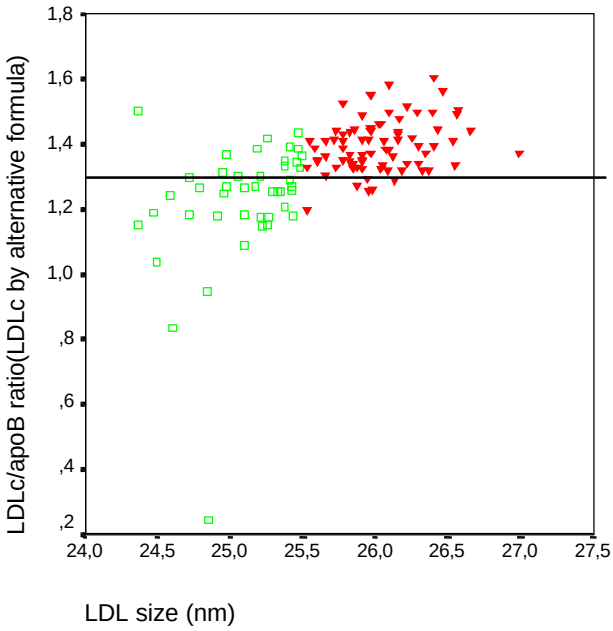
[+] all showing $p<0.005$ vs patients with phenotype A.

Fig 1: Correlation between triglyceride and LDL size (a) and between LDLc/apoB ratio and LDL size (b). Phenotype A. Phenotype B.

1a


Triglyceride (mmol/l)
12
10
8
6
4
2
0
24,0
24,5
25,0
25,5
26,0
26,5
27,0
27,5
LDL size (nm)

1b


LDLc/apoB ratio(LDLc by alternative formula)
1,8
1,6
1,4
1,2
1,0
,8
,6
,4
,2
24,0
24,5
25,0
25,5
26,0
26,5
27,0
27,5
LDL size (nm)

POSTPRANDIAL LIPIDAEMIA IS NORMAL IN NON-OBESE TYPE 2 DIABETIC PATIENTS WITH INSULIN RESISTANCE BUT PRESERVED INSULIN SECRETION.

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Postprandial lipidaemia in diabetes

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2894 words

2 tables

2 figures

ABSTRACT:

Objective: To assess postprandial lipidaemia in normotriglyceridaemic type 2 diabetic patients treated with diet.

Research design and methods: We included 12 non-obese, normotriglyceridaemic, normocholesterolaemic type 2 diabetic patients (8 male, HbA1c 6.80±0.67%) treated with diet only, and 14 controls of similar age, BMI and fasting triglyceride (Tg). A meal (58g fat, 100.000 IU vitamin A) was given. LDL cholesterol (LDLc), HDL cholesterol (HDLc) and apolipoprotein B were measured in fasting, and Tg, retinylpalmitate (RP), LDL size, glucose and insulin, in fasting and postprandial samples. The homeostasis assessment model (HOMA) index and lipoprotein (Lpl) and hepatic (HL) lipase activities were estimated.

Results: Patients showed lower fasting HDLc than controls (1.12±0.26 vs 1.40±0.28 mmol/l, p=0.02) and a trend towards smaller LDL particles, significant 4 hours postprandially (25.86±0.40 vs 26.16±0.30 nm, p=0.04). The area under the curve of Tg (AUC-Tg) and of RP, and Lpl activity were similar, but HL activity was higher in patients (156.63±23.89 vs 118±43.27 U/l, p=0.011). Tg at 4-5 hours postprandially were the best predictors of the AUC-Tg (r 0.944 and 0.949, respectively, $p < 0.01$). Hepatic lipase was inversely correlated with LDL size, and directly correlated with the HOMA index.

Conclusions: Normolipidaemic type 2 diabetic patients with insulin resistance but relatively preserved insulin secretion show low fasting HDLc and increased hepatic lipase activity, but normal postprandial lipidaemia.

Key words: Postprandial lipidaemia, type 2 diabetes, normotriglyceridaemic, LDL size, non-obese, insulin resistance, retinyl-palmitate, hepatic lipase.

Patients with type 2 diabetes have an increased risk of developing cardiovascular events which is not completely explained by classical risk factors (1). Diabetic dyslipidaemia, which mainly comprises modest hypertriglyceridaemia, low HDL cholesterol (HDLc) and normal or slightly increased LDL cholesterol (LDLc) has been proposed as an additional candidate to explain this high risk (2). Other disorders which also have been considered to be components of diabetic dyslipidaemia include hyperapoBlipoproteinaemia (3), predominance of small dense LDL particles and postprandial hyperlipidaemia (2). The latter has not only proved to be associated with coronary heart disease in several cross-sectional studies (4-6), but studying the postprandial state may also provide information on lipid metabolism and the other components of diabetic dyslipidaemia which is not available in the fasting state.

Studies on postprandial lipidaemia performed on diabetic subjects provide conflicting results. Fasting triglyceride is closely related to, and the main determinant/consequence of postprandial lipidaemia (7), and many studies which show increased postprandial triglyceridaemia in diabetic subjects also show higher fasting concentrations (8-10). Nevertheless, when patients and control subjects are matched for age, body mass index (BMI) and fasting triglyceride concentrations, most studies show comparable areas under the curve (AUC) of triglyceride (8,11-13) and retinyl-palmitate, (10), but display minor abnormalities in diabetic patients (11,13). In addition, some of the drugs used in the treatment of diabetes may also influence postprandial lipidaemia.

The aim of this study was to define postprandial lipidaemia, and its relationship with other components of diabetic dyslipidaemia in a specific group of normotriglyceridaemic, type 2 diabetic patients, well-controlled with diet only.

RESEARCH DESIGN AND METHODS

Patients:

A total of 12, non-obese (BMI < 30 Kg/m^2), normolipidaemic (LDLc < 4,13 mmol/l and triglyceride < 2,25 mmol/l) type 2 diabetic patients with HbA1c < 8%, treated with diet only, were

included in the study. Their main features are displayed in table 1. Patients taking drugs or being in situations known to interfere with lipoprotein metabolism were excluded (patients with established nephropathy, or taking lipid-lowering drugs, steroids, non-selective beta-blockers or high-dose diuretics). Postmenopausal women on replacement therapy were not excluded. None of the patients or controls was taking vitamin supplements.

A group of 14 non-diabetic normolipidaemic, non-obese, control subjects of similar age, body mass index and fasting triglyceride, was also included. Women with a history of gestational diabetes were excluded. Their main features are displayed in table 1.

A history was taken, which included cardiovascular risk factors in the case of the control subjects, and also diabetes duration and complications in the patients. Physical examination, which included anthropometric parameters and blood pressure, was performed. A test meal was given, and laboratory determinations were made.

The protocol was approved by the local Ethics Committee and all of the subjects signed written, informed consent before being included in the study.

Test meal:

After a 10-12-hour overnight fast, a peripheral intravenous catheter was inserted and a first blood sample was obtained in Vacutainer® tubes (Beckton Dickinson), containing EDTA or fluoride.

A test meal was given to be ingested in 20 minutes. It consisted of 600 ml of vanilla- or nut-flavoured shake (NEPRO®, Abbot Laboratories, Columbus, USA), and contained 1200 Kcal as fat (58g), protein (42g) and carbohydrate (134g). A total of 100.000 units of vitamin A (Dif Vitamin A, Roche ®) were given with the meal in the form of a pill.

Patients were asked not to perform unusual exercise or drink alcohol the day before the test. Water was allowed, but no other beverages or food were permitted during the test. Walking, but no strenuous exercise or smoking were allowed, either. Periodic blood samples were taken during the

eight hours following the meal. The tubes were covered with foil, and all handling was done away from direct light.

Laboratory tests:

Total triglyceride and cholesterol, LDL, HDL and VLDL cholesterol (VLDLc), apolipoprotein B (apoB), free fatty acids, LDL particle size, retinyl-palmitate, HbA1c, insulin and glucose, were determined in the fasting state. Insulin and glucose were also measured at 1,2,3,4,5 and 6 hours after the meal. Retinyl-palmitate, triglyceride and LDL particle size were measured at 2,3,4,5,6 and 8 hours after the meal.

Total triglyceride and cholesterol and free fatty acids were measured from fresh plasma by automatic enzymatic methods. Net triglyceride were calculated, after measuring and substracting glycerol concentrations. LDLc and VLDLc were determined by betaquantification, and HDLc by a direct method. ApoB was measured by an immunoturbidimetric method calibrated against the WHO/IFCC reference standard SP3-07. Glucose was measured in plasma by the glucose oxidase method, and samples taken in tubes containing fluoride and kept at 4ºC for no more than two hours before separation (all automated measurements from Roche Diagnostics, Basel; Switzerland). Insulin was determined by immunochemoluminiscence (Immulite 2000®, Diagnostic Products Corp, Los Angeles, CA, USA), with a lower detection limit of 14,3 pmol/l. HbA1c was measured in an automatic DCA 2000 reader (Bayer, Elkhart, IN, USA, ref nº 5035B; latex immunoagglutination inhibition; normal values 4.3-5.7%), following the instructions provided by the manufacturer. The homeostasis assessment model (HOMA) was used to estimate insulinresistance (14). Retinyl palmitate was determined by HPLC, using retinyl acetate as an internal standard, following the method described by Ruotolo (15). Total areas under the curves and incremental areas above baseline were calculated for triglyceride and retinyl-palmitate by the trapezoid rule (16).

LDL size was detemined by electrophoresis on gradient (2-16%) polyacrylamide gel, cast in the laboratory, according to the method described by Nichols, with modifications (17). A volume of 10µl of plasma samples was applied on lanes 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 120V, followed by 30 minutes at 20V, 30 minutes at 70V and 16 hours at 100V. 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 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%.

Lipoprotein lipase and hepatic lipase activities were determined at baseline and 15 minutes after the intravenous administration of 100 IU/Kg of sodium heparin. This test was performed on a different day, separated by at least 48 hours from the meal test. Blood was drawn into chilled lithium-heparin tubes kept on ice, and plasma was immediately separated and kept at -80ºC until processing. Lipoprotein lipase and hepatic lipase activities against artificial substrates were measured in post-heparin plasma using a radiolabelled glycerol tri[9,10-(n)-^{3}H]oleate emulsion (Amersham Life Science, Bristol, UK) (18).

Statistical analysis

Analysis was performed using SPSS 8.0 statistical package for Windows (SPSS, Illinois, USA). Quantitative data are expressed as mean and standard deviation (gaussian distribution) or as median and range (non-gaussian distribution), and qualitative data, as percentages. Comparison between groups was performed using Student's t (gaussian distribution) and Mann-Whitney's U

test (non-gaussian distribution) for quantitative data and chi-squared for qualitative variables. Comparisons within a group were made using Student's t for paired data or Wilcoxon's test. Bivariate correlations were analysed between continuous data (Pearson's r o Spearman's Rho depending on normality distribution). Multivariate analysis was performed to ascertain the best independent markers and combination of markers of the AUC of triglyceride and retinyl-palmitate. Partial correlations were used to correct for interference between variables with high colineality.

Results

Fasting laboratory results in patients and control subjects are displayed in table 2. Lower HDLc in the diabetic patients was the only lipidic component which was significantly different from the control group. Postprandial glucose was, as expected, higher in patients than in controls, whereas no differences were found in postprandial insulin concentrations between the groups (see figures 1a and b). Postprandial triglyceride and retinyl-palmitate curves are depicted in figures 2a and 2b. The AUC of triglyceride (10.77 (5.73-24.13) vs 12.61 (6.57-22.77) mmol/l x h, respectively), and peak triglyceride concentration (1.85± 0.64 vs 1.94±0.80 mmol/l) were similar for patients and controls. The AUC of retinyl-palmitate was also similar between the groups (2291.25 (1380.5-4721) vs 2733.25 (1795.5-7717) ng/ml x h, respectively). Although retinyl-palmitate concentrations at baseline and 2 hours after the meal were higher in the diabetic patients (see figure 2b), their incremental areas under the curve for retinyl-palmitate were similar. Postprandial LDL size in patients and controls are displayed in figure 2c. No differences were found between postprandial and fasting LDL sizes within the diabetic group, but a small reduction of LDL size was found in the control group 2 hours after the meal (26.33 (25.34-26.7) vs 26.23 (25.34-26.56), p = 0.04). Nevertheless, diabetic patients showed a trend towards smaller LDL size than control subjects, both at baseline (p=0.069) and postprandially (0.13, 0.15, 0.041, 0.057, 0.19 and 0.20, at

2,3,4,5,6 and 8 hours, respectively). No differences were found in lipoprotein lipase, whereas hepatic lipase activity was higher in diabetic patients (see table 2).

The AUC of triglyceride in diabetic patients was correlated with retinyl-palmitate at 5 (r=0.657, p=0.020) and 8 hours (r=0.620, p=0.020) and all postprandial triglyceride concentrations. It was best correlated with triglyceride 4 hours after the meal (R 0.967, p< 0.0005), whereas fasting triglyceride concentrations showed a somewhat weaker correlation (r = 0.844, p<0.0005). In multivariate analysis, the combination of triglyceride and retinyl-palmitate at 5 hours produced the best correlation with AUC of triglyceride (r 0.990, p<0.0005), and triglyceride at 5 hours remained correlated even after adjusting for retinyl-palmitate at 5 hours (r 0.982, p <0.005). The AUC of retinyl-palmitate was correlated with fasting free fatty acids (R 0.599, p = 0.040) and retinyl-palmitate (R = 0.741, p = 0.006) and with all postprandial retinyl-palmitate concentrations between 2 and 6 hours after the meal, but most strongly with its concentrations at 3 and 4 hours postprandially (R = 0.881 and 0.916, respectively, p<0.0005).

When all subjects (patients and controls) were included into the analysis, fasting LDL particle size correlated with LDLc/apoB ratio (r 0.512, p 0.008), but no correlation was found in the fasting or postprandial states between LDL size and triglyceride concentrations. Hepatic lipase activity was inversely correlated with LDL size at baseline (r = -0.376, p=0.058), 2 (r = -0.441, p = 0.024), 3 (r = -0.40, p = 0.043), 4 (r = -0.422, p = 0.032) and 5 hours (r = -0.473, p = 0.015) after the meal, and directly correlated with the HOMA index (R = 0.455, p<0.05).

Discussion

Differences between patients and control subjects

This specific group of normotriglyceridaemic type 2 diabetic patients, with insulin resistance but with a relatively preserved insulin secretion, show lower HDLc concentrations than the control group. However, their postprandial lipidaemia, quantified as the AUC of plasma triglyceride and retinyl-palmitate, is normal. This supports the idea that postprandial lipidaemia, in diabetic as well

as in non-diabetic subjects, is closely related to fasting triglyceride concentrations, either as a predictor or as a consequence of postprandial changes in triglyceride. Although HDLc concentrations have been described as predictors of postprandial lipidaemia, non-diabetic subjects with isolated low HDLc have normal postprandial lipidaemia, as do the type 2 diabetic patients included in the present study (19). Although some previous studies performed in type 2 diabetes show increased postprandial lipidaemia in these patients, groups are not always matched for interfering factors (8-10). The patients included in the present study show very specific features, due to the selection criteria used to minimise the influence of associated variables. Non-obese patients with normal fasting triglyceridaemia, well controlled without the need of oral agents or insulin were selected. As a result, the study group comprises 12 "mildly" diabetic patients with insulin resistance, but relatively preserved insulin secretion, who displayed normal postprandial lipidaemia. However, only whole plasma was assessed, and retinyl-palmitate, but not apolipoprotein B48, was measured. Even in the absence of gross postprandial whole plasma differences, minor changes are seen in diabetic patients in chylomicron remnant fractions, (11,13) which were not measured in this study. Although retinyl-palmitate is not the ideal marker for the evaluation of chylomicrons and their remnants (20), it has been the most frequently used determination until recently. On the other hand, qualitative disorders have been described in type 2 diabetic patients both in LDL and HDL particles (11,13), and a trend towards smaller LDL particles was seen in the diabetic patients included in the present study. There are, to our knowledge, only two previous studies which assess postprandial LDL size in diabetic patients. In disagreement with our results, one study displays a postprandial shift in LDL size towards smaller denser particles 4-6 hours after a meal in normotriglyceridaemic, normocholesterolaemic type 2 diabetic patients, despite fasting features which are similar to those of control subjects (11). The other study, in consonance with ours, shows a good correlation between both fasting and postprandial LDL size and hepatic lipase activity, but, on the other hand, shows no differences

between normotriglyceridaemic diabetic subjects and controls (8). Lipoprotein lipase activity did not differ between the groups included in our study, in agreement with previous data (7,8), but a higher hepatic lipase activity in the diabetic subgroup, and its correlation with LDL size in the total of subjects, suggests an influence on LDL size. Furthermore, the correlation of hepatic lipase with insulinresistance, which has also been described previously, especially in the presence of low HDLc (21,22), might reflect a link between insulinresistance and the predominance of small LDL particles. Nevertheless, whether enrichment of LDL in triglyceride, despite similar fasting and postprandial triglyceride, stimulates hepatic lipase, or whether enzymatic activity, per se, related to insulin resistance, causes a decrease in size in LDL particles in type 2 diabetes is still to be determined. However, the trend towards higher fasting triglyceride seen in the diabetic patients supports the former hypothesis.

Markers of postprandial lipidaemia

Triglyceride at 4 and 5 hours postprandially were the best predictors of the AUC-tg. To our knowledge, this is the first study to assess postprandial markers of the AUC of triglyceride in order to avoid the performance of this cumbersome test in type 2 diabetic patients. The complexity of postprandial studies does not allow the inclusion of large samples of patients, which on the other hand limits the conclusions that can be drawn from them. Notwithstanding, the measurement of serum triglyceride 4 or 5 hours after a standard test meal would give reliable information about the postprandial state in these subjects, and would be less cumbersome than the performance of a complete meal test. Alternatively, capillary triglyceride measurements have been suggested, which allow more patients to be studied (23).

No consistent correlations were found at any point between LDL size and triglyceridaemia, probably because the subjects included were all normotriglyceridaemic, and most displayed an LDL phenotype A (11). On the other hand, LDLc/apoB ratio proved to be the best and most consistent predictor of LDL size. This ratio has previously been evaluated as a marker of LDL

particle size, but the results from different studies disagree, mainly depending on the accuracy of the method used for the determination of LDLc (24-27). In the present study, where betaquantification, which is the designated comparison method for the estimation of LDLc, was used, a fair correlation was found between LDLc/apoB ratio and LDL size.

In summary, normotriglyceridaemic, non-obese subjects with "mild" type 2 diabetes (well controlled with diet alone, insulinresistant but with relatively preserved insulin secretion), show low HDLc concentrations and increased hepatic lipase activity, but normal postprandial lipidaemia. Thus, insulin resistance by itself does not seem to be sufficient to cause postprandial lipidemia, as long as insulin secretory capacity is preserved.

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Table 1: Clinical features of the type 2 diabetic patients and control subjects.

	Type 2 diabetic patients	**Non-diabetic controls**
N (Male/female)	12 (8/4)	14 (8/6)
Age (years)	52.42±10.36	54.0 ± 6.14
Body mass index (Kg/m^2)	25.77±2.64	24.31±2.40
Waist circumference (cm)	91.58±2.99	85.82±3.2
Waist/hip ratio	0.97 (0.78-1.00)	0.87 (0.76-1.00)
Diabetes duration (years)	3.5 (2-13)	-
HbA1c (%)	6.80±0.67*	5.41±0.34
Smoking	5	2
Hypertension	5	4
Menopause	1†	6
HRT	0	1
Retinopathy	0	-
Microalbuminuria	1	-
Polyneuropathy	0	-
Coronary heart disease	3	0
Cerebrovascular disease	1	0
Peripheral vascular disease	1	0

Qualitative variables are expressed as number of subjects affected, and continuous variables are expressed as mean ± standard deviation (gaussian distribution) or as median (range) (non-gaussian distribution). *$p< 0.0005$ and † $p<0.05$ vs controls. HRT: Hormone replacement therapy.

Table 2: Main results obtained in the type 2 diabetic patients and control subjects.

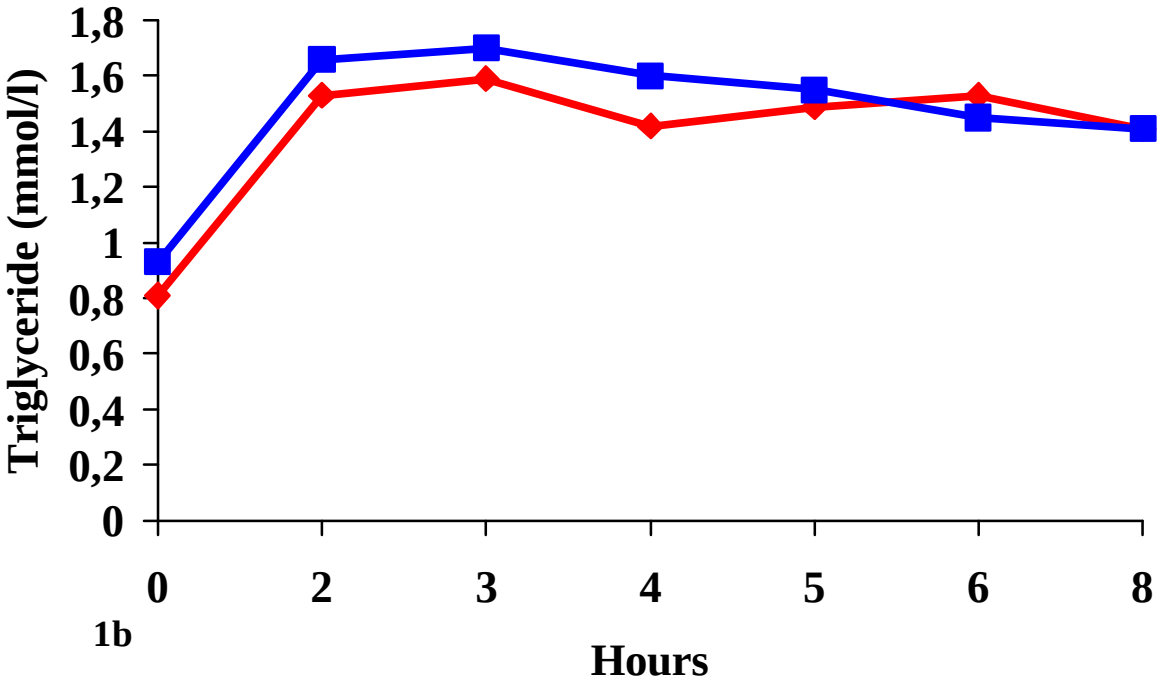
	Type 2 diabetic patients	**Non-diabetic controls**
Fasting triglyceride (mmol/l)	0.92±0.31	0.81±0.20
Total cholesterol (mmol/l)	4.71±0.85	4.99±0.64
HDLc (mmol/l)	1.12±0.26*	1.40±0.28
LDLc (mmol/l)	3.25±0.72	3.28±0.60
VLDLc (mmol/l)	0.32±0.17	0.30±0.11
Apolipoprotein B (g/l)	0.95±0.22	0.95±0.13
Free fatty acids (mmol/l)	0.50±0.16	0.42±0.13
LDL size (nm)	25.90±0.39	26.19±0.38
HOMA index	4.90 (3.91-14.87) †	3.61 (3.02-10.86)
Lipoproteinlipase (U/l)	106.41±30.73	90.03±24.99
Hepatic lipase (U/l)	156.63±23.89*	118±43.27

Qualitative variables are expressed as number of subjects affected, and continuous variables are expressed as mean ± standard deviation (gaussian distribution) or as median (range) (non-gaussian distribution). * $p<0.05$ and † $p< 0.0005$ vs controls.

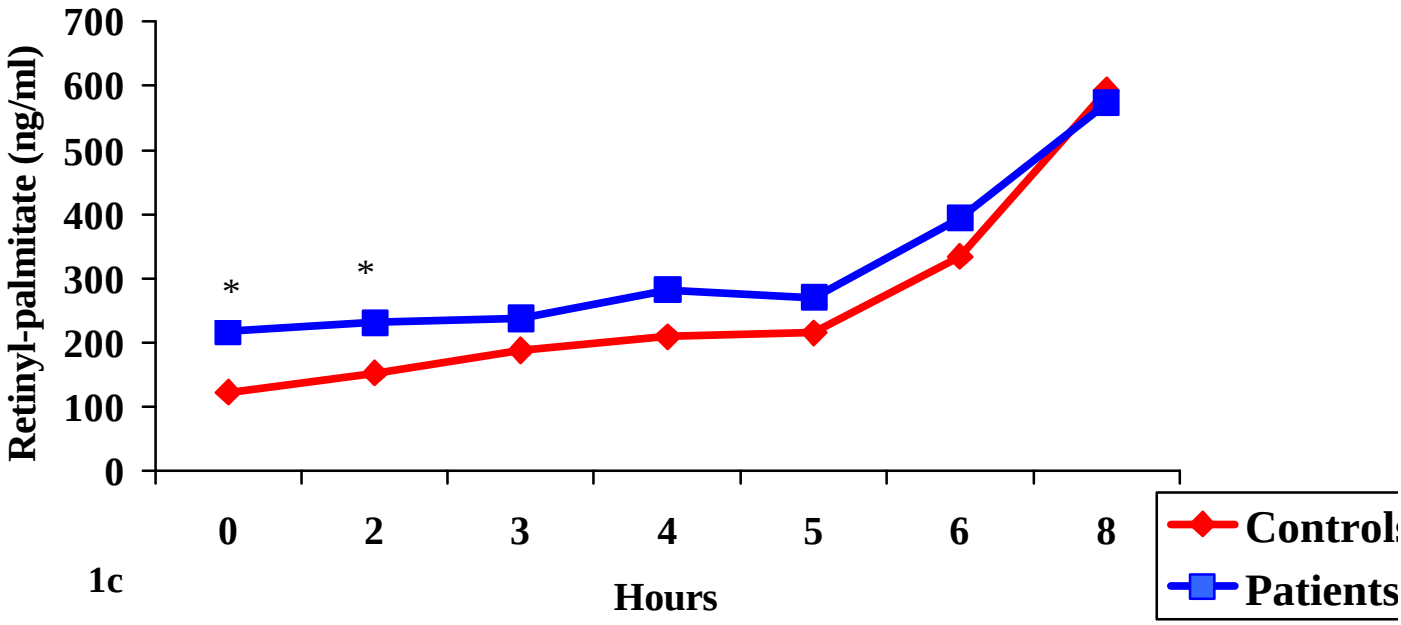
Figure 1: Postprandial glucose (1a) and insulin (1b) curves in the type 2 diabetic patients (squares) and control subjects (circles). * $p < 0.05$ patients vs controls.

Figure 2: Postprandial triglyceride (2a), retinyl-palmitate (2b) and LDL size (2c) curves in the type 2 diabetic patients (squares) and control subjects (circles). * $p < 0.05$ patients vs controls.

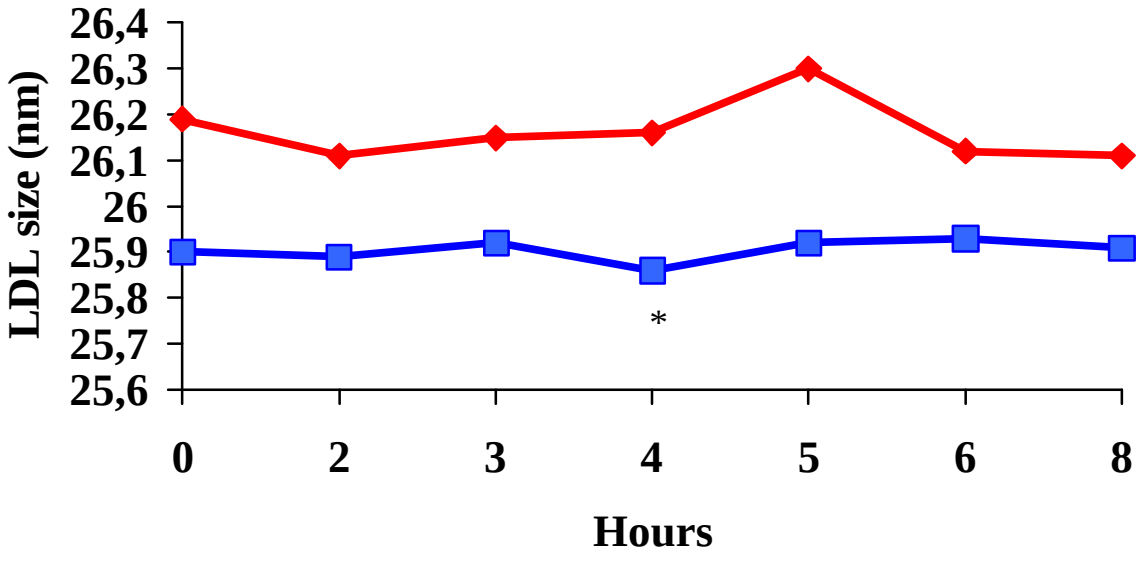
1a


Triglyceride (mmol/l)
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
2
3
4
5
6
8
Hours

1b


Retinyl-palmitate (ng/ml)
700
600
500
400
300
200
100
0
*
*
0
2
3
4
5
6
8
Hours
Control
Patients

1c


LDL size (nm)
26,4
26,3
26,2
26,1
26
25,9
25,8
25,7
25,6
*
0
2
3
4
5
6
8
Hours

COMBINATION THERAPY WITH LOW DOSE ATORVASTATIN AND GEMFIBROZIL IMPROVES ALL OF THE COMPONENTS OF DIABETIC DYSLIPIDEMIA

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Statins and fibrates in diabetic dyslipidemia

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ABSTRACT

Background: Optimal drug treatment for diabetic dyslipidemia is a matter of debate.

Objective: To compare the effect of atorvastatin vs gemfibrozil vs combined treatment on the components of diabetic dyslipidaemia, including apolipoprotein B (apoB) concentrations and LDL size.

Design: Randomized, open, cross-over study.

Setting: Diabetes outpatient clinic at a University Hospital.

Patients: 44 type 2 diabetic patients (LDL cholesterol (LDLc) > 2.6 mmol/l and triglyceride< 4.51 mmol/l).

Intervention: 12 weeks' treatment with atorvastatin (10-20 mg/day) and gemfibrozil (900-1200 mg/day) in a randomized cross-over study. If patients did not show significant side-effects, both drugs were combined (atorvastatin 10mg + gemfibrozil 900 mg) for 12 additional weeks.

Measurements: Triglyceride, LDLc, HDL cholesterol (HDLc), non-HDL cholesterol (non-HDLc), apoB and LDL size were measured at baseline and after each treatment.

Results: As compared with gemfibrozil, atorvastatin was more effective ($p<0.001$) in lowering LDLc (32.7% vs 4.7%), non-HDLc (30.1% vs 10.0%) and apoB (24.8% vs 6.69%), but less effective in lowering triglyceride (7.69% vs 26.49%, $p<0.001$). Only gemfibrozil increased LDL size (from 25.59±0.06 to 25.69±0.06, $p<0.05$). Both drugs were equally effective in increasing HDLc (5.16% vs 4.16%). Combined treatment with both drugs reduced LDLc by 28.0%, triglyceride by 24.6%, apoB by 23.2% and increased HDLc by 4.79% and LDL size by 0.13 nm. Only one patient withdrew due to unspecific side-effects.

Conclusions: Atorvastatin and gemfibrozil are useful, and complementary, in the treatment of diabetic dyslipidemia. The combination of both drugs achieves most

favourable modifications in all of its components and shows a a good safety profile.

Diabetic dyslipidemia typically comprises moderately increased triglyceride, low HDL cholesterol (HDLc), normal or slightly increased LDL cholesterol (LDLc), a predominance of small dense LDL particles (phenotype B) and increased apolipoprotein B (apoB) (1-3). The management of diabetic dyslipidemia is based upon a step-wise type of treatment, starting with life-style modifications and improvement of glycemic control. Lipid-lowering drugs should be used when targets are not met with the previous measures (4). The two main drug groups used in the management of dyslipidemia are "statins" and "fibrates", and both have proved to reduce the risk of coronary heart disease in diabetic patients (5-7). Statins have proved more efficient in lowering LDLc and apoB (8), but fibrates have a more potent effect on triglyceride, they increase LDL size and may induce a shift in LDL subtype distribution from small, dense to intermediate particles in diabetic hypertriglyceridemic subjects (8-10). The combination of statins and fibrates has an additive effect and seems an attractive treatment for diabetic patients, given the features of diabetic dyslipidemia. However, data on their efficacy in diabetic patients are scarce, and do not include the assessment of LDL size (11).

This study was undertaken to compare the efficacy of atorvastatin and gemfibrozil and the combination of both drugs in patients with type 2 diabetes displaying the most common dyslipidemic pattern.

Patients and methods

Study design:

The study was designed as a randomized, open, cross-over study, comparing the effect of atorvastatin and gemfibrozil, on the different components of diabetic dyslipidemia. All patients received either atorvastatin or gemfibrozil for 12 weeks, followed by the alternative treatment. If they did not suffer clinically or biologically significant side-

effects to any of the drugs, they received 12 weeks of combined treatment thereafter. Each treatment period was separated from the next by 4 weeks of wash-out. Measurement of liver enzymes and creatine kinase and recording of side effects was performed at 6 weeks into each treatment period in order to assess safety. If any of the following goals was not achieved: LDLc < 2.6 mmol/l (100 mg/dl), triglyceride < 1.7 mmol/l (150 mg/dl) and apoB < 1.0 g/l, and in the absence of significant side-effects, atorvastatin was increased from 10 to 20 mg, and gemfibrozil from 900 to 1200 mg/day. Combination treatment consisted of 10 mg atorvastatin and 900 mg gemfibrozil for 12 weeks, without titration. The study protocol was accepted by the local Ethics Committee. Pfizer was not involved in the collection nor interpretation of data, nor in the decision as whether to publish the results.

Patients

To be included in the study, patients had to fulfill the following criteria: men and women with type 2 diabetes, aged 35-75 years, no treatment known to interfere with lipid metabolism (non-selective beta-blockers, high-dose diuretics, systemic steroids, lipid-lowering drugs other than the study treatment in the month preceding the inclusion in the study), plasma LDLc >2.6 mmol/l (100 mg/dl) and triglyceride < 4.51 mmol/l (400 mg/dl). Patients were excluded if pregnant or if no reliable contraceptive method was used, or if they displayed serum creatinine > 150 μmol/l, hepatic dysfunction (transaminases > 1.5 times upper normal limit at inclusion), creatine kinase > 3 times upper normal limit or acute or chronic disorders that might interfere with compliance. All patients signed informed written consent before being included in the study. Randomization was made following a computer-generated table.

Physical examination, including anthropometric parameters and blood pressure, was performed at baseline and at each visit. Blood samples were drawn at baseline and at 6 and 12 weeks after administration of each treatment.

Laboratory measurements:

Total cholesterol and triglyceride were measured by enzymatic methods, and HDLc, was measured by a direct method (Roche Diagnostics). LDLc was calculated using Friedewald's formula (12) when triglyceride did not exceed 3.45 mmol/L (300 mg/dl). Otherwise, ultracentrifugation was performed, and LDLc 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 WHO/IFCC reference standard SP3-07 for apoB and SP1-01 for apoAI (13). LDL size was determined by electrophoresis on gradient (2-16%) polyacrylamide gel, cast in the laboratory, according to the method described by Nichols, with modifications (14). A volume of 10µl of plasma samples was applied on lanes 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 120V, followed by 30 minutes at 20V, 30 minutes at 70V and 16 hours at 100V. 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 microscopy was used as control. The gels were scanned (Gel-DOC 2000, Bio-Rad, Hercules, CA, USA), 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)(15).

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 4.6-5.8% (mean 5.1±0.3%).

Efficacy and safety

Changes in lipid and apoprotein concentrations and LDL size were assessed for each treatment, and then compared. In addition, achievement of therapeutic goals was evaluated and compared among treatments. Side effects were registered, as were increases in liver enzymes and creatine kinase concentrations.

Statistical analysis

Analysis was performed using the SAS statistical package, version 8.2. Continous variables were expressed as mean±standard deviation or median (interval), depending on their normality distribution, and qualitative data, as percentages. Comparison for changes in lipid and lipoprotein concentrations with each treatment was done using analysis of variance (ANOVA). Frequencies between groups were compared using chi-squared. Tests were bilateral, and a p value below 0.05 was considered significant.

Results

Patient features and follow-up

A total of 46 patients were initially included in the study. Two dropped out immediately after randomization (before starting treatment): one was excluded because the second determination of LDLc was below 2.6 mmol/l (100 mg/dl), and the other, for personal reasons. A total of 22 started atorvastatin and 22 gemfibrozil. One patient was excluded during the study because of unspecific side-effects to gemfibrozil. Two patients participated in the comparison between both drugs, but dropped out thereafter due to personal and family health problems, unrelated to the study treatment. Thus, 43 patients

were available for single drug comparison, and 41 also for combination therapy. Their baseline features are displayed in tables 1 and 2.

Efficacy

Drug dose was increased from 10 to 20 mg/day at 6 weeks in 45% of the patients when treated with atorvastatin (mean final dose 14.5 mg/day), and from 900 to 1200 mg/day in 88% of them when treated with gemfibrozil (mean final dose 1161 mg/day) ($p<0.0001$). Baseline and posttreatment body mass index, HbA1c and lipidic components are displayed in table 2. The changes in lipid and apoprotein concentrations with each drug and with the combination of both are shown in figure 1. Baseline lipid and apoprotein concentrations were similar in all treatment groups, except for triglyceride, which was higher before combination therapy. Atorvastatin was superior to gemfibrozil in the reduction of LDLc, non-HDLc and apoB. Gemfibrozil caused a greater reduction in triglyceride concentrations, while both drugs showed a similar effect on HDLc concentrations. Combination therapy improved all of the components of diabetic dyslipidemia.

Mean LDL size increased only after treatment with gemfibrozil (either alone or combined with atorvastatin) (see table 2). In the subgroup of 18 patients (40 %) who displayed an LDL phenotype B at baseline, the 1.45% increase in LDL size obtained with gemfibrozil was significantly different ($p<0.05$) from the absence of change seen after atorvastatin and combination therapy. A shift towards phenotype A was seen in 12%, 47% and 29% of the patients with phenotype B treated with atorvastatin, gemfibrozil, or combination therapy, respectively ($p=0.07$ for atorvastatin vs gemfibrozil).

After 12-weeks' treatment, the percentage of patients achieving the targets of LDLc < 2.6 mmol/l, non-HDLc < 130 mg/dl, triglyceride < 1.7 mmol/l and apoB < 1.0 mmol/l

were 60, 65, 70 and 67% with atorvastatin, 5, 16, 78 and 25% with gemfibrozil ($p<0.0005$ for LDLc and non-HDLc, and <0.05 for apoB vs atorvastatin), and 51, 54, 78 and 71% with combination therapy ($p<0.01$ for LDLc and <0.05 for apoB vs gemfibrozil).

Side effects

No new cardiovascular events were suffered by the patients during the study period. Gastrointestinal manifestations including abdominal discomfort, constipation, loose stools and nausea were referred by 6, 11 and 8 of the patients while treated with atorvastatin, gemfibrozil and combination therapy, respectively. Drowsiness was suffered by 1 patient on atorvastatin, and nightmares, by another. Muscle aches, with normal creatine kinase, were referred by one patient during treatment with atorvastatin, 2 while on gemfibrozil, and none while receiving combination therapy. Only one patient dropped out from the study due to unspecific side effects during the treatment with gemfibrozil.

Slight increases in liver enzymes were observed in 5, 2 and 2 of the patients treated with atorvastatin, gemfibrozil and combination therapy, respectively, and in creatine kinase in 2, 3 and 4 patients, respectively. Nevertheless, no patient experienced an elevation in liver enzymes greater than 3 times the upper normal limit, or an elevation in creatine kinase greater than 10 times the upper normal limit, and no patients were excluded for these reasons.

Discussion

In the present study, we confirm that in well-controlled type 2 diabetic patients with the most frequently shown lipid pattern, atorvastatin (10-20 mg/day) is more efficacious than gemfibrozil (900-1200 mg/day) in lowering LDLc, apoB and non-HDLc. Gemfibrozil provided greater reductions in triglyceride than atorvastatin, and also

increased LDL size. In addition, we have shown that low-dose treatment with atorvastatin (10 mg/day) and gemfibrozil (900 mg/day) combines the effects of each of them and improves all of the features of diabetic dyslipidemia. To our knowledge, this is the first time a statin and a fibrate are compared and then combined in a randomized cross-over study assessing LDL size in type 2 diabetic patients.

According to the recently released Adult Treatment Panel III guidelines of the National Cholesterol Education Program (NCEP), diabetes is considered a coronary heart disease risk equivalent, because subjects with diabetes have as high a risk of dying of a myocardial infarction as non-diabetic subjects who have already suffered a coronary event (16,17). For this high-risk group, the LDLc target is < 100 mg/dl (2.6 mmol/l), which is supported by clinical evidence showing that LDLc is atherogenic (18,19), and that lowering its concentrations slows the progression of coronary artery disease (5, 20-22). However, the most typical pattern of lipid abnormalities shown by diabetic patients comprises moderate hypertriglyceridemia, low HDLc, a high proportion of small dense LDL particles and increased apoB (a crude marker of the number of atherogenic particles) (2,3,23). All of these disorders are atherogenic, and results from 2 clinical trials suggest that the reduction in triglyceride and the increase in HDLc are also linked to a reduction in coronary heart disease (6,7). On the other hand, both apoB and non-HDLc (a surrogate of the former, (24)), have been proved to be better predictors of outcome than LDLc in several studies (20,25-27). Taken together, these findings suggest that whenever a lipid disorder is detected in type 2 diabetic patients, it should be corrected (7). When NCEP goals are not achieved by life-style intervention and improvement of glycemic control, as was the case of the patients included in this study, lipid-lowering drugs should be prescribed. The two main drug-groups used in the management of diabetic dyslipidemia are "statins" and "fibrates". As expected, in the

present study, atorvastatin was more potent than gemfibrozil in the reduction of LDLc, apoB and non-HDLc, whereas gemfibrozil was more effective in the reduction of triglyceride (8,11,21,28-30). Despite that the increase in HDLc with gemfibrozil depends on baseline triglyceride concentrations and the ability of fibrates to reduce triglyceride concentration, in the present study, HDLc increased similarly with atorvastatin and gemfibrozil, in accordance with the 4-7% increase obtained in large clinical trials (5,6,21,22,31). In addition, in agreement with previous studies, the fibrate but not the statin, increased LDL size (8,32-35).

The idea of using the combination of a statin and a fibrate is very attractive as a way of improving, not only LDLc, but also the other components of diabetic dyslipidemia. As has previously been shown in patients with combined hyperlipidemia (36,37), in the present study, the combination of low doses of a statin and a fibrate was not only highly effective in reducing LDLc, but also more effective than the statin alone in the reduction of triglyceride concentrations, and increasing LDL particle size. The fact that the combined effect was slightly less than would be expected from the addition of the effects of each single treatment was probably related to the dose administered, which was not titrated, as the individual drugs were. Although combination therapy with statins and fibrates increase the risk of myopathy, data from previous (11,36,37) and the present studies show that combined treatment is well tolerated and safe. Thus, excluding patients with high risk of myopathy, a good therapeutic response can be achieved with an appropriate safety profile.

Although studies including diabetic patients have shown that statins and fibrates may reduce coronary heart disease, based on NCEP recommendations, our results confirm that statins are the choice drug-group for diabetic patients with LDLc >2.6 mmol/l (100 mg/dl), because they are most effective at lowering LDLc, non-HDLc and apoB, and

moderately effective at increasing HDLc. In addition, low-dose combination therapy with atorvastatin and gemfibrozil improve all of the features of diabetic dyslipidemia, including small dense LDL. Actually, we do not know whether statins or fibrates are more effective at reducing cardiovascular events, or whether combined treatment has additional benefit and will not know until data from trials are available (39). However, existing data already support that desirable lipid levels should be obtained. Thus, we believe that the subgroup of patients whose dyslipidemia cannot be controlled with monotherapy, should receive combined treatment.

In conclusion, atorvastatin and gemfibrozil are complementary in the treatment of diabetic dyslipidemia, but based on NCEP recommended goals, the statin is more effective. Combined therapy with both treatments is safe, and may be the most effective therapy in the management of diabetic dyslipidemia.

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Table 1: Main baseline features of the 44 type 2 diabetic patients included in the study.

Male/female (%)	70.5/29.5
Age (years)	57.4±8.8
Body mass index (Kg/m^2)	27.8±3.5
Waist circumference (cm)	97.9±8.8
Waist/hip ratio	0.99±0.06
Diabetes duration (years)	9.5 (0.25-35.0)
Treatment (diet/oral/insulin) (%)	29.5/29.5/41
Hypertension (%)	57
Smoking (%)	16
Retinopathy (%)	29.5
Microalbuminuria (%)	20.5
Polyneuropathy (%)	20.5
Coronary heart disease (%)	20.5
Stroke (%)	0
Peripheral artery disease (%)	20.5
HbA1c (%)	6.9±0.6
Total cholesterol (mmol/l)	5.8±0.8
Triglyceride (mmol/l)	1.4 (0.4-4.4)
LDL cholesterol (mmol/l)	3.5±0.5
HDL cholesterol (mmol/l)	1.1±0.3
Non-HDL cholesterol (mmol/l)	4.6±0.8
Apolipoprotein B (g/l)	1.2±0.1

Apolipoprotein AI (g/l)	1.3±0.2
LDL size (nm)	25.6±0.5
LDL phenotype B (%)	40

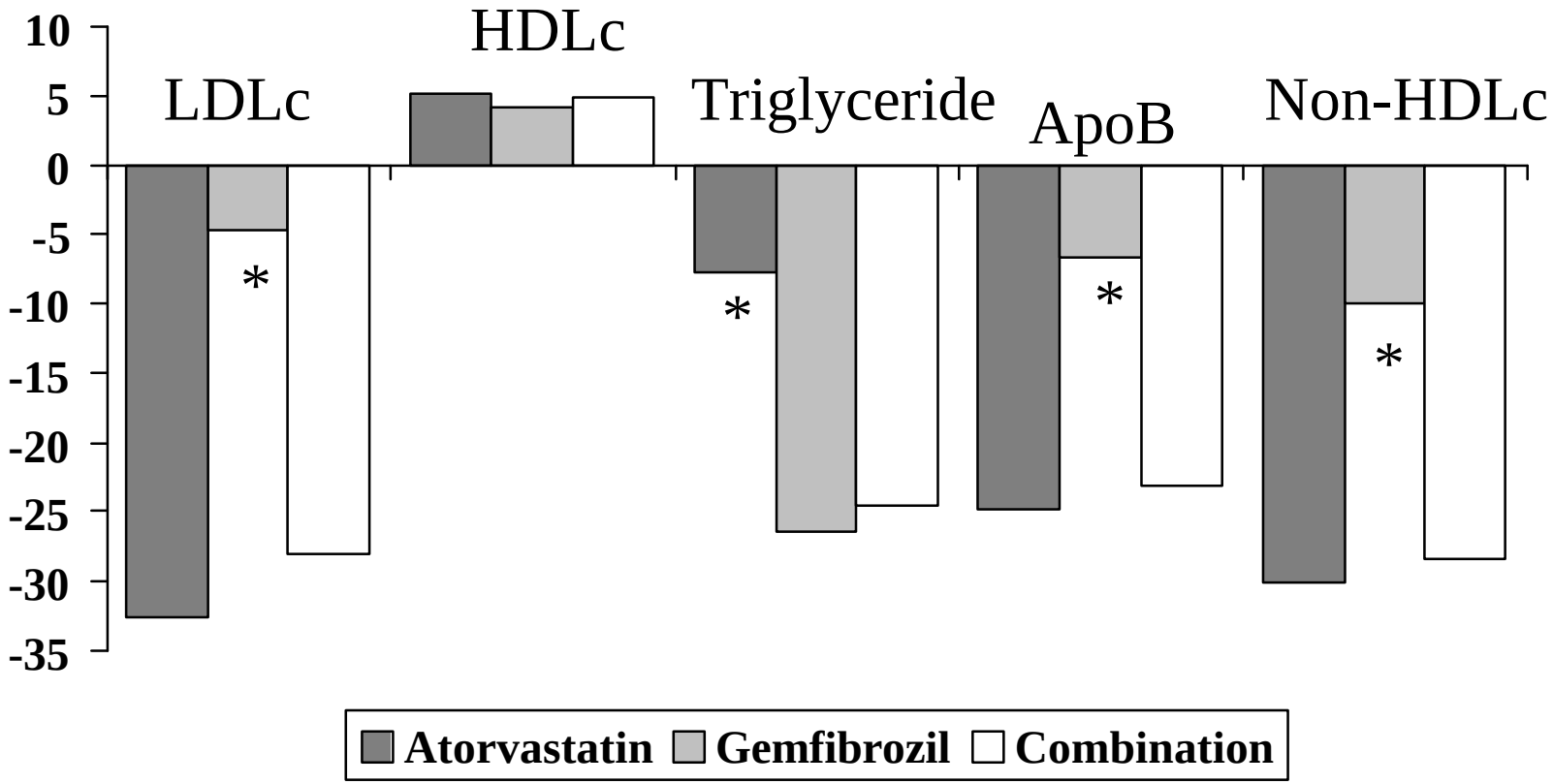
Table 2: Baseline and post-intervention data.

Treatment	Atorvastatin	Gemfibrozil	Combination
LDLc Baseline	3.92±0.07	3.82±0.07	3.83±0.07
(mmol/l) Post-treatment	2.55±0.07*	3.63±0.07†‡	2.74±0.07*
HDLc Baseline	1.20±0.02	1.18±0.02	1.18±0.02
(mmol/l) Post-treatment	1.23±0.02‡	1.23±0.02§	1.24±0.02‡
Triglyceride Baseline	1.83±0.11	1.88±0.11	2.15±0.12**
(mmol/l) Post-treatment	1.62±0.09 ¶	1.28±0.09*	1.32±0.09*,**
ApoB Baseline	1.24±0.01	1.22±0.01	1.26±0.02
(g/l) Post-treatment	0.93±0.02*	1.15±0.02*†	0.94±0.02*
ApoA1 Baseline	1.35±0.01	1.34±0.01	1.37±0.01
(g/l) Post-treatment	1.38±0.01	1.34±0.01	1.39±0.02§‡‡
non-HDLc Baseline	4.76±0.07	4.70±0.07	4.83±0.07
(mmol/l) Post-treatment	3.30±0.08*	4.25±0.08*†	3.36±0.08*
LDL size Baseline	25.57±0.06	25.59±0.06	25.51±0.06
(nm) Post-treatment	25.54±0.06	25.69±0.06§	25.68±0.06§
HbA1c Baseline	7.03±0.08	7.15±0.08	7.32±0.09**
(%) Post-treatment	7.41±0.10§	7.31±0.09	7.42±0.10§
BMI Baseline	27.78±0.07	28.00±0.07**	28.11±0.08§§
(Kg/m^2) Post-treatment	28.03±0.08	27.90±0.07	28.17±0.08‡‡

Concentrations are expressed as mean±standard error. To convert cholesterol and triglyceride into mg/dl, multiply by 38.7 and 88.6, respectively. *p<0.0001 vs baseline, § p<0.05 vs baseline, ‡ p<0.01 vs baseline, ¶ p<0.001 vs baseline, † p<0.0001 vs the

other two treatment groups, **p<0.05 vs atorvastatin, ‡‡ p<0.05 vs gemfibrozil, ¶ p<0.01 vs gemfibrozil, §§ p<0.01 vs atorvastatin.

Fig 1: Percentage lipid changes obtained with the different treatments.* $p<0.001$ vs the other two treatment groups.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Atorvastatin
Gemfibrozil
Combination

DISCUSIÓN

Y

RESUMEN DE RESULTADOS

Al igual que la introducción, la discusión ha sido dividida en apartados, y seguirá un orden similar al de aquélla, centrándose en los resultados de los estudios que componen la presente tesis, que tratan la evaluación de la dislipemia diabética en varios aspectos, y su tratamiento con fármacos hipolipemiantes.

I. EVALUACIÓN DE LA DISLIPEMIA DIABÉTICA

Teniendo en cuenta que las alteraciones lipídicas presentes en los pacientes con diabetes tipo 2 constituyen uno de los factores de riesgo cardiovascular más relevantes, tanto por su prevalencia como por su potencial aterogénico, la evaluación lo más adecuada posible de estas alteraciones constituye una prioridad para la mejora de la predicción del riesgo y del tratamiento de esta población, con objeto de disminuir su morbimortalidad cardiovascular. Aunque existen otros aspectos de interés para evaluar la dislipemia diabética, los estudios incluídos en esta tesis van dirigidos a mejorar la determinación del considerado principal factor de riesgo lipídico (cLDL), así como a la valoración de otros parámetros, no incluídos en las recomendaciones actuales (componentes no clásicos), que pueden ser de importancia por su prevalencia y potencial aterogénico: la apoB, el tamaño de las LDL y la lipemia postprandial.

1. Optimización de la determinación del cLDL

En la evaluación de la dislipemia diabética, los organismos internacionales recomiendan la determinación en ayunas de los triglicéridos, el colesterol total y el cHDL (American Association of Clinical Endocrinologists 2000, Expert Panel 1994, Expert Panel 2001, European Diabetes Policy Group 1999). El cLDL, calculado mediante la fórmula de Friedewald cuando los triglicéridos son inferiores a 400 mg/dl (4,5 mmol/l) (Friedewald 1972), es el principal objetivo en la estimación del riesgo y en el tratamiento de la dislipemia diabética (Expert Panel 1993, American Diabetes

Association 2001). No obstante, la validez de la fórmula de Friedewald ha sido muy discutida, especialmente en sujetos con diabetes (Rubíes 1993, Hirani 1997, Branchi 1998), dado que parte de una premisa (una razón prácticamente constante de colesterol/triglicéridos en las VLDL) no siempre correcta en estos pacientes. Por otro lado, el método designado de comparación (la betacuantificación), que se basa en la separación de las VLDL por ultracentrifugación, y la medición del cHDL en el infranadante (Bachorik 1997) (ver figura 1), es caro y laborioso. Por tanto, sería útil contar con un método de estimación o medición del cLDL que fuera sencillo y automatizable y cuya exactitud superara a la de la fórmula de Friedewald, que presenta una inexactitud superior a la recomendada por el NCEP (±4%) incluso en presencia de triglicéridos inferiores a 400 mg/dl (Wägner 2000). Trabajos previos ya han propuesto fórmulas alternativas para la estimación del cLDL (De Long 1986, Planella 1997, Rao 1988) y métodos directos para su medición (McNamara 1995, Esteban 2000, Nauck 2000, Ordóñez 2001). No obstante, ninguno de los métodos alternativos propuestos ha sido ampliamente aceptado.

Los trabajos incluídos en la presente tesis muestran una infraestimación del cLDL calculado por la fórmula de Friedewald cuando se compara con la betacuantificación en pacientes con diabetes tipo 2, de acuerdo con numerosos trabajos previos (Hirani 1997, Branchi 1998), aun con concentraciones normales de triglicéridos, hecho que pone en duda la validez de la citada fórmula. Con el objeto de mejorar la exactitud de la estimación del cLDL, proponemos dos métodos alternativos a dicha fórmula: un método directo, previamente evaluado por nuestro grupo en población general (Ordóñez 2001), y una fórmula que incluye triglicéridos, colesterol total y apoB, similar a la estudiada previamente por nuestro grupo en sujetos dislipémicos (Planella 1997).

Si ningún método directo de cLDL ha sido ampliamente aceptado en población general, los datos en población diabética son aún menos uniformes. El método utilizado en la presente tesis (LDLc-Plus), identifica tanto el colesterol de IDL (cIDL) como el cLDL, tal y como lo hace la betacuantificación (Ordóñez 2001). Por tanto,

dado que el aumento de partículas ricas en triglicéridos, entre las que se encuentran las IDL, forma parte de la dislipemia diabética (Patti 1987, Kasama 1987, Syvänne 1997) y constituye el principal inconveniente para aplicar la fórmula de Friedewald en el cálculo del cLDL, decidimos evaluar dicho método en pacientes con diabetes tipo 2. Los datos sobre métodos directos de cLDL en pacientes con diabetes existentes en la literatura son dispares: la inmunofiltración muestra ventajas sobre la fórmula de Friedewald (Hirani 1997) en un estudio, pero sobreestima el cLDL cuando se compara con la betacuantificación en otro (Whiting 1997). Por otro lado, un método directo basado en la acción de determinados detergentes, como el utilizado en la presente tesis, muestra resultados prometedores (Ragland 2000). Nosotros observamos que el método LDLc-Plus sobreestima las concentraciones de cLDL cuando se compara con la betacuantificación, especialmente a concentraciones altas de colesterol. En la evaluación del método realizada en sujetos no diabéticos, observamos que el método cLDLc-Plus estaba infraestandarizado, y que medía únicamente el 80% del cLDL y el 65% del cIDL (Ordóñez 2001). En los sujetos con diabetes tipo 2, la fuerte correlación encontrada entre el cLDL-Plus y tanto la apoB como el cociente VLDLc/triglicéridos sugiere que este método mide el colesterol de estas mismas partículas en estos pacientes. Asimismo, la correlación entre aquellos mismos constituyentes y el error del método cLDL-Plus respecto a la betacuantificación apoya el hecho de que la proporción de estas partículas tenga un efecto relevante sobre dicho error. La reasignación de un nuevo valor al calibrador utililizado corrigió el error constante del método cLDL-Plus en sujetos no diabéticos (Ordóñez 2001). Sin embargo, en los sujetos con diabetes encontramos un error proporcional, lo que también va a favor de un efecto de la proporción de las partículas, más que de la calibración, ya corregida, sobre el error observado con el cLDL-Plus. Este efecto también podría verse amplificado en los sujetos con diabetes, por la presencia de una alta proporción de partículas de IDL. Sin embargo, a concentraciones bajas de cLDL (puntos de corte de 2,59 y 3,36 mmol/L, (100 y 130 mg/dl)), pero ante las cuales se plantea tratamiento

farmacológico en los sujetos con diabetes (American Diabetes Association 2001), sólo el cLDL-Plus muestra un error inferior al 4% (máximo aceptado por el NCEP (Bachorik 1995)), cuando se compara con la betacuantificación. A concentraciones más altas de cLDL no ocurre lo mismo: el método directo no supera a los cálculos que estiman el cLDL; no obstante, a estas concentraciones de cLDL, muy por encima de los objetivos terapéuticos en los sujetos con diabetes, este hecho se hace clínicamente irrelevante.

Cuando utilizamos una fórmula alternativa a la de Friedewald, que incluye triglicéridos y colesterol totales y apoB (cLDL = 0,385*colesterol total + 2,010*apoB - 0,342*triglicéridos), mostramos que su exactitud es superior a la de aquélla, tanto para la correcta clasificación de los pacientes en las categorías de riesgo recomendadas por el NCEP como en la actitud terapéutica que se deriva de la misma. En nuestro conocimiento, esta es la primera vez que se evalúa el impacto de la inexactitud de la fórmula de Friedewald sobre la actitud terapéutica tras una valoración individualizada de los pacientes, teniendo en cuenta la existencia o ausencia de enfermedad cardiovascular previa. Cuando se estima la concentración de cLDL mediante esta fórmula alternativa, el 94% de los pacientes son correctamente tratados, y en ninguno se omite el tratamiento farmacológico de forma inadecuada, frente a un 10% de pacientes que quedan sin tratar cuando se aplica la fórmula de Friedewald. Además, aunque en el presente estudio sólo 4 pacientes muestran concentraciones de triglicéridos superiores a 400 mg/dl, datos previos sugieren la utilidad de esta fórmula incluso en pacientes hipertrigliceridémicos (Planella 1997). Por tanto, hasta que se disponga de un método directo de medición del cLDL estandarizado, creemos que la fórmula propuesta para el cálculo del cLDL ofrece ventajas suficientes para recomendar su utilización en pacientes con diabetes tipo 2.

2. Apolipoproteína B

Aunque el cLDL es el principal objetivo para la evaluación del riesgo y para el tratamiento de la dislipemia diabética, sus concentraciones suelen ser normales o

estar sólo levemente aumentadas en los pacientes con diabetes tipo 2 (Syvänne 1997). Muchos estudios han utilizado la fórmula de Friedewald en la estimación del cLDL (Long-term Intervention with Pravastatin in Ischaemic Disease Study Group 1998, Sacks 1998, Pedersen 1998) y, como puede desprenderse del apartado anterior, esto puede tener como consecuencia una infraestimación del cLDL real en los pacientes con diabetes. Sin embargo, también se ha demostrado que las partículas de LDL en los sujetos con diabetes son pobres en colesterol (Attia 1995) y, por tanto, el cLDL no refleja el total de partículas aterogénicas, que también incluye a las partículas ricas en triglicéridos y sus remanentes. La valoración del cHDL y de los triglicéridos además del cLDL mejora la estimación del riesgo en los pacientes con diabetes (Laakso 1993), pero no explica en su totalidad la alta incidencia de eventos y muerte cardiovasculares de esta población. Por tanto, parece necesario buscar otros marcadores de riesgo cardiovascular que puedan incorporarse a la práctica clínica rutinaria.

La apoB es el principal componente proteico de las partículas VLDL, IDL y LDL, y sus concentraciones reflejan la masa total de partículas aterogénicas (Sniderman 1997). Por otro lado, la concentración de apoB es un marcador independiente de riesgo cardiovascular (Westerveld 1998, Lamarche 1996, Graziani 1998), y el aumento de sus concentraciones conlleva un riesgo similar de desarrollar un evento coronario como el aumento de cLDL (Lamarche 1995). Además, es el mejor marcador de progresión de la arteriosclerosis coronaria durante la intervención terapéutica con fármacos hipolipemiantes (Van Lennep 2000, Gotto 2000).

En el estudio realizado por nosotros, un 45% de los pacientes con diabetes tipo 2 normocolesterolémicos muestran fenotipos de dislipemia con aumento de apoB (hiperapoB), una frecuencia que multiplica más de 3 veces a la del grupo control, y que supera incluso a la encontrada en el estudio de Québec en los sujetos con cardiopatía isquémica (Lamarche 1995). No obstante, dado que el punto de corte utilizado para la definición de hiperapoB difiere entre ambas poblaciones, y que la medida de apoB fue

realizada por métodos distintos, estas frecuencias son poco comparables. A pesar de la estandarización del método (Marcovina 1994), dadas las posibles diferencias entre poblaciones (Jungner 1998, Bachorik 1997, Contois 1996) de momento sólo podrán hacerse comparaciones frente a poblaciones control propias. La inclusión de la apoB en la evaluación y clasificación de la dislipemia en los pacientes con diabetes tipo 2 estudiados por nosotros permite identificar pacientes con elevado riesgo cardiovascular (29,3% hiperapoB-normotrigliceridémicos y 16% hiperapoB-hipertrigliceridémicos) (Lamarche 1996), que no habría sido detectados con el perfil lipídico clásico.

Por otro lado, observamos que el grupo de pacientes diabéticos con hiperapoB muestra mayor frecuencia de cHDL bajo y de triglicéridos elevados, que forman parte de la llamada dislipemia aterogénica, típica de pacientes con diabetes tipo 2 o síndrome metabólico (Taskinen 1996). En este sentido, un análisis reciente de los resultados del estudio de Québec muestra concentraciones más altas de apoB y de triglicéridos en el subgrupo de pacientes que tenían cHDL bajo (Sniderman 2001). Todo ello concuerda con el hecho de que la hipertrigliceridemia moderada, el cHDL bajo y la hiper-apoB están metabólicamente relacionadas. La síntesis de apoB es necesaria para la secreción hepática de VLDL, y cada molécula de apoB se mantiene ligada a una partícula hasta que ésta es aclarada del plasma como IDL o LDL (Berman 1978). Cuando el catabolismo de las lipoproteinas ricas en triglicéridos está alterado, como pasa en la diabetes tipo 2, se produce un aumento de remanentes de estas partículas, y un incremento en el intercambio de triglicéridos por colesterol entre las lipoproteinas ricas en triglicéridos y las partículas de LDL y HDL (Syvänne 1995). Como consecuencia, se originan partículas de LDL ricas en triglicéridos, que son hidrolizadas por la lipasa hepática, dando lugar a LDL pequeñas y densas, altamente aterogénicas. Asimismo, las partículas de HDL enriquecidas en triglicéridos son rápidamente aclaradas del plasma (Lamarche 1999), dando lugar a una concentración de cHDL baja (Syvänne 1997). La hipertrigliceridemia y el cHDL bajo son detectados

mediante métodos analíticos elementales, pero el aumento de los remanentes de VLDL y de la proporción de partículas de LDL pequeñas y densas, no. La medida de la apoB podría ser de utilidad en la identificación, aunque fuera indirectamente, de estas alteraciones (Campos 1992). Como cada partícula de LDL, IDL y VLDL contiene una única molécula de apoB (Young 1990), la concentración de ésta podría constituir una buena estimación de la masa de partículas secretadas por el hígado, especialmente las de LDL, que contienen más del 90% de la apoB circulante (Durrington 1978). Por tanto, para concentración normal o moderadamente eleveda de colesterol, la presencia de concentraciones elevadas de apoB indicaría la presencia de un alto número de partículas con apoB, relativamente pobres en colesterol (Austin 1990). En el estudio realizado por nosotros, los pacientes con diabetes tipo 2 normocolesterolémicos muestran concentraciones más altas de apoB que los controles (1,07 vs 0,95 g/l, $p < 0,05$), a pesar de concentraciones semejantes de cLDL, lo que sugiere una mayor masa de partículas aterogénicas en los sujetos con diabetes. Teniendo en cuenta estos datos, y que la razón cLDL/apoB es, de hecho, menor en las subpoblaciones más densas de las LDL (Campos 1992), creemos que la apoB también podría dar información sobre las alteraciones cualitativas de las partículas de LDL.

3. Tamaño de la LDL

El fenotipo B de las LDL (predominio de partículas pequeñas y densas) se asocia con mayor riesgo de enfermedad coronaria (riesgo relativo de hasta 3) (Austin 1988, Campos 1992a, Tornvall 1991, Gardner 1996, Stampfer 1996, Lamarche 1996a, Mykkänen 1999, Kamigaki 2001) carotídea (Skoglund-Andersson 1999, Hulthe 2000) y femoral (Hulthe 2000), y está presente en hasta un 30-50% de los pacientes con diabetes tipo 2 (Caixàs 1997, Mykkänen 1999, Abate 1995). Por tanto, la valoración del fenotipo de las LDL puede aportar información importante para la evaluación del riesgo de estos pacientes. Sin embargo, la medida del tamaño de las partículas de LDL es laboriosa, y no está al alcance de cualquier laboratorio clínico, por lo que sería

de utilidad contar con marcadores clínicos o bioquímicos que nos permitieran predecir el fenotipo de las LDL.

En el grupo de pacientes con diabetes tipo 2 estudiado por nosotros, el 36% presenta un fenotipo B de las LDL. Cuando estudiamos los posibles marcadores clínicos y bioquímicos del fenotipo B, las características clínicas y antropométricas no permiten predecirlo. De los marcadores lipídicos analizados como potenciales predictores del fenotipo de las LDL, el cHDL, los triglicéridos y la apoB son los mejores predictores del tamaño de la LDL en nuestros pacientes con diabetes tipo 2. Estos hallazgos están de acuerdo con los resultados de estudios previos, que sugieren que son las alteraciones lipídicas que la acompañan, más que la diabetes en sí, las que condicionan el tamaño de la partícula. Algunos (Tchernof 1996, Singh 1995), pero no todos los estudios previos (Lahdenperä 1995) muestran una asociación del tamaño de las LDL con la resistencia insulínica y, cuando existe, esta asociación no es independiente de la dislipemia que coexiste con la insulinresistencia (Singh 1995). Por otro lado, la mejora del control glucémico se acompaña de un cambio favorable en la densidad de las partículas de LDL, una disminución en la concentración de triglicéridos y un incremento en el cHDL, lo que también apoya la interrelación entre las concentraciones de triglicéridos y cHDL con el tamaño de la LDL (Caixàs 1997).

En el análisis multivariante realizado en nuestros pacientes, los triglicéridos resultan ser el mejor predictor independiente del tamaño de la LDL, y explican un 40% de la variabilidad del mismo, lo que está en consonancia con estudios previos, que muestran que los triglicéridos plamáticos justifican entre 20-50% de la varianza del tamaño de la LDL tanto en pacientes con como sin diabetes (Tchernof 1996, Singh 1995, Lahdenperä, Rainwater 2000). La concentración límite de triglicéridos que permite distinguir entre los fenotipos A y B de las LDL en nuestros pacientes (2,1 mmol/l; 186 mg/dl) está cercano al recomendado como objetivo terapéutico hasta hace poco por el NCEP y la Asociación Americana de Diabetes (< 2,25 mmol/l; 200 mg/dl) (Expert Panel 1993, ADA 2001). Sin embargo, el punto de corte que mejor diferencia

ambos fenotipos (150 mg/dl, 1,7 mmol/l) es el que recomiendan el European Diabetes Policy Group (European Diabetes Policy Group 1999) y las nuevas recomendaciones del NCEP (Expert Panel 2001), y coincide con el obtenido en estudios previos (Caixàs 1997, Superko 1992). No obstante, existen autores que muestran que el fenotipo de las LDL cambia cuando los triglicéridos superan concentraciones de 1,1-1,5 mmol/l (95-130 mg/dl), tanto en sujetos sin (Griffin 1994) como con diabetes (Syvänne 1996). A pesar de que los triglicéridos son el mejor predictor del tamaño de la LDL, existe un solapamiento entre las concentraciones de triglicéridos observados en sujetos con fenotipo A y fenotipo B, lo que convierte a los triglicéridos en un marcador de moderado valor para la predicción del fenotipo de las LDL. Por tanto, creemos que es necesario buscar predictores clínicos o biológicos alternativos del fenotipo B de las LDL. Las concentraciones de cLDL, principal objetivo terapéutico en el manejo de la dislipemia diabética, son frecuentemente normales o están sólo ligeramente elevadas en la dislipemia diabética, y no informan sobre el tamaño de la partícula, como muestra el presente estudio. Sin embargo, la dislipemia diabética también comprende un aumento de las concentraciones de apoB y una alta frecuencia de fenotipos con hiperapoB. Dado que más del 90% de la apoB se encuentra en las LDL, sería de esperar que los pacientes con LDL pequeñas y densas (relativamente pobres en colesterol) mostraran una razón cLDLc/apoB baja, como han sugerido estudios previos (Campos 1992, Tchernof 1996). En nuestro estudio, la razón cLDL/apoB no es tan buen predictor del tamaño de la LDL como los triglicéridos (r = 0,561 vs -0,632), pero una razón cLDL/apoB inferior a 1,3 es la mejor herramienta diagnóstica para la identificación del fenotipo B de las LDL (kappa = 0,611 vs 0,591). Esta circunstancia podría estar influida por la elevada variablilidad biológica de las concentraciones de triglicéridos, mucho mayor que la de la razón cLDL/apoB (Ortolá 1992). Por otro lado, la exactitud de la razón cLDL/apoB depende de cada uno de sus componentes. La medida de la apoB está estandarizada y automatizada, así que el cLDL viene a ser el factor limitante. La utilización de una fórmula para la estimación del cLDL que incluye a

la apoB, alternativa a la fórmula de Friedewald para la estimación del cLDL, mejora el valor predictor de la razón. La ausencia de valor predictivo del tamaño de las LDL de la razón cLDL/apoB en estudios previos que utilizan métodos para determinar el cLDL que se ven interferidos por las concentraciones de triglicéridos, apoya esta hipótesis (Furuya 2000, Griffin 1999).

Finalmente, el hecho de que la adición de otros parámetros lipídicos a los triglicéridos o a la razón cLDL/apoB no mejore la estimación del tamaño de la LDL, probablemente sea debido a que todos estos componentes son distintas manifestaciones de una alteración común en el metabolismo lipoproteico de la diabetes tipo 2, además de su alta colinealidad.

En resumen, a pesar de que los triglicéridos constituyen el mejor marcador de tamaño de las LDL, una razón cLDL/apoB inferior a 1,3 es la mejor herramienta para diagnosticar el fenotipo B de las mismas.

4. Lipemia postprandial

Aunque existe la creencia de que los pacientes con diabetes tipo 2 muestran hiperlipemia postprandial, los resultados obtenidos en estudios previos son controvertidos, dado que, aunque algunos trabajos muestran un aumento de la lipemia postprandial en estos pacientes (Chen 1993, Syvänne 1994), no todos coinciden en esta afirmación (Attia 1995, Tan 1995, Durlach 1996). Además de algunas situaciones patológicas como la obesidad (Lewis 1990), especialmente si ésta es de predominio central (Mekki 1999, Coulliard 1998), o el tabaquismo (Mero 1997, Eliasson 1997, Axelsen 1995), la lipemia postprandial se ve influida por características individuales de los sujetos estudiados, como el sexo (Coulliard 1999) o la edad (Cohn 1988, Krasinski 1990). No obstante, el principal predictor de la lipemia postprandial, tanto en sujetos con diabetes (Lewis 1991, Reznik 1996) como sin diabetes (Karpe 1999, O'Meara 1992, Cohn 1988, Potts 1994), es la concentración basal de triglicéridos, incluso cuando ésta se halla el rango considerado como normal (Cintora 1999). De hecho, muchos de los demás factores condicionantes de la lipemia postprandial

probablemente lo hacen fundamentalmente a través de la concentración de triglicéridos en ayunas (Vansant 1999, Coulliard 1999). Por otro lado, también el control glucémico y el tratamiento de la diabetes tienen influencia sobre la lipemia postprandial (Jeppesen 1994, Jeppesen 1994a). Para evitar estas interferencias, en la presente tesis se seleccionaron pacientes con diabetes tipo 2 no obesos, normotrigliceridémicos, tratados con dieta, y se compararon con un grupo de sujetos no diabéticos normolipidémicos, de similar edad, índice de masa corporal, distribución por sexos y triglicéridos en ayunas. Las diferentes concentraciones basales de cHDL (más bajas en los sujetos con diabetes), que en teoría podrían interferir con los resultados de la lipemia postprandial en nuestros pacientes, no parecen modificar la lipemia postprandial en estudios diseñados para evaluar esta influencia en sujetos normotrigliceridémicos (Coulliard 2000, Miller 1993, O'Meara 1992), a pesar de que existe una correlación inversa entre el cHDL en ayunas y los triglicéridos postprandiales (Coulliard 2000, Patsch 1992, Cohn 1988).

No encontramos diferencias en la lipemia postprandial (cuantificada como área bajo la curva de triglicéridos totales y retinil-palmitato plasmático) entre los pacientes con diabetes y los controles. Estudios previos realizados en pacientes normotrigliceridémicos con diabetes tipo 2 muestran triglicéridos postprandiales normales, pero una disminución en el aclaramiento de los quilomicrones cuantificado como área bajo la curva de retinil-palmitato en la fracción libre de quilomicrones (Durlach 1996, Attia 1995, Tan 1995). El menor tamaño de las partículas de LDL hallado en nuestro grupo de pacientes con diabetes se asocia y correlaciona con una mayor actividad de lipasa hepática. El menor tamaño postprandial observado en las LDL no sólo ha sido descrito previamente en pacientes con diabetes tipo 2 (Attia 1995), sino que también se ha correlacionado con una mayor actividad de lipasa hepática (Tan 1995), propia de la dislipemia diabética, especialmente en presencia de cHDL bajo (Kasim 1987, Baynes 1991).

La selección de un grupo de pacientes no obesos normotrigliceridémicos hace que, evidentemente, los resultados no sean extrapolables a toda la población con diabetes tipo 2, pero sí permite evaluar el efecto de la diabetes sobre la lipemia postprandial, independientemente de las concentraciones lipídicas basales. Aún queda por evaluar, no obstante, si las alteraciones observadas tras la ingesta son causa o consecuencia de las encontradas en ayunas.

Aunque son necesarios más estudios para determinar la relevancia de la evaluación de la lipemia postprandial en sujetos diabéticos para su aplicación clínica, dada la evidente dificultad y consumo de tiempo que conlleva el estudio de la lipemia postprandial, es necesario simplificar el proceso e investigar buenos y asequibles marcadores de la misma. En los pacientes con diabetes estudiados por nosotros, la trigliceridemia a las 4-5 horas de la ingesta es el mejor marcador de la lipemia postprandial. La buena correlación que existe entre ambos parámetros permitiría estudiar la lipemia postprandial de un individuo únicamente midiendo los triglicéridos 4-5 horas tras la ingesta de la comida de prueba, aunque otros grupos han sugerido incluso el estudio de la curva de triglicéridos en sangre total capilar (Halkes 2000). El tamaño basal y postprandial de las LDL se correlacionó con la razón cLDL/apoB observada en ayunas, lo que también apoya la utilización de este parámetro de fácil determinación en la evaluación de la dislipemia diabética.

5. Resumen

Tras la exposición de los resultados obtenidos, podríamos hacer nuevas recomendaciones para la evaluación de la dislipemia diabética. Para tener una información más completa del riesgo cardiovascular de los pacientes con diabetes tipo 2, e incluso del tratamiento hipolipemiante más adecuado (ver siguiente apartado), sería recomendable determinar, no sólo el colesterol y los triglicéridos totales y el cHDL, sino también la apoB, especialmente en sujetos con cLDL entre 100 y 130 mg/dl y/o hipertrigliceridemia. La apoB proporciona información adicional sobre el riesgo de estos pacientes, a la vez que permite calcular el cLDL de forma más exacta

que la fórmula de Friedewald y predecir de forma aceptable el fenotipo B de las LDL, utilizando la razón cLDL/apoB. Ésta última, no sólo predice el tamaño de la LDL en ayunas, sino también el mismo en situación postprandial. Por otro lado, el estudio de la lipemia postprandial, aunque podría simplificarse midiendo solamente los triglicéridos a las 4-5 horas tras la comida de prueba, aún no está estandarizada, por lo que debería reservarse a la investigación de la fisiopatología de la dislipemia diabética.

II. TRATAMIENTO DE LA DISLIPEMIA DIABÉTICA

La dislipemia diabética, como ya se ha mencionado, se caracteriza por una hipertrigliceridemia moderada, un cHDL bajo, un cLDL normal o levemente aumentado, un aumento de la apoB y un predominio de partículas de LDL pequeñas y densas (fenotipo B de las LDL) (Wägner 1999, Syvänne 1997). Su tratamiento se basa en un abordaje escalonado, que empieza por la instauración de medidas higiénico-dietéticas y la optimización del control glucémico. Cuando no se obtienen los objetivos terapéuticos con estas medidas, deberá iniciarse tratamiento farmacológico. Si la alteración predominante es un aumento del cLDL, las estatinas son el grupo farmacológico de elección (American Diabetes Association 2001). No obstante, no existe consenso sobre el fármaco óptimo en el tratamiento de pacientes con cLDL normal o levemente aumentado (inferior a 160 mg/dl, 4,13 mmol/l).

Las estatinas reducen más eficazmente el cLDL (principal objetivo terapéutico) y la apoB que los fibratos, pero éstos reducen la concentración de triglicéridos e incrementan el tamaño de las LDL (Frost 2001, Lahdenperä 1993, O'Neal 1998). Por otra parte, la combinación de fibratos y estatinas tiene un efecto aditivo de sus componentes y, aunque proscrita durante años, se acompaña de una baja toxicidad en la mayoría de los estudios (Ellen 1998, Gavish 2000). En nuestro conocimiento, los estudios comparativos entre fibratos, estatinas y su combinación, en pacientes con

diabetes tipo 2, son escasos, se limitan a pacientes con hiperlipemia combinada (colesterol y triglicéridos elevados), y no evalúan los cambios en el tamaño de las LDL (Gavish 2000). No obstante, la mayoría de los pacientes con diabetes tipo 2 suele mostrar cLDL normal o levemente aumentado, triglicéridos moderadamente aumentados y cHDL bajo (Syvänne 1997), y un predominio de partículas de LDL pequeñas y densas (fenotipo B de las LDL) en hasta un 30-50% de los pacientes (Siegel 1996, Caixàs 1997, Abate 1995, Mykkänen 1999, Boizel 2000).

En la presente tesis comparamos el tratamiento con atorvastatina, gemfibrocilo y su combinación en un grupo de pacientes con diabetes tipo 2 y concentraciones lipídicas típicas de la dislipemia diabética.

1. Comparación entre un fibrato y una estatina

Los resultados de este estudio muestran que la atorvastatina es más eficaz en la reducción del cLDL y la apoB, pero que el gemfibrocilo lo es en la reducción de los triglicéridos, y es el único fármaco capaz de modificar el tamaño de la LDL. Los datos concernientes a los componentes clásicos de la dislipemia diabética (triglicéridos, cLDL y cHDL) están en consonancia con los previamente publicados en estudios no randomizados (Gavish 2000) o realizados en pacientes con concentraciones altas de cLDL y triglicéridos (Frost 2001).

El tratamiento con estatinas se acompaña de una reducción en el cLDL que oscila entre el 14% y el 46%, dependiendo de la dosis y principio activo prescritos (Stewart 1994, Jeck 1997, Tikkanen 1998, Gavish 2000, Frost 2001, Goldberg 1990). La reducción del 33% del cLDL observada en nuestros pacientes tratados con atorvastatina está de acuerdo con los datos de los estudios previos. No obstante, es la primera vez que, en un estudio de estas características, se titula la dosis del fármaco para alcanzar un determinado objetivo terapéutico. Un 60% de los pacientes tratados con atorvastatina alcanzaron el objetivo de cLDL propuesto (< 2,6 mmol/l, < 100 mg/dl), frente a un 5% de los tratados con gemfibrocilo. Los resultados procedentes de

estudios previos concernientes a los fibratos son dispares, en función, sobre todo, de las concentraciones iniciales de triglicéridos de los sujetos incluídos en el estudio. Algunos (Jeck 1997), aunque no todos los estudios que incluyen pacientes con hipertrigliceridemia (Gavish 2000) muestran aumentos en la concentración de cLDL. Por otra parte, aquellos pacientes que muestran una trigliceridemia normal o levemente aumentada al inicio del tratamiento (triglicéridos medios ≤ 2,9 mmol/l, ≤ 260 mg/dl) presentan reducciones entre el 4% y el 18% del cLDL (Stewart 1994, Tikkanen 1998, Frost 2001). En el presente estudio, en el que los pacientes incluídos mostraban concentraciones lipídicas iniciales inferiores a la mayoría de los incluídos en estudios previos, encontramos una reducción del 5% en el cLDL durante el tratamiento con gemfibrocilo, significativamente inferior a la obtenida con la atorvastatina.

Según estudios previos, las concentraciones de triglicéridos pueden ser reducidas tanto mediante el tratamiento con estatinas como con fibratos, aunque estos últimos son más eficaces (Stewart 1994, Jeck 1997, Tikkanen 1998, Goldberg 1990, Gavish 2000). Nosotros no encontramos una reducción significativa en los triglicéridos con el tratamiento con atorvastatina. De hecho, el efecto hipotrigliceridemiante de las estatinas depende no sólo de la potencia hipocolesterolemiante de las mismas, sino también de las concentraciones basales de triglicéridos (Stein 1998). Por otra parte, el tratamiento con gemfibrocilo produjo una reducción del 26% en los triglicéridos plasmáticos, también menor de lo obtenido en otros estudios que estudian a pacientes hipertrigliceridémicos (Jeck 1997, Tikkanen 1998, Frost 2001), pero similar a los que incluyen pacientes normolipémicos (Stewart 1994).

Existe la creencia de que los fibratos son más eficaces en el aumento del cHDL que las estatinas. No obstante, los estudios multicéntricos que evalúan estos fármacos muestran incrementos parecidos con ambos tratamientos, que oscilan entre el 4 y el 7% (Koskinen 1992, Long-Term Intervention with Pravastatin in Ischaemic Disease Study Group 1998, Rubins 1999, Goldberg 1998, Pyörälä 1997), en consonancia con

los resultados obtenidos en nuestro estudio, que mostró un incremento de un 4% en las concentraciones de cHDL, tanto con la atorvastatina como con el gemfibrocilo.

La concentración de apoB, que refleja la masa total de partículas aterogénicas, independientemente de su composición, puede ser reducida farmacológicamente, tanto con fibratos como con estatinas, pero no existen diferencias consistentes entre los efectos de ambas drogas en estudios previos (Stewart 1994, Frost 2001, Jeck 1997). En los pacientes estudiados por nosotros, la atorvastatina fue más eficaz en la reducción de apoB, ya que ocasionó una disminución del 25% en su concentración, superior al 7% que se obtiene con el tratamiento con gemfibrocilo ($p<0.0001$ entre ambos).

En estudios previos, solamente los fibratos han demostrado incrementar el tamaño de la partículas de LDL, efecto que no se observa durante el tratamiento con estatinas (Frost 2001, Chapman 1996, Yuan 1994, Tsai 1992, Cheung 1993). No obstante, hasta ahora no se habían comparado entre sí en un estudio cruzado. En el presente trabajo, aunque no se encontraron diferencias significativas entre ambos tratamientos, solamente el tratamiento con gemfibrocilo logró modificar el tamaño de las partículas.

El cLDL es el mejor predictor de eventos cardiovasculares en los pacientes con diabetes mellitus tipo 2 (Turner 1998), incluso a concentraciones bajas (Howard 2000), y la apoB es el mejor marcador de eventos nuevos en pacientes con enfermedad coronaria previa tratados con fármacos hipolipemiantes (Van Lennep 2000, Gotto 2000). Por tanto, cabría esperar que las estatinas constituyeran el tratamiento de elección de la dislipemia diabética. Sin embargo, también los triglicéridos y el tamaño de la LDL son predictores de eventos cardiovasculares (Austin 1998, Mykkänen 1999), y la reducción de los triglicéridos e incremento del cHDL que se obtiene con el tratamiento con fibratos reduce la aparición de nuevos eventos, incluso en ausencia de cambios en el cLDL (Rubins 1999, Rubins 2001). Las estatinas reducen todas las

subfracciones de LDL, sin mostrar efectos sobre el tamaño predominante de las partículas, y los fibratos reducen selectivamente las sufracciones pequeñas y densas (Frost 2001). Aunque los datos que se desprenden del presente estudio no difieren en gran medida de los resultados de estudios previos, no comparativos, ésta es la primera vez que se evalúan el efecto un fibrato y una estatina sobre el tamaño de las partículas de LDL, en un estudio randomizado y cruzado.

En resumen, tanto la atorvastatina como el gemfibrocilo son eficaces en el tratamiento de la dislipemia diabética, pero ninguno de dichos tratamientos, por sí mismo, es capaz de alcanzar los objetivos terapéuticos recomendados para los pacientes con diabetes. Por tanto, la combinación de ambos parece una alternativa más eficaz para modificar favorablemente los componentes, tanto clásicos como no clásicos, de la dislipemia diabética.

2. Tratamiento combinado

Tras evaluar la atorvastatina y el gemfibrocilo por separado en un diseño cruzado y randomizado, a aquéllos pacientes que no habían sufrido efectos secundarios importantes los tratamos con la combinación de ambos.

La asociación del fibrato y la estatina combina los efectos terapéuticos de ambos: una reducción del cLDL y de la apoB similares a las obtenidas con la atorvastatina, una reducción en la concentración de triglicéridos similar a la obtenida con el gemfibrocilo, un aumento del tamaño de la LDL y una incidencia de efectos secundarios baja, no superior a la aparecida con cada principio activo por separado. De hecho, con el tratamiento combinado se logró el máximo cumplimiento de los objetivos terapéuticos in nuestros pacientes con diabetes tipo 2. La alta eficacia y baja toxicidad del tratamiento combinado ya ha sido descrita previamente en un estudio no randomizado, que incluyó un grupo de 148 pacientes con glucemias en ayunas superiores a 6 mmol/l (108 mg/dl) tratados inicialmente con atorvastatina o bezafibrato y posteriormente con ambos tratamientos combinados (Gavish 2000) y, previamente, en estudios realizados en sujetos sin diabetes (Ellen 1998). En los pacientes estudiados por nosotros, la

combinación de atorvastatina y gemfibrocilo consiguió una reducción del 26,5% en las concentraciones de cLDL, del 24% en los triglicéridos y del 22% en la apoB, y un incremento del 5% en el cHDL, y un aumento de 0.1nm en el tamaño de la LDL. A diferencia del tratamiento con cada fármaco aislado, el tratamiento combinado no fue titulado, lo que podría explicar una respuesta algo inferior a la esperada por la adición las de ambos principios activos.

Resumen:

La atorvastatina y el gemfibrocilo son eficaces y complementarios en el tratamiento de la dislipemia diabética, y su combinación consigue las modificaciones más favorables en los componentes clásicos y no clásicos de la dislipemia diabética, optimiza la consecución de los objetivos terapéuticos y tiene un buen perfil de seguridad. Sin embargo, hasta ahora no se han comparado los efectos de los fibratos y las estatinas sobre la reducción de eventos cardiovasculares, información que probablemente será porporcionada por estudios actualmente en marcha (Betteridge 2000).

CONCLUSIONES FINALES

Una vez planteados los objetivos de la presente tesis y expuestos y discutidos sus resultados, podemos concluir lo siguiente:

1. La evaluación de la dislipemia diabética debería incluir la determinación de la apoB, fundamentalmente por tres razones:

    - Proporciona información sobre el riesgo cardiovascular que no se obtiene con el perfil lipídico clásico.
    - Formando parte de la fórmula expuesta, permite estimar el cLDL, principal objetivo terapéutico, con mayor exactitud que la fórmula de Friedewald.
    - Permite diagnosticar, con aceptable exactitud, el fenotipo B de las LDL.

2. La medición del cLDL con un método directo basado en la utilización de detergentes, es una alternativa al cálculo del cLDL, especialmente a concentraciones bajas-moderadas de cLDL.

3. Los pacientes con diabetes tipo 2 normotrigliceridémicos muestran un cHDL bajo, una lipemia postprandial normal y partículas de LDL más pequeñas y densas que la población no diabética, asociados a una mayor actividad de lipasa hepática y una mayor resistencia a la insulina.

4. La concentración de triglicéridos a las 4-5 horas postprandiales es un buen predictor de la lipemia postprandial en pacientes con diabetes tipo 2 normotrigliceridémicos.

5. Las estatinas y los fibratos son eficaces y complementarios en el tratamiento de la dislipemia diabética. Su combinación es segura y optimiza la consecución de objetivos terapéuticos.

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Tablas 1a y 1b: Concentraciones medias de lípidos en hombres (1a) y mujeres (1b) con y sin diabetes.

1a

Estudio (Año)	Cita	N	cLDL	cHDL	TG	apoB
PROCAM	Assmann 1988	302 2458	150±42,2 155±36,8	44,6±13,5 47,1±12,7	201±169,8 142±102,1	NE
Framingham Offspring	Siegel 1996	120 1878	132±43,8* 137±35,1	38,8±12,4* 44,5±12,0	180±119,9* 142±112,2	101,2±35,2 96,0±29,3
UKPDS	UKPDS 27 1997	2139 52	139 129	39,0* 42,8	160* 103	NE
San Antonio	Haffner 1998	43	137	37,2	266	NE

1b

Estudio (Año)	Cita	N	cLDL	cHDL	TG	apoB
PROCAM	Assmann 1988	302 2458	173±43,4 165±38,7	51,1±12,6 60,8±14,8	166±125,2 105±65,1	NE
Framingham Offspring	Siegel 1996	120 1878	134±38,7 132±38,2	42,1±11,8* 57,1±14,8	295±538* 102±77,2	106,0±33,5* 82,8±27,3
UKPDS	UKPDS 27 1997	1574 143	151* 134	42,1* 54,4	160* 95	NE
San Antonio	Haffner 1998	46	139	46	207	NE

En la fila superior de cada celda se muestran los datos correspondientes a los sujetos diabéticos.*****p<0,05 vs controles. NE: No evaluado. Del estudio de San Antonio se ha seleccionado el subgrupo de sujetos "blancos no hispanos", para que los resultados sean comparables con los otros estudios de la tabla.

Tabla 2: Concentraciones lipídicas medias basales en los estudios de intervención que comparan pacientes con y sin diabetes

Estudio (Año)	Cita	N	cLDL	cHDL	TG
HHS -Diabetes	Koskinen 1992	135	201*	45,5*	239*
-No diabetes		3946	206	48,6	182
4S -Diabetes	Pyörälä 1997	202	185	43,6*	154*
-No diabetes		4242	188	45,9	132
CARE -Diabetes	Goldberg 1998	586	136*	37,6*	164*
-No diabetes		3573	139	39,0	154
PCABG -Diabetes	Hoofberg 1999	116	152	35,6*	185*
-No diabetes		1235	156	39,3	157

***p<0,05 vs controles.

Tabla 3: Principales trabajos que evalúan la relación entre apoB y enfermedad coronaria.

Cita	Tipo de estudio	Conclusiones
Durrington 1986	Transversal. N= 48 hombres con y 82 sin infarto	La apoB es el parámetro que distingue mejor a los pacientes de los controles
Brown 1990	Intervención (hipolipemiante) N = 120 hombres	La disminución de apoB se acompaña de una menor progresión angiográfica.
Stampfer 1991	Longitudinal (casos/control). N= 246 con y 246 sin infarto nuevo	La apoB no aporta información nueva a los factores de riesgo clásicos.
Coleman 1992	Longitudinal, observacional N = 3634 mujeres	El aumento de apoB duplica el riesgo de cardiopatía isquémica. Este efecto desaparece tras corregir por colesterol total e índice de masa corporal.
Sigurdson 1992	Longitudinal. N=1332 hombres	El incremento de riesgo asociado a la apoB desaparece al ajustar por colesterol.
Tornvall 1993	Transversal N = 64 hombres con infarto	La apolipoproteina B es el mejor indicador de estenosis coronaria.
Lamarche 1996	Longitudinal, observacional. N= 2103 hombres	El incremento de apoB aumenta el riesgo de eventos coronarios.
Westerveld 1998	Transversal. N= 289 mujeres.	La concentración de apoB se correlaciona con el número de arterias coronarias estenóticas, y es mejor marcador que el colesterol total y el cLDL.
Graziani 1998	Transversal. Casos-control N=553	La concentración de apoB identifica a los pacientes con cardiopatía isquémica mejor que el cLDL.
Sweetnam 2000	Longitudinal 5 años. N = 2225 hombres.	El efecto predictor de eventos coronarios de la ApoB no es independiente del colesterol.
Van Lennep 2000	Longitudinal N= 848 en prevención 2ª y tratamiento hipolipemiante	La concentración de apoB es mejor predictor de nuevos eventos que el cLDL
Gotto 2000	Intervención (lovastatina/placebo) N = 6605 en prevención 1ª	La concentración de apoB durante el tratamiento es el mejor predictor de eventos cardiovasculares.

Tabla 4: Lipemia postprandial en la diabetes mellitus y alteraciones asociadas: posibles mecanismos que pueden contribuir.

Alteraciones	Consecuencias
Aporte de sustrato: - Liberación de ácidos grasos del tejido adiposo no inhibida por insulina (insulinresistencia).(Syvännne 1994, Durlach 1996) - Aporte de ácidos grasos y colesterol desde el hígado (esteatosis) (Thompson 1996) - **Síntesis de apoB100** incrementada. (Cummings 1995)	**Aumento de la producción de VLDL**
Actividad de lipoproteinlipasa disminuida: - Insulinresistencia.(Deman 1996, Coppack 1992) - Enriquecimiento en apoE de las LRT (Syvänne 1994ª) - Enriquecimiento de las LRT en apoCIII (?) - Modificaciones cualitativas de apolipoproteinas (glicación*) (Curtiss 1985) - Saturación por aporte continuo de VLDL (Björkegren 1996). **Efecto sobre receptor**: - LDL pequeñas y densas, menos aclarables (Galeano 1998) - LDL más propensas a la oxidación (Diwadkar 1999)	**Disminución del aclaramiento de las LRT.**
- Partículas LDL y HDL enriquecidas en triglicéridos (Syvänne 1997) - Obesidad (Cominacini 1993) - Insulinresistencia (Baynes 1991)	**Actividad aumentada de la hepático lipasa.**
- Aumento de actividad hepáticolipasa (Tan 1995) - Actividad de la proteina transferidora de ésteres de colesterol aumentada en presencia de triglicéridos altos (Mann 1991, Murakami 1995, Jones 1996, Bagdade 1993) - Predominio de acción PTEC sobre HDL por acción de la proteina inhibidora del transporte de lípidos (?) (Serdyuk 1999, Wang 1999) - HDL pequeñas y densas, rápidamente aclarables (Syvänne 1997) - HDL ricas en triglicéridos (Syvänne 1997), rápidamente aclarables (Lamarche 1999).	**Partículas HDL y LDL pequeñas y densas** **cHDL bajo y partículas HDL pequeñas y densas**

(?) mecanismo no probado en la diabetes
(*) las consecuencias del mecanismo señalado no están demostradas.

Tabla 5: Efectos de las diferentes medidas terapéuticas sobre los componentes clásicos de la dislipemia diabética.

	Referencias	cLDL (%)	cHDL (%)	Triglicéridos (%)
Dieta y pérdida de peso	Henry 1986 Kelley 1993 Low 1996 Markovic 1998 Heilbronn 1999	0-22	ns	0-64
Ejercicio	Vanninen 1992 Dunstan 1997 Rigla 2000	0-7	0-13	0-33
Control metabólico	Lindström 1990 Henry 1993 Wolffenbuttel 1993 y 1996 Caixàs 1997 y 1997[a]	0-6	0-30	15-60
Estatinas*	Pyörälä 1997 Goldberg 1998 Downs 1998 LIPID 1998	25-36	4-7	11-19
Fibratos*	Koskinen 1992 Elkeles 1998 Rubins 1999	0-11	6	27-33
Acipimox	Dean 1992 Koev 1993 Stewart 1994 Davoren 1998	ns	ns	15-28
Resinas	Garg 1994	28	ns	13,5

*Valores obtenidos del análisis *post-hoc* de estudios de intervención.

Tabla 6: Efecto del tratamiento hipolipemiante sobre el riesgo cardiovascular de los pacientes con diabetes según estudios randomizados y controlados.

Estudio	N Total/ Diabetes	Seguimiento (Años)	Fármaco	cLDL mg/dl (%¯)	triglicéridos mg/dl (%¯)	cHDL mg/dl (%-)	Objetivo principal	%¯Riesgo (DM/no DM)
HHS	4081/135	5,0	gemfibrocilo	202 (11)*	238 (27)*	49 (6)*	IAM o muerte coronaria	67,6/57
CARE	4159/586	5,0	pravastatina	136 (27)*	164 (13)*	38 (4)*	IAM o Muerte coronaria	13/26
4S	4444/202	5,4	simvastatina	185 (36)*	153 (11)*	44 (7)*	Mortalidad total IAM	37,5/28 45/29,6**
AFCAPS	6440/155	5,2	lovastatina	150 (26,5)	158 (19,3)	36/40 (4,8)	IAM, angina, o muerte coronaria	33,3/37
PCABG	1351/116	4,3	lovastatina ± colestiramina	155 (27,7)¶	160 (10)¶	39 (0)¶	Progresión angiográfica	51/40
VAHIT	2531/633	5,1	gemfibrocilo	111 (0)	161 (31)	32 (6)	IAM o muerte coronaria	24/24
LIPID	8232/782	6,1	pravastatina	150 (25)	138 (11)	36 (5)	IAM o muerte coronaria	19/25
SENDCAP	164/164	3,0	bezafibrato	142 (9,6)*	198 (32,5)*	39 (6,4)*	Grosor de íntima-media carotídea	no diferencias
DAIS	384/384	3,2	fenofibrato	3,38 (7)*	2,59 (28)*	1,04 (7)*	Progresión angiográfica	40-42

La reducción respecto a la concentración basal es la observada con el fármaco - observada con el placebo. * Datos referentes a los pacientes con diabetes en particular (en los demás estudios se dan las concentraciones y reducciones del grupo total).**La reducción del riesgo es significativa en los pacientes con diabetes. ¶ Se comparan reducción moderada (136 mg/dl) y agresiva (93 mg/dl) del cLDL tras revascularización coronaria. HHS: Helsinki Heart Study (Manninen 1988, Koskinen 1992). CARE: Cholesterol and Recurrent Events (Sacks 1996, Goldberg 1998). 4S: Scandinavian Simvastatin Survival Study (4S Study Group 1994). AFCAPS: Air Force/Texas Coronary Atherosclerosis Prevention Study (Downs 1998). PCABG: Post Coronary Artery Bypass Graft Trial (PCABG Trial Investigators 1997, Hoogwerf 1999). VAHIT: Veterans Affairs Cooperative Studies Program High-density Lipoprotein Cholesterol Intervention Trial (Rubins 1999). LIPID: Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID Study Group 1998). SENDCAP: St Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention Study (Elkeles 1998). DAIS: Diabetes Atherosclerosis Intervention Study (DAIS Investigators 2001). Muestra una reducción del 40% en la progresión del diámetro mínimo de la luz vascular, y del 42% del porcentaje de estenosis.

Tabla 7: Efectos comparativos de distintos fármacos sobre la dislipemia diabética.

Estudio	N	Duración (meses)	Tratamiento	cLDL mg/dl (%↓)	triglicéridos mg/dl (%↓)	cHDL mg/dl (%↑)	apo(B) g/l (%↓)
Garg 1989	10	1	Gemfibrocilo	89 (-33,6)	756 (46,7)	25 (17,9)	-
			Lovastatina+gemfibrocilo	(7,0)	(52,5)	(19,0)	-
Goldberg 1990	102	6	Lovastatina 20	189 (26,2)	204 (1,8)	42 (13,6)	-
			Gemfibrocilo 1200	189 (1,1)	218 (36,0)	42 (21,3)	-
Niort 1992#	16	1	Bezafibrato 400	166 (14,5)	270 (37,0)	45 (20,4)	1,44 (30,4)
		1	Acipimox 500	152 (4,2)	261 (14,6)	46 (5,9)	1,37 (5,2)
Stewart 1994*	44	3	Simvastatina 40	104 (46,6)	110 (10,5)	56 (-14,5)	0,93 (39,8)
			Bezafibrato 400	102 (18,2)	130 (28,1)	50 (0)	0,98 (26,5)
			Acipimox 750	105 (15,1)	163 (30,4)	51 (0)	0,98 (22,4)
			Placebo	99 (5,4)	156 (13,1)	51 (0)	0,95 (4,2)
Jeck 1997	73	3	Simvastatina 10	159 (13,8)	506 (22,3)	43 (9,2)	1,67 (15,4)
			Bezafibrato 400	158 (-20,9)	453 (41,2)	42 (17)	1,73 (4,4)
Tikkanen 1998	96	6	Simvastatina 40	175 (41,6)	218 (15,4)	49 (4)	-
			Gemfibrocilo 1200	177 (6,5)	225 (45,2)	46 (13,7)	-
Gavish 2000*	148	6	Simvastatina 20	174 (31)	337 (7,9)	35 (0)	-
		6	Bezafibrato 400	178 (4)	461 (38)	31 (25)	-
		12	Simvastatina+bezafibrato	174 (29)	381 (42)	31 (25)	-
Frost 2001**	11	1,5	Atorvastatina 10	158 (29)	260 (4)	46,8 (10)	1,52 (28)
		1,5	Fenofibrato 200	170 (11)	259 (39)	50,6 (11)	1,54 (20)

Para transformar en mmol/l dividir colesterol por 38,7 y triglicéridos por 88,7. * Los pacientes inician simvastatina o bezafibrato durante 6 meses y después tratamiento combinado durante 1 año. NOTA: Definen diabetes a partir de glucemia de 6 mmol/l. **Estudio cruzado, abierto, randomizado.

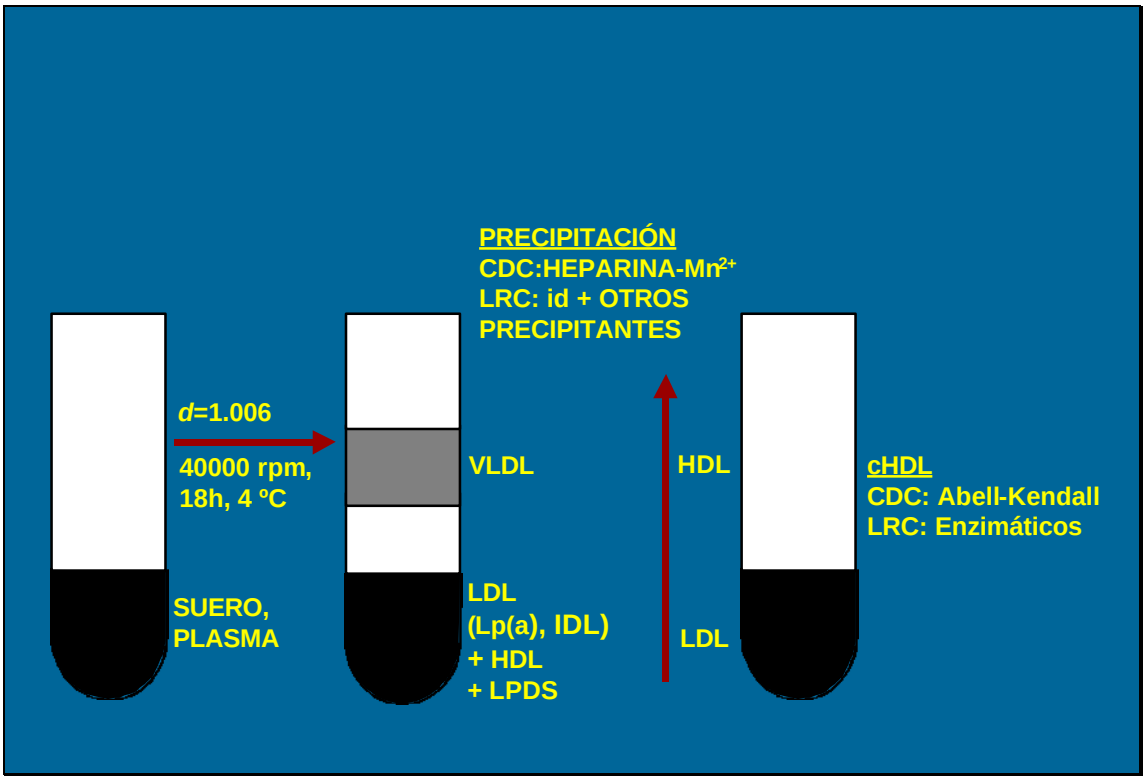

Figura 1: Método designado de comparación para la estimación del cLDL. Tras la separación, mediante ultracentrifugación, de la fracción de densidad inferior a 1,006 Kg/l (VLDL), quedan las partículas de LDL (que incluyen IDL y Lp(a)), de HDL, donde se mide colesterol total. Tras la precipitación de las LDL, se mide el cHDL. El cLDL será el resultado de restar el cHDL del colesterol total medido antes de la precipitación.

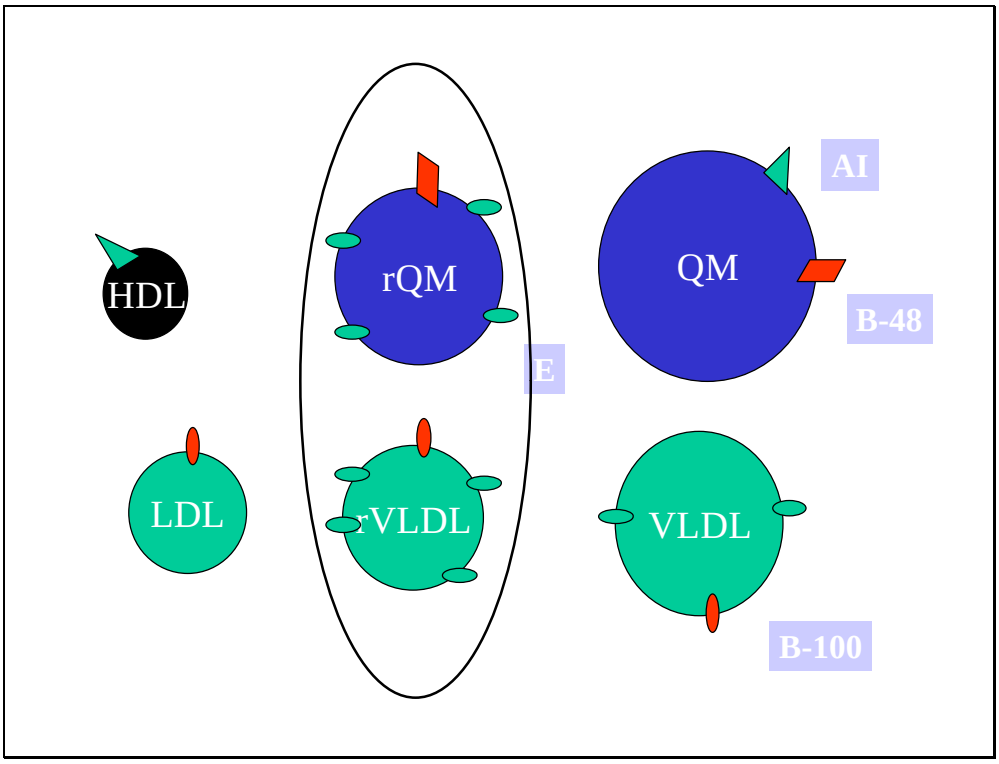

Figura 2: La medida de las lipoproteínas semejantes a remanentes por cromatografía de inmunoafinidad se basa en el distinto contenido de apoproteinas de estas partículas (ricas en apoE). Se utiliza un gel que contiene dos anticuerpos: uno, frente a apoAI (AI) (que permite inmovilizar a los quilomicrones grandes y a las HDL) y otro que reconoce las partículas que contienen apoB-100 (B-100), si no son ricas en apoE (E)(captura LDL y VLDL). En el sobrenadante se miden colesterol y triglicéridos de las partículas remanentes de quilomicrones (rQM) y de VLDL (rVLDL).

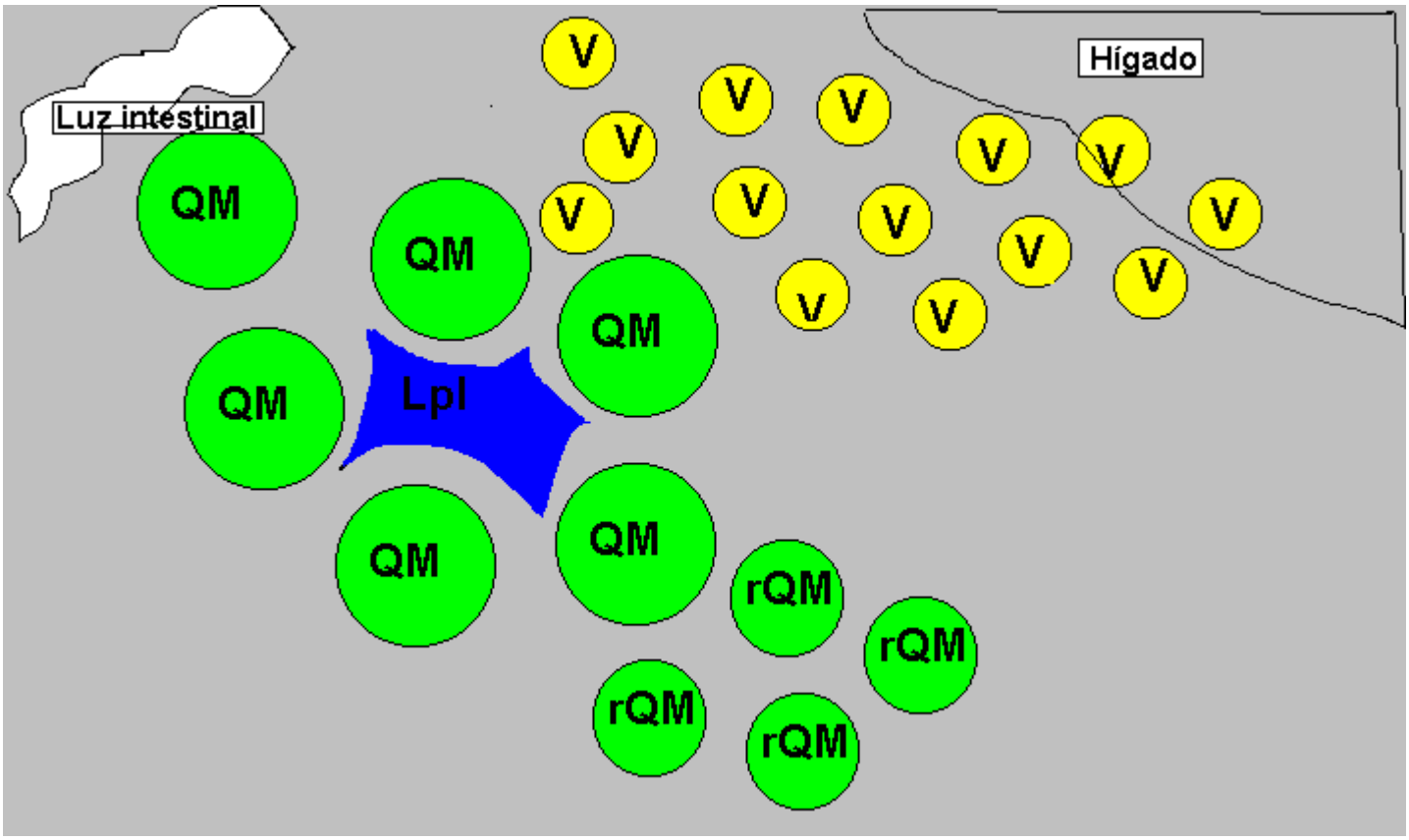

Figura 3: Los quilomicrones (QM) y las VLDL (V) compiten por la lipoprotein lipasa endotelial, pero ésta muestra mayor afinidad por los primeros. Con la llegada de los quilomicrones postprandiales, la degradación de éstos a remanentes de quilomicrones (rQM) satura a la lipoprotein lipasa (Lpl), y ocasiona una acumulación de partículas de VLDL.

ABREVIATURAS

4S: Scandinavian Simvastatin Survival Study

AFCAPS/TexCAPS: Air Force/Texas Coronary Atherosclerosis Prevention Study

ApoB: Apolipoproteína B

ApoE: Apolipoproteina E

CARE: Cholesterol and Recurrent Events

cIDL: colesterol de IDL

cHDL: colesterol de HDL

cLDL: colesterol de LDL

cLDL-Plus: colesterol de LDL determinado por el método directo con el mismo nombre.

cVLDL: colesterol de VLDL

DAIS: Diabetes Atherosclerosis Intervention Study

HHS: Helsinki Heart Study

LIPID: Long-Term Intervention with Pravastatin in Ischaemic Disease

Lp(a): lipoproteina (a)

LRT: Lipoproteínas ricas en triglicéridos.

NCEP: National Cholesterol Education Program

PCABG: Post Coronary Artery Bypass Graft Trial

PROCAM: Münster Heart Study.

PTEC: Proteína transferidora de ésteres de colesterol.

SENDCAP: St Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention Study

UKPDS: United Kingdom Prospective Diabetes Study.

VA-HIT: Veterans Affairs Cooperative Studies Program High-density Lipoprotein Cholesterol Intervention Trial.

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