Non–HDL Cholesterol and Apolipoprotein B in the Dyslipidemic Classification of Type 2 Diabetic Patients

ANA MARIA WÄGNER, MD, PHD[1]
ANTONIO PÉREZ, MD, PHD[1]
EDGAR ZAPICO, MSC[1]
JORDI ORDÓÑEZ-LLANOS, MD, PHD[2,3]

OBJECTIVE — To compare non–HDL cholesterol (HDLc) and apolipoprotein B (apoB) in the identification of nonconventional high-risk dyslipidemic phenotypes in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — Total cholesterol and triglycerides, HDLc, LDL cholesterol, non-HDLc, apolipoprotein B (apoB), and LDL size were determined in 122 type 2 diabetic patients (68% male, aged 59.6 ± 9.7 years, and HbA_{1c} 7.5% [range 5.2–16.0]). They were then classified as normo- and hypertriglyceridemic if their triglyceride concentrations were below/above 2.25 mmol/l, as normo/hyper–non-HDLc if non-HDLc concentrations were below/above 4.13 mmol/l, and as normo- and hyperapoB if apoB concentrations were below/above 0.97 g/l. Both classifications were compared (concordance assessed with the κ index), and low HDLc and LDL phenotype B were identified in each category.

RESULTS — A total of 26 patients were hypertriglyceridemic and 96 were normotriglyceridemic. All hypertriglyceridemic subjects had increased non-HDLc, whereas 24 had increased apoB (κ= 0.95). In the normotriglyceridemic group, 44 had increased non-HDLc, 68 had increased apoB, and 25 of the 52 patients with normal non-HDLc had increased apoB (κ= 0.587). Low HDLc and LDL phenotype B were similarly distributed into the equivalent categories.

CONCLUSIONS — Non-HDLc and apoB are equivalent risk markers in hypertriglyceridemic patients, but apoB identifies additional patients with high-risk dyslipidemic phenotypes in normotriglyceridemic type 2 diabetic patients.

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LDL cholesterol (LDLc) is the main therapeutic target in the treatment of dyslipidemia (1,2). Nevertheless, several epidemiologic studies have shown that both non–HDL cholesterol (HDLc) and apolipoprotein B (apoB) are better predictors of cardiovascular events than LDLc (3–5). The former has, in fact, been included as a therapeutic target for hypertriglyceridemic patients in the most recent National Cholesterol Education Program (NCEP) recommendations (1) and is easy and cheap to calculate. On the other hand, apoB identifies high-risk dyslipidemic phenotypes that are not detected by the standard lipid profile in type 2 diabetic patients, who may present with hyperapoB-dependent dyslipidemic phenotypes (6,7). Because of the high correlation between non-HDLc and apoB in nondiabetic subjects (8), non-HDLc is considered a good surrogate marker for apoB. To our knowledge, however, no comparison has been made between non-HDLc and apoB in the classification of patients into dyslipidemic phenotypes.

The aim of this study was to compare the classification into nonconventional dyslipidemic phenotypes of a group of type 2 diabetic subjects using apoB and non-HDLc.

RESEARCH DESIGN AND METHODS

Patients

A total of 122 type 2 diabetic patients from a university hospital were consecutively included in the study. Those receiving treatments or who were in situations (unrelated to their diabetes) that are known to affect lipid metabolism were excluded. Patients with hypertension were not treated with nonselective β-blockers or high-dose diuretics. A clinical history was taken and physical examination, including anthropometric parameters, was performed. The study group's main clinical and laboratory features are displayed in Table 1.

Laboratory determinations

Total cholesterol and triglyceride were measured by enzymatic methods; HDLc was measured by a direct method using polyethylene-glycol–pretreated enzymes (Roche Diagnostics, Basel, Switzerland). High triglyceride and low HDLc were defined as recommended by the NCEP and the American Diabetes Association (1,2) (triglycerides >2.25 mmol/l and HDLc <1.04 mmol/l for men and <1.30 mmol/l for women), though the cutoff point 1.7 mmol/l (150 mg/dl) was also explored for the definition of hypertriglyceridemia. We calculated LDLc with Friedewald's formula (9) when triglyceride did not exceed 3.45 mmol/l (300 mg/dl), as is the usual procedure in our laboratory, by dividing total triglyceride (in mmol/l) by 2.17. When triglycerides

From the [1]Endocrinology and Nutrition Department, Hospital de Sant Pau, Universitat Autònoma, Barcelona, Spain; the [2]Biochemistry Department, Hospital de Sant Pau, Universitat Autònoma, Barcelona, Spain; and the [3]Biochemistry and Molecular Biology Department, Universitat Autònoma, Barcelona, Spain.

Address correspondence and reprint requests to Ana Maria Wägner, MD, PhD, Endocrinology and Nutrition Department, Hospital de Sant Pau, S Antonio M Claret 167, 08025 Barcelona, Spain. E-mail: awagner@hsp.santpau.es.

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Abbreviations: apoB, apolipoprotein B; HDLc, HDL cholesterol; IDL, intermediate-density lipoproteins; LDLc, LDL cholesterol; NCEP, National Cholesterol Education Program.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying editorial, p. 2207.

Table 1—*Main clinical and laboratory features of the 122 patients included in the study*

Male/female (%)	68/32
Age (years)	59.6 ± 9.7
BMI (kg/m^2)	28.0 ± 3.7
Menopause (women only) (%)	89.5
Hypertension (%)	51.7
Smoking (%)	22.9
Diabetes duration (years)	8 (0–37)
Treatment (%):diet/oral agents/insulin/insulin plus oral agents	26.3/23.7/41.5/6.8
Retinopathy (%)	34.9
Nephropathy (%)	49.5
Microalbuminuria/proteinuria/renal failure	43.2/4.5/1.8
Cardiovascular disease (%)	41.9
Stroke/coronary heart disease/peripheral vascular disease (%)	5.9/20.5/28.7
HbA_{1c} (%)	7.45 (5.2–16.0)
Total cholesterol (mmol/l)	5.64 ± 1.18
Triglyceride (mmol/l)	1.41 (0.56–10.5)
LDLc (mmol/l)	3.61 ± 0.93
HDLc (mmol/l)	1.19 ± 0.29
Non-HDLc (mmol/l)	4.44 ± 1.15
ApoB (g/l)	1.16 ± 0.25

Data are means ± SD or median (range) unless otherwise indicated.

were ≥3.45 mmol/l (n = 11), we determined LDLc by ultracentrifugation in fresh or frozen serum stored at −80°C for no more than 96 h. Non-HDLc was calculated by subtracting HDLc from total cholesterol. High non-HDLc was defined by the cutoff point equivalent to an LDLc >3.36 mmol/l, i.e., when pharmacological intervention is recommended in type 2 diabetic patients, or non-HDLc >4.13 mmol/l (1). ApoB was measured by an immunoturbidimetric method (Tinaquant, Roche Diagnostics) calibrated against the World Health Organization/International Federation of Clinical Chemistry reference standard SP3-07. The apoB cutoff point was calculated according to Contois et al. (10) as the value equivalent to an LDLc value of 3.36 mmol/l in a nondiabetic normolipidemic control group, as described previously (6). Using the equation apoB (g/l) = 0.176 LDLc (mmol/l) + 0.377 (r = 0.712, $P < 0.001$), a value of 0.97 g/l resulted for apoB. LDL size was determined by electrophoresis on gradient (2–16%) polyacrylamide gel, as described elsewhere (11). LDL phenotype B was defined by a predominant LDL diameter <25.5 nm.

Patients were classified according to their triglyceride and apoB concentrations and also according to their triglyceride and non-HDLc concentrations. Patients with low HDLc and LDL phenotype B were identified in each group.

Statistical analysis

Analysis was performed using SPSS version 10.0 statistical package for Windows (SPSS, Chicago, IL). Continuous variables are expressed as mean ± SD (gaussian distribution) or as median and range, and qualitative data is expressed in percentages. Bivariate correlation (Spearman) was performed between apoB and non-HDLc. Concordance between classifications according to apoB and non-HDLc was assessed using the κ index. Values between 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.0 showed fair, moderate, good, and very good concordance, respectively (12). Tests were two tailed, and a P value <0.05 was considered significant.

RESULTS — The 122 patients included in the study had, on average, fair glycemic control (half of them on insulin treatment) and were mildly overweight. Their main laboratory results are displayed in Table 1. Their distribution into the different dyslipidemic phenotypes is depicted in Fig. 1. The correlation between apoB and non-HDLc was strong in the group as a whole (r = 0.916, $P < 0.0005$) and better in the hypertriglyceridemic (r = 0.947, $P < 0.0005$) than in the normotriglyceridemic subgroup (r = 0.773, $P < 0.0005$). In addition, the concordance between both classifications was very good only in hypertriglyceridemic patients (n = 26) (κ= 0.95), but moderate in normotriglyceridemic patients (n = 96) (κ= 0.587). Actually, 25 of the 52 patients considered normolipidemic according to non-HDLc and triglyceride fell into the normotriglyceridemic-hyperapoB phenotype (and only 1 patient was discordant in the opposite way). On the other hand, the frequency of low HDLc and LDL phenotype B was similar in the equivalent dyslipidemic phenotypes and seemed to depend more on the presence of hypertriglyceridemia than on high apoB or high non-HDLc concentrations (Table 2). Nevertheless, the concordance between the classification into apoB and non–HDLc-dependent dyslipidemic phenotypes and the diagnosis of LDL phenotype B was moderate for hypertriglyceridemia-hyperapoB (κ= 0.527) and hypertriglyceridemia-hyper–non-HDLc (κ= 0.571), but fair for normotriglyceridemia-hyperapoB (κ= −0.303) and poor for normotriglyceridemia-hyper–non-HDLc (κ= −0.173). Similar results were obtained when triglycerides >1.7 mmol/l was used for the definition of hypertriglyceridemia (data not shown).

CONCLUSIONS — To our knowledge, this is the first time a comparison has been made between apoB and non-HDLc for the classification of type 2 diabetic patients into nonconventional dyslipidemic phenotypes. The present study reveals that *1)* both hypertriglyceridemia/hyper apoB and hypertriglyceridemia/hyper–non-HDLc are phenotypes with a predominance of small dense LDL particles, and *2)* although apoB and non-HDLc seem equivalent in hypertriglyceridemic patients, in normotriglyceridemic patients, apoB identifies patients at risk better than non-HDLc.

Although LDLc is the main therapeutic target in the treatment of diabetic and nondiabetic dyslipidemia (1,2), its concentrations do not stand for the whole mass of lipoprotein particles, which also include intermediate-density lipoproteins (IDLs) and VLDLs. ApoB is the principal protein moiety of LDL, IDL, and VLDL; its concentrations are a good estimate of the total mass of these particles, especially if LDL particles are predominantly small and dense. Furthermore, there are data from epidemiological (3) and intervention studies (13,14) suggesting that apoB is a better predictor of cardiovascular events than LDLc. Its measurement has gained relevance since an international

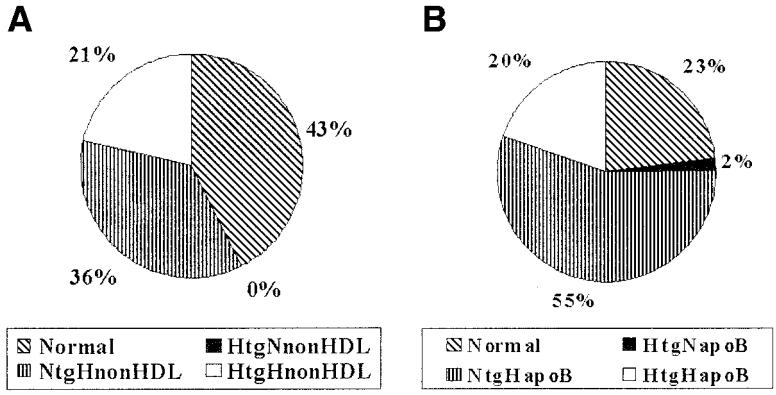

Figure 1—*Phenotype distributions of the 122 type 2 diabetic patients according to non-HDLc (nonHDL) and triglyceride (tg)* (A) *and apoB and triglyceride* (B)*. H, hyper; N, normo.*

standard has become available, making transferability of results from different methods and laboratories possible. Nevertheless, given the differences in normal apoB concentrations among different populations, with the 75th percentile ranging from 1.1 to 1.6 g/l (10,15,16), population-based reference values for this measure are still desirable. In addition, only the Canadian Cardiovascular Society has proposed therapeutic goals based on their population-based studies (17); therefore, values corresponding to LDLc concentrations are recommended (10).

Non-HDLc, calculated by subtracting HDLc from total cholesterol, represents the cholesterol contained in VLDL, IDL, and LDL particles and is considered an acceptable surrogate for apoB (18). It was proposed as an alternative target to LDLc in type 2 diabetes a few years ago (19), but now there are data supporting it as a better predictor of cardiovascular events (5,20) and mortality (4). The most recent recommendations of the NCEP include non-HDLc as a second line (after LDLc) therapeutic target in hypertriglyceridemic patients, with a cutoff point 30 mg/dl (0.78 mmol/l) above the LDLc target (1). In patients with triglyceride concentrations >4.51 mmol/l, when the Friedewald formula is not applicable for the estimation of LDLc, non-HDLc can be used as an alternative. In addition, given the inaccuracy of the Friedewald formula at even lower triglyceride concentrations, non-HDLc might even be an alternative to LDLc in patients with moderate hypertriglyceridemia (21). In type 2 diabetes, the estimation of LDLc carries a higher than recommended bias, even in patients with normal or slightly increased triglyceride concentrations (22). Thus, alternative risk predictors would be useful in all diabetic patients. We, among others, have shown that hyperapoB reveals high-risk phenotypes that are not identified by triglyceride, LDLc, and HDLc (6,7). In the present study, non-HDLc seemed to be a good alternative to apoB in hypertriglyceridemic patients, since a strong correlation and good concordance were found between both parameters in the classification of patients. Nevertheless, this correlation was weaker in the normotriglyceridemic group; almost one-third of the normotriglyceridemic patients, who account for most of the subjects with fair glycemic control (23,24), were misclassified into a low-risk category when non-HDLc was used. On the other hand, although the presence of LDL phenotype B seems to be more related to hypertriglyceridemia than to the increase in apoB or non-HDLc, as stated in previous studies (7), the higher concordance of hyperapoB than hyper–non-HDLc with LDL phenotype B in normotriglyceridemic patients suggests that there might be an increase in small dense LDL particles in normotriglyceridemic type 2 diabetic patients with increased apoB.

The fact that non-HDLc is easy (and cheap) to calculate supports it as a first-line component to be evaluated in diabetic dyslipidemia. ApoB, on the other hand, seems to better identify patients at risk in the normotriglyceridemic group, but its measurement comprises additional cost. Thus, we could propose that non-HDLc be used in all patients with diabetes and that apoB be measured in patients with triglycerides <2.25 mmol/l (or even <1.7 mmol/l) in whom non-HDLc is <4.13 mmol/l. In our group of patients, 42.6% would fall into this category (37.7% if 1.7 mmol/l were to be used for triglycerides). To conclude, non-HDLc and apoB seem to be equally useful in the detection of high-risk phenotypes in hypertriglyceridemic type 2 diabetic patients, whereas apoB seems to be superior in normotriglyceridemic subjects. In addition, recently published data from intervention studies (25) show that apoB is a better predictor of cardiovascular events and carotid intima-media thickness than non-HDLc. Therefore, especially given the difficulties in estimating LDLc in type 2 diabetic patients, our results support the use of non-HDLc in these subjects and apoB in those with normal triglyceride and non-HDLc concentrations for diagnostic and even therapeutic purposes.

Table 2—*Frequency of low HDLc (<1.04 mmol/l for women and <1.30 mmol/l for men) and LDL phenotype B among the different dyslipidemic phenotypes*

	n	Low HDLc	Phenotype B
Normo Tg/normo apoB	28	12 (42.9)	5 (17.9)
Normo Tg/hyper apoB	68	16 (23.5)	15 (22.1)
Hyper Tg/normo apoB	2	2 (100)	2 (100)
Hyper Tg/hyper apoB	24	20 (83.3)	22 (91.7)
Normo Tg/normo non-HDLc	52	17 (32.7)	9 (17.3)
Normo Tg/hyper non-HDLc	44	11 (25)	11 (25)
Hyper Tg/normo non-HDLc	0	—	—
Hyper Tg/hyper non-HDLc	26	22 (84.6)	24 (92.3)

Data are *n* (%). Tg, triglyceridemic.

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