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

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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 HbA1c, 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 0.97 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 normotrigrlyceridemic type 2 diabetic patients.

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DL 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...
were $\geq 3.45$ mmol/l ($n = 11$), we deter-
mimed LDLc by ultracentrifugation in
fresh or frozen serum stored at $-80^\circ$C for
no more than 96 h. Non-HDLc was cal-
culated 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 pharmaco-
gical intervention is recommended in type 2
diabetic patients, or non-HDLc $>4.13$
mol/l (1). ApoB was measured by an
immunoturbidimetric method (Tina-
quant, Roche Diagnostics) calibrated
against the World Health Organization/ 
International Federation of Clinical
Chemistry reference standard SP3-07.
The apoB cutoff point was calculated ac-
cording 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 deter-
mined 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 concentra-
tions and also according to their trigly-
ceride and non-HDLc concentrations.
Patients with low HDLc and LDL pheno-
type B were identified in each group.

### Statistical analysis
Analysis was performed using SPSS ver-
sion 10.0 statistical package for Windows
(SPSS, Chicago, IL). Continuous variables
are expressed as mean $\pm$ SD (gaussian
distribution) or as median and range, and
qualitative data is expressed in percentages.
Bivariate correlation (Spearman) was per-
formed between apoB and non-HDLc. Cor-
discance between classifications ac-
cording to apoB and non-HDLc was as-
essed using the $\kappa$ 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 normo-
triglyceridemic subgroup ($r = 0.773, 
P < 0.0005$). In addition, the concor-
dance between both classifications was
very good only in hypertriglyceridemic
patients ($n = 26$) ($\kappa = 0.95$), but moder-
ate in normotriglyceridemic patients ($n =
96$) ($\kappa = 0.587$). 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 pheno-
types and seemed to depend more on the
presence of hypertriglyceridemia than on
high apoB or high non-HDLc concentra-
tions (Table 2). Nevertheless, the concor-
dance between the classification into
apoB and non–HDLc-dependent dyslipi-
demic phenotypes and the diagnosis of
LDL phenotype B was moderate for hyper-
triglyceridemia-hyperapoB ($\kappa =
0.527$) and hypertriglyceridemia-hyper-
non-HDLc ($\kappa = 0.571$), but fair for nor-
motriglyceridemia-hyperapoB ($\kappa =
-0.303$) and poor for normotriglyceri-
demia-hyper–non-HDLc ($\kappa = -0.173$).
Similar results were obtained when trigly-
cerides $>1.7$ 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 pa-
tients into nonconventional dyslipidemic
phenotypes. The present study reveals
that 1) both hypertriglyceridemia/hyper
apoB and hypertriglyceridemia/hyper-
non-HDLc are phenotypes with a pre-
dominance of small dense LDL particles,
and 2) although apoB and non-HDLc
seem equivalent in hypertriglyceridemic
patients, in normotriglyceridemic pa-
tients, apoB identifies patients at risk bet-
ter than non-HDLc.

Although LDLc is the main therapeu-
tic target in the treatment of diabetic and
nondiabetic dyslipidemia (1.2), its con-
centrations 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 VLDLs;
it 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 interven-
tion studies (13,14) suggesting that apoB
is a better predictor of cardiovascular
events than LDLc. Its measurement has
gained relevance since an international

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

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (%)</td>
<td>68/32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.6 $\pm$ 9.7</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>28.0 $\pm$ 3.7</td>
</tr>
<tr>
<td>Menopause (women only) (%)</td>
<td>89.5</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>51.7</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>22.9</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>8 (0–37)</td>
</tr>
<tr>
<td>Treatment (%): diet/oral agents</td>
<td>26.3/23.7/41.5/6.8</td>
</tr>
<tr>
<td>Insulin/insulin plus oral agents</td>
<td>34.9</td>
</tr>
<tr>
<td>Retinopathy (%)</td>
<td>49.5</td>
</tr>
<tr>
<td>Nephropathy (%)</td>
<td>43.2/4.5/1.8</td>
</tr>
<tr>
<td>Microalbuminuria/proteinuria/renal failure (%)</td>
<td>41.9</td>
</tr>
<tr>
<td>Cardiovascular disease (%)</td>
<td>5.9/20.5/28.7</td>
</tr>
<tr>
<td>HbA$_1$c (%)</td>
<td>7.45 (5.2–16.0)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.64 $\pm$ 1.18</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.41 (0.56–10.5)</td>
</tr>
<tr>
<td>LDLc (mmol/l)</td>
<td>3.61 $\pm$ 0.93</td>
</tr>
<tr>
<td>HDLc (mmol/l)</td>
<td>1.19 $\pm$ 0.29</td>
</tr>
<tr>
<td>Non-HDLc (mmol/l)</td>
<td>4.44 $\pm$ 1.15</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1.16 $\pm$ 0.25</td>
</tr>
</tbody>
</table>

Data are means $\pm$ SD or median (range) unless otherwise indicated.
Non-HDLc versus apoB in type 2 diabetes

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

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

Data are n (%): Tg, triglyceridemic.

References

10. Contois JH, McNamara JR, Lammi-Keefe CJ, Wilson PW, Massov T, Schaeffer E: Reference intervals for plasma apolipo-