Apolipoprotein B and non-high-density lipoprotein cholesterol reveal a high atherogenicity in individuals with type 2 diabetes and controlled low-density lipoprotein-cholesterol

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Abstract

Purpose: Lipid-lowering therapy is guided by Low-density-lipoprotein cholesterol (LDL-c), although the CVD risk could be better reflected by other lipid parameters. This study aimed the evaluation of a comprehensive lipid profile in patients with type 2 diabetes mellitus (T2DM) and comparation of those achieving and not achieving LDL-c control in respect to other non-conventional lipid parameters. Methods: We characterized a comprehensive lipid profile in 96 T2DM patients. ESC/EAS 2016 and 2019 Guidelines for the Management of Dyslipidemias were used to define LDL-c targets. Atherogenic lipoprotein profile was compared in patients with LDL-c within and above the target. Results: Only 28.1% and 16.7% of patients had mean LDL-c levels within the 2016 and 2019 guidelines, respectively. In patients with LDL-c within target by the 2016 guidelines, 22%, 25% and 44% presented levels above the recommended range for non-HDL-c, ApoB and oxidized LDL-c, respectively, whereas accordingly to the 2019 guidelines, 50%, 39% and 44% had elevated levels of -HDL-c, ApoB and oxidized LDL-c, respectively. There was a significant strong association of LDL-c and non-HDL-c (r=0.850), ApoB (r=0.656) and oxidized LDL-c (r=0.508). Similarly, non-HDL-c was significantly strongly correlated with ApoB (r=0.808) and oxidized LDL-c (r=0.588). Conclusions: These findings emphasize the limitations of a sole LDL-c measurement for CV risk assessment. Targeting only LDL-c could result in missed opportunities for CV risk reduction in T2DM individuals. Our data suggest that non-HDL-c, ApoB and oxidized LDL-c could be considered as part of these patients' evaluation allowing a more accurate estimation of CV risk and treatment among these high-risk patients.

Background

Type 2 diabetes mellitus (T2DM) has increased worldwide and consequently, the prevalence of cardiovascular disease has also been growing and it is the main cause of mortality in these patients (1). In addition to glycemic control, the cornerstone of treatment of individuals with T2DM is the control of the several cardiovascular risk factors that commonly are present in these patients. Dyslipidemia is common in T2DM and there is strong evidence that cholesterol lowering improves cardiovascular outcomes, even in patients with apparently unremarkable lipid profiles (2).

LDL-c is recommended as the primary target to guide lipid-lowering therapy(3). However, there is evidence that other lipid parameters are equally or even more important than LDL-c to address in order to reduce coronary heart disease (CHD) risk(4, 5), such as non-high-density lipoprotein cholesterol (non-HDL-c) and Apolipoprotein B (ApoB). Non-HDL-c and ApoB were considered secondary targets for lipid-lowering therapy according to the 2016 European Society of Cardiology /European Atherosclerosis Society (ESC/EAS) Guidelines(3). Nevertheless, studies have suggested that non-HDL-c or ApoB may provide a more accurate measure of CHD risk in comparison to LDL-c(4, 5). In accordance, the 2019 European Society of Cardiology /European Atherosclerosis Society (ESC/EAS) Guidelines(6) has defined new targets for atherogenic lipoproteins and suggested that in order to estimate CV risk non-HDL and ApoB could be preferred when evaluating patients with diabetes, metabolic syndrome, obesity, high triglycerides and very low LDL-c levels.

T2DM patients present an atherogenic dyslipidemia characterized by increased triglycerides, decreased HDL particles, and LDL levels overlapping those of non-diabetics. Nevertheless, they also present small and dense LDL particles that are highly atherogenic and strongly increase these patients' vascular risk. Oxidized lipids in circulating LDL-c are also shown to be strongly associated with coronary atherosclerosis, arterial dysfunction, and mortality(7–9). Therefore, the severe nature of diabetic dyslipidemia is not always revealed in the routine lipid profile, that usually evaluates LDL-c, which in many cases remains within the normal range(3), to estimate CVD risk.

The aim of this study was to evaluate a comprehensive lipid profile in patients with T2DM and compare those achieving and not achieving LDL-c control in respect to other non-conventional lipid parameters. Secondarily, we aimed to compare the atherogenic profile of patients with LDL-c within target accordingly to the 2016 and 2019 guidelines.

Materials And Methods

We performed a retrospective data evaluation of our institution's database and electronic medical records system for patients included in the outpatient department at a Portuguese University Hospital, with information regarding an extended lipid profile. Patients followed in the Dyslipidemia outpatient department between January 2010 and September 2017 were included. After analysis of the medical records of 345 patients, 96 patients with the diagnosis of type 2 diabetes were included in this study. Diabetes was defined as plasma glucose ≥ 126 mg/dL in at least two measurements, A1c ≥ 6.5% or prescription of any antidiabetic medication(10).

Patients were classified as smokers if they consumed any quantity of tobacco. A former smoker was defined as having quit smoking at least 6 months before assessment. Alcohol consumption was considered when patients reported drinking daily at least 1 or 2 glasses of an alcoholic beverage, for women and men respectively. Patients with conditions that could interfere with the lipid profile, such as genetic dyslipidemia, hepatic or renal moderate to severe disease, cancer, viral infection (hepatitis B or C, HIV), genetic metabolic disease, hyper or hypothyroidism were excluded. Pediatric patients and pregnant women were also excluded as well as patients treated with drugs that could exacerbate lipid profile, such as glucocorticoids.

In order to stratify risk categories, patients were classified as having "very high risk" and "high risk" accordingly to the 2016 ESC/EAS Guidelines for the Management of Dyslipidemias(3). The 10-year risk of fatal CVD was calculated using Systemic Coronary Risk Estimation (SCORE). LDL-c targets were defined according to the following ESC/EAS Guidelines(3), LDL-c < 70 mg/dL for very high-risk patients and LDL-c < 100 mg/dL for high risk. In order to analyze other lipid parameters and their agreement with LDL-c levels, targets were also defined for these parameters. The 2016 ESC/EAS Guidelines for the Management of Dyslipidemias was used to define targets such as ApoB, Apolipoprotein A1 (ApoA1), non-HDL-c and Lipoprotein (a) [Lp(a)](3): ApoB: < 80 mg/dL in very high risk individuals and < 100 mg/dL in high risk individuals; Non-HDL-c: < 100 mg/dL in very high risk individuals and < 130 mg/dL in high risk

individuals; ApoA1: low if < 120 mg/dL in men and < 140 mg/dL in women; Lp(a) < 50 mg/dL. For the remaining parameters, the following laboratory reference values were used: oxidized LDL-c: 26–117 mg/dL and ApoB/ApoA1 ratio: 0.45–1.25. After the 2019 guidelines publication, risk categories were reviewed and new targets were defined accordingly to patient's CV risk: LDL-c < 55, <70 and < 100 mg/dL for very-high-, high-, and moderate-risk patients, respectively; ApoB < 65, 80, and 100 mg/dL for very-high-, high-, and moderate-risk patients, respectively; and, non-HDL-c < < 85, 100, and 130 mg/dL for very-high-, high-, and moderate-risk patients, respectively.

Biochemical laboratory tests were conducted after an 8-hour night fast. Lipid parameters included total cholesterol, HDL-c, triglycerides, ApoB, ApoA1, oxidized LDL-c and Lp(a). LDL-c levels were calculated through Friedewald's formula (11): LDL-c (mg/dL) = total cholesterol (mg/dL) − HDL-c (mg/dL) − triglycerides (mg/dL)/5, unless triglycerides ≥ 400 mg/dL, where direct LDL-c measurement was performed. Non-HDL-c was calculated by subtracting HDL-c to total cholesterol. Other parameters such as creatinine, uric acid, glucose and homocysteine were also evaluated. A1c was assessed in most diabetic patients. Creatinine, glucose, uric acid, total cholesterol, HDL-c and triglycerides were measured using an enzymatic colorimetric method, with intra and inter-assay coefficients of variation of < 2.8% and < 3.9%, respectively. These parameters were measured using an automated autoanalyzer (Cobas 8000, Roche Diagnostics, Mannheim, Germany). ApoA1, ApoB and Lp(a) were evaluated through immunoturbidimetry, with intra and inter-assay coefficients of variation of < 1% and < 2.4% for ApoA1, < 1.2% and < 3.2% for ApoB and < 3.7% and < 3.8% for Lp(a), respectively. These parameters were evaluated using an automated autoanalyzer (Cobas Integra 400, Roche Diagnostics, Mannheim, Germany). Oxidized LDL-c was measured by Sandwich Enzyme-Linked ImmunoSorbent Assay (Mercodia, Uppsala, Sweden), with intra and inter-assay coefficients of variation of < 7.3% and < 8.3%. Sd-LDL particles were measured using an enzymatic colorimetric method (Randox Laboratories, UK) with intra and inter-assay coefficients of variation of < 3% and < 3%. A1c was evaluated using an ion-exchange chromatography method (Variant II turbo, BioRad Laboratories, CA, USA), with intra and inter-assay coefficients of variation of < 0.78% and < 0.66%. Homocysteine was measured by nephelometry (Dimension Vista 500, Siemens Healthcare, Germany), with intra and inter-assay coefficients of variation of < 3.3% and < 8.2%.

Statistical analysis was performed using IBM SPSS® version 21.0 and a p value below 0.05 was considered statistically significant. For continuous quantitative variables, distribution normality was tested through histogram observation and kurtosis and skewness analysis. Results are presented as mean values ± standard-deviation and median values (25–75 percentiles). The chi-square test was used to analyze differences between groups in categorical variables. The Student t-test for independent variables and the Mann Whitney test were used to compare continuous variables with normal and non-normal distribution between groups, respectively. Correlations were evaluated using the Pearson and the Spearman correlation test for continuous symmetrical and asymmetrical variables, respectively.

This study was approved by the local Ethics committee (150-DEFI/149-CES). Due to the retrospective nature of this study, consent to participate was waived by the Ethics Committee.

Results

This study enrolled 96 patients with T2DM (56 men and 40 women) with a mean age of 58.9 ± 9.0 years, median diabetes duration of 10 years (IQR: 4–7) and mean HbA1c of 8.1 ± 1.9%. The clinical characteristics of the patients are presented in Table 1. Mean LDL-c levels were 102.4 ± 38.6 mg/dL. The majority of patients (n = 48, 66.7%) were under lipid-lowering drugs and statins were the most common. Ten (13.9%) individuals were on fibrates and 4 (5.6%) on ezetimibe. Patients were not taking other drugs for treating dyslipidemia or any anti-diabetic drugs that could affect the lipid profile such as thiazolidinediones, glucagon-like peptide-1 agonists, dipeptidyl peptidase 4 inhibitors or sodium-glucose co-transporter-2 inhibitors. According to the ESC/EAS guidelines, patients were divided in two groups: patients with LDL-c levels within target (n = 27) and patients with LDL-c levels above target (n = 69). There were no statistically significant differences between the groups regarding gender, age, BMI, blood pressure, waist circumference, HbA1c, triglycerides, HDL-c or glucose levels. We found no statistically significant differences between groups in relation to alcohol ingestion, smoking or the use of drus for dyslipidemia such as statins, fibrates or ezetimibe. Uric acid and homocysteine levels, a significant predictor of new CV events, were not statistically different between the groups.

Table 1

Comparison of clinical and laboratory variables between type 2 diabetes patients with LDL-c within and above target

	n	LDL-c within target (n = 27)	n	LDL-c above target (n = 69)	p
Male n (%)	27	16 (59.3%)	69	40 (58.0%)	0.908
Age (years)	27	59.8 ± 9.7	69	58.6 ± 8.8	0.571
Duration of diabetes* (years)	22	10.0 (3.5–15.3)	55	10.0 (5.0–19.0)	0.412
Cardiovascular disease n (%)	26	7 (26.9%)	68	15 (22.1%)	0.618
Cerebrovascular disease n (%)	26	5 (7.4%)	68	4 (15.4%)	0.256[1]
Peripheral vascular disease n (%)	27	5 (7.4%)	68	2 (7.7%)	0.955[1]
Body mass index (Kg/m^2)	20	29.9 ± 3.7	56	30.6 ± 4.8	0.553
Systolic BP (mmHg)	24	142.0 ± 20.9	59	141.5 ± 18.3	0.928
Diastolic BP (mmHg)	11	71.8 ± 12.0	26	78.2 ± 15.4	0.229
Waist circumference (M) (cm)	12	102.1 ± 9.5	17	105.0 ± 11.9	0.492
Waist circumference (F) (cm)	3	96.0 ± 13.4	15	98.0 ± 12.7	0.438
Triglycerides* (mg/dL)	27	146.0 (106.0–295.0)	69	146.0 (108.0–207.5)	0.300
HDL-c (M) (mg/dL)	16	35.2 ± 9.68	40	41.7 ± 12.4	0.064
HDL-c (F) (mg/dL)	11	50.5 ± 10.7	29	46.6 ± 10.2	0.301
Glucose* (mg/dL)	22	137.0 (108.0–182.3)	59	151.5 (119.0–226.8)	0.062
A1c (%)	18	7.8 ± 2.6	48	8.2 ± 1.6	0.538
Uric acid (mg/dL)	21	5.1 ± 0.4	44	4.9 ± 0.2	0.656
Homocysteine (µmol/L)	20	11.4 ± 1.2	55	12.2 ± 0.8	0.632
Statin use n (%)	20	14 (70.0%)	52	30 (57.7%)	0.337

Data are presented as mean ± standard deviation, unless otherwise indicated by ∗ corresponding to data presented as median, 25th and 75th percentiles and [1] corresponding Fisher´s exact test. LDL-c Low-density lipoprotein cholesterol; BP Blood pressure; HDL-c High-density lipoprotein cholesterol, M male; F female

	n	LDL-c within target (n = 27)	n	LDL-c above target (n = 69)	p
Fibrates use n (%)	20	2 (10%)	52	8 (15.4%)	0.716[1]
Ezetimibe use n (%)	20	3 (15%)	51	1 (2%)	0.065[1]
Alcohol drinking n (%)	8	1 (12.5%)	20	7 (35.0%)	0.371[1]
Smoking n (%)	21	2 (9.5%)	61	7 (11.5%)	0.805[1]
Hypertension n (%)	27	14 (51.9%)	68	56 (82.4%)	0.020

Data are presented as mean ± standard deviation, unless otherwise indicated by ∗ corresponding to data presented as median, 25th and 75th percentiles and [1] corresponding Fisher´s exact test. LDL-c Low-density lipoprotein cholesterol; BP Blood pressure; HDL-c High-density lipoprotein cholesterol, M male; F female

The lipid profile of type 2 diabetes patients with LDL-c within and above target is presented in Table 2. Mean LDL-c concentration was 64.8 ± 14.7 mg/dL in patients with LDL-c within target and 117.1 ± 34.9 mg/dL in those with LDL-c above target ($p < 0.001$). The latter group also presented statistically significant elevated levels of total cholesterol, ApoB, non-HDL-c, ApoB/ApoA1 ratio and oxidized LDL-c. No statistically significant differences were found between the two groups regarding Lp(a) and ApoA1 particles. Despite having an LDL-c within the goal defined by the guidelines, these patients presented elevated levels of other atherogenic lipoproteins. In fact, 22.2%, 25% and 44.4% of patients presented non-HDL-c, ApoB and oxidized LDL-c levels, respectively above the threshold for CV risk.

Table 2
Comparison of lipid profile between type 2 diabetes patients with LDL-c within and above target classification accordingly to the 2016 guidelines

	n	LDL-c within target	n	LDL-c above target	P
LDL-c (mg/dL)	27	65 (53–70)	69	109.0 (92.5–133.0)	< 0.001
Total cholesterol (mg/dL)	27	146 (125–162)	69	180.0 (74.3–107.8)	< 0.001
ApoB (mg/dL)	24	67.0 (58.8–79.0)	64	85.0 (74.3–107.8)	< 0.001
ApoA1 (F) (mg/dL)	8	167.5 (123.0–193.5)	27	155 (140–180)	0.556
ApoA1 (M) (mg/dL)	15	130 (102–143)	33	134.0 (116.5–139.5)	0.772
ApoB/ApoA1 ratio	22	0.54 (0.41–0.64)	60	0.63 (0.54–0.76)	0.004
Non-HDL-c* (mg/dL)	27	99.0 (85.0–118.0)	69	142.2 (119.5–166.5)	< 0.001
Oxidized LDL-c* (U/L)	27	115.7 (94.5–162.1)	69	150.8 (117.3–193.4)	0.007

Data are presented as mean ± standard deviation, unless otherwise indicated by ∗ corresponding to data presented as median, 25th and 75th percentiles. LDL-c Low-density lipoprotein cholesterol; ApoB Apolipoprotein B; ApoA1 Apolipoprotein A1; M male; F female; Non-HDL-c non-nigh-density lipoprotein cholesterol

After reclassification accordingly to the 2019 guidelines, only 16 (16.7%) patients were defined has having LDL-c within target. The lipid profile of patients with LDL-c within and above target is exhibited in Table 3. In these patients, 50%, 38.8% and 43.8% presented elevated non-HDL-c, ApoB and oxidized-LDL-c, respectively. Of note, in patients with ApoB within target, 16.7% had elevated LDL-c.

Table 3
Comparison of lipid profile between type 2 diabetes patients with LDL-c within and above target reclassification accordingly to the 2019 guidelines

	n	LDL-c within target	n	LDL-c above target	P
LDL-c (mg/dL)	16	55.5 (49–66)	80	104.5 (85.3–128.8)	< 0.001
Total cholesterol (mg/dL)	16	143 (125.8–150.8)	80	177 (159–213)	< 0.001
ApoB (mg/dL)	13	70 (54.5–86.5)	75	83 (70–106)	0.037
ApoA1 (F) (mg/dL)	3	160 (NA)	32	157.5 (140.3–180.8)	0.976
ApoA1 (M) (mg/dL)	9	135 (107.5–145.0)	39	134 (115–140)	0.792
ApoB/ApoA1 ratio	12	0.56 (0.39–0.68)	70	0.60 (0.51–0.74)	0.172
Non-HDL-c* (mg/dL)	16	95.5 (84.3–117.3)	80	138 (115.0–164.8)	< 0.001
Oxidized LDL-c* (U/L)	16	113.8 (76.2–160.2)	80	148 (113.9–185.5)	0.024

Data are presented as mean ± standard deviation, unless otherwise indicated by ∗ corresponding to data presented as median, 25th and 75th percentiles. LDL-c Low-density lipoprotein cholesterol; ApoB Apolipoprotein B; ApoA1 Apolipoprotein A1; NA not applicable; M male; F female; Non-HDL-c non-nigh-density lipoprotein cholesterol

LDL-c exhibited positive correlations with several lipid particles. After Bonferroni correction, we found statistically significant positive correlations between LDL-c and total cholesterol ($r = 0.895$, $p < 0.001$), non-HDL-c ($r = 0.850$, $p < 0.001$), ApoB ($r = 0.656$, $p < 0.001$), ApoB/ApoA1 ratio ($r = 0.291$, $p = 0.008$) and oxidized-LDL-c ($r = 0.508$, $p < 0.001$). Non-HDL-c was also significantly and positively correlated with ApoB ($r = 0.808$, $p < 0.001$) and oxidized-LDL-c ($r = 0.588$, $p < 0.001$).

Discussion

Cardiovascular disease is the leading cause of death in patients with diabetes (12), therefore aiming cardiovascular risk reduction is a priority in these individuals. The LDL-c control is recommended as a primary target to reduce CV risk by the 2016 and 2019 European Society of Cardiology /European Atherosclerosis Society (ESC/EAS) Guidelines (3). Nonetheless, even with LDL-c levels in the considered target range, T2DM patients still suffer CVD events, indicating there is a residual CV risk that is not detectable by LDL-c assessment. Accordingly, the 2019 guidelines update have also highlighted the importance of ApoB and non-HDL-c measurements in patients with diabetes. In fact, ApoB is also recommended to assess CV risk, particularly in individuals with hypertriglyceridemia, diabetes, obesity, metabolic syndrome and very low LDL-c levels and it can be used as an alternative to LDL-c for screening, diagnosis and management (6, 13). The present study shows that in type 2 diabetes individuals there are several atherosclerotic lipoproteins that remain elevated even when patients present an optimal LDL-c level. We found that 25% and 22.2% of type 2 diabetes individuals had high ApoB levels and non-HDL-c,

respectively, regardless of having an LDL-c below the cut off for which treatment initiation is recommended. Moreover, when updating the patients' targets accordingly to the 2019 guidelines, 38.8% and 50% of patients presented ApoB and non-HDL-c above target. Thus, in patients with diabetes, targeting only LDL-c seems to be insufficient to estimate the real CV risk and accordingly to the new 2019 guidelines it is clearly insufficient to estimate the real CV risk.

ApoB and non-HDL

ApoB and non-HDL were not routinely evaluated, since they are considered secondary targets(3). However, several studies have demonstrated an important role of non-HDL and ApoB when compared with LDL-c regarding the occurrence of CHD events(14, 15). There is evidence that the non-HDL cholesterol and ApoB may be better predictors of CVD incidence among diabetic men than LDL-c(16). This could occur because all major atherogenic particles originated in liver (very-low density lipoproteins (VLDL) and intermediate-density lipoproteins and LDL have a apolipoprotein B100 (ApoB) molecule (17). Therefore, the ApoB evaluation could work as a direct proxy when assessing the number of atherogenic particles (LDL and non-LDL). Non-HDL-c has also been suggested as a better marker of CVD risk and coronary atherosclerosis(18) and non-HDL lipoproteins, were associated with atherogenic dyslipidemia, insulin resistance, portal hyperinsulinemia and the metabolic syndrome phenotype(15, 17, 19-23). Patients with higher levels of triglycerides were shown to have lower LDL-c levels for any given ApoB concentration compared with subjects with lower levels of triglycerides by Leroux et al (24). Thus, patients with hypertriglyceridemia, such as T2DM patients, may present lower LDL-c concentrations since these values are falsely diminished by elevated triglycerides levels. Nevertheless, these patients still have an high atherogenicity risk since ApoB levels remain elevated, even when there is a decrease of LDL-c levels(24). In our study, we found that 22% and 25% of T2DM individuals had high non-HDL-c and ApoB levels, respectively, regardless of having an LDL-c considered as within target. When updating our results accordingly to the 2019 guidelines, where atherogenic lipoproteins targets are even lower, the proportion of patients with elevated ApoB and non-HDL-c, despite LDL-c within target increased. Friedewald et al. recognized in their original publication that at lower LDL-c levels, even small errors in very low-density lipoprotein cholesterol estimation resulted in significant errors in LDL-C estimation[(11) thus in patients with LDL-c considered within target by the recent guidelines and who have low LDL-c levels, the atherogenicity could be underestimated if only LDL-c is considered. To overcome this, the 2019 guidelines reinforce that in patients with very low LDL-c levels, ApoB can be a better alternative to LDL-c in order to assess CV risk and guide treatment. Our results suggest that in T2DM patients when only LDL-c is considered for deciding treatment, some patients may not be identified, causing a missing oportunity to adjust therapy and thus reduce CV risk. As guidelines have established even lower cutoffs for LDL-c levels, the mismatch LDL-c/ApoB increases and thus, ApoB should also be included obligatory in the evaluation of an individual with T2DM. Of note, in this study even in patients with ApoB within target according to the 2019 guidelines, some patients (16.7%) still present elevated LDL-c.

HDL-c and ApoA1

HDL-C has a cardioprotective role that has been attributed to its role in reverse cholesterol transport, its effects on endothelial cells, and antioxidant activity(25). Similarly, HDL molecules also present anti-inflammatory anti-apoptotic, vasodilatory, antithrombotic, and anti-infectious roles and can modulate directly the glucose metabolism[(26). Although an association between low HDL-c concentrations and an increased risk of type 2 diabetes (T2D) has been shown at an epidemiological level (27, 28), the benefits of raising HDL-C are still not a primary target for CV risk reduction therapy and by them self, serum HDL-C levels are also not sufficient to predict cardiometabolic risk, especially in type 2 diabetes(29). In our study, we did not find any differences in HDL-c levels between patients with LDL-c within and above target. Low levels of ApoA1 also seem to independently associated with new T2DM(30). Nonetheless, according to the ESC/EAS guidelines, our patients evidenced normal ApoA1 concentrations and we did not find any differences in ApoA1 levels between patients with LDL-c within and above target.

ApoB/ApoA1 ratio

ApoB/ApoA1 ratio is a simple and accurate risk factor for CVD (31, 32). Carnevale et al. (2011) describe that a low ApoB/apoA1 ratio reflects a less atherogenic lipid profile, regardless of LDL-c (32). Several studies have also suggested that an elevated ApoB/ApoA1 ratio is a more powerful predictor than other lipid fractions for metabolic disorders, including type 2 diabetes(33-38). Recently, it has been demonstrated that ApoB/ApoA1 ratio is independently associated with carotid atherosclerosis in T2DM with well-controlled LDL cholesterol levels(39). In this study, both groups presented a mean ApoB/ApoA1 ratio within the reference range. Patients with LDL-c levels above target according to the 2016 guidelines showed a significantly higher ratio than those within target, which was not seen when analyzing patient's status accordingly to the 2019 guidelines, indicating that the ApoB/ApoA1 ratio is not a good indicator of atherogenicity in these patients.

Oxidized LDL-c

In our study, 44.4% of patients with type 2 diabetes and LDL-c within target had oxidized LDL-c above the reference range. When updating the results according the 2019 guidelines, 43.8% of patients remained elevated with oxidized LDL-c. Oxidized LDL-c has been associated with progression of atherosclerosis and CHD(40-42). Holvoet et al. suggested that the predictive value of oxidized LDL-c seems to be additive to that of the Framingham global risk assessment score for CV risk (40). Chronic hyperglycemia triggers the production of excess free radicals causing peroxidation of lipid molecules in a chain reaction fashion(43). Malondialdephyde (MDA), oxidized LDL, oxidized LDL/LDL and oxidized LDL/HDL-c levels are significantly elevated in type 2 diabetes patients versus controls in several studies(44-46). MDA is a recognized marker of end products of lipid peroxidation and is reported to modify ApoB, increasing the susceptibility of LDL-c to oxidation and production of oxidized LDL-c (41, 47). In our study, patients presented elevated oxidized LDL-c levels, even patients with LDL-c within target, suggesting a high risk of progression of atherosclerotic CVD in these patients. The results were maintained even when lowering the LDL-c cutoffs in the 2019 guidelines, suggesting that the oxidized LDL-c levels are not affected by LDL-c levels and could be a reliable index of atherogenicity in T2DM individuals, regardless of the LDL-c status.

Lipid parameters correlations

In T2DM patients dyslipidemia presents itself with an high flux of free fatty acids, hypertriglyceridemia, low HDL-c values, increased sd-LDL particles and high ApoB (48). This study correlated LDL-c levels with other lipid parameters according to LDL-c status in patients with T2DM, and presents an positive correlation between LDL-c and total cholesterol and non-HDL-c, with non-HDL-c and ApoB being the most strongly correlated to LDL-c. Non-HDL considered a better risk marker for coronary heart disease had also a marked correlation with ApoB and with oxidized LDL-c, Although these correlations were present for LDL-c, they were weaker than in non-HDL-c. While ApoB and oxidized LDL-c assessment may not be routinely available, non-HDL-c levels could an important tool for atherogenic profile characterization. Since, non-HDL-c levels are inexpensive and easy to obtain and may represent an appropriate index of CV risk better than LDL-c, patients with T2DM included(22). Additionally, non-HDL cholesterol seems also able to predict CVD over a wider range of triglyceride concentrations(22) than LDL-c, where calculation trough either Friedewald's formula or direct measurement are affected by triglycerides concentrations.

Limitations

Our study presents some methodological limitations relatable to his cross-sectional approach, as the non-assessment of long-term outcomes, such as the occurrence of CVD according to lipid levels parameters. Thus, a prospective follow-up approach is required to evaluate medical interventions and lipid goal attainment in relation to mortality in T2DM patients.

Summary

Our study also has strengths. A small number of studies have assessed non-conventional lipid parameters such as ApoA1, ApoB, Lp(a) or oxidized LDL-c in type 2 diabetes patients. Our study is an important contribution to the characterization of a comprehensive lipid profile in patients with this condition. Finally, this study has important clinical implications since it confirms that in T2DM patients solely assessing LDL-c is insufficient for the correct assessment of CV risk, possibly leading to a suboptimal treatment of these patients. We found a high mismatch LDL-c/ApoB thus we agree with the 2019 guidelines recommending ApoB evaluation in patients with T2DM in order to better estimate the atherogenicity of these patients and to guide adjustment of lipid-lowering therapy after achievement of the recommended LDL-c goal. Since a few patients with ApoB within target presented high LDL-c, LDL-c and ApoB should be additive regarding CV risk evaluation.

Conclusions

LDL-c levels are currently accepted as the main therapeutic target for the prevention of CV events in T2DM patients. Nevertheless, even in patients with LDL-c considered by the guidelines within target, some patients still present atherosclerosis progression and CVD-related events. In this study, we confirmed that although having LDL-c within target, patients presented elevated levels of molecules known for their

atherogenic character, namely oxidized LDL-c, non- HDL-c and ApoB. The present study contributes to our understanding of lipid metabolism in T2DM patients and has important clinical implications: LDL-c is insufficient to estimate CV risk, especially in diabetic individuals where other lipoproteins reveals a high atherogenicity status. Our data suggest that combining LDL-c with ApoB, oxidized LDL-c and non-HDL-c may add important information in order to better estimate CV risk in individuals with T2DM.

Abbreviations

ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
BP	Blood pressure
CHD	Coronary heart disease
CV	Cardiovascular
CVD	Cardiovascular disease
EAS	European. Atherosclerosis Society
ESC	*European Society of Cardiology*
F	Female
HbA1c	Glycated hemoglobin
HDL-c	High-density lipoprotein cholesterol
LDL-c	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
M	Male
MS	Metabolic Syndrome
Non-HDL-c	Non-high-density lipoprotein cholesterol
SCORE	Systemic Coronary Risk Estimation
SD	Standard deviation
sd-LDL-c	Small and dense low-density lipoprotein-cholesterol
T2DM	Type 2 diabetes mellitus

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research

committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the local Ethics committee (150-DEFI/149-CES). Due to the retrospective nature of this study, consent to participate was waived by the Ethics Committee.

Consent for publication: Liliana Fonseca assigns Lipids in Health and Disease all rights of copyright.

Availability of data and materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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