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Concentration and relative distribution of low-density lipoprotein subfractions in patients with metabolic syndrome defined according to the National Cholesterol Education Program criteria

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Abstract

It has been proposed that the most common lipid abnormalities associated with the metabolic syndrome are elevated triglyceride and low high-density lipoprotein concentrations as well as the existence of small, dense low-density lipoprotein (LDL) particles. However, so far there are only limited clinical data concerning the distribution of LDL particles in patients with this syndrome. The aim of our study was to directly determine the concentration and relative distribution of LDL subfractions in patients with metabolic syndrome. One hundred seventy-five individuals were included. Patients with metabolic syndrome (n = 105) exhibited higher concentrations of dense LDL particles and lower mean LDL particle size than the control population (n = 70). Both of these parameters were significantly correlated with the number of components of metabolic syndrome. Multivariate analysis revealed that serum triglyceride concentration was the most important determinant of the presence of small, dense LDL particles. In conclusion, patients with metabolic syndrome exhibit higher concentrations of small, dense LDL subfractions than individuals who do not fulfill the criteria for the diagnosis of this syndrome. This increase is directly related to the number of components of metabolic syndrome and is mainly determined by the serum concentrations of triglycerides.
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1. Introduction

The term metabolic syndrome is used to describe a constellation of cardiovascular risk factors that recently have become a problem of epidemic proportions [1]. The main components of this syndrome involve abdominal obesity, dyslipidemia, disturbed carbohydrate metabolism, hypertension, as well as a prothrombotic and proinflammatory profile [1]. At least 3 organizations have recommended the clinical criteria for the diagnosis of the metabolic syndrome [2-4]. However, the most applicable set of criteria in everyday clinical practice is that of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III, because it includes simple measurements that can be performed by any health care provider.

Dyslipidemia is a hallmark of the metabolic syndrome and may substantially contribute to the increased cardiovascular risk observed in this population [5]. The most common lipid abnormalities associated with the metabolic syndrome are elevated triglyceride and low high-density lipoprotein cholesterol (HDL-C) concentrations as well as the existence of small, dense low-density lipoprotein (sdLDL) particles [1]. Nevertheless, so far there are only limited clinical data supporting the presence of increased concentrations of these particles in patients with metabolic syndrome when this is defined according to the NCEP criteria [6]. In addition, in the few clinical studies that have examined this issue, the presence of sdLDL particles was not directly assessed [6].

Several experimental studies have shown that sdLDL particles are more atherogenic than large-buoyant LDL. In this regard, it has been demonstrated that sdLDL has easier access to the subendothelial space in the arterial wall and exhibits enhanced binding to intimal proteoglycans [7,8]. Furthermore, sdLDL exhibits increased susceptibility to oxidation and increased uptake by the macrophages and therefore facilitates the formation of foam cells [9]. Despite

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the abundance of experimental data showing that sdLDL subfractions are characterized by increased atherogenicity, clinical studies that tested the impact of the concentration of these particles on the determination of total cardiovascular risk revealed contradictory results. Thus, although some studies showed that the preponderance of sdLDL particles is associated with increased cardiovascular risk [10-13], others failed to detect an independent effect on the risk for subsequent development of cardiovascular events [14,15].

The distribution of LDL subfractions is determined by both genetic and environmental factors [16,17]. However, it has been proposed that the most important single determinant of LDL particle distribution is the size of the pool of triglyceride-rich lipoproteins. Thus, serum triglyceride levels greater than 200 mg/dL are associated with a greater than 90% prevalence of sdLDL [18].

The purpose of our study was to evaluate LDL subfraction profile in patients with metabolic syndrome defined according to the NCEP ATP III criteria. In addition, the impact of other metabolic parameters (such as triglyceride levels) on the concentration and relative distribution of LDL particles in this patient population was also examined.

2. Patients and methods

2.1. Patients

One hundred five otherwise healthy individuals who fulfilled the NCEP criteria [3] for the diagnosis of metabolic syndrome were included in the study. Seventy age- and sex-matched individuals with less than 3 criteria for the diagnosis of the metabolic syndrome served as controls. Blood pressure was measured at the sitting position after a 5-minute rest. Two consecutive measurements were done with an interim of 3 minutes and the mean value was used. Individuals were excluded if they were found to be diabetic (fasting glucose levels of >126 mg/dL) or if they had a history of cardiovascular disease. In addition, patients with thyroid dysfunction, liver or kidney diseases (defined as a positive medical history or a threefold increase in serum aminotransferases and serum creatinine levels of >1.6 mg/dL, respectively), as well as those receiving drugs that may interfere with glucose or lipid metabolism were also excluded from the study. All participants gave a written informed consent before their enrollment in the study, which was approved by the Ethics Committee of the University Hospital of Ioannina. The investigation conforms with the principles outlined in the Declaration of Helsinki (*Cardiovasc Res* 1997;35:2-4).

2.2. Analytical methods

All lipid and lipoprotein determinations were carried out after an overnight fast. Serum levels of total cholesterol, HDL-C, and triglycerides were determined enzymatically on the Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). Serum LDL-cholesterol (LDL-C) was calculated using the Friedewald formula (provided that triglyceride levels were < 350 mg/dL). Serum apolipoprotein AI and B (apoAI and apoB, respectively) levels were measured with a Behring Holding BN 100 Nephelometer (Liederbach, Germany). Insulin levels were determined by a microparticle enzyme immunoassay on an AXSYM analyzer (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany) with a coefficient of variation of 4.2% to 9%. Homeostasis model assessment (HOMA) index was calculated as follows: fasting insulin (mIU/L) * fasting glucose (mg/dL)/405.

2.3. Low-density lipoprotein subclass analysis

Electrophoresis was performed using high-resolution, 3% polyacrylamide tube gel and the Lipoprint LDL System (Quantimetrix, Redondo Beach, Calif) according to the manufacturer's instructions. Briefly, 25 μL of the sample was mixed with 200 μL of Lipoprint loading gel and placed upon the upper part of the 3% polyacrylamide gel. After 30 minutes of photopolymerization in room temperature, electrophoresis was performed for 60 minutes with 3 mA for each gel tube. Each electrophoresis chamber involved 2 quality controls (sample provided by the manufacturer). For quantification, scanning was performed with ScanMaker 8700 digital scanner (Mikrotek, Carson, CA) and iMac personal computer (Apple Computer, Cupertino, CA). After scanning, electrophoretic mobility (Rf) and the area under the curve were calculated qualitatively and quantitatively with the Lipoprint LDL System template and the Lipoware software (Quantimetrix), respectively. Low-density lipoprotein subfraction was calculated with the Rf between the very low density lipoprotein (VLDL) fraction (Rf, 0.0) and the HDL fraction (Rf, 1.0). Low-density lipoprotein is distributed from Rf 0.32 to 0.64 as 7 bands, whose Rfs are 0.32, 0.38, 0.45, 0.51, 0.56, 0.6, and 0.64 (LDL-1 to LDL-7, respectively). LDL-1 and LDL-2 are defined as large, buoyant LDL, and LDL-3 to LDL-7 are defined as sdLDL. Each LDL subfraction's cholesterol concentration (in milligrams per decaliter) is determined by multiplying the relative area (area under the curve) of each subfraction by the total cholesterol concentration of the sample. Small, dense LDL proportion was defined as the percent of sdLDL (from band 3 to band 7) over the whole LDL. According to the data provided by the manufacturer, the intra-assay coefficients of variation (%) for apoB-containing lipoprotein subfraction determination were as follows: VLDL, 5.58 to 7.28; intermediate density lipoprotein (IDL), 2.94 to 11.14; LDL-1, 1.67 to 3.58; LDL-2, 2.19 to 16.8; LDL-3, 1.65 to 11.79; LDL-4, 2.45 to 4.53; LDL-5, 1.72; LDL-6, 4.62; LDL-7, 17.89. The corresponding interassay values were as follows: VLDL, 7.12 to 9.40; IDL, 4.73 to 13.63; LDL-1, 3.67 to 3.92; LDL-2, 3.85 to 13.5; LDL-3, 5.59 to 19.21; LDL-4, 3.45 to 6.05; LDL-5, 2.58; LDL-6, 12.06; LDL-7, 33.9. Low-density lipoprotein peak particle diameter (LDL-PPD) was determined using the Rf at the highest peak of LDL bands as a

peak size of LDL particles according to the equation proposed by Kazumi et al [19]: LDL-PPD = (1.429 − Rf) * 25, whereas mean particle size was provided by the Lipoprint LDL System.

2.4. Statistical analysis

Data are mean ± SD. An unpaired t test was used for comparisons between study groups, whereas differences in proportions were assessed with χ^2 test. One-way analysis of variance was used for comparisons between patients with different numbers of components of metabolic syndrome. Finally, correlations between sdLDL particle indices and other metabolic parameters were estimated using linear regression analysis, whereas multiple regression analysis was used for the multivariate assessment of the correlations of these variables.

3. Results

The clinical characteristics of the study participants are shown in Table 1. There were no differences in age and sex distribution between the study groups. On the contrary, patients with metabolic syndrome exhibited significantly elevated waist circumference values as well as higher blood pressure readings than control subjects. In addition, these patients displayed higher fasting glucose and insulin concentrations, elevated values of the HOMA index, as well as an adverse lipid profile characterized by elevated concentrations of non–HDL-C, triglycerides, and apoB, as well as by lower levels of HDL-C and apoAI. Total cholesterol and LDL-C concentrations did not differ significantly between the 2 groups (Table 1). Triglyceride values were significantly correlated with apoB concentrations in control individuals (r = 0.57, P < .001). The same correlation (although weaker) was also observed in patients with metabolic syndrome (r = 0.3, P < .05). ApoB concentrations were significantly correlated with non–HDL-C values in both study groups (r = 0.63 and r = 0.87 for patients with metabolic syndrome and control individuals, respectively; P < .01 for both correlations).

Table 1
Clinical and biochemical characteristics of the study population

	Metabolic syndrome	Controls	P
Number	105	70	
Sex (male/female)	37/68	27/43	NS
Age (y)	53 (21-78)	48 (23-70)	NS
SBP (mm Hg)	141.6 ± 15.7	122.9 ± 17.5	<.001
DBP (mm Hg)	90.5 ± 10.1	79.2 ± 8.8	<.001
Waist circumference (cm)			
Men	113.7 ± 11.3	100.3 ± 9.1	<.001
Women	111.2 ± 12.5	89.1 ± 15.7	<.001
Glucose (mg/dL)	104 ± 17	94 ± 9	<.001
Insulin (μU/mL)	15.3 ± 8.4	10.1 ± 6.8	<.001
HOMA index	3.97 ± 2.69	2.38 ± 1.67	<.001
Total cholesterol (mg/dL)	237 ± 46	226 ± 40	NS
Triglycerides (mg/dL)	213 ± 104	124 ± 100	<.001
HDL-C (mg/dL)			
Men	42 ± 8	51 ± 10	<.001
Women	52 ± 10	63 ± 11	<.001
Non–HDL-C (mg/dL)	188 ± 41	168 ± 37	<.001
LDL-C (mg/dL)	145 ± 39	143 ± 32	NS
ApoAI (mg/dL)			
Men	117 ± 26	135 ± 20	<.001
Women	124 ± 32	145 ± 31	<.001
ApoB (mg/dL)	112 ± 30	98 ± 28	<.010

Values are mean ± SD except for age which is presented as median (range). Comparisons between groups were performed using an unpaired t test or a Mann-Whitney U test (for age values). Differences in proportions were assessed by χ^2 test. A P value of less than .05 was considered significant. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

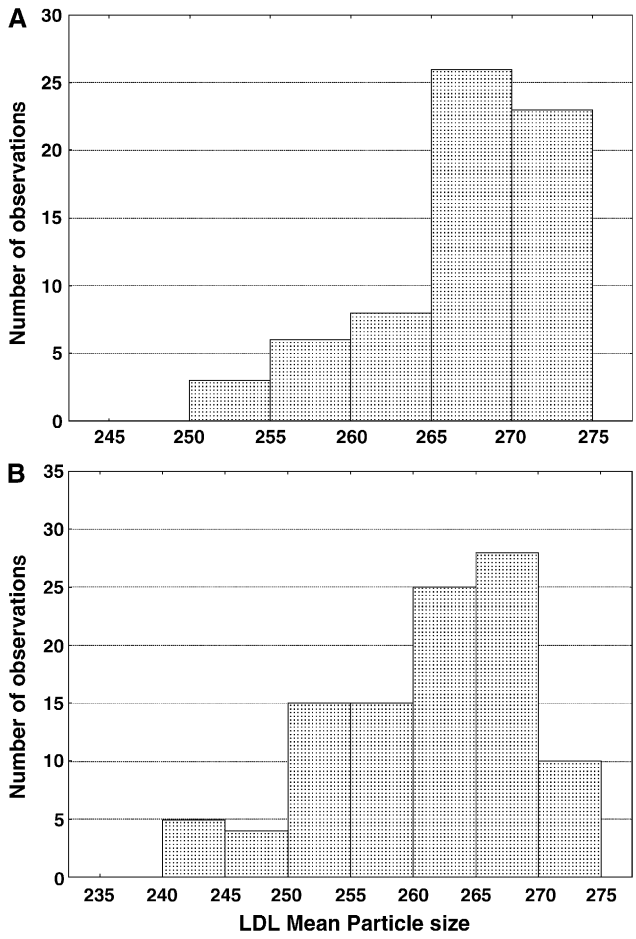

Fig. 1. Distribution plot of LDL mean particle diameter in control population (A) and in patients with metabolic syndrome (B).

The distribution pattern of LDL subfractions was significantly different in the study groups (Fig. 1). Indeed, 73% of the patients in the metabolic syndrome group had measurable quantities of sdLDL particles as compared to 40% of the individuals in the control group (P < .001).

Subfractionation of apoB-containing lipoproteins revealed that patients with metabolic syndrome exhibited significantly higher cholesterol concentrations in the VLDL and sdLDL subfractions (Fig. 2). On the contrary, cholesterol values in the large, buoyant LDL particles as well as in

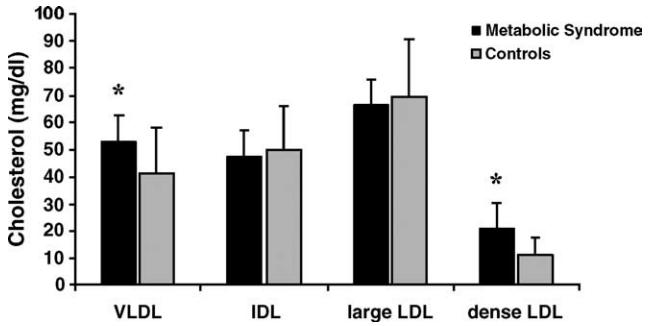

Fig. 2. Cholesterol concentrations in the subfractions of apoB-containing lipoproteins. Data are mean ± SEM. *P < .01 compared to control individuals.

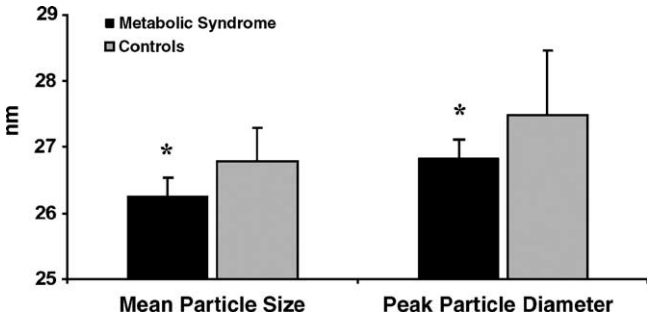

Fig. 4. Mean particle size and PPD of LDL particles in the study population. Data are mean ± SEM. *P < .01 compared to control individuals.

the IDL subfractions did not differ significantly among the study groups. When cholesterol concentrations in the sdLDL subfractions were expressed as a percentage of total LDL concentration, patients with metabolic syndrome exhibited significantly higher values than subjects without this syndrome (15.15% ± 7.93% vs 7.67% ± 4.31%, respectively; P < .001). A limited number of individuals in each group (n = 15 in the control group and n = 17 in the metabolic syndrome group) exhibited elevated concentrations of aminotransferases. The concentrations of cholesterol in sdLDL particles in these individuals did not differ significantly from those observed in individuals of the same group with normal liver function tests (data not shown). It is worth mentioning that only one participant in each group had serum creatinine value greater than 1.2 mg/dL (the upper normal limit). The concentrations of cholesterol in sdLDL subfractions after the study participants were classified according to their total number of components of metabolic syndrome are shown in Fig. 3. There was a strong linear increase in cholesterol concentrations as the number of components of metabolic syndrome increased. The same results were obtained when cholesterol concentrations in sdLDL subfractions were expressed as a percentage of total LDL cholesterol values (data not shown).

Determination of mean particle size in both study groups revealed that patients with metabolic syndrome have relatively smaller LDL particles than individuals who do not fulfill the criteria for the diagnosis of this syndrome (Fig. 4). The same results were also obtained when PPD (a parameter that has been widely used in clinical studies for the evaluation of LDL particle distribution) was used instead of mean particle size (Fig. 4). Assessment of the differences with analysis of covariance using the serum concentrations of aminotransferases and creatinine as covariates revealed the same results. Both mean particle size and PPD were negatively correlated with the number of components of metabolic syndrome (data not shown).

Linear regression analysis revealed that the cholesterol concentrations in sdLDL particles were positively correlated with age, systolic blood pressure values, as well as with total cholesterol, LDL-C, and triglyceride concentrations. On the contrary, LDL mean particle size was negatively correlated with all these parameters, whereas it was also positively correlated with HDL-C values. Fig. 5 displays the correlation between LDL particle size and triglyceride values. It must be noted that neither sdLDL concentration nor LDL mean

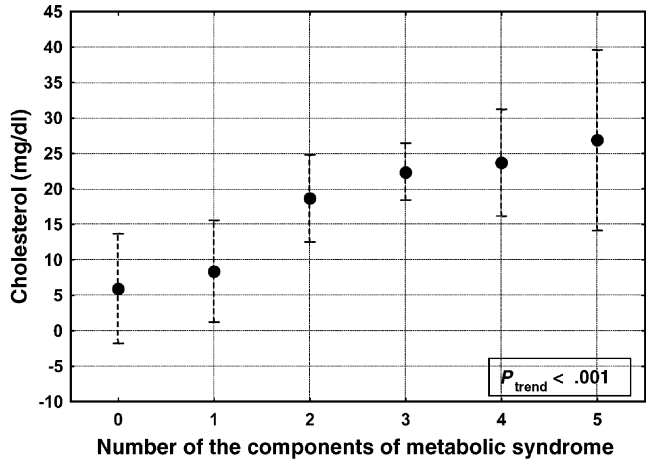

Fig. 3. Cholesterol concentrations of sdLDL particles in individuals with different numbers of the components of metabolic syndrome. Data are mean ± SEM. Differences between groups were assessed by 1-way analysis of variance and a P value of less than .05 was considered significant.

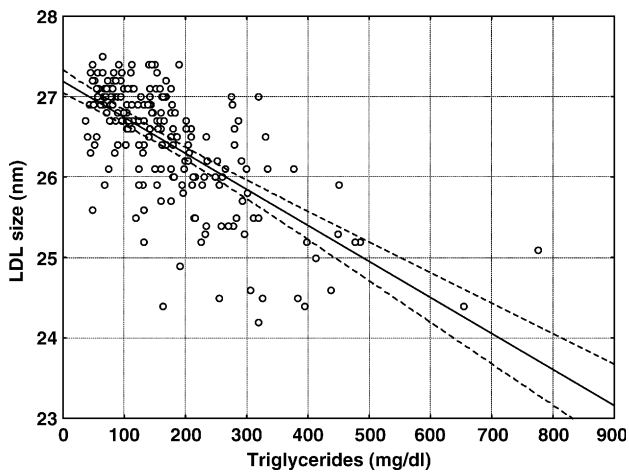

Fig. 5. Correlation between triglyceride values and LDL mean particle size. r = −0.67, P < .001.

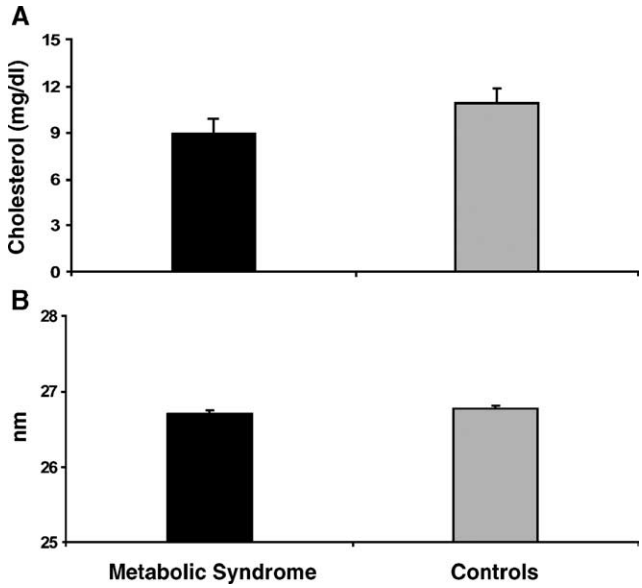

Fig. 6. Cholesterol concentrations in sdLDL particles (A) and mean particle diameter of LDL particles (B) in the subpopulation of patients with metabolic syndrome with normal triglyceride levels and in the control individuals.

particle size was significantly correlated with insulin resistance indices (fasting insulin concentrations and HOMA index values). Multiple regression analysis included all the 5 components of the metabolic syndrome as well as some other potential confounders such as age, sex, HOMA index values; and the serum concentrations of aminotransferases and creatinine showed that serum triglyceride concentration was the most important determinant of both cholesterol concentrations in sdLDL particles and mean particle size (β = .49 and −.51, respectively; $P < .0001$ for both correlations). Except for triglycerides, LDL-C values were also an important determinant of sdLDL cholesterol (β = .345, $P <$ .01); by contrast, LDL-C values were not correlated with LDL mean particle size. The inclusion of other lipid parameters, such as apolipoprotein values, in the multivariate models did not significantly affect the results of the analyses.

To further investigate the role of triglycerides in the determination of LDL subfraction profile, we selected patients with metabolic syndrome and serum triglyceride levels lower than 150 mg/dL (n = 27). This subpopulation of patients with metabolic syndrome exhibited similar triglyceride concentrations with the control subjects as a group (114 ± 21 vs 124 ± 100 mg/dL, respectively). Given the strong dependence of sdLDL indices on serum triglycerides, the cholesterol values in sdLDL particles as well as the mean particle size in this group of patients were similar to the corresponding values of the control population (Fig. 6A and B, respectively).

4. Discussion

Dyslipidemia of metabolic syndrome is usually described as having 3 major components: elevated triglyceride levels, reduced HDL-cholesterol values, and a preponderance of sdLDL particles [1]. However, because the concentrations of these particles have not been previously determined in patients with metabolic syndrome defined according to the NCEP criteria, their presence was indirectly extrapolated by the coexistence of other metabolic abnormalities such as insulin resistance and hypertriglyceridemia [20]. Nevertheless, it is well known that not all patients with metabolic syndrome have elevated triglyceride values and that NCEP ATP III criteria may not provide a sensitive approach to identifying insulin-resistant individuals [21]. Consequently, the former extrapolation cannot be used for the assessment of LDL subfraction distribution in patients with metabolic syndrome defined according to the NCEP ATP III diagnostic criteria.

In this study, we showed for the first time that patients with metabolic syndrome diagnosed by NCEP criteria exhibit significantly higher concentrations of the atherogenic sdLDL subfractions than individuals who do not fulfill the criteria for the diagnosis of this syndrome. This increase is directly related to the number of components of metabolic syndrome and is mainly determined by the serum concentrations of triglycerides. Surprisingly, and in contrast to what was commonly believed, the LDL subfraction distribution was not significantly correlated with insulin-resistance indices.

In their landmark study, Austin et al [18] proposed that the distribution of LDL subfractions as assessed by gradient gel electrophoresis shows a bimodal pattern. Thus, in the majority of healthy individuals, the large, buoyant, less atherogenic LDL particles predominate (phenotype A), whereas in the remainder there is a preponderance of the atherogenic sdLDL particles (phenotype B) [18]. However, this approach has several important limitations. Firstly, the use of PPD for the assessment of the distribution of LDL particles, although convenient for clinical purposes, does not allow the quantitative determination of the concentration of each LDL subfraction. In addition, recently published studies have shown that there is a linear correlation between the concentration of sdLDL particles and the risk for the development of cardiovascular events [13], thus suggesting that even small increases in the concentration of these subfractions may substantially contribute to the determination of total cardiovascular risk [11,13]. Consequently, with the classification proposed by Krauss et al [18], individuals with moderate increases (but no predominance) in the concentrations of sdLDL subfractions may be incorrectly diagnosed as having a low risk for coronary events. On the other hand, the Lipoprint LDL System uses more strict cutoff points for the classification of patients into different phenotypes, which is based on the presence of measurable quantities or on the total absence of sdLDL particles [22]. This approach is based on the assumption that even small increases in the concentrations of sdLDL subfractions may be harmful and are more relevant to the results of the clinical studies mentioned above [11,13].

Low-density lipoprotein–cholesterol values in the group of patients with metabolic syndrome were similar to those found in individuals who do not fulfill the criteria for the diagnosis of this syndrome. This observation implies that from a quantitative point of view, LDL metabolism is not severely affected by the presence of metabolic syndrome. On the other hand, our results indicate that patients with metabolic syndrome exhibit important qualitative alterations of LDL. These disturbances are more pronounced as the severity of the metabolic syndrome (as assessed by the number of its components) increases and, at least in part, could be attributed to the pathophysiological mechanisms that lead to the development of metabolic syndrome. In this context, it is rather surprising that in our population none of the indices used for the evaluation of LDL subfraction profile (concentration or relative proportion of sdLDL particles, mean and PPD) were significantly correlated with the surrogate markers of insulin resistance (fasting insulin levels or HOMA index values). The existence of a correlation between insulin resistance and the presence of sdLDL particles is a subject of debate. Thus, some previous studies showed that insulin resistance is an important determinant of LDL subfraction distribution [20,23,24], whereas others failed to reveal a significant association between these 2 parameters [25,26]. The differences in the patients' selection criteria as well as in the methodologies used for the evaluation of insulin resistance and the assessment of LDL subfraction profile may, at least in part, explain these discrepancies.

The regulation of LDL subfraction distribution is a very complicated process and its details still remain indeterminate. However, it is well known that the exchange of triglycerides for cholesterol esters between triglyceride-rich lipoproteins and LDL particles represents a key step for the formation of sdLDL particles [27,28]. The rate of this exchange is mainly regulated by the size of the triglyceride-rich lipoprotein pool, and thus patients with hypertriglyceridemia usually exhibit elevated concentrations of the sdLDL subfractions [29,30]. Our observation that triglyceride levels were the most important determinant of the concentration of dense LDL particles is consistent with this hypothesis. A consequence of this observation, which may have important therapeutical implications, is that the presence of elevated quantities of sdLDL subfractions is confined to those patients with metabolic syndrome who also exhibit high concentrations of triglycerides. On the contrary, the LDL subfraction profile in patients with metabolic syndrome and normal triglyceride values does not differ significantly from that of individuals without this syndrome.

So far it is not known whether the elevated concentrations of cholesterol in sdLDL subfractions may contribute to the increased cardiovascular risk observed in patients with metabolic syndrome. However, from a pathophysiological point of view, one could expect that the increased cholesterol content in particles that are more readily oxidized compared to the larger LDL subfractions [31] could just represent increased concentrations of substrate that is available for oxidation. The abundance of this substrate combined with the reduced antioxidant capacity that has been observed in patients with metabolic syndrome [6,32,33] may lead to the acceleration of the atherosclerotic process in this patient group.

In conclusion, patients with metabolic syndrome exhibit significantly higher concentrations of the sdLDL subfractions than individuals who do not fulfill the criteria for the diagnosis of this syndrome. This increase is directly related to the number of components of metabolic syndrome and is mainly determined by the serum concentrations of triglycerides. Further studies are needed to delineate the contribution of elevated concentrations of dense LDL particles to the increased cardiovascular risk observed in this population.

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