Analysis of LDL and HDL size and number by nuclear magnetic resonance in a healthy working population: the LipoLab Study

Short title: LipoLab Study

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ABSTRACT

Background and aim

Atherosclerosis is the underlying process in cardiovascular disease (CVD), the first cause of death in developed countries. We aimed to identify people with no known CVD and normal values of LDL-C and HDL-C, but with alterations in the number and size of lipoprotein particles (as measured by nuclear magnetic resonance [NMR]) and to analyze their sociodemographic, clinical, and biochemical characteristics.

Methods

Cross-sectional study in occupational risks prevention center in Castellón (Spain) in 2017 and 2018, in consecutively recruited adults (18-65 years) with no known CVD. Sociodemographic, clinical and biochemical variables were collected. Lipid profiles were analyzed (Liposcale test), along with the concentration, size and number of the main types of lipoprotein particles, determined by 2D diffusion-ordered NMR spectroscopy. Using contingency tables, we analyzed the characteristics of people with normal LDL and HDL cholesterol but abnormal levels of LDL and HDL particles. The magnitude of association between explanatory variables and abnormal levels of each kind of lipoprotein was assessed with multivariable logistic regression models.

Results

Of the 400 total participants (31.3% women; age 46.4±4.3 years), 169 had normal LDL and HDL cholesterol. Abnormal lipoprotein particle values depended on the subtype: prevalence of abnormal LDL levels ranged from 8.3% to 36.7%; and of HDL, from 28.4% to 42.6%. High systolic blood pressure and total cholesterol were significantly associated with

abnormal LDL levels. Male sex and high systolic blood pressure were associated with abnormalities in HDL.

Conclusions

An extended lipids profile, obtained by NMR, enables the identification of people with normal HDL-C and LDL-C levels who present abnormal levels of LDL-P and/or HDL-P. Higher total cholesterol, systolic blood pressure, BMI, and male sex were significantly associated with these abnormal values.

Keywords: LDL particles, HDL particles, NMR, cardiovascular disease, family practice.

What's already known about this topic?

- Concentration of LDL and non-HDL are associated with a high risk of atherosclerotic cardiovascular disease.
- There is evidence that the LDL particle concentration (nmol/L) is a better indicator of cardiovascular risk than LDL concentration (mg/dL).

What does this article add?

- Adults with no known cardiovascular disease and normal HDL and LDL concentration levels, presented abnormal levels of LDL particle and/or HDL particle.
- Higher total cholesterol, systolic blood pressure, BMI, and male sex were significantly associated with abnormalities in the number of LDL and HDL particles.

INTRODUCTION

Atherosclerosis is the underlying process in cardiovascular disease (CVD), the first cause of death in developed countries [1]. Concentration of low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDL-C) are correlated, with high values of either associated with a higher risk of atherosclerotic CVD [2]. Consequently, the European societies for atherosclerosis and cardiology [3] and the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NECTP-ATPIII) [4] have established LDL-C and non-HDL-C as primary and secondary treatment targets, respectively, for reducing cardiovascular risk. The therapeutic reduction of LDL-C has been shown to reduce cardiovascular event rates in patients with or without cardio-metabolic risk [5, 6] and previous studies have demonstrated that there is an inverse association between the HDL-C level and cardiovascular risk [7]. However, in the setting of primary prevention of cardiovascular disease and in the presence of very low concentrations of LDL-C, the HDL-C might not predict cardiovascular risk [8].

In Spain, cardiovascular risk scales like REGICOR [9] and SCORE [10] are the usual methods for assessing cardiovascular risk in asymptomatic populations. These multifactorial indexes generate a score based on classic cardiovascular risk factors in people over the age of 40, stratifying the population according to cardiovascular risk. However, most initial cardiovascular events occur in people who are not considered at high risk [11], reflecting the poor discriminatory capacity of these traditional risk factors. Indeed, a large proportion of patients in treatment, as well as those with no diagnosis, can experience cardiovascular events despite meeting all therapeutic targets, including LDL-C. Furthermore, cardiovascular events are more frequent in patients stratified as being at moderate to intermediate risk, as well as in those with diabetes, obesity and metabolic syndrome [12]. Thus, it is crucial to identify new biomarkers that can help detect additional risks for subclinical CVD.

On top of the standard lipid profile, it is currently possible to obtain a detailed characterization of lipoprotein particles with advanced lipoprotein tests (ALT) [2]. There are different techniques that provide additional parameters, including the size and number of lipid particles, which have been shown to be associated with the appearance of CVD and could improve the evaluation of cardiovascular risk as well as guide lipid-lowering treatments [13]. There is controversy on the inclusion of NMR-based ALTs in clinical practice because some studies have reported that cardiovascular risk is similarly associated with both the lipoprotein profile and with the standard lipid (and/or apolipoprotein) panel [14,15]. However, other authors support using indicators like apolipoprotein B (apo-B) and the LDL

particle (LDL-P) counts, measured by nuclear magnetic resonance (NMR), to assess cardiovascular risk [16,17]. Some studies even suggest that LDL-P is a better indicator of CVD risk than LDL-C [18, 19]. Assuming these findings, this study aimed to identify people with no known CVD and normal values of LDL-C and HDL-C, but with alterations in the number and size of lipoprotein particles (as measured by NMR) and to analyze their sociodemographic, clinical, and biochemical characteristics.

MATERIAL AND METHODS

This was a cross-sectional study in a center for prevention of occupational risks in Castellón (Spain), with consecutive recruitment of adults (aged 18 to 65—the maximum working age in Spain) with no known CVD, who presented to the clinic in 2017 and 2018 for an initial or periodic occupational health check. Exclusion criteria included a prior cardiovascular event or refusal to participate. The ethics committee of the Clinical Research Institute of Valencia (INCLIVA) approved the study, and all participants signed informed consent.

Data collection

Once patients agreed to take part, and during the initial visit at recruitment, the investigator collected the study variables, recording data in an electronic data extraction form. Variables related to sociodemographics (age, sex), anthropometry (waist circumference, body mass index [BMI]), clinical characteristics (blood pressure and metabolic syndrome), and behavior (tobacco use, physical activity), which are typically recorded in patients' medical records during routine clinical practice. A fasting blood sample was taken in all participants—also in line with usual practice—to analyze parameters that the clinician deemed to be of interest according to the participant's occupation. In addition, the cardiovascular risk of participants was calculated by using the SCORE function and participants were asked to complete the seven-item International Physical Activity Questionnaire (IPAQ-7) [20]. Data were stored for subsequent tabulation in an electronic file, using anonymous patient identifiers linked to individuals' electronic medical records. On the information sheet given to participants, they were informed that they would be contacted by phone if necessary.

Advanced lipoprotein test

Lipid profiles were analyzed using the Liposcale test, an advanced lipoprotein test based on 2D diffusion-ordered NMR spectroscopy, which determines the size and number of the main types of lipoprotein particles (very low-density lipoprotein [VLDL], LDL, and HDL) as well as the size and concentration of each subtype [21].

Serum blood samples were preserved with dry ice and sent to the INCLIVA Molecular and Metabolomic Image Lab in Valencia for lipoprotein analysis by NMR. Obtained spectra were

then sent to Biosfer Teslab for processing. Using this technique, described elsewhere [13], 500 μL of serum from each patient was analyzed to obtain the complete lipids profile (size, lipid content, and number of VLDL-P, LDL-P, and HDL-P). For the present study, we analyzed only the concentration of LDL and HDL particles; the concentration of large (L-LDL-P), medium-sized (M-LDL-P) and small (S-LDL-P) LDL particles; and the concentration of M-HDL-P. In order to evaluate the reproducibility of the measurements, we reported the Inter-Assay CV and the Intra-Assay CV, defined as the standard deviation of a set of measurements divided by the mean of the set. Supplemental Table 1 illustrates the variation coefficients for NMR measurements.

We classified participants as having normal or abnormal levels of LDL-Ps and HDL-Ps, according to published cutoffs associated with an increase in cardiovascular risk [22–26], using the equivalents of the 25th and 75th percentiles of LDL-C and HDL-C in the study sample. Thus, the minimum cutoffs for abnormal concentration levels of LDL-P were: for total LDL-P, 1300 nmol/L, or 1000 nmol/L in patients with diabetes; for L-LDL-P, 200 nmol/L, or 160 nmol/L in patients with diabetes; for M-LDL-P, 400 nmol/L, or 260 nmol/L in patients with diabetes; and for S-LDL-P, 690 nmol/L, or 560 nmol/L in patients with diabetes. On the other hand, maximum cutoffs for abnormal concentration levels of HDL-P were: for total HDL-P, 24 μmol/L; and for M-HDL-P, 8.5 μmol/L. Participants' standard lipid profile (LDL-C, HDL-C) was also analyzed based on the Liposcale measurement. Based on European guidelines for managing dyslipidemia and preventing atherosclerosis [11], LDL-C was considered high at levels exceeding 130 mg/dL, or 100 mg/dL in patients with diabetes. HDL-C was considered low at levels of 40 mg/dL or less in men and 50 mg/dL or less in women.

Sample size

We assumed that 40% of the reference population would have normal levels of LDL-C and HDL-C. Of these, we estimated that 45% could have abnormal LDL-P values. To estimate a confidence interval of 95%, with a precision level of 8%, a sample size of 149 participants would be necessary. Assuming 7% missing data, this number would rise to 160 participants. Thus, the estimated final sample size was 400 patients.

Statistical analysis

A descriptive analysis of all the variables was undertaken, with categorical variables expressed as frequencies and quantitative variables expressed as means (standard deviation [SD]) and ranges. We analyzed the characteristics of participants with normal LDL-C and HDL-C levels but with abnormalities in the values of LDL-P and HDL-P, using contingency

tables and the chi-squared or Fisher's exact test, as appropriate, for categorical variables. The mean values of quantitative variables were compared using the student's t test or the non-parametric Mann-Whitney U test, depending on the normality of the distribution. Multivariable logistic regression models were fitted to assess the magnitude of associations between explanatory variables and abnormal levels of each kind of lipoprotein. A stepwise process was used to select variables for inclusion in the model based on the Akaike Information Criterion. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to measure associations. All analyses were undertaken with SPSS (v.25) and R (v.3.5.1) software.

RESULTS

A total of 400 participants were included in the study. Their mean age was 46.4 (SD 4.3) years, and just 31.3% (n = 125) were women. Four patients were being treated for diabetes and two patients had a CV risk SCORE ≥ 5%. Table 1 shows participants' baseline characteristics.

Patients were classified as having normal or abnormal values for each type and subtype of lipoprotein assessed in the lipid profile (ALT) (table 2). Of the total, 44 showed abnormal values for both HDL-C and LDL-C, while 169 showed normal values on both of these measures. Following this determination, we identified the participants with normal LDL-C and HDL-C but with abnormalities in LDL-P and/or HDL-P, according to guideline cutoffs (see the upper left quadrant of Figure 1). Of these patients, abnormal levels of total LDL-P were present in 14 (8.3%) cases, L-LDL-P in 29 (17.2%), M-LDL-P in 62 (36.7%), S-LDL-P in 23 (13.6%), total HDL-P in 48 (28.4%), and M-HDL-P in 72 (42.6%). We then analyzed the characteristics of the subsample of participants with normal values of LDL-C and HDL-C (table 3): most of them were men (69.8%) and did not smoke (77.5%), and they had a mean CT of 180.22 (SD 23.72) mg/dL and a mean age was 46.07 (SD 4.29) years. Based on the results of the bivariable and multivariable analyses, table 4 presents the variables showing statistically significant associations with abnormal levels of any of the lipoprotein particles analyzed in this study. In the multivariable analysis, higher total cholesterol and systolic blood pressure were significantly associated with abnormalities in the number of LDL-Ps. Male sex, higher systolic blood pressure and leukocyte level were significantly associated with abnormalities in the number of HDL-Ps.

DISCUSSION

Our study involved a sample of healthy, working adults with no known CVD and normal HDL-C and LDL-C levels, who presented abnormal levels of LDL-P and/or HDL-P. Higher total cholesterol, systolic blood pressure, BMI, and male sex were significantly associated with abnormalities in the number of LDL-Ps and HDL-Ps.

Previous studies in the USA, like the Cardiovascular Health Study, the Women's Health Study, and the Framingham Offspring Study, support the predictive role of the LDL-P count for cardiac events, especially in women [14,15,18,27]. In Europe, the FINRISK study [28] included more than 7000 individuals with no CVD at baseline, and it showed that the concentration of LDL particles, as measured by NMR, was associated with cardiovascular events. Another study with more than 2800 participants in the UK showed that the concentration of LDL-P was related to coronary disease in healthy people [29]. In regard to HDL-P, in the setting of potent statin therapy, HDL particle number may be a better marker of residual risk than chemically measured HDL-C or apoA-I [25]. In addition, HDL functionality (e.g. cholesterols efflux capacity) has been demonstrated to predict CVD risk better than HDL-C [30].

The present study contributes evidence that in a healthy population at low risk for CVD, there are discrepancies in terms of the plasma concentration of lipids and particles (size). Similarly, other authors have found individuals with discordant levels of LDL-P and LDL-C [14, 19, 31] or non-HDL-C [18,32]. Among them, Otvos et al. [19] reported that when LDL-P and LDL-C levels were discordant, LDL-P indicated atherosclerotic risk better than LDL-C, and Cromwell et al. [18] concluded that LDL-P was a better indicator of low CVD risk than either LDL-C or non-HDL-C. In ARIC study participants [33], small, dense LDL particles predicted risk for incident coronary heart disease in individuals who would be considered at low cardiovascular risk based on their LDL-C level. A subanalysis of the JUPITER trial compared the predictive value of HDL-C, apoA-I, HDL-P and cholesterol efflux and HDL-P was the strongest biomarker as an inverse predictor of incident CVD events [34]. Thus, the upper left quadrant of Figure 1 shows a large proportion of participants who would be considered at low to very low risk in clinical practice, based on the standard lipid profile, but who nevertheless could run higher risks according to the number and size of LDL or HDL particles. Regarding the characteristics of this group of people with normal HDL-C and LDL-C and abnormal levels LDL-P and/or HDL-P, higher total cholesterol and systolic blood pressure were significantly associated with abnormalities in the number of LDL-Ps. Male sex,

higher systolic blood pressure and leukocyte level were significantly associated with abnormalities in the number of HDL-Ps. The leukocyte level was associated with abnormal HDL-P because, probably, HDL/ABCA1/ABCG1 inhibit the proliferation of hematopoietic stem and multipotential progenitor cells, and connect expansion of these populations with leukocytosis and accelerated atherosclerosis [35]. Otvos et al. [19] compared the subgroup with LDL-P > LDL-C with the concordant group, and the former comprised more men and had higher prevalence of diabetes and other known markers of CVD risk. Only one individual in our study sample had diabetes, so we could not assess this factor.

The lipids profile offers an indirect estimate of the cholesterol contained in the group of LDL and HDL particles and the triglyceride-rich lipoproteins, but not of the biochemical characteristics of the wide spectrum of lipoprotein particles in plasma. Today, it is possible to characterize the latter using NMR, integrating this information into patients' risk diagnosis. In fact, these parameters could even be used in population-based screening to identify individuals who would be considered healthy based on traditional measures, but whose risk would be reclassified according to tests like Liposcale. These results could signal the need for very early monitoring, behavioral interventions, and even pharmacological treatments if appropriate [36].

There are many reasons for advancing research into dyslipidemia, including the fact that the conventional lipids profile fails to detect abnormalities in a significant proportion of patients who suffer initial or recurring ischemic episodes. These people are considered to have a residual risk of CVD, attributable to factors other than lipids that have not been adequately detected or controlled, but also to lipidic abnormalities that classic lipids profiles do not identify [37]. In relation to these cases, the number and composition of lipoprotein particles have demonstrated a predictive role in CVD. In our study, we identified healthy individuals with normal values for total cholesterol, LDL-C, and HDL-C, who nevertheless showed abnormalities in levels of LDL-Ps and HDL-Ps that could increase their risk of CVD. This risk is higher in those with a predominance of S-LDL-Ps and M-LDL-Ps compared to those with a predominance of L-LDL-Ps [38]. Moreover, there are clinical situations in which the conventional lipids profile is even less sensitive, providing scarce information on a wide range of lipoprotein abnormalities, associated for example with diabetes, insulin resistance, chronic inflammatory diseases, or kidney failure [37]. These clinical situations are associated with high cardiovascular risk due to different physiopathological mechanisms, including

abnormalities in the structure and function of lipoproteins, which are not reflected in the conventional lipids profile.

Limitations of the present study include the lack of a temporal sequence, precluding a cause-effect evaluation. However, in this study, LDL-P and HDL-P were measured and assessed according to the size of particles, allowing a better characterization of lipoprotein particles in healthy individuals. Cross-sectional studies have the advantage of being performed in a short time period and enabling prevalence estimates and, studies such as ours may constitute a first step for supporting the performance of prospective studies. To minimize the risk of non-response bias, we have estimated a 5% rate of missing data. The ultimate objective of this kind of descriptive study is to describe the frequency and characteristics of a health problem in a given study population and to generate hypotheses that should be investigated through analytical studies. On the other hand, the present study used indistinctly NMR and standard biochemistry derived cut points assuming a high degree of concordance between techniques - in agreement with previous studies using both techniques [39, 40]. However, it should be further demonstrated by using specific calibration material in accordance with good clinical laboratory practices.

In conclusion, our results bring into relief the profile of a person considered healthy, who presents a normal blood lipids profile, but who may presents abnormal levels of LDL-P and/or HDL-P when assessed via NMR using the Liposcale test. Further research is needed to confirm this finding and use this information to take early action on the prevention of lipid cardiovascular disease risk.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript. NA and XC are employed by, are stockholders of, and serve on the board of directors of Biosfer Teslab, a diagnostic laboratory company that performed the lipoprotein subtype analyses described herein. The remaining authors report no relationships that could be construed as a conflict of interest.

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Table 1. Description of the total study sample (N = 400)

Qualitative study variables		**n**	**%**
Sex	Women	125	31.3
	Men	275	68.8
Tobacco use	No	300	75.0
	Yes	100	25.0%
Diabetes mellitus	No	396	99.0
	Yes	4	1.0
Physical activity[a]	None	113	30.9
	Moderate (1–3)	176	48.1
	High (4–7)	77	21.0
Metabolic Syndrome	No	135	82.3%
	Yes	29	17.7%
Categorical variables	**N**	**Mean**	**SD**
Age (years)	400	46.4	4.3
Waist circumference (cm)	351	95.9	12.6
BMI (kg/m^2)	400	26.0	4.3
Systolic BP (mmHg)	400	123.9	15.6
Diastolic BP (mmHg)	400	80.9	10.1
Triglycerides (mg/dL)	191	116.56	66.40
Total cholesterol (mg/L)	400	199.26	35.27
Red blood cells (mil/mm^3)	384	4.89	.40
Hemoglobin (g/dL)	384	14.33	1.29
Hematocrit (%)	384	43.52	3.39
eGFR (mL/min/1.73 m^2)	398	88.86	14.60
Glucose (mg/dL)	398	87.62	11.93
Creatinine (mg/dL)	399	0.92	0.17
LDL-C (mg/dL)	400	128.40	22.74
HDL-C (mg/dL)	400	48.69	9.59
LDL-P (nmol/L)	400	1315.50	230.60
L-LDL-P (nmol/L)	400	198.75	31.71
M-LDL-P (nmol/L)	400	440.52	114.78
S-LDL-P (nmol/L)	400	676.12	123.61
HDL-P (µmol/L)	400	25.76	4.41
M-HDL-P (µmol/L)	400	8.76	1.80
CV risk SCORE	398	0.67	0.70

[a] As measured by the seven-item International Physical Activity Questionnaire.

BMI: body mass index; BP: blood pressure; LDL-C: concentration of low-density lipoproteins; HDL-C: concentration of high-density lipoproteins; eGFR: estimated glomerular filtration rate; LDL-P: concentration of LDL particles; L-LDL-P: concentration of large LDL particles; M-LDL-P: concentration of medium-sized LDL particles; S-LDL-P: concentration of small LDL particles; HDL-P: concentration of HDL particles; M-HDL-P: concentration of medium-sized HDL particles; CV: cardiovascular; SD: standard deviation.

Table 2. Classification of study participants according to recommended values for each lipids parameter (N = 400)

Lipids parameters			**n**	**%**	**95% CI**
Cholesterol content (mg/dL)	LDL-C	normal	225	56.3	(51.5-61.3)
		abnormal	175	43.8	(38.8-48.5)
	HDL-C	normal	300	75.0	(70.3-78.5)
		abnormal	100	25.0	(21.5-29.7)
Concentration of number of LDL particles (nmol/L)	LDL-P	normal	207	51.7	(46.8-57.0)
		abnormal	193	48.3	(43.0-53.3)
	L-LDL-P	normal	217	54.3	(49.3-59.5)
		abnormal	183	45.8	(40.5-50.7)
	M-LDL-P	normal	158	39.5	(35.0-44.5)
		abnormal	242	60.5	(55.5-65.0)
	S-LDL-P	normal	248	62.0	(57.5-66.3)
		abnormal	152	38.0	(33.8-42.5)
Concentration of number of HDL particles (µmol/L)	HDL-P	normal	246	61.5	(56.5-66.2)
		abnormal	154	38.5	(33.8-43.5)
	M-HDL-P	normal	190	47.5	(43.0-52.3)
		abnormal	210	52.5	(47.8-57.0)

CI: confidence interval; LDL-C: concentration of low-density lipoproteins; HDL-C: concentration of high-density lipoproteins; LDL-P: concentration of LDL particles; L-LDL-P: concentration of large LDL particles; M-LDL-P: concentration of medium-sized LDL particles; S-LDL-P: concentration of small LDL particles; HDL-P: concentration of HDL particles; M-HDL-P: concentration of medium-sized HDL particles.

Table 3. Characteristics of the subsample of participants with normal levels of HDL-C and LDL-C according to recommendations (N = 169)

Categorical study variables		n	%	IC 95%
Sex	Women	51	30.2	(23.1-37.3)
	Men	118	69.8	(62.7-76.9)
Tobacco use	No	131	77.5	(71.6-84.0)
	Yes	38	22.5	(16.0-28.4)
Diabetes mellitus	No	168	99.4	(98.2-100)
	Yes	1	0.6	(0.0-1.8)
Physical activity[a]	None	37	23.7	(16.7-30.8)
	Moderate (1–3)	80	51.3	(43.6-59.0)
	High (4–7)	39	25.0	(17.9-32.7)
Metabolic Syndrome	No	63	95.5%	(90.1-100.0)
	Yes	3	4.5%	(0.0-9.9)
Concentration of total LDL particles (nmol/L)	normal	155	91.7%	(87.6-95.3)
	abnormal	14	8.3%	(4.7-12.4)
Concentration of large LDL particles (nmol/L)	normal	140	82.8%	(76.9-88.2)
	abnormal	29	17.2%	(11.8-23.1)
Concentration of medium-sized LDL particles (nmol/L)	normal	107	63.3%	(55.6-69.8)
	abnormal	62	36.7%	(30.2-44.4)
Concentration of small LDL particles (nmol/L)	normal	146	86.4%	(81.1-91.1)
	abnormal	23	13.6%	(8.9-18.9)
Concentration of HDL particles (µmol/L)	normal	121	71.6%	(64.5-78.1)
	abnormal	48	28.4%	(21.9-35.5)
Concentration of medium-sized HDL particles (µmol/L)	normal	97	57.4%	(49.7-64.5)
	abnormal	72	42.6%	(35.5-50.3)

Quantitative variables	n	Range		Mean	SD
Age (years)	169	38.00	60.00	46.07	4.29
Waist circumference (cm)	144	65.00	140.00	92.84	11.73
BMI (kg/m^2)	169	17.90	41.60	24.94	3.82
Systolic BP (mmHg)	169	83.00	188.00	123.24	15.62
Diastolic BP (mmHg)	169	52.00	107.00	79.53	10.17
Triglycerides (mg/dL)	80	40.00	421.00	104.49	67.81
Total cholesterol (mg/L)	169	111.40	237.40	180.22	23.72
Red blood cells (mil/mm^3)	163	3.80	5.70	4.86	0.40
Hemoglobin (g/dL)	163	9.70	17.00	14.23	1.26
Hematocrit (%)	163	32.50	51.80	43.24	3.29
eGFR (mL/min/1.73 m^2)	168	58.60	144.00	90.96	15.08
Glucose (mg/dL)	168	70.00	167.00	86.58	12.85
Creatinine (mg/dL)	169	0.50	1.40	0.90	0.16

[a] As measured by the seven-item International Physical Activity Questionnaire.

[b] Values obtained in the general blood test using conventional methods.

BMI: body mass index; BP: blood pressure; LDL-C: concentration of low-density lipoproteins; HDL-C: concentration of high-density lipoproteins; eGFR: estimated glomerular filtration rate; SD: standard deviation.

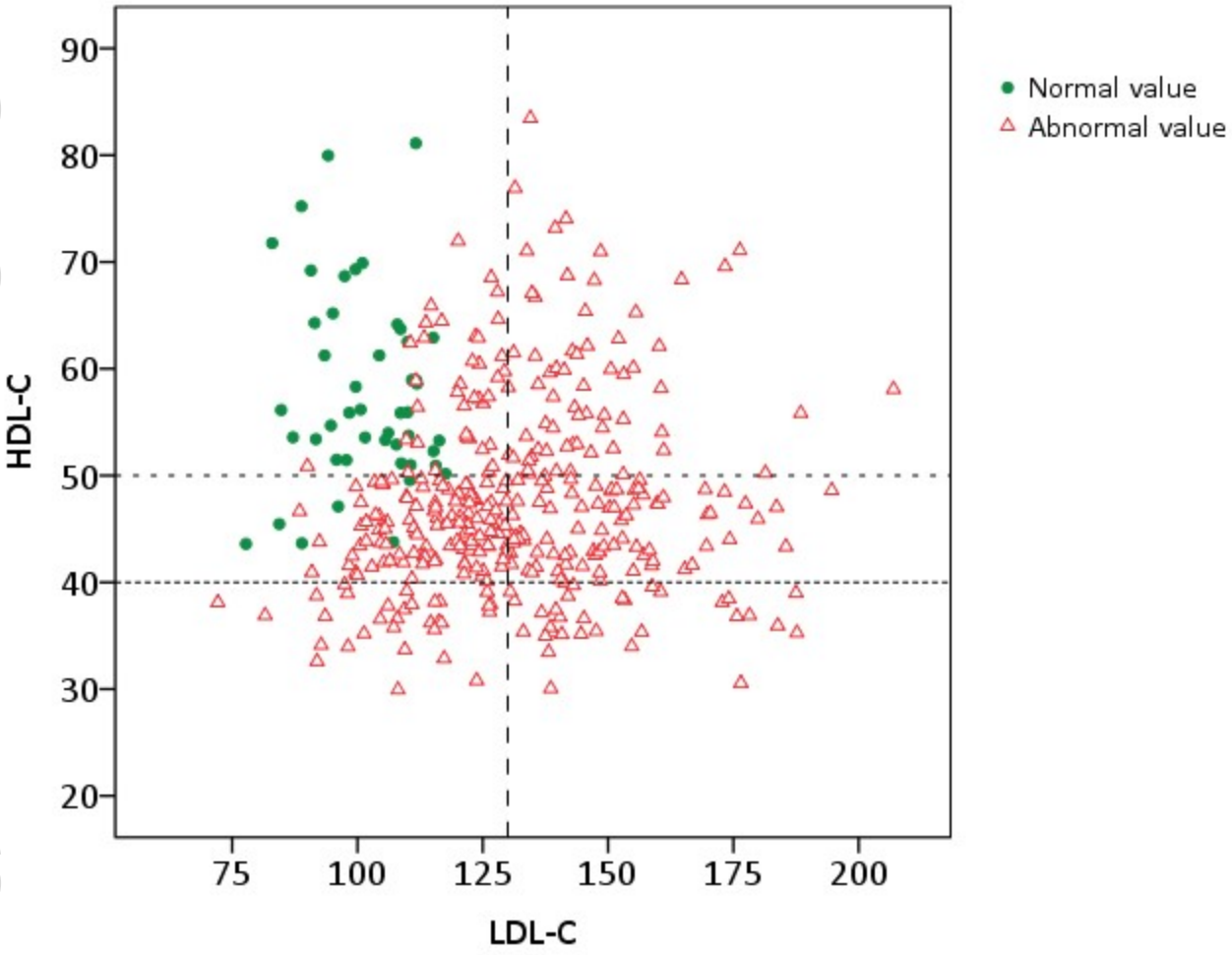
Table 4. Factors associated with abnormal concentrations of lipoprotein particles, as measured by NMR (n=169)

		Bivariable analysis		Multivariable analysis		
n with abnormalities (%)		**Variable**	**p-value**	**OR (95% CI)**	**p-value**	**AUC (95% CI)**
LDL-P	14 (8.3%)	Men	0.010			0.801
		Larger waist circumference	0.037			(0.707-0.899)
		Higher systolic BP	0.008	1.06 (1.02-1.10)	< 0.001	
		Higher diastolic BP	0.043			
		Higher total cholesterol	0.004	1.05 (1.02-1.08)	<0.001	
		Higher hemoglobin	0.002			
L-LDL-P	29 (17.2%)	Higher total cholesterol	<0.001	1.05 (1.02-1.07)	<0.001	0.749
						(0.661-0.837)
M-LDL-P	62 (36.7%)	Older age	0.048			0.785
		Higher total cholesterol	<0.001	1.06 (1.03-1.08)	<0.001	(0.715-0.855)
S-LDL-P	23 (13.6%)	Men	<0.001			0.835
		Larger waist circumference	0.030			(0.747-0.921)
		Higher BMI	0.002	1.25 (1.08-1.43)	0.002	
		Higher triglycerides	0.006	-		
		Higher total cholesterol	<0.001	1.06 (1.03-1.09)	<0.001	
		Higher hemoglobin	<0.001			
		Higher hematocrit	0.023			
		Higher MCH	0.031			
		Higher MCHC	<0.001	2.85 (1.40-5.79)	0.004	
HDL-P	48 (28.4%)	Men	<0.001	21.66 (2.82-166.5)	<0.001	0.790
		Larger waist circumference	<0.001			(0.716-0.859)
		Higher BMI	0.002			
		Higher systolic BP	<0.001	1.03 (1.01-1.06)	0.010	
		Higher diastolic BP	0.001			
		Higher red blood cells	<0.001			
		Higher hemoglobin	<0.001			
		Higher l hematocrit	<0.001			
		Higher MCHC	<0.001			
		Higher leukocytes	0.032	1.31 (1.03-1.68)	0.030	
		Higher creatinine	<0.001			
		Higher GPT	0.004			
M-HDL-P	72 (42.6%)	Men	<0.001	17.08 (4.94-59.0)	<0.001	0.796
		Larger waist circumference	<0.001			(0.729-0.862)
		Higher BMI	<0.001			
		Higher systolic BP	<0.001	1.04 (1.01-1.06)	0.011	
		Higher diastolic BP	0.002			
		Higher red blood cells	<0.001			

Higher level of hemoglobin	<0.001
Higher level of hematocrit	<0.001
Higher MCHC	0.003
Higher creatinine	<0.001
Higher GPT	<0.001

OR: odds ratio; CI: confidence interval; AUC: area under the receiver operating characteristics curve; BMI: body mass index; BP: blood pressure; LDL-C: concentration of low-density lipoproteins; HDL-C: concentration of high-density lipoproteins; LDL-P: concentration of LDL particles; L-LDL-P: concentration of large LDL particles; M-LDL-P: concentration of medium-sized LDL particles; S-LDL-P: concentration of small LDL particles; HDL-P: concentration of HDL particles; M-HDL-P: concentration of medium-sized HDL particles; SD: standard deviation; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; GPT: glutamate-pyruvate transaminase.

Figure 1. Participants with normal and abnormal values in some subtype of lipoprotein, according to levels of LDL-C and HDL-C.



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