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Original article

Advanced lipoprotein profile in individuals with normal and impaired glucose metabolism

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ABSTRACT

Introduction and objectives: Several types of lipoproteins beyond low-density lipoproteins (LDL) are causally related to cardiovascular disease. We aimed to analyze an advanced lipoprotein profile in individuals with normal and impaired glucose metabolism from different cohorts of a Mediterranean region.
Methods: Cross-sectional study in 929 participants (463 normoglycemia, 250 prediabetes, and 216 type 2 diabetes mellitus) with normal renal function, free from cardiovascular disease, and without lipid-lowering treatment. Conventional and advanced (nuclear magnetic resonance [NMR] spectroscopy) lipoprotein profiles were analyzed.
Results: Compared with men, normoglycemic women showed lower serum triglyceride and LDL cholesterol concentrations, lower total LDL particles (P) as well as their subclasses and their cholesterol and triglyceride content, higher high-density lipoproteins (HDL)-P and all HDL-related variables ($P \leq .05$ for all comparisons). Compared with normoglycemic participants, diabetic participants showed higher large and small very LDL-P concentrations ($P < .05$) and lower total HDL-P and medium HDL-P concentrations ($P < .05$). Waist circumference and Fatty Liver Index were positively associated with a proatherogenic profile.
Conclusions: Women had a better advanced lipoprotein profile than did men. Adiposity indexes related to insulin-resistance were positively associated with a proatherogenic lipid profile. NMR revealed altered lipoprotein particles other than LDL in participants with diabetes, frequently associated with an increased cardiovascular risk. Our findings support the usefulness of extended lipoprotein analysis by NMR spectroscopy to uncover new therapeutic targets to prevent cardiovascular events in at-risk participants.

Perfil lipoproteico avanzado en individuos con metabolismo glucémico normal y alterado

Palabras clave:
Lipoproteínas
Mediterráneo
Diabetes tipo 2
Prediabetes
Resistencia insulínica
Resonancia magnética espectroscópica

RESUMEN

Introducción y objetivos: Varios tipos de lipoproteínas, aparte de las lipoproteínas de baja densidad (LDL), tienen relación causal con la enfermedad cardiovascular. Se analizó el perfil lipoproteico avanzado de individuos con metabolismo glucémico normal y alterado provenientes de una región mediterránea.
Métodos: Estudio transversal en 929 participantes (463 normoglucémicos, 250 prediabéticos y 216 con diabetes tipo 2) sin insuficiencia renal, enfermedad cardiovascular ni tratamiento hipolipemiante. Se analizaron los perfiles lipoproteicos convencional y avanzado (resonancia magnética [RM] espectroscópica).

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Resultados: En comparación con los varones, las mujeres normoglucémicas mostraron menores concentraciones de triglicéridos y cLDL, menos partículas (P) de LDL y todas sus subclases y menos contenido en colesterol y triglicéridos, mayor concentración de P de lipoproteínas de alta densidad (HDL) y de todas sus variables relacionadas ($p \leq 0,05$ para todas las comparaciones). En comparación con los normoglucémicos, los diabéticos mostraron una mayor concentración de P-VLDL grandes y pequeñas ($p < 0,05$), además de una menor concentración de P-HDL totales y medianas ($p < 0,05$). Se halló relación directa del perímetro de la cintura y el *fatty liver index* con un perfil proaterogénico.
Conclusiones: Las mujeres mostraron un mejor perfil lipoproteico avanzado que los varones. Se halló relación directa de los índices de adiposidad relacionados con resistencia insulínica con un perfil lipídico proaterogénico. La RM mostró alteraciones en partículas lipoproteicas distintas de las LDL en los diabéticos, a menudo asociadas con mayor riesgo cardiovascular. Nuestros hallazgos confirman la utilidad del análisis lipoproteico avanzado mediante RM espectroscópica para descubrir nuevas dianas terapéuticas con que prevenir eventos cardiovasculares en los individuos en riesgo.

Abbreviations

BMI: body mass index
CVD: cardiovascular disease
NAFLD: nonalcoholic fatty liver disease
NMR: nuclear magnetic resonance
TG: triglyceride
T2DM: type 2 diabetes mellitus

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) and its clinical manifestations are the leading cause of morbidity and mortality globally.[1] A range of clinical and genetic studies have unequivocally established that LDL is an etiopathogenetic factor in the pathophysiology of atherosclerotic CVD.[2] Low-density lipoprotein cholesterol (LDL-C)- lowering therapy has been shown to reduce the rate of CVD events in participants with or without cardiometabolic risk.[3] However, CVD events remain prevalent among individuals with low or normal LDL-C, a phenomenon referred to as residual risk.[4] In this regard, recent studies have reported the association of different lipoprotein subclasses particle concentrations, other than LDL, with the risk of incident CVD events.[5,6] Thus, it has been proposed that CVD risk may also be associated with higher concentrations of atherogenic lipoproteins, which may not be readily apparent from LDL-C concentrations.

Triglycerides (TG) are the principal components of TG-rich lipoproteins and their remnants. TG are predominantly transported by very LDL (VLDLs) and, to a lesser extent, their remnants under fasting conditions. Under postprandial conditions, circulating TG are mainly found in chylomicrons and their remnants (also defined as TG-rich lipoproteins). Compelling evidence suggests that chylomicrons and VLDL remnants are highly proatherogenic, by virtue of their progressive enrichment in cholesterol.[7] There are strong and consistent epidemiologic associations between hypertriglyceridemia and TG-rich lipoproteins and incident CVD events.[8] Mild to moderate hypertriglyceridemia is particularly common in participants with insulin-resistant conditions such as visceral obesity, type 2 diabetes mellitus (T2DM) and chronic kidney disease, all of them associated with an increased risk of CVD.[9] In these circumstances, as well as at very low levels of LDL-C in the presence of high TGs, the calculated or directly measured LDL-C level may underestimate both the total concentration of cholesterol carried by LDL and, more importantly, underestimate the total concentration of atherogenic lipoproteins, thus underestimating the risk of atherosclerotic CVD.[10] In these individuals, the typical lipid phenotype is the condition known as atherogenic dyslipidemia characterized by hypertriglyceridemia, increased concentration of small-dense LDL-P and low high-density lipoprotein (HDL)-C concentrations.[11] The same lipid pattern also occurs in another clinical condition associated with an increased risk of CVD, nonalcoholic fatty liver disease (NAFLD). Persons with NAFLD show many proatherogenic changes in the lipoprotein profile.[12] Therefore, assessing the lipoprotein profile beyond what is currently used in clinical practice may be important in assessing CVD risk in participants with clinical conditions such as those previously mentioned in which LDL-C is usually not elevated and show subtle defects in routine lipid panel analysis.

To the best of our knowledge, there are few data in the literature on the lipoprotein profile in participants with different degrees of impaired glucose metabolism (ie, prediabetes or T2DM) and normoglycemic participants residing in the same geographic area.

The aim of the present study was to analyze serum lipoprotein subclasses and their cholesterol and triglyceride content in normal and impaired glucose metabolism in Mediterranean participants from southern Europe.

METHODS

Study population

A total of 1217 participants, 510 with normoglycemia, 318 with prediabetes, and 389 with T2DM, were identified from different cohorts of 4 participating institutions belonging to the same health care organization in the north-northeast region of Spain. After excluding those participants who were under lipid-lowering therapy, 929 participants were analyzed (463 with normoglycemia, 250 with prediabetes, and 216 with T2DM). Normoglycemic and prediabetic groups were selected from 3 previously published cross-sectional studies[13–15] (2 university hospital cohorts and one primary care cohort in Spain). The study participants with T2DM were selected from the same 2 cross-sectional studies performed at the university hospitals from where participants with normoglycemia and prediabetes were recruited. T2DM participants were recruited from the outpatient clinic of 1 of the participating centers as well as from those identified by screening patients enrolled in the diabetic eye disease program.[16] Some of them were new-onset T2DM patients recruited from the outpatient clinic of the department of endocrinology.[14]

The inclusion criteria for all 3 groups (normoglycemic, prediabetic, and T2DM) were as follows: absence of established impaired renal function (defined as an estimate glomerular filtration rate < 60 mL/min/1.73 m^2), and absence of known heart disease (defined as any known peripheral artery disease, stroke, heart failure, coronary artery disease, including previous myocar-

dial infarction, angina, previous coronary artery bypass surgery, or percutaneous coronary intervention). Exclusion criteria were active lipid-lowering treatment (defined as statin or fibrate drugs) and a diagnosis of type 1 diabetes mellitus (T1DM) or suspicion of having any other specific type of diabetes secondary to genetic defects, endocrinopathies, pancreatic exocrine dysfunction or chemically-induced diabetes.

We recorded participant characteristics, anthropometric parameters, and laboratory measurements (glucose, HbA1c, TGs, total/HDL/LDL-C, creatinine, estimated glomerular filtration rate, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), insulin and homeostatic Model Assessment of Insulin Resistance [HOMA-IR]). To evaluate the presence of NAFLD, the Fatty Liver Index (FLI) was also calculated in all study participants. An FLI $>$ 60 was considered suggestive of NAFLD.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committees. All participants signed a written informed consent form.

Diabetes and prediabetes diagnosis

In the present study, the diagnosis of diabetes was made according to the criteria established by the American Diabetes Association. Prediabetes was also defined according to the American Diabetes Association criteria.[17] We considered as prediabetic any participant who met 1 of the 2 following criteria: *a)* impaired fasting plasma glucose, defined as fasting plasma glucose between 100 and $<$ 126 mg/dL (5.55-6.99 mmol/L), or *b)* HbA1c levels between 5.7% and $<$ 6.5% (39-48 mmol/mol). Participants in the normoglycemic group had fasting plasma glucose and HbA1c values below 100 mg/dL and 5.7%, respectively.

Anthropometric parameters and blood pressure

Participants' weight, height and waist circumference were measured using standardized methods, and their blood pressure (mean of 2 measurements, 5 minutes apart) was measured after 10 minutes in a seated position using a blood pressure monitor (HEM-7001E, Omron, Barcelona, Spain). Body mass index (BMI) was calculated as weight (kg)/stature (m^2).

Biochemistry and laboratory methods

Serum and spot urine samples were collected in the fasting state, and all serum and urine tests were performed using standard laboratory methods. LDL-C was estimated using the Friedewald formula, and estimate glomerular filtration rate was estimated using the Modification of Diet in Renal Disease-4 formula.[18] HbA1c levels were determined using HPLC (Variant, Bio-Rad Laboratories SA, Spain), and its concentrations are expressed in the National Glycohemoglobin Standardization Program/Diabetes Control and Complications Trial units. Urine albumin was measured using an immunoturbidimetric method and a Roche/Hitachi Modular P analyzer (Roche Diagnostics, Spain). Insulin-resistance was assessed by homeostatic model assessment (HOMA)-IR.

NMR analysis of lipoproteins

Blood serum samples were shipped on dry ice to the Biosfer Teslab facilities (Reus, Spain) for advance lipoprotein testing by using the Liposcale test, an advanced lipoprotein test (CE) based on 2D NMR diffusion-ordered spectroscopy that enables exhaustive analysis of lipoprotein particles.[19] We determined the lipid composition and the mean size (nm) for each particle class, and the particle concentration of 9 lipoprotein subclasses, namely large, medium, and small VLDL, LDL, and HDL-P. As previously described, the within-assay precision of the method for the determination of cholesterol and TG concentrations, and particle numbers for the LDL class and its small, medium, and large subclasses, is $\leq$ 5%. The interassay precision for the same parameters is $\leq$ 8%. Similarly, the within-assay and interassay precision for cholesterol and TG concentrations, and VLDL and HDL-P, are $\leq$ 6%. Finally, both within-assay and interassay precision for the mean particle size for each lipoprotein class is $\leq$ 1%.[19]

Statistical analysis

Data management and analyses were conducted using the free R statistical software version 3.6.0. Descriptive statistics are summarized with median, interquartile ranges [25th-75th], mean and standard deviation for continuous variables, or frequency and percentage for categorical variables. The Student *t* test or chi-square test was used to explore the differences between groups. *P*-values corresponding to pairwise comparisons were calculated by multiple testing with the Tukey method. Pearson correlation coefficients were computed between NMR-assessed advanced lipoprotein profiles and clinical and laboratory parameters in the normoglycemic control group. To assess the adjusted differences between groups from each lipoprotein subclass, we used multivariate regression models. The covariate variables included in the adjusted analysis were age, sex, and BMI. Furthermore, we applied multi testing correction according to the Bonferroni method to control the family-wise error rate prefixed to 0.05.

Participants were classified according to their serum concentration of LDL-P and HDL-P particles and lipid content, ie, normal and abnormal, according to previously published cutoffs in relation to CVD risk established in previous studies.[20,21] Thus, the cutoffs for abnormal level of lipoprotein particle concentrations in the normoglycemic group were as follows: total LDL-P $>$ 1300 nmol/L, and $<$ 24 μmol/L for total HDL-P concentration; the LDL-C levels were considered elevated when they exceeded 130 mg/dL, whereas HDL-C was considered low below 40 mg/dL in men and 50 mg/dL in women, respectively.

RESULTS

Population characteristics

Serum lipoprotein particle concentrations were analyzed in 929 participants. From this cohort, 463 (49.8%) were participants with normoglycemia, 250 (26.9%) were participants with prediabetes, and 216 (23.3%) were participants with T2DM. A descriptive analysis of clinical and analytical variables by group is shown in table 1. Compared with the normoglycemic control group, participants with prediabetes and T2DM groups were older, had higher BMI and waist circumference, and a higher percentage had hypertension. They also had a higher fasting plasma glucose and insulin, HbA1c and a higher median HOMA-IR. Regarding the lipid profile, there were some differences in fasting cholesterol subclasses across the 3 groups, with lower serum HDL-C concentrations in participants with T2DM compared with normoglycemic participants and higher serum LDL-C in the prediabetic group than in the normoglycemic group.

Lipoprotein analysis by age in the normoglycemic group

Table 2 and table 3 describe the lipoprotein values according to the age range in both sexes. The analysis of the whole

Table 1
Descriptive analysis of clinical variables by group

Variable	NG	Prediabetic	T2DM	*P*	
	(n = 463)	(n = 250)	(n = 216)	NG vs Prediabetic	NG vs T2DM
Age, y	43.0 [35.8-51.0]	54.0 [46.0-61.5]	59.0 [51.8-66.0]	< .001	< .001
Sex, men	196 (42.3)	111 (44.4)	120 (55.6)	.651	.005
Race, caucasian	446 (96.5)	240 (96)	205 (94.9)	.877	.877
Hypertension	50 (10.8)	52 (20.8)	104 (48.1)	< .001	< .001
Smoking	115 (24.9)	68 (27.2)	41 (19)	.571	.158
Body mass index, kg/m²	24.6 [22.4-27.0]	26.6 [24.5-29.9]	30.1 [27.1-33.7]	< .001	< .001
Waist, cm	90.0 [82.0-99.0]	96.0 [88.0-104]	104 [97.0-112]	< .001	< .001
Systolic blood pressure, mmHg	119 [109-128]	125 [115-136]	135 [124-149]	< .001	< .001
Diastolic blood pressure, mmHg	75.0 [69.0-81.0]	79.0 [73.0-86.0]	79.0 [72.0-86.0]	< .001	< .001
Glucose, mg/dL	86.0 [81.0-92.0]	97.0 [88.0-106]	145 [120-175]	< .001	< .001
HbA1c, %	5.30 [5.10-5.40]	5.80 [5.70-6.00]	7.20 [6.50-8.30]	< .001	< .001
Glycemic control				NE	NE
HbA1c < 7%	463 (100)	250 (100)	94 (43.5)		
HbA1c 7-8%	0 (0.0)	0 (0.0)	51 (23.6)		
HbA1c > 8%	0 (0.0)	0 (0.0)	71 (32.9)		
Triglycerides, mg/dL	84.0 [61.0-112]	89.0 [71.0-135]	116 [81.0-172]	.099	< .001
Total cholesterol, mg/dL	193 [170-219]	200 [184-227]	193 [174-219]	.001	.818
HDL cholesterol, mg/dL	58.0 [48.0-69.0]	57.0 [49.0-68.0]	48.0 [41.0-59.0]	.795	< .001
LDL cholesterol, mg/dL	115 [93.8-137]	124 [105-146]	116 [100-140]	.001	.681
MRDR-4, mL/min/1.73 m^2	94.8 [84.4-107]	89.6 [78.8-104]	90.7 [81.8-104]	.052	.956
Creatinine, mg/dL	0.78 [0.69-0.90]	0.79 [0.70-0.93]	0.80 [0.69-0.92]	.362	.366
Alanine transaminase, U/L	17.0 [13.0-22.0]	18.0 [14.0-25.0]	22.0 [17.0-32.0]	.850	< .001
Aspartate transaminase, U/L	26.0 [22.0-30.5]	24.0 [19.0-30.2]	19.0 [17.0-25.5]	.025	.001
Gamma-glutamyltransferase, U/L	16.5 [12.0-24.8]	19.0 [13.0-28.0]	25.0 [17.8-41.0]	.969	< .001
Insulin, ug/mL	7.35 [5.47-10.1]	8.75 [6.50-13.5]	12.2 [7.88-16.4]	< .001	< .001
Fatty liver index	21.7 [9.30-48.2]	39.9 [17.3-69.6]	73.8 [47.9-90.1]	< .001	< .001
HOMA-IR	1.57 [1.14-2.20]	2.11 [1.51-3.29]	4.27 [3.00-6.04]	< .001	< .001

HBA1c, glycosylated hemoglobin; HDL, high-density lipoproteins; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoproteins; NE, not evaluated; NG, normoglycemic; T2DM, type 2 diabetes mellitus.
Results are expressed as median [25th-75th] for continuous variables and No. (%) for categorical variables.

normoglycemic group showed a significant increase of several lipid parameters with increasing age with a *P* trend < .001 for total cholesterol, *P* < .01 for LDL-C, *P* < .001 for total LDL-P, *P* < .001 for large LDL-P, *P* < .001 for medium LDL-P and *P* < .001 for small LDL-P. HDL particles also increased with increasing age: *P* = .03 for total HDL-P and *P* < .001 for medium HDL-P. Interestingly, the increase in age was more marked in younger and middle-aged age ranges than between middle-aged and older age ranges.

Between-sex differences in lipid parameters and the NMR advanced lipoprotein profile in the normoglycemic control group

Lipoprotein profile was strongly dependent on the participants' sex. Compared with men, women had lower mean serum TG and LDL-C concentrations (82.5 mg/dL vs 114 mg/dL, *P* = .001; 110 mg/dL vs 118 mg/dL, *P* = .010, respectively). In line with serum TG, women showed concomitantly lower concentrations of VLDL-P and lipids, accompanied by elevated concentrations of HDL-P and lipids (*P* < .001 for all comparisons except for medium VLDL-P, which was *P* = .010). LDL characteristics also showed significant sex-related differences. Compared with men, total concentrations of LDL-P and small LDL-P were significantly lower in women (1256 [697-11 981] vs 1319 [572-12 179] nmol/L; *P* = .014; 638 [324-1047] nmol/L vs 735 [401-1171]; *P* < .001, respectively). Similarly, serum concentrations of non-HDL-P, including VLDL-P and LDL-P, were significantly lower in women than in men (*P* = .001). Women also showed lower cholesterol and TG content in VLDL particles and higher cholesterol and TG content in HDL particles (table 1 of the supplementary data).

Only 75 out of 462 normoglycemic participants (16%) showed abnormal values for both HDL-C (with values < 40 mg/dL in men and < 50 mg/dL in women) and LDL-C (with values > 130 mg/dL), with the rest of the participants (84%) showing normal values for both parameters. Interestingly, among normoglycemic participants with normal LDL-C and HDL-C, nearly 50% showed higher than recommended LDL-P concentrations (50.4% with high levels of total LDL-P). In contrast, only 1.8% of normoglycemic participants showed lower than recommended HDL-P concentrations (table 4).

Correlation between the NMR-assessed advanced lipoprotein profile and clinical parameters in the normoglycemic control group

Figure 1 shows the correlation between different NMR-derived lipoprotein characteristics and different clinical variables. The most notable observations were that FLI, waist circumference and BMI were positively related to VLDL-related characteristics (total number, size and cholesterol and TG content), LDL-P small subclass and non-HDL-P. The same clinical variables were inversely related

Table 2
Lipoprotein values according to the age terciles in women

	[18.0-44.3] years (n = 146)		[44.3-56.4] years (n = 76)		[56.4-83.0] years (n = 45)	
	Mean ± standard deviation	Median [25th-75th]	Mean ± standard deviation	Median [25th-75th]	Mean ± standard deviation	Median [25th-75th]
VLDL-P, nmol/L	34.5 ± 17.1	30.1 [23.4-40.7]	38.4 ± 21.3	31.3 [24.5-45.3]	40.0 ± 20.7	37.6 [25.5-50.4]
Large VLDL-P, nmol/L	0.89 ± 0.37	0.84 [0.64-1.06]	0.93 ± 0.47	0.85 [0.60-1.04]	0.98 ± 0.42	0.88 [0.67-1.26]
Medium VLDL-P, nmol/L	3.6 ± 1.9	3.20 [2.13-4.47]	3.78 ± 1.84	3.33 [2.49-4.83]	4.04 ± 2.29	3.20 [2.73-5.05]
Small VLDL-P, nmol/L	30.0 ± 15.3	25.6 [20.1-35.2]	33.7 ± 19.4	27.3 [20.8-40.5]	35.0 ± 18.4	31.3 [21.7-44.1]
LDL-P, nmol/L	1180 ± 231	1170 [1010-1340]	1410 ± 274	1380 [1230-1610]	1440 ± 241	1450 [1290-1580]
Large LDL-P, nmol/L	180 ± 31.6	176 [157-202]	205 ± 34.1	203 [178-228]	209 ± 37.2	213 [178-234]
Medium LDL-P, nmol/L	389 ± 120	378 [300-461]	490 ± 138	477 [384-563]	499 ± 125	508 [419-561]
Small LDL-P, nmol/L	613 ± 111	604 [536-680]	718 ± 134	702 [624-781]	734 ± 119	727 [661-791]
HDL-P, µmol/L	31.1 ± 6.8	30.4 [26.6-34.7]	31.4 ± 5.58	30.8 [27.7-35.1]	32.4 ± 4.51	31.8 [28.7-35.0]
Large HDL-P, µmol/L	0.3 ± 0.06	0.261 [0.24-0.30]	0.29 ± 0.05	0.29 [0.25-0.33]	0.31 ± 0.05	0.31 [0.29-0.33]
Medium HDL-P, µmol/L	10.5 ± 2.4	10.1 [8.91-11.8]	10.4 ± 2.34	9.92 [8.72-12.2]	10.9 ± 1.82	10.9 [9.94-11.5]
Small HDL-P, µmol/L	20.3 ± 4.8	19.7 [17.0-23.3]	20.7 ± 3.9	20.7 [18.5-23.4]	21.2 ± 3.55	21.1 [18.3-23.7]
VLDL-C	8.4 ± 5.7	7.21 [3.97-11.1]	9.43 ± 6.57	7.96 [4.49-11.7]	9.79 ± 7.09	8.20 [4.45-12.9]
LDL-C	118 ± 23.4	116 [101-133]	141 ± 27.5	137 [120-159]	143 ± 25.0	143 [125-160]
HDL-C	61.1 ± 14.1	59.2 [50.5-69.8]	62.3 ± 13.3	59.5 [53.7-70.2]	64.5 ± 10.2	63.3 [57.1-70.6]
VLDL-TG	49.7 ± 23.7	44.1 [33.8-58.4]	54.6 ± 29.0	44.8 [36.7-62.5]	57.4 ± 28.2	53.3 [35.9-69.9]
LDL-TG	15.1 ± 4.8	14.5 [11.5-17.7]	18.1 ± 4.67	17.3 [14.8-20.6]	18.7 ± 4.54	18.7 [16.2-21.0]
HDL-TG	14.7 ± 5.2	13.7 [11.6-16.4]	13.9 ± 3.80	13.2 [11.4-16.0]	14.7 ± 3.72	14.2 [12.5-16.7]

HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein.

Table 3
Lipoprotein values according to the age tertiles in men.

	[18.0-44.3] years (n = 103)		[44.3-56.4] years (n = 62)		[56.4-83.0] years (n = 31)	
	Mean ± standard deviation	Median [25th-75th]	Mean ± standard deviation	Median [25th-75th]	Mean ± standard deviation	Median [25th-75th]
VLDL-P, nmol/L	46.4 ± 24.0	39.9 [28.1-61.4]	59.2 ± 62.1	45.1 [27.9-69.8]	50.6 ± 35.7	44.0 [29.0-61.9]
Large VLDL-P, nmol/L	1.14 ± 0.53	1.02 [0.765-1.47]	1.39 ± 1.34	1.07 [0.721-1.66]	1.22 ± 0.79	0.974 [0.82-1.39]
Medium VLDL-P, nmol/L	4.82 ± 2.72	4.11 [2.95-6.07]	6.77 ± 12.6	4.29 [2.52-6.04]	4.81 ± 2.71	4.06 [3.02-6.25]
Small VLDL-P, nmol/L	40.4 ± 21.3	33.8 [24.0-53.3]	51.1 ± 49.0	39.0 [24.8-62.5]	44.5 ± 32.6	37.7 [24.7-53.2]
LDL-P, nmol/L	1330 ± 290	1300 [1120-1550]	1390 ± 277	1390 [1230-1530]	1400 ± 252	1370 [1210-1520]
Large LDL-P, nmol/L	185 ± 37.7	181 [159-214]	188 ± 36.4	185 [161-212]	198 ± 39.3	189 [168-226]
Medium LDL-P, nmol/L	407 ± 141	389 [301-492]	420 ± 148	418 [318-505]	442 ± 143	404 [328-527]
Small LDL-P, nmol/L	735 ± 148	718 [608-834]	786 ± 142	783 [693-848]	756 ± 121	746 [683-811]
HDL-P, µmol/L	26.1 ± 4.96	25.7 [22.9-28.5]	28.4 ± 5.90	27.1 [24.7-31.0]	27.8 ± 5.04	26.5 [24.4-32.0]
Large HDL-P, µmol/L	0.25 ± 0.05	0.24 [0.21-0.28]	0.26 ± 0.05	0.26 [0.23-0.29]	0.29 ± 0.05	0.27 [0.25-0.31]
Medium HDL-P, µmol/L	8.11 ± 1.74	7.89 [6.84-9.11]	8.77 ± 2.23	8.35 [7.38-9.85]	9.44 ± 2.27	8.81 [8.10-10.1]
Small HDL-P, µmol/L	17.7 ± 3.67	17.6 [15.3-19.8]	19.3 ± 4.33	19.0 [16.6-21.2]	18.1 ± 3.77	17.5 [16.2-19.9]
VLDL-C	11.7 ± 7.76	9.85 [5.59-16.7]	14.9 ± 16.4	11.3 [5.37-19.0]	13.3 ± 10.4	10.8 [6.42-17.5]
LDL-C	130 ± 28.3	129 [109-152]	134 ± 28.9	134 [115-150]	137 ± 26.8	135 [118-153]
HDL-C	50.2 ± 10.0	49.6 [43.2-56.1]	54.4 ± 13.3	50.9 [45.4-62.2]	54.6 ± 11.0	51.2 [46.8-62.1]
VLDL-TG	66.8 ± 33.9	57.3 [43.3-84.7]	87.4 ± 105	63.6 [39.6-96.4]	71.4 ± 48.8	58.4 [41.5-89.0]
LDL-TG	15.5 ± 5.29	14.5 [11.3-19.0]	16.8 ± 4.92	16.9 [12.8-19.3]	17.2 ± 4.58	17.1 [13.6-20.4]
HDL-TG	11.3 ± 4.19	10.6 [9.03-13.0]	12.8 ± 5.30	11.6 [9.42-15.0]	13.2 ± 5.79	12.0 [11.0-13.5]

HDL, high-density lipoprotein; HDL-C, high-density lipoprotein colesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein colesterol; VLDL, very low-density lipoprotein

Table 4
Conventional vs NRM-derived lipoprotein particle concentrations in the normoglycemic group

	Conventional lipid parameters	
	Abnormal	Normal
NMR-derived lipid parameters	n = 75	n = 387
Total LDL-P		
Abnormal	39 (52.0)	195 (50.4)
Normal	36 (48.0)	192 (49.6)
Total HDL-P		
Abnormal	0 (0.00)	7 (1.81)
Normal	75 (100)	380 (98.2)

HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol.
The data are presented as No. (%).
Data regarding lipoprotein particles is lacking in 1 participant due to the absence of LDL-C and HDL-C values in this participant.
Abnormal NMR-derived lipid parameters: total LDL-P > 1300 nmol/L, and < 24 μmol/L for total HDL-P concentration.
Abnormal conventional lipid parameters: LDL-C > 130 mg/dL, HDL-C < 40 mg/dL in men and, < 50 mg/dL in women.

to total HDL-P, medium, and small subclasses, as well as their cholesterol content.

Lipoprotein particles values in T2DM and prediabetes and comparison with the normoglycemic control group

The comparison between lipoprotein subclass sizes between the normoglycemic, prediabetic and T2DM groups are shown in table 5. Multivariate analysis (adjusted for age, gender, and BMI) showed that almost all the VLDL subclasses were significantly higher in participants with T2DM than in normoglycemic participants, with higher serum concentrations of large and small VLDL-P ($P = .040$ and $P = .042$, respectively). No significant differences in the LDL-P distribution were observed when comparing the 3 groups. Furthermore, we found a statistically significant reduction in LDL size (LDL-Z) in participants with T2DM compared with that of normoglycemic participants ($P = .018$). There was a trend toward a higher content of cholesterol in VLDL particles in participants with T2DM than in normoglycemic participants ($P = .083$). Regarding HDL-related characteristics, total HDL-P levels in the T2DM group were lower than in the normoglycemic group ($P = .017$), especially medium particles ($P = .013$). Median cholesterol content in HDL-P was concomitantly lower in participants with T2DM than in controls ($P = .002$).

DISCUSSION

The present study reports the advanced characteristics of circulating lipoproteins, assessed by NMR spectroscopy, in a large number of participants with different degrees of impaired glucose metabolism without previous CVD events not receiving lipid-lowering treatment. The main findings were that in normoglycemic participants, female sex was strongly associated with an improved lipoprotein profile. Women also showed lower cholesterol content in VLDL particles compared with men. Interestingly, nearly 50% of normoglycemic participants with apparently normal conventional lipid values showed abnormal LDL-P concentrations. In addition, compared with normoglycemic participants, participants with T2DM showed abnormal levels of lipoprotein particles other than LDL, such as HDL and VLDL-P concentrations, reported to be associated with increased CV risk. Of note, participants with T2DM showed a marginal trend toward a higher cholesterol content in VLDL particles.

To our knowledge, few studies have reported lipoprotein subclass particle concentrations and their cholesterol and TG content in a large sample of participants residing in the same geographical area. Notably, none of them has been performed in a representative Mediterranean region. In the present study, LDL-P and HDL-P concentrations increased with age, with the increase being more marked in women. This result concurs with previously reported studies showing that LDL-P concentrations show a stronger correlation with age among women than among men.[22] In contrast, in concordance with previously published studies, the increase in LDL-P concentrations observed with increasing age was more evident between young- and middle-aged participants than in middle- and older participants.[23] Finally, similar to a recently published study, total HDL-P in women increased with age.[24] This study suggested that, although the absolute number of HDL-P increases with age in women, their ability to promote the cholesterol efflux capacity from macrophages is compromised with increasing age.[24] The latter is entirely consistent with the current notion that HDL function, rather than HDL-C quantity, may be the target for future HDL therapies for CVD. On the other hand, in line with previous reports,[22] our data support the notion that female sex is strongly associated with an improved advanced lipoprotein profile compared with men. Indeed, women had a lower total amount of atherogenic particles (ie, VLDL-P and LDL-P) and significantly increased concentrations of antiatherogenic particles (ie, HDL-P). In agreement with previous studies,[25] we found that both BMI and waist circumference were positively correlated with proatherogenic alterations in the NMR-assessed lipoprotein subclass profile, with a positive correlation with VLDL-P-related variables and LDL-P, especially smaller particles. Interestingly, nearly 50% of normoglycemic participants with normal conven-

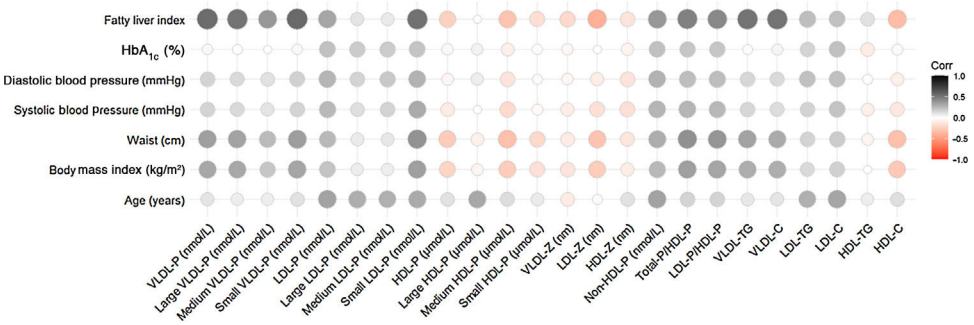

Figure 1. Correlation between NMR-assessed advanced lipoprotein profile and clinical parameters in the normoglycemic control group. HbA_{1c}: glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Table 5
Comparison between lipoprotein subclass sizes between normoglycemic, prediabetic and T2DM group

Variable	NG	Prediabetic	T2DM	*P**	
	(n = 463)	(n = 250)	(n = 216)	NG vs prediabetic	NG vs T2DM
VLDL particles, nmol/L	34.6 [24.8-50.0]	40.8 [28.1-65.5]	55.8 [35.4-85.7]	1.000	.073
Large, nmol/L	0.90 [0.70-1.18]	1.03 [0.80-1.41]	1.35 [0.98-1.89]	1.000	.040
Medium, nmol/L	3.62 [2.50-5.37]	4.24 [2.83-6.35]	4.80 [3.30-7.93]	1.000	1.000
Small, nmol/L	29.7 [21.7-44.1]	35.3 [24.6-57.0]	49.5 [30.6-77.4]	1.000	.042
Cholesterol content, mg/dL	8.59 [4.66-13.8]	10.1 [5.56-17.0]	14.1 [8.63-25.1]	1.000	.083
Triglyceride content, mg/dL	51.1 [36.4-71.4]	57.7 [42.4-93.4]	76.6 [51.0-122]	1.000	.179
VLDL-C/VLDL-TG	0.17 [0.12-0.20]	0.17 [0.12-0.20]	0.18 [0.15-0.21]	1.000	1.000
VLDL-Z, nm	42.1 [42.0-42.3]	42.1 [42.0-42.3]	42.0 [41.9-42.2]	1.000	1.000
LDL particles, nmol/L	1302 [1128-1498]	1374 [1213-1548]	1395 [1245-1561]	1.000	1.000
Large, nmol/L	188 [163-214]	195 [169-217]	186 [165-215]	1.000	1.000
Medium, nmol/L	415 [320-513]	430 [345-518]	422 [339-512]	1.000	1.000
Small, nmol/L	692 [598-786]	731 [648-838]	785 [694-871]	1.000	1.000
Cholesterol content, mg/dL	128 [111-149]	136 [118-152]	133 [118-153]	1.000	1.000
Triglyceride content, mg/dL	16.0 [12.6-19.5]	16.8 [13.9-19.4]	17.7 [15.1-20.9]	1.000	1.000
LDL-Z, nm	21.1 [20.9-21.2]	21.0 [20.8-21.2]	20.9 [20.7-21.1]	1.000	.018
HDL particles, μmol/L	28.7 [25.2-33.1]	28.2 [25.3-31.2]	26.5 [23.4-30.5]	.967	.017
Large, μmol/L	0.27 [0.23-0.31]	0.27 [0.24-0.30]	0.27 [0.24-0.30]	1.000	.999
Medium, μmol/L	9.34 [7.98-11.0]	9.15 [8.10-10.3]	8.42 [7.43-9.52]	.814	.013
Small, μmol/L	19.1 [16.7-22.4]	19.0 [16.4-21.4]	17.7 [15.1-21.0]	1.000	.165
Cholesterol content, mg/dL	56.6 [48.4-66.7]	53.7 [48.4-61.0]	49.1 [43.5-57.9]	.913	.002
HDL-C/HDL-TG	4.49 [3.69-5.52]	4.33 [3.46-5.31]	3.68 [3.05-4.74]	1.000	.863
Triglyceride content, mg/dL	12.6 [10.3-15.4]	12.7 [10.8-15.2]	13.5 [11.1-15.7]	1.000	1.000
HDL-Z, nm	8.23 [8.19-8.27]	8.23 [8.19-8.27]	8.22 [8.18-8.26]	1.000	1.000

HDL, high-density lipoprotein; LDL, low-density lipoprotein; NG, normoglycemic; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoprotein.
Results are expressed as median [25th-75th].
* *P* value resulted in the multivariate analysis after adjusting by age, sex, and body mass index.

tional lipid parameters and without previous CVD showed abnormal lipoprotein subclass concentrations, mostly attributed to the LDL subclasses. These results are similar to those reported in a recent study.[21] Notably, a similar phenotype has been associated with an increased CVD risk.[26]

An atherogenic lipoprotein profile is frequently observed in participants with abdominal obesity[25] and in NAFLD.[12] Consistently, in the present study both BMI and waist circumference as well as FLI were positively associated with proatherogenic lipoproteins, particularly VLDL-P and LDL-P-related characteristics, especially the smaller particles, and were negatively associated with the antiatherogenic HDL-P.

In the present study, the results obtained in normoglycemic participants were compared with those of participants with impaired glucose metabolism. In T2DM, increased hepatic secretion of large TG-rich VLDL and impaired clearance of VLDL appears to be of central importance in the pathophysiology of atherogenic dyslipidemia.[27] Indeed, it has been described that lipoprotein subclass alterations can be attributed primarily to the underlying insulin-resistance.[28] Our results are in general consistent with those of prior studies,[29] as we also identified that in T2DM participants the serum concentrations of HDL-P, particularly the medium HDL subclass, was reduced compared with the control group, while VLDL-P, both large and small subclasses, were increased. The reductions in smaller and medium HDL could be, at least in part, explained by an improper biogenesis and initial enlargement of diabetic HDL-P. On the other hand, as previously reported in other studies,[30] in the present study, there is a trend toward a higher content of cholesterol in VLDL-P in participants with T2DM compared with normoglycemic participants. This is concordant with the increase in the activity of cholesteryl ester transfer protein reported in participants with T2DM.[31] We found no differences in HDL-TG concentrations or the HDL-C/HDL-TG ratio between T2DM and normoglycemic participants. However, it should be pointed out that in T2DM, there was a lower HDL-C/HDL-TG ratio than in normoglycemic participants after adjustment for age, sex, and BMI ($P < .001$). These differences disappeared after the multi testing adjustment. It is probable that with a larger number of participants, the differences in the content of HDL-TG and HDL-C/HDL-TG became more evident. Because we found no differences in the lipoprotein profile of prediabetic individuals compared with normoglycemic individuals, this could be attributed to the lower degree of insulin-resistance observed in the prediabetic participants in our study, which is reflected by their low HOMA-IR index values. Finally, this study had a clinically relevant proportion of participants with LDL-C concentrations that would justify statin therapy. However, some of these participants are not treated with statins because study participants belong to different cross-sectional cohorts from different levels of care and have already been shown not to be optimally managed in daily-clinical practice.[32]

Limitations

Our findings should be interpreted within the context of some potential limitations. First of all, we could not distinguish participants with an impaired fasting glucose from those with impaired glucose tolerance (IGT) among the prediabetic group since study participants did not undergo an oral glucose tolerance test. This may be important because distinct lipoprotein and apolipoprotein changes in individuals with impaired fasting

plasma glucose and IGT have been reported, with the latter being associated with lipoprotein changes similar to those previously described in insulin-resistance.[33] The authors hypothesized that these differences could be the result of distinct pathophysiologic mechanisms in these distinctive states of glucose tolerance, which may be related to the site of insulin-resistance (skeletal muscle or hepatic). Another limitation is that all T2DM participants were recruited mainly from the outpatient clinic, and there may have been a selection bias toward including healthier patients with fewer complications than in the general T2DM population.

CONCLUSIONS

Our results show a better antiatherogenic lipoprotein profile in women than in men. BMI and FLI are important factors positively associated with a proatherogenic profile in normoglycemic participants. T2DM participants showed lower concentrations of total HDL-P and medium HDL-P and increased VLDL-P concentrations, mainly due to the smaller subclass, compared with the normoglycemic group. Changes in these lipoprotein subclasses highlight the potential importance of this subtype of lipoproteins, which are associated with CVD risk and are not currently a target of therapeutic intervention or a tool for CVD risk prediction. These issues highlight the need for new tools, such as lipoprotein profile assessed by NMR, to better characterize the CVD risk. This approach offers a more detailed approach to profound lipid abnormalities. It may therefore help to better characterize CVD risk profile, especially in populations at high risk of CVD, such as those with T2DM and visceral obesity in which LDL-C appears unreliable to guide therapy for atherosclerosis prevention completely.

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AUTHORS' CONTRIBUTIONS

D. Mauricio and N. Alonso conceived, designed and supervised the study. M. Falguera, M. Hernández, M. Barranco-Altirriba, and B. Soldevila participated in patient and material collection. C. Puig-Jové, A. Teis, J. Julve, and N. Alonso interpreted the results. C. Puig-Jové, and E. Castelblanco drafted the manuscript and contributed equally to this study. D. Mauricio, N. Alonso, N. Amigó, J. Franch-Nadal, and E. Ortega revised and edited the manuscript. All the authors revised the manuscript and gave their final approval of the submitted version.

CONFLICTS OF INTEREST

N. Amigó is a stockholder of and serves on the board of directors of Biosfer Teslab, a diagnostic laboratory company that performed the lipoprotein analyses described herein. The remaining authors declare they have nothing to disclose regarding conflict of interest with respect to this manuscript.

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WHAT IS KNOWN ABOUT THE TOPIC?

- Several types of lipoproteins apart from LDL-C are causally related to CVD.
- Conventional LDL-C levels underestimate the total concentration of cholesterol carried by LDL and, more importantly, the total concentration of atherogenic lipoproteins, thus underestimating the risk of atherosclerotic CVD.
- Hyperglycemia and insulin-resistance are the cause of proatherogenic changes in lipoprotein profile.

WHAT DOES THIS STUDY ADD?

- Participants with T2DM show altered lipoprotein particles other than LDL.
- Nearly 50% of participants with normal LDL-C and HDL-C values show an altered lipoprotein profile, mainly LDL particles.
- Women have a better overall advanced lipoprotein profile than men.
- Waist circumference and FLI are positively associated with a proatherogenic lipoprotein profile.

APPENDIX. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.rec.2021.02.006

REFERENCES

1. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2018;392:1736–1788.
2. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J.* 2017;38:2459–2472.
3. Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. *Lancet.* 2015;385:1397–1405.
4. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet.* 2005;366:1267–1278.
5. Lawler PR, Akinkuolie AO, Chu AY, Shah SH, Kraus WE, et al. Atherogenic Lipoprotein Determinants of Cardiovascular Disease and Residual Risk Among Individuals With Low Low-Density Lipoprotein Cholesterol. *J Am Heart Assoc.* 2017;6:e005549.
6. Martin SS, Khokhar AA, May HT, et al. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. *Eur Heart J.* 2015;36:22–30.
7. Nordestgaard BG. Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. *Circ Res.* 2016;118:547–563.
8. Duran EK, Aday AW, Cook NR, et al. Triglyceride-Rich Lipoprotein Cholesterol, Small Dense LDL Cholesterol, and Incident Cardiovascular Disease. *J Am Coll Cardiol.* 2020;75:2122–2135.
9. Hegele RA, Ginsberg HN, Chapman MJ, et al. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol.* 2014;2:655–666.
10. Langlois MR, Chapman MJ, Cobbaert C, et al. Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and Very Low

Concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM. *Clin Chem.* 2018;64:1006–1033.
11. Valensi P, Avignon A, Sultan A, Chanu B, Nguyen MT, Cosson E. Atherogenic dyslipidemia and risk of silent coronary artery disease in asymptomatic patients with type 2 diabetes: a cross-sectional study. *Cardiovasc Diabetol.* 2016;15:104.
12. Bril F, Sninsky JJ, Baca AM, et al. Hepatic Steatosis and Insulin Resistance, But Not Steatohepatitis, Promote Atherogenic Dyslipidemia in NAFLD. *Clin Endocrinol Metab.* 2016;101:644–652.
13. Carbonell M, Castelblanco E, Valldeperas X, et al. Diabetic retinopathy is associated with the presence and burden of subclinical carotid atherosclerosis in type 1 diabetes. *Cardiovasc Diabetol.* 2018;17:66.
14. Vilanova MB, Falguera M, Marsal JR, et al. Prevalence, clinical features and risk assessment of pre-diabetes in Spain: the prospective Mollerussa cohort study. *BMJ Open.* 2017;7:e015158.
15. Amor AJ, Catalan M, Pérez A, et al. Nuclear Magnetic Resonance Lipoprotein Abnormalities in Newly-Diagnosed Type 2 Diabetes and Their Association With Preclinical Carotid Atherosclerosis. *Atherosclerosis.* 2016;247:161–169.
16. Alonso N, Traveset A, Rubinat E, et al. Type 2 diabetes-associated carotid plaque burden is increased in patients with retinopathy compared to those without retinopathy. *Cardiovasc Diabetol.* 2015;14:33.
17. ADA. American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes-2016. *Diabetes Care.* 2016;39:S13-S22.
18. Levey AS, Bosch JP, Lewis JB, et al.A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999;130:461–470.
19. Mallol R, Amigó N, Rodríguez JM, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered1H NMR spectroscopy. *J Lipid Res.* 2015;56:737–746.
20. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. *Circulation.* 2014;129:553–561.
21. Analysis of LDL and HDL size and number by nuclear magnetic resonance in a healthy working population: The LipoLab Study. Int J Clin Pract. 2021;75:e13610.
22. Freedman DS, Otvos JD, Jeyarajah EJ. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem.* 2004;7:1189–1200.
23. Wahl PE, Warnick GR, Albers JJ, et al. Distribution of lipoproteins triglyceride and lipoprotein cholesterol in an adult population by age, sex, and hormone use- The Pacific Northwest Bell Telephone Company health survey. *Atherosclerosis.* 1981;39:111–124.
24. El Khoudary SR, Chen X, Nasr A, et al. HDL (High-Density Lipoprotein) Subclasses. *Lipid Content and Function Trajectories Across the Menopause Transition: SWAN-HDL Study Arterioscler Thromb Vasc Biol.* 2020. http://doi.org/10.1161/ATVBAHA.120.315355.
25. Guardiola M, Solà R, Vallvé JC, et al. Body mass index correlates with atherogenic lipoprotein profile even in nonobese, normoglycemic, and normolipidemic healthy men. *J Clin Lipidol.* 2015;9:824–831.
26. Mora S, Otvos J, Rifai N, et al. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation.* 2009;119:931–939.
27. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation.* 2002;106:1930–1937.
28. Goff DC, D'Agostino RB, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. *Metabolism.* 2005;54:264–270.
29. Wang J, Stančáková A, Soininen P, et al. Lipoprotein subclass profiles in individuals with varying degrees of glucose tolerance: a population-based study of 9399 Finnish men. *J Intern Med.* 2012;272:562–572.
30. Fielding CJ, Reaven GM, Liu G, Fielding PE. Increased free cholesterol in plasma low and very low density lipoproteins in non-insulin-dependent diabetes mellitus: its role in the inhibition of cholesteryl ester transfer. *Proc Natl Acad Sci USA.* 1984;81:2512–2516.
31. Kahri J, Syvänne M, Taskinen MR. Plasma cholesteryl ester transfer protein activity in non-insulin-dependent diabetic patients with and without coronary artery disease. *Metabolism.* 1994;43:498–502.
32. Vinagre I, Mata M, Hermosilla E, et al. Control of glycemia and cardiovascular risk factors in patients with type 2 diabetes in primary care in Catalonia (Spain). *Diabetes Care.* 2012;35:774–779.
33. Lorenzo C, Hatnett S, Hanley AJ, et al. Impaired fasting glucose and impaired glucose tolerance have distinct lipoprotein and apolipoprotein changes: The Insulin Resistance Atherosclerosis Study. *J Clin Endocrionol Metab.* 2013;98:1622–1630.