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Obesity alters VLDL profile and remnant cholesterol differently in metabolically healthy men and women: a ^{1}H-NMR study

Victoria Santisteban[1,2], Anallely López-Yerena[1,3,4], Natàlia Muñoz-Garcia[1], Gemma Vilahur[1,5], Lina Badimon[1,5,6†] and Teresa Padro[1,5*†]

Abstract

Background and aims Lipoprotein particle (p) size and count beyond lipoprotein lipid content [triglycerides (tg) and cholesterol (c)] are critical for their atherogenicity. This study characterized lipoprotein profiles in metabolically healthy individuals with overweight or obesity and assessed the impact of sex, obesity, and lipid background.

Methods Proton-nuclear magnetic resonance (^{1}H-NMR) was used to assess the composition of very low-, low-, intermediate-, and high-density lipoproteins (VLDL, LDL, IDL, HDL), and particle number and size of VLDL, LDL, and HDL in 101 healthy subjects with overweight and obesity.

Results Men showed significantly higher VLDLc and VLDLtg levels, counts of VLDLp (all subfractions) and LDLp (total and small), and smaller LDLp size, compared to women. Men had lower HDLc and HDLp (total and medium). In Obesity (Ob) compared to overweight (Ov), VLDLp number, VLDLtg and remnant cholesterol (RC) levels were significantly increased [Fold changes (FC) Ob.vs.Ov: 1.45, 1.39, and 1.26, respectively]. When stratified by sex, obesity-related VLDL and IDL profile deterioration was evident only in women. Individuals with LDLc ≥ 130 mg/dL showed increased RC compared to those with LDLc < 130 mg/dL (FC:1.26). The median 10-year cardiovascular disease (CVD) risk REGICOR was low (2%), but higher in men and in obesity. Individuals with higher CVD risk showed increased VLDLc, VLDLtg, VLDLp, and RC levels.

Conclusion Men had a higher 10-year CVD risk and a less favorable triglyceride-rich lipoprotein and RC profile, while obesity aggravated these patterns, particularly in women. These findings support considering high-risk lipoprotein patterns in targeted CVD prevention for overweight and obese populations.

Keywords Lipoproteins, Sex, Obesity, Remnant-cholesterol, Nuclear magnetic resonance, Cardiovascular diseases

†Lina Badimon and Teresa Padro contributed equally.

*Correspondence:
Teresa Padro
tpadro@santpau.cat
[1]Institut Recerca Sant Pau, Carrer de Sant Quintí, 77, Barcelona 08041, Spain
[2]School of Pharmacy and Food Sciences, University of Barcelona (UB), Barcelona 08036, Spain
[3]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[4]Department of Internal Medicine, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain
[5]Centro de Investigación Biomédica en Red Cardiovascular (CIBER-CV), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[6]Universitat de VIC-UCC and Cardiovascular Research Foundation for Health Prevention and Innovation (FICSI), Barcelona, Spain

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Introduction

Lipoproteins are essential molecules that carry lipids like cholesterol and triglycerides (TG) through the bloodstream to tissues based on their metabolic needs. They play a vital role in lipid balance and cardiovascular health, with significant clinical relevance. Classified by size, lipid content, and apolipoproteins, they include chylomicrons, very low-, intermediate-, low-, and high-density lipoproteins (VLDL, IDL, LDL, HDL), each serving specific functions. The lipoproteins may undergo alterations that affect their structure, functionality, composition, and plasma concentrations and favor their accumulation in the arterial wall triggering pro-atherogenic processes [1–3].

While LDL cholesterol (LDLc) is the primary target of lipid-lowering therapies to reduce cardiovascular disease (CVD) risk [4]. Growing evidence suggests that additional variables, such as number and size of lipoprotein particles, may provide valuable information and perform better than LDLc in predicting CVD risk [1, 5]. This is the case of small and dense LDL particles (LDLp), which are strongly associated with atherosclerotic CVD (ASCVD) [3], and when present in high concentrations increase CVD risk even when LDLc is considered optimal [3]. Indeed, a significant proportion of cardiovascular events occur in subjects whose LDLc levels are well controlled, according to guidelines [6, 7].

The residual CVD risk has been related to alterations in the lipid metabolism, particularly in the triglyceride-rich lipoproteins (TRL) (VLDL, IDL) and the cholesterol they contain, known as remnant cholesterol (RC), which represents a major contributor [8, 9].

In the clinic, remnant cholesterol, has been suggested as the causal factor of the TRL-associated risk for ischemic heart disease, rather than TGs themselves [10].

HDLc is considered protective for CVD risk [11]. However, HDL particles are heterogeneous in size and composition [1] and their contribution to atherosclerosis and CVD risk is directly related to their structural characteristics, molecular composition and functional properties [11, 12].

In this respect, the Women's Health Study (WHS), with 27,909 women, demostrated that only large HDL particles (HDLp), assessed using nuclear magnetic resonance (NMR), were associated with lower CVD risk [1]. Similarly, a greater number of large and medium size HDLp, along with lower concentration of small HDLp, has been associated to a better cardiometabolic risk profile [13, 14]. Furthermore, a prospective study conducted on a large cohort of healthy women found that an association between reduced cardiovascular risk and adherence to Mediterranean diet through the assessment of HDL and VLDL measures [15] Smaller VLDL are more strongly associated with ASCVD due to their propensity to be retained in the endothelium [16] (An exception is the large triglyceride-rich, buoyant VLDL, which are precursors of remnant particles with high atherogenic capacity [17, 18].

The particle (p) number, size and, composition [triglycerides (tg) and cholesterol (c)] of lipoproteins are relevant determinants of their atherogenic potential. NMR has emerged as an advanced tool to accurately assess these variables, providing deeper insights into their contribution to vascular risk [5].

Several factors are known to influence the lipoprotein profile, including sex [19–21], body mass index (BMI) [19, 20], age [19] and diet [21]. Differences in lipoprotein subclasses have been observed between type 2 diabetes mellitus (T2DM) and normoglycemic individuals [19, 22], and between subjects with obesity and normoweight [23]. However, little is known about the differences that exist within healthy individuals. In a large cohort of apparently healthy men and women, LDLp were closely associated with the occurrence of future coronary artery disease (CAD), however, the VLDL and HDL profile was not evaluated in this study [24]. To address the existing gap, the current study aims to characterize the lipid content, size, and number of lipoproteins using proton nuclear magnetic resonance (^{1}H-NMR) in a cohort of metabolically healthy subjects with overweight and obesity, who had no known CVD risk factors and were not under regular pharmacological treatment. Additionally, the study aimed to evaluate the interaction between the lipoprotein subclass patterns and variables such as sex, BMI, background LDLc, and calculated CVD risk.

or obesity, without known cardiovascular risk factors or regular pharmacological treatment.

Materials and methods

Subjects and study design

The study population comprises 101 apparently metabolically healthy men and women with overweight or obesity [BMI 25–37 kg/m^2] and age aged from 25 to 60 years. None of the subjects in the study population were under pharmacological treatment for any chronic condition, including lipid-lowering or other therapeutic agents. Individuals were excluded if they reported eating disorders, cardiovascular risk factors, a history of cardiovascular disease or cancer among other. Pregnancy was also a reason for exclusion. Additional exclusion criteria included consuming more than 60 g/day of alcohol, or being on a hypocaloric diet or weight loss program within 2 months prior to enrollment. Of 101 subjects, 14 (13.86%) were regular smokers (men: 16% and women: 12%).

Participants underwent a comprehensive physical examination by a physician at the time of biological sample collection to confirm their health status. All

included participants were classified as metabolically healthy according to the Adult treatment panel III (ATP-III) guidelines [25], which define metabolic health as the presence of fewer than three of the following criteria for metabolic syndrome: abdominal obesity (waist circumference > 102 cm in men or > 88 cm in women), elevated triglycerides (≥ 150 mg/dL), low HDL cholesterol (< 40 mg/dL in men or < 50 mg/dL in women), high blood pressure (≥ 130/85 mm Hg), and elevated fasting glucose (≥ 100 mg/dL). In this study, participants with overweight or obesity were considered metabolically healthy since they did not meet the threshold for metabolic syndrome. As shown in Table S1, clinical and biochemical parameters for the study population were within normal physiological ranges. These included a median systolic blood pressure of 123.5 mmHg (IQR: 114–129) and a median diastolic pressure of 70 mmHg (IQR: 62–75), a median fasting glucose level of 4.7 mmol/L (IQR: 4.5–5.0) and a median fasting triglycerides 94.8 mg/dL (IQR: 62.8–134.5).

Overweight and obesity were defined according to the World Health Organization (WHO) criteria [26], with overweight classified as a body mass index (BMI) between 25.0 and 29.9 kg/m², and obesity as a BMI of 30.0 kg/m² or higher [27]. For comparisons, participants were grouped by sex (51 women and 50 men) and by BMI category, distinguishing individuals with overweight ($N = 55$) and those with obesity ($N = 46$), based on the BMI cut-off of 30.0 kg/m². Anthropometric, hemodinamic and biochemical data, including those of liver and kidney function markers are provided in Table S1.

This study utilized retrospective baseline samples from participants enrolled in previous nutritional trials conducted between 2015 and 2021 at the Research Institute Sant Pau (IR-HSCSP), Barcelona, Spain. All participants had provided written informed consent, explicitly allowing the retrospective use of their samples for future cardiovascular research. The study protocols, including the retrospective use of these samples, were reviewed and approved by the Human Ethics Review Committee of the Hospital de la Santa Creu i Sant Pau in Barcelona [28–31].

Biological samples

Twelve-hour fasting blood samples were collected between 8:00 and 11:00 am in tubes without anticoagulant for serum preparation. After collection, samples were allowed to coagulate for 30 min at 37 °C, followed by a further 30 min at 4 °C. Serum was then recovered by centrifugation at 1816 × *g* for 30 min. Serum samples were aliquoted, immediately frozen, and stored at –80 °C without thawing until analysis.

Anthropometric data, blood pressure, biochemical measurements and serum lipid profile

Anthropometric measurements (height, weight, waist, and blood pressure) were taken by trained personnel at the time of the biological sample collection. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Serum biochemical measurements were performed at the centralized laboratory for analysis of the Hospital de la Santa Creu I Sant Pau (Barcelona, Spain) using routine commercially available assays for glucose, hepatic and renal markers, standard serum levels of triglycerides (TG), total cholesterol (TC), and HDLc (Roche Diagnostics, Basel, Switzerland) to characterize the metabolically healthy population used in the study, as previously reported.

^{1}H-NMR for lipoprotein characterization

Lipoprotein (VLDL, LDL, HDL) pattern for particle size distribution, quantity and diameter and lipid composition (TG, cholesterol) of VLDL, IDL, LDL and HDL was determined in serum samples by high-resolution ^{1}H-NMR spectroscopy and the Liposcale® test in vitro diagnostic medical device with Conformité Européenne marking (IVD-CE) [22]. Analysis was performed with a BrukerAvance III 600 Nuclear Magnetic Resonance (NMR) spectrometer (Bruker Biospin, Rheinstetten, Germany), set at proton frequency of 600.20 MHz (14.1 T) and at 310 K. The Liposcale® test (IVD-CE) utilizes 2-dimension (2D) diffusion-ordered ^{1}H NMR spectroscopy to directly quantify lipoprotein subclasses by measuring particle diffusion coefficients. This method decomposes NMR signals into nine distinct Lorentzian functions (F1–F9) corresponding to large, medium, and small subclasses of VLDL, LDL, and HDL [22]. Briefly, the methyl signal from a longitudinal eddy-current delay (LED) pulse spectra was surface-fitted with 9 Lorentzian functions associated with each lipoprotein subtype: large, medium, and small VLDL, LDL, and HDL. The area of each Lorentzian function was related to the lipid concentration of TG and cholesterol in lipoprotein subclasses that were estimated using partial least squares (PLS) regression models based on the total NMR signal intensity. The regression models have been previously calibrated with reference samples purified by ultracentrifugation and enzymatically analyzed.

Lipid concentration units were converted to lipid volume units using common conversion factors. The size of VLDL, LDL and HDL particles were calculated from its diffusion coefficient using the Stokes-Einstein equation. Specifically, the approximate particle size ranges defined for each subclass are as follows: VLDL (Small: 38.6–45.0 nm, Medium: 45.0–60.0 nm, Large: 60.0–81.9 nm); LDL (Small: 18.9–20.5 nm, Medium: 20.5–23.0 nm,

Large: 23.0–26.5 nm); HDL (Small: 7.8–8.2 nm, Medium: 8.2–9.4 nm, Large: 9.4–11.5 nm) [22].

The particle number of each of the 9 lipoprotein subtypes was calculated by dividing the lipid volume by the mean volume of each lipoprotein subclass. Coefficients of variation for the particle sizes were less than 0.3% and for particle numbers between 2% and 4%.

Particle number and size for IDL are not reported by this method due to overlap in diffusion coefficients with small VLDL and large LDL particles, which limits resolution [22].

Cardiovascular risk assessment

Cardiovascular risk was estimated using the REGICOR (Registre Gironí del Cor) score, a validated adaptation of the Framingham equation to adjust it to the epidemiological and risk characteristics of the Spanish population [32]. The variables included in the REGICOR equation age, sex, total cholesterol, HDLc, systolic blood pressure, smoking status, and the presence of diabetes mellitus [33].

Statistical analysis

Statistical analyses were performed using STATA 17.0 (College Station, TX, USA) and StatView 5.0.1 software (SAS Institute, Cary, NC, USA). Normality of the data distribution was assessed using the Shapiro-Wilk test. Statistical differences between comparison groups were calculated using the Wilcoxon-Mann-Whitney test. Chi-square tests were used for categorical variable distribution. Correlations between continuous variables were assessed by the Spearman coefficient. The data are expressed as the median and the interquartile range [IQR]. Statistical significance was assumed when *P*-values were < *0.05*.

Table 1. Lipoprotein lipid composition assessed by ^{1}H-NMR

(mg/dL)	Women	Men	P-Value
VLDLc	12.5 [7.3–17.7]	16.7 [11.4–24.4]	***0.002***
VLDLtg	47.9 [34.9–75.1]	72.6 [51.1–101.0]	***0.000***
IDLc	8.5 [6.4–13.3]	10.2 [7.6–11.7]	0.352
IDLtg	9.3 [7.8–12.5]	10.4 [9.0-11.4]	0.443
LDLc	124.3 [116.2-149.8]	135.4 [120.3-152.1]	0.207
LDLtg	13.0 [11.1–16.4]	13.9 [11.6–16.1]	0.532
HDLc	58.9 [54.9–65.1]	54.0 [49.6–57.8]	***0.000***
HDLtg	14.7 [11.2–16.6]	13.5 [10.9–16.0]	0.254
(mg/dL)	Overweight	Obese	***P-Value***
VLDLc	12.7 [8.0–20.0]	16.0 [11.8–22.3]	0.052
VLDLtg	50.7 [37.0-88.6]	70.3 [50.1–94.2]	***0.024***
IDLc	8.7 [6.4–11.3]	10.7 [7.1–12.8]	0.080
IDLtg	9.7 [7.6–11.4]	10.4 [8.7–12.2]	0.052
LDLc	133.8 [116.2-147.2]	132.3 [119.5-154.6]	0.357
LDLtg	13.4 [11.3–16.3]	13.9 [11.4–17.3]	0.198
HDLc	56.4 [51.6–64.4]	55.9 [53.2–60.9]	0.623
HDLtg	13.5 [10.7–16.1]	14.9 [12.1–16.5]	0.193

Values are shown as median [IQR]. Lipoprotein cholesterol and triglycerides are expressed as mg/dL. P-Value: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance (P < 0.05). ^{1}H-NMR: Proton nuclear magnetic resonance; Very low-, low-, intermediate- and high- density lipoprotein (VLDL, LDL, IDL and HDL, respectively). c: Cholesterol; tg: Triglycerides

Results

Study population

Both groups had similar age and sex distribution (P > 0.050). Individuals with obesity had higher serum concentrations of TC, TG and TG/HDLc compared to individuals with overweight (P = 0.029, P = 0.029 and P = 0.024, respectively). No differences were found in blood pressure, glucose levels (Table S1), or in smoking habits (overweight: 42.86% vs. obesity 57.14%, P > 0.050).

Cholesterol and triglyceride content in lipoproteins: studies by ^{1}H-NMR

The triglyceride and cholesterol content of lipoproteins, assessed by ^{1}H-NMR in the healthy study group is shown in Table S2**.** When discriminated by sex (Table 1.), men had significantly higher levels of VLDL cholesterol (VLDLc) and VLDL triglyceride (VLDLtg) than women with a similar BMI (men vs. women: 29.9 [28.3–31.6] vs. 29.4 [27.4–31.6] kg/m^2, P = 0.525) (Table S1). HDLc levels were > 50 mg/dL in both sexes, although levels in women exceeded those in men (P < 0.001). No differences were found between the sexes in HDLtg, LDLc and LDLtg, or in the IDL fraction (IDLc, IDLtg) (Table 1.).

When the study population was analyzed according to the BMI (overweight vs. obesity, Table 1.), subjects with obesity had significantly higher serum VLDLtg levels than subjects with overweight (*P* = 0.024). A trend towards higher levels of VLDLc was also found in obesity (*P* = 0.052). Statistically significant differences between obesity and overweight were observed in women but no in men (Fig. 1). Similarly, IDLtg and IDLc tended to be higher in obesity although differences did not achieve statistical significance (*P* = 0.052 and P = 0.080, respectively) (Table 1.).

Triglyceride levels in LDL and HDL did not differ between healthy subjects with overweight and obesity, nor did cholesterol levels in the 4 types of lipoproteins (VLDL, IDL, LDL, HDL) (Table 1.).

Serum remnant cholesterol levels: differences by sex, BMI and LDLc background

Median level of remnant cholesterol (RC), cholesterol contained in triglyceride-rich lipoproteins (calculated as the sum of cholesterol carried in VLDL and IDL, as obtained by ^{1}H-NMR), in the healthy study population was 24.7 [16.4–32.2] mg/dL, with values significantly higher in men than in women (Men: 27.0 [21.1–35.0] mg/dL, women: 21.4 [14.8–29.7] mg/dL; *P* = 0.001), matched by age and BMI. Moreover, plasma levels of RC were

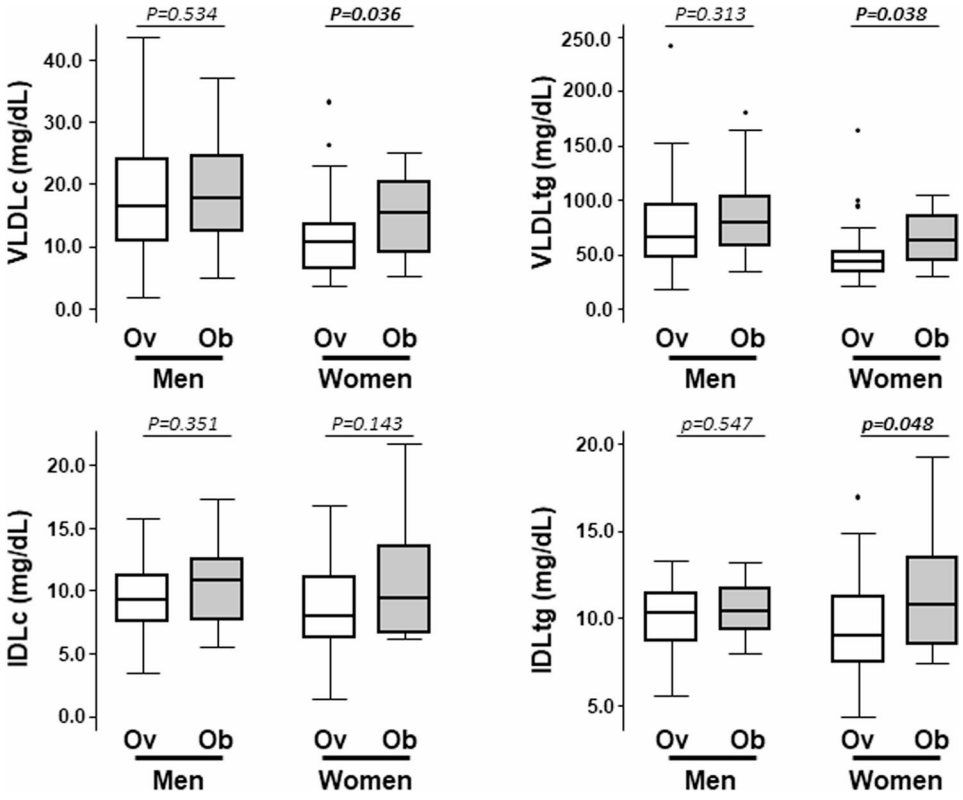


Fig. 1 Triglyceride-rich Lipoproteins in relation to sex and obesity. Box plot of remnant cholesterol assessed by ^{1}H-NMR levels in the total cohort ($N = 101$) and according sex and overweight/obesity. *P-Value*: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance ($P < 0.05$). Ov: with Overweight; Ob: with Obesity; ^{1}H-NMR: Proton nuclear magnetic resonance

significantly higher in the group of people with obesity than in the group of people with overweight (27.4 [19.5–35.0] mg/dL vs. 21.7 [15.3–30.3] mg/dL, respectively; $P = 0.031$). When groups were stratified by sex, remnant cholesterol levels were 1.5fold higher in women with obesity than with overweight *P* but no differences were observed between men with obesity and with overweight (Fig. 1).

40% of the participants had background LDLc levels classified as borderline-high range (130–159 mg/dL) or in the high range (160–189 mg/dL), according the ATPIII guidelines [34], and were defined as High-LDLc group, in contrast to those subjects with background LDLc < 130 mg/dL, who were defined as the Low-LDLc group. The two subgroups, with mean serum LDLc levels of 143.9 [138.8–160.1] mg/dL and 101.6 [91.6–114.9] mg/dL, respectively, significantly differed in levels of remnant cholesterol (Table S3. $P = 0.041$), with the differences being associated to the female sex (Low- vs. High-LDLc background groups: RC-levels 18.4 [12.9–26.1] mg/dL vs. 28.7 [16.4–35.2] mg/dL; $P = 0.011$), whereas no differences were found in males with Low- and High-LDLc background (RC-levels: 26.9 [20.6–34.9] mg/dL vs. 27.0 [21.7–35.0] mg/dL). Among subjects with LDLc < 130 mg/dL, women had lower levels of remnant cholesterol than men *P*.

The subgroup with a ratio TG/HDLc above the median level of the study population (median ratio, 1.95 [1.17–3.02]) showed 2-fold higher RC levels (32.1 [26.8–38.5]) than the subgroup with TG/HDLc ratio below the median value (16.4 [13.9–21.7], $P < 0.001$ for differences between groups).

Levels of plasma RC were not correlated with age (Spearman correlation analysis, (rho = 0.052, $P = 0.604$) in the total healthy cohort, nor when men and women were analyzed separately (men: rho= −0.043, $P = 0.768$; women: rho = 0.076, $P = 0.594$).

Particle number and size of VLDL, LDL and HDL

Size and number of particles for each lipoprotein subfraction were measured by ^{1}H-NMR spectroscopy. Values for the total study population ($N = 101$) are given in Table S2. The diameter and particle number of VLDL, LDL, and HDL lipoproteins were analyzed based on sex and BMI. Table 2. shows the size and total number of VLDLp, LDLp, and HDLp, and Figs. 2 and 3 show the distribution of small, medium, and large subclasses for each lipoprotein type, also in relation to sex and BMI, respectively.

Table 2. Number particles and diameter of lipoproteins assed by ^{1}H-NMR according to sex and BMI

A	Women	Men	p-Value
VLDLp (nmol/L)	34.6 [26.0–53.0	51.9 [38.6–72.5]	***0.001***
VLDLø (nm)	42.1 [42.0–42.3]	42.2 [42.0–42.3]	0.423
LDLp (nmol/L)	1222.6 [1114.6–1466.2]	1368.8 [1212.2–1526.0]	***0.042***
LDLø (nm)	21.2 [21.0–21.3]	21.0 [20.8–21.1]	***0.000***
HDLp (µmol/L)	29.6 [27.4–32.7]	27.4 [25.2–29.5]	***0.001***
HDLø (nm)	8.3 [8.2–8.3]	8.3 [8.2–8.3]	0.557
B	Overweight	Obese	***p-Value***
VLDLp (nmol/L)	36.1 [27.3–61.6]	52.3 [36.6–67.0]	***0.028***
VLDLø (nm)	42.2 [42.0–42.3]	42.2 [42.0–42.3]	0.616
LDLp (nmol/L)	1305.3 [1126.1–1439.9]	1319.9 [1176.7–1536.5]	0.233
LDLø (nm)	21.0 [20.8–21.2]	21.0 [20.9–21.2]	0.900
HDLp (µmol/L)	28.8 [26.1–31.5]	28.3 [25.6–30.4]	0.790
HDLø (nm)	8.3 [8.2–8.3]	8.3 [8.2–8.3]	0.592

Values are shown as median [IQR]. P-Value: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance ($P < 0.05$). ^{1}H-NMR: Proton nuclear magnetic resonance; VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; p: Particles; ø: Diameter

Compared with men, women had lower numbers of total VLDLp *P* and LDLp (*P* along with higher concentrations of HDLp *P* and a larger median LDLp size *P* Table 2.A. As shown in Fig. 2, the lower number of VLDLp in women was observed across all lipoprotein particle size. In contrast, differences in LDLp and HDLp between women and men were size-dependent. Women had significantly fewer small LDLp particles than men *P*, whereas no differences were found for medium and large LDLp between sexes. For HDLp, women showed a trend toward higher numbers across all size subfractions; however, the difference was significant only for the medium-size subfraction *P*.

When participants were stratified by BMI, subjects with obesity had a significantly higher concentration of VLDLp (Table 2.B), which statistically significant differences observed in both the large and small subfractions (Fig.3) compared to subjects with overweight. A similar, though not significant, trend was found for the medium VLDLp subfraction. The statistically significant difference in VLDLp pattern between individuals with overweight and obesity was only observed in women when the analysis was performed separately by sex (Table3).

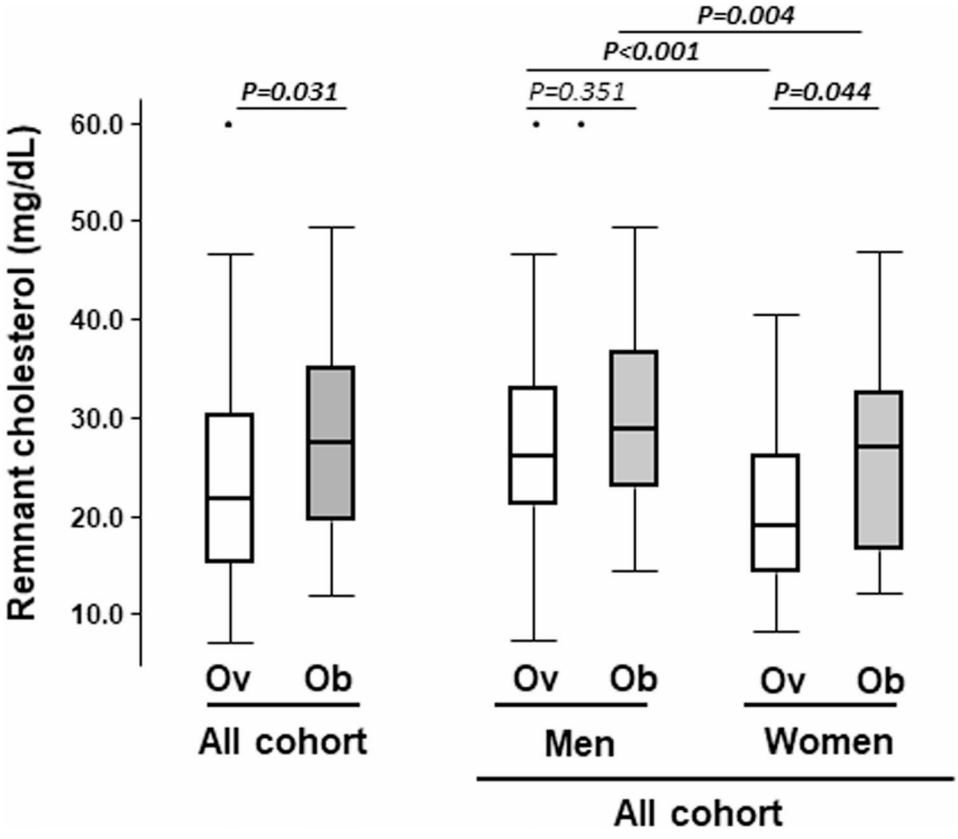


Fig. 2 Number of lipoprotein particles by sex. Violin plot representing differences of the lipoprotein subclasses between men and women. *P-Value*: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance *P*. Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein. p: Particles. Specifically, the approximate particle size ranges defined for each subclass are as follows: VLDL (Small: 38.6–45.0 nm, Medium: 45.0–60.0 nm, Large: 60.0–81.9 nm); LDL (Small: 18.9–20.5 nm, Medium: 20.5–23.0 nm, Large: 23.0–26.5 nm); HDL (Small: 7.8–8.2 nm, Medium: 8.2–9.4 nm, Large: 9.4–11.5 nm)

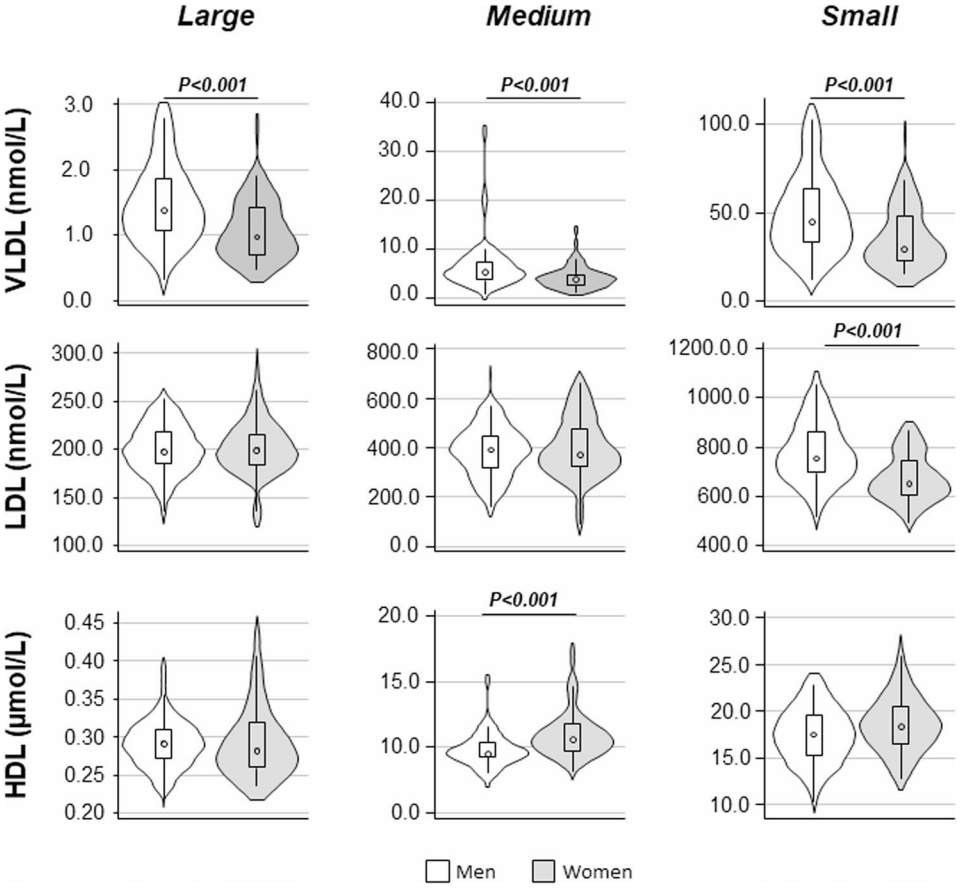


Fig. 3 Number of lipoprotein particles by body mass index. Violin plot representing differences of the lipoprotein subclasses between subjects with overweight and obesity. *P-Value*: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance *P*. Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; p: Particles. Specifically, the approximate particle size ranges defined for each subclass are as follows: VLDL (Small: 38.6–45.0 nm, Medium: 45.0–60.0 nm, Large: 60.0–81.9 nm); LDL (Small: 18.9–20.5 nm, Medium: 20.5–23.0 nm, Large: 23.0–26.5 nm); HDL (Small: 7.8–8.2 nm, Medium: 8.2–9.4 nm, Large: 9.4–11.5 nm)

Table 3 Comparison of VLDL profile between subjects with overweight and obesity in men and women groups

	Men. Ov (N = 26)	Men. Ob (N = 24)	P-Value	Women. Ov (N = 29)	Women. Ob (N = 22)	P-Value
VLDLc (mg/dL)	16.6 [11.1–24.2]	17.8 [12.6–24.5]	0.534	10.6 [6.6–13.5]	15.5 [9.1–20.3]	***0.036***
VLDLtg (mg/dL)	65.8 [47.7–96.5]	79.2 [57.9–103.9]	0.313	44.1 [34.0–52.6]	63.0 [44.7–84.9]	***0.038***
VLDLp (nmol/L)	47.1 [34.3–72.5]	57.0 [42.0–73.3]	0.382	33.0 [24.4–38.1]	47.3 [31.0–61.0]	***0.035***
Large	1.2 [1.0–1.9]	1.5 [1.1–1.8]	0.351	0.9 [0.7–1.1]	1.3 [0.9–1.5]	***0.023***
Medium	5.1 [3.7–7.1]	6.0 [3.8–8.6]	0.426	3.5 [2.2–4.6]	4.2 [3.3–5.5]	0.287
Small	40.7 [29.2–65.0]	50.4 [36.9–63.4]	0.404	27.7 [21.3–32.9]	42.4 [26.5–51.7]	***0.029***
VLDL ⌀ (nm)	42.2 [42.0–42.4]	42.2 [42.1–42.3]	0.756	42.2 [42.1–42.3]	42.1 [41.9–42.3]	0.258
LDLc (mg/dL)	134.1 [125.2–140.0]	143.9 [119.9–161.1]	0.252	123.7 [116.2–149.8]	127.5 [118.7–147.4]	0.747
LDLtg (mg/dL)	13.5 [11.4–15.2]	15.2 [12.1–16.7]	0.260	12.5 [10.8–16.3]	13.1 [11.2–17.7]	0.518
LDLp (nmol/L)	1326.0 [1228.7–1410.2]	1444.4 [1202.1–1579.0]	0.341	1222.6 [1097.1–1461.5]	1248.2 [1166.6–1517.8]	0.494
Large	197.2 [183.0–211.1]	202.1 [185.3–232.6]	0.294	206.5 [179.3–219.8]	194.8 [187.9–213.2]	0.924
Medium	390.2 [289.0–409.6]	419.6 [321.0–511.7]	0.174	372.1 [313.6–474.6]	378.0 [326.3–475.9]	0.849
Small	755.3 [688.1–842.7]	771.1 [711.0–872.8]	0.449	641.7 [617.8–705.9]	660.5 [606.5–772.3]	0.361
LDL ⌀ (nm)	21.0 [20.7–21.1]	21.0 [20.8–21.1]	0.691	21.1 [21.0–21.4]	21.2 [21.0–21.2]	0.690

Values are shown as median [IQR]. P-Value: Wilcoxon Mann Whitney-test. Boldface indicates statistical significance ($P < 0.05$). VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein; OV: with overweight; OB: with obesity C: Cholesterol; Tg: triglycerides; p: Particles. ⌀: diameter

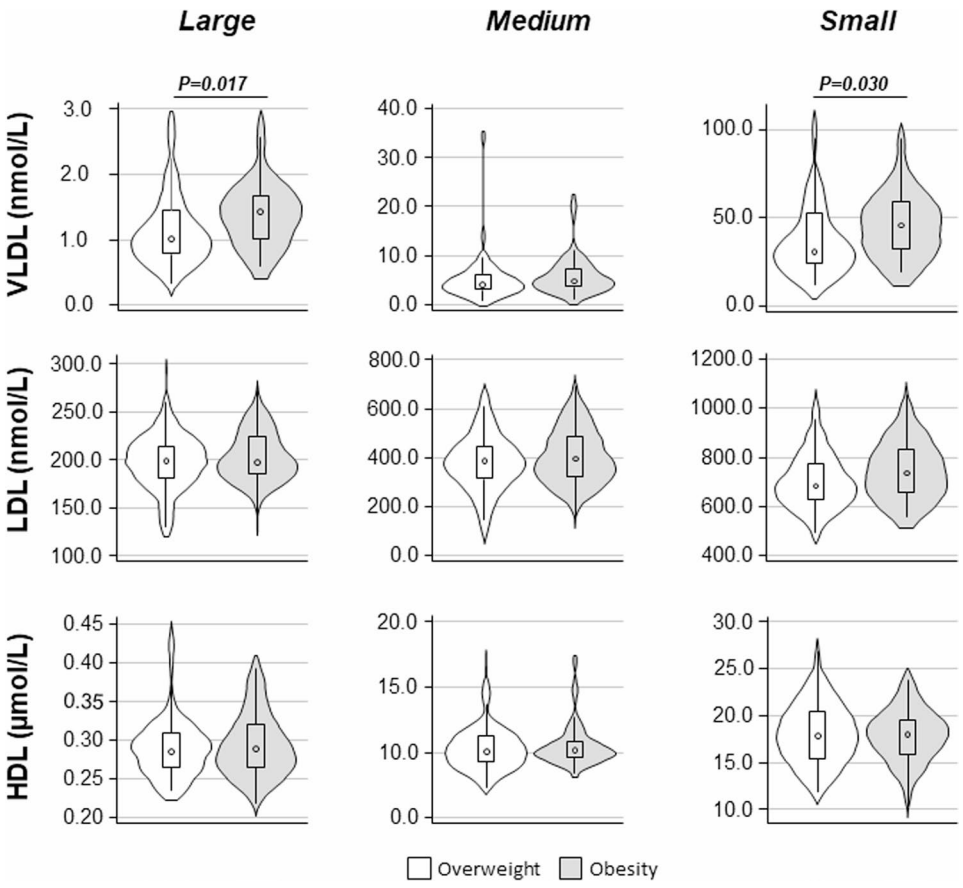


Fig. 4 Comparison of other atherogenic variables between men and women. *P-Value*: Wilcoxon Mann Whitney-test. Statistical significance: $P < 0.05$. Data between subjects with overweight and obesity are not presented because no statistically significant differences were observed for these variables

In contrast, the number and size of circulating LDLp and HDLp did not differ between groups with overweight and obesity when the total study population was considered (Table 2.B), nor when group comparisons were made across size subfractions (small, medium, large) of LDLp and HDLp.

Additional lipoprotein-related variables, including non-HDLp and the non-HDLp/HDLp ratio were calculated (Fig. 4). The non-HDLp/HDLp ratio was statistically higher in men compared to women ($P = 0.001$), whereas no significant sex-related differences were observed in levels of non-HDLp ($P = 0.052$). These variables did not differ between subjects with overweight and with obesity ($P > 0.050$, data not shown).

A comparison of HDL composition, number, and diameter (⌀) between subjects with a TG/HDLc ratio below or above the median is given in Table S4. Subjects with a TG/HDLc ratio above the median value had a lower number of total HDLp, and HDLp of medium-size, and higher concentration of large HDLp (all $P < 0.050$) compared to those with the TG/HDLc ratio below the median value.

Impact of age on sex differences in the lipoprotein profile

Participants were stratified according to the median age of the study population, which was 45 years. The younger group (≤ 45 years) included 24 women (median age: 37 [34–40] years) and 27 men (38 [32–43] years), while the older group (> 45 years) comprised 26 women (55 [49–57] years) and 24 men (51 [48–54] years).

As shown in Table S5, sex differences in RC levels between sexes were evident in participants younger than 45 years, with men showing significantly higher levels than women (median RC: 26.5 [22.9–36.0] mg/dL vs. 19.7 [11.9–29.3] mg/dL; $P = 0.017$). However, these differences were not observed in the group older than 45 years (27.5 [20.4–32.4] mg/dL in men vs. 23.9 [15.4–31.3] mg/dL in women; $P = 0.275$). Sex differences in other lipoprotein traits, including VLDLp, small LDLp, LDL particle size, and HDLp, remained significant across both age groups. When comparing participants below and above 45 years within each sex, no statistically significant differences were observed in RC, VLDLp, LDLp, small LDLp, LDL size, or HDLp levels. However, in women, RC levels showed a non-significant trend toward higher values with age, whereas no such trend was observed in men.

VLDL profile in relation to cardiovascular disease risk

REGICOR (the Registre Gironí del cor) risk equation was applied to estimate the 10-year CVD risk for the entire cohort and separately by sex and BMI. Nine subjects were excluded from the REGICOR analysis as they did not meet the minimum age requirement of 37 years or had cholesterol levels, HDLc, or blood pressure values outside the range considered for the risk calculation.

Among the remaining 92 participants, the median 10-year CVD risk was 2.0 [1.0–3.0] %, classifying this population as the low risk (< 5.0%). When analyzed by sex, women had a significantly lower 10-year CVD risk than men (Men: 3.0 [2.0–3.0] %, Women: 2.0 [1.0–3.0] %, P = 0.001). Although not statistically significant, 10-year CVD risk tended to be higher in individuals with obesity compared to those with overweight (P = 0.097).

Since remnant cholesterol levels and VLDL were the variables showing the greatest differences related to sex and BMI, changes in remnant cholesterol and the VLDL profile (including TG and cholesterol content, size, and particle number) were further analyzed in association with the REGICOR-estimated CVD risk. The total population was divided into two groups based on the median 10-year CVD risk of 2.0%. As shown in Table S6, subjects with a 10-year CVD risk above the median (REGICOR risk 2.0%) had significantly higher levels of VLDLc and VLDLtg, and larger number of VLDLp. RC levels were 1.5 times higher (P < 0.001) in the group with a REGICOR value > 2% compared to those with a risk below the median value.

Differences in the VLDL profile and RC content were compared according to 10-year CVD risk, separately analyzing men and women (Table S7A) as well as subjects with obesity and with overweight (Table S7B). Regardless of sex or degree of obesity, subjects with a 10-year CVD risk > 2% had higher levels of VLDLc, VLDLtg, VLDLp, and RC compared to those with lower CVD risk (REGICOR value < 2%). While this pattern was consistent across all subgroups, the difference in VLDLc levels between women with lower and higher CVD risk did not reach statistical significance (P = 0.124).

Using a non-parametric Spearman rank test, small, medium, and large VLDL particle subfractions were correlated with the REGICOR score, as well as with BMI and waist circumference (Table S8). In the case of LDL particles, the correlation with REGICOR was observed only for the small subfraction, whereas for HDL particles, it was found in the large subfraction.

DISCUSSION

In this study, an in-depth characterization of the plasma lipoprotein profile was conducted using ^{1}H-NMR in a cohort of metabolically healthy men and women with overweight and obesity, a group often underrepresented in research, yet crucial for understanding early lipoprotein changes in CVD risk.

The present study advances the field by reporting on particle size (small, medium and large) and count across lipoprotein subclasses including VLDL, LDL and HDL and assessing lipid content within each lipoprotein fraction. In addition, the association between these lipoprotein features, remnant cholesterol, and cardiovascular risk was investigated as estimated by the REGICOR score.

To the best of current knowledge, prior NMR-based studies [35, 36] have primarily focused on populations with established metabolic disorders (e.g., diabetes, hypertension, or dyslipidemia). In contrast, the present study provides novel insights by isolating the impact of overweight and obesity in individuals with no comorbidities, allowing us to detect subtle, early alterations in the lipoprotein profile.

Based on this healthy cohort, the number of VLDLp was identified as a differential factor between women and men of the same age and BMI range. The higher number of VLDLp together with their higher content of cholesterol and TG in men suggests a less favorable VLDL profile associated increased cardiovascular risk [35, 37]. Consistent with the obtained results, both the Framingham Offspring Study and the STRRIDE study reported higher VLDLp concentrations in men than in women [38, 39]. However, a key difference is that this Spanish cohort is composed exclusively of asymptomatic, untreated adults from a Mediterranean population, with generally lower baseline cardiovascular risk and distinct lifestyle patterns compared to the predominantly North American cohorts of Framingham and STRRIDE, which include a broader range of metabolic profiles and treatment exposures.

In addition, it was found that men exhibited a distinct lipoprotein profile characterized by higher concentrations of LDLp, particularly in the smaller size, resulting in a reduced median LDL particle diameter. This pattern is clinically significant given strong evidence that small, dense LDL particles are potent atherogenic risk indicators [40] and high LDLp counts are consistently associated with increased CVD risk [41]. Importantly, this investigation provides novel evidence that within a healthy Spanish cohort, a population with Mediterranean lifestyle influences, men display not only quantitative (higher LDLp) but also qualitative (smaller particle size) lipoprotein alterations, highlighting the value of advanced profiling for early atherogenic risk detection.

HDL cholesterol (HDLc) levels are typically regarded as inversely related to cardiovascular disease (CVD) risk, although recent observational studies have reported a U-shaped association, with very high HDLc levels also linked to increased all-cause mortality [42]. Despite this

complexity, there is a broad consensus that higher concentrations of total HDLp are generally associated with reduced risk of CVD [43]. In the current healthy cohort both men and women had HDLc levels predominantly in the range from 50 to 60 mg/dL, with women typically exhibiting higher levels than men. Interestingly, while no differences were observed in the triglyceride content of HDL between sexes, women had a higher number of HDLp than men, a difference that was particularly evident in the medium-sized HDL subclass. Medium-sized HDLp are believed to play a key role in the functional properties of HDL that contribute to their overall cardioprotective effects [44]. Small HDL particles are generally considered the most functional [45], and are associated with a lower CVD risk profile [46]. However, the literature remains mixed, as some studies have also linked small HDL particles with greater severity of coronary artery disease (CAD) [47] and an adverse cardiometabolic risk profile [14], while larger HDL particle size have been associated with both with higher [48] and, paradoxically lower CAD risk [13, 47] in different reports [48].

It is important to note that apparent inconsistencies among studies may be due to differences in the methods used to identify HDL subclasses. Discrepancies arise because techniques like NMR spectroscopy, density gradient ultracentrifugation (classifying HDL2/HDL3), and non-denaturing gradient gel electrophoresis (defining HDL2a/2b, HDL3a/3b/3c) apply distinct particle size thresholds and compositional criteria for subclass definitions.

Several factors may explain the higher levels of VLDLp and LDLp, and lower levels of HDLp observed in men compared to women. Premenopausal women typically produce less VLDL and clear it more efficiently, partly due to higher estrogen levels, which reduce hepatic VLDL production and upregulate LDL receptor expression, enhancing LDL clearance [49–51]. Women also tend to have greater insulin sensitivity, which lowers hepatic fatty acid accumulation and reduces the formation of VLDL, IDL and LDL [52]. The higher insulin sensitivity in women is often associated with a pear-shaped distribution of subcutaneous fat in the lower body, which, in turn, is linked to reduced TG and VLDL production [53, 54].

Thus, in healthy subjects, obesity was associated with an increase in serum VLDLp (total, large and small), and VLDL-associated triglycerides. This reflects the known link between obesity and VLDLp overproduction, as VLDLp carry the highest triglyceride content among lipoproteins [55]. Obesity is associated with elevated cholesteryl ester transfer protein (CETP) activity, which promotes a lipid exchange that shifts HDL and LDL toward smaller, denser particles. HDL and LDL size decreases due to triglyceride enrichment and hepatic lipase action [56, 57].

Interestingly, in this study in healthy men and women matched for BMI and age, obesity significantly affected the VLDL profile in women, while no differences were observed between overweight and obese men. Remnant cholesterol (RC), measured by ^{1}H-NMR under fasting conditions, also showed a sex-specific and obesity-specific pattern, with larger increases in men and subjects with obesity. Notably, the obesity effect on RC persisted only in women.

While RC is known to increase ASCVD and mortality risk similarly in both sexes [58, 59], there is no clear evidence that it is a better predictor in women. However, growing data suggest that TRLs may play a key role in premature coronary disease in women [60] and that sex differences in RC vary with age [61]. RC levels are significantly higher in men than in women at early ages but differences between sexes narrowed in the group above 45 years, with women showing a more male-like lipid phenotype. In contrast, the ongoing research found no correlation between RC and age, possibly due to the low overall CVD risk in the cohort. Differing from RC levels, sex differences in VLDLp, LDL size, and HDLp persisted in both age groups.

Sex-related effect of ageing on RC levels may be due to hormonal or metabolic changes in women since menopause is associated with changes in lipid metabolism towards a more atherogenic lipid profile including increased levels of TC, LDLc, and TG levels, while HDLc decreases [62]. Unfortunately, menopausal status was not clinically verified in this study. However, the reported findings suggest the need for a more specific age-related study on RC levels in women, investigating how these levels evolve during the postmenopausal period.

RC levels exhibit a complex relationship with LDLc, with emerging evidence highlighting RC as an independent cardiovascular risk factor, particularly when LDLc is low [63]. In this investigation, although all subjects showed LDLc levels below the pathological range and none of them were under statin treatment, higher RC concentration was evidenced in the group with higher LDLc background (plasma levels above the median of the study group), particularly in women, where levels were notably higher compared to those with low LDLc. This suggests that the increase in LDLc levels may have a more pronounced impact on women, with implications for cardiovascular risk assessment in both sexes [64].

The ratio TG/HDLc, has been proposed as a strong predictor of CVD comparable to LDLc [65]. In the present study, men and individuals with obesity had higher TG/HDLc ratios than women and subjects with overweight. Further analysis showed that a higher TG/HDLc ratio was also associated with a HDL profile characterized by

low HDLc and HDLp concentrations and more elevated levels of HDLtg. In a study in young individuals with obesity, an elevated TG/HDLc ratio has been associated with higher levels of proatherogenic lipoprotein subclasses, such as VLDL, IDL, and LDL [66].

VLDL is known to contribute to atherosclerotic CVD [37]. Therefore, these results suggest that metabolically healthy individuals with obesity may have a less favorable CVD risk profile. A recent, cross-sectional study of 5,301 participants found that increased RC levels were associated with increased visceral adipose tissue [67]. As VLDL showed the most significant changes by BMI and sex in this study, the combined impact of these factors on VLDL profiles was examined. These findings strongly support the view that obesity affects women more than men, as women with obesity had a worse VLDL profile than women with overweight, a pattern not observed in men. This is consistent with the Framingham Heart Study, where obesity increased CVD risk by 64% in women compared with 46% in men [68].

The cardiovascular risk score REGICOR, specifically adapted and validated for the Spanish population [32], evidences that all subjects included in the study were at low or very low risk. To notice those with a risk above 2% were the subjects with higher concentrations of VLDLp and remnant cholesterol, aligning with previous findings in Spanish healthcare workers showing strong associations between REGICOR scores and lipid profiles, age, smoking, and adherence to a Mediterranean diet [69].

Strengths and Limitations

This study has strengths and weaknesses. A key strength is the use of ^{1}H-NMR to accurately analyze lipoprotein particles and their lipid content, crucial for assessing CVD risk. Including both subjects with overweight and obesity, men and women, also allows for direct comparison of sex- differences in lipid profiles. However, the study has some limitations, such as the cross-sectional design, which precludes establishing causal relationships, and the sample size, which may have reduced the ability to detect significant differences in certain subgroups and might affect the generalizability of these findings. Nevertheless, the study was designed as an exploratory investigation to generate hypotheses and provide initial insights into lipoprotein profiles and cardiometabolic risk markers in relation to sex, age, and menopausal status in a metabolically healthy cohort. In addition, data on lifestyle, diet and physical activity, and individual menopause age in women were unavailable for the study.

Conclusion

In conclusion, the results of this study provide evidence that in a metabolically healthy population with overweight and obesity, men and individuals with obesity exhibit a more pro-atherogenic lipoprotein profile than women and individuals with overweight, respectively. Specifically, the more unfavorable CVD risk profile in men and individuals with obesity is primarily characterized by an elevated content of cholesterol and TG carried by VLDL (large, medium and small particles), along with higher levels of RC. Interestingly, obesity-related differences in the VLDL profile and RC levels are particularly evident in women but not in men. This suggests that an increase in BMI above 30 kg/m^2 has a greater impact on women, independently of the age, leading to a higher CVD risk profile.

These findings highlight the importance of identifying VLDL and remnant particles as indicators of cardiovascular risk in otherwise healthy individuals who are overweight or obese. Such profiling could facilitate more personalised risk stratification and contribute to preventive strategies before clinical disease develops. This approach could lead to targeted lifestyle recommendations and monitoring for individuals who are overweight or have early-stage obesity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-025-02723-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Authors' contributions

Author Contributions: Conceptualization, T.P. and L.B.; methodology, V.S., N.M.-G., T.P., and L.B.; formal analysis, V.S., A.L.-Y., N.M.-G., T.P. and L.B.; investigation, T.P. and L.B.; data curation, V.S., N.M.-G., A.L.-Y., T.P. and L.B.; writing—original draft preparation, V.S., A.L.-Y., T.P. and L.B.; writing—review and editing, V.S., N.M.-G., A.L.-Y., T.P., G.V. and L.B.; visualization, V.S., A.L.-Y., T.P. and L.B.; supervision, V.S., L.B. and TP.; project administration, T.P. and L.B.; funding acquisition, T.P., L.B., and G.V. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

LB declares to have acted as SAB member of Sanofi, Ionnis and NovoNordisk; to have received speaker fees from Sanofi and NovoNordisk and to have founded the Spin-off Ivastatin Therapeutics S (all unrelated to this work).T.P. discloses to have received speaker fees from AB-BIOTICS S.A. and to be a co-founder of Spin-off Ivastatin Therapeutics S.L. (both unrelated to this study).G.V. discloses to be a co-founder of Spin-off Ivastatin Therapeutics S.L. (unrelated to this study).The remaining authors have no competing interests to declare.

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