Advanced lipoprotein parameters could better explain atheromatosis in non-diabetic chronic kidney disease patients

Marcelino Bermudez-Lopez1, Hector Perpiñán2, Nuria Amigo3, Eva Castro1, Nuria Alonso4,5,6, Didac Mauricio5,6, Elvira Fernandez1, and Jose M. Valdivielso1, on behalf of the NEFRONA Investigators

1Vascular and Renal Translational Research Group, Spanish Research Network for Renal Diseases (REDINREN del ISCIII), IRBLleida, Lleida, Spain, 2Conselleria de Sanitat Universal i Salut Pública, Generalitat Valenciana, Valencia, Spain, 3Biosfer Teslab SL, Reus, Spain, 4Endocrinology and Nutrition Department, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain, 5Center for Biomedical Research on Diabetes and Associated Metabolic Diseases (CIBERDEM), Barcelona, Spain and 6Endocrinology and Nutrition Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Correspondence to: Jose M. Valdivielso; E-mail: valdivielso@medicina.udl.es and Marcelino Bermudez-Lopez; E-mail: mbermudez@irblleida.cat

ABSTRACT

Background. Chronic kidney disease (CKD) patients have a high burden of atheromatous cardiovascular disease (ASCVD) not fully explained by traditional lipid parameters. Lipoprotein composition and subclass particle number information could improve ASCVD risk assessment. The objective of this study is to investigate the association of advanced lipoprotein parameters with the risk of atheromatosis in a subpopulation of the NEFRONA study.

Methods. This was a cross-sectional study in 395 non-diabetic individuals (209 CKD and 186 non-diabetic and non-CKD) without statin therapy. Vascular ultrasound examination assessing 10 territories was combined with advanced lipoprotein testing performed by nuclear magnetic resonance spectroscopy. Logistic regression was used to estimate adjusted odds ratios (ORs) per 1 standard deviation increment.

Results. Atheromatosis was more prevalent in CKD patients (33.9% versus 64.6%). After adjusting for age, gender, smoking habit and CKD stage, the amount of triglycerides (TGs) within low-density lipoprotein (LDL) lipoproteins was independently and positively associated with atheromatosis [OR 1.33; 95% confidence interval (CI) 1.03–1.74; P = 0.03]. Similarly, total and medium LDL particles (LDL-Ps) showed a positive association (OR 1.29; 95% CI 1.00–1.68; P = 0.05 and OR 1.34; 95% CI 1.04–1.75; P = 0.03, respectively). TG-loaded medium LDL-Ps were higher in CKD patients compared with controls and showed an adjusted OR of 1.40 (95% CI 1.09–1.82; P = 0.01) in non-diabetic patients (CKD and non-CKD individuals). In contrast, non-diabetic CKD patients showed a similar coefficient but the significance was lost (OR 1.2; 95% CI 0.8–1.7; P = 0.359).
Conclusions. Non-diabetic CKD patients showed a higher amount of TG-loaded medium LDL-Ps compared with controls. These particles were independently associated with atheromatosis in non-diabetic patients.

Keywords: atherosclerosis, chronic kidney disease, dyslipidemia, LDL cholesterol, lipoprotein subfractions, triglycerides

INTRODUCTION

Atheromatous cardiovascular disease (ASCVD) is the leading cause of mortality and morbidity in many countries [1]. It is a systemic multifactorial disease that has a long silent phase before manifesting as an incident cardiovascular event [2]. Atheromatosis is closely related to lipid abnormalities. In the general population, traditional lipid parameters, such as high total cholesterol (TC), high low-density lipoprotein cholesterol (LDL-C) and low high-density lipoprotein cholesterol (HDL-C), are associated with atheromatosis [3]. The European Society of Atherosclerosis recently stated that LDL-C directly causes ASCVD [4].

Traditional lipid parameters cannot detect changes in lipoprotein size, number and composition. For instance, LDL-C, which refers to the cholesterol content within LDL particles (LDL-Ps), omits information about particle size, subclass particle number and composition [5]. Similarly, conventional triglyceride (TG) levels measurement indicates total TG with no information about its distribution in different types of lipoproteins. On the contrary, advanced lipoprotein testing (ALT) allows a detailed characterization of lipoprotein particles and it has been proposed as an important new tool for cardiovascular risk assessment [6]. The size, lipid content and number of lipoprotein particles can be measured by nuclear magnetic resonance (NMR)-based advanced lipoprotein profiling and subfractioning. Therefore, we can not only divide the different lipoproteins into large, medium and small particles according to their size, but also determine the total number and its specific lipid load [7].

In the general population, several studies have revealed that ASCVD risk is more closely related to LDL-P number than to total levels of LDL-C [8]. In addition, the TG content in LDLs, HDLs and very-low-density lipoproteins (VLDLs) has been shown to be positively associated with myocardial infarction and ischaemic stroke [9].

Chronic kidney disease (CKD) is a worldwide concern with an increasing incidence [10]. Compared with individuals with normal renal function, CKD patients show a higher prevalence and burden of ASCVD from early stages [11] and this prevalence rises as kidney disease progresses [12, 13]. CKD patients show an unacceptable high residual cardiovascular risk even with an accurate control of traditional lipid parameters [14]. Indeed, the traditional lipid profile in CKD is different from the one found in a conventional high-risk individual; it is characterized by hypertriglyceridaemia, varying levels of LDL-C and low HDL-C levels [15, 16]. Thus, since these parameters do not explain the excess and the rapid progression of atheromatosis in the CKD population [13, 17], information beyond the traditional lipid profile is needed to fully understand the underlying cause of the accelerated atherosclerosis in this population.

Although some studies have performed ALT in patients with CKD, the association of these parameters with atheromatosis remains elusive [18–20]. Thus, the present study combines traditional lipid parameters with an advanced lipoprotein profiling performed by NMR spectroscopy (NMRs) [21] to elucidate their association with atheroma plaque presence in a subpopulation of the NEFRONA study [22] composed of non-diabetic individuals without statin therapy.

MATERIALS AND METHODS

Study design and participants

Cross-sectional study in 209 CKD patients at various stages of the disease (86 CKD stage 3, 71 CKD stages 4–5 and 52 dialysis) and 186 controls (non-diabetic and non-CKD individuals) belonging to the NEFRONA study were selected. The design and objectives of the NEFRONA study have already been published in detail [22, 23]. Briefly, the NEFRONA study was designed as a multicentre prospective observational cohort study to evaluate subclinical atheromatosis burden in CKD. A total of 2445 CKD patients were enrolled in 81 Spanish hospitals and dialysis clinics, from October 2010 to June 2012. Inclusion criteria were: patients between 18 and 74 years of age were eligible if they had CKD stage 3 or higher as defined by current guidelines (glomerular filtration rate < 60 mL/min/1.73 m²). In addition, 559 individuals with a glomerular filtration rate > 60 mL/min/1.73 m² were recruited from primary care centres. Exclusion criteria for both groups were: active infections, pregnancy, active neoplasia, life expectancy shorter than 12 months, previous cardiovascular event, carotid surgery or any organ transplantation. To avoid interference with lipid parameters, patients with diabetes and/or on statin therapy were excluded from this study. Thus, 395 non-diabetic individuals (209 CKD and 186 non-diabetic and non-CKD) without statin therapy were selected. The study protocol was approved by the ethics committees of each hospital. It was conducted according to the principles of the declaration of Helsinki and all patients were included after signing an informed consent.

Clinical and laboratory determinations

Diagnosis of dyslipidaemia and hypertension was collected from clinical records. Anthropometrical parameters, smoking habit and blood samples were obtained at the moment of arterial ultrasound. After processing, serum samples were aliquoted to avoid freeze–thaw cycles and stored at −80 °C. Glomerular filtration rate was estimated according to international guidelines using the CKD Epidemiology Collaboration equation [24]. TC, HDL-C and TG were measured by colorimetric methods according to standardized protocols with an AU5800 Analyzer (Beckman Coulter Inc., Fullerton, CA, USA) in the Clinical Analysis Laboratory, Arnau de Vilanova University Hospital, Lleida, Spain. LDL-C was calculated by Friedewald equation if TG < 250 mg/dL.

Advanced lipoprotein profiling by NMR

A detailed protocol of NMRs has been previously published [25]. Briefly, frozen serum samples (250 μL) were shipped on dry ice to Biosfer Teslab (Reus, Spain) for the analysis of lipoprotein profile. Particle concentration and diffusion coefficients were obtained from the measured amplitudes and attenuation of the spectroscopically distinct lipid methyl group NMR signals, using
the 2D diffusion-ordered $^1$H NMRS (DSTE) pulse. Methyl signal was surface fitted with nine Lorentzian functions associated with each lipoprotein subclass of the main lipoprotein groups (VLDL, LDL and HDL). The concentrations of each lipoprotein subclass and their lipid composition were calculated [21]. The different lipoprotein subfractions correspond to the following diameter size ranges: large VLDL, 68.5–95.9 nm; medium VLDL, 47–68.5 nm; small VLDL, 32.5–47 nm; large LDL, 24–32.5 nm; medium LDL, 20.5–24 nm; small LDL, 17.5–20.5 nm; large HDL, 10.5–13.5 nm; medium HDL, 8.5–10.5 nm; and small HDL, 7.5–8.5 nm. Finally, weighted average VLDL, LDL and HDL particle sizes were calculated from various subfraction concentrations by summing the known diameter of each subfraction multiplied by its relative percentage of particle number.

Arterial ultrasound

Arterial ultrasound was performed as previously described in the NEFRONA study [22]. The VIVID BT09 ultrasound system (GE Healthcare), equipped with a 6–13 MHz linear probe, a module for measuring intima–media thickness (IMT) and a pulsed Doppler ultrasound, were used to assess haemodynamic abnormalities. Atheromatous plaques were defined as an IMT >1.5 mm protruding into the lumen, according to the American Society of Echocardiography (ASE) consensus statement and the Mannheim consensus [26, 27]. Plaque presence was considered when it was detected in at least 1 of the 10 territories analysed (both internal, bifurcation and common carotids, and both common and superficial femoral arteries) by a single reader in blinded fashion, using a semi-automatic software (EchoPAC Dimension, General Electric).

Statistical analysis

Characteristics of the study sample were described as frequencies and percentages for qualitative variables, means and standard deviations for normally distributed quantitative variables (assessed by Shapiro–Wilks tests) and medians and quartiles for non-normal quantitative variables. Bivariate analyses by atheromatous plaque presence were performed by means of the Pearson’s chi-squared test for qualitative variables and the ANOVA or the Kruskal–Wallis test for quantitative variables. The associations for traits measured by NMRS with risk of atheromatosis presence were assessed using logistic regression adjusted for age, gender, CKD stage (controls, CKD stage 3, CKD stages 4–5 and dialysis) and smoking habit (non-smoker, former and current). For each measure, adjusted odds ratios (ORs) and 95% confidence intervals (CIs) per 1 standard deviation (SD) higher parameter measure were estimated. All analyses were conducted using R. The statistical significance was set at a P-value <0.05.

RESULTS

Clinical characteristics and traditional lipid parameters

Atheroma plaque prevalence was lower in women and non-smokers, and higher in CKD patients and patients previously diagnosed with dyslipidaemia and hypertension. The median age and the body mass index were higher in participants with atheromatosis, as well as the levels of both systolic and diastolic blood pressure (Table 1).

The traditional lipid profile revealed that TG levels and the cholesterol content in non-HDL lipoproteins (referred as non-HDL-C) were higher in participants with atheroma plaque. In contrast, the cholesterol content in HDL lipoprotein (referred as HDL-C) was lower (Table 1).

| Table 1. Clinical characteristics and traditional lipid profile according to atheromatous plaque presence |
|----------------------------------------------------|----------|----------|
| No plaque (n = 197) | Plaque (n = 198) | P-value |
| Clinical characteristics | | | |
| Women, n (%) | 111 (56.3) | 61 (30.8) | <0.001 |
| Age (years) | 45 (35–56) | 62 (53–69) | <0.001 |
| CKD stage, n (%) | | | <0.001 |
| Control | 123 (62.4) | 63 (31.8) | |
| CKD 3 | 30 (15.2) | 56 (28.3) | |
| CKD 4–5 | 24 (12.2) | 47 (23.7) | |
| Dialysis | 20 (10.2) | 32 (16.2) | |
| Smoking, n (%) | | | 0.001 |
| Non-smoker | 100 (50.8) | 69 (34.8) | |
| Former | 62 (31.5) | 99 (50) | |
| Current | 35 (17.8) | 30 (15.2) | |
| Dyslipidaemia, n (%) | 28 (14.2) | 71 (35.9) | <0.001 |
| Hypertension, n (%) | 78 (39.6) | 150 (75.8) | <0.001 |
| Systolic BP (mmHg) | 125 (115–139) | 135 (127–152.8) | <0.001 |
| Diastolic BP (mmHg) | 77 (71–84) | 81.5 (74–87) | <0.001 |
| Body mass index (kg/m$^2$) | 25.4 (22.8–29.1) | 27.4 (25.1–30.5) | <0.001 |
| Traditional lipid profile | | | |
| TC (mg/dL) | 188.7 (32.8) | 192.6 (38.6) | 0.274 |
| HDL-C (mg/dL) | 51 (43–60.8) | 48 (40–57.8) | 0.045 |
| LDL-C (mg/dL) | 115.1 (27.1) | 119 (32.6) | 0.187 |
| TG (mg/dL) | 97 (71–132.8) | 111.5 (84–154) | 0.001 |
| Non-HDL-C (mg/dL) | 136.6 (29.2) | 143.1 (34.8) | 0.046 |

Values are shown as means and SDs for normally distributed quantitative variables, and medians and percentiles 25% and 75% for non-normal quantitative variables. BP, blood pressure.

Advanced lipoprotein profile

Patients with atheroma plaque had different lipoprotein composition and particle number (Table 2). LDL and HDL particles had higher TG content (LDL-TG and HDL-TG, respectively), VLDL and intermediate-density lipoprotein (IDL) particles had a higher amount of cholesterol (VLDL-C and IDL-C, respectively) and TGs (VLDL-TG and IDL-TG, respectively). Participants with atheromatosis had an overall increase of VLDL particle number and a higher amount of large HDL particles. In addition, they also had more LDL-Ps, specifically, medium and small molecules (Table 2), which agreed with a smaller averaged LDL-P size.

Association of lipoprotein composition with atheromatosis

The relationship of the lipid content (cholesterol and TG) within each lipoprotein class with atheroma plaque presence was independently evaluated. After adjusting for age, gender, smoking habit and CKD stage, the amount of TGs within LDL lipoproteins (LDL-TG) showed an adjusted OR per 1 SD increment of 1.33 (95% CI 1.03–1.74; P = 0.03; Figure 1). Therefore, LDL-TG was independently associated with atheroma plaque presence.

Association of lipoprotein particle number with atheromatosis

Multivariate regression analysis revealed that total LDL-P number was positively associated with atheroma plaque presence.
Furthermore, lipoprotein subclass profiling showed that among LDL-Ps, medium LDL-P was associated with plaque presence (Figure 2).

Table 2. NMR-assessed advanced lipoprotein profile according to atheromatous plaque presence

|                      | No plaque (n = 197) | Plaque (n = 198) | P-value |
|----------------------|---------------------|------------------|---------|
| **VLDL-P composition (mg/dL)** |                     |                  |         |
| VLDL-C               | 11.8 (7.9–17.5)     | 14.6 (10.5–22)   | <0.001  |
| VLDL-TG              | 43.6 (33.1–64)      | 52.5 (38.2–75.5) | 0.001   |
| **VLDL-P number (nmol/L)** |                     |                  |         |
| Total                | 31 (23.4–45.5)      | 36.7 (26.8–53.9) | 0.001   |
| Large                | 1.1 (0.8–1.4)       | 1.2 (0.9–1.7)    | 0.001   |
| Medium               | 4.9 (3.5–6.8)       | 5.8 (4.1–8.3)    | 0.001   |
| Small                | 25 (19.2–37.9)      | 29.8 (21.6–44.1) | 0.002   |
| **VLDL-P size (nm)** | 42.4 (0.3)          | 42.5 (0.4)       | 0.376   |
| **LDL-P composition (mg/dL)** |                     |                  |         |
| LDL_3SR-C            | 104 (18.4)          | 106.2 (21.4)     | 0.279   |
| LDL-TG               | 12.3 (10–14.6)      | 13.7 (11.5–16.2) | <0.001  |
| **LDL-P number (nmol/L)** |                     |                  |         |
| Total                | 732.2 (128.8)       | 760.2 (144.3)    | 0.043   |
| Large                | 105.1 (20.4)        | 105 (22.4)       | 0.969   |
| Medium               | 263.8 (49.9)        | 274.9 (56.5)     | 0.04    |
| Small                | 362.5 (316–409.5)   | 374.5 (322.3–428.7) | 0.038 |
| LDL-P size (nm)      | 21.152 (21.071–21.257) | 21.104 (21.038–21.204) | 0.004 |
| **IDL-P composition (mg/dL)** |                     |                  |         |
| IDL-C                | 8.2 (6.2–10.7)      | 9.9 (8.1–12.4)   | <0.001  |
| IDL-TG               | 8.3 (6.5–10.3)      | 9.8 (8.1–11.5)   | <0.001  |
| **IDL-P number (µmol/L)** |                     |                  |         |
| Total                | 27.5 (24.5–31.2)    | 28.4 (24.2–31.1) | 0.844   |
| Large                | 0.193 (0.139–0.262) | 0.223 (0.154–0.314) | 0.003 |
| Medium               | 8.4 (7.1–9.8)       | 8.0 (6.5–9.8)    | 0.271   |
| Small                | 19.0 (17–21.6)      | 19.4 (17.2–22)   | 0.333   |
| HDL-P size (nm)      | 8.221 (8.199–8.235) | 8.219 (8.193–8.235) | 0.43   |

Values are shown as means and SDs for normally distributed quantitative variables, and medians and percentiles 25% and 75% for non-normal quantitative variables.

Association of LDL-P number and composition with atheromatosis

Since LDL composition and particle number were independently associated with atheroma plaque presence, we decided to investigate the combined effect of both parameters. The combined variable LDL-TG-medium LDL-P, a variable that reflects the TG content in medium LDL-Ps, was higher in CKD patients compared with controls with no CKD (CKD 3650; 95% CI 1233.78–8076.58 versus non-CKD 3334.31; 95% CI 1398.27–7624.04). However, this result had a borderline significance (P = 0.05; Figure 3). CKD patients showed a higher prevalence of atheromatosis (CKD 64.6% versus non-CKD 33.9%; Figure 3).

LDL-TG-medium LDL-P showed an adjusted OR per 1 SD increment of 1.40 (95% CI 1.09–1.82; P = 0.01; Figure 4). Interestingly, this model showed the lowest Akaike Information Criterion, indicating a better likelihood to predict/estimate atheromatosis presence compared with isolated models (LDL-TG-medium LDL-P 385.18 versus LDL-TG 387.21 versus LDL-P 388.10 versus medium LDL-P 386.91). Therefore, medium LDL-Ps loaded with TGs were positively and independently associated with atheromatous disease in our general population (non-diabetic CKD and non-CKD individuals). Finally, a sensitivity analysis was performed in a subsample of only CKD patients to confirm the association of LDL-TG-medium LDL-P with atheromatosis. Although the significance was lost, the coefficient reported for the variable was similar (OR 1.2; 95% CI 0.8–1.7; P = 0.359).
DISCUSSION

This cross-sectional study provides a comprehensive examination of advanced lipoprotein parameters and their association with atheromatosis presence in non-diabetic CKD patients and controls without statin therapy. The main results of the present study were: (i) the amount of TGs within LDL lipoproteins (LDL-TG), (ii) the total and medium LDL-P number and (iii) the medium LDL-Ps loaded with TGs were positively and independently associated with atheromatous disease in non-diabetic CKD and non-diabetic and non-CKD individuals.

Traditional lipid profiling revealed some statistically significant differences according to atheromatous plaque presence. However, those values were extremely close and their clinical relevance could be at best questionable. These data reinforce the importance of an advanced lipoprotein characterization to elucidate clinically relevant differences.

Regarding LDL-P number, previous studies support the predictive role of LDL-P number for cardiac events in the general population [28–30]. Whereas large LDL-Ps showed divergent association with cardiovascular risk (positive [31], negative [32] or null [29]), medium LDL-Ps normally showed a positive association [33, 34]. Similarly, our results are in line with a previous Spanish study in the general population where medium particles showed the strongest association with cardiovascular events [35]. The combined variable LDL-TG–medium LDL-P was positively associated with atheromatosis in our cohort and showed the better likelihood of predicting atheromatosis presence compared with isolated models. Therefore, medium LDL-Ps loaded with TGs are positively associated with atheromatous disease. However, we cannot affirm with certainty whether this lipid profile is only a marker of atherosclerotic disease or, in fact, reducing these values would reduce the risk of cardiovascular disease in these patients.

In haemodialysis patients, LDL-P size seemed to be helpful to identify those patients who would not be considered at risk with traditional lipid parameters [20]. In fact, a higher LDL-P concentration was associated with a higher cardiovascular risk and mortality in CKD patients [19, 36].
Hypertriglyceridaemia is associated to vascular endothelial damage [37]. LDL-P can easily penetrate the artery wall if the endothelium is injured. When LDL-Ps enter artery wall and become modified (such as oxidized), they provoke an inflammatory reaction that triggers atheroma plaque initiation. Oxidized LDL particles are essential players in the pathogenesis of ASCVD [38]. Interestingly, they usually have a higher concentration of TGs [39]. Therefore, since CKD patients show an accumulation of LDL-Ps, a higher TG content in LDL-Ps and a pro-oxidative state [40], medium LDL-Ps loaded with TGs could have the right size to penetrate the artery wall and their lipid cargo could be more prone to initiate atheroma plaque formation.

In this study, we used the Liposcale test, based on 2D-NMRs, which directly measures the size of lipoprotein particles. This method has been previously validated and compared with the Lipoprint test, established 1D NMR method developed by Jeyarajah et al. [41]. The different lipoprotein particle measurements (i.e. VLDL, LDL and HDL) obtained by using 1D and 2D-NMR techniques were highly correlated, although the particle numbers obtained by 2D-NMR method were more in agreement with biochemical values such as the concentration of apolipoproteins A and B (Apo A and Apo B, respectively) in isolated fractions than those obtained using the 1D-NMR test [21].

The present results must be considered with the following caveats. First, the cross-sectional design of the study does not allow establishing a causal relation between atherosclerosis and lipoprotein profiles. Second, there is no information about other lipid variables associated with cardiovascular disease, such as Apo B and Apo A-I. Third, the lack of association of some lipoprotein parameters with atheroma plaque presence may reflect low statistical power due to the relatively small size of the cohort. In addition, the sensitivity analysis using only CKD patients showed the same lack of power. Fourth, there is a substantial collinearity of lipoprotein parameters, which makes the analysis of interactions between individual parameters (i.e. particle composition and number) difficult. Finally, data about dyslipidaemia and hypertension were collected from clinical records, so memory bias could also be a limitation.

On the other hand, our study has several strengths. First, to the best of our knowledge, this is the first study that combines a comprehensive analysis of lipoprotein composition and particle number. Second, CKD patients at various stages of the disease and controls were selected to obtain a representative study cohort. Finally, the absence of patients with diabetes and/or on statin therapy reduces potential bias and drug interference in lipid parameters, but it hinders generalization of results to other populations. Therefore, a non-treated hidden atherogenic dyslipidaemia was revealed.

CKD patients show several lipoprotein alterations, such as (i) an increased number of VLDL-Ps; (ii) a reduced mean LDL-P size; (iii) a higher lipid content in VLDL and IDL; and (iv) a gain of TG and a reduction of cholesterol in LDL and HDL particles [25]. Recently, TG-rich lipoproteins have been associated to cardiovascular events in CKD patients [42]. Very frequently, CKD patients show hypertriglyceridaemia [16]. This is normally due to an increased hepatic production and decreased clearance of TG-rich lipoproteins [43]. In addition, cholesteryl ester transfer protein modifies lipoprotein lipid content. It transfers TG from triglyceride-rich lipoproteins (chylomicrons, VLDL and IDL) to LDL and HDL, and it exchanges cholesterol esters from LDL and HDL to VLDL and IDL particles [44]. Therefore, in hypertriglyceridaemia states, such as CKD, LDL and HDL, particles gain TG, which can increase atheromatous cardiovascular risk in these patients.

High LDL-TG levels have previously been associated with coronary heart disease in the general population [45]. Similarly, they were associated with both ischaemic stroke and coronary heart disease after adjusting for traditional risk factors, including TC and HDL-C [46]. Interestingly, both studies reported increased levels of inflammatory markers. Another study described that TG content within VLDL, LDL and IDL was
associated with higher risk of myocardial infarction and, to a lesser extent, of ischaemic stroke [9, 47, 48].

In conclusion, changes of advanced lipoprotein profile are independently associated with atheromatous disease in non-diabetic CKD and non-diabetic and non-CKD individuals. Although the reported associations provide new insight into the relation of LDL-Ps with atheromatosis, further mechanistic research addressing LDL-P composition change is needed to fully understand the potential implications of our findings.

ACKNOWLEDGEMENTS

The authors would like to thank the NEFRONA team (Eva Castro, Virtudes María, Teresa Moli, Teresa Vidal, Meritxell Soria) and the Biobank of RedInRen for their invaluable support. The NEFRONA study investigator group is composed by the following: Mª José Aladrén Regidor, Hospital Comarcal Ernest Lluch (Calatayud); Jaime Almirall and Esther Ponz, Corporación Parc Taulí (Barcelona); Jesús Arteaga Coloma, Hospital de Navarra (Pamplona); Mª Auxiliadora Bajo Rubio, Raquel Díaz, Raquel Hospital La Paz (Madrid); Montserrat Belart Rodríguez, Sistemes Renals (Lleida); Antonio Gascón, Hospital Obispo Polanco (Teruel); Jordi Bover Sanjuan and Fundació Puigvert, IIB Sant Pau (Barcelona); Josep Bromssons Artero, Clínica Girona (Girona); Juan B. Cabezuelo Romero and Salomé Muray Cases, Hospital Reina Sofia (Murcia); Jesús Cañizo Varela, Hospital Universitario Lucus Augusti (Lugo); Pilar Caro Acevedo, Clínica Ruber (Madrid); Jordi Carreras Bassa, Diaveritas Baix Llobregat (Barcelona); Alexi Cases Amenós and Eliesabet Massó Jiménez, Hospital Clinic (Barcelona); Rosario Moreno López, Hospital de la Defensa (Zaragoza); Secundino Cigarrán Guldris and Saray López Prieto, Hospital Da Costa (Lugo); Lourdes Comas Mongay, Hospital General de Vic (Barcelona); Isabel Conmera, Hospital General de Manresa (Barcelona); Mª Teresa Compte Jove, Hospital Santa Creu Jesús (Tarragona); Marta Cuberes Izquierdo, Hospital Reina Sofia (Navarra); Fernando de Álvaro and Covadonga Hevia Ojanguren, Hospital Infanta Sofia (Madrid); Gabriel de Arriba de la Fuente, Hospital Universitario Guadalajara (Guadalajara); Mª Dolores del Pino y Pino, Complejo Hospitalario Universitario Torrecardenas (Almería); Rafael Díaz-Tejeiro Izquierdo and Ahijado Hormigos, Francisco Hospital Virgen de la Salud (Toledo); Marta Dotori, USP Marbella (Málaga); Verónica Duarte, Hospital de Terrassa (Barcelona); Sara Estupiñán Torres, Hospital Universitario Canarias (Santa Cruz de Tenerife); Mª José Fernández Reyes, Hospital de Segovia (Segovia); Mª Loreto Fernández Rodríguez, Hospital Príncipe de Asturias (Madrid); Guillermina Fernández, Clínica Santa Isabel (Sevilla); Antonio Galán Serrano, Hospital General Universitario de Valencia (Valencia); Cesar García Cantón, Hospital Universitario Insular de Gran Canaria (Las Palmas); Antonio L. García Herrera, Hospital Universitario Puerto Real (Cádiz); Mercedes García Mena, Hospital San Juan de Dios (Zaragoza); Luis Gil Sacaluga and María Aguilar, Hospital Virgen del Rocío (Sevilla); José Luis Górriz, Hospital Universitario Doctor Peset (Valencia); Emma Huarte Loza, Hospital San Pedro (Logroño); José Luis Lerma, Hospital Universitario Salamanca (Salamanca); Antonio Liebana Cañada, Hospital de Jaén (Jaén); Jesús Pedro Marín Álvarez, Hospital San Pedro de Alcántara (Cáceres); Nádia Martín Alemany, Hospital Josep Trueta (Girona); Jesús Martín García, Hospital Nuestra Señora de Sonsoles (Ávila); Alberto Martínez Castelao, Hospital Universitari de Bellvitge (Barcelona); María Martínez Villaescusa, Complejo Hospitalario Universitario de Albacete (Albacete); Isabel Martínez, Hospital Galdakao (Bilbao); Inigo Moina Eguren, Hospital Basurto (Bilbao); Silvia Moreno Los Huertos, Hospital Santa Bárbara (Soria); Ricardo Mouzo Mirco, Hospital El Bierzo, Fonferrada (León); Antonia Munar Vila, Hospital Universitari Son Espases (Palma de Mallorca); Ana Beatriz Muñoz Díaz, Hospital Virgen del Consuelo (Valencia); Juan F. Navarro González, Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife); Javier Nieto and Agustín Carreño, Hospital General Universitario de Ciudad Real (Ciudad Real); Enrique Novoa Fernández, Complejo Hospitalario de Ourense (Ourense); Alberto Ortiz and Beatriz Fernandez, IIS-Fundación Jiménez Diaz (Madrid); Vicente Paraíso, Hospital Universitario del Henares (Madrid); Miguel Pérez Fontán, Complejo Hospitalario Universitario A Coruña (A Coruña); Ana Peris Domingo, Hospital Francesc de Borja (Valencia); Celestino Piñera Haces, Hospital Universitario Marqués de Valdecilla (Santander); Mª Dolores Prados Garrido, Hospital Universitario San Cecilio (Granada); Mario Prieto Velasco, Hospital de León (León); Carmina Puig Mari, Hospital d’Igualada (Barcelona); Maite Rivera Gorrín, Hospital Universitario Ramón y Cajal (Madrid); Esther Rubio, Hospital Puerta del Hierro (Madrid); Filar Ruiz, Hospital Sant Joan Despí Moisés Broggi (Barcelona); Mercedes Salgueira Lazo and Ana Isabel Martínez Puerto, Hospital Virgen Macarena (Sevilla); José Antonio Sánchez Tomero, Hospital Universitario de la Princesa (Madrid); José Emilio Sánchez, Hospital Universitario Central de Asturias (Oviedo); Ramon Sans Lorman, Hospital de Figueres (Girona); Ramon Saracho, Hospital de Santiago (Vitoria); Maria Sarrias and Daniel Serón, Hospital Universitari Vall d’Hebron (Barcelona); María José Soler and Clara Barrios, Hospital del Mar (Barcelona); Fernando Sousa, Hospital Rio Carrión (Palencia); Daniel Toran, Hospital General de Jerez (Cadiz); Fernando Tornero Molina, Hospital de Sestre (Arganda del Rey); José Javier Usón Carrasco, Hospital Virgen de la Luz (Cuenca); Ildeson Valera Cortes, Hospital Virgen de la Victoria (Málaga); Mª Merce Vilaprinyo del Perugia, Institut Catala d’Urologia i Nefrologia (Barcelona); Rafael C. Virto Ruiz, Hospital San Jorge (Huesca); Vicente Pallarés Carratalà, Clínica MEDEFIS (Vila-real, Castellón), Carlos Santos Altozano, CS Azuqueca de Henares (Guadalajara); Miguel Artigao Ródenas, CS Zona III (Albacete); Inés Gil Gil Área Básica Sanitaria de Arán, CAP Viella (Lleida); Francisco Adán Gil, CS Alfaro (La Rioja); Emilio García Criado, Centro de Salud del Carpio (Córdoba); Rafael Durá Belinchón, CS Godella (Valencia); Jose Mª Fernández Toro, CS Zona Centro (Cáceres); and Juan Antonio, División Garrote Centro de Salud de Casas Ibáñez, Consultorio de Fuentevalbilla (Albacete).

FUNDING

This work was supported by the intramural program of the IRB Lleida, the Instituto de Salud Carlos III [RETIC RD16/0009/0011, FIS PI18/00610], the Ministerio de Ciencia,
Innovación y Universidades [IJC2018-037792-I] and the FEDER funds.

AUTHORS’ CONTRIBUTIONS

M.B.-L. and J.M.V. were responsible for conception and design, analysis and interpretation of the data, drafting of the paper, revising it critically for intellectual content and final approval of the version to be published. E.F. provided resources, methodology and supervision. H.P. performed statistical analysis, original draft writing, editing and interpretation of the data. N.Amigo carried out NMR analysis and drafting of the manuscript. E.C., N.Alonso and D.M. were involved in draft writing and data analysis.

CONFLICT OF INTEREST STATEMENT

N.Amigo is a stock owner of Biosfer Teslab, the company that commercializes the lipoprotein profiling described in the present manuscript, and has a patent for lipoprotein profiling. The other authors have no conflict of interest.

REFERENCES

1. Roth GA, Forouzanfar MH, Moran AE et al. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med 2015; 372: 1333–1341
2. Benjamin EJ, Virani SS, Callaway CW et al.; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2018 update: A report from the American Heart Association. Circulation 2018; 137: e67–e492
3. Boekholdt SM,Arsenault BJ,Mora S et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA 2012; 307: 1302–1309
4. 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
5. Musliner TA, Krauss RM. Lipoprotein subspecies and risk of coronary disease. Clin Chem 1988; 34: B78–B83
6. Stein JH, McBride PE. Should advanced lipoprotein testing be used in clinical practice? Nat Clin Pract Cardiovasc Med 2006; 3: 640–641
7. Williams PT, Zhao XQ, Marcovina SM et al. Comparison of four methods of analysis of lipoprotein particle subfractions for their association with angiographic progression of coronary artery disease. Atherosclerosis 2014; 233: 713–720
8. Otvos JD, Mora S, Shalaurova I et al. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. J Clin Lipidol 2011; 5: 105–113
9. Holmes MV, Millwood IY, Kartsonaki C et al.; China Kadoorie Biobank Collaborative Group. Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke. J Am Coll Cardiol 2018; 71: 620–632
10. Coresh J, Selvin E, Stevens LA et al. Prevalence of chronic kidney disease in the United States. JAMA 2007; 298: 2038–2047
11. Betriu A, Martínez-Alonso M, Arcidiaco MV et al.; on behalf of the Investigators from the NEFRONA Study. Prevalence of subclinical atheromatosis and associated risk factors in chronic kidney disease: the NEFRONA study. Nephrol Dial Transplant 2014; 29: 1415–1422
12. Arroyo D, Betriu A, Martínez-Alonso M et al. Observational multicenter study to evaluate the prevalence and prognosis of subclinical atheromatosis in a Spanish chronic kidney disease cohort: Baseline data from the NEFRONA study. BMC Nephrol 2014; 15: 168
13. Gracia M, Betriu A, Martínez-Alonso M et al.; NEFRONA Investigators. Predictors of subclinical atheromatosis progression over 2 years in patients with different stages of CKD. Clin J Am Soc Nephrol 2016; 11: 287–296
14. Kon V, Yang H, Fazio S. Residual cardiovascular risk in chronic kidney disease: Role of high-density lipoprotein. Arch Med Res 2015; 46: 379–391
15. Barter P. Lipoprotein metabolism and CKD: Overview. Clin Exp Nephrol 2014; 18: 243–246
16. Bermúdez-López M, Betriu A, Valdivielso JM et al. Beyond the traditional lipid parameters in chronic kidney disease. Nefrologia 2018; 38: 109–113
17. Navab KD, Elboudwarej O, Gharif M et al. Chronic inflammatory disorders and accelerated atherosclerosis: chronic kidney disease. Curr Pharm Des 2011; 17: 17–20
18. Al-Shahrioui HZ, Ramirez F, Fantí P et al. NMR identifies atherogenic lipoprotein abnormalities in early diabetic nephropathy that are unrecognized by conventional analysis. Clin Nephrol 2010; 73: 180–189
19. Shen H, Xu Y, Lu J et al. Small dense low-density lipoprotein cholesterol was associated with future cardiovascular events in chronic kidney disease patients. BMC Nephrol 2016; 17: 143
20. Bowden RG, Wilson RL, Beajuan AA. LDL particle size and number compared with LDL cholesterol and risk categorization in end-stage renal disease patients. J Nephrol 2011; 24: 771–777
21. Malloir R, Amigó N, Rodríguez MA et al. Liposcale: A novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res 2015; 56: 737–746
22. Junyent M, Martínez M, Borràs M et al. Predicting cardiovascular disease morbidity and mortality in chronic kidney disease in Spain. The rationale and design of NEFRONA: A prospective, multicenter, observational cohort study. BMC Nephrol 2010; 11: 14
23. Junyent M, Martínez M, Borràs M et al. Usefulness of imaging techniques and novel biomarkers in the prediction of cardiovascular risk in patients with chronic kidney disease in Spain: The NEFRONA project. Nefrologia 2010; 30: 119–126
24. Levey AS, Stevens LA, Schmid CH et al.; for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604–612
25. Bermúdez-Lopez M, Forne C, Amigo N et al. An in-depth analysis shows a hidden atherogenic lipoprotein profile in non-diabetic chronic kidney disease patients. Exp Opin Ther Targets 2019; 23: 619–630
26. Stein JH, Korcarz CE, Hurst RT et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the American Society of Echocardiography Carotid Intima–Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008; 21: 93–111, quiz 189–190
27. Touboul PJ, Hennerici MG, Meairs S et al. Mannheim carotid intima–media thickness and plaque consensus (2004–2006–
An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis 2012; 34: 290–296

28. Cromwell WC, Otvos JD, Keyes MJ et al. LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—implications for LDL management. J Clin Lipidol 2007; 1: 583–592

29. Mora S, Otvos JD, 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

30. El Harchaoui K, van der Steeg WA, Stroes ES et al. Value of low-density lipoprotein particle number and size as predictors of coronary artery disease in apparently healthy men and women: The EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007; 49: 547–553

31. Parish S, Offer A, Clarke R et al.; Heart Protection Study Collaborative Group. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation 2012; 125: 2469–2478

32. Blake GJ, Otvos JD, Rifai N et al. 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

33. Würtz P, Havulinna AS, Soininen P et al. Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. Circulation 2015; 131: 774–785

34. Goliasch G, Oravec S, Blessberger H et al. Relative importance of different lipid risk factors for the development of myocardial infarction at a very young age (<40 years of age). Eur J Clin Invest 2012; 42: 631–636

35. Pichler G, Amigo N, Tellez-Plaza M et al. LDL particle size and composition and incident cardiovascular disease in a South-European population: The Hortega-Liposcale Follow-up Study. Int J Cardiol 2018; 264: 172–178

36. Noori N, Cauflfeld MP, Salameh WA et al. Novel lipoprotein subfraction and size measurements in prediction of mortality in maintenance hemodialysis patients. Clin J Am Soc Nephrol 2011; 6: 2861–2870

37. Ke LY, Law SH, Mishra VK et al. Molecular and cellular mechanisms of electronegative lipoproteins in cardiovascular diseases. Biomedicines 2020; 8: 550

38. Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. Circulation 2004; 109: III2–III7

39. Ke LY, Stancel N, Bair H et al. The underlying chemistry of electronegative LDL’s atherogenicity. Curr Atheroscler Rep 2014; 16: 428

40. Tucker PS, Dalbo VJ, Han T et al. Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers 2013; 18: 103–115

41. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med 2006; 26: 847–870

42. Lamprea-Montealegre JA, Staplin N, Herrington WG et al.; SHARP Collaborative Group. Apolipoprotein B, triglyceride-rich lipoproteins, and risk of cardiovascular events in persons with CKD. Clin J Am Soc Nephrol 2020; 15: 47–60

43. Sacks FM. The crucial roles of apolipoproteins E and C-III in apoB lipoprotein metabolism in normolipidemia and hypertriglyceridemia. Curr Opin Lipidol 2015; 26: 56–63

44. Shrestha S, Wu BJ, Guiney L et al. Cholesteryl ester transfer protein and its inhibitors. J Lipid Res 2018; 59: 772–783

45. MÄrz W, Scharnagl H, Winkler K et al. Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: The Ludwigshafen Risk and Cardiovascular Health Study. Circulation 2004; 110: 3068–3074

46. Saeed A, Feofanova EV, Yu B et al. Remnant-like particle cholesterol, low-density lipoprotein triglycerides, and incident cardiovascular disease. J Am Coll Cardiol 2018; 72: 156–169

47. Girona J, Amigó N, Ibarretxe D et al. HDL triglycerides: A new marker of metabolic and cardiovascular risk. Int J Mol Sci 2019; 20:3151

48. Amigó N, Mallol R, Heras M et al. Lipoprotein hydrophobic core lipids are partially extruded to surface in smaller HDL: “Herniated” HDL, a common feature in diabetes. Sci Rep 2016; 6: 19249