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Lipoprotein profile assessed by 1H NMR, BMI and blood pressure are associated with vascular alterations in children with familial hypercholesterolaemia

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KEYWORDS
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Abstract *Background and aims:* Children with familial hypercholesterolaemia (FH) have elevated low-density lipoprotein cholesterol (LDL-C) concentrations since birth, which increases the risk of cardiovascular disease in adulthood. Arterial injury and stiffness parameters, including carotid intima media thickness (cIMT), pulse wave velocity (PWV) and distensibility (DIST), can be detected early in childhood. We studied the associations between cIMT, PWV and DIST with the lipoprotein profile assessed by proton nuclear magnetic resonance (^{1}H NMR) and with influential variables such as blood pressure (BP) or body mass index (BMI) in children with FH.
Methods and results: In this cross-sectional study, we included 201 children (96 with FH and 105 non-FH controls). Clinical history, physical examination and standard biochemical studies were performed. FH genetic testing was performed when clinically indicated. Carotid ultrasonography and an advanced lipoprotein profile by ^{1}H NMR were performed. Multivariate and classification methods were used.

There were no differences between cIMT, PWV and DIST between FH and non-FH children. FH children presented more total LDL and large, medium and small particles. Small LDL particles, BMI and systolic BP determined the presence of pathological IMT in the FH group. LDL size, high-density lipoproteins and very low-density lipoprotein particles together with blood pressure determined the presence of pathological arterial wall elasticity.
Conclusions: Alterations in lipoprotein parameters assessed by are associated with early structural and functional arterial characteristics in children with FH. BMI and BP act as boosting factors. Cardiovascular prevention should start early in children with FH, encompassing all components of a healthy lifestyle.
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1. Introduction

Familial hypercholesterolaemia (FH) is an autosomal dominant genetic disorder characterized by an elevated plasma LDL cholesterol (LDL-C) concentration from birth, which implies an increased risk of premature development of atherosclerotic coronary heart disease [1,2]. It is estimated that one out of 250 children has FH, but the diagnosis is very often delayed, leading to late treatment [1,3]. This disease is mainly caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene [4], but it can also be caused by mutations in other genes, such as apolipoprotein B100 [5] (*APOB*) and proprotein convertase subtilisin-like kexin type 9 (*PCSK9*) [6].

FH patients develop early vascular lesions that remain subclinical for decades. Several surrogate markers based on the assessment of arterial structure and function are helpful for stratifying patient risk [7]. Arterial injury parameters, determined by ultrasound techniques, have been found to be useful predictors of cardiovascular (CV) mortality, coronary events, and fatal strokes [8]. Carotid intima media thickness (cIMT) and arterial stiffness, measured by pulse wave velocity (PWV) and distensibility (DIST) [9,10], are established and widely used techniques for CV risk prediction. A thicker cIMT, a higher PWV and lower DIST represent early signs of arterial injury and stiffness, which are highly associated with atherosclerosis [11–14]. Atherosclerosis is a progressive disease that in subclinical form is usually present in the first decades of life in FH patients. The mentioned surrogate parameters provide a noninvasive measurement of vascular alterations and are thus markers of cardiovascular risk [10]. These surrogate markers are influenced by multiple factors both in adults and in children. One of the most influential factors are the different lipoprotein levels, with LDL being the most important in terms of atherogenicity. However, other factors such as body mass index, systolic and diastolic blood pressure, or other diseases such as diabetes mellitus play an important role in vascular health [15].

LDL and its cholesterol cargo are the main cause of atherosclerosis. In addition to LDL-C levels, the quality and quantity of LDL particles have an important impact on CV risk. Several studies have addressed the importance of LDL particle quality in adults, showing that small dense LDL particles are sensitive predictors of CV risk in FH [16]. The small size of LDL allows them to infiltrate more easily into the arterial intima, favouring the arteriosclerotic process [17]. In addition, due to their small size, they tend to have less affinity for the LDL receptor [18]. Furthermore, we also observed that small LDL particles were associated with cIMT in children with FH, suggesting that the quality of LDL particles is also important in hypercholesterolemic children [19]. However, more evidence is needed in how lipoprotein quantity, but also quality affects vascular physiology. Serum nuclear magnetic resonance (NMR) allows us to analyse the total particle number and the quality of the different lipoprotein groups (VLDL, LDL and HDL) and subgroups according to their class and size [20].

Several studies have demonstrated that adult patients with FH present an early cIMT increase and greater arterial stiffness, measured as PWV and DIST, which is associated with a higher CV risk [21–24]. It has been described that cIMT increases faster in children with FH [25,26], although some studies did not observe this fact [19]. Published data regarding PWV in children with FH are scarce and sometimes contradictory. For instance, while some studies reported increased PWV values in children with FH compared to age- and sex-matched controls [27–29], Ershova et al. did not find any differences in either local carotid PWV or carotid femoral PWV between 10- to 29-year-old patients with FH and control subjects [30]. In addition, a meta-analysis published in 2019 also found no differences between subjects with FH and controls under 20 years of age [31]. Regarding DIST, there is even less evidence, although two studies showed decreased DIST in children with FH compared to age- and sex-matched controls [27,32].

Very few studies have addressed the differences in the quantity and quality of lipoprotein particles assessed by NMR between children with and without FH [33]. Moreover, there is little evidence on how the quality of the different lipoproteins impacts the vascular mechanism and the arterial stiffness in children with FH [19], with no previous data on the association with PWV and DIST in these type of patients.

Therefore, the purpose of the present study was to evaluate cIMT, PWV, and DIST in a large cohort of children with FH and to assess the impact of lipid subfractions measured by serum NMR with the mentioned arterial injury and stiffness parameters in order to understand better the role of the quality of the lipoproteins on the atherosclerotic process of the children with FH. More information on the impact of different lipoprotein quality on vascular parameters would allow clinicians to stratify children's risk and thus individualise lipid-lowering therapies. Moreover, we have also studied how influential factors such as BMI and systolic and diastolic blood pressure affect the different vascular markers.

2. Methods

2.1. Study design and patients

This cross-sectional study included 201 children and teenagers between 4 and 18 years attending the Lipid Unit of our Hospital. They were part of the "Early Familial Hypercholesterolemia Detection Project" (DECOPIN Project), which focuses on the implementation of opportunistic, direct and reverse cascade FH screening. Children were considered FH if they had a positive genetic test or LDL-C $>$ 150 mg/dL and one of the parents with the Dutch Lipid Clinic Network scale $>$8 in the case of no available or negative genetic test result. The control children group (CCh) included children and adolescents attending our unit because of FH suspicion but not fulfilling FH criteria and non-affected siblings of FH children. When patients were included, they were not receiving lipid-lowering

therapy. Children with chronic renal, hepatic or thyroid disease and type 1 diabetes mellitus, eating disorders, autoimmune disease, homozygous FH and other chronic diseases were excluded from the study. Medical history, physical examination and anthropometric data were collected. Blood pressure was measured using an oscillometric technique with the Press-Mate BP-8800C (COLIN medical instruments, San Antonio, United States) on the left arm, while the child remained seated with the arm resting comfortably, using the most adequate cuff size for each participant. Systolic (SBP) and diastolic (DBP) blood pressure were each measured twice, with a time interval of at least 5 min between the measurements. The average of both measurements was used. The body mass index score (BMI) was calculated by the following equation: [(BMI children - BMI 50th percentile of Orbegozo's growth curves)/standard deviation (SD) 50th percentile of Orbegozo's growth curves] [34].

The Ethics Committee of our Research Institute approved the study protocol. All participants or participant's tutors provided written consent, and the study protocol conformed the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Blood sample collection and standard biochemical analysis

Blood samples were collected after overnight fasting. BioBanc of our centre stored the plasma and serum aliquots at −80 °C until further use.

Enzymatic colorimetric tests were used for measuring serum cholesterol and triglyceride levels (CHOD-POD for cholesterol and GPO-POD for triglycerides). Immunoturbidimetric assays were used for measuring apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB). HDL cholesterol was measured using a direct enzymatic colorimetric method, Spintrol H CAL GC-MS reference methods were assessed to analyse the lipid profile. Spintrol "H" Normal was used as a quality control. Reagents were from Spinreact SA (Spain) and were performed in a Cobas Mira Plus autoanalyser (Roche diagnostics, Spain). LDL cholesterol was calculated by the Friedewald formula.

2.3. 2D-1H NMR lipid profile evaluation

Lipoprotein analysis of serum samples was performed with the Liposcale® test (Biosfer Teslab, Reus, Spain), a 2D diffusion-ordered [1]H NMR spectroscopy-based method, as previously described [35]. A 500-ml aliquot of plasma was shipped on dry ice to Biosfer Teslab for lipoprotein analysis. The particle size and the total particle number of LDL (*low-density lipoprotein*), HDL (*high-density lipoprotein*) and VLDL (*very low-density lipoprotein*) were determined as previously reported [35]. Particle concentrations and diffusion coefficients were obtained from the measured amplitudes and attenuation of their spectroscopically distinct lipid methyl group NMR signals using the 2D diffusion ordered NMR spectroscopy pulse. By this method, the hydrodynamic characteristics of the molecules can be measured as is the case of the diffusion coefficient associated with each subclass of lipoprotein. From the diffusion coefficients, the sizes of different subclasses of lipoproteins are directly calculated through the Stokes-Einstein equation. The direct measurement of the size, as in this method, is of particular importance since it is used to calculate the number of lipoprotein particles. The methyl signal was surface fitted with 3 Lorentzian functions associated with each lipoprotein subtype. The area of each Lorentzian function reflected the lipid concentration of each lipoprotein subtype, and the size of each subtype was calculated from the diffusion coefficient. The particle number of each lipoprotein fraction was calculated by dividing the lipid volume by the particle volume of a given class. Lipid volumes were determined using common conversion factors to convert concentration units obtained from the partial least-squares models into volume units. The variation coefficients for particle number were between 2% and 4%. The variation coefficients for particle size were lower than 0.3%

2.4. cIMT, PWV and distensibility measurement

The cIMT of the right and left common carotid arteries was determined using a MyLab 60-X Vision sonographer (Esaote, Genova, Italy). A 7,5e10 MHz linear transducer and semiautomatic radio frequency software was used (QIMT©, Esaote). The images were obtained and measured by a single operator to reduce observer variability. We averaged the measurements of the left and right carotid arteries to obtain the mean cIMT.

Arterial stiffness expressed by the PWV and DIST was measured directly at both common carotid arteries using the ultrasound linear probe (5−12 MHz) as a tonometer and analysed in vivo by the Quality Arterial Stiffness (QAS©) radiofrequency software (Esaote SpA, Genova, Italy). The PWV was obtained from brachial blood pressure and the accurate measurements of diameter and change in diameter of carotid arteries. DIST was the change in diameter of the carotid artery secondary to intravascular volume expansion caused by the left ventricle systole. Vascular stiffness parameters were calculated after calibration for blood pressure and final values were the median measurements of the right and left carotid arteries.

2.5. Statistical analysis

The results are expressed as the mean ± SD for normally distributed data, as the median (interquartile range (IQR)) for data that were not normally distributed and as frequencies for categorical data. Shapiro test was used to ensure that data was normally distributed. T-tests were used to determine significant differences when the data were normally distributed. Mann-Whitney tests were used to detect significant differences when the data were not normally distributed. cIMT, PWV and DIST were categorized into binary variables, named pathological cIMT (PAT-cIMT), pathological PWV (PAT-PWV) and pathological DIST (PAT-DIST), respectively. Values above the 75th percentile

of the cIMT and PWV were categorized as pathological. Regarding DIST, the values categorized as pathological were the ones below the 25th percentile. Linear and logistic regression analyses were used to analyse the associations of the studied variables with cIMT, PWV and DIST. Models were initially adjusted for age, sex, BMI score, SBP, DBP, triglycerides, ApoA1, ApoB, ApoA-ApoB Ratio, and all the NMR variables, which included total particle number, large, medium and small particles and the diameter of VLDL, LDL and HDL. To avoid multicollinearity and to select the most import features, elastic net regressions and Random Forest (RF) models were performed. Elastic net regressions include in one equation LASSO and ridge penalizations, which not only select the most important features but also perform correctly with collinear data. On the other hand, RF is a supervised classification algorithm based in the growth of conditional inference trees that allows evaluating the importance of each variable in the classification. The importance of each variable is measured in terms of mean decrease in Gini coefficient, which measures how each variable contributes to the homogeneity of the nodes and leaves in the resulting RF. Finally, only the statistically associated variables were selected. The final models for cIMT, PWV and DIST are shown in Supplementary Table 1. The receiver operating characteristic (ROC) curves and area under the ROC curve (AUC) values were calculated as a measure of the classification accuracy of each adjusted logistic regression. The R-squared (R^2) statistic is provided to estimate the amount of variability explained by the model. Akaike information criteria (AIC) is also provided to evaluate the goodness of fit of the different models. A lower AIC value implies a better quality of the model. β coefficients and odds ratios (OR) are shown standardised. All analyses were performed using R Studio, version 4.0.1. $P < 0.05$ were considered statistically significant.

3. Results

3.1. Baseline characteristics

The anthropometric, clinical and standard biochemical data of FH children and controls (CCh) are presented in Table 1. The median age of the participants was approximately 10 years, without significant differences between groups. No significant differences regarding sex, BMI score, waist circumference, systolic blood pressure (SBP) or diastolic blood pressure (DBP) were observed. The FH and control children (CCh) groups presented significant differences in total cholesterol, HDL-C, LDL-C, ApoA1, ApoB, and the ApoB/ApoA ratio ($p < 0.05$).

3.2. 2D-1H NMR lipid profile evaluation

2D-1H NMR data for FH and CCh are presented in Table 2. FH children presented significantly higher total LDL and all subclasses (large, medium and small) ($p < 0.0001$ for all tests) than CCh children. The total particle LDL number was 40% higher in FH children than in CCh children, with small particles being 49% higher. FH children also presented increased values of large and medium HDL particles and medium VLDL particles in comparison with CCh. The mean particle sizes of LDL and HDL were also higher in children with FH ($p < 0.0001$).

3.3. Arterial injury and stiffness parameters

cIMT.

Although there was a trend of higher cIMT in children with FH, the difference was not statistically significant (Table 1) ($p = 0.37$).

Regarding the variables associated with cIMT in the FH group, elastic net regressions and RF models selected large HDL particles, SBP, age, sex and BMI score as important variables. When a multivariate linear regression model was fit with the selected variables, we observed that large HDL particles (β = 0.24, $p = 0.02$), SBP (β = 0.32,

Table 1 Characteristics of the FH and control children groups. Data are presented as mean ± SD for normally distributed data and as median and interquartile range (IQR) for non-normally distributed data. The percentage is used for categorical variables. The Mann-Whitney test was used for data with a non-normal distribution. T-tests were used for data with a normal distribution and X^2 tests were used for categorical variables.

	FH (n = 96)	CCh (n = 105)	p-value
Clinical data			
Age (years)	9 (6–12)	10 (7–12)	0.33
Male (%)	54.16	57.14	0.77
BMI score	0.21 ± 0.93	0.15 ± 0.97	0.45
Waist circumference (cm)	62 (53–70)	61 (55.88–71.25)	0.54
SBP (mm Hg)	108.35 ± 12.93	108.9 ± 11.43	0.75
DBP (mm Hg)	64 (58–70)	62 (57–69)	0.42
Biochemical data			
Total cholesterol (mg/dL)	259.5 (228.8–295.2)	190 (170–204)	<0.0001
HDL-C (mg/dL)	59.50 (51–70)	65 (56–73)	0.039
LDL-C (mg/dL)	182 (149–219)	112 (96–124)	<0.0001
Triglycerides (mg/dL)	63.50 (49.50–87.25)	59 (45–74)	0.09
ApoA1 (mg/dL)	146.5 (131–159.8)	154.5 (139.2–171.8)	0.006
ApoB (mg/dL)	129 (113.5–153.8)	91 (79–97.75)	<0.0001
ApoB/ApoA ratio	0.94 (0.69–1.12)	0.55 (0.47–0.67)	<0.0001
Lp(a) (mg/dL)	15.83 (7.08–66)	11.23 (4.48–40.83)	0.11
Glucose (mg/dL)	81.54 ± 8.83	82.15 ± 7.95	0.60
Creatinine (mg/dL)	0.5 (0.43–0.62)	0.51 (0.44–0.61)	0.82
GGT (U/L)	11 (9–12)	12 (10–13)	0.13
TSH (U/L)	2.54 (1.89–3.27)	2.36 (1.72–3.13)	0.27
Arterial stiffness markers			
cIMT (μm)	429.5 (384–458.5)	417.5 (383–450.5)	0.37
PWV (m/s)	4.79 (4.10–5.31)	4.8 (4.23–5.31)	0.86
Distensibility (kPa)	6.22 ± 0.46	6.23 ± 0.52	0.86

FH, familial hypercholesterolaemia; CCh, Control children.

$p = 0.004$), age ($\beta = 0.26$, $p = 0.01$) and BMI score ($\beta = 0.25$, $p = 0.02$) were statistically associated with cIMT in the FH group. This model explained 35.03% of the cIMT variability, and large HDL particles accounted for 11% of the total amount of variability explained by the model. Model quality also increased when large HDL particles were introduced into the model, as the AIC decreased from 226.81 to 202.95 (Table 3A). However, when we fit the same model for CCh and the overall cohort, large HDL particles were not statistically associated with cIMT (Supplementary Table 2). The variables associated with PAT-cIMT according to the elastic net regressions and RF models were small LDL particles, SBP, age and BMI score. When a logistic regression model was fit with the selected variables, we observed that increased small LDL particles (OR = 1.36, $p = 0.02$), increased BMI score (OR = 1.42, $p = 0.01$), increased age (OR = 1.61, $p = 0.001$) and increased SBP (OR = 1.61, $p = 0.001$) increased the odds of being in the PAT-cIMT group in the FH group of children. The model explained 13.21% of the PAT-cIMT variability. The smaller LDL particles accounted for 2.01% of the mentioned variability, increased the AUC of the ROC curves from 0.72 to 0.74 (Fig. 1A) and decreased the AIC from 377.45 to 336.14, which overall increased the quality of the model (Table 3A). This association was not maintained in CCh or in the overall cohort (Supplementary Table 2). Interestingly, LDL and HDL cholesterol were not statistically associated with either cIMT or PAT-cIMT in children with FH.

3.4. PWV

Table 1 shows the PWV values sorted by FH children and CCh ($p = 0.09$). No significant differences were observed.

Elastic net regressions and RF models selected LDL diameter, SBP, DBP and ApoA1 as important variables associated with PWV in children with FH. When a multivariate linear model was fit with the mentioned variables, we observed that LDL diameter ($\beta = 0.27$, $p = 0.008$), SBP ($\beta = 0.45$, $p = 0.0008$), DBP ($\beta = -0.37$, $p = 0.008$) and ApoA1 ($\beta = 0.20$, $p = 0.04$) were associated with PWV. The model explained 26% of the PWV variability, and LDL diameter accounted for 5% of the mentioned variability and reduced the AIC from 224.07 to 211.92, increasing the quality of the model (Table 3B). However, this association was not maintained in CCh or in the overall cohort (Supplementary Table 3). Elastic net regressions and RF models selected LDL diameter, SBP and DBP as being associated with PAT-PWV. When a logistic regression was fit, we observed that large LDL diameter (OR = 1.82, $p = 0.005$) and SBP (OR = 3.76, $p = 0.007$) increased the odds of being in the PAT-PWV group and that increased DBP (OR = 0.41, $p < 0.0001$) decreased the odds of being in the PAT-PWV group. The model explained 16.11% of the PAT-PWV variability, and LDL diameter accounted for 2.9% of the variability explained by the model, increasing the AUC from 0.73 to 0.78 (Fig. 1B) and decreasing the AIC from 163.27 to 151.76, which overall increased the model quality (Table 2B). This association was not maintained in CCh, but it was maintained in the overall cohort (OR = 1.36, $p = 0.01$) (Supplementary Table 3).

3.5. Distensibility

Distensibility was not different in children with FH and CCh ($p = 0.87$) (Table 1).

Elastic net regressions and RF models selected HDL particle number, DBP, age, ApoB and BMI score as the main variables influencing DIST in FH children. When a multivariate linear model was fit with these variables, we observed that HDL particle number ($\beta = 0.22$, $p = 0.042$), DBP ($\beta = -0.21$, $p = 0.039$) and age ($\beta = 0.22$, $p = 0.049$) were significantly associated with DIST. This model

Table 2 Lipoprotein subclass analysis assessed by 2D-1H NMR. Data are presented as median and interquartile range (IQR) as all the data is non-normally distributed. The Mann-Whitney test was used to compare subgroups.

	FH (n = 96)	CCh (n = 105)	p-value
Lipoprotein particle number and composition			
VLDL (nM)	24.82 (18.61–38.92)	23.93 (17.51–31.83)	0.3218
Large VLDL (nM)	0.75 (0.51–1)	0.75 (0.51–1.03)	0.73
Medium VLDL (nM)	3.30 (2.31–4.25)	2.75 (1.67–3.63)	0.005
Small VLDL (nM)	20.61 (15.54–33.33)	20.28 (15.52–27.34)	0.5
LDL (nM)	1757 (1488.5–2059.6)	1256.8 (1112.2–1350.6)	<0.0001
Large LDL (nM)	258.2 (216–296.7)	176.42 (151.17–196.62)	<0.0001
Medium LDL (nM)	610 (463.5–755.8)	363.2 (281.8–423.6)	<0.0001
Small LDL (nM)	851.8 (775.2–1008)	694.2 (630.1–753.3)	<0.0001
HDL (μM)	27.35 (23.98–30.61)	26.21 (23.70–30.24)	0.44
Large HDL (μM)	0.28 (0.25–0.31)	0.22 (0.21–0.25)	<0.0001
Medium HDL (μM)	9.44 (8.60–10.24)	8.43 (7.57–9.89)	<0.0001
Small HDL (μM)	17.61 (15.17–20.33)	17.61 (15.69–20.80)	0.43
Lipoprotein size (diameter, nm)			
VLDL (nm)	42.31 (42.01–42.26)	42.18 (41.85–42.45)	0.043
LDL (nm)	21.33 (21.14–21.52)	21.07 (20.89–21.22)	<0.0001
HDL (nm)	8.29 (8.24–8.33)	8.24 (8.2–8.27)	<0.0001

FH, familial hypercholesterolaemia; CCh, Control children.

Table 3 Summary of the different linear and logistic regressions for children with FH. **A** cIMT and PAT-cIMT **B** PWV and PAT-PWV **C** DIST and PAT-DIST.

A					
cIMT					
	β	p	R^2 (%)	AIC	
Large HDL particles	0.24	0.02			
SBP	0.32	0.004			
Age	0.26	0.01			
BMI score	0.25	0.02	35.03	202.95	
PAT-cIMT					
	OR	**p**	**R^2 (%)**	**AIC**	**AUC**
Small LDL particles	1.36	0.02			
BMI score	1.42	0.01			
SBP	1.61	0.001			
Age	1.61	0.001	13.21	336.14	0.74
B					
PWV					
	β	**p**	**R^2 (%)**	**AIC**	
LDL diameter	0.27	0.007			
SBP	0.45	0.0008			
DBP	−0.37	0.008			
ApoA1	0.20	0.04	26	211.92	
PAT-PWV					
	OR	**p**	**R^2 (%)**	**AIC**	**AUC**
LDL diameter	1.82	0.005			
SBP	3.76	0.007			
DBP	0.41	<0.0001	16.11	151.76	0.78
C					
DIST					
	β	**p**	**R^2 (%)**	**AIC**	
Total HDL particle number	0.22	0.043			
Age	0.22	0.049			
DBP	−0.21	0.039	9.88	235.74	
PAT-DIST					
	OR	**p**	**R^2 (%)**	**AIC**	**AUC**
Age	0.36	<0.001			
Large HDL particles	0.40	0.03			
Total VLDL particle number	2.39	0.0009			
Large LDL particles	2.46	0.02	17.19	153.33	0.78

OR = odds ratio, p = p-value, AIC = Akaike information criteria, SBP = systolic blood pressure, DBP = diastolic blood pressure.

explained 9.88% of the DIST variability. HDL particle number accounted for 5.11% of this variability and reduced the AIC from 258.01 to 235.74, which increased its quality (Table 3C). Only age remained statistically significant in CCh and in the overall cohort (Supplementary Table 4). Elastic net regressions and RF models selected BMI score, age, large HDL particle number, LDL diameter, total VLDL particle number, large LDL particle number and ApoB as determinants of PAT-DIST. When a logistic regression was fit, we observed that increased age (OR = 0.36, $p < 0.001$), and large HDL particle number (OR = 0.39, p = 0.03) decreased the odds of being in the PAT-DIST group, and increased VLDL particle number (OR = 2.39, p < 0.001) and large LDL particles (OR = 2.46, p = 0.02) increased the odds of being in the PAT-DIST group. The model explained 17.19% of the PAT-DIST variability, and large HDL particles, VLDL particle number and large LDL particles accounted for 9.29% of the variability explained by the model, increasing the AUC from 0.68 to 0.78 (Fig. 1C) and

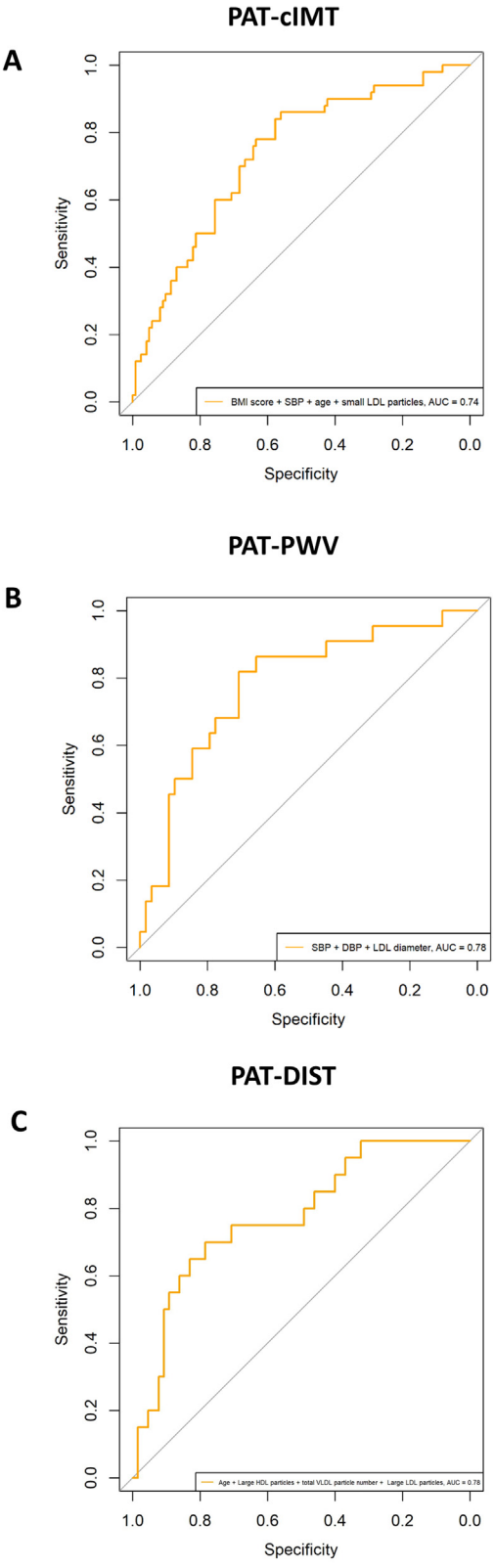

Figure 1 ROC curves of the different logistic regressions of the models studying the associations on children with FH. **A** model for PAT-cIMT; **B** model for PAT-PWV; **C** model for PAT-DIST.

decreasing the AIC from 175.01 to 153.33, thus increasing the model quality (Table 3C). Regarding CCh, age and large LDL particles were also associated with PAT-DIST. In the overall cohort, only age was associated with PAT-DIST (Supplementary Table 4). HDL cholesterol was also statistically associated with PAT-DIST in FH children but with less classification power than total HDL particles (AUC = 0.73).

4. Discussion

Patients with familial hypercholesterolaemia are at high risk of premature CV disease events due to their genetically determined lifelong exposure to high LDL-C levels. Several of the alterations in the arterial wall, a product of high exposure to LDL-C, can already be observed during childhood [32]. Furthermore, not only the quantity but also the quality of the LDL particles is important. Lipoproteins vary in size, density, lipid and apolipoprotein composition, with the small dense LDL being known to be particularly atherogenic due to their susceptibility to chemical modifications and to their less affinity for the LDL receptor [16]. The impact of the sizes and densities of the different lipoproteins, especially on LDL, on CV disease is well known in adults. However, less results have been published in this matter regarding children with FH and would be an interesting tool to stratify patient's risk. Increased cIMT, assessed by ultrasonography, and arterial stiffness, as measured by PWV and DIST, are representatives of arterial injury and stiffness and are frequently used as surrogate CV disease endpoints in clinical studies [12,36]. The present work evaluates cIMT, PWV and DIST as markers of arterial injury and stiffness in a cohort of FH children, studying the impact of these parameters with clinical variables and advanced lipid measurements obtained with NMR lipoprotein profile analysis.

Carotid IMT is considered a marker of cholesterol content in the artery wall. We did not find statistically significant differences in cIMT between FH and non-FH children, although FH children had slightly higher cIMT values. This result is consistent with a preliminary publication by our group [19]. Other studies have found that children with FH show increased cIMT values compared to non-FH children [25,26]. The causes of these conflicting results could include biases in the study design, sample size, or differences in measurement techniques. Additionally, the impact of FH on cIMT may not be uniform across all children. In our study, age, blood pressure and obesity had the main impact on cIMT. Furthermore, beyond lipid concentrations, the number and size of lipoprotein particles also influence this vascular parameter. The variable selection methods employed in our study selected large HDL particles as a relevant variable positively associated with cIMT. Age, SBP and BMI are well known and widely accepted predictors of cIMT [37–39]. Regarding HDL, children with FH had lower HDL-C and ApoA1 but similar HDL particle numbers than controls, suggesting differences in structure and composition. In fact, children with FH had more large and medium HDL particles, leading to a larger HDL mean size. The direct association between larger HDL and vascular risk was observed in the IDEAL and EPIC-Nortfolk cohorts, suggesting that these HDL particles could be less functioning [40]. On the other hand, along with BMI, age and blood pressure, the best lipoprotein parameter to identify individuals with PAT-cIMT was the number of small LDL particles, as has been previously published [19]. Small LDLs have higher affinity for subendothelial component and less affinity for the LDL receptors and consequently exhibit longer retention times in the arterial wall, leading to increased susceptibility to atherosclerosis [41]. These results are consistent with the fact that artery wall thickness, which is determined by the lipid and cell content, is modulated by the quantity and quality of atherogenic lipoproteins.

Arterial stiffness was evaluated with PWV and DIST. Arterial rigidity is determined by several factors, such as artery wall structure and composition but also by regulating factors such as endothelium function. In this regard, a recent study showed that PCSK9 plasma levels could increase the negative impact of LDL mechanical vascular impairment. In addition, it was also observed that reducing PCSK9 levels decreased the PWV in patients with FH [42]. Therefore, PWV and DIST can be influenced by many anatomical, histological, and physiological factors. We did not observe differences in these values between the FH and non-FH groups. Controversial results have been published on this issue, and some studies have detected increased rigidity in FH groups [27–29]. However, methodological differences could explain the controversial results. A recent meta-analysis concluded that there were no differences in the PWV between adolescent FH patients and controls [31]. However, in the FH group, variable selection methods showed that quantitative and qualitative LDL and HDL particle characteristics along with blood pressure and age were the main factors associated with artery rigidity evaluated by PWV or DIST. These observations agree with data in the literature where SBP, DBP and ApoA1 have been associated with PWV in FH [43]. On the other hand, the role of HDL-C and ApoA1 in arterial stiffness is controversial, as contradictory results have been published [44,45]. As there are few publications focusing on the relationships between ApoA1 and PWV and even fewer with DIST [24,29], unanimous conclusions cannot be drawn. In our study, the total number of HDL particles was associated with higher DIST [44,45]. The impact of LDL particles on arterial stiffness was suggested by the positive association between LDL size and PWV and the odds of being classified as PAT-PWV and PAT-DIST.

In our study, age showed a positive association with DIST. Age is in general a predictor of lower DIST in adults reflecting the senescence process [46]. However, in children has to be seen as a physiological process of growth associated to physiology optimization. We also observed that increased VLDL particle number increased the odds of being in the PAT-DIST group. Our results are in line with the published evidence that triglycerides are highly correlated with inflammation and have been widely associated with arterial injury and stiffness markers, both

in adults and in children [47,48]. Furthermore, an increase in the total number of HDL particles was also positively associated with distensibility, which is in the direction that HDL particles show an anti-atherogenic nature [49].

This study shows that in children with FH, lipoprotein particle number and size measured with NMR present associations with cIMT and arterial stiffness that traditional lipid parameters do not show. Interestingly, in FH children, beyond lipoprotein values, blood pressure and adiposity parameters have a significant influence on arterial structure and functionality even in this group of otherwise healthy FH young kids with blood pressure and weight within normal ranges. Therefore, in children with FH, cardiovascular preventive actions should involve all components of a healthy lifestyle beyond LDL-C. Furthermore, most of the associations found in the children with FH were not found on our controls, which suggest that children with FH may exhibit changes in the lipoprotein composition secondary to the LDL receptor pathway impairment.

The study has some limitations. First, we cannot conclude causality of the associations observed in the ultrasonographic variables due to the cross-sectional design of our study. However, the next step in this work is performing a longitudinal study to confirm the causal relationship of our associations as well as evaluating the impact of lipid lowering therapy on mechanical vascular impairment in children with FH. Secondly, the age range of our groups coincided with puberty, when body size and the impact of hormonal variations in lipid parameters are difficult to control. Finally, although this is one of the largest cohorts of children with FH, the absolute sample size is still limited. Our control group was formed by children and adolescents suspicious of FH; therefore, *sensu stricto,* they cannot be considered representative of the general healthy population.

In conclusion, in the larger lipidomic study performed in an accurate controlled FH population, we showed significant associations between clinical variables and lipid particles measured by NMR and structural and functional markers of arterial injury and stiffness. Blood pressure and adiposity act as cardiovascular risk-enhancing factors in these genetically driven hypercholesterolaemic patients. Our findings might contribute to understanding the underlying mechanism that influences these associations and are interesting tools to stratify children's risk. However, further studies are needed to definitively clarify these points.

Author contributions

DLL, JCV and LM performed the conceptualization of the study and carried out the data curation and the formal analysis. DI and NP measured the ultrasonographic parameters. DI, NP and CRB selected the patients. DLL performed the statistical analyses. DLL, JCV and LM wrote the original draft. JCV, LM, AF and JE reviewed and approved the final version of the manuscript. All authors supervised and validated the study.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **LM:** fees for lectures/advisory work from AMGEN, AMARIN, AMRYT, SANOFI, NOVARTIS, SERVIER, DAIICHI-SAMKYO. DLL, AF, DI, JE, NP, CBR and JC declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.06.012.

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