Atherosclerosis 373 (2023) 38–45

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Association between Nordic and Mediterranean diets with lipoprotein phenotype assessed by ^{1}HNMR in children with familial hypercholesterolemia

Cèlia Rodríguez-Borjabad [a,b,c], Ingunn Narveud [c,d], Jacob Juel Christensen [c], Daiana Ibarretxe [a,b], Natalia Andreychuk [a,b], Josefa Girona [a,b], Kristin Torvik [c], Guro Folkedal [c], Martin P. Bogsrud [d,e], Kjetil Retterstøl [c,f], Núria Plana [a,b], Luis Masana [a,b,*], Kirsten B. Holven [c,d]

[a] Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, Sant Joan University Hospital, Universitat Rovira i Virgili, IISPV, Reus, Spain
[b] Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain
[c] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[d] Norwegian National Advisory Unit on Familial Hypercholesterolemia, Oslo University Hospital, Rikshospitalet, P. O Box 4950, Nydalen, Norway
[e] Unit for Cardiac and Cardiovascular Genetics, Oslo University Hospital, P. O Box 4956, Nydalen, Norway
[f] The Lipid Clinic, Oslo University Hospital, Rikshospitalet, P. O Box 4950, Nydalen, Norway

ARTICLE INFO

Keywords:
Familial hypercholesterolemia
Children
Nordic diet
Mediterranean diet
NMR lipid profile

ABSTRACT

Background and aims: Both Nordic and Mediterranean diets are considered healthy despite notable regional differences. Although these dietary patterns may lower cardiovascular risk, it is unclear if they improve the lipoprotein phenotype in children with familial hypercholesterolemia (FH). The aim is to determine the impact of Nordic and Mediterranean diets on the advanced lipoprotein profile in children with heterozygous FH (HeFH).
Methods: This was a cross-sectional study performed in children with FH recruited from the Lipid Clinics at Sant Joan University Hospital in Reus (Spain) and Oslo University Hospital (Norway).
Two-hundred fifty-six children (mean age 10 y/o; 48% girls): 85 Spanish and 29 Norwegian FH children, and 142 non-FH healthy controls (119 from Spain and 23 from Norway) were included in the study. A pathogenic FH-associated genetic variant was present in 81% of Spanish children with FH and all Norwegian children with FH. An ^{1}H NMR based advanced lipoprotein test (Nightingale®) providing information on the particle number, size and lipid composition of 14 lipoprotein subclasses was performed and correlated to the dietary components.
Results: Levels of LDL-C, HDL-C and triglycerides were not significantly different between the Nordic and Mediterranean FH groups. Spanish children with FH had more LDL particles, mainly of the large and medium LDL subclasses, than Norwegian FH children. Spanish FH children also had more HDL particles, mainly medium and small, than Norwegian FH children. The mean LDL size of Spanish FH children was larger, while the HDL size was smaller than that of the Norwegian FH children. The HDL particle number and size were the main determinants of differences between the two groups. In Norwegian children with FH, dietary total fat and MUFAs showed a significant correlation with all apolipoprotein B-containing lipoproteins and LDL size, whereas there was no correlation to SFA. A weaker association pattern was observed in the Spanish children.
Conclusions: The lipoprotein profiles of Spanish and Norwegian children showed differences when studied by ^{1}H NMR. These differences were in part associated with differences in dietary patterns.

1. Introduction

Heterozygous familial hypercholesterolemia (HeFH) is a relatively common inherited disorder caused by genetic variants in the low-density lipoprotein (LDL) receptor or functionally related genes [1]. Children born with this genetic condition are at increased risk for atherosclerotic cardiovascular disease (CVD). During childhood, HeFH is asymptomatic and is detected by high cholesterol levels and family history. Implementing screening strategies for familial hypercholesterolemia (FH) detection in children leads to early diagnosis and

* Corresponding author. Facultat de Medicina, Universitat Rovira i Virgili, C/. Sant Llorenç, 21, 43007, Reus, Spain.
E-mail address: luis.masana@urv.cat (L. Masana).

https://doi.org/10.1016/j.atherosclerosis.2023.04.009
Received 21 November 2022; Received in revised form 20 April 2023; Accepted 21 April 2023
Available online 22 April 2023
0021-9150/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

appropriate treatment, resulting in a better prognosis [2,3].

Although LDL-C elevation is unequivocally the main determinant of cardiovascular risk in FH, a significant variability in the incidence of CVD events has been reported in FH patients, even among those carrying the same genetic mutations and comparable LDL-C levels [4,5]. In addition to routine measurements, in recent years, proton-nuclear magnetic resonance (^{1}H NMR) techniques have been implemented to determine the number and size of lipoproteins [6,7]. These techniques allow us to obtain more extensive and detailed information on lipoprotein metabolism. It has been shown that knowing the amount and size of lipoprotein particles provides more detailed information about CVR [8–10] even though the predictive use still remain to be explored. Current guidelines reinforce the importance of lipoprotein number on atherosclerosis pathogenesis and recommend the measurement of apolipoprotein B (ApoB), a surrogate of atherogenic particle number, for a better CVR definition [8,9,11].

Even though the high cholesterol levels in HeFH children are driven by gene variants, environmental factors could modulate the lipoprotein phenotype. The influence of diet on CVD factors and CVD was well established many years ago [12–14]. There are several dietary components affecting the plasma concentration and function of lipoproteins. Among them, the type of fat in the diet appears to be an important determinant. Both Nordic and Mediterranean diets have been proposed to improve cardiovascular risk despite notable differences between them. The Nordic diet is characterized by the intake of berries, apples and pears, root and cruciferous vegetables, whole grain and rye bread as cereals, high intake of fish, low-fat dairy products and potatoes [15] and Mediterranean diet is characterized by high intake of fruits and vegetables, nuts and seeds, legumes, fish, low intake of meat and dairy products, and extra virgin olive oil as a major fat source [16]. These differences were confirmed in a previous study [17], where we showed that Norwegian children consumed more polyunsaturated fatty acids (PUFAs) and, in contrast, Spanish children consumed more monounsaturated fatty acids (MUFAs), cholesterol and fiber.

It is unknown to which degree dietary patterns affect the lipoprotein phenotype in children with FH. Therefore, the aim of this study was to assess the differences in the lipoprotein subclass particle in children with FH from Norway and Spain and to evaluate the association between Nordic and Mediterranean diet components with the lipoprotein profile.

2. Patients and methods

2.1. Study design and participants

This is a cross-sectional study of children with and without FH from two established cohorts from Spain and Norway. We obtained information about the full lipoprotein profile, as assessed by ^{1}H NMR [7] and dietary intake by FFQ/food record respectively, and compared the differences between the Spanish and Norwegian children.

The recruitment method and clinical characteristics of the study participants have been described previously [18–20]. Two hundred fifty-six children with and without FH (n = 114 and n = 142, respectively), aged 4 to 18, were recruited from the lipid clinics at Oslo University Hospital (Oslo, Norway) and the "Sant Joan" University Hospital (Reus, Spain). Twenty-nine untreated Norwegian HeFH children with FH-associated genetic variants were recruited between September and December 2013 and healthy children (non-FH) included in (2014–2015) with full diet composition data available were included as controls (n = 23). Eighty-five Spanish children with FH were recruited from the DECOPIN Project (The Early Familial Hypercholesterolemia Detection Project) [21] from March 2013 to May 2019. The diagnosis criteria were a positive genetic test (81%) or if a genetic test was unavailable LDL-C >4.14 mmol/L and one parent with definite FH. Children evaluated for suspected FH who did not meet the FH criteria and had an LDL-C below 3.5 mmol/L composed the Spanish non-FH group (n = 119). The exclusion criteria were the presence of secondary hyperlipidemia, severe chronic disease and other conditions that could alter nutrition or lipid metabolism.

Data on anthropometry, medical antecedents and physical examination were obtained from medical records. Standard biochemical measurements and plasma lipid profiles by ^{1}H NMR were performed. A comprehensive study of the participants' diets was also performed (see below for details).

All participants were lipid-lowering therapy naive. Before study participation, one of the parents (or the child, if older than 16 years old) signed the informed consent form. The study was approved by the Regional Committee for Medical and Health Research Ethics (East region of Norway) and by the Ethics Committee of the Hospital Research Institute in Reus (Spain).

2.2. Standard laboratory measurements

In the Norwegian group, the non-fasting blood samples for biochemical tests were obtained, while in the Spanish group, analyses were performed in samples obtained after overnight fast. TC and TG levels were determined by enzymatic colorimetric tests, HDL-C was measured by a direct enzymatic colorimetric method, and apolipoprotein levels were measured by immunoturbidimetric assays. LDL-C levels were calculated by the Friedewald equation for the Spanish samples and by a direct enzymatic method for the Norwegian group.

2.3. Advanced lipoprotein profiling by ^{1}H NMR

Nightingale's Health ® (Finland) method based on serum ^{1}H NMR analysis was used to assess the number and size of the different lipoprotein subclasses, and a targeted set of metabolites. This method gives a detailed snapshot of the metabolic profile, including the particle number of the main lipoprotein subclasses (VLDLP, IDLP, LDLP, and HDLP) and their mean size (VLDL-Z, LDL-Z, and HDL-Z). It also provides the particle number of 14 different lipoprotein subclasses: 6 VLDL [extremely large (XXL), very large (XL), large (L), medium (M), small (S) and extra small (XS)], 1 IDL, 3 LDL [L, M, S] and 4 HDL [XL, L, M, S]). The lipid cargo of all these fractions, including free and esterified cholesterol, triglycerides and phospholipids, was also determined. The method has been extensively described and used in several recent publications [19,21–24].

2.4. Dietary intake evaluation

The diet assessment method has been previously reported [17]. In brief, the diet data were collected from children and families by registered nutritionists in both cohorts during medical visit. The diets of both countries were analyzed based on validated food frequency questionnaires (FFQs). The Norwegian FFQ contained a list of 277 food items [25–28], and the Spanish FFQ contained a list of 137 food items [29–32].

In the Norwegian cohort, the daily intake of energy and nutrients was computed using the AE-14 food database and the "Kost beregningssystem" software (version 7.1, year 2014).

In the Spanish cohort, the frequencies of consumption were reported on an incremental scale with nine levels (never or almost never, 1–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, 2–3 times per day, 4–6 times per day and more than 6 times per day). The reported frequencies of food consumption were converted to the number of intakes per day and multiplied by the weight of the portion size indicated.

Validated photographic booklets were used for the estimation of portion size in both cohorts. To compare the diet components in both cohorts, the macronutrient composition of the diets was calculated from the questionnaire information.

Detailed information about the diet content has been previously described [17].

Table 1
Comparison of demographic characteristics and biochemical measurement between Norwegian and Spanish cohorts in children with and without FH.

	NORWEGIAN COHORT (n = 52)			SPANISH COHORT (n = 204)			*p*-value FH Norway *vs* Spain	*p*-value Non FH Norway *vs* Spain
	FH	Non FH	*p*-value	FH	Non FH	*p*-value		
	(n = 29)	(n = 23)		(n = 85)	(n = 119)			
Demographic characteristics								
Age (y)	11.00 (8.00–13.00)	10.50 (7.80–12.20)	0.448	9.00 (6.00–12.00)	11.00 (8.00–13.00)	**0.006**	**0.024**	0.706
Sex gender (% girls)	55.2	52.2	0.520	47.05	55.17	0.589	0.589	0.825
Z-score-BMI	0.32 (−0.24-0.91)	0.17 (−0.51-0.53)	0.587	0.27 (−0.42-0.83)	−0.07 (−0.65-0.67)	0.129	0.552	0.408
Biochemical measurements								
TC (mmol/L)	5.80 (5.20–7.10)	3.80 (3.40–4.50)	**<0.0001**	6.81 (5.93–7.82)	4.90 (4.35–5.28)	**<0.0001**	0.153	**<0.0001**
LDL-C (mmol/L)	4.10 (3.40–5.20)	2.00 (1.62–2.52)	**<0.0001**	4.82 (3.86–5.70)	2.73 (2.33–3.16)	**<0.0001**	0.249	**<0.0001**
HDL-C (mmol/L)	1.40 (1.30–1.70)	1.50 (1.30–1.80)	0.644	1.53 (1.32–1.73)	1.71 (1.40–1.97)	**0.001**	0.538	**0.024**
TG (mmol/L)	0.80 (0.60–1.10)	0.70 (0.50–1.00)	0.389	0.72 (0.56–0.99)	0.67 (0.52–0.84)	**0.035**	0.190	0.217
ApoB (g/L)	1.20 (0.90–1.40)	[a]	[a]	1.33 (1.15–1.56)	0.89 (0.74–0.97)	**0.001**	0.066	[a]
ApoA (g/L)	1.4 (1.3–1.6)	[a]	[a]	1.46 (1.30–1.57)	1.55 (1.39–1.73)	**<0.0001**	0.422	[a]
Lp (a) (mg/dL)	293.00 (154.00–581.00)	[a]	[a]	156.26 (64.26–675.87)	129.18 (45.84–425.03)	0.194	0.934	[a]

The values are presented as the median (25th-75th percentile) and percentage. Differences between demographic characteristics and lipid categories were tested using Independent *t*-test for normally distributed continuous variables, Mann–Whitney *U* test for skewed continuous variables, and Chi-square test for dichotomous variables. All the *p-value* were adjusted by age. A 2-sided *p*-value <0.05 is considered statistically significant.
FH; familial hypercholesterolemia; z-score-BMI; z-score body mass index; TC; total cholesterol; LDL-C; low-density lipoprotein cholesterol; HDL-C; high-density lipoprotein cholesterol; Triglycerides; TG; ApoB; apolipoprotein B100; ApoA; apolipoprotein A1; Lp (a); lipoprotein (a).
[a]Data not available.

2.5. Statistical analysis

Continuous data are shown as the median (25th-75th percentile) for skewed data or as frequencies (percentage) for categorical data. Kolmogorov-Smirnov tests were used to examine normality. The Mann-Whitney test was used to compare continuous variables, whereas the Chi square test was used to compare categorical variables. The lipid profile and lipoprotein particle number and size comparisons between groups were analyzed by ANCOVA and adjusted by age. The associations between lipoprotein profile and diet components were analyzed using a partial correlation adjusted for age. Significant correlations were defined by a $p < 0.05$. Those p-values that were less than 0.05 and presented a significant partial correlation coefficient >0.400 was considered of clinical interest.

To assess the relationships between lipid variables and patient provenance in order to seek differences between Norwegian and Spanish FH children, we carried out a series of multivariate models. In these models, we predicted the Cohort (Norway or Spain) using all the lipid and lipoprotein variables as predictors, which allowed us to analyse lipid differences between cohorts.

To assess the effect of the predictive variables on the target, we carried out a dual approach. Random forests and boosted models proved to be more accurate models for such complex scenarios [33] and furthermore allowed us to assess the relative importance of each variable against all others via out-of-bag accuracy before and after variable permutation [34]. Both models are slightly different in variable selection, but close enough to be able to combine them to draw meaningful conclusions. A receiver operating characteristic (ROC) curve based on a model including the particle number of all lipoprotein subclasses was performed to estimate the differences between both cohorts. For further details, please see Supplementary Material 1.

All analyses were performed using the SPSS 25.0 statistical package for Windows (SPSS, IBM®, Chicago, IL) and the R statistical program version 4.0 (R Core Team, 2014). A *p*-value <0.05 was considered statistically significant in all analyses.

3. Results

In Table 1, we show the baseline characteristics of children with and without FH in the Norwegian and Spanish cohorts. Spanish children with FH were younger than the Norwegian group. Sex and z-score body mass index (z-score BMI) was similar in the two groups. Regarding the standard lipid profile, nonsignificant differences were observed between Norwegian and Spanish FH children. Regarding the non FH children, the Spanish group showed higher LDL-C and HDL-C while TG were similar.

Despite showing no differences in the standard lipid profile, the Spanish FH children had a significantly higher number of LDL particles ($p = 0.038$), mainly the L_LDL ($p = 0.032$) and M_LDL subclasses ($p = 0.031$), compared to Norwegian FH children (Table 2). Although the S_LDL also showed a trend to be higher in the Spanish cohort, the difference did not achieve statistical significance ($p = 0.126$*);* moreover, the proportion of S-LDL was not different between Spanish and Norwegian FH cohorts and similar to the nonFH group (Supplementary Fig. 1). The Spanish cohort showed a higher number of HDL particles ($p < 0.0001$), mainly the M_HDL ($p = 0.004$) and S_HDL subclasses ($p < 0.0001$). Regarding the particle size, LDL-Z was smaller in the Norwegian FH children ($p < 0.0001$), while they had larger HDL-Z ($p = 0.003$) compared to Spanish children.

The abovementioned differences were further confirmed by a gradient boosting analysis, where the HDLP number, mainly the S-HDL particle, but also the L- and XL-HDL particle, was the main determinant of lipoprotein metabolism differences between Norwegian and Spanish FH cohorts (Fig. 1). Fig. 2 focused in the differences in the number of total and S-HDL particle between FH cohorts, sorted by sex.

An ROC curve based on a model, including only the particle number of VLDL, LDL and HDL and their subclasses, discriminated Norwegians

Table 2
Lipoprotein particle number and size of Norwegian and Spanish non-FH and FH children.

	NORWEGIAN COHORT (n = 52)			SPANISH COHORT (n = 204)			*p*-value FH Norway *vs* Spain	*p*-value Non FH Norway *vs* Spain
	FH (n = 29)	Non FH (n = 23)	*p*-value	FH (n = 85)	Non FH (n = 119)	*p*-value		
Lipoprotein particle number								
VLDL (nmol/L)	175.00 (157.00–235.00)	126.00 (100.00–158.00)	**<0.0001**	191.97 (167.31–236.43)	154.70 (128.07–188.42)	**<0.0001**	0.768	**0.009**
XXL-VLDL (nmol/L)	0.14 (0.00–1.00)	0.02 (0.01–1.07)	1.000	0.01 (0.00–0.52)	0.01 (0.00–0.34)	1.000	1.000	1.000
XL-VLDL (nmol/L)	2.55 (1.74–4.64)	1.90 (1.16–3.50)	0.274	2.56 (1.72–3.87)	1.78 (0.91–3.08)	**0.005**	1.000	1.000
L-VLDL (nmol/L)	8.96 (6.11–13.70)	6.69 (5.01–11.20)	0.316	8.66 (6.50–12.67)	7.22 (4.56–10.44)	**0.013**	1.000	1.000
M-VLDL (nmol/L)	49.50 (43.00–65.50)	31.20 (22.50–38.80)	**<0.0001**	58.16 (47.10–71.91)	43.33 (33.59–52.51)	**<0.0001**	0.226	**<0.0001**
S-VLDL (nmol/L)	44.90 (39.10–57.50)	34.10 (26.50–43.70)	**0.002**	47.77 (41.04–60.18)	40.40 (32.92–52.42)	**<0.0001**	0.589	**0.017**
XS-VLDL (nmol/L)	73.30 (65.30–91.40)	51.90 (45.90–62.20)	**<0.0001**	77.29 (64.64–88.41)	63.49 (50.77–73.55)	**<0.0001**	0.657	**0.004**
IDL (nmol/L)	405.00 (355.00–530.00)	256.00 (220.00–297.00)	**<0.0001**	479.00 (386.55–558.37)	364.17 (291.70–425.00)	**<0.0001**	0.397	**<0.0001**
LDL (nmol/L)	1535.75 (1300.49–1995.76)	928.00 (779.00–1149.56)	**<0.0001**	1859.52 (1517.26–2160.51)	1361.62 (1153.34–1691.57)	**<0.0001**	**0.038**	**<0.0001**
L-LDL (nmol/L)	883.00 (739.00–1121.59)	502.00 (425.00–642.00)	**<0.0001**	1086.94 (867.68–1265.81)	781.25 (657.19–968.41)	**<0.0001**	**0.032**	**<0.0001**
M-LDL (nmol/L)	396.00 (344.00–543.00)	240.00 (205.00–305.00)	**<0.0001**	492.75 (406.48–563.44)	359.83 (30.30–443.01)	**<0.0001**	**0.031**	**<0.0001**
S-LDL (nmol/L)	253.00 (220.00–319.00)	177.00 (147.00–199.00)	**<0.0001**	290.97 (245.08–332.20)	225.54 (191.76–268.59)	**<0.0001**	0.126	**<0.0001**
HDL (μmol/L)	21.23 (20.14–22.68)	21.23 (20.14–22.68)	0.212	25.53 (23.42–27.60)	25.85 (23.31–27.98)	0.423	**<0.0001**	**<0.0001**
XL-HDL (μmol/L)	0.44 (0.38–0.50)	0.37 (0.31–0.41)	**<0.0001**	0.40 (0.33–0.46)	0.35 (0.28–0.46)	0.215	0.074	0.356
L-HDL (μmol/L)	2.57 (2.21–3.12)	2.51 (1.84–2.81)	0.102	2.61 (2.08–2.98)	2.39 (1.96–3.33)	0.446	0.783	0.112
M-HDL (μmol/L)	5.20 (4.84–6.12)	5.50 (4.97–5.98)	0.565	6.05 (5.41–6.88)	6.34 (5.56–7.46)	**0.023**	**0.004**	**<0.0001**
S-HDL (μmol/L)	13.49 (12.79–14.21)	12.86 (12.03–14.24)	0.185	16.28 (14.82–17.39)	15.84 (14.77–17.54)	0.739	**<0.0001**	**<0.0001**
Lipoprotein size								
VLDL-Z (nm)	38.01 (37.47–38.64)	37.68 (37.09–38.64)	0.780	38.06 (37.64–38.48)	37.82 (37.38–38.45)	**0.040**	0.497	0.814
LDL-Z (nm)	23.72 (23.68–23.77)	23.59 (23.55–23.64)	**<0.0001**	23.77 (23.74–23.80)	23.73 (23.68–23.77)	**<0.0001**	**<0.0001**	**<0.0001**
HDL-Z (nm)	9.75 (9.64–9.83)	9.77 (9.65–9.82)	0.704	9.66 (9.58–9.73)	9.67 (9.59–9.80)	0.270	**0.003**	0.203

The values are presented as the median (25th-75th percentile). Differences between lipoprotein particle number and size in two cohorts were tested using Independent *t*-test for normally distributed continuous variables and Mann–Whitney *U* test for skewed continuous variables. Significant *p*-values (in bold) were obtained by ANCOVA (adjusted by age). A 2-sided *p*-value < 0.05 is considered statistically significant.
FH; familial hypercholesterolemia; XXL; extremely large; XL; very large; L; large; M; medium; S; small; XS: extra small; VLDL; very low-density lipoprotein; IDL; intermediate-density lipoprotein; LDL; low-density lipoprotein; HDL; high-density lipoprotein; Z; size.

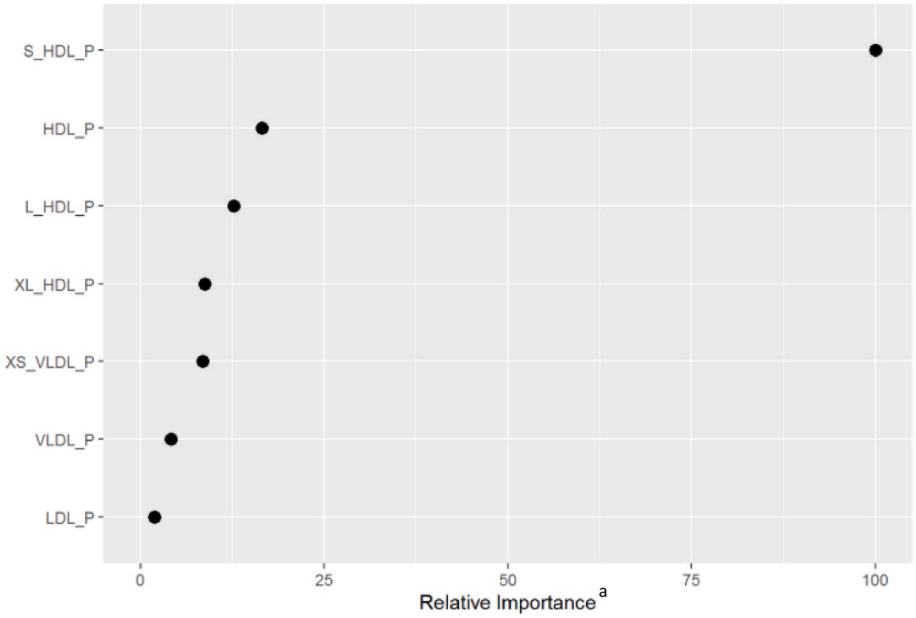

Fig. 1. Random forest analysis of the relative importance of the most influence variables from the gradient boosting model.

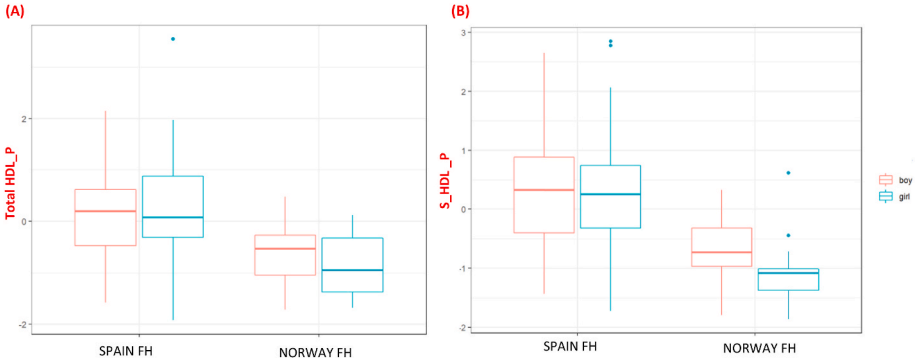

Fig. 2. Box plot of total HDL particles (A) and S_HDL_P (B) in Norwegian and Spanish FH children.

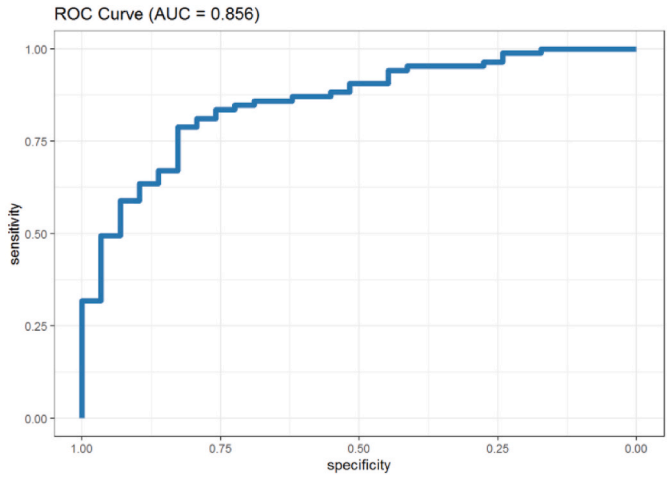

Fig. 3. Receiver Operating Characteristic (ROC) curves.
Model included VLDL_P (L_VLDL_P; S_VLDL_P; XS_VLDL_P), IDL_P, LDL_P and HDL_P (XL_HDL_P; L_HDL_P; M_HDL_P; S_HDL_P) particles variables. Area under the curve (AUC = 0,856).

and Spanish FH cohorts with a significant specificity and sensitivity (AUC: 0.856) (Fig. 3). The lipid cargo of lipoprotein subfractions was also assessed. All data on lipid concentrations derived from them were aligned with the information obtained from the particle number, consequently we focus our work on lipoprotein particle characteristics. (Supplementary Table 1).

Fig. 4 illustrates the correlations between lipoprotein particle number and size and the main differential macronutrients defined in a previous report [17] (total percentage of fat, MUFAs, PUFAs and daily consumption of fiber and cholesterol) that we had already identified as significantly different between cohorts in a previous study [17]. The association between nutrients and lipoprotein parameters was rather weak; therefore, we focused our results on associations with an "r" value above 0.400. While in the Spanish groups the associations were smaller, in the Norwegian FH group, total fat, MUFAs and fiber showed associations ($r > 0.400$) with all ApoB-containing lipoprotein particle number [VLDL (mainly smaller subfractions), IDL and all LDL subfractions]. In the same cohort, the fiber consumption was directly associated with VLDL size and inversely associated with mean HDL-Z and M_HDL.

4. Discussion

In this study, we provide new information on the lipoprotein profile as assessed by ^{1}H NMR in children with FH from two different regions,

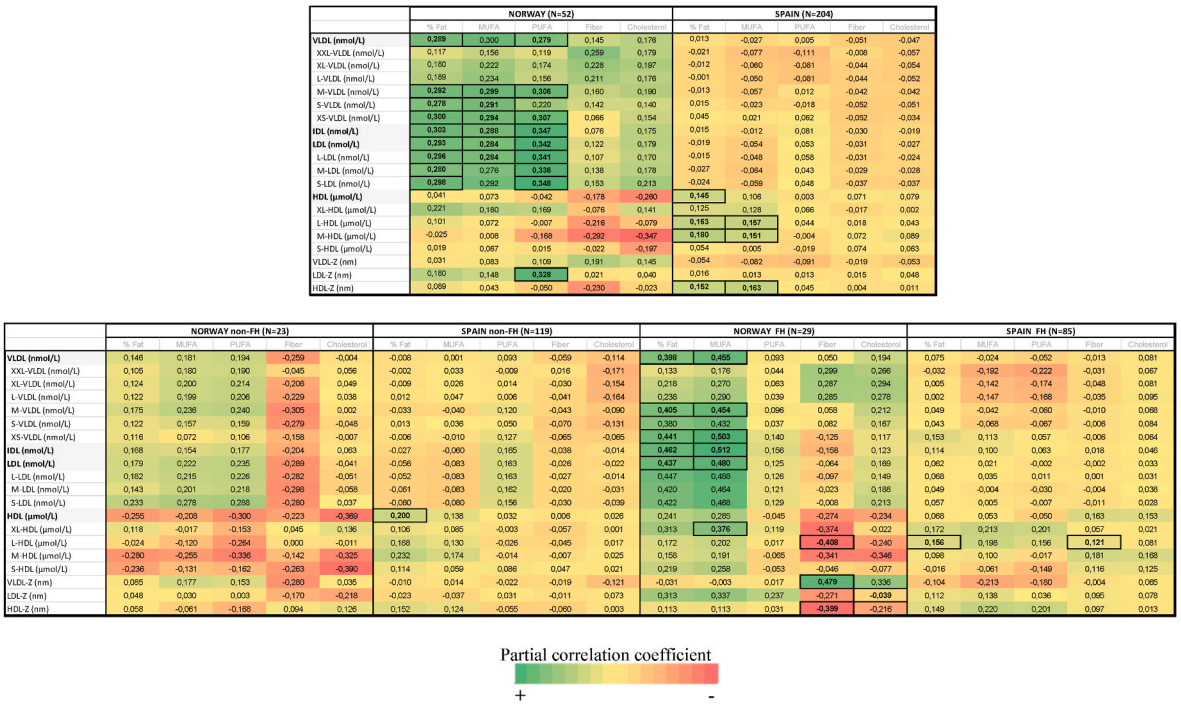

Fig. 4. Heatmap showing the correlations between lipoprotein particle number and size and consumption of the more informative macronutrients as previously defined [17].
All values were adjusted by age. Significant *p*-values in bold.

Nordic and Mediterranean. There were no significant differences in the standard lipid profile including total, LDL, HDL cholesterol and triglycerides between Norwegian and Spanish FH children. A non-significant trend to lower LDL cholesterol values in the Norwegian FH children could be explained by age differences, because teenagers show a slight drop in their LDL [35]. Despite a similar standard lipid profile, ^{1}H NMR analysis detected significant differences between the Norwegian and Spanish children. Both the Norwegian and Spanish FH children, as expected, had a higher absolute number of LDL particles than the non-FH children [36]. These differences were observed in all subclasses, including S_LDL. However, there were no differences in the overall distribution within subclasses [37] between the two FH groups. FH children had more S_LDL particles due to higher overall LDL (Supplementary Fig. 1). In fact, the mean LDL size is significantly larger in children with FH than in children without FH in both countries, supporting that the vascular effects in FH patients are driven by an increased number of all LDL subclasses. On the other hand, we also observed a higher level of VLDL and IDL particle number in FH children, which was not detected by standard measurements. Taking into account that LDLR also takes part in remnant and IDL clearance, this result should not be surprising despite not usually being captured by standard analyses. While the increased levels of LDL is the main cause of CVD in FH patients, the higher concentration of triglyceride-rich lipoproteins (TRL), even at subclinical levels in children, could account for an added CVD risk in FH. Recently, several studies have confirmed the impact of cholesterol carried by TRL on CVR [38,39]. Furthermore, the Copenhagen study, using the same NMR method, showed that VLDL particles are more atherogenic than LDL in terms of particle to particle [40].

Spanish children had statistically more LDL particles of all subclasses what is in accordance with differences in LDL-C concentrations that were higher in the Spanish group although non-statistically significant according to standard methods. Interestingly, this difference was not observed in the S_LDL; moreover, the mean diameter of LDL was larger in the Spanish group. An important difference between the Norwegian and Spanish children with FH was the HDL particle number concentration. The Spanish children with FH had a nonsignificant trend toward high HDL-C and ApoA concentrations (Table 1); however, the HDL particles, particularly M_HDL and S_HDL, and the mean HDL diameter were significantly higher (Table 2 and Fig. 2). In the random forest analysis, the main identifier of the Spanish vs. Norwegian FH groups was the concentration of HDL particles and S_HDL, as confirmed by the gradient boosting model (Fig. 1). According to the ROC curve, a model based on lipoprotein subclasses predicted either the Norwegian or Spanish FH groups with a significant sensitivity and specificity (AUC = 0.856) (Fig. 3). Again, the HDL particle number plays the main role in this calculation. Although more HDL and large LDL particles could be associated with a better vascular prognosis, the reasons and clinical significance of these differences remain to be clarified. On the other hand, the protective effect of HDL seems to be restricted to larger and medium sized particles, thus, the higher proportion of smaller HDL particle in the Spanish FH group lowers the possible positive effect of a higher HDL particle number [41].

Several differences were observed between the non-FH groups from both countries. However, the recruitment differences in both countries (general population in Norway and attending the lipid clinic in Spain) preclude the discussion of these differences.

The main aim of our study was to assess the association of two diverse diets (Nordic and Mediterranean) on the lipid profile of children with FH. We reported that Norwegian children consumed more PUFAs, while Spanish children consumed more MUFAs, fiber and cholesterol in their diets [17]. The discrepancy between these two dietary patterns could contribute to the lipoprotein profile differences. However, when we studied the associations between macronutrients and advanced lipoprotein profile components, we observed several robust correlations only in the Norway FH group and only weak associations in the Spanish cohort. After adjusting for age, total fat, but mainly MUFAs, was directly correlated with all ApoB-containing lipoproteins in the Norwegian cohort. The direct correlation between MUFAs and LDL-C was recently reported in a study based on UK Biobank data [42]. This correlation was not observed in the Spanish FH group, where the association between MUFAs and atherogenic particles was very weak or even inverse. This discrepancy can probably be explained by the total amount of MUFAs

consumed by Spanish children. They consumed *more* MUFAs than Norwegian children, which could drive the differences in cholesterol levels between cohorts, but intragroup variation is probably not enough to determine differences in the lipid profile subclasses. Another, more likely explanation could be the type of foods providing fats in both dietary patterns. While in the Nordic diet, MUFAs are provided by foods also rich in saturated fats, in the Mediterranean diet, MUFAs are mainly provided by olive oil and nuts.

Although the association between macronutrients and lipoprotein profile components could provide some clues based on observed differences, the main conclusion to draw from the present data is that, beyond isolated macronutrients, two different dietary patterns, Nordic and Mediterranean, may affect the lipoprotein profile of children with FH. Recent epidemiological data have confirmed that both European regions are considered low risk for cardiovascular disease based on standardized incidence data [43], suggesting that lifestyle factors, including dietary habits, in both Norway and Spain are healthy.

Our work has several limitations and some unique strengths. The small FH sample size may result in spurious false or positive findings, but there are few cohorts of FH children that have the complete lipoprotein profile by NMR. The recruitment methods and sample collection were not equally designed between groups because we analyzed pre-existing cohorts, which complicates the interpretation of comparisons between cohorts. One of the differences to take into account is that the sampling was obtained in fasting and nonfasting state in the Spanish and Norwegian children respectively. The fasting and nonfasting sampling has a very limited impact on the lipid profile affecting mainly to TG and VLDL particles [44]. However, no differences on TG were observed between groups while the VLDL particles tend to be higher in the Spanish group. On the other hand, the comparison of FH children between two different environments of Nordic and Mediterranean gives a unique strength to our results. The comprehensive diet composition analysis also provides additional value. Finally, advanced lipoprotein analysis with ^{1}H NMR gives a unique deeper view of lipoprotein metabolism in these FH groups.

In conclusion, this is the first study that compares the effect of two types of diet patterns (Nordic and Mediterranean) claiming to be healthy, on an advanced lipoprotein profile assessed by $^{1H-}$NMR in FH children. Children with FH from different countries, are exposed to different regional food traditions, culture and social context that influence dietary patterns and despite the genetic alteration modulated the disease expression. Nordic *vs.* Mediterranean diet, are associated to subtle metabolic differences than are not detected by standard analysis, involving all atherogenic particles. HDL particles are also different between Spanish and Norwegian children with FH. The beneficial impact of Nordic and Mediterranean dietary patterns on CVD risk is probably mediated by different effects on the lipoprotein profile. Healthy diets based on food patterns rather than nutrient proportions should be designed according to local cultural traditions to increase adherence and effectiveness.

Financial support

This study was funded by the University of Oslo, the National Advisory Unit on FH (Oslo), the Throne-Holst Foundation for Nutrition Research (Oslo), the South-Eastern Regional Health Authority (Oslo), "Marató de TV3" (tittle of the project: Preventing Premature Coronary Heart Disease in Catalonia by Expanding Familial Hypercholesterolemia Diagnosis - grant number 20152430) (Catalonia), a Rovira Virgili University mobility grant (AEE2019-Biomedicina) co-financed by the "FI-DGR 2018" grant and the European Social Fund.

Declaration of interest

Prof. Holven reports grants and/or personal fees from Tine SA, Mills DA, Olympic Seafood, Amgen, Sanofi, Kaneka and Pronova, none of which are related to the content of this manuscript. Prof. Masana has received personal fees for lectures and advisory work from Sanofi, Amgen, Mylan, and Servier, none of which are related to the content of this manuscript. The other authors have no relevant financial relationships to disclose.

CRediT authorship contribution statement

Cèlia Rodríguez-Borjabad: Conceived and designed the research, Methodology, Performed the statistical analyses, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Ingunn Narveud:** Methodology, Performed the statistical analyses, Writing – review & editing, Visualization, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Jacob Juel Christensen:** Methodology, Performed the statistical analyses, Formal analysis, Writing – review & editing, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Daiana Ibarretxe:** Methodology, Writing – review & editing, Funding acquisition, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Natalia Andreychuk:** Methodology, Writing – review & editing, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Josefa Girona:** Methodology, Performed the statistical analyses, Formal analysis, Writing – review & editing, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Kristin Torvik:** Methodology, Writing – review & editing, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Guro Folkedal:** Methodology, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Martin P. Bogsrud:** Methodology, Writing – review & editing, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Kjetil Retterstøl:** Methodology, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Núria Plana:** Methodology, Writing – review & editing, Funding acquisition, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Luis Masana:** Conceived and designed the research, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. **Kirsten B. Holven:** Conceived and designed the research, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition.

Acknowledgements

We would like to thank grouping the dietary data, nurses and technicians for the blood samples, and we obviously thank all the children and families participating in the study. We also thank the entire primary care paediatrician group (The DECOPIN Group) for their assistance in the recruitment of participants in Catalonia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2023.04.009.

References

[1] A. Wiegman, S.S. Gidding, G.F. Watts, M.J. Chapman, H.N. Ginsberg, M. Cuchel, et al., Familial hypercholesterolæmia in children and adolescents: gaining decades of life by optimizing detection and treatment, Eur. Heart J. 36 (2015) 2425–2437, https://doi.org/10.1093/eurheartj/ehv157.

[2] I.K. Luirink, A. Wiegman, D.M. Kusters, M.H. Hof, J.W. Groothoff, E. De Groot, et al., 20-Year follow-up of statins in children with familial hypercholesterolemia, N. Engl. J. Med. 381 (2019) 1547–1556, https://doi.org/10.1056/NEJMoa1816454.
[3] H. Tada, M. Takamura, M. Kawashiri, Familial hypercholesterolemia: a narrative review on diagnosis and management strategies for children and adolescents, Vasc. Health Risk Manag. 17 (2021) 59–67, https://doi.org/10.2147/VHRM.S266249.
[4] R.D. Santos, Phenotype vs. genotype in severe familial hypercholesterolemia: what matters most for the clinician, Curr. Opin. Lipidol. 28 (2017) 130–135, https://doi.org/10.1097/MOL.0000000000000391.
[5] V. Bianconi, M. Banach, M. Pirro, Why patients with familial hypercholesterolemia are at high cardiovascular risk? Beyond LDL-C levels, Trends Cardiovasc. Med. 31 (2021) 205–215, https://doi.org/10.1016/j.tcm.2020.03.004.
[6] R. Mallol, N. Amigó, M.A. Rodríguez, M. Heras, M. Vinaixa, N. Plana, et al., Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy, J. Lipid Res. 56 (2015) 737–746, https://doi.org/10.1194/jlr.D050120.
[7] P. Soininen, A.J. Kangas, P. Würtz, T. Suna, M. Ala-Korpela, Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics, Circ. Cardiovasc. Genet. 8 (2015) 192–206, https://doi.org/10.1161/CIRCGENETICS.114.000216.
[8] F. Mach, C. Baigent, A.L. Catapano, K.C. Koskinas, M. Casula, L. Badimon, et al., ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Eur. Heart J. 41 (2019) (2019) 111–118, https://doi.org/10.1016/j.atherosclerosis.2019.08.014.
[9] P.S. Jellinger, Y. Handelsman, P.D. Rosenblit, Z.T. Bloomgarden, V.A. Fonseca, A. J. Garber, et al., American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease, Endocr. Pract. 23 (2017) 1–87, https://doi.org/10.4158/EP171764.APPGL.
[10] S. Mora, J.E. Buring, P.M. Ridker, Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events, Circulation 129 (2014) 553–561, https://doi.org/10.1161/CIRCULATIONAHA.113.005873.
[11] American Diabetes Association, Classification and diagnosis of diabetes: *Standards of medical Care in diabetes-2021*, Diabetes Care 44 (2021) S15–S33, https://doi.org/10.2337/dc21-S002.
[12] J.E. Dalen, S. Devries, Diets to prevent coronary heart disease 1957-2013: what have we learned? Am. J. Med. 127 (2014) 364–369, https://doi.org/10.1016/j.amjmed.2013.12.014.
[13] R.H. Eckel, J.M. Jakicic, J.D. Ard, J.M. De Jesus, N. Houston, V.S. Hubbard, et al., AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of cardiology/American Heart Association task force on practice guidelines, Circulation 129 (2014) (2013) S76–S99, https://doi.org/10.1161/01.cir.0000437740.48606.d1.
[14] H. Dai, A.A. Much, E. Maor, E. Asher, A. Younis, Y. Xu, et al., Global, regional, and national burden of ischaemic heart disease and its attributable risk factors, 1990–2017: results from the Global Burden of Disease Study 2017, Eur. Heart. J. Qual. Care. Clin. Outcomes. 8 (2022) 50–60, https://doi.org/10.1093/ehjqcco/qcaa076.
[15] V. Adamsson, A. Reumark, T. Cederholm, B. Vessby, U. Riserus, G. Johansson, What is a healthy Nordic diet? Foods and nutrients in the NORDIET study, Food Nutr. Res. 56 (2012), https://doi.org/10.3402/fnr.v56i0.18189.
[16] A. Bach-Faig, E.M. Berry, D. Lairon, J. Reguant, A. Trichopoulou, S. Dernini, et al., Mediterranean diet pyramid today, Sci. Cult. Updates Public. Health. Nutr. 14 (2011) 2274–2284, https://doi.org/10.1017/S1368980011002515.
[17] C. Rodríguez-Borjabad, I. Narveud, J.J. Christensen, S.M. Ulven, A.I. Malo, D. Ibarretxe, et al., Dietary intake and lipid levels in Norwegian and Spanish children with familial hypercholesterolemia, Nutr. Metabol. Cardiovasc. Dis. 31 (2021) 1299–1307, https://doi.org/10.1016/j.numecd.2020.12.002.
[18] K. Torvik, I. Narverud, I. Ottestad, A. Svilaas, J.M. Gran, K. Retterstøl, et al., Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia, Atherosclerosis 252 (2016) 21–27, https://doi.org/10.1016/j.atherosclerosis.2016.07.913.
[19] J.J. Christensen, S.M. Ulven, K. Retterstøl, I. Narverud, M.P. Bogsrud, T. Henriksen, et al., Comprehensive lipid and metabolite profiling of children with and without familial hypercholesterolemia: a cross-sectional study, Atherosclerosis 266 (2017) 48–57, https://doi.org/10.1016/j.atherosclerosis.2017.09.021.
[20] D. Ibarretxe, C. Rodríguez-Borjabad, A. Feliu, J. Bilbao, L. Masana, N. Plana, et al., Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: the DECOPIN project, Atherosclerosis 278 (2018) 210–216, https://doi.org/10.1016/j.atherosclerosis.2018.09.039.
[21] P. Soininen, A.J. Kangas, P. Würtz, T. Suna, M. Ala-Korpela, Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics, Circ. Cardiovasc. Genet. 8 (2015) 192–206, https://doi.org/10.1161/CIRCGENETICS.114.000216.
[22] P. Würt, A.S. Havulinna, P. Soininen, T. Tynkkynen, D. Prieto-Merino, T. Tillin, et al., Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts, Circulation 131 (2015) 774–785, https://doi.org/10.1161/CIRCULATIONAHA.114.013116.
[23] U.M. Kujala, V.P. Mäkinen, I. Heinonen, P. Soininen, A.J. Kangas, T.H. Leskinen, et al., Long-term leisure-time physical activity and serum metabolome, Circulation 127 (2013) 340–348, https://doi.org/10.1161/CIRCULATIONAHA.112.105551.
[24] Q. Wang, P. Würtz, K. Auro, V.P. Mäkinen, A.J. Kangas, P. Soininen, et al., Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence, BMC Med. 14 (2016) 1–14, https://doi.org/10.1186/s12916-016-0733-0.
[25] I.T.L. Lillegaard, L.F. Andersen, Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters, Br. J. Nutr. 94 (2005) 998–1003, https://doi.org/10.1079/bjn20051587.
[26] I.T.L. Lillegaard, N.C. Øverby, L.F. Andersen, Can children and adolescents use photographs of food to estimate portion sizes? Eur. J. Clin. Nutr. 59 (2005) 611–617, https://doi.org/10.1038/sj.ejcn.1602119.
[27] I.T.L. Lillegaard, E.B. Løken, L.F. Andersen, Relative validation of a pre-coded food diary among children, under-reporting varies with reporting day and time of the day, Eur. J. Clin. Nutr. 61 (2007) 61–68, https://doi.org/10.1038/sj.ejcn.1602487.
[28] L.F. Andersen, M.L. Pollestad, D.R. Jacobs, A. Løvø, B.E. Hustvedt, Validation of a pre-coded food diary used among 13-year-olds: comparison of energy intake with energy expenditure, Publ. Health Nutr. 8 (2005) 1315–1321, https://doi.org/10.1079/phn2005751.
[29] J.M. Martin-Moreno, P. Boyle, L. Gorgojo, P. Maisonneuve, J.C. Fernandez-Rodriguez, Development and validation of a food frequency questionnaire in Spain, Int. J. Epidemiol. 22 (1993) 512–519, https://doi.org/10.1093/ije/22.3.512.
[30] C. Vázquez, R. Alonso, M. Garriga, A. de Cos, J.J. de la Cruz, F. Fuentes-Jiménez, et al., Validation of a food frequency questionnaire in Spanish patients with familial hypercholesterolaemia, Nutr. Metabol. Cardiovasc. Dis. 22 (2012) 836–842, https://doi.org/10.1016/j.numecd.2011.01.007.
[31] J. Vioque, M. Garcia-de-la-Hera, S. Gonzalez-Palacios, L. Torres-Collado, L. Notario-Barandiaran, A. Oncina-Canovas, et al., Reproducibility and validity of a short food frequency questionnaire for dietary assessment in children aged 7−9 Years in Spain, Nutrients 11 (2019) 933, https://doi.org/10.3390/nu11040933.
[32] R. Estruch, E. Ros, J. Salas-Salvadó, M.I. Covas, D. Corella, F. Arós, et al., Primary prevention of cardiovascular disease with a Mediterranean diet, N. Engl. J. Med. 368 (2013) 1279–1290, https://doi.org/10.1056/NEJMoa1200303.
[33] R. Couronné, P. Probst, A.L. Boulesteix, Random forest versus logistic regression: a large-scale benchmark experiment, BMC Bioinf. 19 (2018) 270, https://doi.org/10.1186/s12859-018-2264-5.
[34] L. Breiman, Random forests, Mach. Learn. 45 (2001) 5–32, https://doi.org/10.1023/A:1010933404324.
[35] M.A. Eissa, N.L. Mihalopoulos, R. Holubkov, S. Dai, D.R. Labarthe, Changes in fasting lipids during puberty, J. Pediatr. 170 (2016) 199–205, https://doi.org/10.1016/j.jpeds.2015.11.018.
[36] C. Rodríguez-Borjabad, A.I. Malo, D. Ibarretxe, J. Girona, M. Heras, R. Ferré, et al., Efficacy of therapeutic lifestyle changes on lipid profiles assessed by NMR in children with familial and non-familial hypercholesterolemia, Clin. Invest. Arterioscler. 32 (2020) 49–58, https://doi.org/10.1016/j.arteri.2019.10.001.
[37] C. Rodríguez-Borjabad, D. Ibarretxe, J. Girona, R. Ferré, A. Feliu, N. Amigó, et al., Lipoprotein profile assessed by 2D-1H-NMR and subclinical atherosclerosis in children with familial hypercholesterolaemia, Atherosclerosis 270 (2018) 117–122, https://doi.org/10.1016/j.atherosclerosis.2018.01.040.
[38] O. Castañer, X. Pintó, I. Subirana, A.J. Amor, E. Ros, Á. Hernáez, et al., Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease, J. Am. Coll. Cardiol. 76 (2020) 2712–2724, https://doi.org/10.1016/j.jacc.2020.10.008.
[39] M. Balling, B.G. Nordestgaard, A. Langsted, A. Varbo, P.R. Kamstrup, S. Afzal, Small dense low-density lipoprotein cholesterol predicts atherosclerotic cardiovascular disease in the copenhagen general population study, J. Am. Coll. Cardiol. 75 (2020) 2873–2875, https://doi.org/10.1016/j.jacc.2020.03.072.
[40] M.Ø. Johansen, S. Vedel-Krogh, S.F. Nielsen, S. Afzal, G.D. Smith, B. G. Nordestgaard, Per-particle triglyceride-rich lipoproteins imply higher myocardial infarction risk than low-density lipoproteins: copenhagen general population study, Arterioscler. Thromb. Vasc. Biol. 41 (2021) 2063–2075, https://doi.org/10.1161/ATVBAHA.120.315639.
[41] A. Kontush, HDL particle number and size as predictors of cardiovascular disease, Front. Pharmacol. 6 (2015) 218, https://doi.org/10.3389/fphar.2015.00218.
[42] R.K. Kelly, C.Z. Watling, T.Y.N. Tong, C. Piernas, J.L. Carter, K. Papier, et al., Associations between macronutrients from different dietary sources and serum lipids in 24 639 UK Biobank Study participants, Arterioscler. Thromb. Vasc. Biol. 41 (2021) 2190–2200, https://doi.org/10.1161/ATVBAHA.120.315628.
[43] SCORE2 working group and ESC Cardiovascular risk collaboration, SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe, Eur. Heart J. 42 (2021) 2439–2454, https://doi.org/10.1093/eurheartj/ehab309.
[44] B.G. Nordestgaard, A. Langsted, S. Mora, G. Kolovou, H. Baum, E. Bruckert, et al., Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine, Eur. Heart J. 37 (2016) 1944–1958, https://doi.org/10.1093/eurheartj/ehw152.