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META-ANALYSIS

Adherence to a Mediterranean diet, dyslipidemia and inflammation in familial hypercholesterolemia

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Cholesterol;
Inflammation;
Mediterranean diet

Abstract *Background and aims:* Familial Hypercholesterolemia (FH) is characterized by elevated LDL-cholesterol (LDL-C) and high atherosclerosis risk. The impact of different dietary patterns on atherosclerosis biomarkers has been poorly studied in FH.

This study verified the association of adherence to a Mediterranean diet with biomarkers of dyslipidemia and low-grade inflammation in molecularly proven FH adults from Brazil (BR) and Spain (SP).

Methods and results: In this cross-sectional study adherence to the Mediterranean diet was assessed by a validated score and generalized estimating equations were used to evaluate its association with plasma LDL-C, apolipoprotein-B (ApoB) and high sensitivity C-reactive protein (hs-CRP) concentrations. We included 92 (mean age 45 years, 58.7% females) and 98 FH individuals (mean age 46.8 years, 60.2% females) respectively from BR and SP. FH causing variants did not differ between countries. LDL-C, ApoB and hs-CRP concentrations were higher in BR than in SP: 179 (135–250) and 161 (133–193) mg/dL; 141 (109–181) and 103 (88–134) mg/dL; and 1.6 (0.8–4.0) and 0.8 (0.4–1.5) mg/L respectively (all $p < 0.001$). Most of BR had low adherence ($n = 77$, 83.7%), while the majority of SP were divided into moderate ($n = 35$, 35.7%) and strong adherence to the Mediterranean diet ($n = 37$, 37.8%), $p < 0.001$. There was a significant inverse association of adherence to the Mediterranean diet score with higher LDL-C, ApoB, and hs-CRP after adjusting for socio economic parameters, caloric and fatty acid intakes as well as pharmacological lipid lowering therapies.

Conclusions: Higher adherence to a Mediterranean diet was associated with better dyslipidemia and low-grade inflammation profiles in FH.

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Introduction

Familial hypercholesterolemia (FH) is a genetic autosomal dominant disorder characterized by reduction in the removal from plasma of low-density lipoprotein (LDL) and is associated with early atherosclerotic cardiovascular disease (ASCVD) onset [1]. In addition to pharmacological LDL-cholesterol (LDL-C) lowering therapies, dietary patterns associated with lower risk of ASCVD are recommended for individuals with FH [2]. Adequacy of eating habits may not only help reduce high concentrations of LDL-C [3], the most important causative factor of atherosclerosis in FH [4], but also influence additional risk factors, such as hypertension, obesity and type 2 diabetes [5]. In addition, diet may modulate pro-atherogenic mechanisms, such as oxidative stress, inflammation and endothelial dysfunction and therefore could prevent development of ASCVD.

The Mediterranean diet as followed in Spain among other countries has been associated with lower ASCVD risk [6]. This diet characterized by a high consumption of fats, especially monounsaturated fatty acids derived from olive oil as its major source of fat and foods derived from high-fiber plants contrasts to the classic saturated-fat restricted cholesterol-lowering diet [5,7] as recommended in Brazil and other countries in the Americas.

Recently Antoniazzi et al., [8]. studied the association of dietary components with dyslipidemia and low-grade inflammation biomarkers in molecularly confirmed FH individuals from Brazil (BR) [9] and Spain (SP) [10]. In that study greater consumption of monounsaturated and polyunsaturated fats and their ratios to saturated fats were associated with lower concentrations of plasma LDL-C, apolipoprotein B (ApoB) as well as high sensitivity C reactive protein (hs-CRP) even after adjustment for pharmacological therapies [8]. On the other hand, greater consumption of carbohydrates was associated with higher hs-CRP levels. That study suggested a greater benefit of components of a Mediterranean diet than simple restriction of saturated fats alone in dyslipidemia and inflammation profiles of FH individuals. Considering that, and the fact that dietary patterns are much more representative of the impact of diet rather than its components [5], the present study aimed to test the association of the degree of adherence to the Mediterranean diet with dyslipidemia and low-grade inflammation biomarkers in molecularly characterized heterozygous FH individuals participating in cascade screening programs from BR and SP.

Methods

Study design

As previously described [8] this is a multicenter cross-sectional study evaluating dietary patterns of Brazilian and Spanish individuals with molecularly confirmed FH who participated, respectively, in the Genetic Screening Program for Familial Hypercholesterolemia (Hipercol Brasil) and the Spanish Study of Familial Hypercholesterolemia - (SAFEHEART). The study subjects were followed up respectively at a tertiary cardiology center in BR (InCor-HCFMUSP) and in primary and specialized care facilities in SP. To calculate the study sample size a 10% hypothetical difference in LDL-C concentration between BR and SP was predicted considering the differences in dietary patters between the 2 countries and mean LDL-C at screening of 170 mg/dL (±standard deviation of 60 mg/dL). The level of significance and the study power were established, respectively, at 5% and 80%. These premises generated the number of 90 subjects to be included in either BR or SP groups.

Adults (≥20 years old) of both sexes presenting FH pathogenic or possibly pathogenic LDL receptor genetic (*LDLR*) variants, who agreed to participate and signed an informed consent, were included in the study from October 2013 to October 2016. Pregnant women, patients with homozygous FH, individuals with thyroid disorders, cancer, heart failure, liver disease and those whose questionnaires were incomplete were excluded.

Initially, 249 individuals undergoing cascade screening in Brazil were analyzed. Of these, 92 (36.9%) had a confirmed defect in the *LDLR* and were included in the study. BR subjects were matched for age, sex and body mass index (BMI) with Spanish individuals.

This study was approved by the Commission and Ethics for Research Project Analysis - CAPPesq of Hospital das Clínicas da FMUSP (CAAE: 46269815.1.0000.0068, number: 1,172,951).

Data collection

Study subjects with clinical suspicion of FH were evaluated in the first consultation of the genetic cascade screening programs, when molecular diagnosis was unknown. Data collection on socio-demographic characteristics, medical and personal history and pharmacological treatment was carried out using standardized questionnaires for both countries [9,11]The following information was collected: age, sex, education (without formal education, primary, secondary or university level), smoking (smoker, ex-smoker, never smoked), referred diagnosis of type 2 diabetes and hypertension, current and previous pharmacological treatment for hypercholesterolemia, history of coronary heart disease (angina pectoris, myocardial infarction and surgical or percutaneous coronary revascularization), peripheral artery disease and ischemic stroke. An early cardiovascular event was considered if occurring before the age of 55 and 65 years respectively for men and women. The level of physical activity was measured using the International Physical Activity Questionnaire - IPAQ and classified as very active, active or insufficiently active [12]. Pharmacological treatment was classified as effective for FH if it had the power to reduce LDL-C $\geq$ 50% as previously described [13].

Blood pressure measurements were performed twice in a sitting position with a digital sphygmomanometer and after a 5-min rest to calculate the mean value of

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systolic and diastolic pressures. The anthropometric assessment included measures of waist circumference, weight and height that enabled the calculation and assessment of BMI.

Dietary assessment

To assess food intake, food frequency questionnaires (FFQ) validated respectively for Brazil [14] and Spain [15] were applied. Food composition tables for each country were used to calculate the values of energy and nutrients, based on the quantities collected by the FFQ, applied in Hipercol Brasil and SAFEHEART cascade screening programs respectively. Individuals who had extreme caloric intakes, <500 or> 3500 calories for women and <800 or> 4200 calories for men, were excluded from the analysis as a previously recommended [16].

To evaluate adherence to the Mediterranean diet, 14 items of food consumption by Brazilian and Spanish individuals were scored as proposed by Estruch et al. [17,18], which assigns 1 point for the desired response in each of the items evaluated, as shown in Supplementary Table 1. The score has a maximum value of 14 points, the higher the score the greater the adherence to the Mediterranean diet and therefore the following categories were considered: strong (score $\geq$ 9 points), moderate (score 7–8 points) and poor (score $\leq$ 6 points) adherence.

Genetic evaluation

Only individuals with *LDLR* variants were included in this study. In BR, individuals with suspected FH had their diagnosis confirmed as previously described [19] by means of a genetic study with automatic sequencing of the promoter region and the 18 exons, including the intronic limits of the *LDLR* by next generation sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) if necessary. In SP, the molecular diagnosis was performed using the LIPOchip array platform followed if necessary, by gene sequencing and or adapted quantitative multiplex PCR methodology (QMFSP) or MLPA as previously described [10]. All variants were verified using Mutalyzer v 2.0. The molecular defects of the *LDLR* in both populations were classified as defective and negative, respectively, according to the JOJO genetics database (https://www.jojogenetics.nl/wp/).

Biochemical analyzes

Similar procedures were performed in BR and SP. Blood samples were collected, and plasma and serum samples were placed in Eppendorf tubes and stored at −80 °C until biochemical analyzes were performed in central laboratories. In BR most individuals were not fasting, differently from SP therefore, to minimize the influence of the non-fasting state on the triglycerides that would be used to calculate LDL-C by the Friedewald formula, LDL-C was determined by a direct enzymatic method. However, there are no data available on total cholesterol and HDL-C and triglycerides for this study. In SP, the serum LDL-C concentration was calculated using the Friedewald formula for triglyceride levels up to 350 mg/dL. To minimize methodological errors in relation to possible differences in LDL-C determinations, ApoB concentrations were determined in both countries by the same immunoturbidimetry method (Roche laboratory). For the entire population studied (n = 190), a significant 0.89 Spearman correlation coefficient was encountered between LDL-C and ApoB determinations. The high-sensitivity C-reactive protein (hs-CRP) was determined by the same immunoturbidimetry in both countries, using Roche laboratory kits.

For statistical purposes, the concentrations of LDL-C, ApoB and hs-CRP were considered altered if greater than: 100 mg/dL, 130 mg/dL and 2 mg/L, respectively.

Statistical analysis

Since most continuous variables did not exhibit Gaussian distribution (Kolmogorov–Smirnov test), they were presented as medians and interquartile intervals (IQR), corresponding to the 25th and 75th percentiles, respectively. Categorical variables were presented in absolute numbers and percentages (%). The Kruskal Wallis test was used to compare the medians of continuous variables. The Chi [2] test was used to check for differences between categorical variables. Due to the non-Gaussian distribution of the hs-CRP values, this variable was transformed and used on a base 10 logarithmic scale for the regression models. The nutrients and dependent variables were normalized by one standard deviation (SD) in the linear regression analyzes.

Generalized estimation equations (GEE) considering the country as a group variable and adjusted for pharmacological treatment (untreated, treated with low doses, treated with effective doses), type of *LDL*R variant (defective or negative), smoking, education, physical activity status, BMI and caloric intake were used to assess the association between dietary patterns and the dependent variables (LDL-C, ApoB and hs-CRP).

For linear regressions with the Mediterranean diet adherence score as a continuous variable, two different models were used:

Model 1 - pharmacological treatment (untreated, treated with low doses, treated with effective doses), type of *LDLR* variant (defective or null), smoking, education, physical activity, BMI and caloric intake.

Model 2 - pharmacological treatment (untreated, treated with low doses, treated with effective doses), type of LDLR variant (defective or null), smoking, education, physical activity, BMI and fatty acid (saturated, monounsaturated and polyunsaturated) intake.

The same adjustment variables of the two models mentioned above were used in the logistic regression model that tested association with the Mediterranean diet adherence categories. The category of poor adherence to the Mediterranean diet (score $\leq$ 6 points) was considered as reference, compared to moderate and strong adherence. In all tests, the level of significance considered was 5%.

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Statistical analyzes were performed using SPSS 20.0 and Stata 13.1 software.

Results

Table 1 shows the socio-demographic and clinical characteristics of participants in BR (n = 92) and SP (n = 98). There were no differences regarding age and sex but distinctions in education levels (p = 0.001) and physical activity between SP and BR (p < 0.001) were encountered. No differences were seen on BMI, and frequencies of type 2 diabetes and hypertension. Groups were also similar regarding *LDLR* variants categorized as defective, negative or unclassified: 56.6% (n = 52) and 54.1% (n = 53); 34.8% (n = 32) and 39.8% (n = 39); and 8.7% (n = 8) and 6.1% (n = 6), respectively in BR and SP (p = 0.671). There were no differences regarding the current use of pharmacological treatment, however SP had a greater use of ezetimibe (p = 0.002) and effective pharmacological treatments to reduce LDL-C (p = 0.004). No patient was in use of PCSK9 inhibitors.

Table 2 shows that the prevalence of previous coronary heart disease events (p = 0.001) and peripheral artery disease (p = 0.014) was higher in BR.

Table 3 shows that median values of LDL-C (p = 0.007), ApoB (p < 0.001) and hs-CRP (p < 0.001) were higher in BR. There were no differences in the proportions of individuals with LDL-C> 100 mg/dL, but in BR there was a greater number of subjects with ApoB>130 mg/dL (p = 0.001) and hs-CRP> 2 mg/L (p = 0.001).

As previously shown [8] there were marked differences in relation to the consumption of macronutrients in the BR and ESP groups. There was a greater consumption of calories (Kcal) 2137 (1779–2688) vs. 1673.0 (1494.0–1992.0), p < 0.001; protein (as % of energy) 20.0 (16.8–23.2) vs. 18.6 (17.1–20.6), p = 0.031; carbohydrates (as % of energy) 57.1 (51.4–62.1) vs. 42.5 (38.5–47.8), p < 0.001; cholesterol (mg) 283.0 (224.0–367.0) vs. 189.0 (1520–225.0), p < 0.001 but a lower consumption of fats (% of energy) 22.2 (19.0–26.5) vs. 38.3 (33.1–41.8), p < 0.001 and fibers (g/1000 Kcal) 13.6 (10.4–18.4) vs. 16.6 (14.2–18.9), p = 0.001, in BR than in SP.

Table 4 shows food consumption according to adherence to the Mediterranean diet. There were significant differences on consumption of the dietary components comprising the adherence score between BR and SP. The most remarkable was the use of olive oil as the main cooking fat source for almost all SP and none of BR individuals respectively (p < 0.001). In SP there was also a higher consumption of sofrito preparations (olive oil-based seasoning, p < 0.001), nuts (p = 0.023), vegetables (p = 0.001), fish and shellfish (p < 0.001), wine (p = 0.026) and sweet or carbonated beverages

Table 1 Socio-demographic and clinical characteristics of Brazilian (BR) and Spanish (SP) FH individuals.

Parameters	BR (n = 92)	SP (n = 98)	p
Age (years)	45.0 (34.3–59.0)	46.8 (35.2–58.6)	0.661
Sex			
Male, % (n)	41.3 (38)	39.8 (39)	0.832
Female, % (n)	58.7 (54)	60.2 (59)	
Education			
No formal education, % (n)	3.3 (3)	7.1 (7)	0.001
Primary level, % (n)	17.6 (16)	26.5 (26)	
Secondary level, % (n)	34.1 (31)	48.0 (47)	
University level, % (n)	45.1 (42)	18.4 (18)	
Physical Activity			
Sedentary, % (n)	43.5 (40)	12.2 (12)	<0.001
Moderate, % (n)	35.9 (33)	60.2 (59)	
High, % (n)	20.7 (19)	27.6 (27)	
Smoking			
Active smoker % (n)	6.5 (6)	6.1 (6)	0.073
Ex-smoker, % (n)	17.4 (16)	31.6 (31)	
Non-smoking, % (n)	76.1 (70)	62.2 (61)	
Diabetes mellitus, % (n)	8.7 (8)	5.1 (5)	0.327
Hypertension, % (n)	26.1 (24)	15.3 (15)	0.066
BMI (kg/m^2)	26.1 (23.9–29.9)	26.0 (24.0–29.0)	0.698
Normal weight (BMI 18.5–25), % (n)	37.0 (34)	32.7 (32)	0.647
Overweight (BMI 25–29.9), % (n)	40.2 (37)	46.9 (46)	
Obesity (BMI ≥ 30), % (n)	22.8 (21)	20.4 (20)	
Waist circumference (cm)	91.2 (83.9–100.5)	88.0 (80.0–96.3)	0.079
Patients on prior statin use, % (n)	58.7 (54)	84.7 (83)	<0.001
Patients on prior effective lipid lowering therapy, % (n)	15.2 (14)	26.5 (26)	0.056
Current on pharmacological treatment, % (n)	84.8 (78)	90.8 (89)	0.203
Current on use of ezetimibe, % (n)	22.8 (21)	43.9 (43)	0.002
Current on effective lipid lowering treatment, % (n)*	38.0 (35)	59.2 (58)	0.004

Results presented in medians and interquartile ranges (IQR) – 25th and 75th percentiles – (Kruskal Wallis test) or absolute number and percentage (Chi-square test); WC = waist circumference; BMI = body mass index; Lipid lowering treatment was considered effective if medications used could reduce LDL-C≥ 50%.

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Table 2 Previous cardiovascular events of Brazilian (BR) and Spanish (SP) FH individuals.

Parameters	BR (n = 92)	SP (n = 98)	P
Coronary Heart Disease, % (n)	21.7 (20)	5.1 (5)	0.001
Early cardiovascular event	15.2 (14)	6.1 (6)	0.041
Myocardial infarction, % (n)	14.1 (13)	3.1 (3)	0.003
Angina pectoris, % (n)	21.4 (18)	3.1 (3)	<0.001
Surgical or percutaneous coronary revascularization, % (n)	10.9 (10)	4.1 (4)	0.073
Peripheral artery disease, % (n)	5.9 (5)	(0) 0	0.014
Ischemic stroke, % (n)	2.4 (2)	2.0 (2)	0.876

Absolute number and percentage (Chi-square test).

Table 3 Laboratory parameters of Brazilian (BR) and Spanish (SP) FH individuals.

Biochemical parameters	BR (n = 92	SP (n = 98)	p
LDL-C (mg/dL)	179 (135–250)	161 (134–193)	0.007
LDL-C ≥ 100 mg/dL, % (n)	98 (90)	92 (90)	0.056
ApoB (mg/dL)	141 (109–181)	103 (88–134)	<0.001
ApoB > 130 mg/dL, % (n)	63 (58)	26.5 (26)	<0.001
hs-CRP (mg/L)*	1.6 (0.8–4.0)	0.8 (0.4–1.5)	<0.001
hs-CRP > 2 mg/L, % (n)*	45.6 (42)	23.5 (23)	0.001
Lipoprotein(a) mg/dL **	28.8 (13.6–52.0)	19.0 (5.3–48.0)	_

Results presented in medians and interquartile ranges (IQR) – 25th and 75th; ApoB = apolipoprotein B; LDL-C = low density lipoprotein cholesterol; hs-CRP = high sensitivity C-reactive protein; * hs-CRP- was measured in 88 (95.6%) individuals from BR and 94 (95.9%) from SP. ** available for n = 31 (36.7%) in BR and n = 98 (100%) in SP, statistical tests not performed.

Table 4 Food consumption assessed according to Mediterranean diet adherence score in Brazilian (BR) and Spanish (SP) FH patients.

Food consumption	BR (92)	SP (98)	p
Olive oil as main cooking fat n (%)	0 (0)	96 (98)	<0.001
Olive oil ≥ 4 tablespoon/day. n (%)	0 (0)	7 (7.1)	0.002
Vegetables ≥ 2 servings/day. n (%)	9 (9.8)	29 (29.6)	0.001
Fruits≥ 3 servings/day. n (%)	34 (37.0)	16 (16.3)	0.001
Red meat, hamburger, or meat products <1 serving/day. n (%)	84 (91.3)	81 (82.7)	0.075
Butter, margarine, or cream <1 serving/day. n (%)	55 (59.8)	98 (100)	<0.001
Sweet or carbonated beverages <1 serving/day. n (%)	69 (75.0)	85 (86.7)	0.038
Wine ≥ 3 glasses/week. n (%)	9 (9.8)	21 (21.4)	0.026
Beans≥ 2 servings/week. n (%)	61 (66.3)	41 (41.8)	0.001
Fish or shellfish ≥ 3 servings/week. n (%)	13 (14.1)	62 (63.3)	<0.001
Commercial sweets or pastries <3 times/week. n (%)	38 (41.3)	29 (29.6)	0.091
Nuts ≥ 1 serving/week. n (%)	25 (27.2)	42 (42.9)	0.023
Preferentially consume white meats n (%)	57 (62.0)	46 (46.9)	0.037
Preparations seasoned with *sofrito* ≥2 times/week. n (%)	0 (0)	96 (98)	<0.001

(p = 0.038). Furthermore, in SP there was a greater restricted consumption of other sources of fat like butter, margarine or cream than in BR (p < 0.001). Brazilians on the other hand had a greater consumption of white meats (p = 0.037), fruits (p = 0.001) and beans (p = 0.001). There were no differences regarding consumption of red meat products, commercial sweets and pastries between the groups.

Considering these differences, the median adherence to Mediterranean diet score was higher in SP than in BR 7 (6–9) vs. 5 (4–6), p < 0.001. Most individuals of the BR group were concentrated on the poor adherence category (83.7%) while the majority of individuals on the SP group were divided in moderate and strong adherence (73.4%, p < 0.001 as shown in Fig. 1).

Table 5 shows the multivariate associations between the Mediterranean diet adherence score and biochemical parameters. There were independent inverse and statistically significant associations of the score points as a continuous variable with LDL-C, ApoB and hs-CRP after

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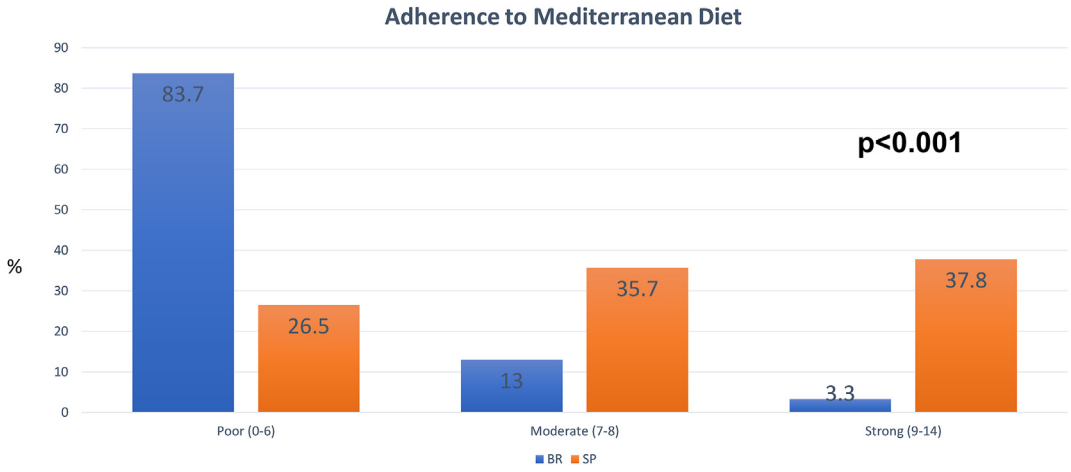

Figure 1 Adherence to the Mediterranean diet in Brazilian (BR) and Spanish (SP) heterozygous FH patients.

Table 5 Multivariate association between adherence to the Mediterranean diet and biochemical parameters in the whole studied population.

	Model 1			Model 2		
	β	95% CI	P	β	95% CI	p
LDL-C (n = 190)						
+1 point (continuous)	−0.08	−0.15−−0.02	0.015	−0.07	−0.13−−0.02	0.005
7-8 points	−0.21	−0.72−0.30	0.411	−0.14	−0.65−0.38	0.606
≥9 points	−0.30	−0.77−0.17	0.210	0.28	−0.66−0.10	0.152
ApoB (n = 190)						
+1 point (continuous)	−0.14	−0.17−−0.10	<0.001	−0.12	−0.17−−0.07	<0.001
7-8 points	−0.32	−0.76−0.12	0.156	−0.23	−0.78−0.31	0.404
≥9 points	−0.51	−1.00−−0.02	0.040	−0.48	−0.94−−0.01	0.044
hs-CRP (n = 182)						
+1 point (continuous)	−0.09	−0.12−−0.06	<0.001	−0.08	−0.10−−0.05	<0.001
7-8 points	−0.09	−0.24−0.4	0.167	−0.01	−0.11−−0.08	0.770
≥9 points	−0.30	−0.48−−0.12	0.001	−0.27	−0.35−−0.18	<0.001

Model 1: linear regression adjusted for pharmacological treatment, type of *LDLR* variant, smoking, education, level of physical activity, BMI and caloric intake.
Model 2: adjusted linear regression for pharmacological treatment, type of *LDLR* variant, smoking, education, level of physical activity, BMI and fatty acid intake.
Significant differences considered with $p < 0.05$. ApoB = apolipoprotein B; LDL-C = low density lipoprotein cholesterol; hs-CRP = high sensitivity C-reactive protein.

adjustment for confounders including caloric and fatty acid intake. Scores of 7−8 points and ≥9 points reflecting moderate and strong adherence to the Mediterranean diet respectively were not associated with LDL-C concentrations. On the other hand, strong adherence to the Mediterranean diet was independently and inversely associated with ApoB and hs-CRP concentrations.

Discussion

This is the first study comparing dietary patterns of individuals with molecularly confirmed heterozygous FH from different countries. Indeed, there were marked differences between FH individuals from BR and SP with the latter showing a lower ASCVD risk profile and greater adherence to the Mediterranean diet. Of importance, there was an inverse association of a score measuring adherence to the Mediterranean diet with dyslipidemia and low-grade inflammation biomarkers and strong dietary adherence was associated with lower ApoB and hs-CRP concentrations after adjustment for confounders.

Previously on the same population [8] we had encountered that plasma concentrations of dyslipidemia and low-grade inflammation biomarkers were associated with dietary components e.g., fatty acids profile and fibers despite the use of pharmacological LDL-C lowering therapies, the mainstay treatment for FH [1]. In this study we have expanded the findings to dietary patterns.

This study suggests that dietary patterns may play a role in dyslipidemia and inflammation profiles even after adjustment for strong confounders like pharmacological treatment even in a disease with a robust autosomal dominant genetic component like FH. Since dyslipidemia and inflammation are risk factors for atherosclerosis it may be possible that a Mediterranean style diet as followed in

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SP is superior to one low in saturated fat as the one recommended in BR in preventing atherosclerosis in FH individuals. Whether this is implicated with the greater severity of ASCVD seen in BR individuals than in SP remains to be determined.

Previous evidence from the same SAFEHEART registry from which individuals from SP originated showed that FH individuals had greater adherence to the Mediterranean diet pattern than their unaffected relatives [11]. The average score observed was 6.1 for men and 6.4 for women. In the present study, the median of the adherence score was 7 for the SP group, slightly above that observed by Arroyo-Olivares et al. In addition, 35.7% of the SP group had a moderate adherence score and 37.8% strong adherence. In the study by Arroyo-Olivares et al. these percentages were lower, respectively, 31.8% and 10.2% for men, and 35.7% and 12.5% for women. At any rate they were all strikingly higher than the ones encountered in BR patients were only 13.3% and 3.3% showed moderate and strong adherence respectively.

The Mediterranean diet has been associated in several studies with a reduced incidence of cardiovascular events and mortality [6,17,20]. In the present study, increase in adherence to the Mediterranean diet was negatively associated with LDL-C and apoB, biomarkers of pro-atherogenic lipoproteins, after adjustment for clinical, socio-demographic, pharmacological treatment and caloric and fatty acid intake.

Data from 3775 adults participating in the Hellenic National Nutrition and Health Survey, showed that higher adherence to the Mediterranean diet was associated with lower LDL-C values (-6.39 mg/dL, 95% CI: −12.60, −0.17) [21]. Estruch et al. in a sample from the PREDIMED study found that intervention with a Mediterranean diet supplemented with olive oil (n = 257) and nuts (n = 257) reduced LDL-C by −5.8 mg/dL (95% CI −9.8 to −1.8) and −3.80 mg/dL (95% CI −7.30 to −0.39), respectively in comparison with controls [17]. On the other hand, a cross-sectional study with 1290 participants in the Aragon Workers Health Study cohort did not observe variation in LDL-C according to quintiles of adherence to the Mediterranean diet [22].

In the present study, in addition to the inverse association observed between adherence to the Mediterranean diet and LDL-C, a stronger relation was seen with ApoB. Indeed, individuals categorized with strong adherence showed a significant negative association with ApoB concentrations a fact not seen for LDL-C. This is important since ApoB is a stronger marker of ASCVD risk than LDL-C since it encompasses not only LDL particles but also other pro-atherogenic lipoproteins like VLDL-remnants and IDL [23]. This is particularly important since there were some differences between BR and SP subjects regarding fasting status, not followed by most BR group, and also on the methods used to determine LDL-C, despite the high correlation coefficient encountered between LDL-C and ApoB concentrations. Indeed, there is evidence also from PREDIMED that a Mediterranean diet reduces ApoB levels in high cardiovascular risk individuals in comparison with a low-fat diet [24].

If reduction in pro-atherogenic lipoproteins by a Mediterranean diet in comparison with a low saturated fat diet is operational in prevention of ASCVD events in individuals with FH it remains to be determined. In a randomized controlled study with 7447 participants Estruch et al. [6] compared the effects of a Mediterranean diet supplemented with extra virgin olive oil or with mixed nuts with one with reduced fat content. After a mean follow-up of 4.8 years, Mediterranean diet groups were associated with a 30% reduced relative risk of ASCVD events. In that study however differences in clinical endpoints were not ascribed to possible changes on lipid profile. This suggests that other factors may be modulated by dietary patterns.

There is evidence that beneficial effects of the Mediterranean diet may come from olive oil, which is a food that predominates in this type of diet and contains antioxidant polyphenols, which together with other components obtained synergistically in this diet pattern could impact on a better anti-inflammatory profile and improvement in endothelial function, independently from the blood lipid profile [25,26]. In addition to the beneficial composition of olive oil, mainly monounsaturated fatty acids that may reduce either LDL-C or its oxidation when substituted for saturated fatty acids [27,28], its use in the Mediterranean cuisine improves the extraction of bioactive compounds such as polyphenols and carotenoids from the food matrix [29]. The beneficial effects of the Mediterranean diet may be not only due to consumption of certain foods, but also by use of culinary techniques such as sofrito (olive oil, garlic and tomatoes) [29,30]. Carotenoids in tomatoes can also be transferred to the oily fraction, due to their structure and solubility, and this could improve their bioavailability. The presence of these compounds in the oil fraction after the cooking process indicates stability in the oil matrix, preventing oxidation [31].

In the present study, a significant and inverse association was observed, regardless of caloric and fatty acids intake, between adherence to the Mediterranean diet and hs-CRP. A strong adherence to the Mediterranean diet was inversely associated with hs-CRP levels. This occurs even after adjustment for statin and ezetimibe use, medications that have been shown to reduce blood hs-CRP levels [32]. In a systematic review conducted by Fernandes et al. [33] olive oil consumption showed a negative association with hs-CRP values in studies that evaluated that component within a Mediterranean diet pattern, reinforcing the synergistic effect of nutrients consumed in different dietary patterns. In Italy a olive oil vegetable diet pattern was associated with lower hs-CRP than a pasta, and meat and egg, and sweet patterns [34]. A randomized study found that a Mediterranean diet reduced hs-CRP levels in comparison with one reduced in fat in subjects with the metabolic syndrome [35]. Finally, in the Women's Health Study moderate and strong adherence to a Mediterranean diet pattern were associated with lower hs-CRP levels and

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most important ASCVD events in comparison with women presenting low adherence [36].

Our study suggests that diet may indeed influence the pro-atherogenic biomarker profile even in a disease with a strong genetic effect as FH. Previously Pimstone et al. [37] had encountered a less severe FH phenotype including lipid profiles, in individuals living in rural China compared to Chinese living in Canada, despite presenting similar genetic variants. Part of these differences was attributed to distinctions in diet between the 2 countries suggesting that lifestyle may affect the FH phenotype.

This study has several limitations: i) Its cross-sectional design does not allow showing causality, but only associations; ii) Different nutritional questionnaires were used, however, they were validated for each population, considering cultural and nutritional differences between Brazil and Spain; iii) There were differences in the methods of determining LDL-C between countries that were minimized by the concomitant determination of ApoB. In addition, HDL-C and triglycerides were not determined in BR individuals, preventing further analysis of the influence of diet on the entire lipid profile. On the other hand, the well characterized molecular defects, clinical and nutritional data and the robust statistical methods reduce uncertainly of study results.

In conclusion, there were marked differences in dietary patterns between individuals with FH from both countries. The higher adherence to a Mediterranean-style diet was associated with a more favorable profile of dyslipidemia and low-grade inflammation biomarkers, this may have clinical implications supporting the role of lifestyle in preventing ASCVD in FH.

Declaration of competing interest

RDS has received honoraria related to consulting, research and or speaker activities from: Abbott, Amgen, Aché, Astra Zeneca, Esperion, GETZ Pharma, Kowa, Libbs, Merck, MSD, Novo-Nordisk, Novartis, PTC Therapeutics, Pfizer, and Sanofi. Others none to declare.

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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.2021.04.006.

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