Atherosclerosis 252 (2016) 21–27

Contents lists available at ScienceDirect

Atherosclerosis

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

Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia

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ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form
7 June 2016
Accepted 20 July 2016
Available online 22 July 2016

Keywords:
Familial hypercholesterolemia
Children
Diet
Dietary fat
Plasma cholesterol levels

ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). Guidelines recommend cholesterol-lowering medication from 8 to 10 years of age and dietary recommendations. Little is known about the diet of FH children and the effect of dietary counseling. The aim of the study was to describe the diet of FH children with respect to fat quality, and to investigate if dietary counseling improved lipid profile.

Methods: Fifty-four FH children (5–18 years) were included in the study and dietary intake was recorded with a pre-coded food diary for four days. Information about plasma lipid levels was obtained.

Results: Median intake of total fat, monounsaturated fat, polyunsaturated fat (PUFA) and saturated fat (SFA) was 30.8, 10.4, 5.9 and 12.0 E %, respectively. Among non-statin treated FH children, SFA intake was significantly correlated with TC, LDL-C and apolipoprotein (apo) B ($r_{sp} = 0.55$; $p = 0.004$, $r_{sp} = 0.46$; $p = 0.02$, and $r_{sp} = 0.45$; $p = 0.02$, respectively), and PUFA/SFA ratio significantly inversely correlated with TC ($r_{sp} = -0.42$; $p = 0.03$). Compared to the first visit, non-statin and non-plant sterol treated FH children ($n = 10$) had significantly reduced levels of TC ($p < 0.01$), LDL-C ($p = 0.01$), high-density lipoprotein cholesterol ($p = 0.02$), apo B ($p = 0.05$) and apo A-1 ($p = 0.02$) levels at a later visit.

Conclusions: FH children had a higher intake of SFA than recommended and the SFA intake was positively correlated with plasma TC, LDL-C and apo B levels in FH children not using statins. Importantly, the plasma lipid profile was improved in FH children after dietary counseling where focus was on reducing intake of SFA and dietary cholesterol.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominantly inherited disease, mainly caused by a mutation in the gene encoding the low-density lipoprotein (LDL) receptor [1] resulting in a two-to threefold increase in circulating LDL cholesterol (LDL-C) levels [1,2]. Treatment of FH is a lifelong process, as it is the total life-long exposure to LDL-C that may eventually lead to events of coronary heart disease [3]. Both medical and dietary treatments are recommended. The treatment target for primary prophylaxis in heterozygous FH is LDL-C below 3.5 mmol/L for children and below 2.5 mmol/l for adults [3]. However, in children cholesterol-lowering medication is not recommended until they reach the age of 8–10 years [4]. Total cholesterol (TC) and LDL-C levels can be modulated by dietary intake of fatty acids and cholesterol [5] and dietary recommendations are the first-line therapy before cholesterol-lowering medication may be initiated in children [6]. The dietary recommendations are according to the National Cholesterol Education Program (NCEP) and include the principles of a cholesterol-lowering diet, emphasizing a reduced intake of fat, especially saturated fat (SFA), and dietary cholesterol [7,8].

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http://dx.doi.org/10.1016/j.atherosclerosis.2016.07.913
0021-9150/

Studies have shown that dietary adjustments can reduce plasma cholesterol levels by 10–30% in non-FH subjects [9–11]. However, a recent Cochrane review found that data were not adequate to conclude about the effectiveness of dietary modifications in FH subjects, except for that of plant sterols [12]. A review by Katan et al. concluded that the LDL-C can be lowered by 10% if consuming two grams of plant sterols per day, and that there was sufficient evidence to encourage the use of plant sterols in persons with elevated plasma cholesterol levels [13]. Little is known about the recommended cholesterol-lowering diet on plasma cholesterol in FH children. The aim of the study was therefore 1) to characterize the diet in children with FH with particular focus on dietary fat quality, 2) to investigate the relation between fat intake and plasma cholesterol levels, and 3) to investigate the effect of the dietary advices on plasma cholesterol levels in non-statin and non-plant sterol treated FH children.

2. Patients and methods

2.1. Study sample

All children with FH aged 5–18 years that had an appointment at the outpatient Lipid Clinic, Oslo University Hospital, from September to December 2013 were invited to participate in the study. The children were invited to attend one study visit, subsequent to the already pre-scheduled appointment at the Lipid Clinic. Some of the children had never been to the Lipid Clinic before, other children had previously had an appointment at the Lipid Clinic (hereafter named *follow-up patients*). Invitations were sent by mail along with four pre-coded food diaries, a photographic booklet for estimation of portion sizes, and information on how to correctly record food intake. Before study participation, informed consent was signed by one of the parents or the child if 16 years or older. A flow chart of the study sample is shown in Fig. 1. Exclusion criteria were homozygous FH or other known diseases or conditions other than heterozygous FH. Throughout the recruitment period, 103 children were invited to participate. Out of these, 54 completed the study (52%). All FH children except three had genetically-verified FH. The remaining three children were assessed to have FH based on clinical examination and the Simon Broome criteria [14]. The study was approved by the Regional Committee for Medical and Health Research Ethics, Oslo, Norway.

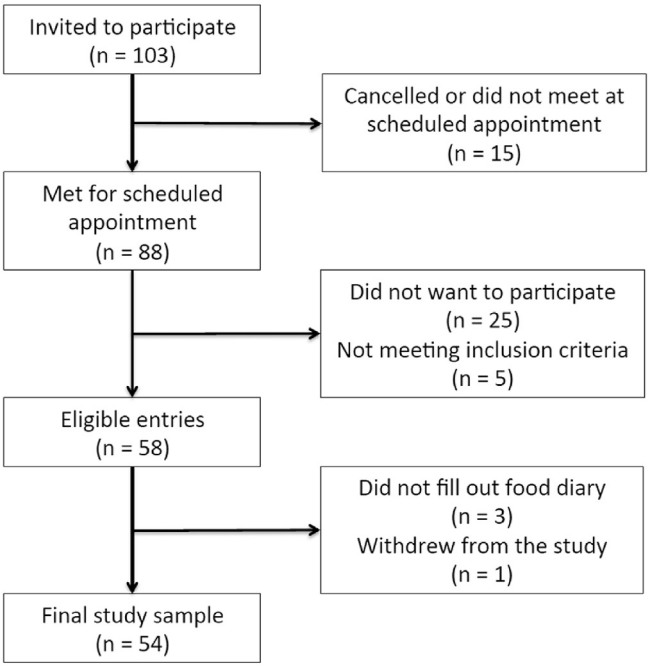

Fig. 1. Flow chart of the study sample.

2.2. Dietary counseling at the Lipid Clinic

At the Lipid Clinic at Oslo University Hospital, all patients meeting to a first time visit receive dietary counseling by a registered dietician. They all fill out a simple dietary registration (SmartDiet questionnaire) [22,23] before the consultation with the doctor and the dietician. The results from the SmartDiet questionnaire form the basis of the dietary counseling. This dietary counseling with a registered dietician lasts for approximately one hour at the first visit. Posters and food packages illustrating differences in fat quality between different food items are used during the session. Exchange list (suggestions for exchanging food items to items with improved fat quality) are reviewed. The patients are also given a booklet with dietary advices to subjects with high cholesterol or triglycerides. The Lipid Clinic and the National Advisory unit on FH have recently revised this booklet. At follow-up visits, the dietary counseling lasts 30 min. Usually, the dietary counseling is given for children together with one or both or the parents and siblings (family consultation). However, from the age of 15 years, the children may receive dietary counseling alone. If the patients appear to be well educated on the topic and when the SmartDiet score is satisfactory, a dietary consultation may not also be necessary at each visit but dietary counseling of children is of high priority. FH patients are usually followed-up on a 1–2 year basis.

2.3. Study visit

At study visit, a clinical examination was performed. Blood sampling was voluntary and was not a criterion for participation, if lipid analysis had been carried out within one month prior to the visit at the Lipid Clinic and the data were available in the medical journal. The following clinical information was extracted from medical record: mutation type, family history of cardiovascular disease, peripheral cholesterol deposits, weight, height, clinical information and pre-treatment plasma lipid values. For all follow-up patients, we also collected data from medical journals from the first visit at the Lipid Clinic, including biochemical data. For this group of children, data was compared between the first visit and the follow-up visit. Because of age-related variation in body composition and body mass index (BMI; kg/m^2), cut-off values change with age [15]. Iso-BMI is BMI adjusted for age [16] and was used to assess the relation between weight and height in the participants [16]. We used the BMI-curves suggested by Júlíusson et al. containing cut off curves for underweight and overweight and different curves for iso-BMI in girls and boys [17].

2.4. Dietary registration

To record dietary intake, we used a pre-coded food diary (PFD) previously validated in a Norwegian children cohort (9 and 13 years of age) [18–21]. The PFD contained 18 pages listed with pre-coded beverages, food items and dishes, as well as dietary supplements, in an arranged pattern. Household units and a validated photographic booklet were used for estimation of portion sizes [18–21]. Throughout the PFD, there were open spaces where participants could fill in consumed items that were not listed as alternatives. Participants were asked to write down everything they ate and drank in the PFD for four consecutive days, of which one day was a weekend day. The participants were interviewed either in person

or by phone after their filling of the PFD, and they were asked whether they used full-fat or low-fat version of products like meat, milk and cheese, and products with an improved fatty acid composition. Since the pre-coded dishes in the PFD consisted mainly of full-fat recipes, we made new recipes for the common-used dishes taco, pita, pasta Bolognese and lasagna. The PFD-sets was sent to the participants in advance, however, only approximately half of the study participants filled out the PFD before the study visit (n = 24). The other half (n = 30) filled out the PFD after their study visit. The dietary data was calculated using KBS (version 7.1, year 2014), which is a food database and software system developed at the Department of Nutrition, University of Oslo. In addition, a dietary score was also calculated by the use of the Smart Diet questionnaire for both children and the parent accompanying the child for the visit. Smart Diet questionnaire is a short self-instructing questionnaire on diet and lifestyle, and was developed by the Lipid Clinic, Oslo University Hospital, to easily assess diet and lifestyle habits in clinical settings. The questionnaire has been validated in 2002 among adult subjects [22]. The total score forms the basis for an overall assessment of the diet and food quality as described previously [22,23].

2.5. Routine laboratory assays

Standard blood chemistry, lipid parameters, hormones (estradiol, testosterone, sexual hormone binding protein and 25-hydroxy vitamin D), ferritin, vitamin B 12, folate, vitamin A and C-reactive protein (CRP) were measured in serum/plasma using routine laboratory methods. The LDL cholesterol was measured directly.

2.6. Statistical analysis

Most variables were not normally distributed, and prior to analyses such variables were log-transformed. To account for familial dependency, differences in diet and biochemical markers between children were tested using a random intercept linear mixed model accounting for between-family variation. All linear mixed models were fitted using the package *lme4*, while *p*-values for the fixed effects were calculated using the package *lmerTest*, both available in the open-source statistical software R, version 3.2. [24]. All data are presented as median (25^{th}–75th percentiles) if not otherwise stated. Spearman's correlation coefficient (r_{sp}) was used for describing the correlation between two continuous variables and was performed among non-statin treated and non-siblings FH children. *p*-values <0.05 were considered statistically significant.

3. Results

Characteristics of the study sample are shown in Table 1. The median age was 12 years (5–18 years, min-max). As expected, both TC and LDL-C were higher in the FH children compared to reference values. All other blood values were within normal range for age. There were few differences between girls and boys, except for plasma vitamin D levels which were significantly higher in girls ($p = 0.004$), and as expected, girls had a significantly higher plasma level of estradiol ($p = 0.002$) and a significantly lower plasma level of testosterone ($p < 0.001$) compared to boys. Twelve out of 27 girls had estrogen levels ≥0.15 nmol/L and seven out of 20 boys had testosterone ≥8 nmol/L. Thirty-five percent of the children used statins and 15% used plant sterol enriched margarine.

3.1. Intake of macro- and micronutrients

The intake of macronutrients is shown in Table 2. The total fat intake, monounsaturated, polyunsaturated fat and cholesterol were within NCEPs recommended range in FH children. However, the median intake of SFA was 12.0 E%, which is much higher than the recommended intake of 7 E%. The boys had a significantly higher intake of total unsaturated fat compared to girls ($p = 0.03$), but no other significant differences were observed between girls and boys (Table 2) ($0.06 \leq p \leq 0.82$).

Twenty-nine of 54 participants used one or more dietary supplements during the dietary registration period. Most of these participants used omega-3 supplements (n = 28), including cod liver oil. We found no significant difference between the non-statin treated children taking omega-3 supplements (n = 23) or not (n = 9) with respect to TC, LDL-C, triglycerides and apolipoprotein (apo) B levels ($0.62 \leq p \leq 0.89$), whereas high-density lipoprotein cholesterol (HDL-C) and apo A-1 levels seemed to be influenced by omega-3 supplementation ($p = 0.02$ and $p = 0.003$, respectively). Children using omega-3 supplements had lower HDL-C (median (25th –75th percentile) 1.4 (1.3–1.5) mmol/L and 1.8 (1.3–2.1) mmol/L; respectively) and apo A-1 levels (1.4 mmol/L (1.3–1.5) and 1.8 mmol/L (1.5–1.8); respectively) than children who did not.

The intake of most micronutrients was within the recommended range [25], but the median intake of vitamin D and iron were lower than the recommended value for both genders, whereas folate intake was lower only for the girls (data not shown). There were no significant differences in the intake of micronutrients or dietary supplements between girls and boys ($0.10 \leq p \leq 0.70$).

3.2. Relation between dietary fats, plasma lipid levels and other blood parameters

We investigated the association between intake of different types of dietary fats and plasma lipid levels, and the estimated correlation coefficients are shown in Table 3. Twenty-six non-sibling FH children not on cholesterol-lowering medication were included in the analysis. There was no significant correlation between total fat intake and plasma lipid levels ($0.11 \leq p \leq 0.54$). However, significant positive correlation was observed between SFA intake and plasma TC, LDL-C and apo B ($r_{sp} = 0.55$, $r_{sp} = 0.46$ and $r_{sp} = 0.45$, respectively, $p \leq 0.02$). Plasma HDL-C, apo A-1 and triglycerides were not significantly associated with the intake of SFA ($0.07 \leq p \leq 0.39$). No significant correlation was found between polyunsaturated fat (PUFA) intake and plasma lipid levels ($0.26 \leq p \leq 0.85$). For the PUFA/SFA ratio (PS-ratio), we found a significant negative correlation with TC ($r_{sp} = -0.42$, $p = 0.03$).

3.3. Dietary scores

We correlated the Smart Diet score of the parent with that of the non-sibling FH child not on cholesterol-lowering medication, where Smart Diet score was available from both child and parent (n = 21). There was no significant correlation between the Smart Diet score of the children and the score of the parents ($r_{sp} = 0.28$; $p = 0.22$). However, the Smart Diet score of the children (n = 26) correlated negatively with plasma total cholesterol ($r_{sp} = -0.55$; $p = 0.04$), LDL-C (r = −0.47; $p = 0.02$ and apo B (r = −0.44; $p = 0.03$), whereas there was no significant correlation between the parent Smart Diet score and the lipid levels of the child ($0.080 \leq p \leq 0.93$, for all). Furthermore, we also correlated the Smart Diet score of the FH children (n = 26) with macronutrients calculated from the four-day dietary registration. Significant negative correlations were found for Smart Diet and total fat E% ($r_{sp} = -0.39$; $p = 0.05$), saturated fat E% ($r_{sp} = -0.67$; $p < 0.001$), trans fat E% ($r_{sp} = -0.65$; $p < 0.001$) and positive correlations were found for polyunsaturated fat E% ($r_{sp} = 0.48$; $p = 0.013$). No significant correlations were found for Smart Diet score and E% of

Table 1
Characteristics (All FH children).

	Total, n = 54	Girls, n = 31	Boys, n = 23	Reference values[a]	*p*
Age, years	12 (10–15)	13 (10–15)	11 (10–15)		
Girls, %	57	–	–		
Siblings, %	46	55	35		
iso-BMI[b] normal, %	74	74	74		
iso-BMI ≤ 18.5, %	7	7	9		
iso-BMI ≥ 25, %	19	19	17		
Cholesterol-lowering therapy, % medication, %	35	36	35		
Use of plant sterols, %	15	13	17		
TC, mmol/L	5.6 (5.0–7.1)	5.6 (5.2–7.1)	5.5 (5.0–7.0)	3.0–5.5	0.28
LDL-C, mmol/L	4.1 (3.3–5.4)	4.1 (3.4–5.2)	3.6 (3.3–5.4)	<3.0	0.43
HDL-C, mmol/L	1.4 (1.2–1.7)	1.5 (1.3–1.8)	1.4 (1.2–1.6)	0.8–2.7	0.24
Triglycerides, mmol/L	0.8 (0.6–0.9)	0.8 (0.6–0.8)	0.8 (0.6–1.1)	0.5–2.6	0.31
Apo A-1, g/L	1.4 (1.3–1.5)	1.4 (1.3–1.6)	1.4 (1.3–1.5)	1.0–2.3	0.68
Apo B, g/L	1.1 (0.9–1.4)	1.2 (1.0–1.4)	1.1 (0.9–1.4)	0.5–1.3	0.09
Lp (a), mg/L[c]	287 (99–623)	269 (120–548)	289 (93–643)	–	0.45
CRP, mg/L	0.6 (0.6–1.1)	0.6 (0.6–1.4)	0.6 (0.6–1.0)	<4	0.31
Estradiol, nmol/L[d]	0.10 (0.05–0.17)	0.15 (0.06–0.29)	0.05 (0.04–0.12)	–	**0.002**
Testosterone, nmol/L[d]	0.6 (0.5–2.5)	0.5 (0.5–1.4)	5.2 (0.5–12.9)	–	**<0.001**
SHBG, nmol/L[4]	58 (39–85)	65 (47–87)	49 (33–74)	–	0.11
Glucose non-fasting, mmol/L[c]	4.9 (4.7–5.4)	4.8 (4.7–5.2)	5.1 (4.8–5.6)	4.0–6.0[e]	0.26
Ferritin, μg/L	31 (24–46)	32 (22–58)	31 (28–42)	10–140	0.27
Vitamin B_{12}, pmol/L[f]	393 (332–534)	398 (336–526)	367 (325–525)	150–650	0.55
Folate, nmol/L[f]	19 (16–23)	19 (15–25)	19 (17–23)	>10	0.56
Vitamin A, μmol/L[d]	1.3 (1.1–1.6)	1.4 (1.1–1.6)	1.3 (1.1–1.4)	1.2–3.6	0.36
Vitamin D (25-hydroxy), nmol/L[d]	48 (39–59)	56 (45–63)	43 (36–50)	37–131	**0.004**

Baseline data for the whole FH children group including new and follow-up subjects.
Data are presented as percentage or median (25th-75th percentile). Blood samples are non-fasting.
BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo (A-1, B), apolipoprotein (A-1, B); Lp(a), lipoprotein (a); CRP, C-reactive protein; SHBG, sex hormone-binding globulin. P-value indicate difference between boys and girls.
Significant *p* values in bold.
[a] The reference values are the reference values for girls and boys aged 12 years, given by the Department of Medical Biochemistry at Oslo University Hospital.
[b] iso-BMI is BMI adjusted for age.
[c] Total n = 50, girls = 28, boys = 22.
[d] Total n = 47, girls = 27, boys = 20.
[e] Reference value for fasting glucose.
[f] Total n = 48, girls = 27, boys = 21.

Table 2
Intake of macronutrients in children with familial hypercholesterolemia.

	Total, n = 54	Girls, n = 31	Boys, n = 23	Recommendations[a]	*p*
Energy, kJ	7276 (6204–8700)	6953 (5719–7970)	7956 (6908–8706)	balanced intake	0.33
Protein, E%	16.8 (15.5–18.7)	16.7 (15.7–19.0)	16.8 (14.9–18.1)	15	0.20
Fat, E%	30.8 (28.7–33.8)	30.6 (28.5–33.3)	31.9 (29.2–35.0)	25–35	0.28
Saturated fat, E%	12.0 (10.0–13.8)	11.9 (10.4–13.3)	13.1 (9.8–14.3)	<7	0.68
Trans fatty acids, E%	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.3 (0.3–0.5)	<1[b]	0.69
Monounsaturated fat, E%	10.4 (9.2–11.0)	10.1 (9.0–10.8)	10.6 (9.6–11.5)	up to 20	0.22
Polyunsaturated fat, E%	5.9 (5.3–6.7)	5.7 (5.0–6.7)	6.1 (5.6–7.5)	up to 10	0.06
Total unsaturated fat, E%	16.6 (14.9–17.4)	15.7 (14.7–16.8)	16.9 (15.7–18.2)	–	**0.03**
PS-ratio	0.50 (0.38–0.66)	0.45 (0.37–0.60)	0.51 (0.41–0.71)	–	0.15
Cholesterol, mg	184 (138–253)	169 (133–223)	197 (164–272)	<200	0.16
Carbohydrates, E%	48.0 (45.7–52.2)	48.0 (46.0–51.9)	48.0 (45.4–52.2)	50–60	0.82
Added sugar, E%	10.2 (6.4–14.0)	9.0 (6.3–11.5)	13.7 (7.4–16.3)	<10[c]	0.52
Fiber, g	18 (13–22)	18 (14–24)	16 (13–21)	20–30	0.24

Baseline data for the whole FH children group including new and follow-up subjects.
Data are presented as median (25^{th}-75th percentile).
kJ, kiloJoule; E%, energy percent. PS-ratio; ratio between polyunsaturated fat and saturated fat.
p-values indicate difference between boys and girls. The nutrient composition of the diet recommended by the National Cholesterol Education Program (NCEP) [7][a], the European Society of Cardiology and the European Atherosclerosis Society (52)[b], the Nordic Council of Ministers [25][c].
Significant *p* values in bold.

protein, carbohydrates and monounsaturated fat.

3.4. Effects of dietary counseling on plasma lipid levels

To investigate the effect of dietary counseling on blood parameters in FH children who did not use cholesterol-lowering medication or food containing plant sterols, plasma lipid levels from first visit at the outpatient clinic were compared to plasma lipid levels at the current follow-up visit (n = 10). The median age at the current visit was 11 (9.5–14.5) years, 80% were girls, and the median time between first and current follow-up visit was 2 years (2 years for 80% of the participants). Children living in Oslo usually meet once yearly including a 15–30 min dietary counseling while children from distant areas may not meet every year. Consequently for some children, there may have been more visits in-between the first visit and the current visit (including dietary counseling), whereas for

Table 3
Correlations between dietary intake of saturated and polyunsaturated fat and plasma lipid levels.

	Non sibling and non-statin users, n = 26							
	Total fat intake		Saturated fat intake		Polyunsaturated fat intake		PS-ratio	
	r	*p*	r	*p*	r	*p*	r	*p*
TC	0.32	0.11	**0.55**	**0.004**	−0.23	0.26	**−0.42**	**0.03**
LDL-C	0.25	0.21	**0.46**	**0.02**	−0.23	0.27	−0.38	0.06
HDL-C	0.13	0.54	0.18	0.39	−0.05	0.82	−0.07	0.73
Triglycerides	0.17	0.40	0.18	0.39	0.12	0.57	−0.02	0.91
Apo A-1	0.32	0.11	0.37	0.07	0.04	0.85	−0.14	0.48
Apo B	0.28	0.17	**0.45**	**0.02**	−0.21	0.31	−0.36	0.07

r is Spearman's correlation coefficient. Children using cholesterol-lowering medication were excluded. Siblings are not included in the analysis.
TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo (A-1, B), apolipoprotein (A-1, B). PS-ratio is the ratio between polyunsaturated fat and saturated fat. Significant *p* values in bold.

other children, the current visit was the second visit. We found a significant reduction in plasma TC ($p < 0.01$), LDL-C ($p = 0.01$), HDL-C ($p = 0.02$), apo B ($p = 0.05$) and apo A-1 ($p = 0.02$) levels, but no difference in plasma triglyceride level from first visit to current follow-up visit ($p = 0.84$) (Table 4). The median reduction in plasma TC level was 1.1 mmol/L (16%) and the median reduction in plasma LDL-C was 1.1 mmol/L (22%). Among the 10 subjects included in this analysis, two were boys and both had plasma testosterone levels ≤3 nmol/L. Among the eight girls, seven had plasma estradiol levels ≤0.15 nmol/L and one girl had plasma estradiol levels of 0.40 nmol/L. Median estradiol level at the current follow-up visit was 0.06 (0.04–0.14) nmol/L and median testosterone level was 0.50 (0.50–1.8) nmol/L.

4. Discussion

In the present study, intake of saturated fat was positively associated with plasma TC, LDL-C and apo B levels in FH children. A new and important finding was that the plasma lipid profile was improved in FH children after dietary counseling where focus was on reducing intake of SFA and dietary cholesterol despite the fact that FH children had higher intake of SFA than what is recommended.

Studies in children with FH have shown that initiation of statin therapy is safe when started from the age of 8–10 years, which is also according to the current guidelines [4]. Statin therapy has been shown to reduce plasma cholesterol levels in the range 10–60%, depending on type and dose of statin [26–28]. Previous studies in FH children have shown that dietary changes may reduce plasma cholesterol levels by 7–23% [29–32], which is equivalent to the effect observed in non-FH subjects. This magnitude of cholesterol-lowering effect may therefore be important for young FH children as first line therapy (not yet on statin treatment), but also contribute supplementary on top of statin therapy for older children and adolescents. Food items containing plant sterols or stanols, such as plant sterol-enriched yoghurt or margarine, have been shown to reduce plasma LDL-C concentration by 10–15% [33–36] and should be included in the dietary advices for FH patients.

Table 4
Plasma lipids at first visit and follow-up visit in children with FH.

	Follow-up visitors, n = 10		*p*
	First visit	Follow-up visit	
TC, mmol/L	6.8 (6.5–7.7)	5.6 (5.1–7.0)	**<0.01**
LDL-C, mmol/L	5.1 (4.7–5.8)	4.1 (3.4–4.8)	**0.01**
HDL-C, mmol/L	1.4 (1.2–1.7)	1.3 (1.2–1.4)	**0.02**
Triglycerides, mmol/L	0.7 (0.7–0.8)	0.8 (0.6–1.0)	0.84
Apo A-1, mmol/L	1.4 (1.3–1.6)	1.4 (1.3–1.5)	**0.02**
Apo B, mmol/L	1.3 (1.3–1.4)	1.2 (0.9–1.3)	**0.05**

Data are presented as median (25th–75th percentile).
Children using cholesterol-lowering medication and plant sterols were excluded.
TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo (A-1, B), apolipoprotein (A-1, B).
n = 7 for apo A-1 and apo B, first visit.
Significant *p* values in bold.

The composition of diet in FH children in terms of total fat, protein and carbohydrates was consistent with the NCEP and Nordic Nutrition recommendations (NNR) [7,25] and consistent with other dietary surveys conducted on children and adolescents [37–41]. Even though the total fat intake was within the NCEP recommendations, the FH children in this study had an intake of SFA of 12 E%, considerably higher than the NCEP recommendation of dietary intake of 7 E% from SFA [7] and at the same level as found in healthy Norwegian children [37,40,41]. The intake of added sugar among FH children was slightly above the recommended level [25].

The intake of vitamin D, iron and folate was suboptimal, whereas the intake of the remaining micronutrients was adequate, when comparing the intake to the NNR [25]. This is in concordance with other studies [37,38], and therefore probably not a problem isolated to the FH population, but rather a public health problem. However, the blood values reflecting the status of vitamin D, iron and folate were within reference values.

Previous studies in FH subjects have shown changes in plasma cholesterol levels in response to intake of dietary fatty acids [30,42–45]. We found that SFA intake was associated with higher levels of TC, LDL-C and apo B whereas the PS-ratio was associated with lower levels of TC. Our data indicate that replacing SFA with PUFA will reduce plasma total cholesterol in children with FH. Our results are in accordance with the findings of Nuotio et al., who found significant association between SFA, PUFA and PS-ratio and plasma LDL-C and apo B in subjects from the Cardiovascular Risk in Young Finns Study [46], and Sala-Vila et al. who found that intake of the omega-3 PUFA eicosapentaenoic acid was associated with a beneficial lipid profile in adults with FH [47]. In contrast, Tonstad et al. [48] did not find any correlation between dietary intake and plasma lipid levels in children with FH. In accordance with our results, Molven et al. [23] also found a long-lasting effect of dietary counseling with regard to healthier food choices specifically with regard to fat quality in children with FH. Our findings clearly suggest that encouraging an increased intake of PUFA at the expense of SFA is reasonable, and associated with lower levels of TC and LDL-C.

When we looked at the plasma cholesterol levels at the very first visit at the outpatient clinic of the FH children who had previously visited the Lipid Clinic, and compared them to the current follow-up visit, we found that the plasma levels of TC, LDL-C and HDL-C were reduced in FH children. Studies with statin treatment have

shown that LDL-C reductions between 0.6 and 1.0 mmol/L may reduce expected ischemic heart disease events by 20–56% [27], and that early initiation of treatment is necessary for optimal prevention. The IMPROVE-IT study showed that a 0.4 mmol/L reduction of LDL-C concentration with ezetimibe for median 6 years reduced the cardiovascular disease risk by approximately 6% in a secondary prevention trial [49]. Moreover, a lifelong reduced plasma LDL-C of 0.31 mmol/L compared to controls were associated with a relative reduction of 53% in the risk of coronary heart disease [50]. Based on these data, it is therefore conceivable that the difference, though modest, observed in cholesterol concentrations in the present study may be of clinical relevance, especially if maintained throughout life.

The level of plasma lipids will change in puberty, due to the alteration of gonadal hormones [51]. Unfortunately, we do not have hormone measurements from their first visit, but the plasma median estradiol level and testosterone level at the current visit were low, suggesting that puberty may probably not be the cause of the reduced levels of TC and LDL-C observed here. It is therefore likely that the observed decrease in plasma cholesterol is a result of dietary counseling.

As shown in Table 1, a large proportion of the children were siblings (46%), thus we had to take familial dependency into account. Since FH is an autosomal dominant disease, 50% of the children will inherit the disorder. In addition to sharing similar genes, children in the same family are likely to be more similar to each other than to individuals in different families. Siblings also share a similar environment as regards dietary influences. Such dependencies within families were therefore taken into account in the statistical analysis. A limitation of the study is the relatively small number of subjects in the sub-groups and that only 52% of the invited FH children accepted the invitation to participate in the study. However, there was no bias in the selection of subjects as all FH children scheduled for ordinary outpatient visit in the study period were invited to participate in the study. Moreover, another limitation in the study is that we only registered regular use of omega-3 supplements, and not the dose. We therefore cannot differentiate between different doses of omega-3 supplements. Finally, the lack of any measurements of early atherosclerosis progression like carotid IMT and the limited longitudinal assessment must also be pointed out as limitations of the study. A major strength of the study is the use of a food diary validated for use in children [18–21]. However, the pre-coded dishes in the PFD consisted mainly of full-fat recipes, which may have led to an overestimation of the intake of total and SFA, although we made new recipes for the most common eaten dishes. Furthermore, we cannot rule out the possibility that selective misreporting has occurred, in terms of pleasing bias. It is possible that the participants who filled out the PFD after the study visit were influenced by the dietary advice they received at their consultation. However, the participants made an impression of being quite honest, and reporting of unhealthy foods like cakes, sweets and sugar-sweetened beverages was common. Moreover, when investigating the fatty acid composition of the diet among those who filled out the dietary registration prior to the visit ($n = 24$) versus after the visit ($n = 30$), a tendency to increased level of SFA in the diet were observed among the subjects who filled out the registration after the visit (11.3 E% *vs*. 13 E%, median before and after visit respectively; $p = 0.054$) suggesting that this was not the case.

In conclusion, dietary recommendations are first-line therapy in FH children before cholesterol-lowering therapy is initiated at the age of 8–10 years, and adjunct treatment for all FH patients, but the effectiveness of dietary modifications in FH subjects is uncertain [12]. In the present study we found that intake of total fat, monounsaturated fat and PUFA was within the recommended range in children with FH, but intake of SFA was higher than recommended. Intake of SFA was significantly correlated with plasma TC and LDL-C levels, and non-statin non-plant sterol treated FH children had lower plasma TC and LDL-C at the later visit to the outpatient clinic compared to the first visit, suggesting beneficial and clinical relevant effects of dietary treatment in FH children.

Conflict of interest

During the past five years, Retterstøl has received honoraria for lectures or expert meetings from Merck, Pfizer, Mills DA, Melk.no, Apotek1, Pronova, Amgen, Genzyme, and Sanofi; none of which are related to the contents of this manuscript. During the past five years Holven has received research grants or honoraria from Tine, Mills, and Olympic Seafood, and Amgen; none of which are related to the contents of this manuscript. The rest of the authors declare no conflict of interest.

Financial support

This work was supported by grants from The Throne-Holst Foundation for Nutrition Research, Oslo, Norway, The National Advisory Unit on FH, Oslo University Hospital and the University of Oslo, Oslo, Norway.

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