

Original Research

Effect of a Rapeseed Oil Substituting Diet on Serum Lipids and Lipoproteins in Children and Adolescents with Familial Hypercholesterolemia

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Key words: familial hypercholesterolemia, rapeseed oil, monounsaturated fatty acids, cholesterol, triglycerides, children, LDL-cholesterol, HDL-cholesterol

Objective: Familial hypercholesterolemia (FH) is a predominantly inherited disorder, which contributes to a defect of the LDL-cholesterol receptor. For adults with familial hypercholesterolemia (FH), it is known that a supplementary diet of monounsaturated fatty acids reduces elevated levels of total cholesterol and LDL-cholesterol and may further increase HDL-cholesterol. In particular the reduced intake of dietary fat reduces total serum cholesterol and LDL-cholesterol in the range of 10% to 15% and inhibits LDL-oxidation. Once the diagnosis of familial hypercholesterolemia is made in early childhood a supplementary diet with rapeseed oil should be started as early as possible to prevent development of atherosclerosis and subsequent complications. So far there are no reports of a lipid lowering diet enriched with rapeseed oil in children and adolescents.

Methods: Seventeen children and young adolescents (male = 6, female = 11, ages 4 to 19 years) diagnosed with FH were enrolled in this study. They received dietary training and a classical low fat/low cholesterol diet enriched with rapeseed oil over five months. In the first two months they received orally mean 15 g/day (8–23 g/day), for the remaining three months mean 22 g/day (15–30 g/day) rapeseed oil. The calculation of the three-days dietary protocols showed the following characteristics: 29.5% calories from fat, 14.3% calories from protein and 54.6% calories from carbohydrates. The subjects had six sessions of dietary counseling, and serum lipids levels and lipoprotein(a) were estimated; each month's diet adherence was controlled by a dietitian and discussed with the patients and their families during this five-month study.

Results: During five months of rapeseed oil diet serum triglycerides decreased by 29% (119.2 ± 62.8 mg/dL vs. 84.9 mean ± 39.7 mg/dL), VLDL-cholesterol by 27% (23 ± 12 mg/dL vs. 17 ± 8 mg/dL), total cholesterol by 10% (233 ± 35 mg/dL vs. 213 ± 36 mg/dL), LDL-cholesterol by 7% (151 ± 31 mg/dL vs. 142 ± 31 mg/dL), HDL-cholesterol (59 ± 15 mg/dL vs. 57 ± 11 mg/dL) and Lp(a) (29.8 ± 36.3 mg/dL vs. 32.6 ± 40.7 mg/dL) were not changed significantly. The diet was well accepted; in most families a sustained change was reported.

Conclusions: Our results indicate that in children and adolescents with FH a lipid-lowering diet with rapeseed oil has a similar effect on total serum cholesterol and LDL-cholesterol compared to classical cholesterol reduction diets (step I). However, an additional pronounced effect on lowering of triglycerides and VLDL-cholesterol can be observed.

INTRODUCTION

Heterozygous form of familial hypercholesterolemia is one of the most frequent metabolic disorders with an incidence of approximately 1:500 in the general population. The underlying cause, an LDL receptor defect, is located on the short arm of

chromosome 19, and more than 400 mutations have been described so far. FH is due to a lack of functional receptors for LDL on the cell surface, whereby LDL cannot be taken up and catabolized by the cells, especially by the hepatocytes. Myocardial infarction already may occur at the mean age of 45 to 48 years in males and 55 to 58 in females. Relatives of patients

Abbreviations: FH = familial hypercholesterolemia, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low-density lipoprotein-cholesterol, VLDL-C = very low-density lipoprotein-cholesterol, TC = total cholesterol, TG = triglycerides, Lp(a) = lipoprotein (a), BMI = body mass index.

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Journal of the American College of Nutrition, Vol. 21, No. 2, 103–108 (2002)

Published by the American College of Nutrition

with familial hypercholesterolemia may suffer from coronary heart disease before the sixth decade at a significantly higher rate when compared to a healthy population [1–6]. To reduce the LDL-cholesterol in patients with FH it has been shown useful to replace the intake of saturated fats by polyunsaturated and monounsaturated fats. Within the last few years several studies have shown that the replacement of saturated fats by monounsaturated fats significantly lowers cholesterol and LDL-cholesterol in patients with hypercholesterolemia [7–15]. For the latter a beneficial effect in hyperlipidemic patients has been confirmed [16].

We chose a low fat/low cholesterol diet with rapeseed oil as the only visible fat used, produced in Austria. Rapeseed oil has a composition similar to that of olive oil, which is typical for the Mediterranean diet. Our goal was therefore to investigate how adolescents and families with familial hypercholesterolemia would respond to a lipid lowering diet enriched with rapeseed oil and what effects on serum lipids can be observed.

METHODS

Subjects

Patients were selected after careful supervision in our pediatric outpatient clinic, where 43 otherwise healthy children and adolescents with familial hypercholesterolemia entered this study. Heterozygous familial hypercholesterolemia is completely expressed in childhood by significantly increased levels of plasma total cholesterol (>200 mg/dL) and low density lipoprotein-cholesterol (>130 mg/dL). The identification of affected patients is facilitated through a screening of offspring with a positive family history of cardiovascular disease, since different lipoprotein phenotypes reflect the presence of other genetic conditions, including familial hypercholesterolemia [17].

However, 26 patients were not able to adhere to the study protocol or did not provide us with an appropriate dietary protocol. Therefore 17 children and adolescents (males = 6, females = 11) between the ages of 4 and 19 years (median age 12.7 years) were included in this study. Selection criteria that were applied are summarized in Table 1. None of the patients had taken any lipid-lowering drugs during the two months before entering the study.

Table 1. Inclusion Criteria (Characteristics) for Patients Selected

Family History	Cardiovascular disease in one of the parents and/or hypercholesterolemia
Total Cholesterol	>200 mg/dL
LDL-Cholesterol	>150 mg/dL
Triglycerides	<250 mg/dL
Body Mass Index (BMI)	10%–90% percentiles [24]

Study Design/Dietary Program

Starting in October 1997, the study period lasted at least five months. Serum lipids (total serum cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, lipoprotein(a)) were estimated, and body weight and height were recorded at the beginning and on the occasion of each visit. All patients were interviewed individually about their nutritional habits, social environment, potential congenital diseases and their parents' medical history. Informed consent was given to all details of the study. All subjects and their parents were informed about basic energy intake and were instructed how to reduce the consumption of saturated fatty acids and total cholesterol. At each visit a dietary consultation with the same experienced dietitian was performed. Compliance to the diet was checked by routine three-day protocols with children and parents.

During the study all patients met the dietitian seven times and the pediatrician six times. In order to encourage the patients' compliance, all family members were involved in the nutritional education program and followed the diet (Table 2). Diet in combination with rapeseed oil was the basis of our treatment in this study in all patients. A dietitian and a pediatrician gave basic information about the diet, intensive and repeated counseling, as well as motivated and trained the parents and children. Families were advised to weigh food intake only in the period when they wrote their dietary plans. A dietitian explained the dietary plans to all family members and the children. Complete data were obtained from 17 patients. Since the patients were treated as outpatients their parents had to bring monthly detailed protocols of their nutrition habits. These documents comprised the last three days of food composition and fluid intake. These protocols served as a control means and also as a positive factor for continuing the diet.

Diet

The diet was based on two approaches: (1) replacement of as many visible fats as possible by rapeseed oil and (2) a reduction of dietary cholesterol. In order to achieve the first goal children were instructed to avoid meat, sausage, butter and chocolate. The high amount of dietary fiber was achieved by prescribing a high quantity of fruits and vegetables. Protein intake was recommended to be 15% of total daily energy intake, 2/3 consisting of plant and 1/3 of animal protein. This

Table 2. Study Design

Week 0	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20
B		B	B	B	B	B
DT	DT	DT	DT	DT	DT	DT
DR	DR	DR	DR	DR	DR	DR

B = blood sample, DT = dietary training, DR = dietary training including a 3-day dietary recall.

included a fish meal once a week. In order to replace as many visible saturated fats by monounsaturated fats, rapeseed oil (consisting of 59% monounsaturated fatty acids, 7% saturated fatty acids and 34% polyunsaturated fatty acids) was recommended to be used for frying, baking and in salad dressing. All patients together with mothers or fathers were instructed on a low fat and low cholesterol diet containing rapeseed oil, which should provide sufficient energy and micronutrients. The calculation of the three-day dietary protocols showed the following characteristic means: 29.5% calories from fat, 14.3% calories from protein and 54.6% calories from carbohydrates. The mean intake of saturated fat was 19.3 g and cholesterol was 98 g. During this period only rapeseed oil was allowed to be used for preparation of the meals. None of the patients used rapeseed oil before starting the study. Most of the patients normally used sunflower oil for cooking. No patient canceled the study due to dislike of the oil. Rapeseed oil was substituted for all fluid oils, and daily intake was calculated to a mean of 15 g/day (8–23 g/day) during the first two months, raised to a mean of 22 g/day (15–30 g/day) afterwards. Therefore the mean MUFA/PUFA has been calculated to be 1/0.5 (Table 3).

Laboratory Analyses

According to the NCEP [18] and EAS [19] recommendations, total cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol and lipoprotein(a) were determined after a 12-hour fast at the beginning of the study. Cholesterol, triglycerides and HDL-cholesterol were measured by enzymatic methods using an Ektachem Clinical Chemistry Analyzer for spectrophotometrical analysis (Johnson & Johnson). LDL-cholesterol was calculated using the Friedewald equation [20]. Lp(a) was measured by applying immuno latex enhanced immunoassays (Immuno AG, Vienna, Austria).

Statistical Analyses

Statistical evaluation of the patients' data was done by applying declarative statistics tests. Standard deviation was calculated in all cases and assured to be in comparable magnitudes for parameters that were subject to performance tests. We calculated interquartile ranges as required, to lower the effects of outliers. For performance analysis, paired *t* tests were carried out. Trends were calculated with various regression analysis methods, such as linear regression during the whole observation period. The predicted values were then compared to the

patients' blood samples in order to get an impression of compliance. Hence, patients were informed quickly if their dietary behavior seemed to deviate from the program. All serum lipid variables were included in a continuous statistical observation. Body weight (Scale Type, Seca, Hamburg) and height (Stadiometer Type, Holtain Ltd., U.K.) were recorded, but were put in relation to each other using BMI calculations for statistical usability. Finally, absolute and relative changes in all variables over the whole duration of the study were calculated.

RESULTS

Each patient had one parent with definite hypercholesterolemia. Therefore the clinical diagnosis of FH was established. The diagnosis of FH was done according to criteria of the MED-PED program [21]. Five of the seventeen patients were related to a person that had a myocardial infarction between the age of 28 and 55 years. Four of the seventeen patients (three female, one male) showed elevated Lp(a) levels ranging from 29.8 to 104 mg/dL. In two of the seventeen patients, an LDL-receptor gene mutation was detected [22]. One of these had a mutation in exon 17, the other in exon 5 (Cincinnati variant).

The mean (\pm SD) initial total cholesterol was 233 ± 35 mg/dL, LDL-cholesterol was 151 ± 31 mg/dL, HDL-cholesterol was 59 ± 15 mg/dL, VLDL-cholesterol was 23 ± 12 mg/dL, the ratio total cholesterol/HDL-cholesterol was 4.2 ± 1.1 , triglycerides were 119.2 ± 62.8 mg/dL and Lp(a) was 29.8 ± 36.3 mg/dL.

Serum lipid values that were obtained from the study are listed in Table 4. In addition, the mean body weight was measured. At the beginning of the study it was 44.7 ± 17.8 kg, body length was 150.1 ± 19.6 cm. Mean (\pm SD) body mass index (BMI, kg/m²) was 19.4 ± 5.0 at the beginning of the therapy and 19.5 ± 5.0 at the end of the study.

Taking into consideration the standard deviations of the calculated averages, we are able to show that the weight distribution did not change throughout the study. Mean (\pm SDM) of body weight was 46.2 ± 18.5 kg and mean (\pm SDM) of body length was 151.9 ± 19.4 cm after five months of therapy.

Total cholesterol was reduced significantly after five months to 213 ± 36 mg/dL, representing a reduction of 9.5%. After one month of diet, total cholesterol was reduced to 215 ± 35 mg/dL. The major reduction took place within one month of beginning the therapy. After the next four months this reduction reached a steady state.

Table 3. Calculated Values of Macronutrients, Fatty Acids and Cholesterol of the Diet Enriched with MUFA (% of Energy)

E (kcal)	F (%)	SFA (%)	MUFA (%)	PUFA (%)	P (%)	CH (%)	Chol (mg)
1649*	30*	39*	39*	21*	14*	56*	196.4
544.2**							86.7

* Mean.

** Standard deviation.

E = energy, F = Fat, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, P = protein, CH = carbohydrate.

Table 4. Results of the Serum Lipids before and after Application of the Diet

Mean \pm SD	Chol (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	Chol/HDL-C
Before Diet	233 \pm 35	181 \pm 61	59 \pm 15	151 \pm 31	23 \pm 12	4.2 \pm 1.1
After 5 Months	213 \pm 36	85 \pm 40	57 \pm 11	142 \pm 31	17 \pm 8	4.0 \pm 0.9
Delta Value	-9%	-28%	-3%	-6%	-27%	-5%
<i>p</i> -Value	<0.05	<0.03	Not significant	<0.007	<0.043	Not significant

SD = standard deviation, Chol = cholesterol, TG = triglycerol, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol.

A similar trend can be observed in LDL-cholesterol. In eight of seventeen patients LDL-cholesterol reduction reached a level \leq 130 mg/dL. Even after one month, triglycerides were 111 ± 59 mg/dL, and after five months of therapy 85 ± 40 mg/dL. The significant reduction of triglycerides took place after five months. After one month of therapy HDL-cholesterol was 56 ± 12 mg/dL, after five months was 57 ± 11 mg/dL. No change was noticed in the level of lipoprotein(a) (beginning 29.8 ± 36.3 mg/dL, at the end 32.6 ± 40.7 mg/dL).

DISCUSSION

In children and adolescents with familial hypercholesterolemia, a low fat/low cholesterol diet enriched with rapeseed oil led to a reduction of total cholesterol and LDL cholesterol of 10% and of 7% respectively. Moreover, triglycerides were reduced by 29% and VLDL-cholesterol by 28% over a period of five months. HDL-cholesterol remained mainly unaffected during the whole study. To the best of our knowledge, the data presented in this paper represent the first study of a lipid lowering diet with monounsaturated fats in children and adolescents with familial hypercholesterolemia.

The Bogalusa Heart Study demonstrated a high association of specific risk factors (e.g., fat intake in children and adolescents) in childhood with vascular lesions in children and adolescents [23–25].

It was stated by the American Academy of Pediatrics 1998 that a diet low in fat, saturated fat and cholesterol can have positive effects on plasma lipid profiles in children and adolescents [17,26]. Levy *et al.* reviewed studies with a low fat/low cholesterol therapy [27] in children with familial hypercholesterolemia. The described average reduction of the total cholesterol level in blood was between 6% and 20%, and half of the studies showed reductions of more than 10%. In a previous study we were able to show that a standard low fat/low cholesterol diet compared to a soy protein-substituted low fat/low cholesterol diet in 23 children with familial or polygenic hypercholesterolemia was less effective: Total cholesterol decreased by approximately 10% under a classical type II diet and decreased up to 25% under a soy-bean diet [28]. In adults with familial hypercholesterolemia a decrease of total serum cholesterol with a Step I diet can be reached in the range of 10%. With

a low fat/low cholesterol diet supplemented with monounsaturated fats similar effects can be obtained [7–9]. On the other hand Estévez-González *et al.* showed that substituting monounsaturated fatty acids for saturated fatty acids in milk decreases total and LDL-cholesterol significantly and does not affect HDL-cholesterol [29]. The reduction of LDL-cholesterol is generally accepted in order to prevent later premature coronary heart disease even in children [3,27,30]. It is interesting to mention that, in one study that reports on adults treated with a rapeseed oil diet, the reduction was accompanied by an elevation of HDL cholesterol of 9% [31]. However, the decrease of triglycerides reported from adults was not so high that we could observe it in children and adolescents. Recently it has been shown in adults that triglycerides are lowered by a high-monounsaturated fatty acid diet [32].

Having replaced carbohydrate with MUFA in a diet and consequently having observed lowered serum triacylglycerol levels, one might be tempted to conclude that there exists a direct connection between the two exchange elements. However, holding carbohydrate intake in the high MUFA diet constant also lowers TG significantly [32]. Therefore, other reasons must underlie the mechanism of this effect. Up to now, it is not clear, what causes the hypotriacylglycerolemic effect, but we suggest investigating a possible involvement of VLDL that is known to transport serum triacylglycerol. Changes in the VLDL composition [33] or in the expressed activities of the enzymes and proteins involved in intravascular processing and catabolism of VLDL could play an important role in the TG concentration changes. According to [34–36] the composition of VLDL itself is subject to change in a modified dietary fatty acid composition and subsequently converts into other lipoproteins that affect the triacylglycerol metabolism. Indeed, it has been reported that in confined monkey studies, a decreased production of nascent VLDL particles has been observed [37]. This hypothesis is supported by our studies in which we observed significant VLDL reductions ($p < 0.03$). For healthy persons it has been shown that a substitution of monounsaturated fats with polyunsaturated fats has no significant effects on plasma lipid levels [38]. However, as reported earlier by other authors [38,39] we are able to confirm that the reduction of saturated fat supports lowering of low-density lipoprotein levels. This effect has been intensively described in a meta-analysis by Mensink and Katan [40]. Furthermore, it has been

shown that, in persons suffering from coronary heart disease, 12 g/day of rapeseed oil or fish oil led to a reduction of total cholesterol, LDL cholesterol and apolipoprotein B (−14.4%, −20.3%, −15.2% and −12.2%, −16.0%, −14.2%) [41]. With fish oil a triglyceride reduction of even 20.3% was achieved. Lp(a) fell by 14%, and HDL cholesterol rose by 8.3%.

It is known that a diet with olive oil that is frequently used in the Mediterranean area has a similar effect as rapeseed oil. The Mediterranean diet is rich in legumes, fruits, vegetables, grains, salad and fish, but contains very little amounts of meat and visible saturated fats in it. This diet is directly associated with a very low incidence of atherosclerosis and coronary heart disease. Additionally the contribution of natural antioxidants in such a diet should also be taken in consideration [42–44].

The diet used in our study was well adopted, and in all families a sustained change was reported. Rapeseed oil is accepted well because of its odorlessness and tastelessness. Furthermore, it is easily available, not expensive and can be used for any cooking style.

In our region people usually eat very fatty, meaty and less in vegetables and legumes. It takes an intensive effort to change these habits. After five months of therapy and intensive intervention the patients and their families themselves told us that they had changed their habits and lifestyle. In our experience, motivation and compliance in families with children with familial hypercholesterolemia tend to be very good with accompanying guidance.

Standard deviation and overlapping confidence intervals of weight measurements showed that the children had completely normal growth and that weight distribution remained constant throughout the observed period. We could also see that parents, who were affected by this disorder, tend to be more compliant with a diet after hypercholesterolemia was diagnosed in their child. Compliance of adolescents is better when treatment is started in childhood [45,46].

Our results should be interpreted in the light of the fact that we did not use a control group due to practical and ethical reasons. Furthermore, our results do not allow us to confirm the presumption of reduced risk of atherosclerosis in the elderly. Therefore, we recommend performing a long-term study in which saturated fatty acids are exchanged with monounsaturated ones. All possibilities of dietary treatment should be used to avoid an expensive long-term drug therapy.

The additional, significant reduction of triglycerides of 29% and VLDL-cholesterol of 28% was not previously observed in children and adolescents. An early and long-term treatment of hypercholesterolemia should have a positive effect in prevention of vascular disease later in the patients' lives.

We conclude that treatment of familial hypercholesterolemia in children and adolescents with a low fat/low cholesterol diet combined with rapeseed oil is effective and in most of them well accepted and safe, so that medical treatment is affordable later in the childhood.

ACKNOWLEDGMENTS

We want to thank Jack Arisian, Johannes Gutleber, Joachim Kettenbach, Elisabeth Schuller and Harald Städele for their corrections. Also I want to thank my family and Eva Oliva for their patience. We would like to acknowledge the helpful comments of the anonymous reviewers. This work was supported by a grant from the Foundation of the VOG—Linz/Austria.

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Received January 25, 2000; revision accepted December 11, 2001.