Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia

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Abstract In familial hypercholesterolemia (FH) the lowering of serum cholesterol levels should be started in childhood in order to prevent coronary artery disease later in life. However, treatment of children is problematic. We studied the effects of sitostanol (3 g/day) ester dissolved in rapeseed oil margarine as a hypocholesterolemic agent in one homozygous and 14 heterozygous children with FH maintained on a low cholesterol diet for 6 weeks, using a double-blind crossover design. Absorption and synthesis of cholesterol were evaluated by measuring serum plant sterol and cholesterol precursor proportions to cholesterol by gas–liquid chromatography. The compliance was good, and the children could not distinguish by taste the two margarines without and with sitostanol ester. Sitostanol margarine significantly reduced serum total, intermediate density (IDL), and low density lipoprotein (LDL) cholesterol by 11, 26, and 15%, respectively, and increased HDL/LDL cholesterol ratio by 27%. The proportions of serum Δ^8-cholestenol, lathosterol, and desmosterol were significantly increased by 36, 19, and 18%, and those of serum cholestanol, campesterol, and sitosterol were significantly decreased by 9, 42 and 29%, respectively, suggesting that cholesterol absorption was decreased and synthesis was compensatorily increased. High basal precursor sterol proportions predicted a high decrease in LDL cholesterol levels. In conclusion, partial replacement of normal dietary fat consumption by sitostanol ester margarine appears to be an effective and safe hypocholesterolemic treatment in children with FH.—**Gylling, H., M. A. Siimes, and T. A. Miettinen.** Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. *J. Lipid Res.* 1995. **36:** 1807–1812.

Supplementary key words cholesterol • plant sterols • cholesterol precursor sterols

Coronary heart disease develops slowly with advancing age. The rapidity of its development is apparently related to the magnitude of the serum cholesterol level even if the increased levels appear early in life. Thus, in patients with familial hypercholesterolemia (FH) the atheroma formation starts exceptionally early, because even in the heterozygous FH children the low density lipoprotein (LDL) cholesterol level is usually markedly increased already at birth (1). Accordingly, preventive serum cholesterol reduction should be started as early as possible but the measures to achieve this may be difficult. Effective treatment of FH is known to require the use of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, but their benefit in growing children is not documented. Unabsorbable bile acid binders could be used but long-term treatment with these resins may be problematic because of poor compliance due to side effects. Recently, treatment of FH children with sitostanol pastils has given surprisingly promising results, causing a marked decrease in serum cholesterol level through inhibition of cholesterol absorption (2). We have developed a sitostanol ester margarine that includes sitostanol in soluble ester form so that the margarine can replace a small amount of normal dietary fat, causing a 10–15% reduction in serum total and LDL cholesterol level of mildly hypercholesterolemic adult subjects (3–6). Our purpose in this study was to test how children with FH accept this margarine as their normal nutritional ingredient, and how this dietary change alters their serum lipid and noncholesterol sterol levels. The latter include cholesterol precursor sterols, which reflect cholesterol synthesis (7–9), and plant sterols and cholestanol, which reflect cholesterol absorption (9, 10).

MATERIALS AND METHODS

Study group

The population included 14 children with heterozygous FH; seven boys and seven girls with mean age of

Abbreviations: FH, familial hypercholesterolemia; VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; GLC, gas–liquid chromatography; apo, apolipoprotein.

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9.1 ± 1.1 yr (2–15 yr) (**Table 1**). The FH diagnosis was established in children and in one of the parents mostly by DNA technique (11). In addition to the heterozygotes, a 2-year-old FH homozygous child was studied. Accordingly, both of his parents had heterozygous FH.

All the subjects and actually the whole family of the FH children had been advised to use a low animal fat–low cholesterol diet for years, which was quite rich in monoenic fatty acids.

Study design

Two fasting blood samples, 2 weeks apart, were obtained on the low cholesterol–low fat home diet. Subsequently, the children were randomized to replace 24 g of their normal daily fat intake by the same amount of a rapeseed oil-rich margarine without or with sitostanol ester (sitostanol transesterified with rapeseed oil (3), Raisio Inc., Raisio, Finland) for 6 weeks. Sitostanol was actually a sterol mixture containing 81% sitostanol, 7% campestanol, 10% sitosterol, and 2% campesterol. After the 6-week period the diets were switched oppositely. The fat replacement was carefully explained to the children and their mothers by a dietician, and they kept a 7-day dietary recall. The study was performed double blind, but the children were asked to tell their opinions on the fat taste after switching the margarines. Both margarines were delivered in identical-looking 8 g buttons, and the use of the fat was carefully controlled by calculating and weighing the returned buttons. In most cases the fat was used on sandwiches, one each at breakfast, lunch, and dinner. The protocol resulted in daily consumption of 3 g of free sitostanol.

The Ethical Committee of our hospital approved the study protocol.

TABLE 1. Clinical characteristics of 14 children with heterozygous FH and one child with homozygous FH

Variables	Mean ± SE	Range
Age, yr	9.1 ± 1.1	2–15
Sex, m/f	8/7	
Weight, kg	35.6 ± 4.0	11.8–62.8
Height, cm	138.3 ± 6.3	92–176
Body mass index, kg/m²	17.67 ± 0.87	13.6–24.0
ApoE phenotypes		
2/3 (n = 1)		
3/3 (n = 6)		
4/3 (n = 7)		
4/4 (n = 1)		
Serum cholesterol, mmol/l	7.68 ± 0.35	5.6–10.5
Serum triglycerides, mmol/l	0.87 ± 0.10	0.34–1.61

To convert mmol/l cholesterol to mg/dl, multiply by 38.7. To convert mmol/l triglycerides to mg/dl, multiply by 88.2.

Blood sampling and analysis

Blood was drawn twice during the normal home diet, and twice at the end of each treatment period. After separation of red cells, ultracentrifugation was used to separate very low (VLDL), intermediate density lipoprotein (IDL), LDL and high density lipoprotein (HDL) from plasma. The plasma and subfractions were analyzed for total, free, and esterified cholesterol, phospholipids, and triglycerides. In addition, squalene and noncholesterol sterols, including demethylated precursor sterols of cholesterol synthesis chain (Δ^8-cholestenol, desmosterol, and lathosterol), plant sterols (campesterol and sitosterol) and cholestanol, were determined by gas–liquid chromatography (GLC) (12). Serum and lipoprotein cholesterol and triglycerides were determined by commercial kits (Boehringer Diagnostica, Germany). For GLC nonsaponifiable lipids of plasma were analyzed, as shown earlier, on a 50-m long SE-30 capillary column. For all these determinations double values were available on the normal home diet and the means of the two measurements were also calculated during both margarine periods without and with sitostanol ester. Apolipoprotein (apo) E phenotype was determined by electrofocusing from serum (13).

Means and standard errors of the 14 heterozygous FH children were calculated, and paired *t*-test (or in appropriate cases Wilcoxon sign test) was used to show differences in responses. Correlations were calculated with the least-square method. The noncholesterol sterol values are adjusted to serum cholesterol levels and expressed in terms of mmol/mol of cholesterol in order to eliminate the effect of serum cholesterol variation.

RESULTS

The children could not distinguish the two margarines by taste. The respective mean consumptions of margarine without and with sitostanol were 94 ± 4 and 92 ± 5% of the scheduled amount, indicating that the mean sitostanol intake was 2.76 ± 0.15 g/day. The dietary intake of cholesterol was 3.2 ± 0.1 mg/kg per day during both periods, and the energy percentage of fat was 33%, 14% as saturated, 12% as monounsaturated, and 7% as polyunsaturated fatty acids. During the relatively short study period the body weight of the children increased only slightly but similarly during the two periods i.e., from 39.2 ± 3.9 kg to 39.8 ± 4.0 kg, and the respective height increased from 132.8 ± 6.8 to 134.0 ± 6.8 cm.

Table 2 shows that replacement of normal dietary fat by the test margarine had no effect on lipoprotein fractions. However, a significant decrease in the serum

TABLE 2. Effects of margarine without and with sitostanol ester on serum lipids and lipoproteins in 14 children with heterozygous FH

Lipid	Home Diet	Margarine	Margarine + Sitostanol	% Change by Sitostanol
Cholesterol, mmol/l				
Total	7.68 ± 0.36	7.62 ± 0.32	6.81 ± 0.34	−10.6[a]
VLDL	0.21 ± 0.05	0.26 ± 0.06	0.25 ± 0.07	−3.8
IDL	0.32 ± 0.09	0.27 ± 0.08	0.20 ± 0.07	−25.9[a]
LDL	5.64 ± 0.35	5.47 ± 0.30	4.65 ± 0.32	−15.0[a]
HDL	1.17 ± 0.07	1.20 ± 0.07	1.25 ± 0.08	+4.2
HDL/LDL	0.23 ± 0.04	0.23 ± 0.03	0.30 ± 0.04	+26.8[a]
HDL_2	0.62 ± 0.06	0.62 ± 0.08	0.68 ± 0.08	+9.7
HDL_3	0.51 ± 0.03	0.51 ± 0.02	0.50 ± 0.02	+2.2
Phospholipids, mmol/l				
Total	3.18 ± 0.11	3.16 ± 0.11	2.94 ± 0.11	−7.0[a]
VLDL	0.14 ± 0.02	0.17 ± 0.04	0.16 ± 0.04	−6.6
IDL	0.10 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	−21.3[a]
LDL	1.73 ± 0.10	1.67 ± 0.09	1.43 ± 0.09	−14.4[a]
HDL	1.03 ± 0.06	1.06 ± 0.06	1.10 ± 0.06	+3.6
HDL_2	0.43 ± 0.05	0.44 ± 0.07	0.48 ± 0.05	+9.8
HDL_3	0.39 ± 0.02	0.39 ± 0.01	0.40 ± 0.01	+3.9
Triglycerides, mmol/l				
Total	0.87 ± 0.10	1.03 ± 0.13	0.92 ± 0.12	−10.7
VLDL	0.39 ± 0.08	0.52 ± 0.12	0.43 ± 0.09	−17.3
IDL	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	−15.2
LDL	0.26 ± 0.02	0.27 ± 0.02	0.24 ± 0.02	−13.6
HDL	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	−3.4
HDL_2	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0
HDL_3	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	−7.5

Values are given as mean ± SE. To convert mmol/l cholesterol to mg/dl, multiply by 38.7. To convert mmol/l phospholipids to mg/dl, multiply by 75.0. To convert mmol/l triglycerides to mg/dl, multiply by 88.2.
[a]Significant change from margarine by margarine + sitostanol.

cholestanol proportion and increased precursor sterol and campesterol proportions were observed (**Table 3**).

Consumption of sitostanol margarine was associated with a significant decrease in total, IDL, and LDL cho-

TABLE 3. Effects of margarine without and with sitostanol ester on noncholesterol sterol proportions in serum

Components	Home Diet	Margarine	Margarine + Sitostanol	% Change by Sitostanol
Squalene	31 ± 3	37 ± 6	33 ± 3	−10
	2.4 ± 0.2	2.8 ± 0.4	2.2 ± 0.2	
Δ^8-Cholestenol	9 ± 2	11 ± 3[a]	15 ± 4	+36[b]
	0.7 ± 0.1	0.8 ± 0.2	1.0 ± 0.3	
Lathosterol	130 ± 18	141 ± 21[a]	168 ± 29	+19[b]
	10.0 ± 1.4	10.7 ± 1.6	11.4 ± 2.0	
Desmosterol	70 ± 4	73 ± 6	86 ± 5	+18[b]
	5.4 ± 0.3	5.6 ± 0.4	5.8 ± 0.3	
Cholestanol	150 ± 10	143 ± 8[a]	130 ± 7	−9[b]
	11.5 ± 0.8	10.9 ± 0.6	8.8 ± 0.5	
Campesterol	353 ± 30	395 ± 32[a]	229 ± 21	−42[b]
	27.1 ± 2.3	30.1 ± 2.4	15.6 ± 1.4	
Sitosterol	178 ± 15	175 ± 13	125 ± 9	−29[b]
	13.7 ± 1.1	13.3 ± 1.0	8.5 ± 0.6	

Values are given as mean ± SE; upper values in terms of 10^2 × mmol/mol of cholesterol and lower values in terms of μmol/l (μg/dl = 38.7 × μmol/l).
[a]Significant ($P < 0.05$) change from home diet.
[b]Significant ($P < 0.05$) change from margarine.

lesterol (10.6, 25.9, and 15.0%, respectively) and phospholipid (7.0, 21.3, and 14.4%, respectively) levels below the margarine values in the heterozygote FH children (Table 2). The reductions in triglyceride and the increases in HDL cholesterol levels were insignificant. The HDL/LDL cholesterol ratio increased significantly from 0.23 to 0.30. It is interesting to note that the basal cholesterol/triglyceride ratio in IDL (3.18 ± 0.76) was markedly higher than that in VLDL (0.48 ± 0.03), and was slightly but significantly decreased (2.67 ± 0.55 vs. 0.51 ± 0.05) only in IDL by sitostanol. The levels of cholesteryl esters in VLDL, IDL, and HDL were decreased significantly by 3.6%, 8.1%, and 1.6%, respectively. The reductions of cholesterol levels were not related to apoE phenotypes, and the pre- and post-treatment values were not apoE-related.

The mean cholesterol precursor sterol proportions were significantly increased by 36, 19, and 18% for Δ^8-cholestenol, lathosterol, and desmosterol, respectively, and were proportionately larger than the respective decrease of the total cholesterol level. The squalene proportion was not changed. On the other hand, the proportions of cholestanol and especially those of campesterol and sitosterol were decreased by 9, 42, and 29%, respectively, during the sitostanol treatment. The squalene and noncholesterol sterol values or their changes were not related to the apoE phenotypes.

The higher the pre-sitostanol IDL cholesterol level, the greater was its decrease ($r = -0.79$; $P < 0.01$), while no respective associations were observed for total, LDL, and VLDL cholesterol. Also, the higher the plant sterol or precursor sterol proportions, the greater were their respective decreases ($r = -0.74$ for campesterol and $r = -0.64$ for sitosterol; $P < 0.01$ for both) or increases (e.g., $r = 0.64$ for lathosterol; $P < 0.01$). However, the decrease of the LDL cholesterol concentration was not related to the pre-sitostanol plant sterols or their decreases, but was predicted by the high proportion of lathosterol ($r = -0.54$; $P < 0.05$) (or Δ^8-cholestenol, **Fig. 1**). On the other hand, serum lathosterol proportion and VLDL cholesterol levels were significantly related during both periods ($r = 0.88$ and $r = 0.63$), and the sitostanol ester-induced increase in lathosterol proportion predicted an efficient VLDL cholesterol reduction ($r = -0.54$, $P < 0.05$).

In the homozygote FH boy, the respective total, VLDL, IDL, and LDL cholesterol levels were decreased from 20.9, 0.55, 1.62, and 17.7 mmol/l by sitostanol ester to 20.2, 0.54, 1.12, and 16.1 mmol/l, respectively. The respective reductions were 6, 56, and 50% for the cholestanol, campesterol, and sitosterol proportions, respectively; there were respective increases of 10, 28, and 23% for Δ^8-cholestenol, desmosterol, and lathosterol.

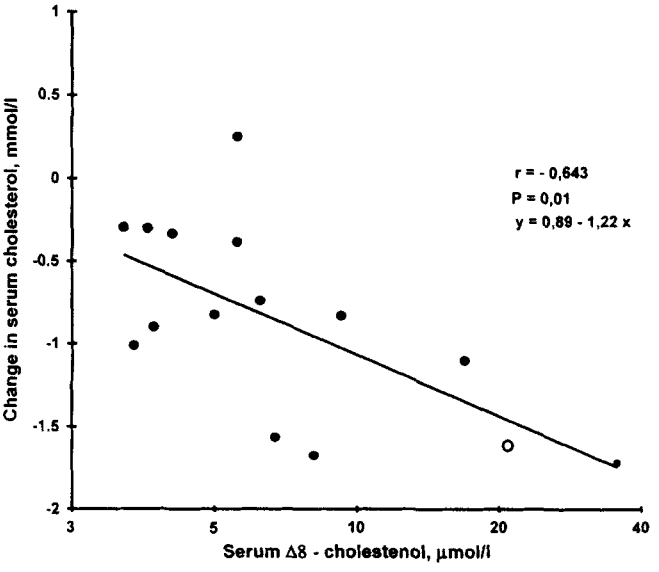

Fig. 1. Correlation between the change in serum cholesterol level by sitostanol ester margarine and the pre-sitostanol serum Δ^8-cholestenol level in 14 children with heterozygous FH (closed circles) and in one homozygous FH child (open circle). To convert mmol/l cholesterol to mg/dl, multiply by 38.7.

DISCUSSION

During the low-cholesterol home diet, a partial replacement of regular daily fat intake by the present sitostanol ester margarine reduced total, IDL, and LDL cholesterol levels by 11, 26, and 15%, respectively, and increased HDL cholesterol by 4% and the HDL/LDL cholesterol ratio by 27% in the heterozygous FH children. The total and LDL cholesterol reduction in the FH children of this series is of the same magnitude as that observed in moderately hypercholesterolemic adults during sitostanol (14) or soluble sitostanol ester intake (3–6). In adult males (15) dietary sitostanol, administered in a poorly soluble suspension, had virtually no effect on serum cholesterol levels. The decrease in the LDL cholesterol level of our FH children was less than that obtained by Becker, Staab, and von Bergmann (2) with 1.5 g/day of free sitostanol fed in three daily doses as pastils. Soluble free sitostanol could cause a more effective inhibition in cholesterol absorption than esterified sitostanol, a conclusion presented for sitosterol and its esters (16). Becker et al. (2) however, observed a significant decrease in HDL cholesterol and an increase in serum triglycerides, while in the present study an opposite trend was noted. The HDL/LDL cholesterol ratio increased from 0.17 to 0.21 and 0.23 to 0.30, respectively, in the two studies.

The sitostanol ester margarine was well tolerated even though the overall fat intake, despite replacement of

corresponding amount of usual dietary fat by the margarine, was occasionally felt to be high in both margarines, especially in the smallest child. The result was that the daily margarine intake tended to be reduced, so that the mean sitostanol intake was 2.8 g/day instead of the planned 3 g/day.

As the basal home diet of the children was apparently low in animal fat, the margarine period without sitostanol resulted in only very small changes in the serum lipid values. Because the rapeseed oil margarine was rich in plant sterols (850 mg/100 g) there was a small increase in serum campesterol level. Apparently the plant sterols of the margarine slightly inhibited absorption of other sterols so that the cholestanol value decreased while that of cholesterol was unchanged. The trend to increased serum precursor sterol values may be attributed to an increased cholesterol synthesis.

The decrease of LDL cholesterol may be related to inhibition of cholesterol absorption by sitostanol. This reduced the IDL and LDL cholesterol levels through diminished entry of intestinal cholesterol as chylomicrons to the liver. The resulting decrease of hepatic cholesterol was apparently balanced by enhanced LDL receptor activity and up-regulation of cholesterol synthesis. Earlier kinetic analyses actually suggested that decreased production rate of LDL apoB was a major reason for serum cholesterol lowering by sitostanol ester, and that the low LDL apoB production rate was caused by up-regulation of LDL apoB receptor activity (6). Also, because serum IDL cholesterol level and its high cholesterol/triglyceride ratio were reduced by sitostanol ester, it can be assumed that the activated LDL apoB receptor picked up IDL remnants so that less of it was converted to LDL (6).

The relative decrease of the serum campesterol proportion was markedly greater than that of cholesterol. The plant sterol and cholestanol proportions in serum are positively related to cholesterol absorption efficiency and negatively to cholesterol synthesis (9, 10, 17). Thus, the small decrease in the cholestanol proportion (–9%) was more likely associated with impaired sterol absorption than enhanced biliary sterol secretion caused by increased cholesterol synthesis. Cholestanol is mostly synthesized from cholesterol (18). A 50% reduction in the serum campesterol proportion was shown to reduce cholesterol absorption efficiency over 60% in non-FH subjects (6). Accordingly, the 42 and 56% reductions in the respective campesterol proportions of the present heterozygous and homozygous FH children suggest that cholesterol absorption efficiency was decreased to about half of the pre-sitostanol value. Yet the respective LDL reductions were 15 and 9%, most likely due to the fact that, in contrast to campesterol, cholesterol synthesis was increased compensatorily to the sitostanol-induced decrease in cholesterol absorption. In the homozygous FH boy a lacking LDL receptor activation apparently only tends to decrease serum cholesterol despite the trends of the noncholesterol sterols, which indicate even larger changes in either absorption or synthesis of cholesterol than in the heterozygotes. Despite the marked increases in fecal elimination of cholesterol by cholestyramine, only small serum lipid changes usually occur in homozygous FH probably owing to direct output of newly synthesized cholesterol to bile (19). This may have occurred in the present homozygous FH boy.

A prediction of sitostanol responders could be important. The decrease of serum total or LDL cholesterol was not significantly related to the pre-sitostanol values. However, the greater the pre-sitostanol lathosterol proportion in serum, the greater was its increase and the greater was the decrease in LDL cholesterol. Thus, those FH children with a high basal cholesterol synthesis responded effectively to sitostanol by lowering serum cholesterol. The plant sterol proportions in serum correlate with cholesterol absorption (9, 10). Thus, the correlations of the plant sterol changes with their pre-sitostanol values suggested that the greater the pre-sitostanol cholesterol absorption the greater was the decrease in absorption, but this decrease was not, in contrast to non-FH subjects (4), related to the decrease in serum total or LDL cholesterol. Overall, the results suggest that the FH children with high baseline lathosterol proportions in serum can be expected to be good responders to LDL cholesterol lowering by dietary sitostanol ester.

The taste of the sitostanol ester margarine was well accepted by the children and could not be distinguished from the margarine without sitostanol. In addition, good compliance by the patients, and effectiveness to lower serum cholesterol and to increase the HDL/LDL cholesterol ratio indicate that the sitostanol ester margarine could be recommended for the basal dietary fat intake of heterozygous FH children. Its use appears to be free of adverse effects, at least, as far as serum fat-soluble vitamins and oxidation of serum lipids are concerned (T. A. Miettinen, P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen, unpublished observations), so that the resulting cholesterol reduction could serve as a basic long-term preventative of forthcoming arterial atheromatosis before combination treatment with some more effective hypolipidemic drug.

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