Hypercholesterolaemia treated by soybean protein diet

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SUMMARY After a period of stabilisation on a controlled low lipid low cholesterol diet with animal proteins a group of 16 children with familial hypercholesterolaemia were given a textured soybean protein based diet, with a similar fat composition. All the children had a highly significant reduction in total cholesterol, averaging -21.8% against the baseline after eight weeks. Compliance became less strict afterwards, but more than half of the patients have regularly continued the diet and results have been maintained for one year. Minimal changes were noted in triglyceridaemia and in high density lipoprotein cholesterol concentrations, which showed a slight rise only at the end of treatment. The children's growth during the trial was normal. In view of the psychological difficulties of prescribing treatment with drugs to children with severe hypercholesterolaemia before puberty and of the relative ineffectiveness of standard low lipid diets in this condition the soybean protein diet may offer a satisfactory alternative.

Hypercholesterolaemia in childhood poses serious therapeutic problems, in that treatment with drugs, although reported as well tolerated,¹ often conflicts with the physician's desire to avoid chronic treatments during growth. Dietary studies, on the other hand, characterised by a moderate cholesterol intake with increased polyunsaturated/saturated (P/S) fatty acid ratios, often lead to fairly modest plasma cholesterol reduction.^{2 3}

More recently, the observation that switching from a low lipid diet with animal proteins to a similar diet with textured vegetable proteins (TVP) extracted from soybean markedly reduces cholesterolaemia in adult patients with type II hypercholesterolaemia has attracted considerable attention. An attempt to modify the type of dietary proteins in children with hypercholesterolaemia has already been carried out, using a vegetarian type of diet (including 60% animal proteins, mostly from milk) with a high linoleic acid content (P/S ratio 1.6).⁵ Compared with a 'control' diet, with a similar P/S ratio, this vegetarian diet lowered cholesterolaemia by roughly 10%.

As it seems possible, based on our extensive experience in adult patients, to provide a reasonably well accepted dietary regimen, by totally substituting animal proteins with TVP from soybean, now

provided with added lecithin (L-TVP),⁶ we decided to test this dietary approach in a selected group of type II children.

Patients and methods

Sixteen prepubertal subjects, with an age range of 3 to 12 years, were included in the study. All came from families known by the investigators of the three participating centres (Bologna, Milan, and Mendrisio) to have a high prevalence of hypercholesterolaemia. All the children presented with a low density lipoprotein (LDL) cholesterolaemia in excess of the 90th centile according to accepted guidelines⁷ as well as for the Italian population of their age.⁸ In addition, they either presented with tendon xanthomatosis or with a hypercholesterolaemic first degree relative with xanthomatosis and coronary disease. In one case (case 4) a diagnosis of homozygous type II disease was made, based on the clinical findings and on the pattern of inheritance. This diagnosis was confirmed by fibroblast typing, showing receptor deficient homozygosity, with 18% of normal high affinity LDL receptors (courtesy of Dr J L Goldstein, University of Texas Southwestern, Dallas, United States of America).

All the children were already on a low chole-

terol, high P/S diet when their families were contacted for participation in the study. In some cases one or both of their parents were on or had been on a soybean protein based diet. On enrolment into the study children and parents were advised to follow their usual therapeutic diet as closely as possible. This had a P/S ratio of about 1.6–1.8, with the following energy distribution: carbohydrates 55%, lipids 25%, and protein 20% (of which about 70% was of animal origin and 30% from vegetable sources); butter and cheese were excluded.

The children's plasma lipid concentrations were monitored after two (–2) and four weeks (0) of controlled low lipid regimen. At each interval plasma samples for lipid-lipoprotein determinations were collected. At time 0 all subjects (and their closest living relatives) received detailed instructions on the use of L-TVP, by following a short course (three days) of instructions for the preparation of the main recipes. The commercial product used was Cholesterol-L (relative percentage composition: protein 52.0, carbohydrate 23.6, fat 4.0, lecithin 6.0, fibre 3.5, minerals 6.5, and moisture 4.4).⁶ By correctly assuming the prescribed diet about 90% of total proteins were derived from vegetable sources versus 10% of animal origin (mostly skimmed milk). Particular care was applied to the design of dietary programmes to allow normal development of the children without causing either undue increases or losses in weight.

At the –4, 0, +4 and, when possible, +8 week intervals the complete lipoprotein separation was carried out by preparative ultracentrifugation.⁹ In four of the participants, all of whom were below the age of 6, the drawing of large blood samples was not

considered appropriate, and lipoprotein distribution was determined, after selective precipitation of LDL and very low density lipoproteins,¹⁰ by the use of the Friedewald formula.¹¹ Plasma cholesterol and triglyceride concentrations were determined at each visit by enzyme methodologies,^{12 13} standardised, within each of the three laboratories, under the care of the World Health Organisation monitoring centre at Prague (Professor D Grafnetter).

Statistical analysis of the data was carried out by multiple paired comparisons with the Dunnett *t* test.¹⁴

Results

The experimental dietary plan was carefully explained to all children and their families, and all seemed capable of following it closely. All 16 children, in fact, successfully completed four weeks of treatment. After this, one child (case 5) withdrew for family reasons. There tended to be less compliance after the first month of dietary treatment. In spite of this, 12 children were still carefully following the regimen after both 12 and 18 weeks of treatment. At this point the study was considered to be complete and the data were analysed. At present, more than one year after the start of the study, eight of the children are still on a soybean diet plan, and two others are on a high P/S diet (around 1.6), with animal proteins being substituted by soy proteins in six meals each week.¹⁵

All subjects followed their regular pattern of weight increase during the study. In no case was there any unexpected decrease or pronounced increase (Table 1). The mean weight of the 12 subjects

Table 1 Clinical characteristics of the participating subjects

Case No	Age (yrs)	Sex	Height (cm)	Centre	Total cholesterol (mg/dl) (–4 weeks)	Weight (kg)	
						At –4 weeks	At end of study (week)
1	5	M	105	Mi	413	14.5	15 (18)
2	11	F	141	Mi	371	31.5	33 (18)
3	12	F	170	Mi	317	53	55 (18)
4	9	M	138	Mi	628	39	40 (12)
5	11	M	139	Mi	517	29.5	30 (4)
6	4	M	108	B	440	15.5	16.2 (12)
7	8	M	136	B	350	34	36.7 (18)
8	6	F	117	B	348	16.2	16.8 (18)
9	10	M	149	B	448	44	45 (18)
10	9	M	138	B	461	36	37.2 (18)
11	7	F	127	B	371	23	23.5 (18)
12	12	M	159	Me	280	45	48 (18)
13	12	F	158	Me	329	49	49.5 (12)
14	10	F	137	Me	268	33.5	34.5 (18)
15	8	M	132	Me	265	29	31 (18)
16	3	F	102	Me	200	19	21 (18)

Conversion: traditional to SI units—Cholesterol: 38.6 mg/dl = 1 mmol/l.
Mi=Milan, B=Bologna; Me=Mendrisio.

Table 2 Plasma total cholesterol, triglycerides, and high density (HDL-cho) and low density (LDL-cho) cholesterol concentrations in all participating patients during the trial. All values are expressed in mg/dl and are mean (SD)

	Weeks of trial					
	-4	0	+4	+8	+12	+18
No of subjects	16	16	16	15	12	12
Total cholesterol	375.8 (107.2)	360.3 (111.1)	291.5 (94.8)	278.4 (100.4)	287.2 (98.6)	287.3 (101.8)
Triglycerides	128.0 (42.6)	115.4 (45.8)	119.6 (47.1)	137.2 (67.9)	110.1 (34.4)	104.9 (28.4)
HDL-cho	36.4 (7.5)	35.6 (6.4)	36.8 (6.5)	35.3 (6.8)	36.1 (8.8)	37.0 (8.6)
LDL-cho	312.1 (102.1)	301.6 (106.4)	230.1 (87.5)	215.7 (94.7)	229.2 (97.1)	229.3 (98.7)

Conversion: traditional to SI units—Cholesterol: 38.6 mg/dl = 1 mmol/l; Triglycerides: 88.6 mg/dl = 1 mmol/l.

completing 18 weeks of dietary treatment increased from 31.8 to 33.9 kg.

During the four pre-trial weeks (-4 to 0), no major changes in plasma lipid concentrations were detected. Mean (SD) plasma total cholesterol dropped from 375.8 (26.8) mg/dl to 360.3 (27.8) mg/dl at the end of the four weeks (not significant), possibly due to the improved compliance to the standard low lipid diet. Similarly, a small drop in triglyceridaemia was also noted (from mean (SD) 128.0 (10.6) to 115.4 (11.4) mg/dl, not significant). No specific changes were noted in high density lipoprotein (HDL) cholesterolaemia (Table 2).

After the start of the dietary treatment, the fall in cholesterolaemia was dramatic, even after two weeks of treatment. As four of the participating children did not report at the two week visit, data

are given as the differences from baseline (0) at each interval (Figure). The mean (SD) reduction of plasma cholesterol concentration (12 children) was 56.0 (19.4) mg/dl after two weeks of L-TVP diet. All children reported at two weeks, when the mean cholesterol fall was 19.2% ($p < 0.001$ v baseline). At this same interval, LDL-cholesterolaemia was reduced by 19.7% (also $p < 0.01$ v baseline); triglyceridaemia had increased slightly (from mean (SD) 115.4 (45.8) to 119.6 (47.1) mg/dl, not significant) as had the HDL-cholesterolaemia (from 35.6 (6.4) mg/dl to 36.8 (6.5), not significant). In the 15 patients continuing treatment after eight weeks there was a further reduction of cholesterolaemia, which reached its minimum at -81.9 (11.1) mg/dl (-21.8%, $p < 0.0001$ v their respective baseline). Little change was noted in triglyceridaemia and in HDL-cholesterol (Figure).

At the 12 and 18 week intervals the 12 remaining children showed a stabilisation of their serum cholesterol concentration, achieving a mean reduction around 70 mg/dl (-21.6% v their respective baselines, $p < 0.001$ in both cases). At these intervals no further significant changes were recorded for triglyceridaemia, whereas a further small rise in HDL-cholesterol was recorded (Table 2).

Discussion

The effect of the addition or subtraction of different dietary nutrients on cholesterolaemia has been repeatedly examined in both normal subjects and patients with hypercholesterolaemia.¹⁶ Specific formulas have been proposed to evaluate quantitatively the effect of varying the P/S ratio or the dietary cholesterol intake, or both.^{17 18} More recently, it has been suggested that the P/S ratio may be of less importance when subjects consume a diet with a fairly low (less than 30%) fat content.¹⁹

Compared with the wealth of knowledge related to adult hypercholesterolaemia, little is known on

Figure Mean (SD) changes in individual cholesterolaemias (Δ mg/dl) in patients presenting at successive intervals during the study (numbers in parentheses = No of subjects) during the low-lipid (L-L) and soybean protein (TVP) diets.

Conversion: traditional to SI units—Cholesterol: 38.6 mg/dl = 1 mmol/l.

the specific sensitivity of children to dietary changes. Apparently, both infants²⁰ and young children³ seem to be remarkably sensitive to dietary cholesterol and fat restriction, generally showing dramatic plasma cholesterol reductions on shifting from a diet of ≥ 400 mg/day cholesterol to a diet with < 150 mg/day. The effects are, however, far less striking after children with hypercholesterolaemia reach 6 years of age. Rose *et al* detected a 20% reduction of serum cholesterol in only eight of 16 children with type II disease treated for one year with a low cholesterol, high P/S diet; no substantial change was found in the other eight. More recently, Fernandes *et al* tested a virtually cholesterol free, high linoleic acid vegetarian diet, with 39% of energy from vegetable protein against 33% in the reference low lipid diet, in children with type II disease.⁵ The mean cholesterol reduction induced in these 39 children (4 to 14 years of age) was around 10%; HDL-cholesterol and apo AI decreased by 4%.⁵ In a study with different objectives MacLean *et al* noted that in malnourished children animal proteins (casein) led to 36% higher cholesterolaemias compared with vegetable proteins (wheat).²¹

Based on our large clinical experience with the total substitution of animal proteins with TVP from soybean in the treatment of adult patients with type II hypercholesterolaemia,^{4,6} this diet was administered to a group of 16 children with an inherited form of hypercholesterolaemia. Some came from families where the disease had exerted an appreciable impact on the cardiovascular morbidity and mortality. Another incentive to this study was the recent observation, by one of our groups, of an up regulatory effect of this diet on liver lipoprotein receptors, down regulated by dietary cholesterol.²² In view of the clear importance of an impaired receptorial regulation in many of these children, this type of diet seemed capable of providing a satisfactory alternative to effective, but at times poorly tolerated,²³ treatment with drugs.

The described results show that children respond to the dietary change in a more or less similar way to adults. Unexpectedly, we found it difficult to maintain many of these children on a strict regimen for a prolonged period. We hoped that children, less exposed to the habitual consumption of animal protein compared with adults, would accept the new regimen better. This was not the case, possibly because of the constant exposure to highly publicised food items, as well as to the school environment. One of the children (case 4), with a homozygous receptor deficient disease, was treated, however, with the regimen during admission to hospital; after just four weeks of a strictly controlled diet he presented with a plasma cholesterol re-

duction of close to 30%. Greater sensitivity of children compared with adults cannot thus be excluded. Moreover, more than half of the children have continued the regimen, either on a total basis or on partial substitution,¹⁵ for over a year.

The cholesterol reduction seems to be limited to changes in LDL concentrations. In contrast to the experience of Fernandes *et al*,⁵ we failed to note any drop in HDL-cholesterol. Indeed, to some extent similar to our previous experience with adult patients with type II hypercholesterolaemia treated with the new TVP regimen containing 6% lecithin,⁶ we noted a slight trend to increase in HDLs. In the other study only patients with a very low HDL-cholesterol responded with a significant increase when placed on the new diet.⁶ Also because of the fairly small number of children with very low HDL-cholesterol in this study we could not show the presence of a similar trend in our patients.

In conclusion, it seems that the soybean diet regimen can provide a safe and effective dietary treatment for children with type II hypercholesterolaemia, even with familial conditions. The potential of this regimen to reduce the incidence and severity of arterial complications needs to be evaluated further.

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