Effect of Dietary Cholesterol upon Serum Lipids in Rural Guatemalan Indian Children

JOSÉ MÉNDEZ, PH.D.,* WITH THE TECHNICAL ASSISTANCE OF SILVIA MORALES AND ROLANDO FUNES

ALTHOUGH the cholesterol content of the diet has been considered to have a relatively unimportant influence on cholesterolemia in man, recent evidence suggests that it is a significant hypercholesterolemic factor. Several efforts to prove this effect, in which crystalline cholesterol was used, have been unsuccessful.[1–3] But it appears that cholesterol in diets containing a good amount of certain fats[4–6] or of lipoprotein complexes[7] is well absorbed by man and produces hypercholesterolemia.

In previous field trials,[8] it was shown that a dietary supplement containing 600 mg. of crystalline cholesterol and 15 ml. of cottonseed oil given to rural Guatemalan Indian school children in one glass of Incaparina[8] daily for four weeks and then increased to 1,200 mg. for another four weeks did not produce significant changes in serum lipid levels, including serum cholesterol. It was shown also,[9] in the same population, that the daily ingestion of 60 gm. of skim milk or 30 gm. of different fats over long periods of time failed to produce changes in serum cholesterol concentration. On the other hand, when the diet commonly consumed by families from the upper socioeconomic groups was given to school age children from lower urban and rural socioeconomic families, there was a significant increase in serum cholesterol levels.[10] One of the factors that may be responsible for this effect is the cholesterol content of the diet.

In view of the previous results with crystalline cholesterol[3] and of the increasing number of reports in the literature on the effect of dietary cholesterol,[11,12] this experiment was designed to test the effect of dietary cholesterol furnished by egg compared with that of crystalline cholesterol in 30 ml. of oil.

MATERIAL AND METHODS

School age children from a rural Indian village in the highlands of Guatemala were divided into three groups: group 1, the "egg" group, included twenty-three children (six boys and seventeen girls) whose average age was 11.6 years; group 2, the "cholesterol" group, included twenty-one children (eighteen boys and three girls) with an average age of 10.6 years; and group 3, the "control" group, included thirty-four children (nineteen boys and fifteen girls) with an average age of 9.4 years.

For a period of four weeks the children in group 1 (the egg group) were given 1 boiled egg and 1 glass of plain Incaparina twice daily as mid-morning and mid-afternoon snacks. The cholesterol content of this supplement was about 600 mg. In group 2 (the cholesterol group) the children were given 600 mg. of crystalline cholesterol and 30 ml. of cottonseed oil daily in two glasses of Incaparina in the same way and for the same period of time. The cholesterol was dissolved in the hot oil and was mixed into the Incaparina gruel. In group 3 (the control group) the children were given only the two glasses of Incaparina with the same amount of oil. All Incaparina

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* Associate Chief, Division of Physiological Chemistry, and Director of Training Programs.

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TABLE I
Estimated Daily Dietary Intake During the Study

Intake	Diet in the Home	Group 1			Group 2			Group 3		
		Supplement	Total	% of Calories	Supplement	Total	% of Calories	Supplement	Total	% of Calories
Calories	1,165	537	1,702	...	656	1,821	...	656	1,821	...
Total protein (gm.)	32	26	58	14	14	46	10	14	46	10
Carbohydrate (gm.)	221	79	300	70	78	299	66	78	299	66
Fat (gm.)	17	13	30	16	32	49	24	32	49	24
Fatty acids (gm.)										
Saturated	5	3	8	4	8	13	6	8	13	6
Oleic	7	5	12	6	8	15	7	8	15	7
Polyunsaturated	3	4	7	4	16	19	9	16	19	9
Cholesterol (mg.)	45	600	645	...	600	645	...	...	45	...

NOTE: Group 1 = the egg group. Group 2 = the cholesterol group. Group 3 = the control group.

supplements were seasoned with sugar and cinnamon making palatable preparations that were well accepted.

Fasting fingertip blood samples were obtained before the start of the experiment and again at the end of four weeks. Total serum lipids, lipid phosphorus and total cholesterol were determined by methods previously cited.[3] The statistical comparisons were made using a t test for correlated pairs.[18]

RESULTS

The estimated daily dietary intakes during the study are presented in Table I. Group 1 consumed more protein (58 versus 46 gm.) and less fat (30 versus 49 gm.) than the other groups. The carbohydrate intake was equal in all groups. The percentage of calories derived from protein, carbohydrate and fat was 14, 70 and 16 per cent, respectively, for group 1 and 10, 66 and 24 per cent for the other two groups. Although the fatty acid intake was higher in groups 2 and 3 than in group 1, the saturated to polyunsaturated fatty acid ratio was higher in the latter group, 1.1 versus 0.68. The daily cholesterol intake was about 645 mg. in group 1 and 2, and 45 mg. in group 3.

TABLE II
Effect of Increased Dietary Cholesterol on Serum Lipid Concentration in Guatemalan Rural Children

Group	No.	Age (yr.)	Height (cm.)	Weight (lb.)	Total Lipids (mg./100 ml.)		Cholesterol (mg./100 ml.)		Lipid Phosphorus (mg./100 ml.)	
					Initial	Final	Initial	Final	Initial	Final
1	23	11.6	127.4	59.7	469	499*	120	131†	7.3	7.6
		1.2	5.6	6.0	49	69	19	22	0.8	1.2
2	21	10.6	123.9	55.0	470	446	128	119	7.7	7.0†
		2.0	9.1	10.1	91	78	21	23	1.0	0.6
3	34	9.4	116.8	48.2	457	438	136	127*	6.9	6.4
		1.7	7.8	7.0	54	68	26	25	0.9	0.9

NOTE: First row of figures represents the mean, the second row the standard deviation.
* $p < 0.05$.
† $p < 0.01$.

The mean age, body height and weight of the children studied, as well as the serum lipid levels obtained at the beginning and at the end of the trial, are given in Table II. In group 1 the children were about one year older than those in group 2 and two years older than those in group 3. This was due to the fact that complete school grades were selected to form each group and assigned to each treatment at random. Working under field conditions and with a relatively large sample, it would be extremely difficult to have children in a single school grade assigned to different treatments. The height and weight differences among the groups are in accordance with the differences in ages and sex distribution.

There was an increase in serum total lipids and cholesterol in group 1. The increase in serum cholesterol was significant at the 1 per cent level of probability. Although no significant change was observed in serum lipid phosphorus, the final values were higher than the initial ones.

In group 2 crystalline cholesterol did not produce significant changes in serum total lipid and cholesterol levels, although the values had a tendency to decrease. There was a significant decrease in serum lipid phosphorus in this group.

In group 3 there was a significant decrease in serum cholesterol. The values in total lipids and lipid phosphorus were lower at the end of the trial, although the differences were not significant.

COMMENTS

Serum cholesterol levels at birth are very low in Guatemalan infants and do not differ between children born to mothers in the upper and lower socioeconomic groups.[14] On the other hand, in school children from families of these two different groups, there is a wide difference in serum cholesterol levels.[15] Environmental factors, mainly dietary, must be largely responsible. Studies in Guatemala[10] have shown that there is a significant increase in the low serum cholesterol levels of rural Indian children when they are given a diet similar to that consumed by children in the upper income families, although the levels do not reach those found among the upper socioeconomic population. One possible cholesterolemic factor in this diet, in addition to the kind and amount of fat, may be the cholesterol intake.

The effect of dietary cholesterol on serum lipid levels has been the subject of repeated discussions and experimentation. In past experiments exogenous cholesterol was shown to have no effect in increasing serum cholesterol.[1,2] These experiments have been criticized on the basis that (1) the cholesterol intake of the subjects was already high, (2) the serum cholesterol levels were elevated at the beginning and (3) the effect of dietary cholesterol was only observed within a limited range of intake. Beveridge et al.[11] observed a good correlation between the amount of cholesterol in the serum and cholesterol increments in the diet up to 800 mg. per 950 cal., but no further significant increases were obtained even when intakes were increased by as much as 1,300 to 4,500 mg.

As already mentioned, in a field trial in Guatemala[3] with rural Indian school children, the increase in the daily cholesterol intake from 45 to 645 mg. for four weeks and then to 1,245 mg. for another four weeks, given with 15 ml. of cottonseed oil in one glass of Incaparina, failed to produce significant changes in serum cholesterol. Several factors might explain this lack of response. It has been stated that children probably do not absorb exogenous cholesterol,[16] that the absorption of cholesterol takes place only in the presence of a good amount of fat and that cholesterol probably needs to be dissolved in fat. The 15 ml. of cottonseed oil used in the previous experiment[3] could be considered small, although taking into consideration the solubility of cholesterol in cottonseed oil of 3.2 gm. per cent,[17] the calculated amount dissolved in the preparation used was around 480 mg., which gives a daily intake of dissolved cholesterol of 314 mg. per 950 cal. Although this intake, according to Beveridge's results,[11] should have produced an increment of 30 per cent in serum cholesterol levels, it failed to do so.

In the present experiment, the administra-

tion of egg containing 600 mg. of cholesterol daily produced a significant increase in serum cholesterol. Although the t test on paired observations is the one of choice in the present case, treating the serum cholesterol data in an analysis of variance and correcting for the disproportion of sex, no significant effect due to sex or to the interaction of sex and time was obtained. The intake of 600 mg. was chosen because this is the usual level of intake among the upper socioeconomic groups in Guatemala. Although the cholesterol levels at the end of the trial increased significantly, they were lower than those observed among upper socioeconomic groups. It was probably not due to the short period of experimentation since Beveridge et al.,[11] and Wells and Bronte-Stewart[12] have observed greater changes than those reported here, in the same length of time. A more likely possibility is the amount of cholesterol absorbed. Cook et al.[7] have estimated that 60 per cent of egg cholesterol is absorbed by man. If absorption is taken into account, then in the present trial only 360 mg. was absorbed. This gives 200 mg. of cholesterol per 950 cal. per day. Beveridge et al.[11] have shown that an increase of 42 mg. per 100 ml. was obtained when the daily cholesterol intake was 634 mg., giving 200 mg. per 950 cal. Another possibility is that these children may have been subsisting on low protein quality diets and, therefore, have been suffering from chronic subclinical protein malnutrition. This could decrease lipid absorption and/or impair blood lipid transport. This has been suggested by Arroyave et al.[18] in relation to vitamin A.

The lack of complete agreement between the present study and Beveridge's is probably due to the fact that his subjects were given a fat-free diet which decreased serum cholesterol and before they were given cholesterol in a diet which contained 30 per cent of the calories in the form of a butter oil distillate. The fact that these subjects showed a rapid decrease in serum cholesterol when they were on the fat-free diet is an indication that they reacted readily to dietary manipulations, including changes in dietary cholesterol.

In the present trial, no previous dietary manipulation was necessary because the habitual diet of the population studied is low in fat and cholesterol content. The small, although significant increase observed in serum cholesterol when egg was given, could in part be due not only to its cholesterol content, but also to its fatty acid contribution to the diet. Wells and Bronte-Stewart,[12] however, have shown that cholesterol-free egg fat fractions did not affect cholesterolemia in man, although cholesterol and egg fat fractions given together produced marked increases in serum cholesterol. They found no difference between uncooked and cooked egg.

The children in group 2 who were given crystalline cholesterol and Incaparina with cottonseed oil showed a decrease in serum cholesterol which was not statistically significant, whereas the children in group 3, given only Incaparina with cottonseed oil, showed a significant decrease. This decrease could be attributed to both the amount of oil used and its fatty acid composition.

Serum total lipids and lipid phosphorus paralleled the changes in serum cholesterol. The significant change in lipid phosphorus in group 2 cannot be attributed to a specific dietary factor.

The present results confirm previous field dietary trials[10] that showed that the very low cholesterol levels of Guatemalan rural children can be increased readily by dietary means. Because of the relatively small change obtained, it seems that these children have an intrinsically low serum cholesterol responsiveness to dietary manipulations. Similar studies with adults of the same population, and children and adults of other socioeconomic groups, may clarify if this responsiveness is characteristic of these children.

SUMMARY

School children from a rural Indian community in the highlands of Guatemala were divided into three groups. Group 1 (the egg group) received 2 boiled eggs and 2 glasses of Incaparina daily. The cholesterol content of this supplement was 600 mg. Group 2 (the cholesterol group) received 600 mg. of crystalline cholesterol daily, plus 2 glasses of

Incaparina containing 15 ml. of cottonseed oil each. Group 3 (the control group) received only the Incaparina with the same amount of oil. A significant increase in serum cholesterol levels was observed in group 1 at the end of four weeks; no increase was observed in the other groups. Although the increase in serum cholesterol levels observed in group 1 was significant, the increase was small, indicating that probably this Indian group has an intrinsically low responsiveness to dietary manipulations.

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