Mendez, Jose, Aida Acosta and Rolando Funes

1963. Response of Rural Guatemalan Indian Children with Hypocholesterolemia to Increased Crystalline Cholsterol Intake.

J. Nutrition, v. 79, no. 2, February.
Pages 200–204.

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA., U.S.A.

Reprinted from THE JOURNAL OF NUTRITION
Vol. 79, No. 2, February 1963

Response of Rural Guatemalan Indian Children with Hypocholesterolemia to Increased Crystalline Cholesterol Intake[1,2]

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Until recently, the idea that dietary cholesterol had little influence upon human serum cholesterol levels has been supported by epidemiological and experimental evidence (Keys et al., '56; Mattil). Dietary cholesterol as a factor contributing to the pathogenesis of atherosclerosis in man, therefore, was given no importance. Recent publications, however, appear to indicate that dietary cholesterol increases serum cholesterol levels (Beveridge et al., '59, '60).[4] The design of the experiments supporting this position depends, in most cases, primarily upon a cholesterol-free period to decrease serum cholesterol levels before cholesterol supplementation is begun. Beveridge et al. ('60), using this procedure, obtained a remarkably good correlation between cholesterol increments, up to 800 mg/950 Cal., and cholesterol concentration in the serum, but no further significant increases in cholesterol concentration have been observed even with daily intakes of as high as 1300 to 4500 mg. Similar increases have also been obtained using diets high in egg yolk or in other sources of dietary cholesterol (Connor et al., '61a, b; Taylor et al., '60). These results have supported the possible role of dietary cholesterol as an important factor influencing serum cholesterol levels in man, and, possibly, in the pathogenesis of atherosclerosis.

It has been shown that members of the rural, lower socio-economic population of Guatemala, who have a low dietary intake of fat, also have low serum cholesterol levels and show very low prevalence of severe aortic atherosclerosis and myocardial infarction. On the other hand, those in the urban upper socio-economic population of Guatemala have a high dietary intake of fat, show high serum cholesterol levels, and exhibit a high prevalence of severe aortic atherosclerosis and myocardial infarction (Mann et al., '55; Scrimshaw et al., '57; Tejada et al., '58). Previous dietary surveys (Méndez et al., '62; Scrimshaw et al., '57) showed that although there is no difference in total protein intake between the groups, the urban, upper socio-economic group consumes 6 times as much animal protein as the rural group. The daily fat intake furnishes 37 and 7% of the caloric intake of the urban and rural group, respectively. The ratio of polyunsaturated to saturated fatty acid is 0.25 in the urban and 1.43 in the rural diet. Also, only 52% of the total caloric intake of the urban diet is furnished by carbohydrates, whereas the percentage in the rural diet is 80. The estimated daily cholesterol intake of the urban group is 626 mg and of the rural population 61 mg. The average serum cholesterol level is 200 mg/100 ml for the urban, and 135 for the rural populations (Mann et al., '55; Méndez et al., '62).

The conditions prevalent among rural Guatemalans are almost ideal for a study of the effect of dietary cholesterol on serum lipid levels. Because of the very low cholesterol intake of these rural people, diets need not be manipulated before

Received for publication August 22, 1962.

[1] This investigation was supported by grants 266 from the Nutrition Foundation and H-2653 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

[2] INCAP Publication I-255.

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[4] Anderson, J. T., F. Grande, C. Chlouverakis, M. Proja and A. Keys 1962 Effect of dietary cholesterol on serum cholesterol level in man. Federation Proc., *21:* 100 (abstract).

testing the effect of increased cholesterol intake, as usually necessary in this type of experiment. Consequently, experiments were carried out to determine the effect of crystalline cholesterol on the low serum cholesterol level of rural Guatemalan children.

MATERIAL AND METHODS

In the rural village of Magdalena, Guatemala, 60 school-age Mayan Indian children were divided into two groups. Group 1, called the "cholesterol group," included 25 boys and 9 girls whose average age was 11.8 years. Group 2, or the "control group," included 13 boys and 13 girls whose average age was 9.3 years.

During two experimental periods the children in the "cholesterol group" received a daily mid-morning snack of a glass of Incaparina, INCAP's all-vegetable protein mixture for human consumption (Scrimshaw et al., '61). The cholesterol was dissolved in the heated oil, incorporated into the Incaparina gruel, seasoned with sugar and cinnamon. This made a palatable preparation that was well accepted. During the first 30 days, 600 mg of crystalline cholesterol and 15 ml of cottonseed oil were given, and during the second 30-day period this amount was increased to 1200 mg/day.

Group 2, the "control group," received a daily glass of Incaparina with only the 15 ml of cottonseed oil added, during both 30-day periods. Table 1 shows the daily intake of the children under study, as well as the total composition of the supplement. The usual daily caloric intake of 1163 was increased to 1493, and the protein intake was increased from 32 to 39 gm. Carbohydrates were also increased from 221 to 260 gm, 26 gm of which were furnished by the sucrose added as flavoring. Total fat was increased from 17 to 33 gm and the calories thus derived amounted to 20%. Saturated fatty acids were increased from 5 to 9 gm, oleic acid from 7 to 11 gm, and polyunsaturated fatty acids from 3 to 11 gm.

None of the subjects studied evidenced severe diarrhea, a few of them experienced a small degree of gastrointestinal discomfort when the supplementation was initiated, but this disappeared within few days.

Height, weight, and fasting finger-tip blood samples were taken before the experiment started and at the end of both the first and second 30-day periods. Total serum lipids were determined by the Bragdon method (Bragdon, '51), lipid phosphorus by the Chen et al. ('56) method, and total cholesterol by the method of Abell et al. ('52) adapted for micromethods.

RESULTS

The height and weight changes of the children throughout the two experimental periods are shown in table 2. The cholesterol group had an average increase in height of 0.8 cm, whereas the height of the control group increased 1.2 cm. On the other hand, the weight changes were 1.3 and 0.6 kg, respectively. The differences in height and weight gain could be

TABLE 1

Estimated daily dietary intake during the cholesterol study

	Home	Supplement	Total	Percentage of calories
Calories	1163	330	1493	—
Total protein, gm	32	7	39	10
Carbohydrate, gm	221	39	260	70
Fat, gm	17	16	33	20
Fatty acids				
Saturated, gm	5	4	9	6
Oleic, gm	7	4	11	7
Polyunsaturated, gm	3	8	11	7
Cholesterol, mg	45	600–1200	645–1245	—

accounted for by the differences in the mean ages of the two groups.

Table 3 shows the total serum lipids, lipid phosphorus, and cholesterol levels at the different periods of observation. The initial level of 598 mg serum total lipids did not change significantly at the end of the first 30-day period of cholesterol treatment, when it was 577 mg, or at the end of the second period, when it was 581 mg. The children in the control group showed a similar response. The initial serum level was 609 mg, and at the end of both the first and second 30-day periods, these values were 616 and 599 mg, respectively.

There also was no effect on serum lipid phosphorus. The initial values in the group receiving cholesterol were 6.8 mg, and the values at the end of the first and second 30-day treatment periods, 6.7 and 6.7, respectively, were not significantly different. The control group reacted similarly with an initial value of 7.2, ending with 7.1 and 6.9 mg, respectively.

No significant change in total serum cholesterol levels occurred in the children receiving crystalline cholesterol. They had 133 mg initially and 134 mg at the end of the first 30 days during which they had received 600 mg daily. After they had received 1200 mg for another 30 days, the value was 132 mg. The control group responded similarly with values of 136, 135 and 135 for the same periods, respectively.

DISCUSSION

The observations presented in this paper agree with those of some investigators who have used crystalline cholesterol (Keys et al., '56; Mattil), although they are at variance with the results of Beveridge et al. ('60), Connor et al. ('61a, b) and Anderson et al.[5] ('62).

[5] See footnote 4.

TABLE 2

Height and weight changes during the dietary cholesterol study

	Age	Height			Weight		
		Initial	Period 1	Period 2	Initial	Period 1	Period 2
		cm	*cm*	*cm*	*kg*	*kg*	*kg*
		Cholesterol group (N = 30)					
Mean	11.8	130.8	130.3	131.6	29.2	29.9	30.5
Standard deviation	1.0	6.3	6.7	6.6	3.9	3.7	4.2
		Control group (N = 22)					
Mean	9.3	118.6	119.4	119.8	23.6	23.9	24.2
Standard deviation	1.8	7.3	7.3	7.4	3.1	3.4	3.3

TABLE 3

Effect of high cholesterol intake on serum lipid levels[1]

	Cholesterol group (N = 34)			Control group (N = 26)		
	Initial	Period 1	Period 2	Initial	Period 1	Period 2
	Total lipids					
Mean	598	577	581	609	616	599
Standard deviation	76	98	111	114	106	117
	Lipid phosphorus					
Mean	6.8	6.7	6.7	7.2	7.1	6.9
Standard deviation	0.9	1.1	1.0	1.4	1.2	1.1
	Total cholesterol					
Mean	133	134	132	136	135	135
Standard deviation	21	26	26	23	22	28

[1] All values are expressed in mg/100 ml of serum.

Since these studies were performed with children, the results may not be applicable to adults. However, the marked differences in serum cholesterol levels observed between adults of lower and upper socioeconomic groups in Guatemala, are also found between school children of the same population groups (Scrimshaw et al., '57).

If dietary cholesterol per se were responsible in part for the high serum cholesterol levels encountered among urban, upper socio-economic population groups in Guatemala (Mann et al., '55; Scrimshaw et al., '57), then the increase in daily cholesterol intake from 45 mg to 645 mg in the first period, and to 1245 in the second period would have elevated the serum levels. This, however, was not the case. Ahrens et al. ('57) failed to increase serum cholesterol levels of a hypercholesterolemic child after giving a corn oil formula diet supplemented daily with 0.6 to 2 gm of crystalline cholesterol; but small increases, although significant, were obtained when 4 to 8 gm of cholesterol were given. A possible explanation for the lack of effect of dietary cholesterol reported here is that the increase of polyethenoid fatty acid content in the diet, with the concomitant decrease in the saturated fatty acid, could have overcome the effect of the crystalline cholesterol. The expected change in serum cholesterol due to the change in fatty acid intake calculated from a prediction equation (Keys et al., '59) did not support this possibility. Connor et al. ('61a, b) failed to lower the serum cholesterol level in subjects given a high cholesterol diet containing increased polyethenoid fatty acids simultaneously.

Finally, there is some evidence to support the idea that the physical state of cholesterol in foods, such as the lipoprotein complexes found in egg yolk, and the levels and kind of dietary fat in the diet (Cook et al., '56) result in greater intestinal absorption and might, therefore, increase the serum cholesterol levels in man. This will be the subject of the next field experiment.

SUMMARY

In the rural village of Magdalena, Guatemala, 60 school-age Mayan Indian children, consuming diets low in fat and cholesterol, were divided into two groups: Group 1 received a 600-mg crystalline cholesterol and 15-ml cottonseed oil supplement daily in a glass of Incaparina for 30 days. The cholesterol intake was then increased to 1200 mg and given for another 30 days. Group 2, the control group, received daily a glass of Incaparina with only 15 ml of cottonseed oil for the same two periods. The results showed that exogenous cholesterol did not increase the low serum lipid and cholesterol levels of these children.

ACKNOWLEDGMENT

The technical assistance of Carlos Enrique Amézquita and Berta Mendizábal is gratefully acknowledged.

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