



Factors Influencing Serum Cholesterol Levels of Central American Children

I. Effect of Adding Fat and Animal Protein to the Diet

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THE rural population of Central America has been shown to have relatively low serum cholesterol levels,¹⁻³ a low dietary intake of both fat and animal protein,¹⁻³ and a very low prevalence of severe aortic atherosclerosis^{4,5} and myocardial infarction.⁶ Although at present there is no conclusive evidence that these phenomena are more than secondarily associated, attention has naturally been directed to the extent to which they are causally related. Although marked differences in serum cholesterol levels between socioeconomic and dietary groups within Guatemala are clearly evident, even among children of school age,³ differences in the severity of aortic atherosclerosis cannot be detected until the fourth decade of life.^{4,5} Differences in activity and psychologic stress have been postulated as accounting for some of the differences observed among adult socioeconomic groups, but this is less likely to be true for children.

Since the diets of rural Guatemalan school children are known to be extremely low in both fat and protein of animal origin, supplementary snacks offer a particularly convenient method of testing the effect of relatively long-

term dietary changes on serum lipid levels. In view of the extensive evidence for the influence of dietary fat on serum cholesterol levels⁷⁻⁹ and the suggestion that under certain circumstances methionine may be a factor affecting serum cholesterol levels,⁹ arrangements were made to give groups of rural school children in different villages supplements designed to provide either milk or one of three different kinds of fat daily, for periods ranging up to nearly a year.

MATERIALS AND METHODS

Two rural villages in the highlands of Guatemala were selected for the administration of skim milk. Forty-one school children in Ciudad Vieja (CVS) and sixty in Santo Tomás Milpas Altas (STM) were given 60 gm. of reconstituted dried skim milk powder daily for twenty-eight weeks as a supplement to the customary diet. All these children were of predominantly Mayan Indian racial origin. The supplement provided approximately 200 calories per day and 20 gm. of protein. Blood samples were taken at the beginning of the trial and at the eighteenth and twenty-eighth week. The variation in number of children sampled at each examination in this and subsequent trials was due largely to the difficulty of obtaining permission to take a blood sample from a rural Indian child.

In the rural village of San Lorenzo el Cubo (SLC) and Santa María Cauqué (SMC), 30 gm. of fat were administered by spreading a fried black bean paste on a fried tortilla and serving it twice daily as a between meals snack

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TABLE I
Average Daily Dietary Intake of Eighty-one Guatemalan Children in 4 Rural Highland Towns*

Nutrients	Intake (gm.)	% of Calories	Nutrients	Intake (mg.)
Calories.....	1,581		Calcium.....	980
Animal protein.....	5.1	1.3	Phosphorus.....	988
Total protein.....	46.9	11.9	Iron.....	17.2
Carbohydrates.....	322	81.5	Thiamine.....	1.05
Total fat.....	13.8	7.9	Riboflavin.....	0.54
Fatty acids			Niacin.....	8.53
Saturated.....	2.6	1.5	Vitamin C.....	34.00
Oleic acid.....	5.8	3.3	Vitamin A.....	2,520†
Polyunsaturated.....	4.0	2.3		

Fatty Acid Content of Daily Supplements

	Saturated (gm.)	Oleic (gm.)	Polyunsaturated (gm.)
Skimmed milk (60 gm.).....	0.6	0.3	0.1
Lard (30 gm.).....	12.0	13.1	3.4
Cottonseed oil (30 gm.).....	7.1	7.1	14.3
Hydrogenated cottonseed oil (30 gm.).....	6.9	13.6	8.0

* SMC and three similar nearby towns not included in the experimental study.

† Expressed in international units.

providing a total of approximately 400 calories daily. In this way, recipients were not aware of differences in the type of fat used. In SLC a group of nineteen school children (group A) were given lard for forty-five weeks. Blood samples were taken at the beginning of the trial and at the nineteenth, thirty-seventh and forty-fifth week. In the same village of SLC, a second group of twenty-seven children (group B) were given hydrogenated cottonseed oil for twenty-six weeks. Blood samples were taken at the start of the trial and at the fifteenth and twenty-sixth week. In Santa María Cauqué (SMC) a group of 105 school children (group C) were given plain cottonseed oil for an eighteen-week period, and blood samples taken only at the beginning and end of the experiment.

Serum cholesterol levels were determined by the method of Abell et al.¹⁰ The statistical analysis was made using the t test to evaluate the significance of the differences in means, and also the significance of the differences in paired data for individual children.

The average daily intake of eighty-seven school children in four nearby rural localities including one of the towns of the present study (SMC), and the composition of the supplements given, are presented in Table I. The food composition tables of INCAP¹¹ were used in determining the nutritional value and those of Hardinge and Crook¹² to calculate the fatty acid composition of the food consumed.

RESULTS

As shown in Table II, the daily administration of 60 gm. of skim milk during twenty-eight weeks to the school children of the rural villages of CVS and STM failed to produce any significant effect on serum cholesterol. Similarly, the data in Table III indicate that the daily administration of 30 gm. of either lard, hydrogenated cottonseed or plain cottonseed oil also failed to cause any change in serum cholesterol levels among the three groups. There was no significant correlation between serum cholesterol values and either age or gain in weight.

TABLE II
Serum Cholesterol Levels of Guatemalan
School Children
(Effect of Daily Administration of 60 Gm. Skim Milk)

Period	No. of Children	Mean (mg./100 ml.)	Standard Deviation
Village CVS			
Initial	41	121	22
18 weeks	31	119	21
28 weeks	28	117	21
Village STM			
Initial	40	119	26
18 weeks	60	114	20
28 weeks	25	123	22

COMMENTS

Low serum cholesterol values have been reported in several population groups in the world and have been related to the amount and kind of fat in the diet^{7,8} and to a variety of other dietary constituents.⁹ The differences in serum cholesterol levels previously observed among different socioeconomic groups in Guatemala are compatible with the hypothesis that they are influenced greatly by the dietary fat. Nevertheless, adding considerable fat to the diets did not raise the serum cholesterol levels of the children in the field trials reported.

Among nondietary influences, racial and familial factors have been considered to influence serum cholesterol levels in man. The studies of Keys et al.¹³ and our own findings in rural Costa Rica² suggest that in determining the average levels of population groups, these are quite secondary to environmental factors.

Despite evidence suggesting the importance of dietary protein in influencing serum cholesterol levels, Keys and Anderson¹⁴ demonstrated that cholesterol levels in adults did not differ when the diet contained either 10 or 20 per cent of the total calories from skim milk. There are, however, conflicting reports in the literature as to the effect of methionine deficiency. Olsen et al.¹⁵ reported a decrease in serum lipid levels with low methionine-low choline diets in both rats and man and an in-

TABLE III
Serum Cholesterol Levels of Guatemalan
School Children
(Effect of Daily Administration of 30 gm. of Lard,
Hydrogenated or Plain Cottonseed Oil)

Period	No. of Children	Mean (mg./100 ml.)	Standard Deviation
<i>Group A (SLC)—Lard</i>			
Initial	17	118	17
19 weeks	19	121	17
37 weeks	14	114	18
45 weeks	11	122	16
<i>Group B (SLC)—Hydrogenated Cottonseed Oil</i>			
Initial	27	106	19
15 weeks	17	102	15
26 weeks	13	105	14
<i>Group C (SMC)—Plain Cottonseed Oil</i>			
Initial	103	117	25
18 weeks	105	116	25

crease in lipid when either methionine or choline was added to such a diet. On the other hand, Mann and associates¹⁶⁻¹⁸ concluded that methionine, as well as certain other sulfur-containing compounds, exert a specific nonlipotropic effect in lowering serum cholesterol levels in such diverse animals as the rat, chick and monkey. In the present investigation, the administration of 60 gm. daily of skim milk, supplying 522 mg. of additional methionine, did not produce any apparent effect on the serum cholesterol levels of rural school children in Guatemala, even though their habitual diet was relatively low in animal protein. In twenty-four well nourished American men, the administration of 3 gm. of methionine daily for six weeks did not alter the serum cholesterol and lipoprotein levels.¹⁹

There are many experimental studies, some of them in man, in which the general increase of fat in experimental diets, especially the proportion of nonessential fatty acids, altered serum cholesterol levels. Thus, the failure to demonstrate any serum cholesterol changes when either animal or saturated vegetable fat

was administered to school children consuming a very low fat diet was unexpected. However, the largest part of the habitual diet of the rural Guatemalan child consists of tortillas made from whole corn. It has been observed in animals that corn starch has a lowering effect on serum cholesterol values⁹ and also that corn contains a steroid, sitosterol, which also has this effect.⁹ Other factors favoring lower serum cholesterol levels are crude fiber and calcium; both are characteristic of a predominantly tortilla diet.⁹ All serum cholesterol levels among the groups studied were very low as compared with similar data for the United States or upper income groups in Guatemala.¹⁻³ It is possible that several different factors in the diets of the rural Guatemalan population act synergistically to maintain low serum cholesterol levels. The supplementary fat was evidently not sufficient to overcome the factors favoring a relative hypocholesterolemia.

While the level of physical activity and possibly also psychological stress are important factors potentially influencing cholesterol levels in the adult population, it is unlikely that children of different socioeconomic groups in Guatemala differ markedly in this regard. We do not believe that these nondietary factors are responsible for the low serum cholesterol levels of rural Guatemalans. On the other hand, we must conclude that manipulating individual dietary factors such as the amount of fat or protein or calories is not enough to affect the balance of all the factors responsible. If indeed these factors determining the low cholesterol levels of rural school children in Guatemala are primarily dietary in nature, a readjustment of the total dietary pattern may be required in order to demonstrate this fact; at least, this is the next field experiment to be tried.

SUMMARY

The administration of 60 gm. of reconstituted dried milk powder daily for twenty-eight weeks had no effect on initial serum cholesterol levels of 101 rural Guatemalan school children. In a related study 30 gm. of fat were given children twice daily in a fried bean and tortilla

supplement providing a total of approximately 400 calories. In one (SLC) village, nineteen children received lard (forty-five weeks) and twenty-seven, hydrogenated cottonseed oil (twenty-six weeks); in another (SMC) village all 105 were given cottonseed oil (eighteen weeks). No significant effect on serum cholesterol levels was noted with any of the supplements. Neither frequency of attendance nor gain in weight influenced these results.

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