Serum Cholesterol in Japanese Coal Miners

A DIETARY EXPERIMENT

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IN CONNECTION with a study of the relationship between the mode of life and the concentrations of cholesterol and lipoproteins in the blood serum in Japan, it was important to decide whether the low serum cholesterol found to be characteristic of the Japanese men could be ascribed to the diet. An independent dietary survey at Shime (near Fukuoka, Kyushu, Japan) confirmed the very low-fat content of the average diet as reported from Japanese national surveys.[1] Only 10.3 per cent of the total calories of the miners were provided by fats in the diet. The sources of the fats are summarized in Table I.

While the diet would seem to be an adequate explanation for the serum cholesterol values, it was decided to see whether the addition of a moderate amount of fat to the diet would, in fact, raise the serum cholesterol level. The results of a brief experiment on a group of these miners are summarized below.

SUBJECTS AND PROCEDURE

Twenty-one apparently healthy Japanese coal miners 22 to 54 years of age were recruited in the small mining town of Shime. All were engaged in their usual hard work underground at the coal face. It was arranged that their customary mode of life would be maintained without change except that for 11 days they would modify their diet to replace about 700 cal of their daily rice with 50 g of butter or margarine, two slices of bread and a serving of water ice (sherbet) all adding up to the calorie equivalent of the displaced rice. They insisted on the bread to help them eat the fat, which they generally dislike, and the water ice (sherbet) was our answer to their request for a sweet (dessert) to offset the taste of the fat.

TABLE I

Fats in the Average Coal Miner's Diet at Shime, Japan, in April 1956. The Table Gives the Percentages of the Total Fat Calories From the Various Food Sources

Rice	17.0%	Fish	32.5%
Soy bean	11.3	Eggs	15.2
Sesame, rape seed	4.9	Marine invertebrates	4.3
Misc. vegetables	4.0	Butter	3.9
Wheat	2.7	Beef	3.0
		Whale oil	1.2
All veg. sources	39.9%	All animal sources	60.1%

Blood samples were taken from arm veins twice, two days apart, just before the diet change, after four and eleven days on the diet and again two weeks after resuming their usual diet.

After the bloods had clotted, the serum was pipetted off and two or more 0.1 ml portions of each sample were measured out on Whatman's No. 1 filter paper and dried by hanging

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up the papers in the air at room temperature (14° to 20° C). Additional 0.1-ml portions were measured out for paper electrophoresis for 15 hr at 185 volts by the method of Anderson and Keys.[2] After the electrophoresis was completed the paper strips were air dried in the same manner as the whole serum samples. The dry paper strips and serum samples were all sent by air mail to the Laboratory of Physiological Hygiene in Minneapolis where they were analyzed for total cholesterol and for cholesterol in the alpha and beta lipoprotein fractions as separated by paper electrophoresis, using the methods of Anderson and Keys.[2]

A detailed study of the ordinary diet of the miners at Shime was carried out by making household inventories and recording food purchases for a week. The diets of different families conformed to a very constant pattern in which proteins provided an average of about 13 per cent and fats about 10 to 12 per cent of the total calories. The extra fat eaten during the experiment raised the average fat intake from 10.3 to 24.9 per cent of the total calories.

The 21 men were divided into three groups of seven men each matched in regard to age, relative body weight, and fatness (judged from the thickness of the subcutaneous fat over the triceps muscle and over the tip of the scapula). In each group there were two men in the twenties and five men in the forties and early fifties. Group B received fresh butter as the added fat. Group AM received margarine made largely or wholly from animal fats, while Group VM received a margarine made exclusively from vegetable oils.

In Japan, as elsewhere, manufacturers are reluctant to disclose the exact composition and details of ingredients in their margarines. We were assured, however, that the hydrogenation and other procedures are exactly the same as used in the United States and in Europe and, indeed, the appearance, texture and taste of both margarines was comparable to the common colored margarines of the United States. The animal fat margarine (Snow Brand) was stated to be made largely from whale oil together with beef and pork fat and some vegetable oils. The vegetable margarine (made by the same manufacturer in Hokaido) was emphatically stated to be made exclusively with vegetable oils; this is an important consideration for some religious groups in Japan.

Samples of these margarines were sent by air mail to Minnesota and there stored in the cold until they were sent to the Research Division of the Procter and Gamble Co., in Cincinnati, Ohio, where detailed analyses were made. The essential data, together with average data for butterfat and for a U. S. all-vegetable-oil margarine for comparison, are summarized in Table II. These materials

TABLE II

Composition of Japanese Margarines A and B, Used in the Present Experiment, and of a Typical U. S. Margarine as Analyzed by the Research Division of Procter and Gamble Co. Typical Values for Butterfat Are Also Listed

	A (Whale)	B (All veg.)	C (U. S. veg.)	Butter fat
	Fat Component			
Iodine Value	39.2	36.6	78.6	35
Saponification Value	226.0	227.7	194.2	245
Free fatty acids, %	0.1	0.6	0.1	?
Unsaponifiable, %	0.4	0.3	0.6	?
Fatty Acid Composition, %				
Saturated	65.0	63.8	21.7	65
Oleic	24.9	30.0	66.0	25
Linoleic	9.9	6.0	11.9	3
Linolenic	0.2	0.2	0.4	1
Arachidonic	0	0	0	1
Conjugated Diene	0.3	0.2	1.2	?
Trans acids, %	17.3	18.1	41.5	0
	Proximate Composition			
Water, %	16.5	14.4	14.4	15.5
Fat, %	79.9	81.2	80.3	81.0
Solids not fat, %	4.5	4.1	4.3	3.5

were analyzed "blind" by the Procter and Gamble Research Division by the methods given in the Official Methods of the American Oil Chemists Society. The following comments are appropriate:

Margarine A: The proximate composition of Margarine A is typical of that which is seen here in the United States, except that the fat content is a little low. On the other hand, the fat used in the preparation of this Margarine is considerably different from that used in the

United States . . . It appears to have been prepared from coconut oil and some other fat. It would appear to contain from 40 to 50 per cent coconut oil.

Margarine B: Margarine B is uuite similar in composition to Margarine A and all the remarks about Margarine A can be repeated here as far as Margarine B is concerned. The only difference between the two is that Margarine B is a somewhat firmer product as indicated by the iodine value and the trans-fatty acid content.

Margarine C: The proximate composition is typical of that of a margarine seen in the United States. The analytic values, too, are typical of an American margarine. On the basis of the analytic values obtained on the fat portion, it would appear that this margarine was made from soybean oil, cottonseed oil, or a similar type of domestic oil that has been hydrogenated. The somewhat higher conjugated level in this fat indicates that it undoubtedly received some mistreatment during its processing.

RESULTS

The belief is widespread in Japan that eating fats will cause diarrhea. The subjects were therefore agreeably surprised to find they were not incapacitated from diarrhea as they had feared. However, one man did develop persistent diarrhea and the diet was discontinued on that account. Another man developed an upper respiratory infection and was also discarded as a subject. Finally, one subject withdrew, leaving 18 men for the study. Some of their control characteristics are summarized in Table III.

TABLE III

Control Means (±S.E.) for the Men in the Diet Groups. "Rel. Wt." = Body Weight as % of Average for Same Age and Height in U. S. Medico-Actuarial Table. "Skinfold" = Sum of Arm and Sub-Scapular Values. "% beta" = % of Total Serum Cholesterol in the beta Lipoprotein Fraction

Group	N	Age	Rel. wt. %	Skinfold *mm*	B.P. *mm* Hg	Serum Cholesterol	
						Total *mg*/100 ml	% in beta
B	7	39.1	90.0	14.8	137/86	145.4 ± 14.4	69.3 ± 3.4
AM	6	37.8	84.8	12.2	138/83	135.7 ± 11.0	67.8 ± 4.1
VM	5	43.1	84.3	13.5	134/83	163.2 ± 16.6	73.8 ± 2.6
AM + VM	11	40.2	84.6	12.8	136/83	148.2 ± 7.9	70.5 ± 2.2
All	18	39.8	86.5	13.6	136/84	147.1 ± 7.2	70.1 ± 2.0

Since the mean difference between the pre- and post-diet control values for total cholesterol was only 2.6 mg per 100 ml with a standard error of ±2.7, the pre- and post-diet values for each man were averaged and these figures are given in Table I and were used as the control values in subsequent computations.

The principal findings in regard to serum cholesterol are summarized in Table IV. All of the groups tended to show a slight rise in total serum cholesterol after four days on the

TABLE IV

Mean (±S.E.) Serum Cholesterol Change after 4 and 11 Days on Diet with 450 cal of Rice Being Replaced with 50 g of Fat: "B" = Butter; "AM" = Animal Fat (Mostly Whale Oil) Margarine; "VM" = All-Vegetable-Oil Margarine

Group	N	Δ Total cholesterol *mg*/100 *ml*		Δ beta%
		4 Days	11 Days	11 Days
B	7	+0.43 ± 6.58	+17.57 ± 5.23	−2.6 ± 1.8
AM	6	+2.17 ± 3.65	+19.00 ± 7.98	−0.3 ± 2.1
VM	5	+8.80 ± 5.09	+14.20 ± 6.92	+1.2 ± 3.6
AM + VM	11	+5.18 ± 3.62	+16.82 ± 4.82	+0.5 ± 1.8
All	18	+3.30 ± 3.11	+17.11 ± 3.66	−0.7 ± 1.4

diet but this change is not statistically significant for any of the groups or for all groups combined. After 11 days, however, the total serum cholesterol concentration had risen in all groups to about the same extent, the grand average being 17.11 ± 3.66 mg per 100 ml which is statistically highly significant (t = 4.68, for N = 18, t = 4.5 for p = 0.0003). There was no significant difference between the responses of any of the groups nor did the butter group (B) differ from the combined margarine groups (AM + VM).

The observed rise in total serum cholesterol represents a grand average increase of 11.6 per cent above the control values, ranging from 8.7 per cent for the VM group to 15.2 per cent for the AM group. This suggests a greater relative effect in the AM than in the VM group but the difference of 6.5 per cent is not statistically significant, the standard error of the difference being ±7.0 per cent.

Most of the rise in the total serum cholesterol on the increased dietary fat is obviously accounted for by the cholesterol in the beta lipoprotein fraction but the data do not allow a secure conclusion as to whether the mean alpha lipoprotein cholesterol also was actually increased. The mean rise in this fraction was + 3.00 ± 2.07 mg per 100 ml. As Table IV shows, there was no significant change in the percentage of the total cholesterol represented by that in the beta lipoprotein.

DISCUSSION

The experiment clearly shows that it is reasonable to attribute the low cholesterol values in these Japanese to their diet and it is unnecessary to invoke theories about race or physical labor as conceivable prime factors. The outstanding peculiarity of the diet, is the fact that total fats provide only about one-fourth the proportion of calories from fats currently used in the average U. S. diet (10.3 vs. 40 + per cent of calories from fats). The total calories in the diet of these miners seem to be adequate. The average intake of 3,297 cal daily is a good deal for these small men (average height 160.5 cm or only 5 feet 3 inches) even though they are doing hard work. There were no complaints of insufficient food and the men showed no signs of underfeeding. Their average relative body weight is low by United States standards but this value has little significance in these men with very small bones and whose body form is so different from that of the American. The skinfold measurements showed that these men are not fat but the values are not indicative of real undernutrition. Moreover, inspection of these men in the nude showed smooth contours and no prominent rib cages, deep supra-sternal notches or winged scapulae. Finally, the observed serum cholesterol changes occurred without changing the dietary calories and no significant weight changes were observed. Such small individual weight changes as were recorded (extremes of + 4 lb and −2.5 lb) were not correlated with the changes in the serum cholesterol.

We have observed similarly low cholesterol values in population samples elsewhere who subsist on relatively low-fat diets. Thirty-five Neapolitan steel workers aged 40 to 49 (average 4.36 years of age), with about 20 per cent of their calories from fats, averaged 158.8 mg of total cholesterol per 100 ml of serum.[3] Forty-six Sardinian coal miners 40 to 49 years of age (average 43.2 years) with an average of 24 per cent of fat in the diet, averaged 173.2 mg/100 ml.[4] Bantu workmen in Cape Province, South Africa, whose diet provides about 17 per cent of calories from fats, averaged 166.3 ± 41 mg/100 ml at average age 46.[5] These values may be compared with the average of 249.8 mg of total cholesterol per 100 ml for 103 members of the Minneapolis Fire Department aged 40 to 49 (average 43.6 years of age).[6] Their diets are estimated to provide more than 40 per cent of the calories from fats, mostly from butter fat and meats. The basic diets of these populations are, of course, very different. The Neapolitans and the Sardinians get most of their total calories from wheat, the Bantu from corn and the Japanese from rice.

It would be interesting, of course, to compare the serum cholesterol response in these men to that produced by a similar dietary change in other groups of men differing in race and occupation. There are no strictly com-

parable data at hand for this purpose but it does appear that the response to diet fat of these Japanese miners is at least roughly similar to our findings in dietary experiments in Minnesota.

Twelve physically healthy men with schizophrenia at the Hastings State Hospital in Minnesota, averaging 41 years of age, were maintained in calorie balance for four weeks on a diet in which 11 per cent of the total calories were provided by fats and on another occasion they were similarly maintained on a diet with 17 per cent fat calories, the difference in the amount of fat in the several diets being made up almost entirely of butterfat and beef fat. Before and for several months in between the experiments they subsisted on a standardized diet with 37 per cent fat calories. Their average intake on the hospital diet was 3.065 cal including 49 g. of butterfat, 70 g of meat fat and lard, and 16 g of vegetable fat (mainly cottonseed oil). On this hospital diet the grand average total cholesterol concentration in the serum prior to the start of the lower fat diets was 220.2 ± 9.4 mg per 100 ml in the fourth week. On the 17 per cent fat diets the mean change of the individuals from their preceding hospital diet values was a fall of 16.3 ± 5.5 mg per 100 ml and on the 11 per cent fat diet the corresponding value was a decline of 24.4 ± 6.9 mg per 100 ml from the previous house diet control. These values indicate a direct relationship between these diet fats and serium cholesterol which responds similarly in both the Minnesotans and in the Japanese coal miners. The picture is summarized in Figure 1.

These are short-time responses, of course, and it is hazardous to insist that the long-time effects would necessarily be identical. It seems reasonable to suggest, however, that some progression of the diet effect over time would explain the differences observed when populations on their habitual diets are compared. The experimental results at Shime and at the Hastings State Hospital indicate a difference of about 25 mg of cholesterol per 100 ml of serum associated with the dietary difference between 11 and 37 per cent fat cal maintained for only a couple of weeks. But comparing the Shime coal miners on their habitual diet of 11 per cent fat cal with the Hastings patients on their hospital diet of 37 per cent fat cal, the average difference is around 70 mg per 100 ml, i.e., almost three times as great. This might indicate the cumulative effect of long-time persistence on the two types of diets.

There is, of course, a major difference between the 11 per cent fat calories diet used by the Japanese and that used in the Hastings experiment. The fatty acids in the Hastings low-fat diet were something like 50 per cent saturated and less than 10 per cent represented linoleic and polyethenoid acids while the corresponding figures for the Japanese diet are less than 20 per cent saturated and perhaps 40 per cent polyethenoid acids. These differences may make an important contribution to the picture in the overall comparison of the populations on their native diets. But it should be observed that both at Hastings and at Shime the cholesterol differences were experimentally produced by changing the amounts of the more saturated fats (butterfat and beef fat) in the diet. Hence the comparison in Figure 1 has validity in demonstrating that

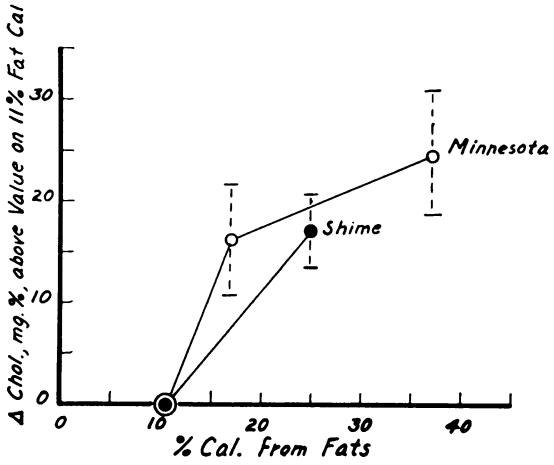

Fig. 1. Serum total cholesterol concentration at different levels of fat in the diet, expressed as the mean difference (and standard error) for the same individuals, from their values on a diet providing 11 per cent of the total calories from fats.

the miners and the patients respond similarly to changes in the amounts of the same types of fats in the diet.

SUMMARY

Eighteen Japanese coal miners at Shime, Japan, were studied before, during and after a dietary experiment in which, for 11 days, their habitual diet was altered by the isocaloric substitution of 50 g of butter or margarine for a part of their customary rice. Seven of the men received butter, six of them had a margarine made from animal fats (mainly whale oil) and five received an all-vegetable margarine. The iodine values of these added fats were: butter 35, margarine A 39, vegetable margarine 37 and the corresponding contents of linoleic acid glyceride were 3, 10 and 7 per cent. The extra fat raised the average intake of these miners from 10.3 to 24.9 per cent fat cal. Their work and other living habits were unchanged.

The dietary change produced an average rise of 17.1 ± 6.6 mg of total cholesterol per 100 ml of blood serum and there were no significant differences between the effects of the several fats. The control cholesterol average was 147.1 ± 7.2 mg. per 100 ml so the rise was 11.6 per cent in 11 days. Separate analysis of the cholesterol in the alpha and beta liporotein fractions showed no significant change in the distribution resulting from the dietary change.

Comparison with dietary experiments in Minnesota indicated that the response seen in these Japanese men was comparable to that observed in Minnesota men.

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