Dietary Protein and the Serum Cholesterol Level in Man*

By ANCEL KEYS PH.D. AND JOSEPH T. ANDERSON, PH.D.

POPULATIONS habitually subsisting on diets low in fats tend to have low concentrations of cholesterol in the blood serum.[1–5] Because isocaloric substitution of carbohydrates for the mixed fats in the ordinary American diet decreases the serum cholesterol level of man in experiments, it seems reasonable to attribute the findings in these populations to their low dietary fat intake. However, many populations who subsist on low-fat diets are also low in their consumption of total protein or of proteins of animal origin, so the question arises as to whether the dietary protein is influential in producing the low cholesterol values.

The present paper is a report of controlled experiments on human subjects on diets constant in calories but differing in protein content. In one experiment the serum cholesterol response to a change from a moderate-fat diet to a high-fat diet was compared at two levels of protein intake (about 83 and 130 g daily). In another pair of experiments two groups of men were maintained on a diet constant in fats (64 to 69 g daily), the protein intake was then doubled for four weeks and subsequently was reduced to the original protein level. In these latter experiments a daily supplement of 1000 mg of cholesterol was also added to the diet, with and without the protein supplement, for eight weeks. Skimmed milk powder was used as the source of the protein supplement.

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis 14, Minnesota, and the Mental Health Research Laboratory, Hastings State Hospital, Hastings, Minnesota.

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SUBJECTS AND METHODS

The subjects in both sets of experiments were physically healthy, schizophrenic men, 32 to 54 years old, who were stable in their mental state and were judged to be metabolically normal. During the experiments, and for a month or more before the dietary manipulations, the men were housed and fed in the Metabolic Unit of the Hastings State Hospital where they were under the constant supervision of a special dietitian and group of attendants in addition to the ordinary medical and supervisory staff. All of the diets were devised to be fully adequate and constant in mineral and vitamin content. The caloric levels were set to maintain balance on the basis of control studies with measured portions of foods for 4 weeks or more before the start of the experiments proper. During the experiments the food served to each man was measured out at each meal, and periodic checks of rejections and plate waste allowed estimation of the actual food eaten by each man. Body weights were recorded weekly and slight adjustments in the food portions were made on this basis.

Blood samples were drawn in the fasting state during the last days of each four-week period. They were analyzed in duplicate for total serum cholesterol by the Bloor method[6] in Experiment 1 and by the method of Abell and others[7] as applied in this laboratory[8] in Experiment 2. The cholesterol in the alpha-lipoprotein fraction in the serum as separated by cold ethanol precipitation was measured by the Bloor method. Beta-lipoprotein cholesterol was computed from the total minus the alpha fraction. In order to make the numerical values for cholesterol in Experiment 1 more precisely comparable to those obtained by the more recent method, all values for choles-

terol in Experiment 1 were multiplied by the factor of 0.85.[8]

Seventeen men were maintained for a month on a relatively low-fat diet providing 3000 calories, 85 g of protein, and 55 g of fat, including about 6 g of linoleic acid. They were then divided into two groups matched in mental state, physical activity, hemoglobin concentration, stability of weight, etc. The nine men in the "control-protein diet" group averaged 40.0 years in age and had a relative body weight averaging 98 per cent of the value for equal height and age in the Medico-Actuarial tables. The eight men in the "high-protein diet" group averaged 42.8 years of age and their mean relative body weight was 94.7 per cent.

For the second part of the experiment the diet was the same except that (1) for both groups additional fat, as butterfat, was isocalorically substituted for carbohydrates to provide a total of 125 g of fats and (2) for the high protein group the protein was increased by 50 g by the isocaloric substitution of skimmed milk powder for carbohydrate. The averages for the major nutrients actually eaten are summarized in Table I.

TABLE I

Average Nutrients Actually Eaten in Experiment 1

Item	Moderate protein group		High protein group	
	Control	Expt.	Control	Expt.
Calories	2,957	2,909	2,954	2,886
Proteins, g	82.2	82.5	83.9	130.9
Protein as % of cal	11.1	11.3	11.4	20.0
Fat, g	52.5	123.6	53.0	123.9
Fat as % of cal	15.6	38.2	16.1	38.8

Both groups of men remained essentially at constant body weight throughout. On the high-fat diet the control protein group lost an average of 0.1 kg while the high-protein group gained an average of 0.7 kg. The serum cholesterol finding are summarized in Table II.

Both groups of men showed substantial increases in the serum total and beta-lipoprotein cholesterol after four weeks on the high-fat diet and these changes are statistically highly significant in each group. But, as is obvious from Table II, there was no significant difference between the responses of the two groups.

Twenty-five men were used in the pair of experiments here summarized as Experiment 2. These experiments were so arranged that each man served as his own control before and after doubling the protein intake. There were four diet periods of four weeks each after a preliminary stabilization and control-testing period. The men were divided into two groups whose dietary plan differed only as to the period during which they received the protein supplement; this allowed for detection of any general time trend. The dietary plan is indicated in Table III which also shows the averages for body weights and for the nutrients actually eaten in each period.

Again the body weights were essentially constant throughout. The serum cholesterol values are summarized in Table IV. The mean cholesterol values show no significant differences between any periods for either group. To examine the possible effect of dietary protein properly the responses of the individuals are analyzed in the last column in Table IV. The men in group A showed an average of 2.92 mg per 100 ml more serum cholesterol when on the high-protein diet (period 2) than when on the control (period 3). The standard error of this change, computed from the changes for the 12 individuals, is ±5.06. The men in Group B showed an average of 3.53 mg ± 3.00

TABLE II

Means (± Standard Error) Serum Total and Beta-Lipoprotein Cholesterol Concentrations of Two Groups of Men After Four Weeks on a Moderate-Fat Diet and Again After Four Weeks of Increased Fat Intake With and Without Increased Protein Intake. All Values Are in Mg per 100 ml

Group	Control value		Δ on high-fat diet	
	Total chol.	β-Chol.	Total chol.	β-Chol.
Moderate-protein	192.9 ± 11.5	160.0 ± 11.5	+23.0 ± 5.6	+18.2 ± 5.3
High-protein	213.0 ± 14.0	175.3 ± 13.7	+25.8 ± 7.5	+18.1 ± 7.5

TABLE III

Mean Body Weight and Nutrients Eaten Daily in Experiment 2

Item	Group A, periods				Group B, periods			
	1	2	3	4	1	2	3	4
Body wt., kg	67.8	67.5	67.8	67.6	65.6	65.5	65.3	64.9
Calories	3,176	3,203	3,246	3,174	2,974	3,010	3,019	3,041
Protein, g	69.9	138.7	68.2	69.4	64.7	63.2	125.6	66.0
Protein, % cal	8.8	17.9	8.4	8.7	8.7	8.4	17.2	8.7
Fat, g	68.9	68.2	68.5	66.7	65.5	66.4	65.5	64.4
Fat, % cal	19.5	19.2	19.0	18.9	20.5	19.9	19.5	19.1
Cholesterol, mg	395	1,436	1,388	477	374	1,369	1,417	452

mg per 100 ml *less* serum cholesterol when on the high-protein diet (period 3) than when on the corresponding control period (period 2). For all 26 men the mean serum cholesterol on the high-protein diet plus diet cholesterol was 0.42 mg per 100 ml less than on the corresponding low-protein plus diet cholesterol control, the standard error of this difference being ±2.27, again computed from the individual differences.

Superficial inspection of the values for the means of the periods in Table IV suggests the possibility that the added dietary cholesterol (periods 2 and 3) may have some tendency to produce a slight rise in the serum. However, this is not verified by detailed statistical computation. The grand mean for periods 2 and 3 for both groups is 210.0 mg per 100 ml while for periods 1 and 4 it is 206.4. The difference, 3.6 mg per 100 ml, is not significant, the standard error being ±2.90, computed from the differences for the 25 individuals between their means for periods 1 and 3 and their means for periods 2 and 4. This conforms to the expectations from many investigations.[9]

DISCUSSION

In the United States at present the total protein in the ordinary average diet supplies from 13 to 14 per cent of the total calories as shown in Table V. Actually, this is not high compared with the diets of most other populations. Some populations known to be characterized by low serum cholesterol values, such as in Japan and in Italy, equal or exceed the U. S. average in total protein. The control ("low-protein") diets used here in experiments 1 and 2, in which proteins supplied, respectively, only 11.3 and 8.6 per cent of the calories are therefore low-protein diets either on a U.S.A. or a world-wide basis.

The common belief that populations whose diets are low in fats always have low-protein intakes is erroneous, as shown by Table V. But it is true that most low-fat diet populations eat relatively small amounts of proteins from animal sources. Japan and Italy are good examples, with averages of about 3.4 and 3.5 per cent of total calories provided by proteins from animal sources, respectively. In comparison,

TABLE IV

Serum Total Cholesterol Concentrations After Four Weeks on Each of the Four Diets and the Differences ("Δ Chol.") Between the Individual Values on the High-Protein Diet and on the Low-Protein Diet (Group A Values for Period 2 Minus Those for Period 3; Group B Values for Period 3 Minus Those for Period 2). Values on High Protein Diets Are in Italics. All Values in Mg per 100 ml

No. of men	Group	Mean cholesterol, periods				Δ Cholesterol	
		1	2	3	4	Mean	S. E.
12	A	200.9	*209.4*	206.5	199.5	+2.92	±5.06
14	B	211.5	213.6	*210.0*	211.4	−3.53	±3.00

TABLE V [10–35]

Percentages of Total Calories Supplied by Fats and by Proteins in the Diets of Populations Listed in Order of Increasing Proportion of Fat

Area	Population	% Ca. from		Rank order		Reference No.
		Fats	Prot.	Fats	Prot.	
Japan	Rural, Nat. Survey, 1949	6	12*	1	7	10
Nigeria	Moslem natives	8	13*	2	14	11
Central America	Rural	8	15*	3	26	12
Japan	Nat. Survey, 1954	9	13*	4	15	13
India	Schools, Dangs, 1951–52	9	15	5	27	14
Greenland	Eskimos, Fishing, 1935–43	9	39	6	29	15
Japan	Urban, Nat. Survey, 1949	10	14*	7	18	10
Uganda	Native school	12	12	8	8	16
Japan	Kyushu small town, 1955	13	14*	8	19	17
South Africa	Bantu near Johannesburg	14	11*	10	4	18
Nigeria	Ibo region	15	8*	11	1	11
Gold Coast	Dome & Mampony children	16	8	12	2	19
Venezuela	Nat. Survey, 1949	18	14	13	20	20
Portugal	Nat. food use, 1949	20	10	14	3	21
Italy	Nat. food use, 1950	20	14*	15	21	22
Spain	Valleeas (Madrid) 1952	27	12*	16	9	3
Greenland	Eskimos, sealing, 1935–43	27	19	17	28	15
Australia	Nat. food supply, 1951–52	33	12	18	10	23
Great Britain	Industrial workers, 1952	35	12	19	11	24
Great Britain	National food use, 1951	36	13	20	16	25
Netherlands	National food use, 1954	36	11	21	5	26
Central America	Urban	36	14	22	22	12
Canada	Nat. Retail, 1951	38	12	23	12	27
Sweden	Nat. food use, 1953	38	12	24	13	28
U.S.A.	Nat. food use, 1952	40	13	25	17	29
Finland	Wood cutters	42	11	26	6	30
U.S.A.	Georgia adults	43	14	27	23	31
U.S.A.	U. S. Army, 44 messes	44	14	28	24	32
U.S.A.	U. S. Army, 2 companies	46	14	29	25	33
Arctic	Primitive eskimos	47	44	30	31	34
Arctic	Primitive eskimos	55	43	31	30	35

* Indicates Populations in Which Samples Have Proved to be Low in Serum Cholesterol Concentrations (General Levels in Middle Age of 200 Mg per 100 ml or less).

the usual figure in the United States is about 8 to 9 per cent of total calories from animal proteins. In this respect, too, the control diets used in the experiments reported here are low. In Experiment 2 during the low-protein diet periods (periods 1, 3 and 4 group A; periods 1, 2 and 4 for group B), proteins from animal sources provided an average of 3.5 per cent of the total calories and in this respect there is close similarity to diets in Japan and in Italy. During the high-protein periods in Experiment 2 animal proteins accounted for 11 per cent of the total calories, a much higher figure than for any ordinary American diet.

The results in the present study do not afford confirmation to the suggestion that the low cholesterol values in populations living on low-fat intakes are in any way related to the amount or kind of protein in their diets. Actually, the idea that dietary protein may influence the serum cholesterol level is supported only by animal experiments that seem to have little relevance to the situation in man. Monkeys on a diet severely restricted in certain amino acids, particularly L-cystine, and which are fed supplements of choline plus large amounts of cholesterol, have been reported to develop hypercholesterolemia and subsequent arterial wall lesions.[36] The suggestion from this result was that protein deficiency, or a specific de-

ficiency of L-cystine or sulfur-containing amino acids in general,[37] contributes to the production of hypercholesterolemia and atherosclerosis.

Comparative studies on populations, however, would suggest precisely the opposite conclusion, i.e., a diet low in proteins from animal sources, which are generally rich in sulfur-containing amino acids, including cystine, may lead to a *low* cholesterol level in the blood. But the present experiments indicate that the low-protein level in such diets is not influential in this regard and that the findings in populations such as the Bantu, in Japan and Italy and so on, are explicable on the ground that low animal protein diets merely happen to be associated frequently with a low fat level which *is* influential.

It should be recognized, of course, that extrapolation beyond the time duration of the present experiments may not be justifiable. What happens over the course of years may not be predictable from experimental studies over a mere matter of weeks or months. However, in the case of dietary fats, the tendency exhibited in populations is in conformity with the findings in short-time experiments and there is no reason to suggest that dietary protein may be involved in different relationships.

SUMMARY

Groups of physically healthy, schizophrenic men were metabolically stabilized on a typical American diet and then were maintained at calorie equilibrium under rigid control in dietary experiments lasting 16 or more weeks.

The response of the total serum cholesterol to a change from a low- to a high-fat intake was the same in one group of men receiving 83 g of protein daily as in a matched group on the same diet except for the isocaloric substitution of an extra 47 g of skimmed milk protein for carbohydrate in the diet. The cholesterol in the beta-lipoprotein fraction in the serum behaved as did the total serum cholesterol.

Two groups of subjects were maintained on a low-protein intake (averaging 8.6 per cent of calories from proteins), were changed for four weeks to a high-protein intake (17.7 per cent protein calories) and then were changed back to the low-protein intake, all at a constant fat intake. There was no significant change in the serum cholesterol level in either group at any time.

An increase, maintained for eight weeks, of 1000 mg per day in the dietary cholesterol intake, had no significant effect on the serum level in these experiments.

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