The Effect of Diet on Serum Cholesterol Levels in the Normal Rat[1]

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IN RECENT YEARS there has been a great deal of research interest in the relationship between specific nutrients in the diet and the development of atherosclerosis. In man, a high frequency of elevated serum cholesterol levels has been observed in atherosclerotic patients (1–3). Atherosclerotic responses can be obtained in animals by using dietary regimens consisting of high cholesterol intakes (4–10). However the rat is highly resistant to the development of experimental atherosclerosis under these conditions (11–15).

It was therefore considered to be of importance to investigate further the relationship between the intake of specific nutrients and the level of serum cholesterol in the rat. In the present work, in the rat, extending over a period of 50 weeks, serum cholesterol measurements were made on rats fed diets varying in protein, fat, and cholesterol content.

Experimental Methods

Twenty-five-day-old male weanling rats (Holtzman) were distributed in groups of 14 animals, equalized by body weight, and placed in individual cages with raised screen floors. The animals were fed *ad libitum* until they achieved body weights of 400 g. Restricted feed intakes were then employed to maintain the body weights of individual animals at 400 g. ± 15 g., for the remainder of the experiment. Weekly weighing and adjustment of the feed intake of individual animals were required to maintain each rat at that weight level.

A series of experimental rations was designed to provide low and high levels of either lard[4] or corn oil,[5] in combination with graded levels of casein. Sucrose provided the dietary carbohydrate. One per cent cholesterol was substituted for an equal amount of the dietary fat in diets 9 and 10. The composition of the experimental rations is shown as ingredient percentages by weight, and by calculated calories in Tables I and II.

Thus there were two basic series of rations in this experiment, comprising the rations numbered 1, 2, 3, and 4, which provided low levels of fat, in combination with progressive increases in the level of casein. In rations 5, 6, 7, and 8, fat provided four times as many calories as in rations 1, 2, 3, and 4. The calories per 100 g. of ration were constant in each series of rations.

Diets 9 and 10 were equivalent to diets 5 and 7, respectively, with the substitution of 1% cholesterol by weight for an equal amount of dietary fat.

Each of the diets was fed with either lard or corn oil, as the sole source of dietary fat, to provide comparative data on the effects of each of these fats. The rates of gain and other nutritional findings have been published elsewhere (16).

After 29, 41, and 50 weeks, blood samples were obtained from individual animals, using tail vein or terminal bleeding methods (17). These individual blood samples were centrifuged, and the serum was stored under nitrogen at −20°C. for subsequent analysis.

Serum cholesterol determinations were made on the serum from individual animals, using the method of Carr and Drekter (18).

Results

The results obtained in these studies are summarized in Table III. There was a large increase in average

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[4] Deodorized lard, Reliable Packing Company, Chicago, Ill.; linoleic acid content: 11.4%.

[5] Mazola brand, Corn Products Refining Company, Chicago, Ill.; linoleic acid content: 49.2%.

TABLE I

Percentage Composition of Experimental Rations by Weight[a]

Ration number	1	2	3	4	5	6	7	8	9	10
Casein	12	24	36	48	14.8	29.5	44.3	59.1	14.8	44.3
Lard or corn oil	5	5	5	5	24.6	24.6	24.6	24.6	23.6[b]	23.6[b]
Sucrose	78.5	66.5	54.5	42.5	55.0	40.2	25.5	10.7	55.0	25.5

[a] Vitamins, minerals, and fish liver oil were provided in a constant ratio to calculated calories, to make 100% (19).
[b] 1% cholesterol substituted for dietary fat.

TABLE II

Calculated Percentage Composition of Experimental Rations by Calories[a]

Ration number	1	2	3	4	5	6	7	8	9	10
Casein	11.8	23.6	35.4	47.2	11.8	23.6	35.4	47.2	12.0	36.0
Lard or corn oil	11.1	11.1	11.1	11.1	44.2	44.2	44.2	44.2	42.2[b]	42.2[b]
Sucrose	77.1	65.3	53.5	41.7	44.0	32.2	20.4	8.6	45.8	21.8
Calories/100 g.	407	407	407	407	501	501	501	501	492	492

[a] Calculated as 4 calories/g. for casein and sucrose, 9 calories/g. for lard or corn oil.
[b] Cholesterol was assumed to contribute no calories *per se*.

TABLE III

The Effect of Diet and Age on Serum Cholesterol Levels [a]

Ration number	1	2	3	4	5	6	7	8	9	10
Lard										
29 weeks	89 ± 19	98 ± 23	93 ± 19	104 ± 20	102 ± 30	102 ± 29	97 ± 30	118 ± 34	182 ± 53	193 ± 53
41 weeks	147 ± 30	161 ± 21	162 ± 37	179 ± 33	155 ± 23	159 ± 25	167 ± 23	170 ± 24	232 ± 50	237 ± 38
50 weeks	138 ± 28	144 ± 25	147 ± 37	156 ± 31	141 ± 18	152 ± 21	164 ± 24	169 ± 24	241 ± 99	248 ± 50
Corn oil										
29 weeks	78 ± 18	.....	92 ± 22	.....	88 ± 27	.....	85 ± 25	.....	143 ± 71	162 ± 41
41 weeks	136 ± 26	.....	146 ± 20	.....	135 ± 13	.....	151 ± 23	.....	220 ± 61	230 ± 41
50 weeks	198 ± 34	.....	142 ± 19	.....	131 ± 24	.....	138 ± 21	.....	198 ± 69	260 ± 62

[a] Values shown are average ± standard deviation, in mg. cholesterol/100 ml. serum.

serum cholesterol levels in the interval between 29 and 41 weeks, in each of the experimental groups, while small decreases in average values were observed from 41 to 50 weeks for all groups except those fed lard in rations 9 and 10 and those fed corn oil in ration 10.

The effects of the level of protein in the diet on serum cholesterol levels are not completely consistent for the data obtained after 29 weeks. The results obtained after 41 weeks and 50 weeks, when either lard or corn oil was fed, indicate that, in the experimental rat, serum cholesterol levels increase with increasing levels of casein in the diet. These increases in serum cholesterol with increased dietary casein intake were small and not statistically significant, but they were consistent for each of the experimental groups, at 41 and 50 weeks.

The increased percentage of the calories from fat in the diet usually resulted in slight increases in serum cholesterol levels, for each of the protein levels tested, when either lard or corn oil was fed. These effects however were less pronounced than those noted with changes in the level of protein in the ration. The results indicate that the average serum cholesterol levels of the animals fed the corn oil rations were somewhat lower than those observed when lard was included in the experimental ration. These differences were consistent with one exception, Group 10 fed corn oil at 50 weeks.

The results shown for groups 9 and 10 indicate that the feeding of 1% cholesterol, substituted by weight for an equivalent amount of fat in the ration, resulted in marked increases in average serum cholesterol levels. However a corresponding increase in variability within the experimental groups was also observed. These studies of serum cholesterol levels in individual animals provide significant information with regard to the normal biological variability among members of a single dietary group. In general, the standard deviation of serum cholesterol levels for each of the groups in Table III is so large that statistical significance of the differences between experimental groups was not obtained. Thus, when serum samples from individual animals in experimental groups are pooled, information relating to biological variability is automatically eliminated and the evaluation of statistical significance between treatments is lost. These results indicate the importance of long-term studies, using large groups of animals in order to evaluate critically the variability in biochemical responses to nutritional factors.

Summary

In a long-term study of the effects of specific nutrients upon the serum cholesterol levels in individual rats, average serum cholesterol levels increased between 29 and 41 weeks in all experimental groups, and small consistent decreases were observed between 41 and 50 weeks. Serum cholesterol levels increased slightly with increasing levels of casein in the diet when increased levels of fat were fed. The substitution of 1% cholesterol for either lard or corn oil resulted in increased levels of serum cholesterol and increased variability among the animals within each experimental group.

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