Diet *and* Plasma Cholesterol Levels

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THE ROLE of diet in affecting plasma cholesterol levels in experimental animals has been clearly demonstrated in certain species.[1-4] This picture is to be contrasted with that found in studies with humans, which have been marked by many conflicting reports and controversies regarding the interpretation of the data obtained. On the one hand, a number of investigators have reported that alteration of dietary cholesterol level has no effect on the plasma content of this lipid,[5,6] and, on the other hand, Messinger *et al.*[7] have claimed that increased dietary cholesterol levels do in fact bring about increased plasma concentrations of this substance. However, these workers also indicate that some other dietary factor present in egg yolk has an important determining influence.

The level of dietary fat has also been implicated in studies utilizing both experimental animals[8,9] and humans.[10,11] There would appear to be a reasonable degree of consistency in the reports on the effect of high levels of animal fat in elevating plasma cholesterol concentrations. At the same time, it must be pointed out that animal fat has invariably associated with it a significant percentage of cholesterol, and hence the possibility exists that the alteration may have been due to this circumstance. There have been conflicting results reported on the effect of high levels of vegetable fat. Kinsell *et al.*,[12] working with various hospital patients who were receiving a variety of treatments, including ACTH and cortisone, both of which have been noted to affect plasma lipids under certain conditions,[13] reported that diets containing high levels of vegetable fat not only did not lead to increased plasma cholesterol levels but actually effected a decrease. Groen and his colleagues,[14] in a long-term investigation using sixty presumably normal subjects, also noted a decrease when a diet essentially free from cholesterol (estimated intake about 1.5 mg./day) and high in vegetable fat was substituted for one high in animal fat. Conflicting conclusions, however, have been reached by Hildreth, Mellinkoff, Blair, and Hildreth,[11] and by Keys.[5] It seems obvious that certain important factors affecting plasma lipid levels are not being properly controlled in these studies. The possibility of such factors as positive or negative calorie balance,[15,16] physical or emotional stress,[13,14] and the various types of vegetable fats has not always been adequately taken into consideration. These and other unknown influences may well serve to resolve the conflicting reports and opinions on this subject.

In the study herewith described, dietary modifications were made with a view to altering the level of natural cholesterol, or animal or vegetable fat, at the expense of primarily carbohydrate foods. The latter were taken in amounts sufficient to satisfy the appetite and to maintain body weight as constant as possible.

EXPERIMENTAL

Methods

The co-operation of five dependable and apparently healthy and emotionally stable

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male subjects (physicians and internes) was obtained. Complete physical examinations and electrocardiograms were carried out and found to be negative. No ill effects of a physical or emotional nature were observed throughout the experimental period. The subjects had been in a state of nutritional balance, without overt weight gain or loss for three to four months prior to our study.

All five subjects ate for some months prior to and during the experimental period in a common dining-room. For each individual the composition of the pre-experimental and subsequent experimental diets was calculated as accurately as possible by Miss F. M. Silverlock, chief dietitian of the Kingston General Hospital, using Bridge's *Food and Beverage Analyses* (1950) by Mattice. All diets were prepared under her direction.

TABLE I

Approximate Composition of the Daily Diet Prior to Our Experiments

Subject	Protein	Fat	Carbohydrates	Cholesterol	Total calories	Calories taken as fat
	Gm.	*Gm.*	*Gm.*	*mg.*		%
A	80	80	240	500	2000	34
B	120	140	315	300	3000	42
C	100	120	180	500	2200	33
D	130	150	210	800	2700	50
E	140	150	190	1000	2700	50

The intake of cholesterol, and of animal and vegetable fat, was controlled as closely as possible. Some leeway was permitted in the intake of carbohydrate foods such as potatoes, sugar, marmalade, bread, and arrowroot biscuits, the principal objective being to maintain body weight fairly constant. Blood samples were taken prior to breakfast at appropriate intervals throughout the experimental period, and total and free cholesterol were determined on the plasma by the Schoenheimer-Sperry procedure.[17] The characteristics of the experimental rations and the order in which they were eaten are given below.

Diet No. 1

This ration, which was ingested for a period of four weeks, was made low in fat and in cholesterol by the partial elimination of vegetable and animal fat with associated cholesterol. Table II gives the approximate composition of the modified diet; it may be noted that the fat content ranged from about 23 to 52 Gm. and protein from 102 to 190 Gm., and that about 90 per cent of both components was of animal origin. These foods supplied 100 to 180 mg. of cholesterol per day.

TABLE II

Approximate Composition of Diet No. 1

Subject	Protein	Fat	Carbohydrate	Cholesterol	Total calories	Calories supplied by fat
	Gm.	*Gm.*	*Gm.*	*mg.*		%
A	102	23.1	323	100	1908	10.9
B	189.5	45.2	449	180	2968	13.9
C	137	46.4	321	140	2249	18.9
D	164.5	51.7	398	150	2605	17.8
E	164.5	43.8	410	150	2674	14.8

Diet No. 2

This ration, designed to be low in fat and high in cholesterol, was obtained by adding three scrambled or boiled eggs calculated to provide about 800 mg. of cholesterol. The animal fat component was decreased by the amount of fat supplied by the eggs. This diet was eaten for a period of one week, the fifth of the experiment.

Diet No. 3

This ration, designed to be high in vegetable fat and low in cholesterol, was obtained by adding about 70 Gm. of fat in the form of peanut oil, corn oil, or margarine to Diet No. 1. The total caloric intake was maintained approximately constant by the reduction of carbohydrate, a change that was made spontaneously by the subjects. This diet was eaten for a period of one week, the sixth of the experimental period.

Diet No. 4

This ration, designed to be high in animal fat and to contain a moderate amount of cholesterol, was obtained by adding about 70 Gm. of animal fat, mainly in the form of lard, to Diet No. 1. The cholesterol intake was calculated to be about 210–300 mg. per day. This diet was eaten for a period of two

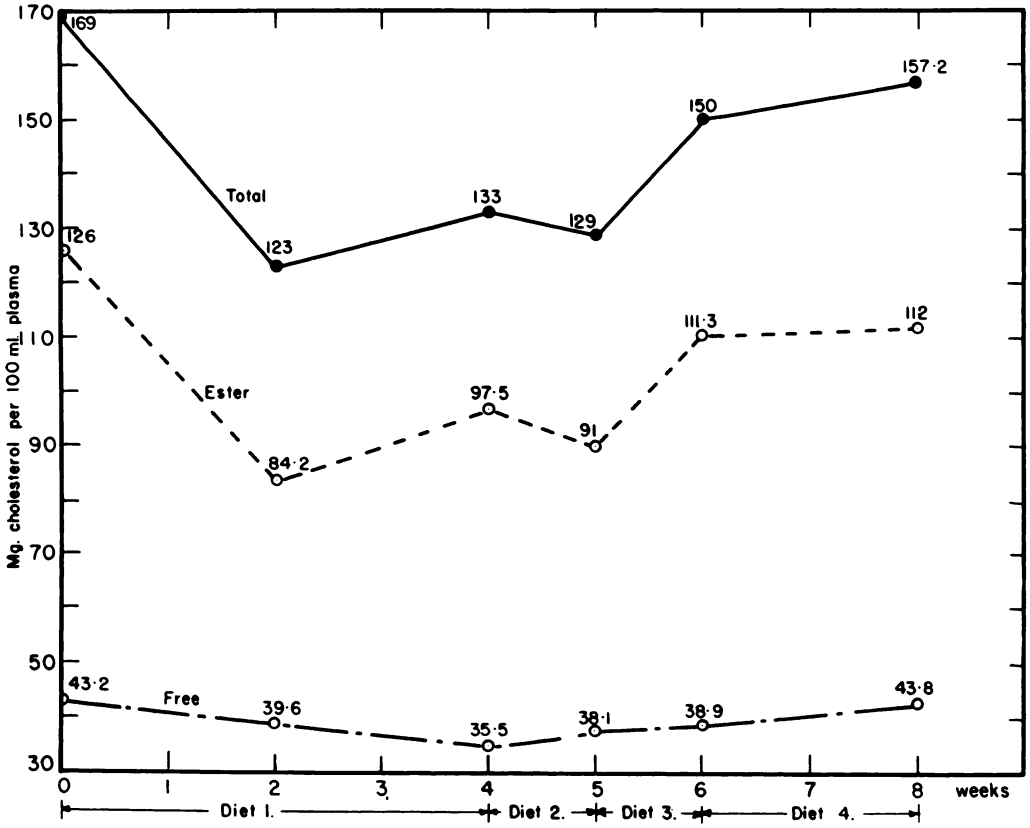

Fig. 1. Mean cholesterol levels of the five subjects throughout the experimental period.

weeks, the seventh and eighth of the experimental period.

Results

Figure 1 shows the mean cholesterol levels during the entire experimental period. The individual data on the five subjects are presented in Table III. A statistical analysis of the results was performed by the procedure outlined by Bernstein and Weatherall,[18] in which the significance of the mean of the differences was tested (see Table IV).

Discussion

The change from a normal mixed hospital diet, which supplied 33–50 per cent of the total calories in the form of fat and between 300 and 1000 mg. of cholesterol per day, to a diet in which fat provided only 11–19 per cent of the total calories and between 100 and 180 mg. of cholesterol led to a rapid and significant decrease in both esterified and free cholesterol (respectively $P = < 0.01$ and 0.02), by far the greater change occurring in the former fraction. It may be noted that in the case of Subject D, despite the fact that his diet provided 18 per cent of the calories in the form of fat, a figure at the upper range of the group values, his cholesterol level decreased from 141 to 83 mg. per 100 ml. plasma. It would appear, therefore, that it is not necessary to subject patients to an extreme restriction of fat in order to effect a significant decrease in plasma cholesterol. Keys[10] noted a lowering of only about 20 per cent when subjects were shifted from a standard diet providing 150 Gm. of fat and 600 to 700 mg. cholesterol per day to a diet devoid of cholesterol and supplying only 15 Gm. fat per day. This response, while qualitatively the same as that noted in our work, is quantitatively different, being much smaller than that noted by us. In this connection, it is of interest that the decreases from individual to individual could not be re-

TABLE III

Plasma Cholesterol Levels of Five Subjects Ingesting Diets Differing in Fat and Cholesterol Content. Individual Data

Subject	Age (years)	Weeks	Diet	Wt. in lb.	Total cholesterol*	Cholesterol*	
						Free	Esterified
A	39	Start	1	186	223.0	63.6	159.4
		2	1	188	186.0	56.1	129.9
		4	1	186	186.5	49.5	137.0
		5	2	183	174.5	51.0	123.5
		6	3	185	193.0	48.7	144.2
		8	4	189	182.0	53.5	122.5
B	33	Start	1	157	167.0	43.4	123.6
		2	1	156	124.5	40.4	84.1
		4	1	156	119.0	32.7	86.3
		5	2	154	127.0	36.8	90.2
		6	3	155	137.0	37.5	99 5
		8	4	157	140.0	41.5	98.5
C	24	Start	1	156	172.0	44.6	127.4
		2	1	156	144.0	42.0	102.0
		4	1	153	144.0	36.2	107.8
		5	2	152	148.0	40.6	107.4
		6	3	153	169.0	42.0	127.0
		8	4	154	173.0	47.9	125.1
D	24	Start	1	164	140.5	31.7	108.8
		2	1	161	83.3	30.9	52.4
		4	1	163	97.0	27.8	66.2
		5	2	157	89.0	30.6	58.4
		6	3	158	130.0	35.0	95.0
		8	4	157	134.0	38.8	95.2
E	23	Start	1	183	146.0	33.1	112.9
		2	1	184	81.0	28.5	52.5
		4	1	184	122.0	31.5	90.5
		5	2	181	107.0	31.7	75.3
		6	3	183	122.0	31.3	90.7
		8	4	180	156.5	37.5	119.0

* Total, free, and esterified cholesterol expressed in terms of mg./100 ml. plasma.

TABLE IV

Comparison of the Significance of the Mean of the Difference in Plasma Cholesterol Levels on Different Dietary Regimes

Comparison between values obtained on following diets	Total cholesterol		Free cholesterol		Ester cholesterol	
	Change in mg./100 ml.	Probability	Change in mg./100 ml.	Probability	Change in mg./100 ml.	Probability
Pre-test vs. Diet 1 at end of 2 weeks	−46.2	< 0.01	−3.7	0.02–0.05	−42.2	< 0.01
Pre-test vs. Diet 1 at end of 4 weeks	−36.5	< 0.01	−7.7	0.02	−28.8	< 0.01
Pre-test vs. Diet 2	−41.0	< 0.01	−5.1	0.05–0.1	−37.4	< 0.01
Pre-test vs. Diet 3	−19.8	≃0.02	−4.4	0.2–0.3	−15.2	≃0.02
Pre-test vs. Diet 4	−12.7	0.2–0.3	+0.6	0.9	−13.9	0.1–0.2
Diet 1 vs. 2	−3.4	0.5	+2.4	≃0.05	−4.6	≃0.2
Diet 1 vs. 3	+17.0	0.02–0.05	+3.3	≃0.1	+13.7	≃0.05
Diet 1 vs. 4	+24.0	0.02–0.05	+8.3	<0.01	+14.5	0.1–0.2

lated closely to the fat intake. For example, Subject A, whose diet provided 10.9 per cent of the total calories in the form of fat, exhibited a decrease of 37 mg. or 16.6 per cent, whereas Subject D, whose diet supplied 17.8 per cent of the caloric intake in the form of fat, showed a decrease of 57 mg. or 40.6 per cent. It may therefore be concluded that widely varying degrees of fat restriction can produce similar decreases in plasma cholesterol levels. For this reason, any prescription of diets designed to lower blood cholesterol should be controlled by determinations of this lipid, and the least troublesome dietary modification that produces the desired decrease should be prescribed. A similar conclusion has also been reached by Hildreth *et al.*[11]

Both Keys[10] and Mellinkoff, Machella, and Reinhold[19] have reported that when dietary alterations are made that affect cholesterol levels, the major change occurs during the first week to 10 days. An examination of the data in Table III provides some confirmation of this statement, inasmuch as the greatest alteration was noted after an interval of two weeks on Diet 1.

Despite the fact that approximately 800 mg. cholesterol per day were taken in the form of egg yolk in the subsequent period, no demonstrable change was observed in plasma cholesterol levels. This agrees with the findings of Keys *et al.*[5] and Hildreth *et al.*,[11] but is in conflict with those of Messinger *et al.*[7] However, the proper interpretation of the latter work is made difficult by the fact that no attempt was made by these investigators to control the level of dietary fat. The addition of 150 Gm. of egg yolk powder would add approximately 95 Gm. of fat per day to the diet, and the effect of animal fat in increasing plasma cholesterol levels appears to be fairly well substantiated, as indicated below. Furthermore, the latter workers utilized about five times as much egg yolk as was eaten by the subjects in our study, and this may explain the wide difference in the results obtained.

The addition of 70 Gm. per day of vegetable fats to Diet 1 led to a significant increase in plasma cholesterol. This finding is in agreement with that of Hildreth *et al.*[11] and Keys *et al.*,[5] and emphasizes once more the importance of the level of dietary fat, no matter what the source. However, both Groen *et al.*[14] and Kinsell *et al.*[12,20] present rather convincing evidence that the change from a diet containing animal fat to one containing vegetable fat is associated with a significant decline in plasma cholesterol values. Additional information supporting this view has been recently published by Hardinge and Stare[21] who found that "pure" vegetarians exhibited lower serum cholesterol levels despite a free intake of vegetable fat. These conflicting conclusions indicate the necessity for further work in this field in order to elucidate the role of dietary fats in affecting plasma cholesterol levels.

The substitution of animal fat for the vegetable fat resulted in a further but insignificant increase in plasma cholesterol to levels that almost undoubtedly would have been reached had the diet high in vegetable fat been continued for a similar length of time. Under the conditions used here, therefore, no distinction could be made between vegetable and animal fat in effecting an elevation of plasma cholesterol.

Summary

Five apparently healthy male subjects were placed upon diets having the following characteristics:

(1) 100 to 180 mg. cholesterol per day and 10.9 to 18.9 per cent of the total calories in the form of fat.

(2) 900 to 980 mg. cholesterol daily and with the same proportions for fat.

(3) 100 to 180 mg. cholesterol per day and 34 to 46 per cent of the total calories in the form of vegetable fat.

(4) 210 to 300 mg. cholesterol per day and 34 to 46 per cent of the total calories in the form of animal fat.

The diet low in fat and in cholesterol led to a highly significant decrease in plasma cholesterol levels, whereas the addition of cholesterol in the form of egg yolk did not cause any change. When the proportion of dietary fat was increased in the form of vege-

table fat, a significant increase occurred in plasma cholesterol levels, and these values were not further significantly increased by a substitution of animal fat for the vegetable fat. The results of this investigation indicate that, within the limits used here, dietary cholesterol has no effect on plasma cholesterol levels, whereas alterations in the level of dietary fat, whether of animal or vegetable origin, led to parallel changes in plasma cholesterol.

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Resumen

Dieta y niveles plasmáticos de colesterol

Cinco sujetos de sexo masculino gozando, según las apariencias, de buena salud, fueron sometidos a dietas con las siguientes características:

(1) 100 a 180 mg. colesterol/día y 10,9 a 18,9 por 100 de las calorías totales en forma de grasas.

(2) 900 a 980 mg. colesterol/día y con las mismas proporciones de grasas.

(3) 100 a 180 mg. colesterol/día y 34 a 46 por 100 de las calorías totales en forma de grasa vegetal.

(4) 210 a 300 mg. colesterol/día y 34 a 46 por 100 de las calorías totales en forma de grasa animal.

La dieta pobre en grasas y en colesterol indujo un descenso muy significativo de los niveles plasmáticos de colesterol, mientras que la adición de colesterol en forma de yema de huevo no causó ningún cambio. Cuando la proporción de grasa dietética fué aumentado por la adición de grasa vegetal, hubo un aumento significativo de los niveles plasmáticos de colesterol, y estos valores no fueron significativamente aumentados por la sustitución por grasa animal de la grasa vegetal. Los resultados de esta investigación indican que, dentro de los límites observados aquí, el colesterol dietético no afecta en nada los niveles plasmáticos de colesterol, mientras que cambios en el nivel de grasa dietética, sea ésta de origen animal o vegetal, resultan en cambios paralelos del colesterol plasmático.