Effect of Mixed Fat Formula Feeding on Serum Cholesterol Level in Man

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IN EXPERIMENTAL STUDIES with cholesterol–cholic acid-fed rats, Hegsted *et al.*[1] found an apparent synergism between the completely saturated fatty acids and linoleic acid in the lowering of the levels of serum cholesterol. The product of the saturated fatty acid content times the linoleic acid content of the dietary fat had a high inverse correlation with the serum cholesterol level, when a number of oils and mixtures of oils were tested. This finding is apparently incompatible with the results obtained by Ahrens and associates,[2] Keys *et al.*,[3] and others who have studied the effects of dietary fats upon the level of serum cholesterol in man. In general, increasing the saturated fatty acids in the diet apparently causes an increase in the serum cholesterol level, although all studies have not uniformly shown this effect.

In the studies upon rats an equal mixture of coconut oil and safflower oil gave the highest product of the mixtures studied and provides about maximum amounts of linoleic acid and saturated fatty acids in readily obtainable forms. The admixture results, of course, in an oil with approximately half the iodine number and about half the linoleic acid content of safflower oil, since coconut oil is nearly devoid of unsaturated acids.

The present paper presents the results obtained upon a series of 10 patients in which a formula diet was used and in which the two fats, safflower oil and an equal mixture of safflower and coconut oils, were compared.

The difficulties encountered in interpreting changes in serum cholesterol are well known to those interested in this field of investigation. We believe the experimental design used is efficient and useful for the comparison of two dietary effects, since it avoids the subjective determination of "plateau" levels of serum cholesterol and randomizes the effects of variables which may depend upon the time determinations are done.

MATERIAL

The formula diet which was used in these experiments was similar to that of Ahrens *et al.*[2] Safflower oil or an equal mixture of safflower and coconut oils constituted 42 per cent of the total daily calories, while carbohydrate in the form of glucose, and protein derived from milk proteins, contributed 43 per cent and 15 per cent of the calories, respectively. Sodium chloride (2 g) and two multivitamin capsules were given as daily supplements.

The pertinent clinical data on the patients who participated in this study are presented in Table I. All of the subjects were hypercholesteremic; two had xanthomata and 8 had compensated coronary heart disease and healed myocardial infarction. All patients were ambulatory. During the experimental period, body weights were kept constant by adjustment of the formula intake. Preced-

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TABLE I
Clinical Data on Male Patients with Hypercholesterolemia

Case no.	and initials	Age	Occupation	Height (inches)	Present weight (lb)	Clinical diagnosis	Maximum weight (lb)
1	W. M.	35	Draftsman	70	153	C.H.D., M.I.	194
2	J. P.	35	Policeman	67	145	C.H.D., M.I.	186
3	J. G.	50	Gardener	65	146	C.H.D., M.I.	190
4	S. S.	40	Police captain	69	180	T.X.	220
5	J. B.	32	Postal clerk	73	197	C.H.D., M.I.	235
6	F. S.	45	Lawyer	70	150	C.H.D., M.I.	160
7	J. F.	42	Mechanic	68	163	C.H.D., M.I.	190
8	J. D.	41	Photographer	63	180	M.X.	201
9	T. F.	50	Clerk	66	163	C.H.D., M.I.	194
10	J. D.	42	Heavy-machinery operator	64	160	C.H.D., M.I.	169

C.H.D. = coronary heart disease; M.I. = myocardial infarction, stable; T.X. = tendon xanthomata; M.X. = mixed xanthomata.

ing the present study by two months to eight years, a fairly constant body weight had been maintained in all of the patients with coronary heart disease since their convalescence from acute myocardial infarction.

Nine of the patients had been obese prior to the development of their disorders We have obtained careful dietary histories from all of these patients over the years preceding their difficulty. A summary of these dietary intakes is presented in Table II. As revealed from the statistical analysis, a considerable amount of variation occurred in the constituents of these high calorie diets. Nonetheless, the variations were found at higher levels of intake. We emphasize that these figures are approximations derived from dietary histories, yet they undoubtedly played a role in the attainment of each individual's "maximum" obese weight.

METHODS

The studies were made on patients in the metabolic ward. Prior to the start of the formula feeding, two blood samples were drawn approximately 24 hours apart for cholesterol analysis, which was done by the method of Abell *et al.*[4] in duplicate. Alternate patients were assigned to either group A or group B, and the experimental study began

TABLE II
Summary of Daily Dietary Intake of the 10 Reported Patients Over the Years Prior to Development of Clinical Disorder

	Calories	Carbohydrate (g)	Protein (g)	Total fat (g)	Animal fat (g)	Vegetable fat (g)	Alcohol (g)
Mean	5,673	545	173	252	197	55	75
S.D.*	±1,919	±201	±64	±68	±83	±27	±75
S.E.†	±606	±63	±20	±21	±26	±8	±24

* Standard deviation. † Standard error.

after the second control sample was taken. Groups A and B were similar except for the order of the two oils tested. Those in group A received the safflower oil formula followed by a similar period of the mixture of safflower and coconut oils, while group B received the mixture first followed by the safflower oil alone. In the beginning it was planned that the feeding periods on each oil would be of three weeks' duration, but in approximately half of the patients the time had to be decreased to two weeks, since a maximum of one month was as much time as could be obtained from many of the men. On the last two days of the feeding period, blood samples were obtained and analyzed as before. The formula was changed and the procedure repeated.

RESULTS

The results of this study are shown in Table III, where the mean cholesterol values for each man at the beginning and at the end of each period are presented. It can be seen that

TABLE III
Mean Cholesterol Values

Group and subject		No. weeks	Mean serum cholesterol (mg/100 ml)		
			Control	Safflower	Mixture
A	1	3	364	215	214
	3	3	358	279	272
	5	2	353	250	281
	7	2	336	232	240
	9	3	315	276	198
Mean			345	250	241
			Control	Mixture	Safflower
	2	3	416	274	251
	4	2	348	245	217
	6	2	331	265	285
	8	3	489	361	351
	10	2	310	289	257
Mean			379	272	287
			Control	Safflower	Mixture
Grand mean			362	268	256

either safflower oil or the mixture of safflower and coconut oils caused a marked decrease in the serum cholesterol levels and approximately to the same extent. Indeed, the mixture of oils caused a slightly greater decrease in the cholesterol level than did safflower oil.

A variance analysis of the results is shown in Table IV. The so-called "treatment effect" including the control and two feeding periods is highly significant. Nearly all of this variance is accounted for by the difference between the control period and the two oil periods. The difference between the two oil periods is not significant. The data also show, as would be expected, that there are significant differences in the serum cholesterol levels of the individuals tested. The variation between pairs of determinations, i.e., samples of blood taken a day apart at the beginning and at the end of each feeding period, gives a standard deviation of about 10 mg/100 ml. This would be representative of the reproducibility of the serum cholesterol value on separate blood samples.

TABLE IV
Analysis of Variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatment	2	133,536	66,768	44.8*
Control vs. oils	1	132,202	132,202	88.7*
Between oils	1	1,334	1,334	—
Interaction	18	26,817	1,489.8	—
Between individuals	9	88,465	9,829.4	88.05*
Within pairs of determinations	30	3,349	111.63	—

* Significant; $p < 0.01$.

DISCUSSION

The results of this study are in essential agreement with the previous results obtained with rats.[1] With the number of patients tested one could not expect to show significant differences in the potency of oils when the results are as closely similar as those obtained. Nevertheless, it is of some interest that the mixture of oils, in both series, gave slightly lower serum cholesterol values than safflower oil alone.

The results appear at the moment to be incompatible with the generalizations proposed by Ahrens *et al.*[2], Keys *et al.*,[3] or other workers in the field. Whereas Ahrens and co-workers[2] found the "serum cholesterol lowering effect" to be closely related to the iodine value of the dietary fat, the mixture of coconut oil with an iodine value of 10 to safflower with an iodine value of 145 yields a mixture with an iodine number of 78. Similarly, applying the formulation of Keys and associates,[3] in which saturated fatty acids counteract the action of polyunsaturated fatty acids, does not account for the results obtained. The mixture provides 51 g of saturated acids and approximately 42 g of polyunsaturated acids per 100 g compared to 12 and 81 respectively in safflower oil and, according to Keys' formulation, should produce considerably higher serum cholesterol levels. Since this work was done with hypercholesterolemic patients the actual coefficients in Keys' formula may not be applicable. Kinsell *et al.*[5] concluded that the major cholesterol lowering ingredient in various vegetable fats was linoleic acid. The results are not compatible with the belief that the linoleic acid content of an oil is proportional to its lowering effects upon serum cholesterol.

This does not appear to be the appropriate place to discuss the many and diverse findings so far reported in the literature. Still, it is worthwhile to note that, contrary to the usually accepted opinion, various workers have found that the ingestion of hydrogenated fats does not invariably result in a rise in the serum cholesterol. Bronte-Stewart and associates[6] also have reported one case in which the addition of hydrogenated ground-nut oil to a highly unsaturated fraction of sunflower seed oil did not apparently effect a rise in the serum cholesterol level. Hydrogenated coconut and whale oils did not affect serum cholesterol when fed individually as the sole source of fat,[7] and hydrogenated corn oil added to butterfat produced a hypocholesterolemic response.[8]

It is apparently true at this time that none of the generalizations so far proposed can account for the diverse results obtained in different laboratories. Nor does it seem likely that this diversity can be explained as being due to "errors." It appears more likely that the differences in conditions in the various laboratories—basal diets, patients used, etc.—may account for some of the discrepancies. Since the conditions of our studies are similar to those employed by Ahrens, it may be worthwhile to inquire whether mixtures of fats behave in a manner similar to natural fats of the same general fatty acid composition. However, we may note that the mixture of safflower and coconut oils falls into an area upon the triangular plot of Ahrens which is blank in his paper. If these data are comparable to those of Ahrens, i.e., safflower oil is essentially the same as corn oil and the mixture is similar to safflower oil, then the curved surface should drop rapidly along the saturated-linoleic coordinate, reaching a minimum by at least the 50-50 point.

In spite of much work in recent years, the adequate determination of serum cholesterol levels remains a problem. In few laboratories are there sufficient controls in operation to determine the deviations from the true levels occasioned from day to day, week to week, between technicians using the same method, etc. Experimental designs should be utilized in which such variation cannot possibly influence the results. The subjective evaluation of "constant" or plateau levels should also be eliminated if possible.

The mechanism of the apparent synergism between the saturated and the essential fatty acids in lowering serum cholesterol remains unknown. Preliminary experiments indicate a difference in the degree of cholesterol response to the mixture of oils related to the initial body weight of the experimental subject. It is not known whether the initial depot fatty acid composition determines the body's response toward a critical mixture of fatty acids. It is possible that the initial cholesterol lowering usually obtained by consuming a generous quantity of corn or safflower oil may be in part due to a synergism between it and the saturated fatty acids of body fat.

We believe that the results obtained here are important insofar as they suggest caution against the belief that the "saturated" fats are "bad" and the essential fatty acids are "good." In addition, they emphasize the need for investigation of the potency of mixtures of oils in man, which may lead to more acceptable dietary regimens which are not a "compromise" but actually desirable.

SUMMARY

Formula-feeding experiments were conducted in 10 patients with hypercholesterolemia, in which the two fats, safflower oil and an equal mixture of safflower and coconut oils, were compared with regard to their effect on serum cholesterol level.

Either the safflower oil or the mixture of safflower and coconut oils caused a marked decrease in serum cholesterol. The mixture effect was obtained regardless of whether it was fed before or after the safflower oil. The results are comparable to those previously obtained with these two oils in rats.

The results are incompatible with the proposed hypotheses that the serum cholesterol-lowering effect of a dietary fat is proportional to the iodine value or the linoleic acid content of the fat. In addition, the results do not support the view that the saturated fats counteract the effect of polyunsaturated fats.

The experimental design used is efficient and useful for the comparison of two dietary effects on serum cholesterol level in man. It eliminates the subjective evaluation of constant or plateau levels of serum cholesterol and randomizes the effects of variables inherent in time and methodology of determination of the lipid.

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