Serum Cholesterol Response to Changes in the Diet. IV. Particular Saturated Fatty Acids in the Diet

By ANCEL KEYS, JOSEPH T. ANDERSON AND FRANCISCO GRANDE

For many dietary changes satisfactory prediction of the average change in the serum cholesterol level of man in mg./100 ml., is given by $\triangle$ Chol. = 1.35 ($2\triangle S - \triangle P$) + $1.5\triangle Z$ where S and P are percentages of total calories provided by glycerides of saturated and polyunsaturated fatty acids in the diet and Z^2 = mg. of dietary cholesterol/1000 Cal. This formula fails, however, when the dietary change involves large amounts of cocoa butter and discrepancies also appear with beef tallow or hydrogenated coconut oil diets. Controlled dietary experiments at the University of Minnesota and at 2 other centers, provide 63 sets of comparisons of serum cholesterol averages for groups of men on each of 2 chemically characterized diets. Least-squares analysis indicates that stearic acid, as well as saturated fatty acids containing fewer than 12 carbon atoms, have little or no effect on serum cholesterol in man. The equation, $\triangle$ Chol. = $1.2(2\triangle S' - \triangle P) + 1.5\wedge Z$, yields good correlation (r = 0.93) with the observed values in these 63 sets of data. This formulation also resolves heretofore puzzling discrepancies in the literature.

FOR ALL ORDINARY DIETS, as well as for most experimental diets, reasonably satisfactory estimates of the average serum cholesterol response to changes of fats in the human diet can be made from data on the percentage of total calories provided from saturated, S, and polyunsaturated, P, fatty acids in the diets concerned (cf. the previous parts of the present communication).[1,2] The formula previously used for this purpose, $\triangle$ Chol. = $2.7\triangle S - 1.3\triangle P$, or $1.35(2\triangle S - \triangle P)$ implies that, in this regard, all saturated fatty acids are equivalent, but this formulation was designed only as an approximation to apply to most practical dietary situations without implications as to particular fatty acids. In other words, the coefficient +2.7 applies to mixtures of the saturated fatty acids as they were represented in the considerable variety of diets used in the derivation of the formula.

The limitations of $1.35\ (2\triangle S - \triangle P)$ are indicated by the experimental diets studied. These included common U.S. "luxus," medium- and low-fat diets of ordinary foods with and without inclusion, in amounts up to about 35 per cent of total calories, of the following experimental fats: corn, soybean, sunflower seed, rapeseed, safflower, cottonseed, coconut and olive oils, "sardine" (pilchard) and menhaden fish oils, and butterfat. From other data it appears that the formulation should also apply to sesame, peanut and mustard seed oils.

With the exception of diets containing unusually large amounts of coconut

From Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota.

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oil and butterfat, in almost all diets palmitic acid dominates the saturated fatty acid group, and in effect, the term "S" mainly refers to this fatty acid. In coconut oil, lauric acid is dominant but fatty acids with fewer than 12 carbon atoms make up about 16 per cent of the total saturates. In butterfat, such short- and medium-chain length fatty acids make up around 10 per cent of the saturated fatty acids.

There is good reason to believe that, in the diet, fatty acids with fewer than 12 carbons have much less effect on serum cholesterol than the longer chain saturated fatty acids. We were unable to find any effect of butyric acid in the diet.[3] Hashim et al.[4] found virtually no serum cholesterol effect of a mixture of fatty acids with fewer than 12 carbons in the chain, even when fed in large amounts to human subjects. Beveridge et al.[5] provided confirmation.

The fatty acids with fewer than 12 carbon atoms in the chain are more polar and less hydrophobic than the other fatty acids and they appear to be metabolized quite differently, being absorbed via the intestinal capillaries and the hepatic portal system rather than via the lymphatics.[6-9] It is reasonable, therefore, to suggest exclusion of such short-chain fatty acids from the S term in estimations. This would have little consequence for calculations except in the case of diets specially devised to emphasize the short-chain fatty acids. In the case of butter, its cholesterol-raising effect (independent of the cholesterol in the butter), may be overestimated by around 10 per cent if all saturated fatty acids are included in the term S. Similarly, in the case of coconut oil, inclusion of all saturated fatty acids in the S term may lead to a small overestimation of cholesterol effect.

At the other end of the scale of chain length among saturated fatty acids, only stearic acid is ever present in the diet in considerable amount. Tristearin is very poorly digestible and it would be expected, therefore, that the effect of tri-stearin on cholesterol metabolism might well differ from that of the more usual fatty acid glycerides. But tri-stearin is present in most natural food fats only in trivial amounts and it would seem safe to neglect consideration of it. Triglycerides containing one or two stearic acid molecules are digestible and are less uncommon in food fats but until recently we saw no reason to distinguish between stearic and palmitic acid, for example, in the diet. However, the pursuit of some discrepancies between observation and predictions from $1.35(2\triangle S - \triangle P)$ led to an unexpected picture.

Experiments with Cocoa Butter

In connection with studies on the effect of dietary cholesterol on the serum cholesterol level, it was desired to make comparisons in diets containing highly saturated fatty acids. For this purpose, cocoa butter was a convenient choice to use in the fat mixture for the saturated fat diet. In the outcome, the effect on the blood of the dietary cholesterol was identical in various types of diets (vide supra, Part II), but the serum cholesterol levels did not correspond to expectations when the 2 diets were compared at the same level of cholesterol intake. For example, in experiment AF, the formula $1.35(2\triangle S - \triangle P)$ predicted an average difference of 65 mg./100 ml., but the difference observed

was only 33; this is far too big an average discrepancy for fully controlled experiments on 22 men subsisting on each of the 2 diets in a switch-back design. Similar discrepancies appeared in the AM series in which cocoa butter was also used in one of the diets.

Experiments AF and AM were completely comparable, except for the use of cocoa butter in the diet, with the long series of previous studies which were also conducted in the Metabolic Unit of the Hastings State Hospital with physically healthy, schizophrenic men. Transfer of the Metabolic Unit to the Faribault State School and Hospital provided opportunity to organize new experiments with men of a very different type; these subjects were also physically healthy but were mentally defective rather than psychotic. The FB series of experiments at Faribault also indicated a similar peculiarity in serum cholesterol response.

While these puzzling findings were under scrutiny, Connor et al.[10] published their data from similar experiments with dietary cholesterol and their results were almost identical with those we had obtained, both in regard to the effect of dietary cholesterol and in the unexpectedly low serum cholesterol levels when the diet included substantial amounts of cocoa butter.

While preparations were under way for further experiments designed to provide critical tests of hypotheses to explain the peculiar results when the diet contains cocoa butter, the paper by Erickson et al.[11] appeared. They too used substantial amounts of cocoa butter in the diet and their data are in agreement with the findings here and at Iowa City.

The outstanding peculiarity of cocoa butter, as indicated by its detailed analysis, is its extraordinarily high content of stearic acid; stearic acid makes up an average of 35 to 36 per cent of the total fatty acid in cocoa butter. For comparison, the percentage of stearic acid in other fats averages as follows: mutton tallow, 30; beef tallow, 20; milk fat, 10; lard, 13; and most ordinary vegetable oils 2 to 4. In usual American diets stearic acid seldom accounts for more than 3 per cent of total calories.

Several explanations may be suggested for the serum cholesterol discrepancies observed when cocoa butter was present in the diet. Conceivably, the active ingredient could be something other than stearic acid, and this possibility deserves investigation. But it is useful to consider the possibilities concerning stearic acid.

(1) Perhaps stearic acid has no cholesterol-promoting effect and simply should be omitted in computing S in the formula, $1.35(2\triangle S - \triangle P)$. If stearic acid glyceride is denoted by S″ and $S = S' + S''$, the formulation could be suggested:

(IV, 1) $$\triangle Y = 1.35(2\triangle S' - \triangle P)$$

in which Y = serum cholesterol (mg./100 ml.), and $S' = S - S''$, i.e., the S′ = saturated fatty acids excluding stearic acid.

(2) Stearic acid might have a direct cholesterol-lowering effect, something like that of polyunsaturated fatty acids, in which case the formulation could be:

(IV, 2) $\triangle Y = 1.35(2\triangle S' - \triangle P) - e\triangle S''$, where e is a constant.

(3) Alternatively, it could be suggested that stearic acid interferes with the effects of the other fatty acids on the serum cholesterol. This hypothesis is expressed by the equation:

$$\triangle Y = 1.35(2\triangle S' - \triangle P)(1 - e\triangle S''). \quad \text{(IV, 3)}$$

Conceivable variants on this latter idea would be that stearic acid interferes with the action of S′ or that it potentiates the action of P. Designs are under consideration for experiments to test these different hypotheses critically. In the meantime, there are available many data suitable for statistical analysis to indicate the possibilities, including practical estimation of the results of dietary changes involving stearic acid.

Substantial differences in stearic acid content were not involved in the dietary comparisons we have analyzed previously.[1,2] However, all natural diets contain glycerides in which stearic acid has at least a small representation. Accordingly, it seemed desirable to reanalyze all of the data from controlled experiments in which reliable dietary details are available.

The experiments summarized in table 1, conform to these requirements. In most cases, the fatty acid composition of the diets was determined by gas-liquid chromatography of the total lipid extract from homogenates of the entire diet as eaten. In the other cases, a constant low-fat basic diet was used to which was added experimental fats of known composition, the fatty acid composition of the basic diet being estimated from tables of average food composition. Accordingly, though in some of the experiments there may be questions about the precise amount of stearic acid in the total diet, within any one set of experiments the differences between diets in stearic acid content are accurately known.

Apart from the foregoing reason for making the analysis in terms of differences between diets within the separate sets of experiments, the men and other conditions were not the same in the various sets of experiments, though these variables were constant within each set. The subjects studied by Connor et al.[10] included several controlled diabetics as well as healthy volunteers; the subjects of Erickson et al.[11] were prisoners in a penitentiary. And, as noted earlier, the Minnesota subjects were schizophrenic men in one state hospital and mentally defective men in another.

For table 1, we did not include experiments in which fish oils provided most of the fat calories because of lack of adequately detailed data on chemical composition directly obtained from the diets as eaten. However, in all, table 1, provides data allowing 63 comparisons between the averages of groups of men where the only variable was the dietary situation indicated in the table. It seemed desirable, therefore, to make no assumptions from the previously obtained coefficients, $+2.7\triangle S$ and $-1.3\triangle P$, and to make a new, independent, multiple regression analysis. The following models were analyzed:

$$\triangle Y = b\triangle S' + d\triangle P, \quad \text{(IV, 1a)}$$

$$\triangle Y = b\triangle S' + d\triangle P + e\triangle S'', \quad \text{(IV, 2a)}$$

$$\triangle Y = (b\triangle S' + d\triangle P)(1 - e\triangle S''). \quad \text{(IV, 3a)}$$

Table 1

Line	Reference	N	F	S′	S″	P	Z²	Serum
1	Minn. HWX-LF	13	11.4	3.3	1.4	1.5	94	186
2	Minn. HWX-CT	13	37.0	9.0	1.9	14.8	94	180
3	Minn. HWX-CO	13	37.4	5.6	1.9	16.3	94	160
4	Minn. HYZ-LF	12	11.8	3.4	1.4	1.6	94	181
5	Minn. HYZ-HYD	12	37.6	20.0*	3.8	0.9	94	224
6	Minn. HYZ-CT	12	37.5	9.2	1.9	14.8	94	178
7	Minn. JWX-LF	14	11.3	3.7	1.4	0.8	94	185
8	Minn. JWX-OL	14	37.4	6.0	1.9	2.8	94	188
9	Minn. JWX-SU	14	37.0	6.5	1.9	16.9	94	170
10	Minn. JWX-CO	14	37.6	5.7	1.9	15.4	94	161
11	Minn. JWX-BU	14	36.8	14.4*	3.7	1.9	214	224
12	Minn. JYZ-CT	12	39.2	10.1	1.9	15.2	94	184
13	Minn. JYZ-OL	12	39.3	6.2	1.9	2.9	94	190
14	Minn. JYZ-CO	12	39.0	5.9	1.9	16.6	94	160
15	Minn. JYZ-BU	12	38.8	15.6*	3.9	1.8	230	219
16	Minn. NWY-SA	12	38.4	6.3	1.9	22.8	94	161
17	Minn. NWY-CO	12	38.9	7.6	1.9	17.4	94	159
18	Minn. NXZ-SA	12	38.3	6.3	1.9	22.8	94	157
19	Minn. NXZ-CO	12	38.4	7.4	1.9	17.3	94	162
20	Minn. P-LF	22	9.5	3.3	0.9	1.1	94	159
21	Minn. P-OL	22	36.3	6.7	1.6	3.7	94	171
22	Minn. P-SM	22	18.3	6.5	1.9	3.7	94	171
23	Minn. AM-LCS	22	8.1	3.1	0.7	2.3	17	205
24	Minn. AM-LCF	22	38.9	21.8	6.1	2.5	17	239
25	Minn. AM-HCS	22	8.1	3.1	0.8	2.2	566	219
26	Minn. AM-HCF	22	40.1	21.0	7.1	3.0	545	259
27	Minn. AF-LCF	22	40.6	24.2	5.4	2.6	18	228
28	Minn. AF-LCE	22	40.9	10.0	1.7	15.8	18	194
29	Minn. AF-HCF	22	39.8	23.7	5.7	2.6	525	257
30	Minn. AF-HCE	22	39.6	9.8	1.7	15.4	523	224
31	Minn. FB-SP80	28	32.9	8.9	1.8	12.0	94	211
32	Minn. FB-OS28	28	15.6	3.5	0.9	2.8	94	206
33	Minn. FB-CHO	28	7.0	2.6	0.8	0.9	94	209
34	Minn. FB-OS80	28	33.9	5.7	1.3	6.6	94	206
35	Minn. FB-BU	28	33.0	13.7*	4.0	2.4	210	244
36	Conn. I	5	40.0	9.1	6.9	3.8	265	222
37	Conn. II	5	39.9	7.3	7.6	3.8	0	180
38	Conn. III	5	40.0	6.0	1.4	12.6	0	174
39	Conn. IV	5	39.9	6.9	1.2	12.4	263	202
40	Eric. A	21	40.4	5.5	2.7	13.3	0	193
41	Eric. A+	22	40.4	5.5	2.7	13.3	306	217
42	Eric. B	21	40.5	5.9	2.7	12.9	0	188
43	Eric. B+	19	40.5	5.9	2.7	12.9	306	215
44	Eric. C	21	40.7	7.3	6.2	9.1	0	190
45	Eric. D	20	40.8	7.3	6.2	9.2	0	188
46	Eric. E	20	40.4	10.2	12.7	2.0	0	195

*Saturated fatty acids with fewer than 12 carbon atoms are not included.

Mean values for serum (mg. cholesterol/100 ml.) and for composition of the diet as eaten: mg. cholesterol/1000 Cal. (=Z²), and percentage of total calories provided by total fat (=F), and by glycerides of stearic acid (=S″), of saturated fatty acids other than stearic (=S′), and of polyunsaturated fatty acids (=P). N = number of men. Conn. = Connor et al.[10] Eric. = Erickson et al.[11]

Expansion of equation (IV, 3a) yields an equation with cross-products

$$(IV, 3b) \qquad \triangle Y = b\triangle S' + d\triangle P - eb\triangle S'\triangle S'' - ed\triangle P\,\triangle S'',$$

and no single unique value for the coefficient e can be guaranteed in a linear model. In the actual analysis the equation is

$$(IV, 3c) \qquad \triangle Y = b\triangle S' + d\triangle P - f\,\triangle S'\triangle S'' - g\triangle P\triangle S''.$$

Many of the experiments summarized in table 1, involved no differences in dietary cholesterol, Z^2, but in others account must be taken of Z^2 if valid comparisons are to be made. For this purpose we have used the estimate of dietary cholesterol effect previously obtained, i.e., $\triangle$ Chol. $= 1.5\triangle Z$, when all other conditions are constant. Hence in those comparisons in which $\triangle Z \neq 0$, the term $1.5\triangle Z$ was added to each of the equations above, Z^2 being mg. of cholesterol/1000 Cal.

The coefficients for saturated (less stearate) and for polyunsaturated fatty acid are + and −, respectively, in every solution and the ratio of these 2 coefficients varies only from 1.70 to 2.34, the average being 1.99. This means that the controlling variable must be very close to $2S' - P$. The least-squares solution for the simplest model, equation (IV, 1a), may be written as $\triangle$ Chol. $= 1.1\ (2.05\triangle S' - \triangle P)$. A close approximation, which we propose for general use, is the still simpler form (IV, 1b), $\triangle$ Chol. $= 1.2(2\triangle S' - \triangle P)$. Where $\triangle Z$ is not zero, the value $+1.5\triangle Z$ must be added to any of the above-noted equations, of course.

The improvement resulting from distinguishing between saturates less stearate, S′, and simply saturates, S, may be seen when the comparisons from table 1, are used to calculate the correlation coefficient between $2\triangle S - \triangle P$ and the observed $\triangle$ Chol.: the result is $r = 0.91$ for the Minnesota data and $r = 0.88$ for all 63 sets of comparisons. These values are in contrast with the corresponding values $r = 0.96$ and $r = 0.93$ when the calculation is made with $2\triangle S' - P$. In all of these calculations the observed serum value was corrected by $1.5\triangle Z$ when $\triangle Z$ was not zero. For the 40 sets of comparisons where $\triangle Z = 0$, i.e., no difference in dietary cholesterol was involved, the coefficients of correlation of $\triangle$ Chol. with $2S - P$ and from $2S' - P$ are $r = 0.91$ and $r = 0.96$, respectively.

These improvements in the coefficient of correlation are more consequential than might appear at first glance. With $r = 0.96$, only 8 per cent of the variance remains unexplained but with $r = 0.91$, the unexplained variance is 17 per cent, i.e., more than twice as great. And when $r = 0.88$, variance explained is only 77 per cent of the total. More important from the standpoint of theory is the fact that results in some experiments, e.g., with cocoa butter and ethyl stearate, are predictable from $2S' - P$ but otherwise would be wholly discrepant.

Figure 1 compares the observed values for $\triangle$ Chol. with those predicted from equation (IV, 1c), i.e., $\triangle$ Chol. $= 1.2(2\triangle S' - \triangle P) + 1.5\triangle Z$.

It is instructive to compute the coefficient of correlation between the

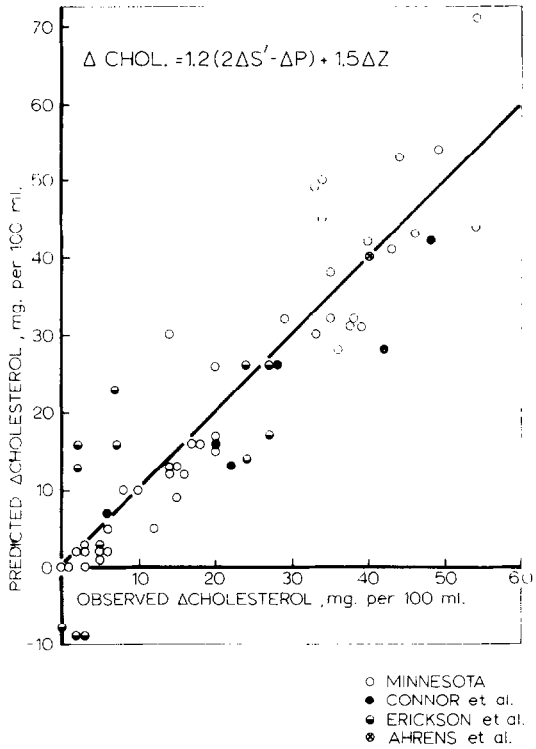

Fig. 1.—Correlation between △ cholesterol observed and the values predicted from $1.2(2\triangle S' - \triangle P) + 1.5\triangle Z$. Each point is the average for a group of men maintained on each of 2 diets. Open circles are Minnesota data, solid circles from Connor et al.,[10] half solid from Erickson et al.,[11] cross from 2 patients fed cocoa butter vs. butter.[12]

observed serum cholesterol differences and the function $\triangle FQ$, where Q is the iodine value and F is percentage of calories from total fats. The result is $r = -0.23$ where $\triangle Z = 0$ and $r = -0.28$ for all comparisons, correcting the observed values by $1.5\triangle Z$ where needed, as in the previous calculations. While these coefficients indicate statistically significant correlations, they account for less than 10 per cent of the variance. If correction for $\triangle Z$ is omitted, the correlation between the iodine value function and △ Chol. is not significant except where $\triangle Z = 0$. But the correlation is much better when no difference in stearic acid is involved, i.e. $\triangle S'' = 0$.

Discussion

The experimental data of Erickson et al.,[11] were included in the present analysis with reservations because of the experimental design. There were 7 groups of 4 to 6 men each and 7 diets were fed but in only 4 experiments so that the cholesterol values on each diet represented different combinations of subjects. In other words, in comparing average values on any different diets about half of the men were the same on the two diets but the other half of the men were different on those two diets and the "treatment effect" is combined

Table 2

(IV, 1a) $\triangle$ Chol. $= a_1\triangle S' + a_2\triangle P$

Source	Diet. Chol.	N	a_1	a_2	a_1/a_2	r	S.E.E.
Minn.	$\triangle Z = 0$	30	2.063	−1.159	1.78	0.97	5.01
	All	45	2.281	−1.269	1.80	0.96	6.66
All	$\triangle Z = 0$	40	1.974	−1.008	1.96	0.94	6.31
	All	63	2.249	−1.098	2.05	0.93	7.58

(IV, 2a) $\triangle$ Chol. $= a_1\triangle S' + a_2\triangle P + a_3\triangle S''$

		N	a_1	a_2	a_3	a_1/a_2	r	S.E.E.
Minn.	$\triangle Z = 0$	30	2.626	−1.199	−2.331	2.19	0.97	4.84
	All	45	3.048	−1.305	−2.960	2.34	0.96	6.39
All	$\triangle Z = 0$	40	2.442	−1.202	−1.583	2.03	0.96	5.05
	All	63	2.762	−1.324	−1.831	2.09	0.95	6.35

(IV, 3c) $\triangle$ Chol. $= a_1\triangle S' + a_2\triangle P + a_3\triangle S'\triangle S'' + a_4\triangle P\triangle S''$

		N	a_1	a_2	a_3	a_4	a_1/a_2	r	S.E.E.
Minn.	$\triangle Z = 0$	30	2.543	−1.299	0.055	0.370	1.96	0.98	3.97
	All	45	2.521	−1.409	0.074	0.270	1.79	0.98	5.22
All	$\triangle Z = 0$	40	1.779	−1.044	−0.067	−0.115	1.70	0.95	5.87
	All	63	2.251	−1.033	0.065	−0.061	2.18	0.94	7.10

Least-squares solutions for equations IV, 1a, IV, 2a, and IV, 3c using the data on all differences between experiments within each of the 12 sets listed in table 1. S.E.E. = standard error of estimate of the regression; r = coefficient of correlation between observed and predicted values.

with interindividual differences. Further, the cholesterol values are reported without indicating confidence limits and had been "adjusted" (for time trends?). Actual observed means and standard deviations were not reported for any of the 7 groups on any of the 7 diets or for the control period.

Proper detailed calculations cannot be from the published data. From the indicated significance of the adjusted means for groupings, it can be inferred that a mean difference of 20 mg. cholesterol/100 ml. serum would be significant at $p = 0.05$ or less in that material. In spite of such limitations, comparisons were made between observed differences and those predicted from $1.2(2\triangle S' - \triangle P) + 1.5\triangle Z$. Twelve sets of differences are available from the material and for these the coefficient of correlation between observed and predicted differences is $r = 0.87$. Treating the observed and predicted $\triangle$ values as 2 estimates of the same thing, the standard error of measurement is S.E.M. $= \pm 7.1$, a value that must be similar to the standard errors between the observed means. It is concluded that the data of Erickson et al.[11] are consistent with the hypothesis that the average serum cholesterol response to a change in lipids in the diet is predictable from $1.2(2\triangle S' - \triangle P) + 1.5\triangle Z$, where S′ does not include stearic acid.

Besides the experiments covered in tables 1 and 2, scattered data in the literature can be examined with the help of table 3, which gives the approximate average composition of important food fats and oils. While different samples of such fats vary considerably, these values provide the basis for rough calculations for the experiments mentioned below, as well as for other purposes.

Ahrens et al.[12] fed patients No. 26 and No. 37 on 40 per cent fat calories, using both ordinary butter (BU) and cocoa butter (CB). Though the BU diet

Table 3

Fat	Q	s′	s″	p	Diet Chol.
Beef tallow	46	32	20	3	90
Butterfat	40	45	9	5	330
Cocoa butter	37	27	35	3	0
Coconut oil	10	72	2	2	0
Beef tallow	46	32	20	3	100
Coconut oil, hydrog.	5	72	8	0	0
Corn oil	130	8	4	56	0
Cottonseed oil	110	23	2	48	0
Lard	70	30	13	10	100
Mustard seed oil	104	4	0.5	18	0
Mutton tallow	42	27	30	4	90
Palm oil	50	49	4	9	0
Peanut butter*	77	14	8	12	0
Peanut oil	92	14	4	22	0
Olive oil	86	8	2	8	0
Rapeseed oil	102	10	1	22	0
Safflower oil	144	9	2	73	0
Sesame oil	116	9	4	42	0
Soybean oil	130	9	4	59	0

*Lightly hydrogenated.

Approximate average characteristics of edible fats and oils. Q = iodine value; Chol. = mg. cholesterol/100 g. s′, s″ and p are percentages of total fat represented by glycerides of, respectively, saturated fatty acids with 12 to 16 carbons in the chain, stearic acid, and polyunsaturated fatty acids.

fat had a slightly higher iodine value and provided less saturated and a little more polyunsaturated fatty acid, the average serum cholesterol was 41 mg./100 ml. *higher* on the BU than on the CB diet. The data are summarized in table 4.

Judging from iodine values, emphasized by Ahrens et al.,[12] it would be expected that the serum cholesterol level would be slightly *lower* on the BU than on the CB diet. And the calculation ignoring dietary cholesterol and stearic acid predicts BU − CB = −3 mg./100 ml. But, as indicated in table 4, prediction from the equations developed in the present paper gives good agreement with the observed difference. From equation IV, 1b, and IV, 3c, (for all 63 sets), the predictions for BU − CB are △ Chol. = 40.8 and △ Chol. = 33.7, respectively.

Ahrens et al.[12] also fed 2 patients, No. 29 and No. 35, corn oil and beef tallow at 40 per cent fat calories and these experiments are interesting because of the high stearic acid content of beef tallow. The serum cholesterol difference observed, tallow diet minus corn oil diet, was △ Chol. = 27, S.E. = ±12 (28 ± 10.9 and 26 ± 13.2). The calculation from,[2] $1.35(2\triangle S - \triangle P)$ would predict △ Chol. = 79 which becomes 89 when allowance is made for 40 mg. of cholesterol/1000 Cal. in the tallow diet ($1.5 \times 6.3 = 9.5$).

The equations developed in this paper also overpredict △ Chol. for the tallow-fed patients but to a much smaller degree: (IV, 1c), △ Chol. = 58; (IV, 1a), △ Chol. = 54; (IV, 3c), △ Chol. = 44. But it must be noted that

Table 4

Diet	I.V.	F	S′	S″	P	Z	Chol.
BU	39.5	40	19.5	3.6	1.6	12.3	245
CB	36.6	40	10.0	14.0	1.2	0	204
△			9.5	−10.4	0.4	12.3	41

Predicted from equations:

IV, 1b $\triangle$ Chol. $= 1.2(19.0 - 0.4) + 1.5(12.3) = 40.8$

IV, 3c $\triangle$ Chol. $= 2.25(9.5) - 1.03(0.4) + 0.06(-10.4)(9.5)$
$-0.06(-10.4)(0.4) + 1.5(12.3) = 33.7$

Means for 2 patients studied by Ahrens et al.[12] on butterfat (BU) and cocoa butter (CB) diets. Symbols as in table 1. I.V. = iodine value. Z^2 = 152 mg. cholesterol/1000 Cal. of diet.

these 2 patients were relatively hypocholesterolemic and adjustment must be made accordingly.[13] If, on the average, these patients had about 72 per cent of the reference serum cholesterol value on a reference diet, i.e., $\pi = 0.72$, the prediction from equation (IV, 1c) would be $\triangle$ Chol. = 28, as will be seen from Keys et al.[13] In any case, allowing for stearic acid in the diet much improves the prediction.

Table 3, suggests that it would be interesting to examine experimental data for diets containing a large amount of hydrogenated coconut oil such as used by Malmros and Wigand.[14] Exact numerical analysis is impossible but it is noted that in their experiments the serum cholesterol levels did not rise significantly when their "free" diet was replaced by a diet in which hydrogenated coconut oil was the only fat and provided 40 per cent of the calories.

A reasonable estimate for an average "free" diet in Malmö, Sweden, is S = 17, S′ = 13, S″ = 3, P = 4 and 250 to 300 mg. cholesterol/1000 Cal., i.e. Z = 17. The hydrogenated coconut oil diet provided 60 per cent of the calories from "bread, cereals, vegetables, potatoes, rice, fruit and sugar" *(op. cit.)* so that the total diet must have been about S = 37, S′ = 28, S″ = 4, P = 3 and Z = 0. Allowing for $\triangle$Z, equation (IV, 1c), predicts $\triangle$ Chol. = 11 mg./100 ml., an expected difference that would be difficult to demonstrate with small groups of subjects.

Finally, we recall Horlick's[15] experiment in which ethyl stearate added to the diet failed to produce a rise in serum cholesterol as expected from the increase in saturation of the dietary fat. This long-puzzling oddity is entirely in keeping with the current analysis.

All available data are consistent with the theory that stearic acid in the diet is not cholesterol-promoting. There is little reason to suggest that some mysterious substance in cocoa butter is responsible for the uniformally surprising result when that fat is a prominent part of the diet. Except for the dominance of stearic acid, there is no obvious peculiarity about the fatty acids in cocoa butter; it contains practically no short-chain fatty acids, and the average composition is palmitic = 25, stearic = 35, oleic = 37, linoleic = 2 per cent according to Eckey;[16] samples analyzed by us are in agreement except that we find more linoleic acid, as high as 8 per cent. The unsaponifiable frac-

tion of cocoa butter ranges only from 0.2 to 1 per cent. If some unknown substance were responsible, a tiny amount must have an extremely powerful action. But the best argument against a special agent in cocoa butter is the fact that other fats besides cocoa butter which are relatively rich in stearic acid—beef tallow and hydrogenated coconut oil—seem to exhibit a similar peculiarity in effect on serum cholesterol.

Generalizations from the analysis of the data in table 1, are properly confined to the range of variation in the diets represented, i.e., from 7 to 41 per cent of calories from total fats. We have little experience with extremely high fat diets but unpublished data obtained by Dr. H. L. Taylor in experiments on students suggest that the relationships shown here extend also to diets as high as 53 per cent in fat calories.

Ahrens et al.[12] reported data from 4 patients who were fed formula diets in which corn oil provided both 40 and 70 per cent fat calories. Patient No. 13 was extraordinary in that his severe hypercholesterolemia was the same on all diets, including variations from 10 to 70 per cent corn oil. The other three patients, Nos. 31, 32, and 34, showed no significant cholesterol response to the extra corn oil, the averages being 165 and 172 mg./100 ml., on 40 and on 70 per cent, respectively, calories from corn oil. Perhaps there is an upper limit for the polyunsaturated fatty acid effect.

The current analysis suggests that the cholesterol-promoting effect of saturated fatty acids in natural human diets is due to lauric, myristic and palmitic acids. Since palmitic acid is much more abundant than lauric and myristic acids in most diets, it may be primarily responsible for the contribution of dietary fat to the serum cholesterol level in man.

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Ancel Keys, Ph.D., Professor, School of Public Health, Director, Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Joseph T. Anderson, Ph.D., Professor, School of Public Health, University of Minnesota, Minneapolis, Minn.

Francisco Grande, M.D., Professor, School of Public Health, University of Minnesota, Director, Jay Phillips Research Laboratory, Mount Sinai Hospital, Minneapolis, Minn.