Special Article

Serum-cholesterol response to dietary cholesterol: a re-evaluation[1-3]

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ABSTRACT The data from the literature in which the serum-cholesterol response has been measured following a change in cholesterol intake have been re-evaluated. The overall data appear to be best explained by exponential equations. However, very large differences in response have been reported for similar changes in cholesterol intake and no predictive equation can explain such values. It is concluded that over the range of cholesterol intakes of practical interest—0 to 400 mg/1000 kcal—the usual response is approximately linear, each 1 mg/1000 kcal resulting in an expected increase of serum cholesterol of ~0.1 mg/dl. With a 2500 kcal diet, an increase in intake of 100 mg/day would be expected to increase serum cholesterol by ~4 mg/dl. *Am J Clin Nutr* 1986;44: 299–305.

KEY WORDS Dietary cholesterol, serum cholesterol

Background

In a recent paper, Keys (1) tabulated the results obtained in 39 apparently adequately designed feeding trials in which the serum-cholesterol response was measured after a change in cholesterol intake. He concludes that the predictive equation developed by Keys et al (2, 3) provides the best possible fit of the data whereas the equation developed from the data of Hegsted et al (4) grossly overestimates the serum-cholesterol response to dietary cholesterol. We have reassessed the data available and conclude that neither of these equations provides a satisfactory prediction of the responses obtained.

The equation of Keys et al (2, 3) was derived from his own data and other values in the literature. Many of the same values were included in his recent evaluation, but more recent reports were also included. The Keys equation is $y = 1.5\ (x_2^{1/2} - x_1^{1/2})$ in which y is the change in serum cholesterol in mg/dl and x_2 and x_1 are the cholesterol content of the two diets being compared, expressed as mg/1000 kcal. The exponent, one-half, was selected by calculating the predicted response with various exponents and concluding that one-half minimized the variance between the predicted and observed responses.

The Hegsted equation was derived from the multiple-regression equation, $\Delta y = 2.16\ \Delta S - 1.65\ \Delta P + 6.77\ \Delta C$, in which Δy is change in serum cholesterol in mg/dl; ΔS and ΔP are changes in intake of saturated and polyunsaturated fatty acids expressed as percentage of calories, and ΔC is change in cholesterol intake expressed as decigrams/2600 kcal. When converted into the same terms as those in the Keys equation, $6.77\ \Delta C$ becomes $\Delta y = 0.176\ (x_1 - x_2)$, which is a linear equation.

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This equation appears to overestimate the response observed when dietary cholesterol alone is changed, but not to the degree that Keys concludes. He believes that the trouble with this equation is that "no high cholesterol diets were used, no comparisons were made with dietary cholesterol as the only variable, and there were only two experiments in which the amount of dietary cholesterol was the primary variable." This is not correct, although it depends, in part, upon the definition of a high-cholesterol diet. There were, in fact, nine diets in which the primary variable was dietary cholesterol. Three different fats—coconut, olive, and safflower oil—were fed with a basal diet that provided 137 mg cholesterol/1000 kcal and, with each of these diets, the eggs in the diet were removed to yield 82 mg/1000 kcal or additional eggs were added to provide 264 mg/1000 kcal. Removal or addition of eggs did cause modest changes in fatty acid content of the diet, but the primary variable was dietary cholesterol. The highest value, 264 mg/1000 kcal, corresponds to about 700 mg cholesterol per subject daily. The serum-cholesterol changes observed are not inconsistent with many values in the literature.

Distribution of variance among independent variables

Contrary to the conclusion of Keys, the overestimation of the Hegsted equation almost certainly lies in the nature of multiple-regression equations, an inherent characteristic that is often overlooked: Such equations do not necessarily distribute the variance appropriately among the independent variables unless these variables are randomly and independently distributed. An examination of the correlation matrix of the Hegsted data reveals modest degrees of correlation, ie, with ΔS and ΔP, $r = 0.59$; with ΔS and ΔC, $r = 0.31$; and with ΔP and ΔC, $r = -0.15$. An r of 0.36 is significant at the 5% level of probability. Just how much such correlations affect the coefficients of S, P, and C is not known but, as was shown in the original paper, elimination of one or another of the variables does affect the size of the regression coefficients. It seems likely that the multiple-regression equation attributes too much of the effect to ΔC and too little to ΔS.

In this regard, it should be noted that when Keys et al (5) developed their original equation to predict the effects of fat composition, $\Delta y = 2.74\ \Delta S - 1.31\ \Delta P$, they ignored the effects of changes in dietary cholesterol. They state that "The low-fat-basal diet provided an average of about 400 mg less cholesterol than the corresponding house diet but variations of twice this amount of cholesterol are without effect on serum cholesterol." It is now known this statement is not true. It is agreed that the effects of dietary cholesterol are small compared to the effects of dietary fat but, whatever the effects of dietary cholesterol were in those studies, their effects are accounted for in the coefficients of ΔS or ΔP, or both. Thus, when the effects of dietary cholesterol, independently determined (3), were simply added to the above multiple regression equation to yield $\Delta y = 1.3\ (2\Delta S - \Delta P) + 1.5\ \Delta Z$), ($\Delta Z = x_1^{1/2} - x_2^{1/2}$), dietary cholesterol effects are apparently included in both parts of the equation. This probably explains some of the differences in the coefficients of ΔS and ΔP in the equations of Hegsted and Keys.

In studies of this kind using natural fats and foods, it is difficult, if not impossible, to design diets in which all variables are independently distributed. Changes in S, for example, will almost invariably be correlated with changes in P. Thus, the coefficients for ΔS and ΔP in either equation may not be very reliable when these are tested alone. This does not necessarily impair the overall predictive capacity of the equations, however, because similar changes will usually occur in practice.

Results reported in the literature

Figure 1 is a plot of the 39 observed values Keys reported from the literature compared with those he predicted by his Minnesota equation (1). With an adequate predictive equation, the predicted and observed values would fall along the line $y = x$ in such a plot. This does not happen, of course, because observed values vary substantially at similar intakes. It is apparent, however, that the values predicted by the Keys equation fall above the

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line with the smaller responses (all but one of the values < 15 are above the line) and fall below the line with greater responses (all but two of the values > 15 are below the line). Thus, this equation does not appear to provide a good prediction of the observed values.

The data considered in this paper are listed in **Table 1.** These include the 39 values tabulated by Keys (1) and the 9 values from the paper of Hegsted et al (4). For reasons that will become apparent, 8 values near the bottom of the table are identified as *low responses* and another 11 values as *relatively high intakes.* The observed responses in serum cholesterol have been plotted against the change in dietary cholesterol in **Figure 2.** The 9 values from Hegsted et al that were not included in Keys' calculations are identified by a different symbol (open circle).

The figure shows clearly that extremely large differences in serum-cholesterol response have been reported at comparable differences in cholesterol intake. For example, increments of dietary cholesterol of 200–250 mg/1000 kcal are reported to cause elevations in serum cholesterol ranging from 4 to 30 mg/dl (Table 1). No equation can satisfactorily describe such variable data. Of particular concern are the eight very low responses, identified in Table 1

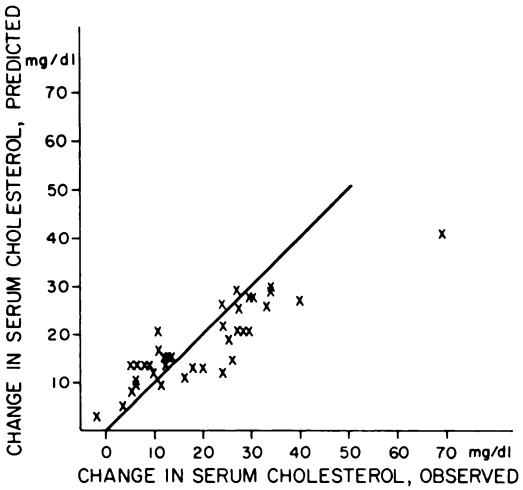

FIG 1. Changes in serum cholesterol predicted by Keys (1) compared to those actually observed. A satisfactory predictive equation should yield values equally distributed around the line, $y = x$, which is shown.

TABLE 1
Reported changes in serum cholesterol at two cholesterol intakes

Reference	Cholesterol levels compared: Low	Cholesterol levels compared: High	Change in dietary cholesterol	Change in serum cholesterol
	mg/1000 kcal	*mg/100 kcal*	*mg/1000 kcal*	*mg/dl*
3	19	200	181	14
3	19	144	125	16
6	5	34	29	4
6	5	55	50	6
6	5	105	100	10
6	5	226	221	25
7	0	300	300	24
7	0	300	300	27
8	0	290	290	33
9	1	92	91	9
9	1	92	91	8
10	0	106	106	13
10	0	212	212	24
10	0	318	318	40
11	36	266	230	12
12	50	292	242	26
13	192	492	300	24
14	94	328	234	18
15	92	343	251	20
16	147	348	201	11
17	0	400	400	34
4*	45	264	219	29
4*	45	264	219	27
4*	45	264	219	22
4*	45	127	83	6
4*	45	127	83	4
4*	45	127	83	11
4*	127	264	137	11
4*	127	264	137	22
4*	127	264	137	25
		(low responses)		
3	200	559	359	13
3	144	559	415	11
3	144	200	56	−2
18	110	309	199	6
14	94	328	234	6
14	94	343	249	4
14	94	563	469	12
16	147	348	201	6
		(relatively high intakes)		
3	19	559	540	27
3	18	523	505	30
3	18	525	507	29
6	5	463	457	34
6	5	891	886	69
6	5	1605	1600	57
19	0	1364	1364	45
13	220	821	601	29
14	94	563	469	28
14	94	563	469	27

* Values not included in Keys' (1) evaluation.

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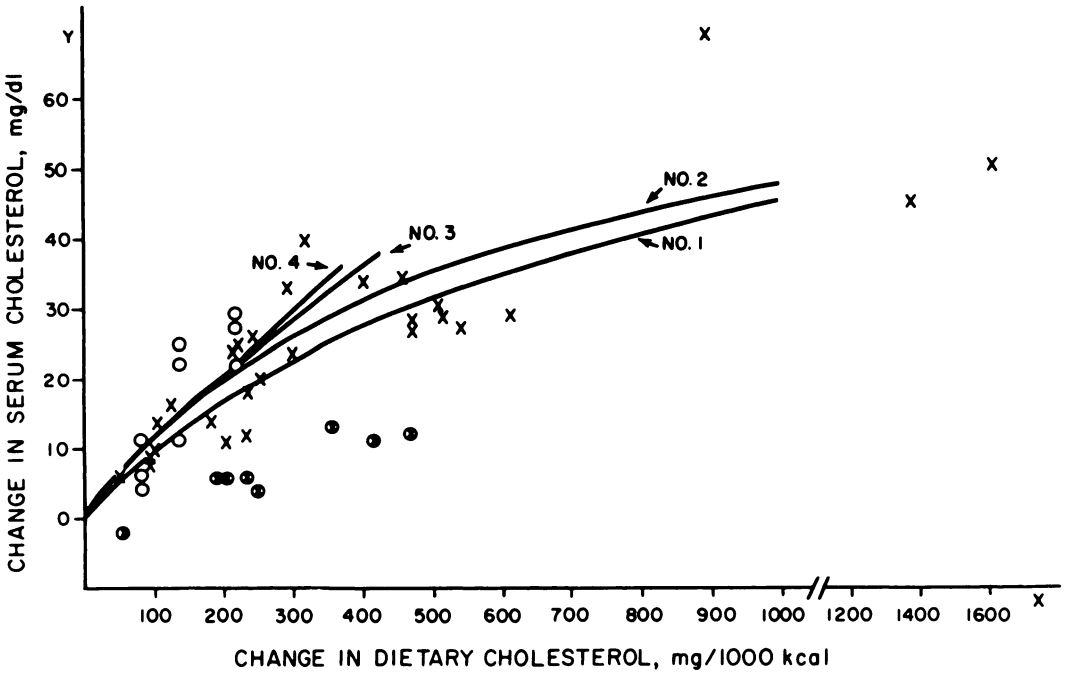

FIG 2. Changes in serum cholesterol versus cholesterol intake reported in the literature (Table 1) with curves predicted by the four equations from Table 2. Values indicated by ⊗ were arbitrarily identified as *low responses* and not included in the calculation of equations 2 to 4. Values indicated by the open circles are those reported by Hegsted et al (4) that were not included in the evaluation by Keys (1).

and on Figure 2. These points, especially, appear to describe more or less a separate family of points. My colleagues and I have searched the original reports to see if we could identify explanations for these apparently aberrant values. No consistent characteristic of these experiments could be found. Bronsgeest-Schoute et al (11, 12) and Schonfeld et al (14) have reported that diets with high P:S ratios attenuated the response to dietary cholesterol. But this effect could not be shown in the studies of Anderson et al (9), Hegsted et al (4), or Connor et al (8). The evidence appears about equally divided and one cannot, apparently, accept or reject the probability that an attenuated response may occur under some conditions.

Some of the studies have been done with crystalline cholesterol, others with eggs or egg yolks. Some have been done with mixed diets, others with formula diets. Samuel and McNamara (20) concluded from their studies on cholesterol absorption that "the rate of cholesterol absorption is critically dependent upon the physiochemical state of the intraluminal contents." Some forms of dietary fiber affect serum-cholesterol levels and bile-acid metabolism (21–23) and it is not unlikely that some dietary constituents or the source of the dietary cholesterol may affect the serum response to dietary cholesterol under some conditions.

Individuals vary in their response to dietary cholesterol. Generally one expects that individuals with higher serum-cholesterol levels will show a greater response to changes in dietary fat or cholesterol than those with lower levels. When small groups are studied, the selection of subjects may greatly influence the response obtained. Also, in the general population, serum-cholesterol levels tend to increase with age to perhaps age 40 yr or so. It would not be surprising if students, for example, were to show a somewhat different response than older adults.

It is generally agreed that, whatever the response to dietary cholesterol may be, response does tend to plateau at some level of intake. This seems fairly clear from the data in Figure

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2, even though one very high cholesterol response has been reported with a dietary increment of over 800 mg cholesterol/1000 kcal. The grouping of values at the 400–600 mg/1000 kcal intake level suggests a plateau near this level where the increase in serum cholesterol was about 30 mg/dl. Thus, one might expect differences in serum response, depending upon the level of dietary cholesterol in the two diets being compared.

Perhaps the most likely cause of the large variation in responses is simply the difficulty of characterizing a change in serum cholesterol accurately. Buzzard et al (16), for example, compared the serum-cholesterol response of two groups to a diet in which ascorbic acid intake was presumably the only variable. The increments in serum cholesterol at 2, 4, and 6 wk were 3.6, 3.5, and 10.3 mg/dl in one group and 7.9, 18.3, and 12.6 mg/dl in the other. Just how one decides what the response is can be a matter of some debate. Bronsgeest-Schoute et al (18) selected 44 subjects and removed all egg from the diet. Of the 44, 16 had an increase in serum cholesterol ranging from near zero to plus 20 mg/dl. The remainder showed decreases ranging from near zero to minus 50 mg/dl.

Whatever the response to eggs may be, it is not likely that removal of eggs actually causes an increase in serum cholesterol. Two bleedings before and after a dietary change do not adequately characterize an individual, although this may be reasonably satisfactory method for characterizing a group if dietary control is adequate. In our laboratory, studies (4) in which two bleedings were analyzed near the end of each dietary period with groups of 9–11 men resulted in a standard error of the mean cholesterol was about 5–6 mg/dl. The standard error of a difference is substantially larger, of course. Thus, it would not be surprising if many of the increments in serum cholesterol that have been reported are in error by 10 mg/dl or so.

Although one apparently cannot identify specific reasons why any particular value in the literature may be atypical, there are obvious opportunities for obtaining aberrant values even in well-designed experiments. I cannot and do not conclude that any particular value is simply wrong, but I do conclude that there is adequate reason to be suspicious of outlying values. No equation can explain outlying values. We are presumably interested in a predictive equation that will best describe the *usual* responses *observed* because this would be the response expected under most conditions.

Predictive equations

Four predecline equations have been applied to data described in this paper. These equations and the data used for calculating the curves in Figure 2 are presented in **Table 2.** The SAS computer system was used for all calculations (24). Table 2 also shows the total sum of squares, the sum of squares accounted for by the regression equation, and the residual sum of squares. The proportion of the sum of squares (percentage) explained by the regression is given in the last column. Many types of curves might be fitted to reported data but it is clear in Figure 2 that the curves derived with the exponential equations characterize much of the biologic data.

The first equation, $y = 51.06 - 54.67 \times e^{-0.00151x}$, is derived from all of the data. This provides quite a good fit and the re-

TABLE 2
Characteristics of predictive equations

Data included	(#)	Equation	Sum of squares			Variance explained by regression
			Total	Regression	Residual	
						%
All data	(1)	$y = 57.08 - 54.67e^{-0.00151X}$	29951	26313	3538	87.9
Less low responses	(2)	$y = 52.29 - 50.73e^{-0.00218X}$	29389	27558	1831	93.8
Less low and all values > 400 mg/1000 kcal	(3)	$y = 93.27 - 93.07^{-0.00121X}$	14123	13333	789	94.4
Same data as for equation 3	(4)	$y = 0.0974X$ (linear)	14123	13303	819	94.2

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gression accounts for 87.9% of the total variance. If the eight apparently low values are excluded, the equation becomes $y = 52.29 - 50.73e^{-0.00218x}$ and the fit is substantially improved, of course. It may be noted that in equations of this type, $y = a - be^{cx}$, the value of a is the asymptotic value, the apparent maximal increase in serum cholesterol at infinite levels of cholesterol intake. This value, of course, is largely determined by the three very high intakes and cannot be well defined.

The upper ends of the curves, however, are of little practical importance. Major interest must lie in the effect of dietary-cholesterol modification within the range of usual intake, say about 0–1 g/day. In a 2500-kcal diet, intake would be 0–400 mg/1000 kcal. The third equation, $y = 93.27 - 93.07e^{-0.00121x}$, represents the data after elimination of all values over 400 mg/1000 kcal as well as the eight *low* values. This provides a good fit, accounting for 94.9% of variance, although the large asymptotic value of 93 indicates that the curve is not appropriate at values above those used to define the curve. Over this particular range of intakes, however, the curve is practically linear and, indeed, the linear equation, $y = 0.097x$, provides an equally satisfactory fit and explains 94.2% of the variance.

Discussion

Exponential equations probably describe the data as well as any that can be developed. Semilog and log-log analyses were also made. These yield nearly linear regression lines, but do not account for as much of the variance as the exponential regression lines. The most appropriate equation will depend upon the level of dietary cholesterol of interest but, because there are few experimental values at very large cholesterol intakes, the serum-cholesterol response to high intakes cannot be well defined. Within the range of practical interest (0–400 mg/1000 kcal) the linear equation appears to be adequate and the spread in the data from which any predictive curve must be developed does not appear to justify further refinements at this time.

When Hegsted et al (4) examined the effects of cholesterol variation alone (Fig 6 of that paper), they estimated that an increase of 100 mg cholesterol per day would increase serum cholesterol by about 5 mg/dl. The linear equation above indicates that an increase in serum cholesterol of about 0.10 mg/dl is expected for each mg/1000 kcal increase in dietary cholesterol. If one assumes a diet of 2500 kcal, then an increase of 100 mg cholesterol/day should yield a serum-cholesterol increase of about 4 mg/dl.

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