Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review[1-3]

Paul N Hopkins

ABSTRACT Attempts to estimate the effects of dietary cholesterol on serum cholesterol by meta-analysis have not previously included baseline together with added dietary cholesterol in a mathematical model. Mean reported changes in serum cholesterol from 27 studies in which controlled diets were supplied by a metabolic kitchen provided 76 data points, each weighted by the number of subjects in nonlinear regression. A good fit to the data ($P < 0.0005$, and $r = 0.617$ between observed and predicted points) was given by the equation $y = 1.22(e^{-0.00384x_0})(1 - e^{-0.00136x})$ where y is the change in serum cholesterol (in mmol/L), x is added dietary cholesterol, and x_0 is baseline dietary cholesterol (both in mg/d). Possible reasons for the hyperbolic shape of the relationship between change in serum cholesterol and added dietary cholesterol, mechanisms for individual responsiveness to dietary cholesterol, and important implications regarding interpretation of prior studies and public health issues are discussed. *Am J Clin Nutr* 1992;55:1060–70.

KEY WORDS Dietary cholesterol, serum cholesterol, serum lipoproteins

Introduction

Investigators have actively attempted to derive a reliable equation to predict the effect of dietary cholesterol on serum cholesterol. If dietary cholesterol does indeed raise serum cholesterol significantly, the public health consequences would obviously be important because each 1% rise in serum cholesterol is predicted to increase the risk of coronary disease by $\approx 2\%$ (1). Most recently, Hegsted (2) reviewed results from a series of studies and proposed a revised predictive equation. The equation, which included each study as a single data point, was $y = 1.47 - 1.41e^{-0.00151x}$, where y is the expected change in serum cholesterol (in mmol/L) and x is the change in dietary cholesterol (in mg/d). This equation reflects what was evident to even the earliest investigators: larger amounts of dietary cholesterol have proportionately smaller effects on serum cholesterol. Alternatively, modest amounts of dietary cholesterol added to a cholesterol-free diet would be expected to most efficiently elevate serum cholesterol. Furthermore, given the form of the Hegsted equation, changes in serum cholesterol would be expected to be minimal if cholesterol was added to a diet already rich in cholesterol. Thus, changes in serum cholesterol when baseline dietary cholesterol intakes were high would be difficult to detect given the wide range of individual responsiveness to dietary cholesterol.

Because diminishing effects of higher dietary cholesterol have long been recognized, it was surprising to find that none of the prior meta-analyses included baseline dietary cholesterol concentrations. These ranged from 0 to well over 300 mg/d. Furthermore, different polyunsaturated to saturated fatty acid ratios (P:S) in these studies had not been examined formally in meta-analysis, and it was not apparent whether analyses had incorporated weighting for the number of subjects in each trial. Accordingly, a reanalysis of cholesterol-feeding trials in humans performed to date was undertaken, incorporating the above variables into the model.

Methods

Data from studies listed in **Table 1** (3–43) were entered into a computer. Initially, studies with completely controlled diets were analyzed separately. Variables shown in the table were included. Only changes in plasma or serum total cholesterol were used in the analysis because many well-conducted early studies had not measured changes in plasma lipoprotein lipids. Data were not available for baseline serum cholesterol concentrations in all studies; therefore, percent change in serum total cholesterol could not always be calculated. Each line of data from Table 1 contributed one data point, which was weighted by the number of subjects in the study. Weighting by the reciprocal of variance was also performed, but, because of incomplete reporting, 28 of the 76 available data points were excluded from studies with controlled diets in which this method was used. Twelve of the 17 studies with uncontrolled diets did not report standard deviations.

The diets used in the available studies varied widely and included formula diets, semipurified solid diets, and diets based on ordinary foods. No attempt was made to distinguish between these kinds of diets, although differential effects are possible. Because detailed descriptions of diet composition were only infrequently

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[3] Address reprint requests to PN Hopkins, University of Utah, Cardiovascular Genetics Research, 410 Chipeta Way, Room 161, Salt Lake City, UT 84108.

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TABLE 1
Effects on serum total cholesterol of adding dietary cholesterol in studies with strict control of dietary intake

Study, year (reference)	Number of subjects*	Dietary cholesterol		Δ Serum total cholesterol	Percent calories from fat	P:S†
		Baseline	Added			
		mg/d	*mg/d*	*mmol/L*	%	
Studies with defined diets						
Beveridge et al, 1960 (3)	6	13	81	0.06 ± 0.47‡	30	0.08
	9	13	140	0.10 ± 0.50	30	0.08
	9	13	280	0.17 ± 0.51	30	0.08
	9	13	621	0.43 ± 0.52	30	0.08
	6	13	1282	0.59 ± 0.36	30	0.08
	10	13	2481	1.20 ± 0.65	30	0.08
	9	13	4490	0.87 ± 0.42	30	0.08
Connor et al, 1961 (4)	2	0	475	1.71 ± 0.48	40	0.76
	2	0	950	1.64 ± 0.02	40	0.76
	2	0	1425	1.99 ± 0.04	40	0.76
Connor et al, 1961 (5)	3	0	2400	1.47 ± 0.94	40	0.88
	1	0	1650	2.43 —	40	0.88
	1	0	1900	2.97 —	40	0.88
	1	0	4800	2.53 —	40	0.88
Wells and Bronte-Stewart, 1963 (6)	3	0	17	0.44 —	15	—
	3	0	42	0.56 —	15	—
	3	0	67	0.66 —	15	—
	3	0	88	0.80 —	15	—
	3	0	142	0.96 —	15	—
	3	0	267	1.03 —	15	—
	3	0	517	1.18 —	15	—
	3	0	1017	1.09 —	15	—
	3	0	1517	1.29 —	15	—
	3	0	3017	1.23 —	15	—
Connor et al, 1964 (7)	6	0	729	1.03 ± 0.49	40	0.25
	5	0	725	0.74 ± 0.39	40	1.7
Steiner et al, 1962 (8)	6	0	3000	1.30 ± 0.58	40	0.68
Erickson et al, 1964 (9)	6	0	742	0.61 —	41	1.6
	6	0	742	0.69 —	41	1.6
Hegsted et al, 1965 (10)	10	116	570	0.75 —	39	5.4
	10	306	380	0.29 —	39	0.05
	10	116	570	0.70 —	39	0.68
Keys et al, 1965 (11)	22	50	470	0.36 —	40	—
	22	50	1410	0.70 —	40	—
	22	50	330	0.41 —	40	—
	22	50	1400	0.80 ± 0.69	40	1.3
	22	50	1410	0.75 ± 0.62	40	0.08
Diet-Heart Study, 1968 (12)	81	126	495	0.12 ± 0.29	30	2.31
	81	126	495	0.27 ± 0.33	39	0.5
	57	401	495	0.32 ± 0.60	40	0.08
	57	154	495	0.18 ± 0.33	40	0.96
Quintão et al, 1971 (13)	4	43	2441	0.96 ± 0.17	40	0.93
	1	43	499	0.88 —	40	0.93
	1	44	197	−0.80 —	40	0.93
	2	53.5	4002	0.13 ± 0.66	40	0.93
Mattson et al, 1972 (14)	14	0	297	0.34 ± 0.31	39	0.31
	14	0	594	0.61 ± 0.23	39	0.31
	14	0	888	1.05 ± 0.29	39	0.31
Anderson et al, 1976 (15)	12	3	291	0.23 ± 0.19	35	0.26
	12	3	291	0.21 ± 0.14	35	4.7
Nestel and Poyser, 1976 (16)	4	210	500	1.56 ± 1.98	40	1.9
	2	257	500	0.25 ± 0.05	40	1.9
	2	334	532	0.76 ± 0.64	40	1.9
	1	103	439	0.67 —	40	1.9
Quintão et al, 1977 (17)	6	0	3250	0.74 ± 1.06	40	0.93

TABLE 1 (Continued)

Study, year (reference)	Number of subjects*	Dietary cholesterol		Δ Serum total cholesterol	Percent calories from fat	P:S†
		Baseline	Added			
		mg/d	*mg/d*	*mmol/L*	%	
Studies with defined diets (continued)						
Bronsgeeste-Sehoute et al, 1979 (18, 19)	20	98	567	0.32 ± 0.30	44	2
	21	98	567	0.25 ± 0.25	44	2
	9	124	607	0.70 ± 0.53	34	0.2
	9	124	607	0.66 ± 0.52	34	0.2
Lin and Connor, 1980 (20)	2	45	1081	2.45 ± 1.90	40	0.8
McMurry et al, 1981 (21)	12	0	600	0.93 ± 0.31	40	0.8
Schonfeld et al, 1982 (22)	11	300	750	0.47 ± 0.39	40	0.32
	9	300	1500	0.72 ± 0.50	40	0.32
	6	300	750	0.13 ± 0.35	40	0.8
	6	300	1500	0.70 ± 0.58	40	0.8
	6	300	750	0.05 —	40	2.5
	6	300	1500	0.26 —	40	2.5
McMurry et al, 1982 (23)	8	0	905	0.88 —	20	0.7
Nestel et al, 1982 (24)	6	200	1500	0.42 ± 0.44	31	1
Maranhão et al, 1983 (25)	13	40	1350	1.19 ± 1.77	40	0.93
Applebaum-Bowden et al, 1984 (26)	9	137	897	0.28 ± 0.48	40	0.82
Beynen and Katan, 1985 (27)	6	114	526	0.25 ± 0.43	42	0.46
Katan et al, 1985 (28)	94	110	500	0.50 ± 0.39	42	0.16
Zanni et al, 1987 (29)	9	130	745	0.58 ± 0.22	31	2.1
	9	130	745	0.39 —	31	0.64
Johnson and Greenland, 1990 (30)	10	200	400	0.26 ± 0.11	30	1.5
Studies with self-selected basal diets (basal cholesterol intakes estimated)						
Slater et al, 1976 (31)	25	314	482	−0.09 ± 0.24	—	—
Kummerow et al, 1977 (32)	21	250	470	0.05 —	40	—
Porter et al, 1977 (33)	55	301	235	0.16 —	38	—
	59	301	235	0.03 —	38	—
Flynn et al, 1977 (34)	56	260	540	0.49 —	38	—
	60	260	540	0.00 —	38	—
Mistry et al, 1981 (35)	37	522	1500	0.75 —	41	—
	14	480	750	0.62 —	41	—
Roberts et al, 1981 (36)	16	196	532	0.40 ± 0.29	40	—
Packard et al, 1983 (37)	7	180	1290	1.47 ± 0.69	38	0.17
Oh and Miller, 1985 (38)	21	474	654	0.27 —	35	0.62
Beynen and Katan, 1985 (39)	6	207	1596	0.48 ± 0.48	46	0.5
	6	207	1596	0.61 ± 0.43	46	0.5
Edington et al, 1987 (40)	33	120	188	0.13 —	26	0.8
	135	120	188	0.12 —	35	0.6
McNamara et al, 1987 (41)	39	192	628	0.16 —	35	1.45
	36	288	575	0.13 —	35	0.27
Kestin et al, 1989 (42)	10	180	686	−0.02 ± 0.56	41	0.37
	15	204	735	0.04 ± 0.44	36	0.85
Clifton et al, 1990 (43)						
Normocholesterolemic control subjects	11	185	681	0.06 ± 0.47	29	0.6
Hypercholesterolemic diet-insensitive	22	185	681	0.19 ± 0.47	29	0.6
Hypercholesterolemic diet-sensitive	23	185	681	0.36 ± 0.37	29	0.6

* All subjects received both amounts of dietary cholesterol listed without changes in other dietary constituents (from refs 3–42).
† Ratio of polyunsaturated to saturated fatty acids in diet.
‡ $\bar{x} \pm$ SD.

available, potential effects of dietary protein, complex carbohydrate, sugars, or fibers were not analyzed. Studies that changed factors other than dietary cholesterol were not included, however. The most consistently reported descriptions of diet composition were total percent calories from fat and the P:S (Table 1).

Rationale for the equation used in nonlinear regression is illustrated in **Figure 1.** When baseline dietary cholesterol is equal to 0, the response to added dietary cholesterol is predicted by a curve with form $y = A(1 - e^{-bx})$, where y is the change in serum total cholesterol concentration, A is an estimated asymptote

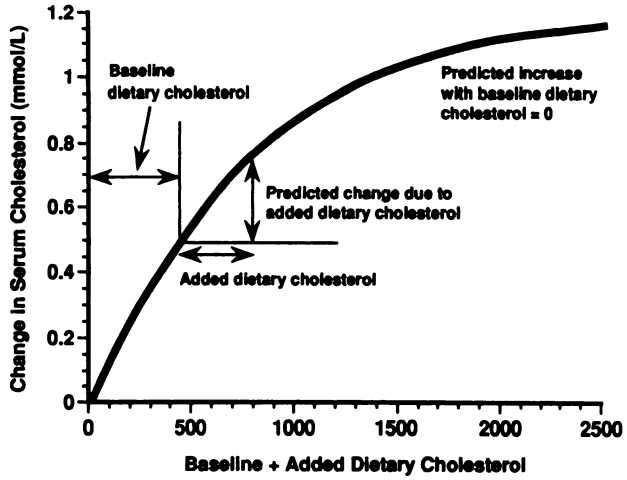

FIG 1. Model for the effect of baseline dietary cholesterol (in mg/d). Baseline dietary cholesterol is predicted to have an effect equivalent to moving the origin of the axes up along the line of predicted increase where baseline dietary cholesterol is 0.

representing maximal cholesterol change, x is added dietary cholesterol, and b is an estimated coefficient. If baseline cholesterol is > 0, then the effects of added dietary cholesterol should be diminished by the height of the curve at the point of baseline dietary cholesterol, as illustrated. That is

$$y = A[(1 - e^{-b(x_0+x)}) - (1 - e^{-bx_0})],$$

which simplifies to

$$y = A(e^{-bx_0})(1 - e^{-bx})$$

where x_0 is baseline dietary cholesterol. In this model therefore, adding baseline dietary cholesterol effectively acts to decrease the asymptote A at an exponential rate.

Parameters of the equation $A(\exp^{-b_0x_0})(1 - \exp^{-bx})$ were estimated by using the *SAS* (SAS Institute Inc, Cary, NC) nonlinear-regression procedure. The Gauss-Newton method of iteration was used to determine the equation parameters that give the minimum sum of squared deviations. The overall significance of the regression is provided by the F statistic, which is equal to the mean squares explained by regression divided by the residual mean squares (as in ordinary multivariable linear regression). The coefficients b_0 and b were tested separately (though in the original equation they would have been defined as equivalent) to test the contribution of baseline dietary cholesterol to the overall prediction; 95% confidence intervals were calculated by using the asymptotic standard error. The significance of the difference between the correlation coefficient for predicted vs observed change in serum cholesterol from the new model compared with a simple exponential model (without baseline cholesterol) was calculated by using a Z statistic for comparison of correlation coefficients derived from a single set of observations (44).

Results

In nonlinear-regression analysis in which only those studies with completely controlled diets (n = 76 data points, studies weighted by number of participants) were used, baseline dietary cholesterol was a statistically stronger predictor of change in plasma cholesterol than was added dietary cholesterol. Obviously, no change would be predicted if no dietary cholesterol was added. Nevertheless, the regression coefficient (b_0) for baseline dietary cholesterol was 0.00384 (95% CI 0.00186–0.00581) whereas that for added dietary cholesterol was 0.00136 (0.000674–0.00204). The asymptote A had a value of 1.22 mmol/L (0.94–1.51) and the model was statistically highly significant overall (R^2 = 0.812, $F_{[3,73]}$ = 105, $P < 0.0005$). Calculating b_0 and b separately rather than as a single parameter b resulted in a better fit to the data.

Thus, when baseline dietary cholesterol was increased, added dietary cholesterol resulted in diminishing increments of serum total cholesterol. This relationship is shown in **Figure 2**—a series of curves showing predicted changes in serum cholesterol vs added dietary cholesterol with a variety of baselines for dietary cholesterol. The correlation between predicted serum cholesterol changes (when the above equation was used) and observed changes from Table 1 was r = 0.617. The use of only the simple exponential without baseline dietary cholesterol resulted in a predicted to observed correlation of r = 0.477, not as good a fit to the observed changes in serum cholesterol ($P < 0.001$ for the difference between the r values). The hyperbolic relationship between added dietary cholesterol and change in serum total cholesterol is well illustrated in **Figure 3** (only studies with three or more participants eating controlled diets, starting with a baseline dietary cholesterol of ≤ 50 mg/d or less, are included). The effects of increasing baseline dietary cholesterol in similar studies is shown in **Figure 4.**

An alternative method of weighting the studies uses the inverse of variance. Because 28 data points were lost, estimates of parameters using this method were less stable. Thus, the asymptote A became 3.64 mmol/L (−1.42 −8.71), b_0 was 0.0186 (0.0126–0.0246), and b_2 was 0.00333 (−0.00833–0.0150); overall $F_{[3,45]}$ = 94.3, and R^2 = 0.86. These results are qualitatively similar to the above analysis in that baseline dietary cholesterol remained a highly significant predictor of serum cholesterol change though the other parameters did become nonsignificant.

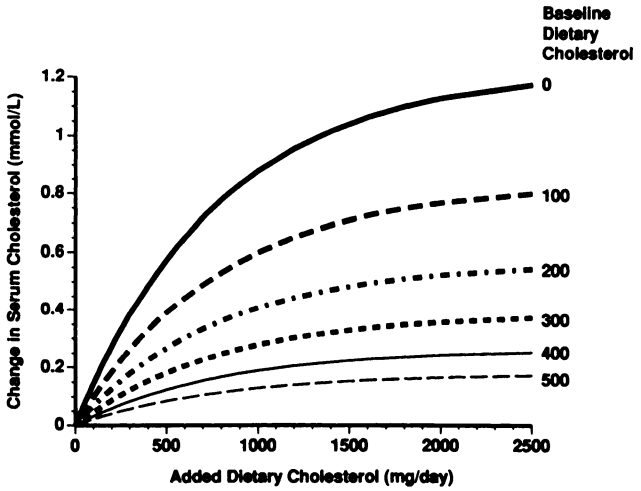

FIG 2. Effects of added dietary cholesterol on serum total cholesterol. Included in the nonlinear regression is baseline as well as added dietary cholesterol. At moderate dietary cholesterol intakes, little additional change in serum cholesterol would be expected.

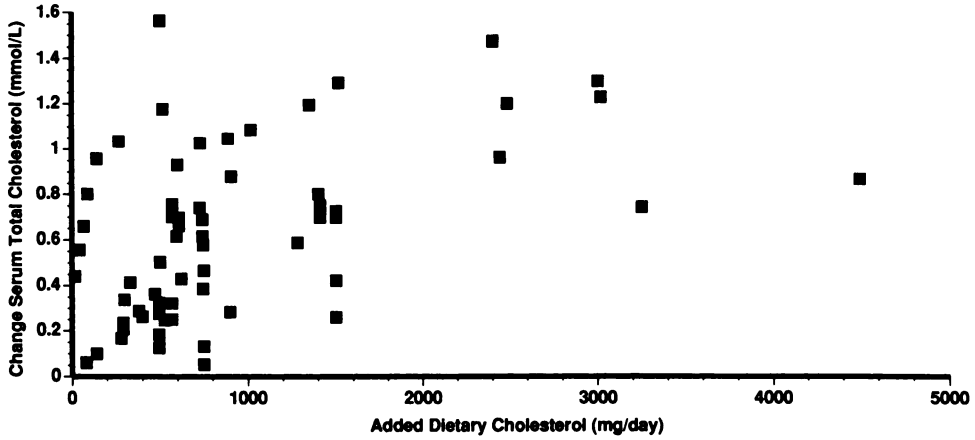

FIG 3. Mean change in serum total cholesterol vs added dietary cholesterol in studies with three or more participants and with baseline dietary cholesterol ≤ 50 mg/d where diets were controlled by a metabolic kitchen.

Inclusion of the 13 studies with self-selected diets into the regression did not materially affect the results. The estimate for the asymptote A became 1.30 mmol/L (0.91–1.69) when the number of subjects in each study was used as the weighting factor; the coefficients for baseline and added dietary cholesterol became 0.00249 (0.00120–0.00378) and 0.000958 (0.000436–0.00148). Most important, the overall significance of the regression did not change ($F_{[3,90]} = 95$, $R^2 = 0.75$) and the correlation between predicted and observed change in serum cholesterol was nearly identical ($r = 0.60$).

The predicted responses to added dietary cholesterol shown in Figure 2 represent absolute changes in total cholesterol (in mmol/L). Most of the studies were performed in normal subjects with serum cholesterol concentrations of ≈5.17 mmol/L. Percent changes of ≈12–15% are therefore predicted when 500 mg cholesterol is added to a cholesterol-free diet.

In a separate set of analyses using a multiple logistic equation rather than an exponential, similar results were obtained. The model used for these analyses was

$$\text{Change} = \frac{A}{1 + \exp(-\beta_0 - \beta_1 \text{ base} - \beta_2 \text{ added} - \beta_3 \text{ P:S})}$$

where change is the change in serum total cholesterol (mmol/L), A is the asymptotic maximum change (mmol/L), base is the baseline dietary cholesterol (mg/d), and added is added dietary cholesterol (mg/d). Estimated regression parameters were A = 1.09 mmol/L (0.81–1.38), $\beta_0 = -1.12$ (−2.00 to −0.234), $\beta_1 = -0.00488$ (−0.00893 to −0.000824), $\beta_2 = 0.00251$ (0.000866–0.00415), and $\beta_3 = -0.320$ (−0.719 to 0.0793), with $F_{[5,58]} = 48$ and $R^2 = 0.805$. Thus P:S was not found to be a significant predictor of change while baseline and added dietary cholesterol remained significant. Total fat did not contribute to the prediction of response to dietary cholesterol (data not shown).

Although P:S did not predict response to dietary cholesterol in this metaanalysis, some of the largest and best-designed studies specifically designed to examine this potential interaction did find reduced responses to dietary cholesterol when P:S was high (12, 18, 19, 22, 29). Other seemingly well-designed studies did not confirm this interaction (7, 10, 11, 15, 42). The studies that did not report a significant difference in response to dietary cholesterol when background diets with high and low P-S ratios were compared tended to be those in which the diets used at baseline were low in cholesterol (range 0–200 vs 124–300 mg/d). Possibly, addition of saturated fatty acids to the background diet exaggerates the response to added dietary cholesterol by shifting the response curve to appear more like that seen with lower baseline dietary cholesterol. When the baseline dietary cholesterol diet is very low or 0, this effect may not be observed at all. Nevertheless, when interaction terms such as baseline dietary cholesterol × P:S) were incorporated into the mathematical models presented here (using either all studies or only those with different P-S ratios incorporated into their design), little (nonsignificant) improvement in prediction occurred. Differences in study design or simply the small number of available data points may have obscured the potential interaction.

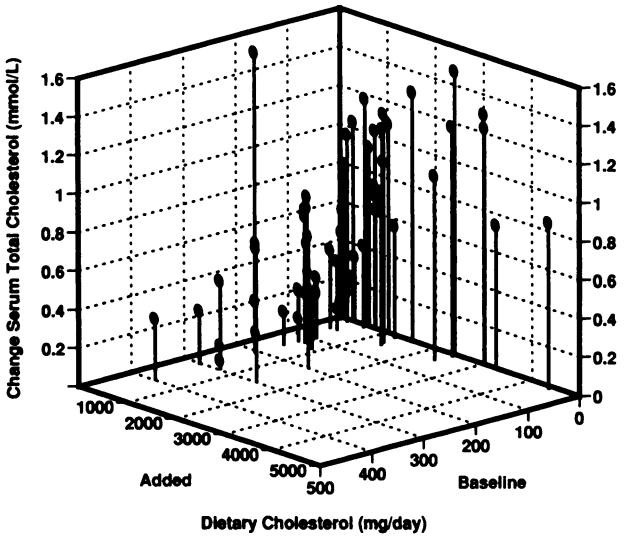

FIG 4. Mean change in serum total cholesterol vs added and baseline dietary cholesterol in studies with three or more participants where diets were controlled by a metabolic kitchen.

Discussion

The studies reviewed here used a variety of baseline diets (formula or natural foods, egg-yolk cholesterol vs purified cholesterol), subtly different study designs, and, of course, different

subjects. Nevertheless, responses to added dietary cholesterol were sufficiently consistent to provide statistically significant and physiologically meaningful findings. The results illustrated in Figure 2 provide important insights. When modest amounts of cholesterol are added to the daily diet, the major predictor of change in serum cholesterol is baseline dietary cholesterol. Thus, when one or two eggs are added to a diet that is typical for the average American (containing ≈400 mg/d), little change would be expected. This is precisely the study design of a number of outpatient studies in which there is little or no control of the diet, either before or after addition of eggs (31–34, 38, 40, 41). Such experimental designs cannot prevent either deliberate or unconscious changes in the intake of other cholesterol-containing foods or other changes in the diet after the addition of eggs. Nevertheless, even the inclusion of these studies into the analysis did not alter the prediction illustrated in Figure 2.

One study in persons with self-selected diets used 118 mL whole eggs or a cholesterol-free egg substitute (Eggbeaters, Nabisco Brands, Inc, East Hanover, NJ) added to the daily diet of 16 normal volunteers for 4 wk in a double-blinded, cross-over design. Mean serum cholesterol was reduced from 6.28 ± 1.01 mmol/L ($\bar{x}$ ± SD) on the whole egg diet to 5.66 ± 1.14 mmol/L with egg substitutes ($P < 0.01$), a 10% decrease (36). Significant but lesser changes in serum cholesterol were also noted in another well-controlled study in which dietary supplements containing different amounts of cholesterol were added to a closely supervised but self-selected low-fat, relatively low-cholesterol diet (43). Thus, well-designed studies find significant differences attributable to dietary cholesterol in outpatients even when the remainder of the diet is self-selected, suggesting the practicality of using cholesterol-restricted diets to lower serum cholesterol.

Sources of individual variability

One of the more striking features of the data in Table 1 is the large standard deviations of reported change in serum total cholesterol. Not surprisingly, studies with few participants may show strikingly different standard deviations, simply by random inclusion of responsive and unresponsive individuals (*see* results for reference 4, Table 1). Potential sources of the large variances include genetic and prior environmental influences as well as inadequate dietary control. About 50% of the population variance of serum cholesterol can be attributed to genetic factors. Furthermore, genetic influence appears to affect response to diet independently from baseline serum cholesterol concentrations (45). By observing changes in serum cholesterol vs changes in diet, some individual variability can be reduced as the baseline serum cholesterol is subtracted out (46, 47). Not surprisingly, no correlation was found in cross-sectional analyses comparing dietary cholesterol estimated by questionnaire or interview and serum cholesterol in populations from Framingham, MA (48) or Tecumseh, MI (49). Any measurable relationship between diet and serum cholesterol was undoubtedly obscured by individual variability as well as relatively high cholesterol intakes (46, 50).

In remarkable contrast to the Framingham and Tecumseh populations, the Tarahumara Indians of Mexico consume little cholesterol, but intakes are sufficiently different between individuals to result in a distinct relationship between dietary and serum cholesterol. Those who do consume cholesterol eat relatively small amounts because of the sparse supply of eggs and chicken (dietary cholesterol concentrations ranged from 0 to only 160 mg/d). The correlation between dietary cholesterol and serum cholesterol was striking ($r = 0.90$, $P < 0.01$) (51). Greater genetic heterogeneity with regard to response to dietary cholesterol might obscure any similar correlation in other populations. Further investigations in this remarkable tribe, cited in Table 1 (23), document remarkable sensitivity to added dietary cholesterol. Addition of dietary cholesterol led to a 30% increase in total cholesterol (from a baseline of 113 mmol/L) and a 31% increase in low-density-lipoprotein (LDL) cholesterol. These observations suggest that persons who are accustomed to a very-low cholesterol diet may be more sensitive to dietary changes. This is further confirmation of the concept illustrated in Figure 2.

Reported dietary intakes are notoriously subject to large day-to-day variation and reporting error (50). Even participants carefully trained in diet reporting varied their cholesterol intake by > 100% day to day (eg, from 50 to 250 mg/d) while on a reportedly very-low cholesterol diet (52). Although averaging daily cholesterol intakes for 9–10 d may decrease calculated intraindividual variation, such a statistical slight-of-hand (41, 52) does not negate the reality of large percent changes in daily dietary intake when diets are not controlled by a metabolic kitchen. Because these variations are within the range of large baseline effects depicted in Figure 2, false-negative results in cholesterol-feeding studies using uncontrolled diets can be anticipated.

Marked differences in individual responsiveness have been documented in animals for many years. Responders and nonresponders have been selectively bred in rabbits, squirrel monkeys, rhesus monkeys, cynomolgus macaques, and baboons (53), clearly showing that at least a large portion of dietary responsiveness to cholesterol feeding is genetically mediated. In humans, individual differences in response to added dietary cholesterol may be marked. Within a single study, responses to added dietary cholesterol may range from essentially 0 to increases in serum total cholesterol of > 100% (13, 16). In other studies, when normal healthy volunteers were changed from a moderately high to a near-zero cholesterol diet, serum cholesterol concentrations quite predictably fell (7, 54).

Some investigators report relatively stable differences in response to dietary cholesterol after retesting normal volunteers after ≈1 y (28, 55). Furthermore, serum cholesterol responses to dietary cholesterol and to saturated fatty acids seem to correlate with each other (43, 56). Associations between apo E phenotype and responsiveness to dietary cholesterol were reported by Finnish investigators (57, 58), but these findings were not confirmed by an Australian group (43).

Animal studies suggest differences in cholesterol absorption efficiency and conversion of cholesterol to bile acids as major determinants of hypo- and hyperresponsiveness to dietary cholesterol (59–62). In humans, increased intestinal sterol absorption efficiency (using plasma concentrations of plant sterols as indirect markers) may also play an important role in determining higher serum cholesterol concentrations (63). Indeed, increased sterol absorption efficiency, evidenced by elevated plasma plant sterols, may be an inherited trait associated not only with higher serum cholesterol concentrations but with increased risk of coronary artery disease (64). Cholesterol absorption efficiency was correlated positively with LDL cholesterol concentration and inversely with LDL fractional catabolic rate in other studies (57, 65).

In two studies (35, 41) an inverse correlation after cholesterol feeding was reported between change in serum LDL cholesterol

and change in freshly isolated mononuclear cell HMG-CoA reductase activity or cell sterol synthesis. Ability to down-regulate HMG-CoA reductase may mitigate the elevation of LDL cholesterol because of added dietary cholesterol. Although some evidence suggests this mechanism as a means of minimizing effects of dietary cholesterol for at least for some individuals (66), in other studies no measure of whole body cholesterol balance or synthesis accurately predicted serum cholesterol response (13, 17, 25).

Hypothetical model of responsiveness to dietary cholesterol

The changes in plasma cholesterol documented in Table 1 primarily reflect changes in plasma LDL cholesterol. In those studies that examined lipoprotein concentrations rather than simply total cholesterol and triglycerides, increases in LDL cholesterol accounted for 80–90% of the increase in serum total cholesterol (22, 24, 29, 39, 42, 43). High-density lipoprotein (HDL) was consistently increased in most studies, with HDL_2 increasing more than HDL_3 after cholesterol consumption (42, 43). Interestingly, the increase in serum HDL cholesterol resulting from cholesterol feeding appears to be greater when the background diet is high in polyunsaturated fatty acids (29). Although serum triglycerides were generally not affected, increases in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) cholesterol concentrations may result from cholesterol feeding (39, 41). Retention of VLDLs in heparin affinity columns (a measure of VLDL-remnant accumulation) also increased after high-cholesterol diets (24). These subtle changes in lipoprotein composition and concentration—apart from changes in serum total or LDL cholesterol concentrations—may help explain recent epidemiologic findings that implicate dietary cholesterol as an independent risk factor for coronary disease after fasting serum total cholesterol and other known cardiovascular risk factors have been controlled for (67, 68). However, because changes in LDL cholesterol accounted for most of the changes in total cholesterol, LDL cholesterol will be the focus of the remaining discussion.

Most human kinetic studies of cholesterol feeding demonstrate decreased LDL removal (or fraction catabolic rate) with little or no effect on LDL synthesis (57, 69–71). Down-regulation of LDL receptors may increase apparent LDL production if VLDL conversion to LDL is enhanced. Only regulated, LDL-receptor mediated transport of LDL seems to be depressed by cholesterol feeding (37). Elevations of serum LDL cholesterol concentrations induced by high-cholesterol diets were accompanied by marked (41–74%) reductions in LDL degradation by freshly isolated mononuclear cells (26, 35, 41). Interestingly, failure to respond to dietary cholesterol when it was increased from 300 to 1000 mg/d was explained by the lack of change in LDL kinetic parameters in a study of five volunteers selected for unresponsiveness to dietary cholesterol (72). Thus, for added dietary cholesterol to produce measurable effects on serum LDL cholesterol, changes in LDL kinetic parameters must occur. During cholesterol feeding, cholesterol concentrations in the liver or other tissues may increase, whole-body cholesterol balance may become positive, or bile sterol excretion may change, but unless LDL synthesis is increased or removal decreased, serum LDL cholesterol will not rise. Because control of the LDL receptor seems to be the primary determinant of serum LDL response to dietary cholesterol, attention will be focused on cellular cholesterol homeostasis and accompanying changes in LDL receptor activity.

Cellular cholesterol stores are regulated by homeostatic mechanisms that safeguard an adequate supply of cholesterol for cellular needs. In any regulated system, however, upper and lower limits to homeostatis exist. In a remarkable series of studies, human liver was obtained from biopsies of patients undergoing cholecystectomy. In hepatocytes thus obtained, cholesterol supply appeared to be regulated preferentially by modulation of HMG-CoA reductase activity with lesser percentage changes seen in LDL-receptor activity after cholesterol homeostasis was challenged by cholestyramine, bile acid feeding, or pravastatin (an HMG-CoA reductase inhibitor) (73–75). Increases in HMG-CoA reductase activity so effectively defended intracellular cholesterol that only after HMG-CoA reductase inhibition did cellular cholesterol concentrations fall measurably (75). Simultaneous up-regulation of LDL receptors accounted for the reduction of serum LDL cholesterol. Less effective means of depleting cholesterol stores (cholestyramine administration) resulted in a threefold induction of LDL receptors and reduced serum LDL cholesterol, but no change in hepatic cholesterol stores because HMG-CoA activity increased more than fivefold (74). LDL-receptor binding and HMG-CoA reductase activity were directly and linearly correlated ($P < 0.01$ in 13 subjects), suggesting coordinate regulation of these two regulatory mechanisms.

Contrary to the abundant reserve for cholesterol repletion, compensation for excessive exogenous cholesterol appears to be limited. Thus, in liver biopsies from volunteers being fed 3100–3400 mg cholesterol/d there was a 62% increase in cholesterol content while serum cholesterol increased only 19% (15). Among 63 middle-aged men, whole-body-cholesterol synthesis was in-

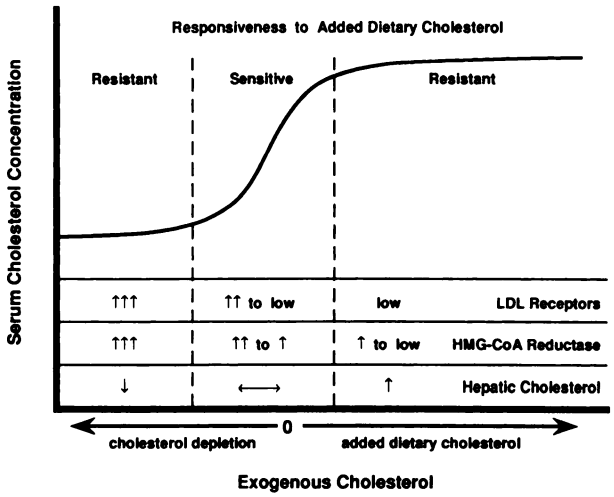

FIG 5. Hypothetical model to explain serum (total or LDL) cholesterol responses to added dietary cholesterol. Individuals may be resistant to added dietary cholesterol either because hepatic cholesterol is sufficiently low that exogenous cholesterol only decreases de novo synthesis but does not affect LDL receptors (presumably less common), or because hepatic cholesterol stores are already high enough to maximally suppress the major homeostatic mechanisms replenishing intracellular stores. Further addition of exogenous cholesterol to hepatic stores would not affect serum cholesterol in this latter (more common) scenario because LDL receptors would already be maximally down-regulated and production of lipoproteins would not be expected to increase.

versely associated with cholesterol absorption. In this whole-body-cholesterol balance study, essentially complete inhibition of cholesterol synthesis was predicted at just 500 mg/d of absorbed dietary cholesterol (for a 70-kg man). Percent absorption of cholesterol ranged from 25 to 74%, with a mean of 47% (65). Neutral sterol excretion is increased by cholesterol feeding (13). Also, prolonged cholesterol feeding may result in increased bile acid synthesis in man (20, 76), although shorter-term cholesterol-balance studies did not confirm this effect (13, 77). Nevertheless, these mechanisms do not usually compensate completely for increased dietary cholesterol in most individuals and in some, total-body cholesterol accumulation can be considerable (13). It would appear, then, that cholesterol needs in humans are rather easily sated.

A hypothetical model incorporating the above considerations is presented in **Figure 5.** Serum cholesterol concentration for a given individual is depicted as a function of exogenous cholesterol (positive for dietary cholesterol, negative for cholestyramine or other bile acid–and cholesterol-binding agents). The range of serum cholesterol concentration reflecting only modulation of LDL-receptor activity is considered (LDL synthesis constant). If for any combination of reasons (low cholesterol absorption efficiency, increased conversion of cholesterol to bile acids or removal as neutral sterols, or increased transport of cholesterol out of the liver as VLDL lipoproteins) an individual had relatively high requirements for hepatic cholesterol, then HMG-CoA reductase activity would presumably be high. In this situation, added dietary cholesterol may act mainly to down-regulate HMG-CoA reductase with little effect on LDL-receptor activity and essentially no change in serum LDL cholesterol concentrations. (Above considerations suggest, however, that LDL receptors may begin to be down-regulated together with HMG-CoA reductase. In this case, the resistant phase depicted in the left portion of Figure 5 may not be observed.) As hepatic cholesterol stores were further replenished by increasing dietary cholesterol, down-regulation of hepatic LDL receptors would begin to occur and responsiveness to dietary cholesterol would begin to be apparent. At the same time, modulation of HMG-CoA reductase and possibly increased bile acid synthesis would continue to blunt changes in LDL-receptor activity as dietary cholesterol increased. If the exogenous cholesterol input was great enough to exceed cellular output, then even if mechanisms of cholesterol replenishment were completely suppressed, hepatic cholesterol content would begin to rise. In this scenario, where hepatic cholesterol stores were sufficiently high to maximally suppress hepatic HMG-CoA reductase activity and LDL receptors, added dietary cholesterol would not be expected to increase serum cholesterol further.

The hyperbolic shape of the response curves seen in studies to date (Figure 3) suggests hepatic cholesterol overload as the primary basis for diminishing responsiveness to increasing loads of dietary cholesterol. Thus, most persons appear to be near the middle or toward the upper shoulder of the S-shaped curve depicted in Figure 5. This finding is also consistent with the excellent responses most diet-resistant patients experience with lipid-lowering drugs and may help explain the absence of any clear lower threshold in studies shown in Figure 3. Occasionally, persons may have inherently low LDL concentrations with very-low-cholesterol absorption efficiency and high bile acid synthetic rates, which may help compensate for even enormous cholesterol intakes (76). However, if the majority of people were unresponsive to increasing dietary cholesterol primarily because of effective feedback inhibition of cholesterol synthesis or ability to upregulate bile acid synthesis, then the expected shape of the response curve would be linear or exponentially increasing rather than hyperbolic.

This hypothesis predicts that many apparently resistant individuals would become sensitive to dietary cholesterol after effective cholesterol depletion with either a near-zero cholesterol diet or bile acid sequestrants. Furthermore, persons whose serum cholesterol concentrations are apparently resistant to dietary cholesterol may experience increased hepatic or other body cholesterol stores with further cholesterol loading. Paradoxically, greater hepatic cholesterol stores are seen in cholesterol-fed animals when the baseline diet is supplemented with polyunsaturated rather than saturated fatty acids (78, 79). This was observed even though LDL-receptor activity was enhanced and serum LDL cholesterol was lower on the polyunsaturated fatty acid-enriched baseline diet (79). Increased biliary neutral sterols and bile acid excretion by human subjects on polyunsaturated fatty acid-enriched diets (80, 81) may also reflect increased hepatic cholesterol stores. Higher hepatic stores of cholesterol, despite lower serum cholesterol concentrations, might explain the relative insensitivity to dietary cholesterol when baseline diets were high in polyunsaturated fatty acids (*see* above). This effect would be exaggerated if baseline dietary cholesterol intakes were already high enough to cause near maximal supression of cholesterol synthesis and LDL-receptor activity. Further investigations to pursue these possibilities are needed.

Increased hepatic cholesterol stores may stimulate net VLDL secretion. In rats, very-high-cholesterol diets (up to 2%) led to marked hepatic cholesterol retention. VLDL protein secretion from perfused liver was stimulated up to 100% compared with the 0-cholesterol diet. Near-maximal stimulation of VLDL secretion was already achieved with the 0.25% cholesterol diet (82). A plausible mechanism involves down-regulation of LDL receptors. Thus, net secretion of apolipoprotein B from HepG2 cells was reduced when increased LDL receptor activity (among other factors) resulted in more rapid reuptake of nascent VLDL immediately after its export across the plasma membrane (83, 84). This may help explain the apparent reduction of VLDL apolipoprotein B secretion observed in human subjects with moderate hypercholesterolemia treated with pravastatin (85). Modulation of net VLDL secretion by changes in LDL-receptor activity would not change the conclusions reached in the foregoing discussion.

Summary

Serum cholesterol concentration is clearly increased by added dietary cholesterol but the magnitude of predicted change is modulated by baseline dietary cholesterol. The greatest response is expected when baseline dietary cholesterol is near zero, while little, if any, measurable change would be expected once baseline dietary cholesterol was > 400–500 mg/d. People desiring maximal reduction of serum cholesterol by dietary means may have to reduce their dietary cholesterol to minimal levels (< 100–150 mg/d) to observe modest serum cholesterol reductions while persons eating a diet relatively rich in cholesterol would be expected to experience little change in serum cholesterol after adding even large amounts of cholesterol to their diet. Despite modest average effects of dietary cholesterol, there are some individuals who are much more responsive (and others who are not

responsive). Individual degrees of response to dietary cholesterol may be mediated by differences in cholesterol absorption efficiency, neutral sterol excretion, conversion of hepatic cholesterol to bile acids, or modulation of HMG-CoA reductase or other key enzymes involved in intracellular cholesterol economy, each ultimately resulting in changes of plasma LDL cholesterol concentration mediated primarily by up- or down-regulation of LDL receptors.

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