Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis

Wanda H Howell, Donald J McNamara, Mark A Tosca, Bruce T Smith, and John A Gaines

ABSTRACT  Quantitative relations between dietary fat and cholesterol and plasma lipid concentrations have been the subject of much study and some controversy during the past 40 y. Previous meta-analyses have focused on the most tightly controlled, highest-quality experiments. To test whether the findings of these investigations are generalizable to broader experimental settings and to the design of practical dietary education interventions, data from 224 published studies on 8143 subjects in 366 independent groups including 878 diet-blood lipid comparisons were subjected to weighted multiple-regression analysis. Inclusion criteria specified intervention studies published in English between 1966 and 1994 reporting quantitative data on changes in dietary cholesterol and fat and corresponding changes in serum cholesterol, triacylglycerol, and lipoprotein cholesterol concentrations. Regression models were reported for serum total cholesterol, triacylglycerol, and low-density- high-density-, and very-low-density-lipoprotein cholesterol, with multiple correlations of 0.74, 0.65, 0.41, 0.14, and 0.34, respectively. Interactions of dietary factors, initial dietary intakes and serum concentrations, and study and subject characteristics had little effect on these models. Predictions indicated that compliance with current dietary recommendations (30% of energy from fat, < 10% from saturated fat, and < 300 mg cholesterol/d) will reduce plasma total and low-density-lipoprotein-cholesterol concentrations by ~5% compared with amounts associated with the average American diet. Am J Clin Nutr 1997;65:1747-64.

KEY WORDS Meta-analysis, plasma lipids, low-density lipoprotein, LDL, high-density lipoprotein, HDL, dietary fat energy, saturation of dietary fat, dietary cholesterol, human nutrition

INTRODUCTION

The prediction of blood lipid responses to multiple-component dietary changes have been investigated extensively by Keys et al (1-3) and Hegsted et al (4, 5). Although their early prediction equations were based on narrow sets of data inclusion criteria, these equations have formed, in part, the foundation for the current National Cholesterol Education Program (NCEP) dietary guidelines for the prevention of coronary artery disease (6). More recent comparisons of these predictive models by their authors, however, have resulted in an ongoing controversy regarding the extent of serum cholesterol response to dietary cholesterol (7, 8). Although the effects of saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) are similar in the two equations, the Hegsted model predicts a substantially greater effect of dietary cholesterol on serum cholesterol than does that of Keys. This difference was extended recently in Hegsted's latest overview of the effects of dietary change, which considered not only changes in serum total cholesterol but also changes in concentrations of plasma low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol (5).

In addition to these efforts, Hopkins (9), Mensink and Katan (10), and McNamara (11) reported prediction equations generated from meta-analytic investigations that also had narrow inclusion criteria. Hopkins combined baseline dietary cholesterol with added dietary cholesterol as the two predictors of change in total serum cholesterol. Mensink and Katan incorporated the effects of changes in carbohydrate and fatty acid (but not dietary cholesterol) intakes on serum total cholesterol, LDL cholesterol, and HDL cholesterol. McNamara pooled data summarizing the effects of changes in dietary cholesterol on serum total cholesterol concentrations.

None of these previously reported quantitative reviews included sensitivity analyses to assess the potential effects of numerous study and subject characteristics on lipid responses to dietary change. This investigation assessed these potential effects with a comprehensive database and rigorous meta-analytic procedures. Our objectives were to determine whether different study and subject conditions significantly influenced reported responses to diet manipulation and to explore similarities and differences in published prediction models. A secondary aim was to use a wide range of data sets to develop models to predict the effects of dietary change on concentrations of very-low-density-lipoprotein (VLDL) cholesterol and triacylglycerol in addition to serum total cholesterol, LDL cholesterol, and HDL cholesterol. Other published reports have not included models to predict VLDL cholesterol and triacylglycerol responses.

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The rather narrow scope of all previously reported quantitative reviews indicated the need for a comprehensive, systematic approach to integrating the diverse scientific literature. This meta-analytic investigation was designed to determine the extent to which study and subject characteristics, initial serum lipid concentrations, interactions of dietary manipulations, and the duration of treatment influenced the predictive models. Our intent was to develop a more broadly applicable model, spanning a diversity of study designs and types of subjects, to predict more appropriately the extent to which meeting the NCEP Steps 1 and 2 national dietary guidelines could be expected to affect changes in blood lipid concentrations of the American population.

METHODS

Study retrieval and selection

The data set consisted of published studies meeting the following inclusion criteria: 1) dietary interventions on adults (> 18 y of age) published in English between January 1966 and February 1994; 2) studies reporting single-group or multiple-group repeated-measures comparisons; 3) studies reporting quantitative measures of manipulated dietary components including one or more of the following: cholesterol, total fat (% of energy), SFAs, PUFAs, and monounsaturated fatty acids (MUFAs) (% of energy); 4) studies reporting group means ± SDs or SEMs for quantitative measures of response variables including any or all of the following: serum total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, and serum triacylglycerol.

Computer-assisted literature searches were undertaken by using MEDLINE (National Library of Medicine, Bethesda, MD) and the new OVID 3.0 system (National Library of Medicine) to locate potentially appropriate studies. The keywords diet, dietary cholesterol, dietary fat, and fatty acid were intersected with the words serum (plasma) cholesterol, serum (plasma) lipoproteins, LDL, HDL, VLDL, triacylglycerol, human, and English. To locate candidate studies not included in the computer-assisted literature searches, manual techniques were used, including a reference scan of primary studies and published narrative reviews and a search of indexes of review publications such as Nutrition Abstracts and Reviews, Nutrition Reviews, and World Review of Nutrition and Dietetics.

These search procedures resulted in 12,520 citations. A scan of their titles led to the elimination of all but 896 (7%) of them. Abstracts of the remaining citations (and, if necessary, the papers themselves) were examined for compliance with the meta-analytic inclusion criteria listed earlier. Discarded citations included letters to editors, reviews of related literature, reports of epidemiologic studies, reports lacking sufficient detail on dietary manipulation, reports of studies using nonexperimental ("one-shot") single- or comparison-group designs, and reports of other descriptive, nonintervention studies. Large clinical trials involving multiple interventions were not included. In addition, studies reporting data on weight-reduction diets, fish oils, trans fatty acids, and hydrogenated fats were excluded. Of the 896 articles passing the title scan, 224 (26%) met the inclusion criteria (12–235).

Data extraction

One of the objectives of this investigation was to consider the extent to which subject and study design characteristics may influence the effect of dietary manipulations on blood lipid concentrations. To address this objective, a coding instrument was designed to facilitate the collection of data relevant to study and subject characteristics. Both substantive and methodologic issues were incorporated into the coding form. Subject group characteristics included location during dietary intervention, sample size, sex, age, recruitment procedure, and inclusion and exclusion criteria.

Design issues were type of design, group assignment procedure, blinding, attrition, and control of selected confounding variables. Location of subjects was classified as free-living, confined to a hospital or metabolic ward, or free-living with institutional meals provided. Internal validity was assessed as high, medium, or low. To be rated as having high internal validity, studies had to use a control group or random assignment of subjects to groups or use repeated-measures designs with multiple observations and treatments and control for confounding variables. Medium ratings were assigned to studies that used matching or pretreatment equation procedures or repeated measures at a limited number of time points with limited control for confounding variables. Studies judged as having low internal validity used cross-sectional single group, pre- and postintervention designs with limited control for confounding variables.

Treatment characteristics included type of dietary manipulation, quantitative levels of dietary components, duration of treatment, compliance with treatment, and method of assessing dietary compliance. Two items were added to the coding form after initial pilot testing: the method used to provide dietary treatment and indication that multiple posttreatment measures were taken and averaged for each reported time point. Before the study data were coded, a list of coding instructions was developed and independent coders were trained with periodic tests to maximize intercoder agreement. The average agreement was 94%.

Data organization

The data extracted during this protocol were organized into three databases. The study-level database included the authors' credentials and affiliations, design type, and internal validity. Each study involved one or more groups of subjects. A second database contained 366 records storing characteristics of those individual groups, such as size (n), percentage of male subjects, mean age, attrition rates, and subject compliance. The third, or observation-level, database included quantitative blood lipid data recorded for a group at a particular time as well as quantitative measures of the subjects' diet in the preceding period and the duration of that treatment.

To identify appropriate comparisons for each group, a schematic diagram of each study design was drawn. For groups that received a single dietary manipulation, each subsequent observation was compared with the initial (baseline) observation. In crossover designs, the last observation before a new diet regimen began became the new baseline for subsequent observations until the next dietary change. In either case, n observations yielded n - 1 comparisons. On the basis of these definitions, a final comparison-level database was constructed.
that included 878 records, each containing an initial and a final set of dietary and serum measures to be compared.

Weighting of comparisons

The study groups differed considerably in the number of subjects and in the number of times that each group was observed. Larger groups provide more statistically reliable information, but multiple observations of a single group are clearly not independent. To allow proportionally greater influence from larger studies and to control for nonindependence of data, study groups were weighted proportionally to their size and inversely to the number of times observed. The final weighting used for each comparison can be described by using the following formula:

\[
G \left( \frac{n_i}{\sum n_i} \right) = \frac{1}{C_i} \tag{1}
\]

where \( G \) is the total number of groups in the meta-analysis, \( n_i \) is the group size, and \( C_i \) is the number of comparisons for each group.

Data analysis

For each comparison, differences between the final and initial values of dietary cholesterol (mg/d) and total fat, PUFA, MUFA, and SFA (% of energy) were computed to create the dietary change variables \( \Delta \)cholesterol, \( \Delta \)fat, \( \Delta \)PUFA, \( \Delta \)MUFA, and \( \Delta \)SFA, respectively. Corresponding differences for serum total cholesterol, VLDL cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerol were derived to create the serum lipid response variables \( \Delta \)serum total cholesterol, \( \Delta \)VLDL, \( \Delta \)LDL, \( \Delta \)HDL, and \( \Delta \)triacylglycerol.

Bivariate Pearson correlations were used to explore the relations among the dietary variables and between these variables and the response variables. Stepwise-multiple-regression analysis was used to identify the best linear prediction equations for each of the response measures, evaluating the combined and independent contributions of specified dietary variables. Each of the dependent serum variables (\( \Delta \)serum total cholesterol, \( \Delta \)VLDL, \( \Delta \)LDL, \( \Delta \)HDL, and \( \Delta \)triacylglycerol) in turn was regressed on the dietary change variables (\( \Delta \)cholesterol, \( \Delta \)fat, \( \Delta \)PUFA, \( \Delta \)MUFA, and \( \Delta \)SFA) by using all the comparisons having complete data for all variables for that regression. Forward stepwise variable selection allowed exploration of all data relations and controlled for problems of linear dependence (e.g., \( \Delta \)fat = \( \Delta \)PUFA + \( \Delta \)MUFA + \( \Delta \)SFA). That is, the stepwise addition stops before all four variables enter, although any three of them could. Each model identified by the stepwise procedure was then reestimated, including only the selected predictor variables; this made maximum use of the available data. SEs of the unstandardized regression coefficients were used to compute CIs.

Effects of other factors (interactions among dietary components, initial values of serum lipid and dietary variables, study and subject traits, etc.) on the basic models were evaluated by constructing appropriate interaction terms, such as \( \Delta \)cholesterol \( \cdot \) \( \Delta \)fat (a dietary interaction), serum total cholesterol \( \cdot \) \( \Delta \)cholesterol (an interaction with an initial serum concentration), and age \( \cdot \) \( \Delta \)cholesterol (a subject-effect interaction), and reestimating the models with these interaction terms available as predictors.

When a person’s diet is changed, the effect of that change would not be immediate but would be expected to emerge over time. One factor recorded for each comparison in these studies was the time that each treatment had been in effect when the serum lipid data were obtained, permitting exploration of the rates at which diet-serum lipid equilibria are reestablished. Although most of these studies were designed to allow sufficient time for the effects of dietary manipulations to express themselves, there is enough variation in treatment durations (ranging from 1 d to 6 y) to warrant exploration of this issue. This was done by modifying a linear prediction model, such as \( \Delta Y = B \cdot \Delta X \), into a nonlinear one of the form \( \Delta Y = B_1(1 - e^{-k \Delta X}) \), where \( t \) is treatment duration and \( k \) specifies the rate at which the influence of \( \Delta X \) on \( \Delta Y \) exponentially approaches a limit, \( B_1 \), from an expected value of 0 at \( t = 0 \). The half-life of the process is given by \( \ln(2)/k \). Nonlinear least-squares estimates of \( k \) significantly different from 0 were taken as indicative of a discernible treatment duration effect.

RESULTS

Study and subject characteristics

Data from 224 published studies of 8143 subjects in 366 independent groups including 878 diet-blood lipid comparisons were presented for the weighted least-squares-regression analyses. An average of 70% of the subjects in the independent groups were men. The combined sex group mean age was 37 y, ranging from 18 to 69 y.

Coding of several other characteristics of the subjects and studies allowed for further descriptive analysis of the database. The descriptive data for some of the coded characteristics are provided in Table 1. Most of the data represent healthy, free-living individuals with reasonably good compliance with dietary intervention.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of studies and subjects (^t)</th>
<th>Percentage of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject selection criteria</td>
<td>Healthy</td>
<td>81</td>
</tr>
<tr>
<td>Coronary artery disease, or at risk</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Subject location</td>
<td>Free-living</td>
<td>61</td>
</tr>
<tr>
<td>Confined to a hospital or metabolic ward</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Method of diet intervention</td>
<td>Total diet provided</td>
<td>67</td>
</tr>
<tr>
<td>Manipulated diet component provided</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Instructions to modify</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Subject compliance with diet</td>
<td>Good</td>
<td>54</td>
</tr>
<tr>
<td>Fair</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No assessment</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Method used to assess compliance</td>
<td>Weighed or measured intake</td>
<td>49</td>
</tr>
<tr>
<td>Subject-reported intake records</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Subject recall</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Method not reported</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

\(^t\) \( n = 224 \) studies.
All experiments used some type of repeated-measures study design: 38% used single-group designs with two or more treatments, 22% used a crossover treatment design with two or more groups, 14% used multiple groups with one or more treatments, and 26% used a single-group, pre- and posttreatment design. Of the experiments with multiple-group designs, 65% randomly assigned subjects to groups, 21% used matching, and 14% used a pretreatment equation. Only 10% of studies were blinded: 7% double- and 3% single-blinded. Control for changes in body weight of subjects during treatment phases was attempted by adjusting energy intake during 83% of the investigations. Subjects were requested to maintain their usual physical activity during participation in 35% of the experiments.

Judgments regarding the level of internal validity of studies were made according to the predetermined criteria outlined earlier. Only 9% of these studies were rated as having high internal validity, 49% had medium internal validity, and the remaining 42% had low internal validity.

**Bivariate relations between variables**

Correlations between changes in dietary variables are presented in Table 2. All relations, except that between ∆fat and ∆PUFA and that between ∆PUFA and ∆MUFA, were significantly different from zero. Correlations between ∆fat and ∆MUFA (r = 0.57) and ∆SFA (r = 0.63) were moderately high, which is to be expected because PUFA, MUFA, and SFA are constituents of fat. The level of correlation between these independent variables was sufficient to require multiple regressions to isolate their independent effects. However, none of the correlations between dietary variables were large enough to induce multicollinearity problems.

The correlation matrix in Table 3 shows significant relations between changes in all dietary variables and corresponding changes in serum total cholesterol, LDL cholesterol, and HDL cholesterol. When the strengths of these correlations for each response variable were compared, it appeared that the change in serum total cholesterol was most strongly associated with the change in SFA (r = 0.80) and PUFA (r = −0.62). Changes in both LDL cholesterol and HDL cholesterol were most highly associated with the change in SFA (r = 0.79 and r = 0.60, respectively), but the strength of the association between changes in LDL and changes in PUFA was greater than that between changes in HDL and PUFA (r = −0.48 and r = −0.24, respectively). Changes in both LDL and HDL were moderately correlated with changes in total dietary fat (r = 0.52 and r = 0.58, respectively). The only significant relation between changes in VLDL cholesterol or plasma triacylglycerol concentrations and changes in dietary variables was for PUFA (r = −0.37 and r = −0.40, respectively).

Bivariate regression coefficients corresponding to the significant correlations in Table 3 are presented in Table 4. Because of the significant and often substantial correlations between the dietary variables, these bivariate regressions only serve as a basis for detailed analysis via multiple regression to isolate the independent effects of dietary manipulations.

**Prediction equations**

The multiple-regression analyses for this data set are complicated by a substantial amount of missing data, as can be seen in the variations among the n values reported in Tables 2 and 3. We first estimated stepwise-multiple-regression models for each of the serum lipid response variables on all the dietary change variables with listwise deletion. The models were then reestimated by using only the predictors selected in the first pass to make maximum use of available data. The results of these analyses are presented in Table 5.

As indicated in Table 5, the best-fitting model for change in serum total cholesterol included changes in SFA (% of total energy), PUFA (% of total energy), and dietary cholesterol (mg). This prediction model explained 74% of the variance in change in serum total cholesterol. These relations indicate that a 1% alteration in total energy from SFA will result in a 49.1-μmol/L (1.9-mg/dL) change in serum total cholesterol. Likewise, a change in PUFA of 1% of total energy will produce a 23.3-μmol/L (0.90-mg/dL) change (in the opposite direction) in serum total cholesterol. Finally, a change of 1 mg/d in dietary cholesterol will produce a change of 0.57 μmol/L (0.022 mg/dL) in serum total cholesterol. The full linear prediction equation can be expressed as follows:

\[
\Delta \text{Serum total cholesterol (μmol/L)} = 49.599 \cdot \Delta \text{SFA} - 23.274 \cdot \Delta \text{PUFA} + 0.5741 \cdot \Delta \text{Cholesterol} \quad (2)
\]

\[
\Delta \text{Serum total cholesterol (mg/dL)} = 1.918 \cdot \Delta \text{SFA} - 0.900 \cdot \Delta \text{PUFA} + 0.0222 \cdot \Delta \text{Cholesterol} \quad (3)
\]

Note that even though all five dietary factors were significantly related to ∆serum total cholesterol at the bivariate level (Tables 3 and 4), neither ∆fat nor ∆MUFA added significant predictive power to this model.

Although the bivariate regression of ∆VLDL on ∆PUFA was significant, no other dietary factors entered the ∆VLDL prediction model in our multivariate analysis. This prediction equation accounted for only 14% of the variance in plasma VLDL.
TABLE 3
Correlations between changes in dietary variables and corresponding changes in blood lipid response variables

<table>
<thead>
<tr>
<th>ΔSerum total cholesterol (μmol/L)</th>
<th>ΔVLDL (μmol/L)</th>
<th>ΔLDL (μmol/L)</th>
<th>ΔHDL (μmol/L)</th>
<th>ΔTriacylglycerol (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔCholesterol (mg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.502</td>
<td>0.050</td>
<td>0.463</td>
<td>0.358</td>
</tr>
<tr>
<td>( n' )</td>
<td>307</td>
<td>61</td>
<td>185</td>
<td>225</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.0005</td>
<td>0.704</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>ΔFat (% of energy)(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.423</td>
<td>-0.201</td>
<td>0.523</td>
<td>0.579</td>
</tr>
<tr>
<td>( n )</td>
<td>345</td>
<td>65</td>
<td>194</td>
<td>237</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.0005</td>
<td>0.109</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>ΔPUFA (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>-0.624</td>
<td>-0.371</td>
<td>-0.479</td>
<td>-0.237</td>
</tr>
<tr>
<td>( n )</td>
<td>221</td>
<td>39</td>
<td>117</td>
<td>146</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.0005</td>
<td>0.020</td>
<td>&lt; 0.0005</td>
<td>0.004</td>
</tr>
<tr>
<td>ΔMUFA (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.177</td>
<td>-0.060</td>
<td>0.120</td>
<td>0.251</td>
</tr>
<tr>
<td>( n )</td>
<td>191</td>
<td>32</td>
<td>107</td>
<td>127</td>
</tr>
<tr>
<td>( P )</td>
<td>0.014</td>
<td>0.747</td>
<td>0.219</td>
<td>0.004</td>
</tr>
<tr>
<td>ΔSFA (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.803</td>
<td>0.051</td>
<td>0.790</td>
<td>0.604</td>
</tr>
<tr>
<td>( n )</td>
<td>244</td>
<td>40</td>
<td>129</td>
<td>169</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt; 0.0005</td>
<td>0.755</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

\(^1\) PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

\(^2\) Pearson correlation coefficient.

\(^3\) Number of independent groups.

\(^d\) % of energy from total fat.

Changes in ΔSFA and ΔPUFA were the best predictors of change in LDL cholesterol and explained a combined total of 65% of its variance. The unstandardized regression coefficients indicated that for every 1% change in SFA (% of total energy), a change in LDL cholesterol of 46.5 mmol/L (1.8 mg/dL) was predicted and an increase of 1% in PUFA (% of total energy) was expected to decrease LDL cholesterol by 12.93 mmol/L (0.50 mg/dL). The combined linear prediction equation for change in LDL cholesterol is as follows:

\[
\text{ΔLDL (μmol/L)} = 46.755 \cdot \text{ΔSFA} - 12.801 \cdot \text{ΔPUFA}
\]

Again, not all the significant bivariate predictors of ΔLDL entered the equation. In particular, Δcholesterol was almost significant enough to enter \( (P = 0.067) \). This suggests that even though Δcholesterol had a significant bivariate relation to ΔLDL, it was its joint relation with ΔSFA and ΔPUFA that

TABLE 4
Bivariate regressions of changes in blood lipid response variables to changes in dietary variables\(^1\)

<table>
<thead>
<tr>
<th>ΔSerum total cholesterol (μmol/L)</th>
<th>ΔVLDL (μmol/L)</th>
<th>ΔLDL (μmol/L)</th>
<th>ΔHDL (μmol/L)</th>
<th>ΔTriacylglycerol (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔCholesterol (mg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta ) (^2)</td>
<td>0.7318</td>
<td>0.0548</td>
<td>0.1267</td>
<td></td>
</tr>
<tr>
<td>( r^2 ) (^3)</td>
<td>0.252</td>
<td>0.214</td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td>ΔFat (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>-61.831</td>
<td>-7.034</td>
<td>-41.531</td>
<td>-5.715</td>
</tr>
<tr>
<td>( r^2 ) (^3)</td>
<td>0.389</td>
<td>0.137</td>
<td>0.230</td>
<td>0.056</td>
</tr>
<tr>
<td>ΔPUFA (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>21.748</td>
<td>6.284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r^2 ) (^3)</td>
<td>0.031</td>
<td>0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMUFA (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>70.210</td>
<td>51.875</td>
<td>11.740</td>
<td></td>
</tr>
<tr>
<td>( r^2 ) (^3)</td>
<td>0.645</td>
<td>0.623</td>
<td>0.365</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

\(^2\) Unstandardized regression coefficient.

\(^3\) Coefficient of determination. Insignificant regressions not shown.

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affected ΔLDL rather than any independent effect it might have had.

The best-fitting model for change in HDL cholesterol included changes in SFA and total fat. The predictive model for HDL cholesterol explained 41% of the variance associated with dietary change. The regression coefficients indicated that for every 1% change in total energy from SFA, a change in HDL cholesterol of 7.4 μmol/L (0.3 mg/dL) was predicted. A 1% change in energy from total fat will produce a 5.0-μmol/L (0.2-mg/dL) change in HDL cholesterol. The combined linear prediction model for change in HDL cholesterol was as follows:

$$\Delta\text{HDL (μmol/L)} = 7.422 \cdot \Delta\text{SFA} + 4.965 \cdot \Delta\text{fat}$$ (6)

$$\Delta\text{HDL (mg/dL)} = 0.287 \cdot \Delta\text{SFA} + 0.192 \cdot \Delta\text{fat}$$ (7)

The prediction model for triacylglycerol presented in Table 5 accounts for 36% of the variance. The full equation is as follows:

$$\Delta\text{Triacylglycerol (μmol/L)} = 0.1626 \cdot \Delta\text{cholesterol}$$

$$- 12.035 \cdot \Delta\text{PUFA} - 10.376 \cdot \Delta\text{fat}$$ (8)

$$\Delta\text{Triacylglycerol (mg/dL)} = 0.0144 \cdot \Delta\text{cholesterol}$$

$$- 1.066 \cdot \Delta\text{PUFA} - 0.919 \cdot \Delta\text{fat}$$ (9)

In this model, Δfat enters with a negative coefficient. That is, a decrease in total fat in the diet will lead to an increase in triacylglycerol, other things being equal. This seemingly anomalous finding may be due to the substitution of simple carbohydrate energy for fat energy in isoenergetic diets.

Observed compared with expected changes for individual comparisons from each of the four prediction models are shown in Figure 1. The relative degrees of scatter reflect variations in R² among the four models. No outliers of undue influence are apparent. Note that in each case there are many comparisons in which the predicted and observed values differ in sign.

Effects of other factors

After these prediction models were identified, additional regressions (sensitivity analyses) were undertaken to explore the effects of other factors, such as dietary interactions, initial dietary and plasma lipid values, subject and study characteristics, and treatment duration. These analyses do not assess the independent effects of these factors but rather their influence on the effects of dietary changes on serum lipid responses.

The first factors considered were interactions among the dietary change variables. The models described earlier assumed that the effects of dietary changes are additive, ie, that if more than one dietary component is changed at once, their combined effect will be the simple sum of their independent effects as specified in the model. This need not be the case because simultaneous changes in multiple dietary components could potentiate or inhibit each other’s effects. To explore these issues, product terms were computed for each pair of dietary variables included in each model. For example, for Δserum total cholesterol they were Δcholesterol ∙ ΔPUFA, Δcholesterol ∙ ΔSFA, and ΔPUFA ∙ ΔSFA. The model equations were then reestimated with these new product terms available for stepwise inclusion.

Only one dietary interaction term entered any of the four equations. The modified model for Δtriacylglycerol became

$$\Delta\text{Triacylglycerol (μmol/L)} = 0.1400 \cdot \Delta\text{cholesterol} - 9.698 \cdot \Delta\text{PUFA}$$

$$- 8.422 \cdot \Delta\text{fat} + 0.892 \cdot \Delta\text{PUFA} \cdot \Delta\text{fat}$$ (10)
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\[ \Delta \text{Triacylglycerol (mg/dL)} = 0.0124 \cdot \Delta \text{cholesterol} - 0.859 \cdot \Delta \text{PUFA} - 0.746 \cdot \Delta \text{fat} + 0.079 \cdot \Delta \text{PUFA} \cdot \Delta \text{fat} \quad (11) \]

This modification increased the \( R^2 \) for the model by 5%, from 0.34 to 0.39. Interpretation of this model can be facilitated by factoring the model equation to give

\[ \Delta \text{Triacylglycerol (\( \mu \text{mol/L} \))} = 0.1400 \cdot \Delta \text{cholesterol} - 9.698 \cdot \Delta \text{PUFA} + (-8.422 + 0.892 \cdot \Delta \text{PUFA}) \cdot \Delta \text{fat} \quad (12) \]

\[ \Delta \text{Triacylglycerol (mg/dL)} = 0.0124 \cdot \Delta \text{cholesterol} - 0.859 \cdot \Delta \text{PUFA} + (-0.746 + 0.079 \cdot \Delta \text{PUFA}) \cdot \Delta \text{fat} \quad (13) \]

Here, the effect of \( \Delta \text{fat} \) on changes in triacylglycerol concentrations is seen as a linear function of \( \Delta \text{PUFA} \). A decrease in total fat energy will increase triacylglycerol only if \( \Delta \text{PUFA} \) is < 9.4% (8.422/0.892) of total energy. For positive changes in \( \Delta \text{PUFA} > 9.4\% \) of total energy, a decrease in fat will result in a reduction in triacylglycerol.

Next, effects of initial dietary values were considered, allowing for the possibility that, for example, the effect of
changes in dietary cholesterol might depend on the amount of dietary cholesterol at the beginning of treatment. Product variables of the form cholesterol, ∆cholesterol were constructed and the models were reestimated. Again, only one such term entered any of the regressions, yielding

$$ \Delta \text{Serum total cholesterol (μmol/L)} $$
$$ = 48.539 \cdot \text{SFA} - 31.549 \cdot \Delta \text{PUFA} $$
$$ + 0.5456 \cdot \Delta \text{cholesterol} + 0.879 \cdot \text{PUFA}_1 \cdot \Delta \text{PUFA} \quad (14) $$

$$ \Delta \text{Serum total cholesterol (mg/dL)} $$
$$ = 1.877 \cdot \Delta \text{SFA} - 1.220 \cdot \Delta \text{PUFA} + 0.0211 $$
$$ \cdot \Delta \text{cholesterol} + 0.034 \cdot \text{PUFA}_1 \cdot \Delta \text{PUFA} \quad (15) $$

This factors to

$$ \Delta \text{Serum total cholesterol (μmol/L)} $$
$$ = 48.539 \cdot \text{SFA} + (-31.549 + 0.879 \cdot \text{PUFA}_1) $$
$$ \cdot \Delta \text{PUFA} + 0.5456 \cdot \Delta \text{cholesterol} \quad (16) $$

$$ \Delta \text{Serum total cholesterol (mg/dL)} $$
$$ = 1.877 \cdot \Delta \text{SFA} + (-1.220 + 0.034 \cdot \text{PUFA}_1) $$
$$ \cdot \Delta \text{PUFA} + 0.0211 \cdot \Delta \text{cholesterol} \quad (17) $$

The effect of ∆PUFA here is a linear function of the initial amount of PUFA in the diet. Increasing ∆PUFA will reduce serum cholesterol only if PUFA, is < 36% of total energy (31.549/0.879); the lower the initial amount of PUFA in the diet, the greater the effect of a given increase in it. For initial concentrations > 36% of energy, increasing ∆PUFA will increase serum cholesterol concentrations. The multiple $R^2$ increases by 0.01 to 0.76. It is questionable whether any of these modified regression equations improve the predictive accuracy relative to the increase in model complexity.

Given that the effects of dietary changes on serum lipid concentrations might differ if the subjects already had unusually high or low serum lipid amounts, interactions of initial lipid concentrations with dietary changes were considered. Two of these entered the models: HDL, ∆fat for HDL, which increased the $R^2$ by 0.03 to 0.44, and triacylglycerol, ∆PUFA for Δtriacylglycerol, which increased the $R^2$ by 0.04 to 0.38.

Next, effects of subject mean age and percentage of male subjects were considered. The only significant interaction to enter any of the models was %male ∆fat for Δtriacylglycerol, increasing the $R^2$ by 0.02 to 0.36.

Design characteristics, such as assessed internal validity, the location of subjects, the use of eggs or liquid diets, and the average of multiple observations were considered next. Only the location of subjects had significant interactions with the dietary change variables. A modified equation for ∆serum total cholesterol was identified:

$$ \Delta \text{Serum total cholesterol (μmol/L)} $$
$$ = 49.134 \cdot \Delta \text{SFA} - 28.317 \cdot \Delta \text{PUFA} + 0.5586 $$
$$ \cdot \Delta \text{cholesterol} + 28.653 \cdot \text{Conf} \cdot \Delta \text{PUFA} \quad (18) $$

$$ \Delta \text{Serum total cholesterol (mg/dL)} $$
$$ = 1.900 \cdot \Delta \text{SFA} - 1.095 \cdot \Delta \text{PUFA} + 0.0216 $$
$$ \cdot \Delta \text{cholesterol} + 1.108 \cdot \text{Conf} \cdot \Delta \text{PUFA} \quad (19) $$

where Conf is a dummy (0/1) variable identifying groups of subjects confined in hospitals or metabolic wards. This indicated that the effect of ∆PUFA for confined subjects was significantly less than that for free-living subjects. The coefficients for confined, free-living, and free-living but institutionally fed subjects are plotted in Figure 2. Note that the plotted limits of the CI for confined subjects do not overlap that for free-living subjects.

Finally, treatment duration effects were considered. These were treated differently, modifying the model equations to include exponential terms in treatment duration, $t$, and by using nonlinear regression to estimate the effects. Only one of these gave a significant result: ∆SFA for ∆serum total cholesterol. The resultant equation was as follows:

$$ \Delta \text{Serum total cholesterol (μmol/L)} $$
$$ = 51.849 \cdot [1 - \exp(-4.888 \cdot t)] \cdot \Delta \text{SFA} - 27.256 $$
$$ \cdot \Delta \text{PUFA} + 0.5715 \cdot \Delta \text{cholesterol} \quad (20) $$

$$ \Delta \text{Serum total cholesterol (mg/dL)} $$
$$ = 2.005 \cdot [1 - \exp(-0.189 \cdot t)] \cdot \Delta \text{SFA} - 1.054 $$
$$ \cdot \Delta \text{PUFA} + 0.0221 \cdot \Delta \text{cholesterol} \quad (21) $$

The corresponding half-life is 3.7 d, indicating 90% of maximal effect in ≈12 d. This effect was barely significant at $P = 0.05$, with a $t$ ratio of 1.97 and an increase of < 0.01 in explained variance. The finding that treatment duration effects were not readily discernible in this data set was not surprising because most studies were designed to allow sufficient time for the effects of dietary manipulations to establish themselves.

**DISCUSSION**

**Comparison of prediction models**

Over the years, several researchers have published models for predicting changes in serum lipids, particularly plasma total cholesterol, from changes in dietary lipid composition. Both the diversity and consistency of these models is remarkable. Several prediction models are given in Table 6. The first six equations listed in Table 6 are multivariate models predicting a change in serum total cholesterol (Δserum total cholesterol) from changes in dietary fat and cholesterol. All models include terms for ∆SFA and ∆PUFA and their coefficients are quite similar. Four of the models also include linear terms for cholesterol, which are somewhat more variable. These coefficients are plotted in Figure 3. Particularly for ∆SFA and ∆PUFA,
these plots display the similarity among the coefficients in the various models, with all falling within the range of the overlapping CIs. In contrast, Hegsted et al.’s 1965 data (4) yield a comparatively high coefficient for dietary cholesterol, whereas the cholesterol coefficient estimated for the present data set falls between those estimated in Hegsted’s 93M (metabolic ward) and 93F (free-living) models. The latter observation was expected because this meta-analysis combined data from both settings.

Because cholesterol and dietary fats are measured in different units, it is difficult to assess their relative effects. However, there is a useful test case available that permits a direct comparison. Data from the second National Health and Nutrition Examination Survey (NHANES II) show that the average American diet provides 385 mg cholesterol/d and 37% of total energy from fat (236). The energy from fat was distributed as 7% from PUFA, 17% from MUFA, and 13% from SFA. The NCEP Step 1 diet recommends reducing both cholesterol and total fat intakes, with a redistribution of types of fat to provide 300 mg cholesterol/d and 30% of total energy from fat (10% from PUFA, 10% from MUFA, and 10% from SFA). The Step 2 diet further reduces cholesterol and SFA to provide 200 mg cholesterol/d and 30% of total energy from fat (10% from PUFA, 13% from MUFA, and 7% from SFA). NCEP Step 1 and 2 diets provide a standard set of dietary changes that can be used to evaluate these multivariate models. Given specific changes in these dietary components, the change in serum total cholesterol each model predicts and how much of that change is attributable to each dietary component can be determined.

Predicted changes in serum cholesterol based on each of these multivariate models, resulting from a change from the average American Diet to the NCEP Steps 1 and 2 diets, along with the proportions of the predicted responses that are a result of each of the dietary factors are presented in Figure 4. [Note that the Keys-57 (3) model included no cholesterol term.]

The last four models in Table 6 were all derived considering changes in dietary cholesterol only, either by ignoring or controlling dietary fat in the treatment designs. The multivariate models presented in the upper panel controlled for dietary fat statistically. All models in Table 6 (except for Keys-57) generate predictions of the serum total cholesterol response to manipulations of dietary cholesterol. These equations are even more difficult to compare directly because several different functional forms are used. Plots of these models’ predicted reductions in serum total cholesterol for a range of reductions in dietary cholesterol from an initial value of 385 mg/d are shown in Figure 5. For a reduction of 100 mg/d, the predictions of the reduction in serum total cholesterol range from 35.43 µmol/L (1.37 mg/dL; Hop-92) to 175.07 µmol/L (6.77 mg/dL; Heg-65). Assuming an initial serum cholesterol of 6.21 mmol/L (240 mg/dL), these predicted reductions range from 0.6% to 3.3%.

On the basis of the predictive equation developed in this analysis of the most comprehensive set of data, a shift from the current diet to the Step 1 diet would reduce the average plasma total cholesterol concentration by 263.8 µmol/L (10.2 mg/dL) and a further reduction of 204.3 µmol/L (7.9 mg/dL) with a shift from a Step 1 to a Step 2 diet. This reduction of 465 µmol/L (18 mg/dL) represents an 8.6%
TABLE 6
Prediction equations for diet-mediated changes in serum total cholesterol concentrations

<table>
<thead>
<tr>
<th>Source</th>
<th>Mnemonic</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keys et al (3)</td>
<td>Keys-57</td>
<td>70.86 - ΔSFA - 33.88 - ΔPUFA (4.65) (4.91)</td>
</tr>
<tr>
<td>Hegsted et al (4)</td>
<td>Heg-65</td>
<td>55.86 - ΔSFA - 42.67 - ΔPUFA + 1.7507 - Δcholesterol (8.02) (9.31) (0.4008)</td>
</tr>
<tr>
<td>Keys and Parlin (2)</td>
<td>Keys-66</td>
<td>67.2 - ΔSFA - 33.6 - ΔPUFA + 24.57 - (cholesterol^{1/2} - cholesterol^{1/2})</td>
</tr>
<tr>
<td>Hegsted et al (5)</td>
<td>Heg-93M</td>
<td>54.31 - ΔSFA - 30.00 - ΔPUFA + 0.6930 - Δcholesterol (3.88) (3.88) (0.1086)</td>
</tr>
<tr>
<td>Metabolic ward</td>
<td>Heg-93F</td>
<td>71.12 - ΔSFA - 26.64 - ΔPUFA + 0.4138 - Δcholesterol (7.50) (11.64) (0.1500)</td>
</tr>
<tr>
<td>Free-living</td>
<td>Heg-90</td>
<td>49.65 - ΔSFA - 23.27 - ΔPUFA + 0.5741 - Δcholesterol (3.62) (4.14) (0.0957)</td>
</tr>
<tr>
<td>Howell et al (1997)</td>
<td>How-97</td>
<td>1220.6 - exp(-0.09930 - cholesterol) - [1 - exp(-0.03517 - Δcholesterol])</td>
</tr>
</tbody>
</table>

1 SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids.
2 When there were multiple models, the particular equation is identified.
3 Defines short-hand tags that will be used in subsequent tables and figures.
4 The coefficients in the equations have been rescaled for application to the measurement units used in this study (μmol/L for serum lipids, % of total energy for dietary fats, and mg/d for dietary cholesterol). SEs for the coefficients are in parentheses.

Plasma cholesterol response to dietary cholesterol

The extent of the plasma cholesterol response to changes in dietary cholesterol has been debated for many years. The report of a 1988 National Institutes of Health conference on the effect of dietary cholesterol on plasma cholesterol (238) suggested that a 100-mg increase in dietary cholesterol per 4185 kJ/d increased plasma cholesterol by 259 μmol/L (10 mg/dL), which for a 10.5-MJ diet predicts an increase in plasma cholesterol of 103 μmol/L (4 mg/dL) per 100-mg/d increase in cholesterol intake. In contrast, all prediction equations published since 1990 presented in Table 6 and Figure 5 suggest a rather narrow range of plasma cholesterol reductions (35.43–69.30 μmol/L, or 1.37–2.68 mg/dL) for a 100-mg/d reduction in dietary cholesterol. The predicted plasma cholesterol response to a 100-mg/d decrease in dietary cholesterol based on the results of this meta-analysis is a decrease of 56.9 μmol/L (2.2 mg/dL), or ≈1% in the average population cholesterol concentration.

Plasma lipoprotein response to dietary change

Although the majority of prediction equations address changes in plasma total cholesterol with changes in dietary fat and cholesterol, changes in specific plasma lipoproteins are of greater importance given their relative atherogenic and antiatherogenic properties. Data from this meta-analysis indicate that plasma LDL-cholesterol concentrations are determined by ΔSFA and ΔPUFA, whereas plasma HDL-cholesterol concentrations are determined by ΔSFA and Δfat. Comparable findings have been reported from epidemiologic studies (239) and the meta-analysis conducted by Mensink and Katan (10). Knuiman et al (239) found that in free-living populations, exchanging carbohydrate for fat energy in the diet significantly reduced plasma HDL-cholesterol concentrations by 10.3 μmol/L (0.4 mg/dL) for every 1% of energy exchanged (239). More specifically, the analysis by Mensink and Katan (10) indicated that all fatty acids elevated HDL cholesterol when substituted for carbohydrates and that the effect diminished with increasing unsaturation of the fatty acids (10). These results are comparable with our finding that the two major dietary determinants of changes in plasma HDL cholesterol were total fatty acids and SFA.

Plasma lipid response to specific fatty acids

Few studies in this meta-analysis included data on the effects of specific fatty acids on plasma total or lipoprotein cholesterol
concentrations. There is strong evidence, however, that the cholesterol-raising SFAs are lauric, myristic, and palmitic acids, whereas stearic acid is not hypercholesterolemic. The hypercholesterolemic SFAs in mixed natural diets are fairly constant at 60–70% of the total saturates, indicating that specific effects are probably not a major concern. Although it has been suggested that the hypocholesterolemic response to dietary linoleic acid reaches a threshold at 5–6% of total energy, this study found no evidence for a threshold response to PUFA intake, indicating that the response factor is therefore linear.

Implications for clinical management

There is little debate regarding the advisability of lowering an elevated LDL-cholesterol concentration to reduce the associated coronary artery disease risk. The efficacy of population-wide dietary modifications to achieve this end is, however, controversial. For patients in the high-risk range for LDL cholesterol (> 4.14 mmol/L, or 160 mg/dL), data from this meta-analysis indicate that 4.5% and 7.7% average reductions in this amount would be predicted from modifying current average intakes of SFAs and PUFAs to NCEP Steps 1 and 2 recommendations, respectively. There is, however, a large degree of individual variability in responses to changes in both dietary fat quality and cholesterol. The existence of precise feedback mechanisms balancing the input of exogenous dietary cholesterol and endogenous synthesis of cholesterol in the majority of individuals is a primary reason why a reduction in dietary cholesterol has a relatively small effect on plasma cholesterol concentrations in most patients who are diet-sensitive.

The variations among individuals in the plasma cholesterol response to dietary changes may also be related to factors such as ethnicity, adiposity, physical activity, and hyperlipidemia. The effects of these variables on the response to dietary interventions are not well defined. In addition, support for a strong genetic influence on the effects of dietary factors on plasma cholesterol is growing and provides evidence that both levels and response genes influence the efficacy of dietary interventions to lower cholesterol concentrations and reduce cardiovascular disease risk.

The data supporting a relation between dietary fat and cholesterol intake and elevated plasma lipid concentrations should be evaluated not as mean values but on the basis of individual patient responses. Some individuals can lower their plasma cholesterol concentrations by decreasing dietary SFA and cholesterol intakes; however, this meta-analysis presents persuasive evidence that there are those for whom dietary change within the range of the NCEP recommendations will have a modest effect on heart disease risk. Clinically, the problem is first to determine who needs intervention, and second, to determine how effective the intervention is for each patient. Effective screening, including multiple plasma lipid determinations, and follow-up evaluation to determine the efficacy of dietary intervention are essential.
FIGURE 4. Reductions in serum total cholesterol, resulting from subjects changing to the National Cholesterol Education Program's Steps 1 and 2 diets, predicted by various models, with proportional contributions of the changes in saturated fatty acids, polyunsaturated fatty acids, and dietary cholesterol. See Table 6 for mnemonic designations.

FIGURE 5. Predicted reductions in serum total cholesterol for given reductions in dietary cholesterol. See Table 6 for mnemonic designations. NCEP, National Cholesterol Education Program.
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