Meta-regression analysis of the effects of dietary cholesterol intake on LDL and HDL cholesterol

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ABSTRACT

Background: Elevated low-density lipoprotein (LDL) cholesterol is a major risk factor for cardiovascular disease. Dietary guidance recommends reducing saturated fatty acid, *trans* fatty acid, and cholesterol intakes to reduce circulating LDL cholesterol. Cholesterol intake may also affect high-density lipoprotein (HDL)–cholesterol concentrations, but its impact has not been fully quantified.

Objectives: The aims of this study were to investigate the dose-response relation between changes in dietary cholesterol intake and changes in lipoprotein-cholesterol markers for cardiovascular disease risk and to provide a reference for clinicians on how changes in dietary cholesterol intake affect circulating cholesterol concentrations, after accounting for intakes of fatty acids.

Methods: We used a Bayesian approach to meta-regression analysis, which uses Markov chain Monte Carlo techniques, to assess the relation between the change in dietary cholesterol (adjusted for dietary fatty acids) and changes in LDL and HDL cholesterol based on the use of data from randomized dietary intervention trials.

Results: Fifty-five studies (2652 subjects) were included in the analysis. The nonlinear Michaelis-Menten (MM) and Hill models best described the data across the full spectrum of dietary cholesterol changes studied (0–1500 mg/d). Mean predicted changes in LDL cholesterol for an increase of 100 mg dietary cholesterol/d were 1.90, 4.46, and 4.58 mg/dL for the linear, nonlinear MM, and Hill models, respectively.

Conclusions: The change in dietary cholesterol was positively associated with the change in LDL-cholesterol concentration. The linear and MM models indicate that the change in dietary cholesterol is modestly inversely related to the change in circulating HDL-cholesterol concentrations in men but is positively related in women. The clinical implications of HDL-cholesterol changes associated with dietary cholesterol remain uncertain. *Am J Clin Nutr* 2019;109:7–16.

Keywords: LDL cholesterol, HDL cholesterol, dietary cholesterol, cholesterol, meta-regression

Introduction

Cardiovascular disease (CVD) continues to be a major cause of morbidity and mortality in the United States and worldwide, and its cause is associated with a constellation of factors; elevated circulating cholesterol, particularly LDL cholesterol, is a major contributor to CVD risk (1, 2). In its 2015 report, the National Lipid Association concluded that elevated concentrations of apolipoprotein (apo) B–containing lipoproteins, namely non-HDL cholesterol and its main component, LDL cholesterol, are a primary cause of atherosclerosis, which is the key underlying process that ultimately leads to clinical CVD events such as myocardial infarction and stroke (3). Health professional organizations and authoritative bodies recommend decreasing circulating LDL cholesterol as a main strategy to lower CVD event risk (2, 3).

Circulating cholesterol concentrations can be modified by diet and pharmacologic agents. Dietary changes are the first recommended means to achieve desirable circulating concentrations of total cholesterol (TC) and its components, including LDL and non-HDL cholesterol (4). Although HDL cholesterol has often been referred to as the "good cholesterol," results from genetic studies and pharmaceutical trials have failed to

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Supplemental Materials 1–3 and Supplemental Tables 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CVD, cardiovascular disease; LOOIC, leave-one-out information criterion; MCMC, Markov chain Monte Carlo; MM, Michaelis-Menten; TC, total cholesterol; TFA, *trans* fatty acid; TG, triglyceride; ΔCHOL, change in dietary cholesterol.

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show that modification of the HDL-cholesterol concentration alters CVD event risk, despite the strong inverse association between HDL-cholesterol concentration and CVD event risk in population studies (5, 6). Accordingly, current recommendations focus on maintaining low concentrations of cholesterol in apoB-containing lipoproteins (non-HDL and LDL cholesterol) to reduce CVD risk (3).

Dietary recommendations in the 2015 Dietary Guidelines for Americans and from professional organizations such as the National Lipid Association have focused primarily on lowering intakes of SFAs, *trans* fatty acids (TFAs), and dietary cholesterol as much as possible, while maintaining a healthy diet, since these dietary factors have all been shown to increase circulating TC and LDL-cholesterol concentrations (4, 7, 8). Higher intakes of PUFAs and, to a lesser extent, MUFAs, as a replacement for carbohydrate, have been found to lower circulating TC and LDL cholesterol while modestly raising HDL-cholesterol concentrations (8). Limited data exist as to how circulating TC, LDL-cholesterol, and HDL-cholesterol concentrations are affected by dietary cholesterol once intakes of SFAs, TFAs, PUFAs, and MUFAs have been controlled for in randomized controlled trials. This study aims to investigate the dose-response relation between changes in dietary cholesterol intake and changes in circulating cholesterol concentrations (i.e., LDL cholesterol, HDL cholesterol, and VLDL cholesterol) through Bayesian meta-regression analysis, a weighted technique that combines information across studies. The overall goal of this analysis was to elucidate the impacts of changes in dietary cholesterol on lipoprotein-cholesterol markers for CVD risk, in order to provide a reference for clinicians on how changes in dietary cholesterol intake affect circulating cholesterol concentrations after accounting for intake of fatty acids.

Methods

Literature search and data selection

We used 2 common biomedical-based search engine databases (PubMed and Cochrane Central) to identify abstracts of potential interest from 1946 to August 2016 (see **Supplemental Material 1**, **Supplemental Table 1** for search terms). Abstracts were imported into and initially screened by the abstract management program Abstrackr (Brown University). Only randomized controlled dietary intervention trials, published in the English language, were reviewed for the following a priori inclusion criteria: *1*) adult (≥18 y) human subjects; *2*) quantitative documentation (milligrams or grams per day or week) of actual dietary cholesterol intake for each unique diet or study condition; *3*) quantitative documentation (i.e., grams or percentage of energy) of dietary SFAs, MUFAs, and PUFAs for each condition (TFA intake was recorded, where available, but not used as an inclusion criterion due to inconsistent and infrequent reporting); *4*) intervention exposure duration for each condition ≥2 wk (14 d); and *5*) quantitative outcome measurements of LDL cholesterol, with respective variability measurements (i.e., the SD or SE of LDL-cholesterol measurements).

Cross-sectional, case-control, cohort, or any other observational studies were excluded. Additional a priori exclusion criteria were as follows: *1*) single-condition design or interventions with no control group; *2*) studies including subjects with chronic disease, with the exception of obesity, type 2 diabetes, or metabolic syndrome (studies including subjects with these conditions were included if the study met all other inclusion/exclusion criteria); *3*) studies including children (<18 y old) or pregnant women; *4*) trials investigating the effect of a weight-loss or lifestyle-modification program or trials that use a weight-loss medication, supplement, or drug therapy or studies not designed to investigate weight loss but in which subjects experienced weight loss of clinical significance (≥4.5 kg); *5*) trials that did not assess the association of cholesterol intake with relevant outcomes of interest (i.e., diet-related changes in LDL cholesterol); and *6*) trials investigating the effects of lipid- or cholesterol-altering medications, supplements, or drug therapy or studies not designed to investigate lipid/cholesterol-altering medications or supplements but in which >25% of subjects were receiving ≥1 of these therapies. In addition, the References sections from relevant peer-reviewed meta-analyses, systematic reviews, and topic reviews (see the Cross-Reference list in Supplemental Material 1) were assessed for other studies that fit the inclusion criteria but were not identified in the search strategy.

Two post hoc changes were made to the inclusion and exclusion criteria, both relating to the definition of the change in cholesterol intake. Initially, the aim was to compare pre- and postintervention lipoprotein measurements within each diet or intervention, regardless of whether the study was of a crossover or parallel design. Upon further consideration, it was determined that the definition of the point of comparison needed to be different for parallel and crossover studies to avoid excessive exclusion of trials from the analysis. For parallel studies, the change in cholesterol intake (and the change in serum lipids) was defined relative to the preintervention concentrations. This meant that the parallel studies needed a reliable measurement of preintervention cholesterol intake and serum lipids, so that the change could be determined for each group. Therefore, any parallel studies that did not quantitatively report prediet cholesterol intake and serum lipid concentrations were excluded.

The second post hoc change related to the studies with crossover designs. For these studies, no separate preintervention concentration for each diet was available, and it was therefore necessary to define a baseline. Therefore, the diet with the lowest cholesterol intake amounts (within the same study) was identified as the reference condition, against which all the other diets in the study were compared. Thus, the change in cholesterol for a given study condition was calculated as the difference between the cholesterol intake in that study condition and in the reference condition, and the postintervention serum lipid concentrations for the study condition of interest were compared with the postintervention results for the reference condition. Comparing the postdiet results of the study condition with the postdiet results of the reference condition appropriately captures the impact of each diet, without being affected by prior diets, because a study inclusion criterion specified that each of the subjects be maintained on the respective diet for at least the minimal time to steady state (≥14 d). Therefore, we were able to remove the inclusion criterion that required baseline (prediet) lipoprotein measurements to be available for crossover studies. Studies originally considered for exclusion based on a lack of background or baseline LDL-cholesterol measurement were Becker et al. (9), Fumeron et al. (10), Klass et al. (11), Lewis

et al. (12), Sabate et al. (13), Sehayek et al. (14), Tholstrup et al. (15), and Weggemans et al. (16). These studies were included due to the change in this criterion.

For each study that met all inclusion criteria and no exclusion criteria, the baseline and outcome measurements, along with respective variability measurements, were extracted, when available, for TC, HDL cholesterol, and VLDL cholesterol. Triglycerides (TGs) were extracted if data on VLDL cholesterol were not available. All information was extracted by 1 of 2 scientists, stored in a Microsoft Excel database, and independently reviewed by ≥1 additional scientist other than the extractor. In the event of a disagreement, ≥1 additional scientist reviewed the article and a discussion took place until consensus was reached. This review process was completed prior to beginning the modeling. Unit conversions and extrapolations from other serum limits were conducted as needed in order to include the maximum number of studies in the analysis. Specifically, studies reporting LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, and TC measurements in millimoles per liter were converted to milligrams per deciliter with the use of a conversion factor of 38.67. TG measurements were converted from millimoles per liter to milligrams per deciliter with the use of a factor of 88.57. When VLDL-cholesterol information was not reported, it was estimated by dividing the measured TG concentrations (in milligrams per deciliter) by 5.0 (17).

Data adjustments

To quantitatively determine the changes in serum lipid responses attributable to dietary cholesterol changes, we adjusted the data to account for the effects of dietary fatty acids, specifically SFAs, PUFAs, MUFAs, and, when possible, TFAs, according to the coefficients from the equation given by Mensink et al. (8). This method calculates a predicted response in LDL and HDL cholesterol based on changes in dietary fatty acids alone. The predicted changes in LDL- or HDL-cholesterol concentrations were subtracted from the observed changes in LDL and HDL cholesterol, respectively, to calculate the dietary cholesterol–associated changes in the lipoproteins (the dependent variable in the meta-regression modeling).

The SD of these predicted changes was used to calculate the variance of the mean lipoprotein measurements, which determines the weight, or impact, on the meta-regression model (i.e., data points with higher variance will have less impact on determining the model). Several of the studies did not report the SD but provided other measures of variability, which were used as the basis for calculating the SD (see **Supplemental Material 2** for additional details on manipulations to measures of variability).

Some studies required additional study-specific adjustments. Cox et al. (18) reported dietary intake information for men and women separately, although the lipoprotein measurements were pooled across sexes. We calculated the weighted mean of the dietary factors for men and women for use in determination of predicted LDL-cholesterol outcomes and model inputs. Burns-Whitmore et al. (19) measured study outcomes as geometric means (GMs). These were adjusted to arithmetic means (AMs) for consistency ($AM = \exp[\mu(L) + \sigma(L)/2]$, where $\mu(L) = \ln(GM)$, and $\sigma(L)$ is the SD on a logarithmic scale).

Meta-regression and Markov chain Monte Carlo implementation

Meta-regression modeling is a statistical approach for combining data from several primary sources and for estimating the relation between some explanatory variables and the response of interest. In our analysis, the primary sources were the controlled trials of dietary cholesterol intake and associated changes in serum cholesterol components. A Bayesian approach that uses Markov chain Monte Carlo (MCMC) techniques [implemented with R version 3.3.1 and the Stan statistical package (20)] was used throughout the analyses.

The primary outcome was the change in LDL-cholesterol concentration. Meta-regression modeling requires reporting of the variance of the effect sizes (magnitude of lipid concentration changes) in order to derive the log-likelihood values that drive the Bayesian updating. For a majority of the studies, the variance of the change in response (i.e., the difference between referent and intervention diet response) was not reported. When the variance of the change in response (change_var) was reported by the primary literature, that value was used. For studies where the variance of the change in response was not reported, that variance was calculated (see Supplemental Material 2 for additional details).

Three models were considered for assessment of the effect of the change in dietary cholesterol (ΔCHOL) on changes in circulating cholesterol-associated lipoproteins (e.g., ΔLDL cholesterol and ΔHDL cholesterol) with and without the modifying impact of baseline cholesterol intake (baselineCHOL in Equation *1*). All 3 models have the following form (for simplicity, the change in lipoprotein-cholesterol concentration is referred to below as Δlipoprotein):

$$\text{cholesterol} - \text{associated } \Delta\text{lipoprotein} = (b + \beta \times \text{baselineCHOL}) \times f_s(\Delta\text{CHOL});\ s = 1, 2, 3 \quad (1)$$

The 3 specific model types corresponding to the 3 choices for $f_s(x)$ are as follows:

$$f_1(x) = x \quad (2)$$

$$f_2(x) = x/(k + x) \quad (3)$$

$$f_3(x) = x^p/(k^p + x^p) \quad (4)$$

Equation *2* represents the set of linear models, Equation *3* represents a family of Michaelis-Menten (MM) models, and Equation *4* represents a family of "Hill" models. These models were selected to evaluate multiple dose-response shapes, specifically linear, low-dose linear (MM), and nonlinear sigmoidal shapes. All models assume an intercept of 0—that is, no cholesterol-associated Δlipoprotein when ΔCHOL = 0. The b parameter represents the slope of the change in lipoprotein response. The β parameter mediates the effect of baseline cholesterol intake—that is, the mean referent cholesterol intake (for crossover studies) or prediet cholesterol intake (for parallel studies). The parameter k represents the half-saturation lipoprotein change. Modeling was conducted with and without the β parameter for each of the 3 equations and for both LDL and HDL cholesterol.

TABLE 1 Prior distributions for model parameters

Parameter	Prior distribution	Parameters of the prior
b	Normal	Mean = 0, SD = 20
β	Normal	Mean = 0, SD = 20
k[1]	Log normal	Log-scale mean = 6.56, log-scale SD = 0.83
p[2]	Half-Cauchy	Location = 1, scale = 15

[1]For Michaelis-Menten and Hill models only.
[2]For Hill model only.

Because sex-based differences in HDL-cholesterol response may be expected (21), sensitivity analyses were conducted to determine if the change in HDL cholesterol associated with ΔCHOL is comparable in men and women who participated in the clinical trials. The proportion of women participating in each diet was incorporated into a modified version of Equation *1* (see **Supplemental Material 3**).

MCMC modeling, in essence, considers the model parameters to be random variables; knowledge or beliefs about the values of those variables is reflected, prior to the analysis, in terms of "prior distributions" describing the relative likelihood of the values of those parameters. The prior distributions are updated, yielding posterior distributions, based on the data included in the data set created for this analysis [see Allen et al. (22) for additional description of the MCMC technique].

The prior distributions assumed for this analysis are defined in **Table 1**. For this analysis, relatively uninformative priors were selected to let the data drive the parameter-updating process. The priors for b and β were normal, with mean = 0. In other words, no a priori assumption was made about the direction of lipid change in response to increased cholesterol intake. The SDs for b and β priors were quite large in relation to the expected change per unit change in cholesterol (intake or baseline, respectively). For the MM and Hill models the prior for k was assumed to be log normal, reflecting the logical constraint that the half-saturation value must be positive. The choice of parameters for that log-normal distribution entails that the mean and SD of the prior for k were both equal to 1000 mg/d [1000 mg/d ≈ exp(log-scale mean + log-scale SD/2)] and that the distribution assigns a reasonably large probability (slightly greater than 0.1) that the half-saturation parameter is >2000 mg/d, which was the upper range of the intake changes included in the database. The half-Cauchy distribution for the parameter p is restricted to values >1 (to prevent supralinear dose-response shapes) and has a maximum density for $p = 1$. The scale parameter for the p prior is somewhat larger than a priori beliefs about how nonlinear the Hill model might be [adapting reasoning presented by Gelman (23)].

Results

Literature search and data selection

The literature search initially identified 3616 abstracts, of which 77 unique publications meeting all inclusion criteria and no exclusion criteria were selected. Data from these 77 unique publications were extracted and used for the initial, unweighted meta-regression analyses for this project (**Figure 1**). Additional screening led to the exclusion of an additional 22 studies. Most of these studies were excluded because the reported metric of cholesterol intake could not be converted into milligrams per day due to various reasons (i.e., cholesterol intake was reported as millimoles per liter or milligrams per megaJoule); these exclusions took place during the extraction phase. Other reasons included duplication of data across 2 studies, lack of data to determine MUFA intake, parallel studies not reporting preintervention cholesterol intake, and variations in LDL-cholesterol calculation, which would render results inconsistent with other studies (see Supplemental Material 1, **Supplemental Table 2** for additional details).

In total, 55 studies with 120 unique diet conditions (after subtracting the reference condition) were included in the meta-regression analysis (see Supplemental Material 1 and **Supplemental Table 3** for the list of studies that were included in the meta-regression analysis and the number of unique diets per study). The complete data set for modeling is shown in Supplemental Material 1, **Supplemental Tables 4** and **5**. Risk of bias is expected to be minimal (see Supplemental Material 1). Descriptive summary statistics for reference diet cholesterol intake and prediet LDL- and HDL-cholesterol concentrations are shown in Supplemental Material 1, **Supplemental Table 6**. Studies that analyzed the impact of the dietary interventions on multiple subgroups have multiple referents, equal to the number of subanalyses in the study [e.g., Greene et al. (24), Herron et al. (25), Kestin et al. (26), Klass et al. (27), Noakes and Clifton (28), and Weggemans et al. (29)]; each subgroup is treated as a separate contributor to the likelihood calculations that underlie the MCMC computations.

Meta-regression modeling

Prior to modeling the dose-response relation between changes in cholesterol intake and changes in lipoprotein-cholesterol concentrations in the blood, serum, or plasma, preliminary analyses evaluated whether a trend of increasing or decreasing lipid concentrations with increasing change in cholesterol intake appears to exist for each of the 4 responses: LDL cholesterol, HDL cholesterol, VLDL cholesterol, and TC. Unweighted linear regression models (i.e., models that did not consider response variance) found that a dose-related trend was observed only for LDL cholesterol and TC (data not shown). This result is consistent with those from previous studies and the underlying biology. VLDL cholesterol is not expected to change in response to changes in dietary cholesterol (21, 30). Although TC does change with dietary cholesterol, Ginsberg et al. (21, 30) found that changes in LDL cholesterol mainly drove the observed change in TC (which includes LDL cholesterol, HDL cholesterol, and VLDL cholesterol). This means that any models of TC would be largely dependent on models of LDL cholesterol and detailed dose-response modeling of TC would not provide additional information beyond that obtained for modeling LDL cholesterol. Although HDL cholesterol did not appear to be affected by cholesterol intake overall in the preliminary analyses, Ginsberg et al. (21) suggested that the menstrual cycle affects HDL-cholesterol responses and that there may be sex-specific differences in response (i.e., a dose-response among women but not men). Based on the results of the unweighted linear regression analyses and the prior results by Ginsberg et al. (21), further

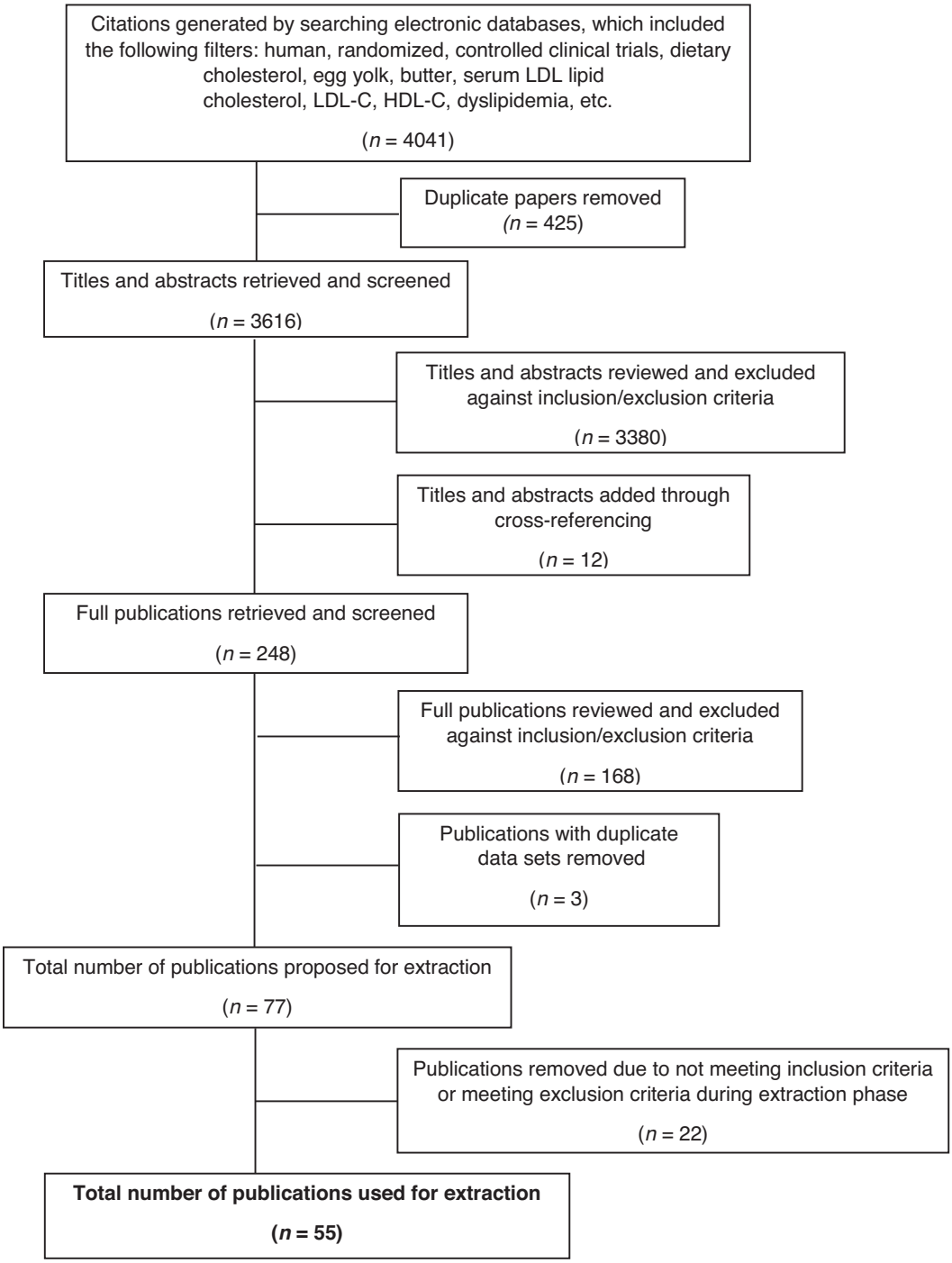

FIGURE 1 Literature search results. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

analyses were conducted only for LDL cholesterol and HDL cholesterol.

LDL-cholesterol analysis.

Convergence was achieved in each of the MCMC runs. Leave-one-out cross-validation was used to estimate relative model performance (31, 32). The nonlinear MM and Hill models fit the data better than the linear model based on leave-one-out information criterion (LOOIC) values, a tool that quantifies this comparison and considers the predictive ability of the model as well as the number of effective parameters; lower values of LOOIC indicate better performance (see Supplemental Material 3, **Supplemental Table 7** for additional details). Overall, the Hill model had the lowest LOOIC estimate, indicating a better fit than the MM or linear models. Inclusion of the baseline parameter (β), which accounts for the study participants' cholesterol intake at the beginning of the study or during the reference diet, did

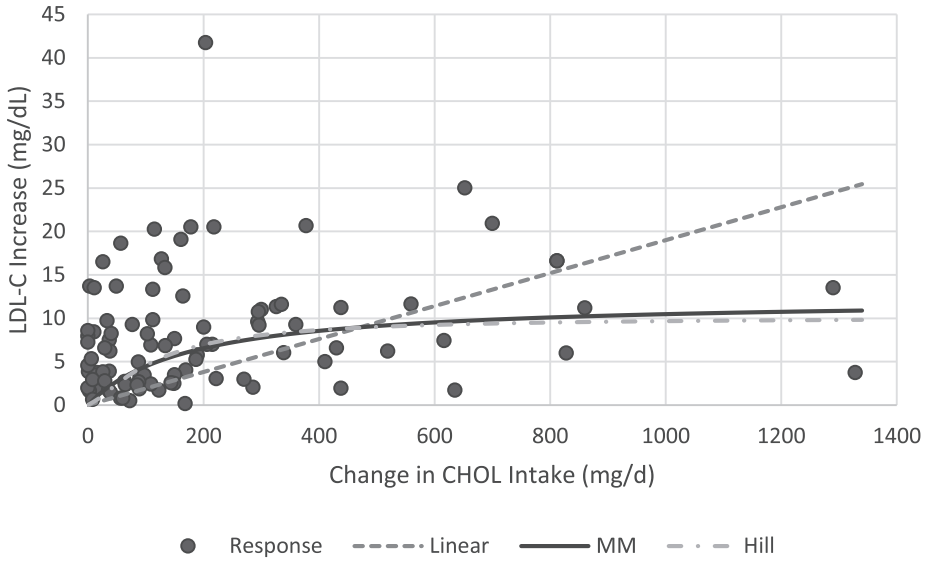

FIGURE 2 The best linear, MM, and Hill models showing the predicted LDL-cholesterol change associated with cholesterol intake change (baseline not included; $n = 55$ studies, 2652 subjects). Note that the MM and Hill models are virtually identical on these graphs and therefore are not easily distinguished. CHOL, cholesterol; LDL-C, LDL cholesterol; MM, Michaelis-Menten.

not improve the model fit. The predicted dose-responses, based on the value of the parameter vector (i.e., the vector of *b*, *k*, and *p*) yielding the maximum posterior density (i.e., the peak in the distribution of posterior parameter values) associated with changes in the full range of assessed dietary cholesterol intakes, are shown in **Figure 2** and **Table 2**. Table 2 also shows, for comparison, the predicted dose-responses associated when the input data for change in dietary cholesterol intake was limited to ≤400 mg/dL.

HDL-cholesterol analysis.

Convergence was achieved in each of the MCMC runs for the linear and MM models (with male and female subjects combined) but not the Hill model. Therefore, results are shown only for the linear and MM models. Inclusion of the baseline parameter, β, again does not improve the model fit (based on LOOIC estimates), and therefore the β parameter was not included in further modeling (see Supplemental Material 3, **Supplemental Table 8** for additional details). The model-predicted dose-responses, based on the value of the parameter vector yielding the maximum posterior density, are shown in **Figure 3** and **Table 3**.

When men and women are pooled together in the study samples, and the model makes no distinction between the sexes with respect to model parameter estimates (Equation *1*), there appears to be no significant increase or decrease in HDL-cholesterol concentrations; the posterior distribution–based 95% credible intervals for the *b* parameter all include 0 (Supplemental Material 3, Supplemental Table 8). A credible interval is a Bayesian equivalent to the CIs used in frequentist approaches. A 95% credible interval indicates that 95% of the posterior probability is within that range. However, sensitivity analyses indicate that there are sex-specific differences in the HDL-cholesterol response relative to dietary changes in cholesterol, as suggested by Ginsberg et al. (21) (Supplemental Material 3, **Supplemental Table 9**). Based on the conclusions from the previous analyses, baseline cholesterol intake was not included in these models. The predicted male and female HDL-cholesterol responses are nearly opposite in their direction and magnitude (**Figure 4**, **Table 4**).

Additional sensitivity analyses investigated the impact of study diets with no (0) change in cholesterol intake between the experimental and referent or prestudy diets. This analysis ensured that there was no bias due to the inclusion of diets where changes in dietary fats alone (e.g., MUFAs, PUFAs, and SFAs) drove the predicted changes in LDL- and HDL-cholesterol responses. Removal of these study conditions had no significant impact on the posterior distribution of the model parameters for either LDL or HDL cholesterol with men and women combined (data not shown).

TABLE 2 Predicted changes in LDL-cholesterol response associated with changes in the full range of assessed dietary cholesterol intakes[1]

	Predicted LDL-cholesterol change (mg/dL) based on		
ΔCHOL, mg/d	Linear model	MM model	Hill model
20	0.38 (0.65)	1.25 (1.11)	0.80 (0.81)
60	1.14 (1.96)	3.13 (2.96)	2.90 (2.75)
100	1.90 (3.27)	4.46 (4.45)	4.58 (4.45)
200	3.80 (6.54)	6.55 (7.16)	6.96 (7.38)
300	5.70 (9.81)	7.76 (8.98)	8.06 (9.11)
400	7.60 (13.1)	8.56 (10.3)	8.65 (10.2)
500	9.50	9.12	9.01
600	11.40	9.53	9.24

[1]Changes are based on the maximum posterior distribution estimates for each model (baseline not included). Predicted changes when modeled data are restricted to changes in cholesterol intake ≤400 mg/d are shown in parentheses. Model parameters are shown in Supplemental Material 3, Supplemental Table 7. MM, Michaelis-Menten; ΔCHOL, change in dietary cholesterol.

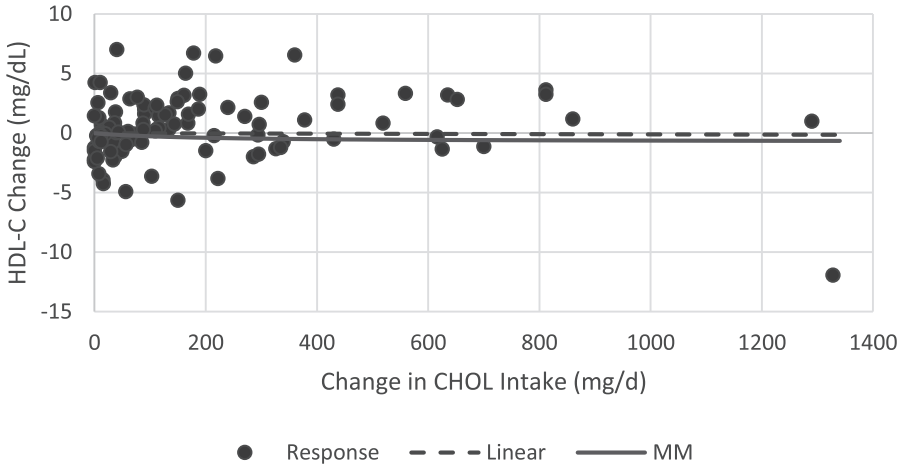

FIGURE 3 The best linear and MM models showing the predicted HDL-cholesterol change associated with cholesterol intake change ($n = 52$ studies, 2492 subjects). CHOL, cholesterol; HDL-C, HDL cholesterol; MM, Michaelis-Menten.

Discussion

The meta-regression analyses presented herein indicate that, after providing a theoretical control for intakes of SFAs, MUFAs, PUFAs, and when possible, TFAs, there is a positive, dose-related trend between changes in cholesterol intake and changes in circulating LDL cholesterol, a finding that is consistent with the published literature (21, 30, 33). The nonlinear dose-response shapes (MM and Hill) best fit the data. For a 100-mg/d increase in dietary cholesterol intake, circulating LDL-cholesterol concentrations are predicted to increase by 1.90 mg/dL (linear model), 4.46 mg/dL (MM model), or 4.58 mg/dL (Hill model). The linear analysis of LDL cholesterol produced a coefficient that is remarkably similar to that reported by Clarke et al. (33) of 1.935 mg/dL. A 200-mg/d increase in dietary cholesterol was predicted to increase circulating LDL cholesterol by 3.9 mg/dL based on the Clarke et al. (33) meta-analysis; the current meta-analysis calculated an increase in circulating LDL cholesterol of 3.8 mg/dL with the linear model. Since a dietary intake change of ≤400 mg cholesterol/d is a more representative range of dietary intakes in the United States, post hoc modeling was conducted to determine the effect of restricting the data to be modeled to that range. The results of modeling these restricted data should be interpreted with caution since they represent a smaller subset of the data. The purpose of this analysis was examination of the influence of "high" (>400 mg/d) changes in cholesterol intake on predicted LDL-cholesterol response. The impact of focusing on changes of ≤400 mg of dietary cholesterol intake/d was small for the nonlinear models and larger for the linear model (see Table 2). As with the primary model that used the full range of dietary cholesterol intake changes, the nonlinear models predict larger increases in LDL cholesterol from increases in dietary cholesterol of ≤400 mg/d compared with the linear model. The nonlinear models would be preferred for research purposes, such as predicting the effects of an experimental diet. However, given the known degree of interindividual variability, the linear model may be sufficient for rapid calculations in clinical settings, even though it may somewhat underestimate the effect of dietary cholesterol on LDL cholesterol, on average.

An additional post hoc sensitivity analysis investigated the impact of baseline, or prestudy, LDL-cholesterol concentration on changes in LDL-cholesterol response through the use of a modification of Equation *1*, where the β parameter is applied to baseline LDL cholesterol instead of baseline cholesterol. LOOIC comparisons indicate that the inclusion of background LDL cholesterol does not statistically improve the predictivity of the model, but the results do indicate that background LDL cholesterol may have a clinically relevant impact. For example, a change in cholesterol intake of 100 mg/d is predicted to increase LDL cholesterol by 2.7, 3.6, 4.6, and 5.5 mg/dL for individuals with baseline LDL-cholesterol values of 100, 125, 150, and 175 mg/dL, respectively. Although the predicted absolute change is numerically larger at higher baseline LDL-cholesterol concentrations, the percentage change is similar (e.g., 2.7% at a baseline LDL-cholesterol concentration of 100 mg/dL and 3.1% at a baseline LDL-cholesterol concentration of 175 mg/dL). However, this investigation does not support the hypothesis of a significant effect, and the predicted differences

TABLE 3 Predicted changes in HDL-cholesterol response associated with changes in dietary cholesterol intake[1]

	Predicted HDL-cholesterol change (mg/dL) based on	
ΔCHOL, mg/d	Linear model	MM model
20	0.00	−0.07
60	−0.01	−0.18
100	−0.01	−0.26
200	−0.02	−0.39
300	−0.03	−0.46
400	−0.04	−0.51
500	−0.05	−0.54
600	−0.06	−0.57

[1]Changes are based on the maximum posterior distribution estimates for the linear and MM models (baseline not included). Both male and female volunteers are included in these models. Model parameters are shown in Supplemental Material 3, Supplemental Table 8. MM, Michaelis-Menten; ΔCHOL, change in dietary cholesterol.

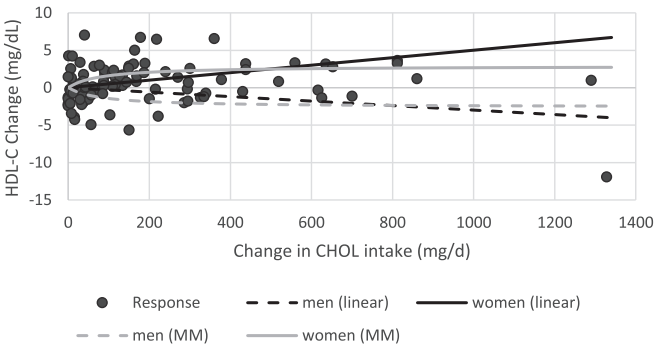

FIGURE 4 The best linear and MM models showing the predicted HDL-cholesterol change associated with cholesterol intake change, where the proportion of male and female subjects are used as parameters in the model (n = 52 studies, 2492 subjects). CHOL, cholesterol; HDL-C, HDL cholesterol; MM, Michaelis-Menten.

in response associated with difference in baseline need further exploration.

The impact of dietary cholesterol intake on circulating concentrations of HDL cholesterol is less clear. There appears to be no significant (positive or negative) relation between the change in dietary cholesterol intake and the change in circulating HDL-cholesterol concentration when data from men and women are pooled. The lack of effect of dietary cholesterol intake on overall HDL-cholesterol response was of interest, and thus additional post hoc analyses were conducted. The results of those analyses indicate that the effects of dietary cholesterol intake on circulating HDL-cholesterol concentration are sex specific, such that the change in dietary cholesterol intake is inversely related to the change in circulating HDL-cholesterol concentration in men and positively related to the change in circulating HDL cholesterol in women. These results remained consistent regardless of whether the linear or MM models were used. Although this dimorphic response is intriguing, the HDL-cholesterol modeling results need to be interpreted with caution for several reasons. First, the HDL-cholesterol models generated by the data in this meta-regression analysis do not allow for making precise predictions of the absolute change in HDL-cholesterol concentration, but rather, serve as a means to indicate a relative effect of increases or decreases.

Furthermore, the clinical relevance of changes in HDL cholesterol remain uncertain. Genetic variants that alter HDL cholesterol without affecting LDL or VLDL cholesterol, and pharmacologic interventions that increase HDL cholesterol, have not been found to affect CVD risk (5, 6, 34). This does not indicate that changes in HDL cholesterol induced by dietary alteration are not clinically important, but rather, that it is not possible at present to predict changes in CVD risk based on such changes. Various methods of altering HDL cholesterol may have differing effects on the functions of HDL. Additional research will be required to determine the potential clinical importance of diet-induced changes in HDL cholesterol. The modest decline in HDL cholesterol observed in men and the modest increase in HDL cholesterol observed in women in response to increasing dietary cholesterol resulted in nearly no change overall when the sex-specific samples were pooled. Thus, the observed alterations in HDL cholesterol were minimal compared with the changes observed in LDL cholesterol in response to cholesterol intake, so it is likely that net changes in TC are driven almost exclusively by changes in LDL cholesterol, particularly in the combined sample of men and women. Additional research will be needed to assess the reproducibility of this finding and to explore both mechanistic explanations and clinical relevance, if any.

Baseline cholesterol intake was initially included in the meta-regression models because it was hypothesized that baseline cholesterol intake would affect the lipoprotein response. The model indications that baseline is not a significant explanatory factor (i.e., the models that include β do not improve fit, based on LOOIC values) are counter to our original hypothesis. More research is needed to better understand the possible modifying impact of baseline cholesterol intake and other factors (e.g., baseline lipoprotein concentrations).

Background diets, particularly the mix of types of fatty acids, affect circulating LDL- and HDL-cholesterol concentrations. The effects of differences between diets in dietary fatty acid intakes were accounted for by adjusting the HDL- and LDL-cholesterol responses to account for the impacts of changes in SFAs, PUFAs, and MUFAs according to the regression coefficients detailed in Mensink et al. (8). This allowed us to theoretically estimate how much of the changes in LDL and HDL cholesterol are attributable to changes in dietary cholesterol alone. Ideally, we would also include TFAs in the adjustments for each of the studies, because changes in TFA intake also affect LDL-cholesterol changes (22, 35). However, the available literature rarely reports quantitative TFA intake; only 6 of the 55 included studies quantitatively report dietary TFA intake as a percentage of dietary energy or grams per day (36–41). Better characterization and reporting of differences in TFA intake is needed in order to examine the impact of dietary cholesterol in isolation. It is possible that residual confounding may still exist, and the correction for the simultaneous effect of cholesterol and fatty acid intake within the food matrix may be incomplete. However, the results obtained for the linear model are very similar to those obtained from the analysis completed by Clarke et al. (33) and to the results obtained by Ginsberg et al. (21, 30) from feeding studies in men and women. Thus, although the limitations of the methods used are acknowledged, the findings

TABLE 4 Predicted changes in HDL-cholesterol response associated with changes in dietary cholesterol intake[1]

	Predicted HDL cholesterol change (mg/dL) based on			
	Linear model		MM model	
ΔCHOL, mg/d	Men only	Women only	Men only	Women only
20	−0.06	0.10	−0.52	0.58
60	−0.18	0.30	−1.11	1.24
100	−0.30	0.50	−1.44	1.61
200	−0.6	1.0	−1.86	2.07
300	−0.9	1.5	−2.05	2.29
400	−1.2	2.0	−2.17	2.42
500	−1.5	2.5	−2.24	2.50
600	−1.8	3.0	−2.29	2.56

[1]Changes are based on the maximum posterior distribution estimates for each model (baseline not included). The proportion of male and female subjects in the study population is included as a model variable. Model parameters are shown in Supplemental Material 3, Supplemental Table 9. MM, Michaelis-Menten; ΔCHOL, change in dietary cholesterol.

appear consistent with those from earlier analyses and the best evidence from tightly controlled feeding trials.

The results from these meta-analyses have potential implications for dietary recommendations. In recent years, there has been inconsistency in the published research about the role of dietary cholesterol in increasing plasma cholesterol concentration (7, 42). Based on the mean of the predictions from the 2 best-fitting nonlinear models, the findings from this meta-regression of randomized controlled trials suggest that a 100-mg/d change in dietary cholesterol would alter LDL cholesterol by 4.52 mg/dL. The corresponding value for a 200-mg/d change would be 6.76 mg/dL. For comparison, the equation given by Mensink et al. (8) predicts a change of 1.23 mg/dL for each 1% increase in SFAs in exchange for carbohydrate. Therefore, based on the nonlinear models, increasing dietary cholesterol by 100 mg/d would be predicted to have an effect comparable to that of increasing dietary SFAs by 3.7%, and increasing dietary cholesterol by 200 mg/d is predicted to be comparable to increasing dietary SFAs by 5.5%. A large egg contains ~185 mg cholesterol (43) and would therefore be expected to increase the LDL-cholesterol concentration by 6.5 mg/dL based on the nonlinear models. However, the majority of dietary cholesterol is not attributable to egg intake in the United States, except for individuals in the highest quartile of TC intake (44). According to a recent publication reporting dietary sources of cholesterol in US adults aged ≥20 y based on NHANES 2013–2014 data, mean dietary cholesterol intake was 293 mg/d and the primary dietary cholesterol source in the overall population was meat (defined as poultry, mixed dishes, red meat, processed meat, and seafood), which accounted for 42% of dietary cholesterol (44). The Institute of Medicine (35) recommends that individuals consume as little dietary cholesterol as possible, consistent with consuming a healthy dietary pattern. The Healthy US-Style Eating Pattern advocates a dietary cholesterol intake of ~100–300 mg /d (7).

In conclusion, the results of this meta-regression, which used data only from randomized controlled dietary intervention trials, indicate that there is a positive, nonlinear relation between the change in dietary cholesterol and the change in LDL cholesterol, after controlling for the effects of changes in fatty acid intakes. The association between change in dietary cholesterol and change in HDL cholesterol is more complex and appears to be modified by sex, showing a modest positive relation in women and a modest inverse relation in men.

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References

1. CDC. Deaths: final data for 2013. Natl Vital Stat Rep 2015;64(2):1–119.
2. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Miller NH, Hubbard VS, Lee IM, Lichtenstein AH, Loria CM, Millen BE, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:2960–84.
3. Jacobson TA, Ito MK, Maki KC, Orringer C, Bays HE, Jones PH, McKenney JM, Grundy SM, Gill EA, Wild RA, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1. J Clin Lipidol 2015a;9(2 Suppl):S129–69.
4. Jacobson TA, Maki KC, Orringer C, Jones PH, Kris-Etherton P, Sikand G, La Forge R, Daniels SR, Wilson DP, Morris PB, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 2. J Clin Lipidol 2015b;9(6 Suppl): S1–122.
5. Boekholdt SM, Arsenault BJ, Hovingh GK, Mora S, Pedersen TR, Larosa JC, Welch KM, Amarenco P, Demicco DA, Tonkin AM, et al. Levels and changes of HDL cholesterol and apolipoprotein A-1 in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. Circulation 2013;128(14):1504–12.
6. Vitali C, Khetarpal SA, Rader DJ. HDL cholesterol metabolism and the risk of CHD: new insights from human genetics. Curr Cardiol Rep 2017;19(12):132.
7. US Department of Health and Human Services; USDA. 2015-2020 Dietary guidelines for Americans [Internet]. 8th ed, Washington (DC); USDA, 2015. [Cited 20 November 2018]. Available from: http://health.gov/dietaryguidelines/2015/guidelines/.
8. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77(5): 1146–55.
9. Becker CC, Lund P, Holmer G, Jensen H, Sandstrom B. Effects of butter oil blends with increased concentrations of stearic, oleic and linolenic acid on blood lipids in young adults. Eur J Clin Nutr 1999;53(7):535–41.
10. Fumeron F, Brigant L, Parra H-J, Bard J-M, Fruchart J-C, Apfelbaum M. Lowering of HDL2-cholesterol and lipoprotein A-I particle levels by increasing the ratio of polyunsaturated to saturated fatty acids. Am J Clin Nutr 1991;53:655–9.
11. Klass DM, Buhrmann K, Sauter G, Del Puppo M, Scheibner J, Fuchs M, Stange EF. Biliary lipids, cholesterol and bile synthesis: different adaptive mechanisms to dietary cholesterol in lean and obese subjects. Aliment Pharmacol Ther 2006;23(7):895–905.
12. Lewis NM, Schalch K, Scheideler SE. Serum lipid response to n-3 fatty acid enriched eggs in persons with hypercholesterolemia. J Am Diet Assoc 2000;100(3):365–7.
13. Sabate J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD. Effects of walnuts on serum lipid levels and blood pressure in normal men. N Engl J Med 1993;328(9):603–7.
14. Sehayek E, Nath C, Heinemann T, McGee M, Seidman CE, Samuel P, Breslow JL. U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans. J Lipid Res 1998;39(12):2415–22.
15. Tholstrup T, Hjerpsted J, Raff M. Palm olein increases plasma cholesterol moderately compared with olive oil in healthy individuals. Am J Clin Nutr 2011;94(6):1426–32.
16. Weggemans RM, Zock PL, Meyboom S, Funke H, Katan MB. Apolipoprotein A4-1/2 polymorphism and response of serum lipids to dietary cholesterol in humans. J Lipid Res 2000;41(10):1623–8.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.
18. Cox CM, Sutherland WH, Ball MJ, Mann JI. Response of plasma lathosterol concentration to change in the quality of dietary fat in men and women. Eur J Clin Nutr 1996;50:358–63.
19. Burns-Whitmore B, Haddad E, Sabate J, Sabaté J, Rajaram S. Effects of supplementing n-3 fatty acid enriched eggs and walnuts on cardiovascular disease risk markers in healthy free-living lacto-ovo-vegetarians: a randomized, crossover, free-living intervention study. Nutr J 2014;13(29):1–9.

20. Stan Development Team, RStan: the R interface to Stan [Internet]. R package version 2.16.2. 2017. [Cited 20 November 2018]. Available from: http://mc-stan.org.
21. Ginsberg HN, Karmally W, Siddiqui M, Holleran S, Tall AR, Blaner WS, Ramakrishnan R. Increases in dietary cholesterol are associated with modest increases in both LDL and HDL cholesterol in healthy young women. Arterioscler Thromb Vasc Biol 1995;15:169–78.
22. Allen B, Vincent M, Liska D, Haber L. Meta-regression analysis of the effect of trans fatty acids on low-density lipoprotein cholesterol. Food Chem Toxicol 2016;98(Part B):295–307.
23. Gelman A. Prior distributions for variance parameters in hierarchical models (comment on article by Browne and Draper). Bayesian Anal 2006;1(3):515–34.
24. Greene CM, Zern TL, Wood RJ, Shrestha S, Aggarwal D, Sharman MJ, Volek JS, Fernandez ML. Maintenance of the LDL cholesterol:HDL cholesterol ratio in an elderly population given a dietary cholesterol challenge. J Nutr 2005;135:2793–8.
25. Herron KL, Vega-Lopez S, Conde K, Ramjiganesh T, Shachter NS, Fernandez ML. Men classified as hypo- or hyperresponders to dietary cholesterol feeding exhibit differences in lipoprotein metabolism. J Nutr 2003;133(4):1036–42.
26. Kestin M, Clifton PM, Rouse IL, Nestel PJ. Effect of dietary cholesterol in normolipidemic subjects is not modified by nature and amount of dietary fat. Am J Clin Nutr 1989;50:528–32.
27. Klass DM, Buhrmann K, Sauter G, Del Puppo M, Scheibner J, Fuchs M, Stange EF. Biliary lipids, cholesterol and bile synthesis: different adaptive mechanisms to dietary cholesterol in lean and obese subjects. Aliment Pharmacol Ther 2006;23:895–905.
28. Noakes M, Clifton P. Oil blends containing partially hydrogenated or interesterified fats: differential effects on plasma lipids. Am J Clin Nutr 1998;68(2):242–7.
29. Weggemans RM, Zock PL, Meyboom S, Funke H, Katan MB. Apolipoprotein A4-1/2 polymorphism and response of serum lipids to dietary cholesterol in humans. J Lipid Res 2000;41:1623–8.
30. Ginsberg HN, Karmally W, Siddiqui M, Holleran S, Tall AR, Rumsey SC, Deckelbaum RJ, Blaner WS, Ramakrishnan R. A dose-response study of the effects of dietary cholesterol on fasting and postprandial lipid and lipoprotein metabolism in healthy young men. Arterioscler Thromb Vasc Biol 1994;14:576–86.
31. Gelman A, Hwang J, Vehtari A. Understanding predictive information criteria for Bayesian models. Stat Comput 2014;24(6):997–1016.
32. Vehtari A, Gelman A, Gabry J. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Stat Comput 2017;27(5):1413–32.
33. Clarke R, Frost C, Collins R, Appley P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ 1997;314(7074):112–7.
34. Burgess S, Harshfield E. Mendelian randomization to assess causal effects of blood lipids on coronary heart disease: lessons from the past and applications to the future. Curr Opin Endocrinol Diabetes Obes 2016;23(2):124–30.
35. Institute of Medicine. Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington (DC): National Academies Press; 2005.
36. Chen SC, Judd JT, Kramer M, Meijer GW, Clevidence BA, Baer DJ. Phytosterol intake and dietary fat reduction are independent and additive in their ability to reduce plasma LDL cholesterol. Lipids 2009;44:273–81.
37. Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43:445–52.
38. van der Made SM, Kelly ER, Berendschot TTJM, Kijlstra A, Lutjohann D, Plat J. Consuming a buttermilk drink containing lutein-enriched egg yolk daily for 1 year increased plasma lutein but did not affect serum lipid or lipoprotein concentrations in adults with early signs of age-related macular degeneration. J Nutr 2014;144(9):1370–7.
39. Tholstrup T, Hjerpsted J, Raff M. Palm olein increases plasma cholesterol moderately compared with olive oil in healthy individuals. Am J Clin Nutr 2011;94:1426–32.
40. Thorning TK, Raziani F, Bendsen NT, Astrup A, Tholstrup T, Raben A. Diets with high-fat cheese, high-fat meat, or carbohydrate on cardiovascular risk markers in overweight postmenopausal women: a randomized crossover trial. Am J Clin Nutr 2015;102:573–81.
41. Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ. Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. New Engl J Med 1999;340(25):1933–40.
42. Berger S RG, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr 2015;102(2):276–94.
43. USDA, Agricultural Research Service, Nutrient Data Laboratory. USDA branded food products database [Internet]. Version current: January 2017. [Cited 20 November 2018]. Available from: http://ndb.nal.usda.gov.
44. Xu Z, McClure ST, Appel LJ. Dietary cholesterol intake and sources among US adults: results from National Health and Nutrition Examination Surveys (NHANES), 2001–2014. Nutrients 2018;10(6):771.