Supplementary Material

Methods for meta-regression analysis

Eligibility criteria

The aim was to examine the independent effect of a change in dietary cholesterol intake on lipids and lipoproteins by conducting a meta-regression analysis of data from controlled feeding studies including adults where the PUFA to saturated fat ratio (P:S) was matched in the referent and intervention arms. Studies were eligible for inclusion if a cross over or parallel controlled feeding design was used, whereby subjects were provided with a complete menu for the duration of the study. In addition, dietary cholesterol intake was the only difference between the treatment and control groups in the included studies. The reported data had to enable comparison of the effect of dietary cholesterol intake at differing levels while the P:S was held constant. And dietary cholesterol had to be reported in mg/day or a manner that enabled calculation of mg/day. Finally, eligible studies included adults (≥18 years), and reported outcomes included either total cholesterol, LDL-cholesterol or HDL –cholesterol.

Studies were not eligible for inclusion if there was no control or referent condition, or there was a difference in the P:S ratio of the control/referent condition vs. the intervention period/arm. In addition, studies where a concomitant intervention (e.g. weight loss or other dietary interventions, drugs or supplements) was administered were ineligible. Studies where only partial provision of food items or total daily caloric intake occurred were excluded.

Search strategy

Tables S3-5 summarize the search strategies that were used to search Medline (Ovid), CINAHL, and the Cochrane Library since the year of index for each database through October 16, 2018.

Table S3: Search strategy used for Medline (Ovid)

1. cholesterol, dietary/ OR cholesterol intake.mp. OR exp dietary fats
2. exp eggs
3. exp meat OR exp seafood OR exp poultry
4. 1 OR 2 OR 3
5. cholesterol, LDL/ OR cholesterol, HDL/ OR cholesterol/
6. 4 and 5
7. limit 6 (English language; human; clinical trial, all; all adult (19 plus years))

Table S4: Search strategy used for CINAHL

1. TX "dietary cholesterol" OR TX "cholesterol intake" OR TX "Dietary fats"
2. TX eggs
3. TX meat OR TX seafood OR TX poultry
4. S1 OR S2 OR S3

5. TX "LDL cholesterol" OR TX "HDL cholesterol" OR TX cholesterol
6. 4 and 5
7. 6 limit to academic journal, adult: 19-44 years, English language)

Table S5: Search strategy used for the Cochrane Library

1. ("dietary cholesterol"):kw OR ("cholesterol intake"):ti,ab,kw OR ("dietary fats"):ti,ab,kw
2. eggs
3. (meat):kw OR (seafood):kw OR (poultry):kw
4. #1 or #2 or #3
5. ("LDL cholesterol"):kw OR ("HDL cholesterol"):kw OR (cholesterol):kw
6. #4 and #5

Study Selection

Figure S1 summarizes the number of studies that were identified by the database search, screened for eligibility, reasons for exclusion, and the total number of studies eligible for inclusion in the meta-regression analysis.

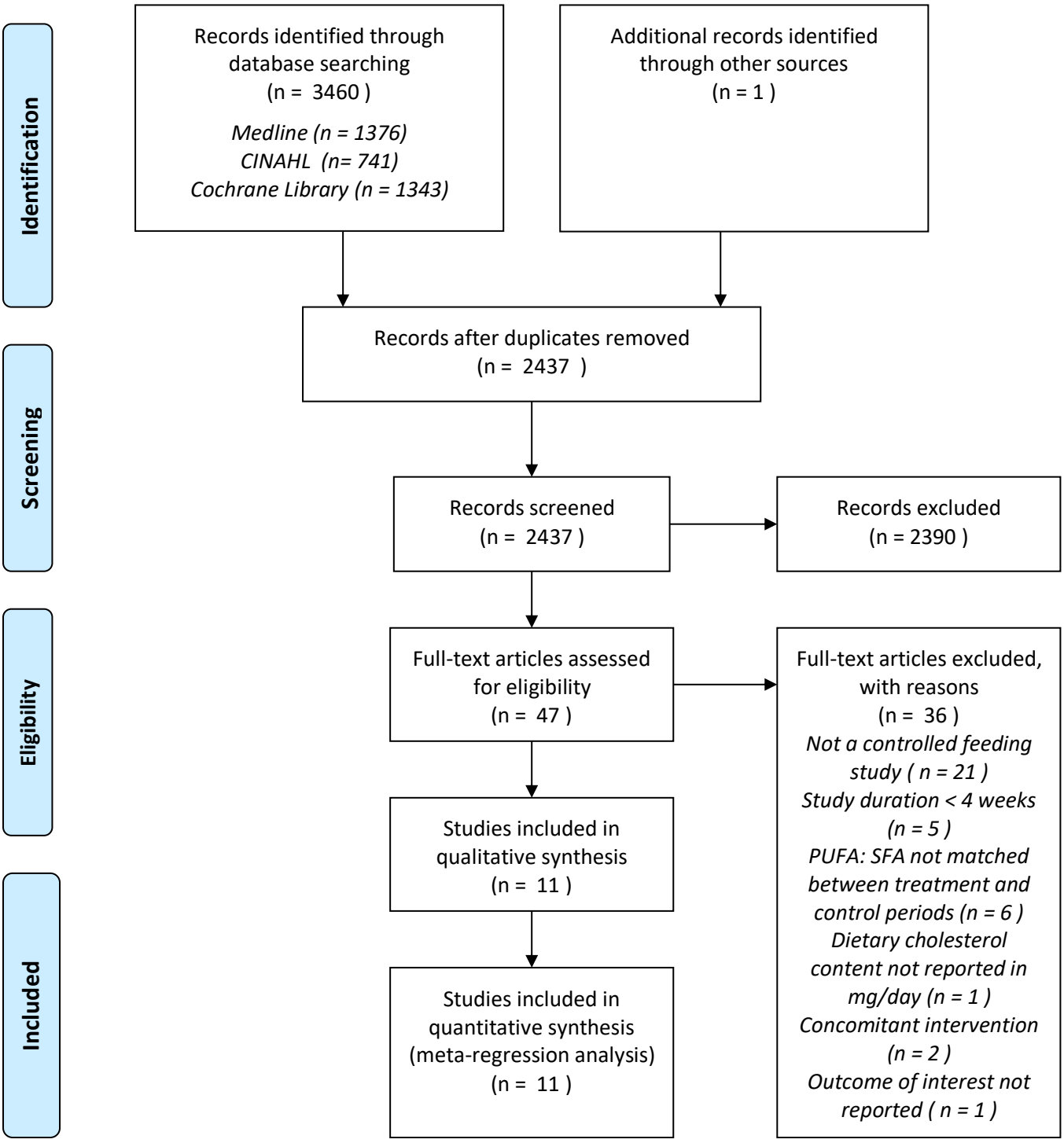

Figure S1: PRISMA flow diagram

Data extraction

Extracted data were entered into a standardized spreadsheet. The following items were extracted: study design (parallel; crossover); number of subjects included in analyses; dietary cholesterol intake in the control/referent condition, and intervention condition; for each outcome measure: mean (or median) and variance, or change from baseline and variance for the intervention and control condition, or the effect size; P:S ratio; presence of hypercholesterolemia (> 200 mg/dl); presence of diabetes.

Data analysis

For cross-over studies, the mean difference in dietary cholesterol intake between the intervention period and the referent/ control period was used for analysis. In the case of more than two levels of intake, the arm with the lowest dietary cholesterol intake was used as the comparator. Blood cholesterol response was defined as the difference between the mean value at the end of the intervention period and the mean value during the referent/control period. Where this was not reported, the within-treatment change from baseline values were used to calculate the between treatment difference. If data were not reported in the required format transformations were made as follows:

Within treatment

$SD = SE \times \sqrt{n}$

Between-treatment

Mean difference = $Mean_{intervention} - Mean_{referent}$

SD of the mean difference = $\sqrt{SD_{intervention}^2 + SD_{referent}^2 - (2 \times corr \times SD_{intervention} + SD_{referent})}$

Correlation was imputed as 0.5 if it could not be derived from the data presented.

SE of the mean difference = SD mean difference/ $\sqrt{N}$

For parallel studies, the within-treatment change in dietary cholesterol intake from baseline was used to calculate the between treatment difference. Similarly, within-treatment change in blood cholesterol was used to calculate the between treatment difference. If data were not reported in the required format transformations were made as follows:

Within group

$SD = SE \times \sqrt{n}$

Within group change from baseline = $mean_{post\ intervention} - mean_{pre\ intervention}$

SD of the within group change from baseline = $\sqrt{SD_{baseline}^2 + SD_{post}^2 - (2 \times corr \times SD_{baseline} + SD_{post})}$

Correlation was imputed as 0.5 if it could not be derived from the data presented.

Between –group
Mean difference = Mean $_{\text{intervention}}$ – Mean $_{\text{referent}}$

SE for the between –group mean difference computed using the metan command in Stata

If outcome measurements occurred at a number of time points the longest duration from baseline was used for analysis. In addition, if follow-up occurred after a period since the active intervention ended, data from end of the active phase were used for analysis.

Meta-regression analyses were conducted using STATA 15.1 (Stata Corp, College Station, Texas, USA). Random-effect models were used due to the between-study heterogeneity present and generated using the metareg command. The presence of a log-linear association between LDL-cholesterol and dietary cholesterol was explored by regressing the natural logarithm of LDL-cholesterol (and the standard error) against dietary cholesterol using metareg. Between study variance was estimated by the residual (restricted) maximum likelihood (REML) method with Knapp- Hartung modification. The analyses were repeated with each individual study removed from the model to assess the impact of each included study. P-values <0.05 were considered statistically significant.

Table S6: Sub-group meta-regression analyses by study design and cut-offs for changes in dietary cholesterol intake

	Total Cholesterol		LDL-cholesterol		HDL-cholesterol	
	n	Coefficient	n	Coefficient	n	Coefficient
All studies	25	0.016 (0.002, 0.03) p=0.024	19	0.007 (-0.008, 0.022) p=0.335	16	0.005 (-0.0004, 0.011) p=0.064
Crossover studies only	19	0.018 (0.004, 0.031) p=0.014	15	0.009 (-0.005, 0.023) p=0.179	12	0.003 (-0.005, 0.011) p=0.42
Parallel studies only	6	0.012 (-0.033, 0.057) p=0.560	4	0.001 (-0.070, 0.072) p=0.942	4	0.009 (-0.013, 0.030) p=0.22
All studies with change in dietary cholesterol ≤ 300 mg/day	7	0.0009 (-0.074, 0.076) p=0.98	7	-0.006 (-0.078, 0.065) p=0.883	6	0.003 (-0.038, 0.044) p=0.84
All studies with change in dietary cholesterol > 300 mg/day	18	0.005 (-0.015, 0.025) p=0.63	12	-0.005 (-0.027, 0.017) p=0.63	10	0.008 (-0.001, 0.017) p=0.075
All studies with a change in dietary cholesterol < 1000 mg/day	23	0.017 (0.001, 0.032) p=0.036	17	0.007 (-0.011, 0.026) p=0.428	14	0.004 (-0.004, 0.012) p=0.294

Table S7: Leave-one-out sensitivity analysis for total cholesterol

	Total Cholesterol	
	n	Coefficient
All studies linear model	25	0.016 (0.002, 0.03) p=0.024
Remove: Chenoweth 1981	23	0.016 (0.001, 0.030) p=0.035
Remove: Ginsberg 1995	23	0.015 (-0.0007, 0.031) p=0.061
Remove: Ginsberg 1994	22	0.017 (0.0008, 0.033) p=0.040

Remove: Illingworth 1995	19	0.015 (-0.0004, 0.031) p=0.056
Remove: Johnson 1990	24	0.016 (0.002, 0.030) p=0.027
Remove: Reaven 2001	22	0.022 (0.006, 0.037) p=0.008
Remove: Connor 1964	23	0.012 (-0.002, 0.027) p=0.082
Remove: Bowman 1988	23	0.017 (0.005, 0.030) p=0.009
Remove: Fielding 1995	23	0.016 (0.002, 0.030) p=0.023
Remove: Flaim 1981	24	0.017 (0.002, 0.032) p=0.024
Remove Quig 1983	24	0.016 (0.001, 0.030) p=0.035

Table S8: Leave-one-out sensitivity analysis for HDL-cholesterol

	HDL-cholesterol	
	n	Coefficient
All studies linear model	16	0.005 (-0.0004, 0.011) p=0.064
Remove: Ginsberg 1995	14	0.006 (-0.0004, 0.012) p=0.065
Remove: Ginsberg 1994	13	0.007 (-0.0009, 0.014) p=0.079
Remove: Illingworth 1995	10	0.006 (-0.0008, 0.012) p=0.079
Remove: Johnson 1990	15	0.005 (-0.0004, 0.012) p=0.065
Remove: Bowman 1988	14	0.005 (-0.001, 0.011) p=0.119
Remove: Flaim 1981	15	0.007 (0.0006, 0.013) p=0.035
Remove Quig 1983	15	0.003 (-0.004, 0.011) p=0.44

Table S9: Leave-one-out sensitivity analysis for LDL-cholesterol

	LDL-cholesterol	
	n	Coefficient
All studies linear model	19	0.007 (-0.008, 0.022) p=0.335
Remove: Ginsberg 1995	17	0.005 (-0.011, 0.022) p=0.493
Remove: Ginsberg 1994	16	0.007 (-0.011, 0.025) p=0.419
Remove: Illingworth 1995	13	0.004 (-0.013, 0.022) p=0.572
Remove: Johnson 1990	18	0.007 (-0.008, 0.022) p=0.339
Remove: Reaven 2001	16	0.01 (-0.007, 0.028) p=0.221
Remove: Bowman 1988	17	0.009 (-0.003, 0.021) p=0.123
Remove: Flaim 1981	18	0.007 (-0.009, 0.023) p=0.359
Remove Quig 1983	18	-0.007 (-0.010, 0.02) p=0.394