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Meta-analyses

High cholesterol intake is associated with elevated risk of type 2 diabetes mellitus – A meta-analysis[1–4]

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SUMMARY

Background & aims: Some foods rich in cholesterol are associated with high risk of type 2 diabetes (T2D). To confirm the association between dietary cholesterol intake and T2D risk, we performed a meta-analysis of observational studies.
Methods: We searched for longitudinal studies that provided data on the relative risk (RR) for T2D in relation to the cholesterol intake level using MEDLINE (from 1950 for July 10, 2013) and EMBASE (from 1974 to July 10, 2013). The RR for the highest vs. lowest cholesterol intake category or for an increment of 100 mg/day in cholesterol consumption was pooled with an inverse-variance method.
Results: Five studies met the inclusion criteria. Compared with the lowest category, the highest category had a significantly higher association with T2D risk (RR [95% confidence interval (CI)], 1.25 [1.16–1.36]). The pooled RR for a 100-mg/day increment was also significant (RR [95% CI], 1.11 [1.06–1.15]).
Conclusion: Current meta-analysis suggested that high intake of cholesterol was positively associated with future T2D risk.

1. Introduction

Cholesterol is one of the most common nutrients; it is recommended that its intake is controlled in relation to the prevention of coronary heart disease (CHD).[1] Indeed, a well-known association exists between dietary cholesterol intake and elevated risk of CHD.[2]

Type 2 diabetes (T2D), as well as CHD, is a global epidemic. Interestingly, both T2D and CHD share insulin resistance as a precursor of disease.[3] Moreover, epidemiological studies have indicated that a high intake of processed meat,[4–7] red meat,[6] and eggs,[5] all of which are rich in cholesterol, is associated with a high risk of T2D. However, some studies did not find an association between cholesterol intake and risk of T2D.[8,9] In addition, the quantitative association between cholesterol and T2D (i.e. T2D risk per incremental increase in cholesterol intake) has not been determined.

The aim of this meta-analysis was to summarize findings in previous longitudinal studies evaluating the association between cholesterol intake and incidence of T2D, and to determine the quantitative association between dietary cholesterol and T2D.

2. Methods

2.1. Search strategies

An electronic literature search was conducted to identify longitudinal studies that investigated the associations between high cholesterol intake and the incidence of T2D using the search engine Embase.com, which incorporates MEDLINE (from 1950 for July 10, 2013) and EMBASE (from 1974 to July 10, 2013) so that these two databases could be searched simultaneously. Details of search terms are provided in Supplemental Table 1. We added a manual search using the reference lists of the relevant articles. This process was repeated until no additional articles could be identified. No language restriction was imposed. For inclusion, a study had to fulfill the following criteria: 1) use of a longitudinal design; 2) T2D as an independent outcome; and 3) relative risk (RR) for the highest

Abbreviations: CI, confidence interval; lnRR, natural logarithm of relative risk; RR, relative risk; T2D, type 2 diabetes.

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category or several categories as compared with lowest category of cholesterol intake. When multiple articles were published on one study,[10,11] the article giving most detailed data was selected.[10]

2.2. Data extraction and quality assessment

Two of the investigators (R.T. and S.K.) independently identified eligible studies, reviewed them, extracted all relevant data and assessed study quality. Discrepancies were resolved by a third investigator (H.S.). The data that was abstracted included the first author's name, year of publication, country of origin, study design, methods for ascertaining T2D, mean or range of follow-up duration, mean or range of participants' age, participants' sex, range of exposure, number of participants and T2D events, and adjusted variables. Study quality was assessed by the Newcastle–Ottawa Scale, which was modified to make it possible to apply to this meta-analysis (Supplemental Table 2). This scale was composed of 9 items, and we awarded each item one point if the study met the criteria for that individual item. If the total score was more than 6, we judged the study quality as high; otherwise it was judged as low. If one article reported two or more RRs, the fully-adjusted RRs were used after excluding the RR adjusted for some fat subtypes (e.g. saturated fatty acid or monounsaturated fatty acid) so that over adjustment due to the strong association between the two intakes of fat and cholesterol could be avoided. We considered that they were strongly correlated with dietary cholesterol intake.

2.3. Data synthesis and analysis

To qualitatively summarize the association of habitual high intake of cholesterol with the risk of T2D, the RR for the highest vs. lowest cholesterol intake category was selected (Qualitative analysis). We added a quantitative analysis to estimate the RR of T2D for an incremental increase of 100 mg/day in cholesterol consumption. For studies that analyzed the cholesterol intake level not on a continuum but as a categorical variable, we used the method for trend estimation (Stata GLST command)[12] to estimate RR in individual studies by regressing the lnRR for the difference in mean exposure between each risk group and the lowest intake group. This method enabled us to correct for covariance between risk estimates from the same study and to estimate the corrected linear trend using generalized least squares if data on the adjusted RR and the number of participants (or person-time) and cases for each category were provided.

In both the qualitative and quantitative analyses, the RR in each study was transformed into a natural logarithm (lnRR) and the lnRR was pooled using an inverse-variance method, where the random-effects model was adopted if between-study heterogeneity which was assessed by Q statistics and I-squared[13] was significant; otherwise the fixed effects model was adopted. Finally, the overall RR was calculated by exponentiation of the pooled RR. We also conducted sensitivity analyses, stratifying the included studies by key factors related to study quality or participant characteristics that we *a priori* identified based on the data extracted from the included studies. These were mean follow-up duration (<10 or ≥ 10 years), participants' sex, methods for identifying T2D (self-report or other), study quality score (<6 or ≥ 6), and adjustment for total energy intake. Meta-regression analyses were conducted to detect the study characteristics that significantly influenced the results.

Publication bias was primarily assessed by funnel plot, where the estimated RR in each study was plotted against its corresponding standard error, assuming that the plot is symmetrical in the absence of publication bias. Statistical assessments were secondarily added to ascertain publication bias, using two formal methods, Begg's rank correlation[14] and Egger's regression test.[15] Data were analyzed using STATA software version 11 (STATA Corporation, College Station, TX, USA). $P < 0.05$ was considered as statistically significant except for the test of publication bias, in which the level of significance was $P < 0.10$.[16]

3. Results

3.1. Study characteristics

Figure 1 shows details of the literature search. Our electronic literature search resulted in retrieval of 3968 citations. Of these, 3946 were excluded based on the title and abstract. This left 22 articles as well as 12 additional articles identified by the manual search for full-text review. After this review, of the 34 papers, 29 were excluded for the reasons shown in Fig. 1. Finally, we identified 5 studies[8–10,17,18] that included a total of 203,903 participants and 7589 incident cases.

Table 1 shows the characteristics of the included studies. All 5 studies were conducted in the U.S. One study included men only[8] and 3 women only.[10,17,18] One study included both sexes and reported separate results according to sex.[9] Mean follow-up duration ranged from 8.8 to 14 years. For assessing habitual dietary cholesterol intake, all studies used validated food frequency questionnaires. Incident T2D cases were identified by self-report in 4 studies.[8,10,17,18] Among these 4 studies, 2 followed up the information with supplementary questionnaires.[8,18] One study used measurements of blood glucose and medication inventory information to identify incident cases.[9]

All included studies adjusted the RR for the following 5 confounders: age, sex, body mass index, physical activity, alcohol intake, and smoking habit. All but one study[9] adjusted for total energy intake. Study quality was assessed using the Newcastle–Ottawa Scale; only 2 studies[9,17] fulfilled the criteria for a score ≥ 6 on the scale of 9.

3.2. Cholesterol intake and risk of T2D

Figure 2 shows pooled RRs for T2D for the highest category of cholesterol consumption as compared with the lowest category of cholesterol consumption. The method for categorization of cholesterol intake varied among studies. Except for one study,[8] the median cholesterol intake in the highest category of the included studies ranged from 273 to 501.2 mg/day and that in the lowest category ranged from 124.9 to 185 mg/day. The lowest category was consistent with the regimen recommended by the Third Report of

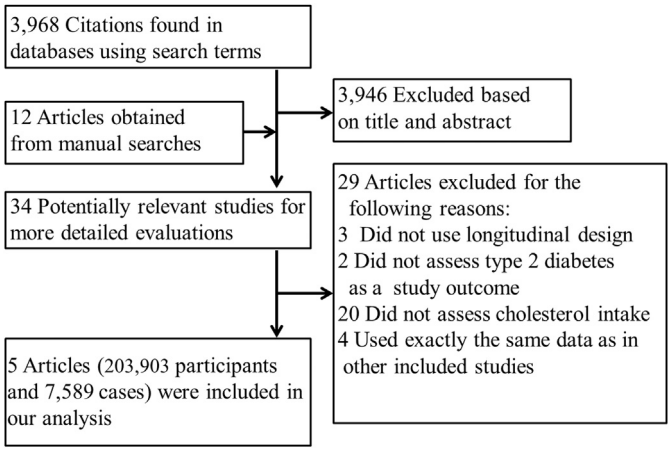

Fig. 1. Flow diagram of the systematic review.

Table 1
Characteristics of studies included in the meta-analysis.

First author Year	Country	% men	Mean age (years) [range]	Participants/ cases	Cholesterol intake (lowest vs. highest)	Follow-up years	Type 2 diabetes ascertainment	Study adjustments
Meyer 2001[17]	U.S.	0	61 [55–69]	35,988/1890	185 vs. 382 mg/day	11	Self-report	Age, sex, BMI, WHR, PA, AL, SM, education, residential area, hormone replacement therapy, cereal fiber intake, TE, Mg intake
Salmeron 2001[18]	U.S.	0	46 [34–59]	84,204/2507	131vs. 273 mg/day	14	Self-report	Age, sex, BMI, PA, AL, SM, FH, time period, protein intake, TE
van Dam 2002[8]	U.S.	100	53 [40–75]	42,504/1321	Not available	12	Self-report	Age, sex, BMI, PA, AL, SM, FH, hyperlipidemia, hypertension, cereal fiber intake, Mg intake, TE, time period
Song 2004[10]	U.S.	0	54 [≥45]	37,309/1558	147 vs. 309 mg/day	8.8	Self-report	Age, sex, BMI, exercise, AL, SM, FH, fiber intake, dietary glycemic load, total fat intake, Mg intake, TE
Djousse 2010[9]	U.S.	43	73 [65–98]	3898/313	125 vs. 501 mg/day	11.3	Medication inventory, blood test	Age, sex, BMI, PA, AL, SM, cereal fiber intake, race

AL: alcohol intake, BMI: body mass index, FH: family history of type 2 diabetes, PA: physical activity, SM: smoking habit, TE: total energy intake, WHR: waist to hip ratio.

the National Cholesterol Education Program (NCEP).[19] The pooled estimate of RR for the highest category of cholesterol consumption compared with the lowest category in individual studies was 1.25 (95% CI: 1.16–1.36). Excluding one study[9] that consisted of 2 data sets and did not adjust the RR for total energy, the pooled RR in relation to high cholesterol intake was 1.27 (95% CI: 1.17–1.38) (Table 2). Quantitative analysis of risk estimates for the development of T2D for incremental increases in cholesterol intake could be performed in all but 1 study[8] (Fig. 3). Similar to the qualitative analysis, higher cholesterol intake was positively associated with T2D risk (per 100 mg/day, RR [95% CI], 1.11 [1.06–1.15]). Table 2 shows the stratified analysis to explore the effects of several study characteristics on the association between high cholesterol intake and risk of T2D. These study characteristics did not significantly modify the risk of T2D, partly because of the limited number of studies.

Egger's test and Begg's test showed no evidence of publication bias in the quantitative ($P = 0.27$ in Egger's test, $P = 0.35$ in Begg's test) or qualitative analysis ($P = 0.24$ in Egger's test, $P = 0.32$ in Begg's test).

4. Discussion

The current meta-analysis indicated that cholesterol consumption is positively associated with the occurrence of T2D from both the qualitative and quantitative viewpoints. In addition, all included studies adjusted the T2D risk for major confounders that were considered to be strongly associated with T2D, suggesting that dietary cholesterol had an independent association with the future risk of T2D.

This meta-analysis was based on observational studies; therefore, we need to emphasize that causality can never be proven in principle. However, there were several studies suggesting a plausible mechanism to explain the causality. In animal studies, dietary cholesterol worsened insulin resistance through promoting the accumulation of macrophage infiltration into adipose tissue,[20] reducing phosphorylation of the insulin receptor subunit,[21] or causing steatohepatitis.[22]

Several limitations should be addressed in the current meta-analysis. First, intake of cholesterol in the reference group and exposure group varied among studies. Second, we *a priori* assumed

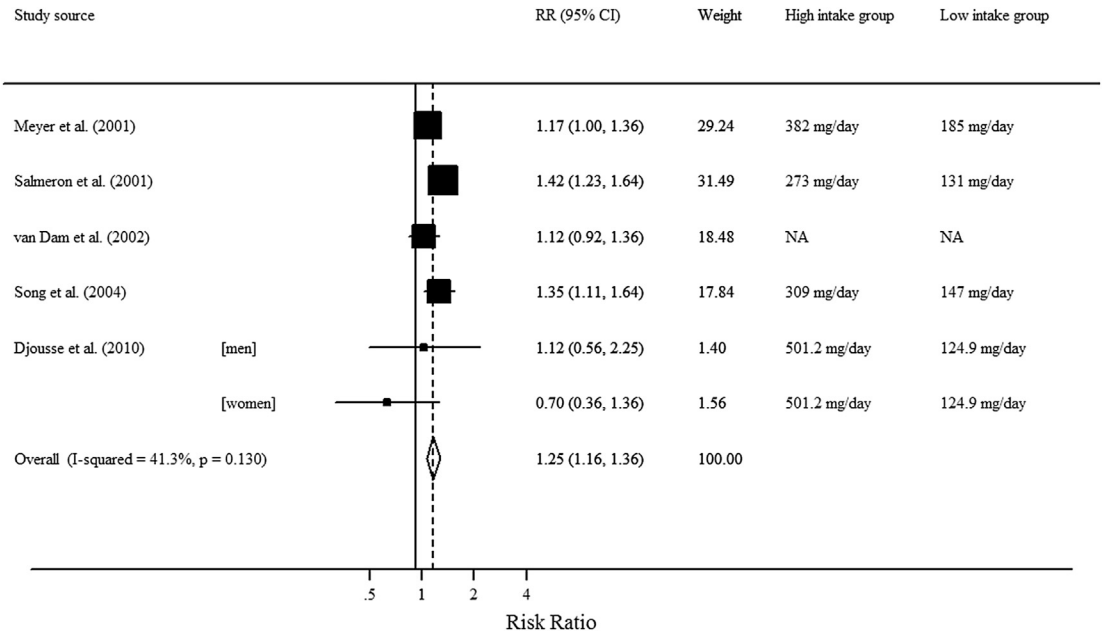

Fig. 2. Qualitative assessment of type 2 diabetes risk: Overall RR (with corresponding 95% CIs) for risk of type 2 diabetes for highest vs. lowest category of cholesterol intake. NA, not available.

Table 2
Estimated relative risk (RR) of type 2 diabetes after stratification by several study characteristics.[a]

	No. of data	Relative risk (95% CI)	Q statistics	I-squared	*P* value of heterogeneity	Meta-regression[b]
Qualitative analysis						
Total	6	1.25 (1.16–1.36)	8.5	41.3%	0.13	
Follow-up periods, years (mean)						
≥10 years	5	1.24 (1.13–1.35)	7.9	49.1%	0.10	0.53
<10 years	1	1.35 (1.11–1.64)	–	–	–	
% men (mean)						
≥50%	2	1.08 (0.90–1.30)	1.8	44.2%	0.18	0.20
<50%	4	1.30 (1.19–1.43)	3.5	15.3%	0.32	
Type 2 diabetes ascertainment						
Self-report	4	1.27 (1.17–1.38)	5.4	44.0%	0.15	0.23
Blood test/Registry	2	0.87 (0.54–1.41)	0.9	0.0%	0.34	
Adjustment for total energy intake						
No	2	0.87 (0.54–1.41)	0.9	0.0%	0.34	0.23
Yes	4	1.27 (1.17–1.38)	5.4	44.0%	0.15	
Quality score (0–9)[c]						
<6	3	1.31 (1.19–1.45)	3.8	47.5%	0.15	0.29
≥6	3	1.14 (0.99–1.32)	2.2	9.3%	0.33	
Quantitative analysis						
Total	5	1.11 (1.06–1.15)	8.3	52.0%	0.08	
Follow-up periods, years (mean)						
≥10 years	4	1.10 (1.05–1.14)	6.8	55.7%	0.08	0.38
<10 years	1	1.18 (1.06–1.32)	–	–	–	
% men (mean)						
≥50%	1	1.01 (0.86–1.19)	–	–	–	
<50%	4	1.11 (1.07–1.16)	7.1	57.9%	0.07	0.57
Type 2 diabetes ascertainment						
Self-report	3	1.13 (1.08–1.18)	0.8	0.0%	0.67	0.08
Blood test/Registry	2	0.96 (0.86–1.08)	0.8	0.0%	0.39	
Adjustment for total energy intake						
No	2	0.96 (0.86–1.08)	0.8	0.0%	0.39	0.08
Yes	3	1.13 (1.08–1.18)	0.8	0.0%	0.67	
Quality score (0–9)[c]						
<6	2	1.13 (1.07–1.20)	0.7	0.0%	0.42	0.28
≥6	3	1.08 (1.02–1.14)	5.9	65.9%	0.05	

[a] To calculate pooled RRs, the random-effects model was used if between-study heterogeneity which was assessed by Q statistics and I-squared was significant; otherwise the fixed effects model was used.
[b] Values indicate *P* value for difference among strata within each subheading representing study characteristic.
[c] Study quality was assessed by using the Newcastle–Ottawa Scale, which was modified to make it possible to apply to this meta-analysis.

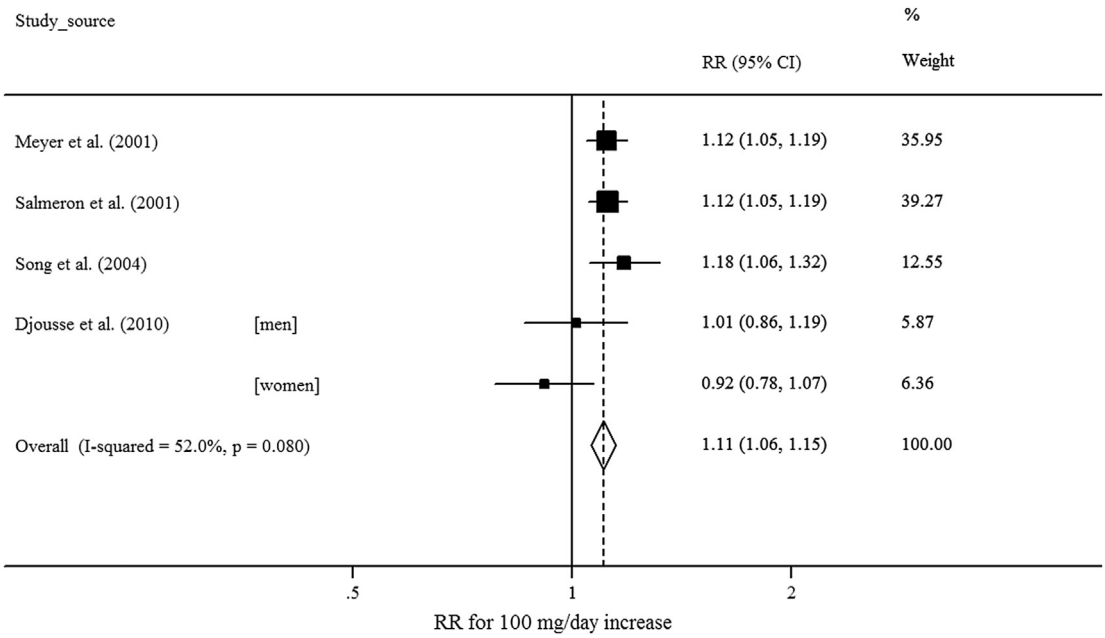

Fig. 3. Quantitative assessment of type 2 diabetes risk: Overall RR (with corresponding 95% CIs) for risk of type 2 diabetes for each 100 mg/day increase in cholesterol intake.

only a binary or linear relationship between cholesterol intake and risk of T2D. Thus, another shape could not be ruled out (e.g. U-shape as is seen for alcohol consumption and risk of T2D).[23] Third, although the included studies considered the major confounders linked with T2D, the possibility for other residual confounding cannot be excluded. For example, prior research proposed that high intake of proteins[5] or iron[10] was associated with high risk of future T2D. It could not be ruled out that such substances consumed with cholesterol had been diabetogenic rather than the cholesterol itself. Lastly, no study has examined the relationship between dietary cholesterol and T2D risk outside the U.S. Considering that consumption of animal products and T2D prevalence has increased in Asia,[24] further studies should be conducted on Asian populations.

In conclusion, results of this meta-analysis suggest that high intake of cholesterol is positively associated with future T2D risk. Further studies should investigate the possibility of extrapolation of the association to various countries outside the U.S.

Statement of authorship

RT and SK played leading roles in the conception and design of the study, devising and carrying out the study methods and drafting all sections of the manuscript. KF, CH, SY, and MH selected studies that met the inclusion criteria and acquired the full papers of studies that met the inclusion criteria and were eligible for further review. HS, KTI, and YY gave various opinions in interpretation of the study results and helped draft the manuscript. RT and SK had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. All authors read and approved the final manuscript.

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Conflict of interest

There are no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.clnu.2014.03.001.

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