Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	No
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	No
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4,5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4,5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5,6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	No
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6,7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6,7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7,8
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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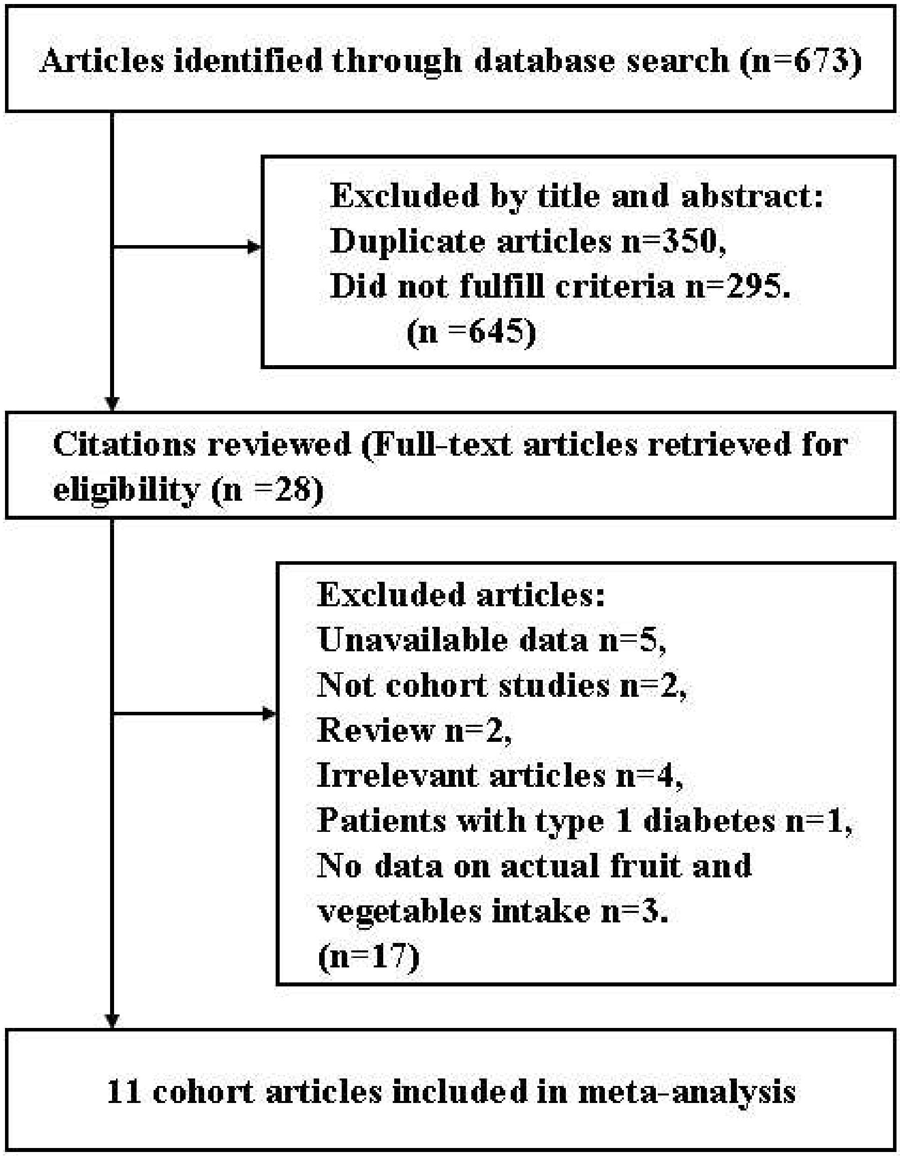


Fig 1 Process of literature search and study selection

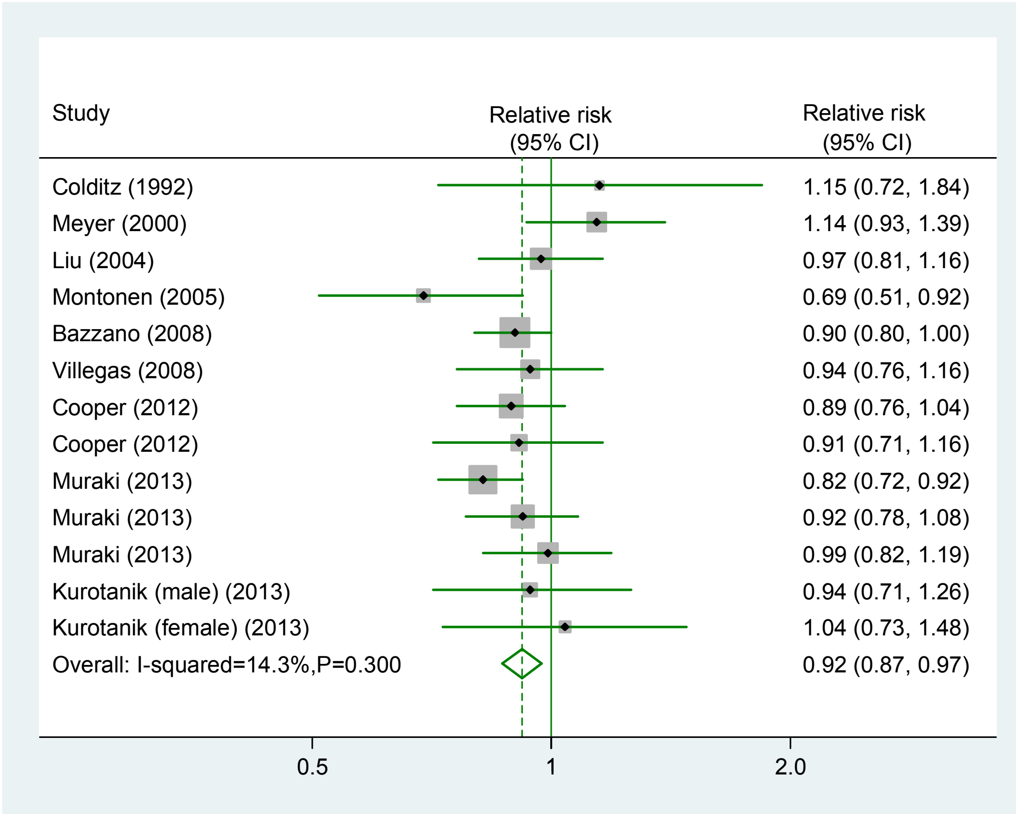


Fig 2 Random effects analysis of fully adjusted studies for the association between fruit intake and risk of type 2 diabetes

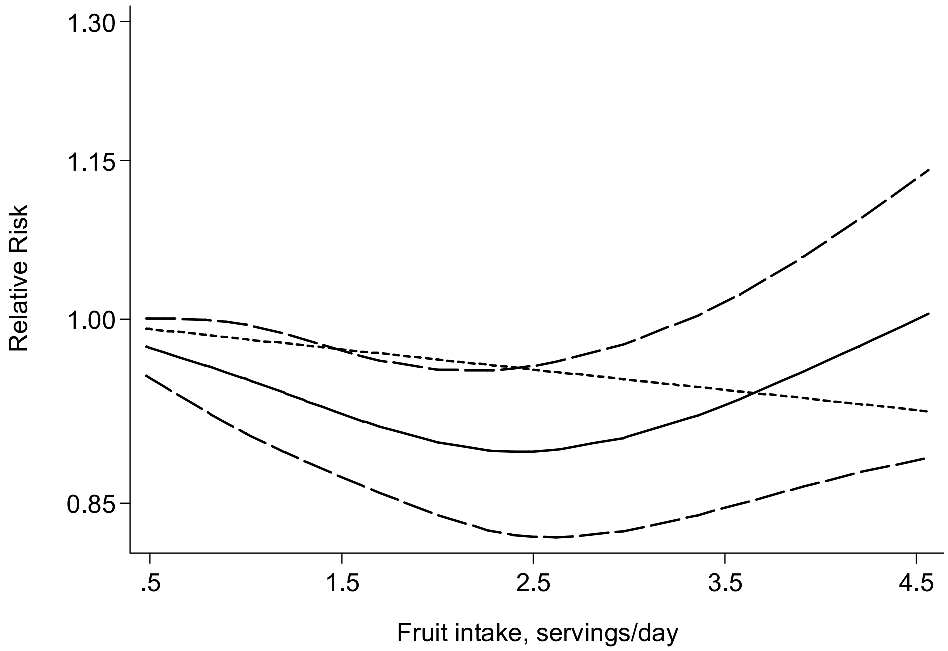


Fig 3 Dose-response analyses of fruit intake and risk of type 2 diabetes

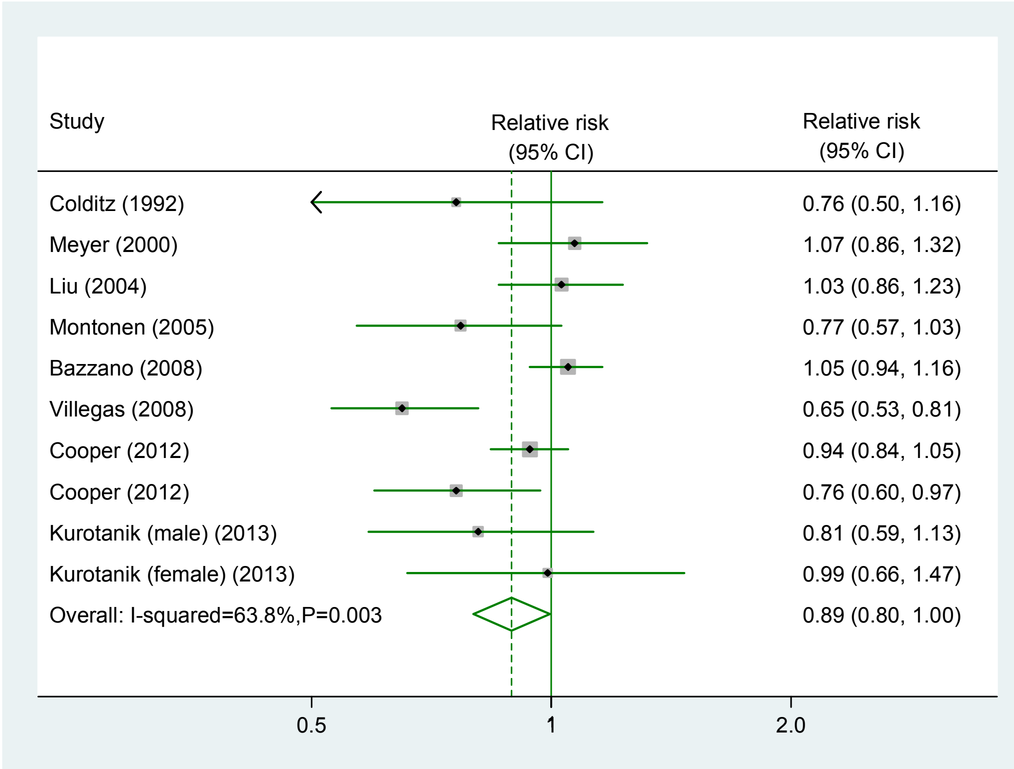


Fig 4 Random effects analysis of fully adjusted studies for the association between vegetables intake and risk of type 2 diabetes

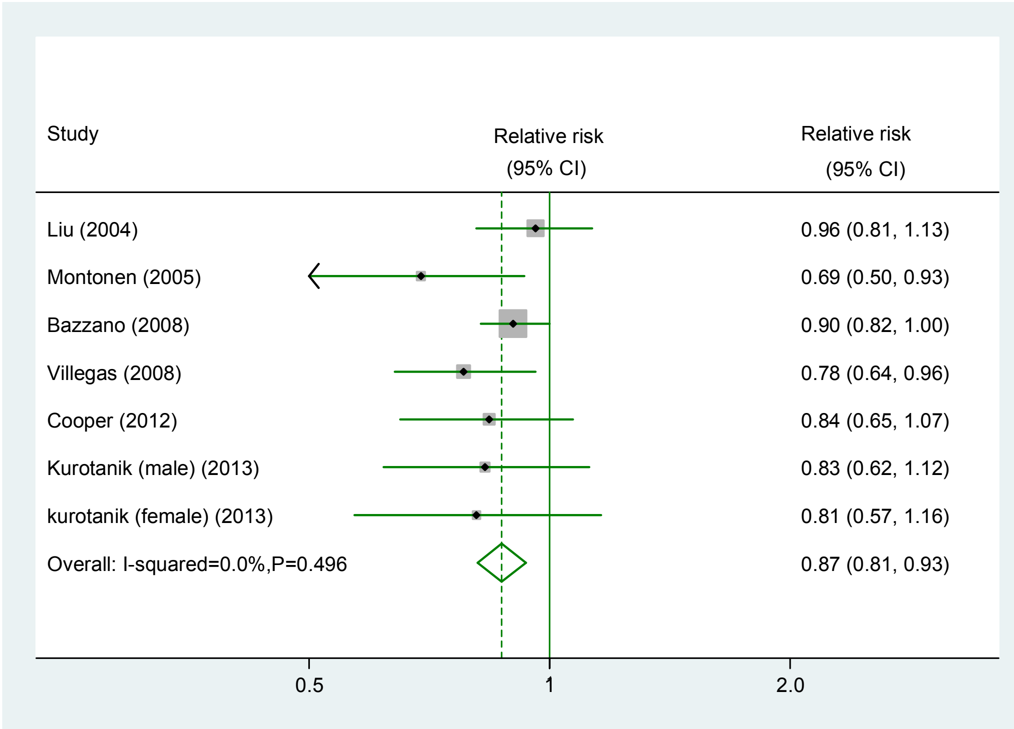


Fig 5 Random effects analysis of fully adjusted studies for the association between green leafy vegetables intake and risk of type 2 diabetes

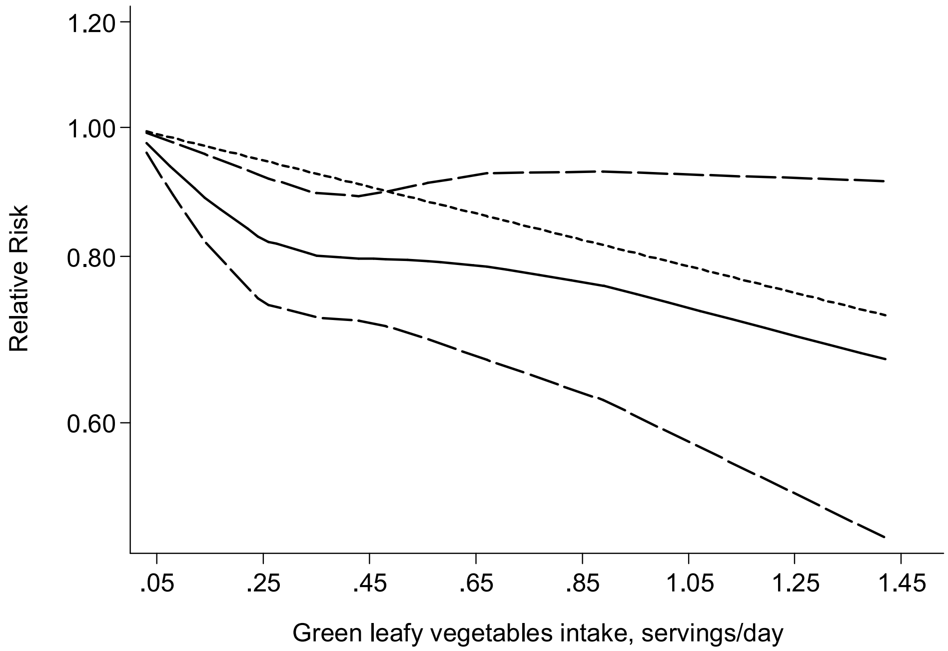


Fig 6 Dose-response analyses of green leafy vegetables intake and risk of type 2 diabetes

Table A. Characteristics of included studies of fruit and vegetables intake in relation to incident type 2 diabetes

First author	Country/ cohort	Age (years) /Sex	No of total/follow -up(years)	No of cases /non-cases	Assessment of type 2 diabetes	Measure of intake	Highest/lowest intakes as servings/day	Adjustments	Quality score
Colditz et al 1992,[43]	USA/Nurses Health Study	30-55/F	84360/6	702/83658	Based on self reported	61 item FFQ. Calculated servings/day for fruit, vegetables. Data divided into fifths	Fruit: ≥3/＜0.6. Vegetables: ≥2.9/＜1.2	Age, BMI, alcohol, family history of diabetes, prior weight change, time period	2
Meyer et al 2000,[44]	USA/lowa Women's Health Study	55-69/F	35988/6	1141/34847	Based on self reported	127 item FFQ, Calculated servings/day for fruit, vegetables, and combined. Data divided into fifths	Fruit: 3.36/0.57. Vegetables: 5.93/1.57. Fruit and vegetables: 8.86/2.57	Age, BMI, total energy intake, WHR, education, smoking, alcohol intake, physical activity	2
Ford et al 2001,[15]	USA/NHANES I	25-74/M and F	9665/20	1018/8647	Confirmed by self report or hospital records or death certificate	24 hour recall. Calculated servings/week for fruit and vegetables combined. Data divided into thirds	Fruit and vegetables: ≥5/0	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004,[45]	USA/Women's Health Study	≥45/F	38018/8.8	1614/36404	Based on self reported	131 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as	Fruit: 3.91/0.62. Vegetables: 6.84/1.47. Fruit and vegetables: 10.16/2.54.	Age, BMI, smoking, total calories, alcohol, exercise, history of hypertension/high cholesterol, family	3

						spinach/kale/lettuce. Data divided into fifths	Green leafy vegetables: 1.42/0.14	history of diabetes	
Montonen et al 2005,[46]	Finland/Finnish Mobile Clinic Health Examination Survey	40-69/M and F	4304/23	383/3921	Confirmed via social insurance register	Dietary history interview. Calculated g/day for fruit and vegetables separately. Data divided into fifths	Fruit: ＞1.47/＜0.31. Vegetables: ＞1.23/＜0.4. Green leafy vegetables: ＞0.4/＜0.1	Age, BMI, sex, smoking, energy intake, family history of diabetes, geographic area	3
Bazzano et al 2008,[48]	USA/Nurses' Health Study	30-55/F	71346/18	4529/66817	Confirmed if met WHO criteria (before 1997) or ADA criteria (after 1998)	61 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce. Data divided into fifths	Fruit: 2.5/0.5. Vegetables: 5.2/1.5. Fruit and Vegetables: 7.5/2.1	Age, BMI, physical activity, smoking, alcohol, hormone therapy, family history of diabetes, total energy intake	4
Villegas et al 2008,[47]	China/Shanghai Women's Health Study	40-70/F	64191/4.6	896/63295	Confirmed by ADA criteria	77 item FFQ. Calculated g/day for fruit and vegetables separately. Defined green leafy vegetables as greens/Chinese greens/spinach. Data divided into fifths	Fruit: 4.56/0.82. Vegetables: 4.04/1.15. Green leafy vegetables: 1.28/0.26	Age, BMI, WHR, education, smoking, alcohol, hypertension, disease history, hormone use, occupational history, physical activity, income, daily energy intake	4

Cooper et al 2012,[5]	8countries/ EPIC-InterAct study	40-79/M and F	24939/11	10821/14118	Based on self reported	Country specific dietary questionnaires. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as chard/endive/lettuce/borage /watercress/beet leaves/spinach. Data divided into quarters	Fruit: 5.39/0.75. Vegetables: 3.94/0.88. Fruit and Vegetables: 8.71/2.13. Green leafy vegetables: 5.93/0.05	Age, BMI, sex, education, centre, physical activity, smoking, total energy intake, alcohol	2
Cooper et al 2012,[10]	England/EPIC-Norfolk	40-79/M and F	3704/11	653/3051	Based on self reported	117 item FFQ. Calculated servings/day for fruit, vegetables and combined. Data divided into thirds	Fruit: 3.4/0.6. Vegetables: 2.6/1.1. Fruit and Vegetables: 5.7/2.1	Age, BMI, sex, waist circumference, education, TDI, occupational social class, smoking, physical activity, family history of diabetes, energy intake, season	2
Muraki et al 2013,[11]	USA/Nurses' Health Study	30-55/F	69554/14	2699/66855	Confirmed by self report or medical records or death certificate	116 item FFQ. Calculated servings/day for fruit. Data divided into fifths	Fruit: ≥3/＜0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
Muraki et al	USA/Nurse	24-44/F	91246/8	741/90505	Confirmed	133 item FFQ. Calculated	Fruit: ≥3/＜0.57	Age, BMI, ethnicity,	4

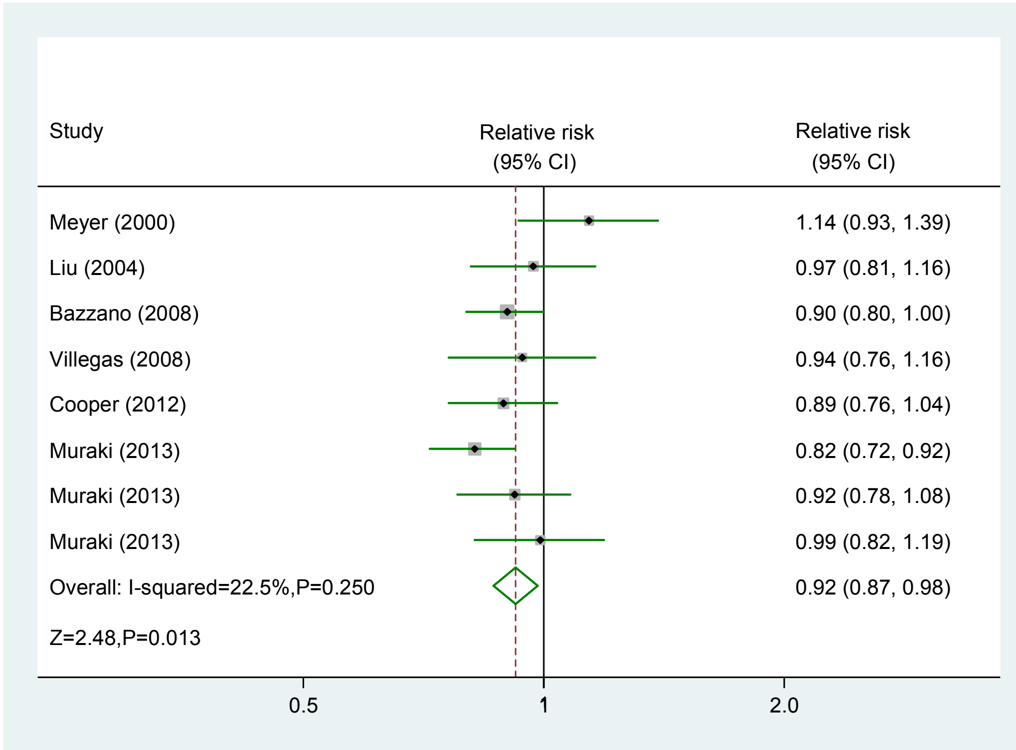
2013,[11]	s' Health Study II				by ADA criteria (after 1998)	servings/week for fruit. Data divided into fifths		smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	
Muraki et al 2013,[11]	USA/Health Professionals Follow-up Study	40-75/M	42504/12	1321/41183	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: ≥3/<0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
Kurotanik et al 2013,[7]	Japan/JPHC Study	40-69/M and F	48437/5	896/47541	Based on self reported	147 item FFQ. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/Chinese chives/garland chrysanthemums/cbingensai/leaf mustard/mugwort/chard/komatsuna. Data divided into quarters	M Fruit: 3.42/0.34. Vegetables: 3.35/0.71. Fruit and Vegetables: 6.48/1.38. Green leafy vegetables: 0.45/0.04. F Fruit: 4.60/0.7. Vegetables: 3.84/0.94. Fruit and Vegetables: 8.1/1.98. Green	Age, BMI, public health centre area, smoking, alcohol, leisure-time activity, history of hypertension, coffee, family history of diabetes, Mg intake, Ca intake, energy intake	3

leafy vegetables: 0.54/0.07

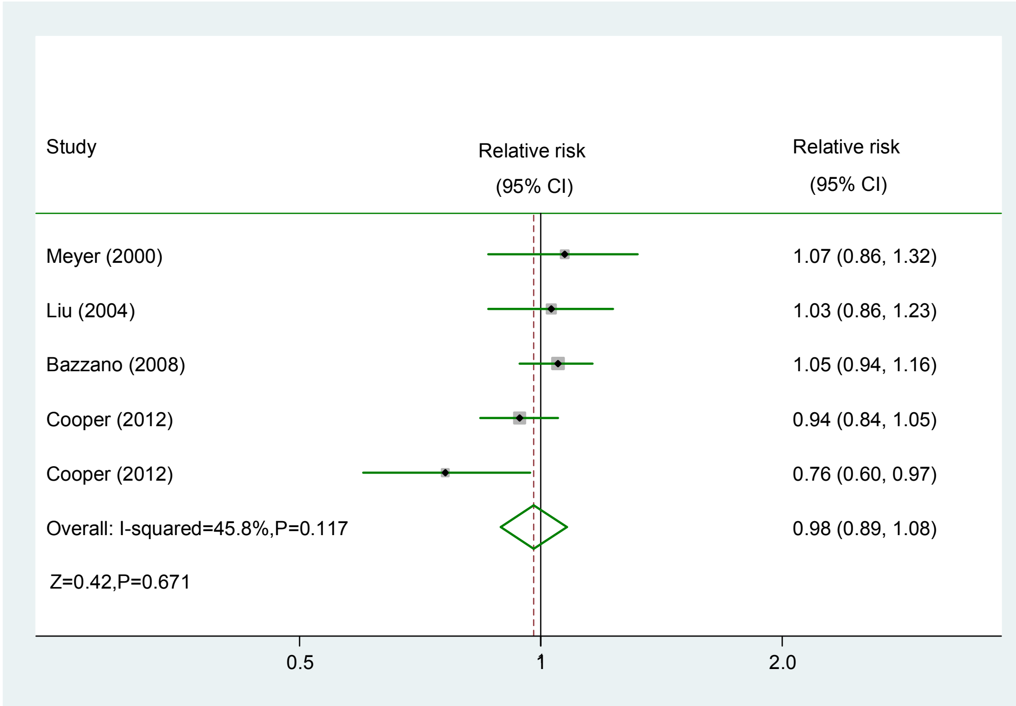
FFQ=food frequency questionnaire, BMI=body mass index, SBP=systolic blood pressure, TDI=townsend deprivation index, WHR=weight:height ratio, ADA=American Diabetes Association, WHO=World Health Organization, M=male, F=female.

Table B. Meta-analysis of intake of fruit and vegetables and risk of type 2 diabetes (highest versus lowest category)

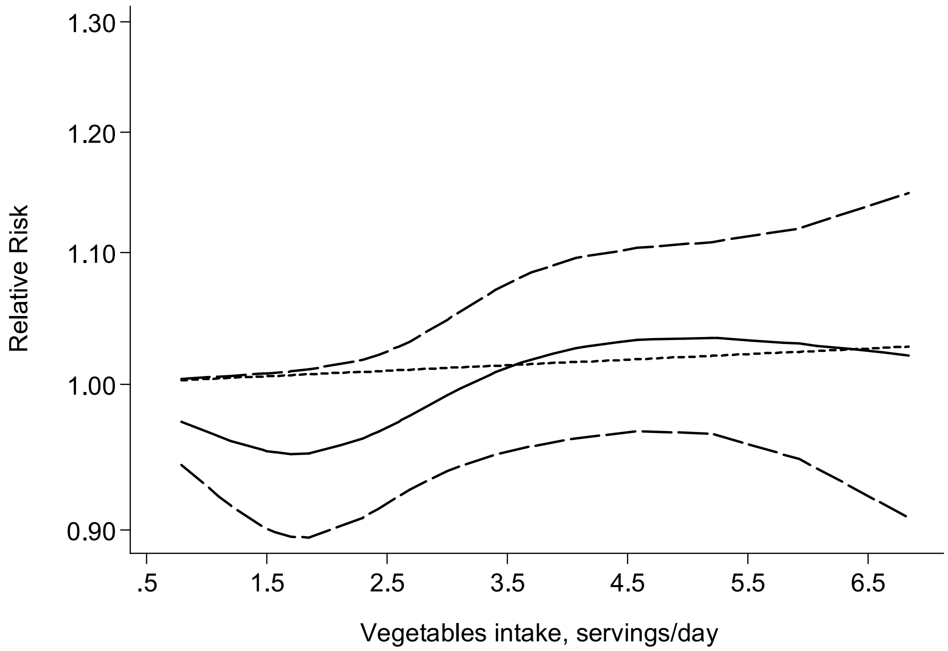
Variables	No of comparisons	Cases/ total	Test of association	Test of heterogeneity	Analysis of publication bias
			Pooled RR (95% CI), P value	Heterogeneity (I^2, %), P value	Begg's test, Egger's test (P value)
Fruit only	13	26396/578591	0.92 (0.87 to 0.97), 0.003	14.3, 0.300	0.127, 0.266
Vegetables only	10	21635/375287	0.89 (0.80 to 1.00), 0.042	63.8, 0.003	0.474, 0.122
Fruit and vegetables	9	20672/232097	0.94 (0.86 to 1.03), 0.202	34.6, 0.141	0.348, 0.609
Green leafy vegetables	7	19139/251235	0.87 (0.81 to 0.93), 0.000	0, 0.496	0.133, 0.101



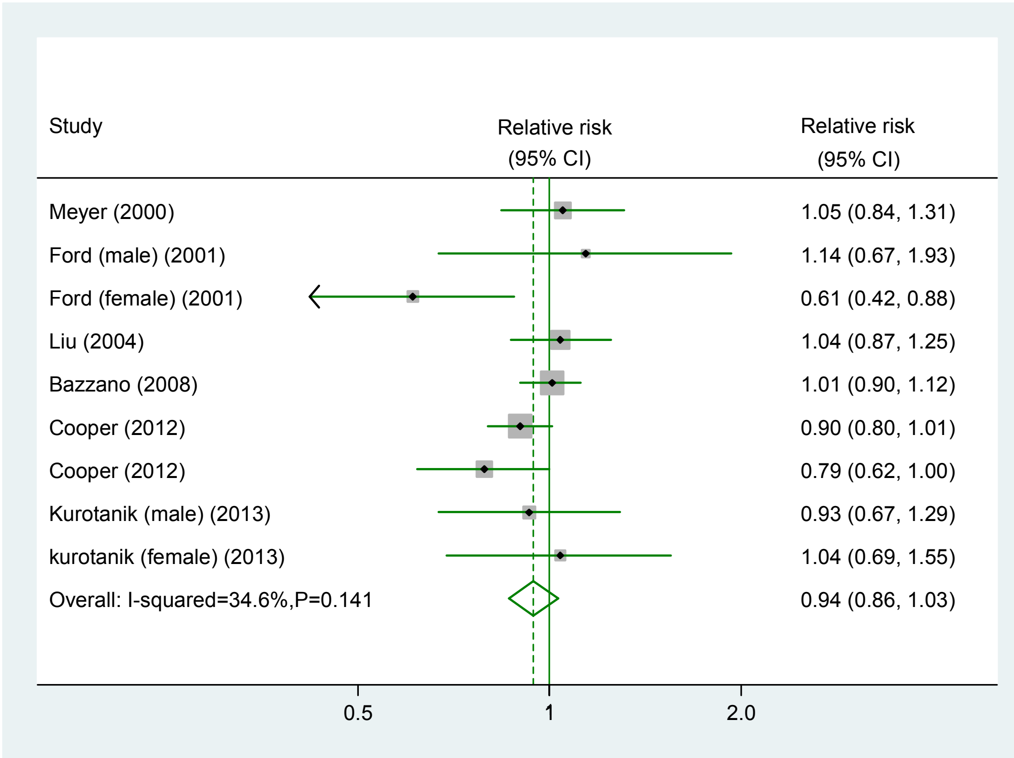
Supplemental fig A Random effects analysis of fully adjusted studies for the association between fruit intake and risk of type 2 diabetes



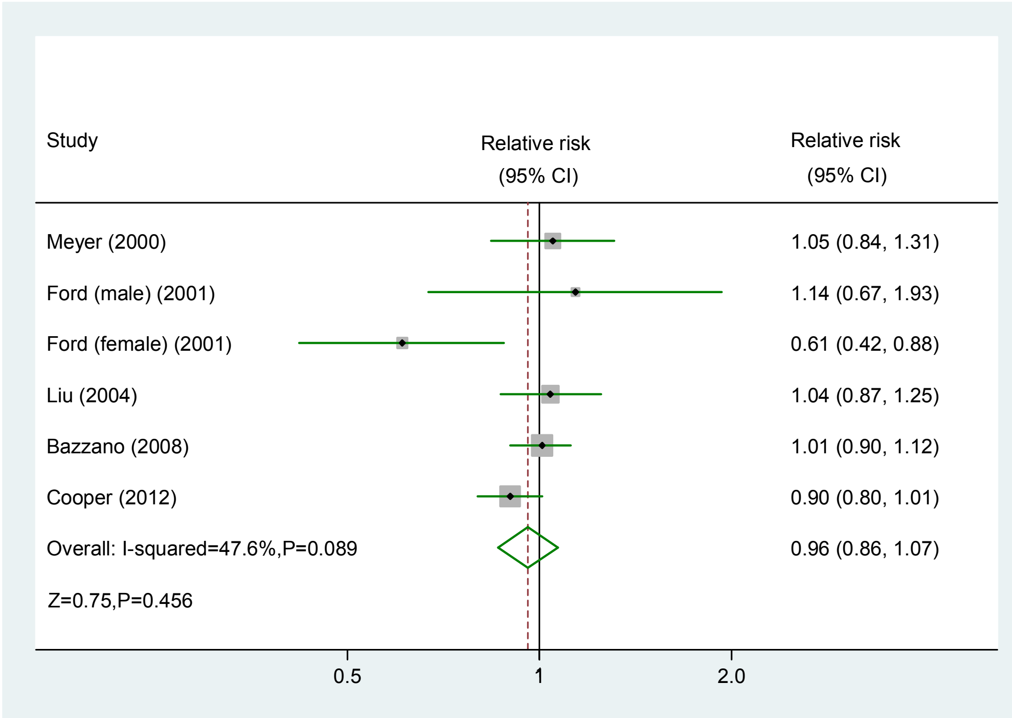
Supplemental fig B Random effects analysis of fully adjusted studies for the association between vegetables intake and risk of type 2 diabetes



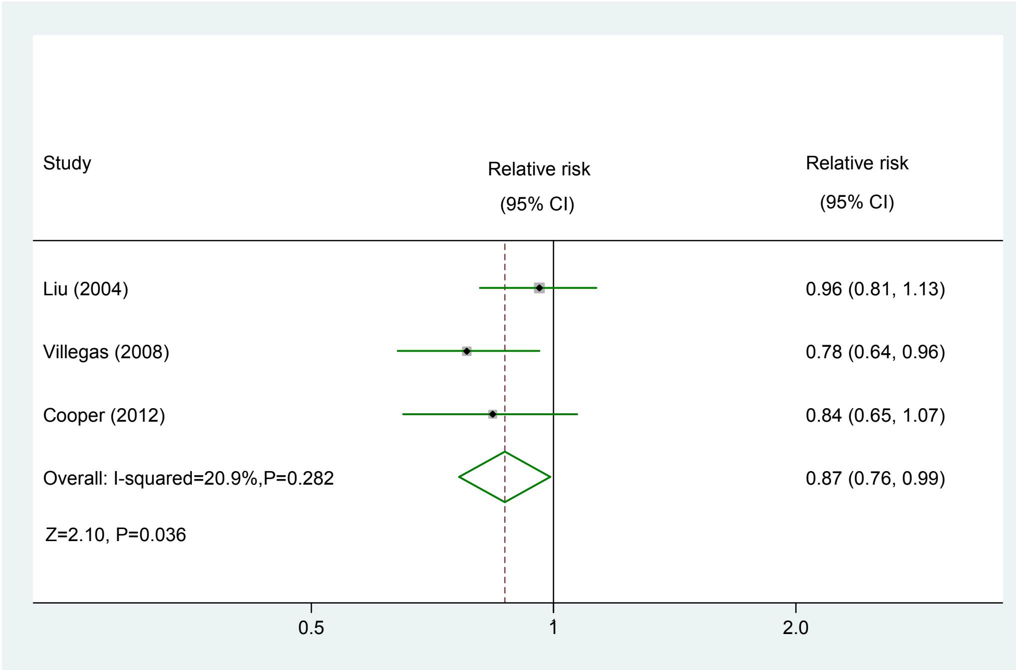
Supplemental fig C Dose-response analyses of vegetables intake and risk of type 2 diabetes



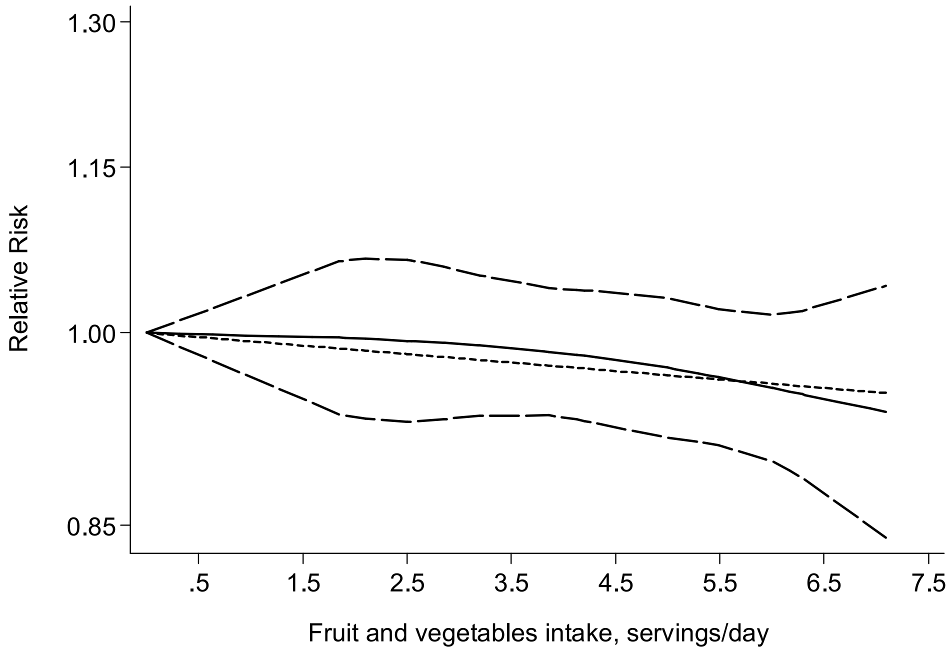
Supplemental fig D Random effects analysis of fully adjusted studies for the association between fruit and vegetables intake and risk of type 2 diabetes



Supplemental fig F Random effects analysis of fully adjusted studies for the association between fruit and vegetables intake and risk of type 2 diabetes



Supplemental fig G Random effects analysis of fully adjusted studies for the association between green leafy vegetables intake and risk of type 2 diabetes



Supplemental fig E Dose-response analyses of fruit and vegetables intake and risk of type 2 diabetes

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Abstract

Objective To clarify and quantify the potential dose-response association between the intake of fruit and vegetables and risk of type 2 diabetes.

Design Meta-analysis and systematic review of prospective cohort studies.

Data source Studies published before February 2014 identified through electronic searches using PubMed and Embase.

Eligibility criteria for selecting studies Prospective cohort studies with relative risks and 95% confidence intervals for type 2 diabetes according to the intake of fruit, vegetables, or fruit and vegetables.

Results A total of eleven articles including fifteen comparisons with 27 414 cases of type 2 diabetes and 588 256 participants were included in the meta-analysis. Evidence of curve linear associations were seen between fruit and green leafy vegetables consumption and risk of type 2 diabetes (P=0.013 and P=0.036 for non-linearity, respectively). The summary relative risk of type 2 diabetes for an increase of 1 serving fruit consumed per day was 0.92 (95% confidence interval 0.87 to 0.97) without heterogeneity among studies (P=0.3, I^2=14.3%). For vegetables, the combined relative risk of type 2 diabetes for an increase of 1 serving consumed per day was 0.89 (95% confidence interval 0.80 to 1.00) with moderate heterogeneity among studies (P=0.003, I^2=63.8%). For green leafy vegetables, the summary relative risk of type 2 diabetes for an increase of 0.2 serving consumed per day was 0.87 (95% confidence interval 0.81 to 0.93) without heterogeneity among studies (P=0.496, I^2=0%). The combined estimates showed no significant benefits of increasing the consumption of fruit and vegetables combined.

Conclusions Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.

Article summary

Strengths and limitations of this study

To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of type 2 diabetes. We also investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of type 2 diabetes.

The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. We cannot exclude the possibility of recall bias in the assessments of diet based on the food frequency questionnaires.

1. Health expenditure on type 2 diabetes is increasing worldwide.
2. Epidemiological studies suggest that the intake of fruit and vegetables is beneficial in delaying or preventing the development of type 2 diabetes, though results from cohort studies are controversial.
3. Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.
4. Dose-response analyses indicated a 8% lower risk of type 2 diabetes per 1 serving/day increment of fruit intake and 13% lower risk of type 2 diabetes per 0.2 serving/day increment of green leafy vegetables intake.
5. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030,[1] with serious consequences for health care expenditure.[2] It has been estimated that the global health expenditure on diabetes is at least $ 376 billion in 2010 and will be $ 490 billion in 2030,[3] this creates a major public health burden. The prevention of T2D is thus clearly an important public health priority. In recent decades, concern has mounted regarding the premature mortality and morbidity associated with T2D, with growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D.[4] Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.[5]

To minimize the risk of dietary factors and reduce the incidence of T2D, a World Health Organization recommended the public to consume more than 400 g or five portions of combined fruit and vegetables per day for the prevention of T2D.[6] Nevertheless, in the Japan Public Health Center-based Prospective (JPHC) Study,[7] after a mean follow-up over five years, participants with the intake of fruit and vegetables may not be appreciably associated with the risk of T2D. Vegetables, especially green leafy vegetables (GLV), have been suggested to explain an apparent beneficial effect on T2D. In addition, several meta-analyses of observational studies have found that an increase in daily intake of GLV could significantly reduce the risk of T2D.[5,8,9] These studies were restricted by language and heterogeneous with respect to sample size. Additionally, recent studies involving relationship between the intake of fruit and vegetables and risk of T2D have been published from then on.[5,7,10,11] Furthermore, whether any dose-response relation exists between the intake of fruit and vegetables and risk of T2D is unknown. Therefore, we systematically reviewed and meta-analysed available studies to quantify the associations between dietary intake of fruit and vegetables and incidence of T2D based on identified prospective cohort studies. We pooled risk estimates for the highest versus lowest category of intake to examine the overall association. We also conducted a dose-response analysis for the trend estimation.

Methods

Search strategy

We carried out a systematic search of PubMed (Medline) and Embase through February 2014 for prospective cohort studies examining the association between the intake of fruit and vegetables and risk of T2D. The following key words were used in our search strategies: (“fruits” OR “vegetables” OR “citrus”) AND (“Type 2 diabetes” OR “non-insulin-dependent diabetes mellitus” OR “Diabetes Mellitus, Type 2” OR “NIDDM” OR “prediabetes” OR “impaired glucose tolerance” OR “impaired fasting glucose” OR “glucose” OR “hyperglycaemia” OR “insulin”) AND (“Follow-up studies” OR “prospective studies” OR “cohort studies” OR “longitudinal studies”). We restricted the search to human studies. No language restrictions were imposed. In addition, we scrutinized possible eligible references from relevant original papers and review articles to identify potential publications. We followed standard criteria for the performing and reporting of the meta-analyses of observational studies.[12]

Study selection

Citations selected from the initial search were subsequently screened for eligibility. Studies were included in this meta-analysis if they satisfied the following criteria: (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design); (3) the exposure of interest was the intake of fruits, vegetables, or fruit and vegetables combined; (4) the outcome was T2D; and (5) reported multivariate-adjusted risk estimates for the association between the fruit, vegetables, or fruit and vegetables combined, assessed as dietary intake, and T2D. Additionally, we excluded animal studies and letters without sufficient data. If data were reported more than once, we included the study with the longest follow-up time.

Validity assessment

Two authors (ML and YF) independently assessed all studies for quality using a modified scoring system, which allowed a total score from 0 to 6 points (6 reflecting the highest quality) on the basis of MOOSE,[12] QUATSO,[13] and STROBE.[14] The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).

Data extraction

Data were carried out independently by two other authors (XZ and WH) using a standard electronic sheets and cross-check to reach a consensus. For each study, the following information was abstracted: name of the first author, publication year, study population, geographical location, sex, age range, sample size (number of T2D cases, number of non-T2D cases, and number of participants), duration of follow-up, methods used to assess fruit and vegetables intake and ascertain T2D cases, highest and lowest of fruit and vegetables intake, and covariates adjusted for in the multivariable model. Study quality was evaluated by using the modified scoring system. All data were extracted from the published papers. If necessary, the primary authors were contacted to retrieve further information. For two studies that expressed data separately for men and women,[7,15] one study that included data from multiple cohorts,[11] we considered the analysis for each sex or cohort as an independent comparison and extracted data separately.

Statistical analysis

Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to be a valid approximation of relative risks, we converted these values in every study by taking their natural logarithms and calculating standard errors and corresponding 95% confidence intervals. Relative risks and their standard errors were pooled with the DerSimonian and Laird random effects model, which takes into account both within-study and between-study variabilities.[16] When some studies included in our meta-analysis used different measurement units (eg, grams per day or portions per day or servings per day),[5,10,15] we standardized fruit and vegetables intake into servings per day using a standard portion size of 106 g.[17] As different studies might use different exposure categories (thirds, quarters, or fifths),[7,11,15] we used the study specific relative risk for the highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the

meta-analysis. We carried out a dose-response analysis for the trend estimation using the method that described by Greenland and Longnecker[18] and Orsini et al.[19,20] This analysis used data from the relative risks and 95% confidence intervals, distributions of cases and person years for exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the median or mean intake was not provided. When the highest category was open ended, we assumed that the average of the category was set at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the distribution.[21] A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to zero. Heterogeneity among studies was evaluated using the chi-square test based on Cochran's Q test and I^2 statistic at $P < 0.10$ level of significance,[16] and quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total variation in point estimates that is due to heterogeneity rather than chance.[22] We considered low, moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively. To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We also performed the Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.[23-25] All the statistical analyses were performed in Stata 12 (Stata Corp, College Station, TX). A threshold of $P < 0.1$ was used to decide whether heterogeneity or publication bias was present.[24] In other ways, P values were 2-sided and $P < 0.05$ was considered statistically significant.

Results

Literature search

Fig 1 shows the results of literature research and selection. We identified 308 articles from PubMed and 365 articles from Embase. After exclusion of duplicate records and studies that did not fulfill our inclusion criteria, 28 articles remained, and we further evaluated the full texts of these 28 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to lack of sufficient data for estimation of relative risks.[26-30] Five articles were excluded because no original data could be extracted (review, type 1 diabetes, or cross sectional studies).[31-35] Another four articles were excluded because we deemed irrelevant.[36-39] We also excluded three articles because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to warrant inclusion within the meta-analysis.[40-42] Finally, eleven articles met the inclusion criteria and were included in the meta-analysis.[5,7,10,11,15,43-48] Among these eleven articles, Ford et al and Kurotani et al study examined male and female separately[7,11] and Muraki et al report included data from three independent cohorts.[15] Thus, our meta-analysis included fifteen comparisons.

Study characteristics

Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted from the included studies, all eleven articles were prospective cohort designs and participants who were free of self reported diabetes at baseline.[5,7,10,11,15,43-48] In aggregate, the included studies consisted of 588 256 participants. Among the participants, we documented 27 414 cases of T2D occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 11 articles, six cohorts were conducted primarily in the United States,[11,15,43-45,48] two articles were done in Asian countries (China and Japan)[7,47] and three cohorts were from European countries.[5,10,46] The number of participants ranged from 3704 in the EPIC-Norfolk study by Cooper et al[10] to 84 360 in the Nurses Health Study by Colditz et al.[43] Five studies included both male and female,[5,7,10,15,46] five studies included only female.[43-45,47,48] One article by Muraki et al reported three independent cohorts, two cohorts included only female, and one cohort only male.[11] The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and vegetables intake separately and combined.[5,7,10,44,45,48] Three papers provided information on fruit and vegetables intake separately,[43,46,47] one paper provided only the combined data,[15] and another paper provided separate data on fruit.[11] Five papers also included separate data on the intake of GLV.[5,7,45-47] In most papers intake of fruit and vegetables was divided into fifths.[11,43-48] All studies provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most studies yielded a score of 3 or below (low quality).

Fruit intake and risk of T2D

13 comparisons from ten studies reported an association between fruit intake and risk of T2D, with 26 396 T2D outcomes and 578 591 participants. Overall, fruit intake was inversely associated with risk (relative risk 0.92, 95% confidence interval 0.87 to 0.97) (fig 2). We saw no heterogeneity among studies (P=0.3, I^2=14.3%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.127) and Egger regression tests (P=0.266) (see supplemental table B in appendix 1). Among 13 comparisons, eight comparisons were eligible for the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.013 for non-linearity, fig 3). Dose-response analysis indicated that a 1 serving/day increment of fruit intake was associated with 8% lower risk of T2D (relative risk 0.92, 95% confidence interval 0.87 to 0.98, I^2=22.5%) (see supplemental fig A in appendix 2).

Vegetables intake and risk of T2D

Nine studies exported an association between vegetables intake and risk of T2D, with 21 635 T2D outcomes and 37 5287 participants. Using a random effects model summarizing all 10 comparisons, we found significant association between vegetables intake and risk (relative risk 0.89, 95% confidence interval 0.80 to 1.00) (fig 4). There was moderate study heterogeneity (P=0.003, I^2=63.8%). However, no evidence of substantial publication bias was observed from the Begg (P=0.474) and Egger regression tests (P=0.122) (see supplemental table B in appendix 1). Among 10 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative risk 0.98, 95% confidence interval 0.89 to 1.08, I^2=45.8%) (see supplemental fig B in appendix 2). No publication bias was observed (P=0.117). We found no evidence of a curve linear association between vegetables intake and risk (P=0.671 for non-linearity, see supplemental fig C in appendix 2).

Fruit and vegetables intake and risk of T2D

Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and

vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies (P=0.141, I^2=34.6%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.348) and Egger regression tests (P=0.609) (see supplemental table B in appendix 1). Among 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and vegetables intake and risk of T2D. We did not find a significant curvilinear association (P=0.456 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval 0.86 to 1.07, I^2=47.6%) (see supplemental fig F in appendix 2).

GLV intake and risk of T2D

7 comparisons from six studies reported an association between GLV intake and risk of T2D, with 19 139 T2D outcomes and 251 235 participants. Overall, GLV intake was inversely associated with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant heterogeneity was detected among studies (P=0.496, I^2=0%). Additionally, we did not observe evidence of substantial publication bias (the Begg and Egger regression tests, P=0.133 and P=0.101, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only three comparisons were eligible for the trend estimation. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.036 for non-linearity, fig 6). Dose-response analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, I^2=20.9%) (see supplemental fig G in appendix 2). No publication bias was observed (P=0.282).

Subgroup analyses

To examine the stability of the primary results, we carried out subgroup analyses (table 1). The association between fruit, vegetables, or fruit and vegetables intake and risk of T2D were similar in subgroup analyses, which were separately defined study quality, length of follow-up, sex, location, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) affected the results. The summary estimates of relative risks from each category were pooled (table 2). We paid close attention to the highest versus lowest category. Almost all subgroups that analysed intake of GLV showed a benefit of consuming greater quantities (fig 5). Table 3 also showed significant reductions in risk of T2D events for consumption of fruit, vegetables, or fruit and vegetables combined.

Discussion

In this meta-analysis dietary intake of fruit, vegetables, and GLV, but not fruit and vegetables combined, were associated with a lower risk of T2D. Dose-response analyses indicated a 8% lower risk of T2D per 1 serving/day increment of fruit intake and 13% lower risk of T2D per 0.2 serving/day increment of GLV intake, but no significant trend for vegetables or fruit and vegetables combined.

Results in relation to other studies

Over the past decades, extensive prospective studies have reported the association of fruit, vegetables, or fruit and vegetables combined with T2D risk.[5,7,10,11,15,43-48] However, the role of dietary factors in T2D is still controversial. Some of the studies failed to find the association between fruit intake or fruit and vegetables combined and risk of T2D.[8,44] However, Bazzano and colleagues analysed data from 11 different U.S. states with 18 years of follow-up and found that consumption of fruit was associated with a lower hazard of diabetes, whereas no significant association for total fruit and vegetables consumption.[48] Similar to previous analysis in the Nurses' Health Study, the results from three prospective longitudinal cohort studies also supported an inverse association between fruit intake and risk of T2D.[11] But these studies have the potential for bias due to measurement error. In addition, two cohort studies have suggested an inverse association between total fruit and vegetables consumption and risk of T2D.[10,15]

A few large cohort studies have found an inverse association between vegetables consumption, especially GLV and risk of T2D.[10,45-47] These findings all agreed with two meta-analyses.[5,9] But another systematic review based on five cohort studies suggested that there was no protective association between vegetables intake and T2D.[8]

Several plausible biological mechanisms have been proposed to explain abovementioned association. Fruit and vegetables are rich in fibre,[49] which has been shown to improve insulin sensitivity and insulin secretion to overcome insulin resistance.[50] However, meta-analyses showed that fruit and vegetables fibre is inconsistently associated with the risk of T2D.[51] On the other hand, it may contribute to a decreased incidence of T2D through their low energy density and glycemic load, and high micronutrient content.[52] In particular, GLV are rich in bioactive phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant properties.[53-55] Antioxidants in fruit and vegetables have been hypothesized to improve insulin sensitivity and protect against diabetes in several supplementation trials.[56,57] In addition, it also might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis detected Mg intake to be inversely associated with the risk of T2D.[58] Taking this evidence into consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. Further investigation is warranted to understand the mechanisms involved in the proposed relation between fruit, vegetables, or GLV and risk of T2D.

Exploration of heterogeneity

Heterogeneity between studies was found, which did not alter much in the subgroup analyses. There are differences in types of vegetable consumed between Asian (such as China) and Non-Asian populations. Therefore, within the subgroup analysis we examined location as a possible source of heterogeneity. As traditional Chinese diets are high in vegetables (such as GLV and cruciferous vegetables), unsurprisingly, vegetables (including GLV) intake were greater in China than the US or Europe. We also examined study quality, length of follow-up, sex, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) as possible sources of heterogeneity, these did not show any significant heterogeneity between studies. Although the subgroup analysis could not explain the level of heterogeneity, in interpreting the results, several differences between the studies are worth discussing.

Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs) to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.[7,10,11,43-45,47,48] It is less suitable for the assessment of absolute intake, which they tend to overestimate.[59,60] However, two studies collected data via a single 24 hour recall and dietary history interviews, respectively.[15,46] These measurements may underestimate true associations between fruit, vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition, calculations of daily consumption were differed (such as servings per week, servings per day, or grams per day). Although we standardized primary data using a standard portion size of 106g, conclusions should be interpreted with caution. Another possible explanation for the differences between the studies might be the classification of food groups. GLVs'criteria was inconsistent: three studies included spinach and lettuce; one included spinach and greens; others did not provide specific description. If they were included with an uniform definition of each groups, the associations might differ.

Strengths and limitations

Compared with the previous meta-analyses,[5,8,9] our study has several strengths. The present meta-analysis included 2.6-times more participants and 2.8-times more T2D cases, which significantly increased the statistical power to detect potential associations. To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of T2D. In addition, to examine the shape of these possible associations, we investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of T2D. Therefore, the results should be more reliable.

In interpreting the results, several limitations of this meta-analysis should also be acknowledged. Firstly, although in the multivariable analysis we considered a multitude of lifestyle and dietary factors. The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. Second, we cannot exclude the possibility of recall bias in the assessments of diet based on the FFQs. However, the prospective study design and exclusion of participants with chronic diseases at baseline should minimize such bias. Third, the noticeable limitation of our study was the potential for bias due to inevitable measurement error, especially for individual with lower consumption levels. We attempted to reduce measurement error in adjusting for energy intake and using of cumulatively averaged intake levels. Fourth, because we had no source of information other than questionnaire for the identification of T2D, we might have underestimated the incidence of T2D. In addition, subclinical diseases at baseline might have distorted our risk estimate to some extent. Finally, the possible limitation is due to language bias. We attempted to minimize this bias by searching major electronic databases with no language restriction. However, several articles published in non-English languages might not appear in international journal databases, and could be omitted by our searches.[61]

Conclusions

In summary, our meta-analysis suggests that higher fruit or vegetables, particularly GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.

Contributors: ML and ZT conceived and designed the study. ML and YF searched the databases and checked them according to the eligible criteria and exclusion criteria. ZT helped develop search strategies. XZ and WH extract quantitative data. YF, XZ, and WH analyzed the data. ML wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper. ZT is the guarantor.

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Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

Variables	Fruit only			Vegetables only			Fruit and vegetables			Green leafy vegetables		
	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value
Location												
Non-Asia	10	0.92 (0.85 to 0.98)	0.013	7	0.95 (0.86 to 1.04)	0.280	7	0.94 (0.84 to 1.04)	0.223	4	0.89 (0.81 to 0.97)	0.012
Asia	3	0.96 (0.82 to 1.12)	0.584	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Quality												
High (≥4)	5	0.89 (0.84 to 0.95)	0.001	2	0.83 (0.52 to 1.33)	0.448	1	1.04 (0.87 to 1.25)	0.671	2	0.86 (0.76 to 0.98)	0.024
Low (<4)	8	0.95 (0.86 to 1.05)	0.288	8	0.92 (0.84 to 1.01)	0.066	8	0.93 (0.84 to 1.02)	0.138	5	0.86 (0.77 to 0.97)	0.010
Duration of follow-up (years)												
≥10	6	0.88 (0.82 to 0.94)	0.736	4	0.91 (0.79 to 1.05)	0.190	5	0.89 (0.77 to 1.02)	0.098	3	0.85 (0.75 to 0.97)	0.014
<10	7	0.99 (0.91 to 1.07)	0.000	6	0.88 (0.73 to 1.06)	0.175	4	1.03 (0.91 to 1.16)	0.674	4	0.87 (0.78 to 0.97)	0.013
Sex												
M and F	5	0.88 (0.79 to 0.98)	0.022	5	0.89 (0.81 to 0.97)	0.010	6	0.87 (0.77 to 0.98)	0.026	4	0.80 (0.69 to 0.92)	0.002
F only	7	0.93 (0.86 to 1.01)	0.076	5	0.92 (0.76 to 1.11)	0.364	3	1.02 (0.94 to 1.11)	0.610	3	0.89 (0.81 to 0.98)	0.014
M only	1	0.99 (0.82 to 1.19)	0.916	0	-	-	0	-	-	0	-	-
Fractions of distribution												
Thirds	1	0.91 (0.71 to 1.16)	0.451	1	0.94 (0.84 to 1.05)	0.277	1	0.90 (0.80 to 1.01)	0.076	1	0.84 (0.65 to 1.08)	0.170
Quarters	3	0.92 (0.81 to 1.04)	0.193	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Fifths	9	0.92 (0.85 to 1.00)	0.046	6	0.94 (0.83 to 1.07)	0.338	6	0.94 (0.82 to 1.08)	0.385	3	0.89 (0.78 to 1.01)	0.062
No of participants												
≥50000	5	0.89 (0.83 to 0.95)	0.001	3	0.82 (0.57 to 1.17)	0.271	1	1.01 (0.91 to 1.13)	0.858	2	0.86 (0.76 to 0.98)	0.024
<50000	8	0.95 (0.87 to 1.04)	0.237	7	0.93 (0.84 to 1.02)	0.109	8	0.92 (0.83 to 1.03)	0.146	5	0.86 (0.77 to 0.97)	0.010
No of cases												
≥1000	6	0.93 (0.85 to 1.01)	0.079	4	1.01 (0.94 to 1.08)	0.810	6	0.96 (0.86 to 1.07)	0.456	3	0.91 (0.84 to 0.98)	0.018
<1000	7	0.91 (0.83 to 1.00)	0.061	6	0.75 (0.67 to 0.84)	0.000	3	0.87 (0.73 to 1.04)	0.119	4	0.78 (0.68 to 0.89)	0.000

M=male, F=female, RR=relative risk.

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Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Abstract

Objective To clarify and quantify the potential dose-response association between the intake of fruit and vegetables and risk of type 2 diabetes.

Design Meta-analysis and systematic review of prospective cohort studies.

Data source Studies published before February 2014 identified through electronic searches using PubMed and Embase.

Eligibility criteria for selecting studies Prospective cohort studies with relative risks and 95% confidence intervals for type 2 diabetes according to the intake of fruit, vegetables, or fruit and vegetables.

Results A total of ten articles including thirteen comparisons with 24 013 cases of type 2 diabetes and 434 342 participants were included in the meta-analysis. Evidence of curve linear associations were seen between fruit and green leafy vegetables consumption and risk of type 2 diabetes (P=0.059 and P=0.036 for non-linearity, respectively). The summary relative risk of type 2 diabetes for an increase of 1 serving fruit consumed per day was 0.93 (95% confidence interval 0.88 to 0.99) without heterogeneity among studies (P=0.477, I^2=0%). For vegetables, the combined relative risk of type 2 diabetes for an increase of 1 serving consumed per day was 0.90 (95% confidence interval 0.80 to 1.01) with moderate heterogeneity among studies (P=0.002, I^2=66.5%). For green leafy vegetables, the summary relative risk of type 2 diabetes for an increase of 0.2 serving consumed per day was 0.87 (95% confidence interval 0.81 to 0.93) without heterogeneity among studies (P=0.496, I^2=0%). The combined estimates showed no significant benefits of increasing the consumption of fruit and vegetables combined.

Conclusions Higher fruit or green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.

Article summary

Strengths and limitations of this study

To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of type 2 diabetes. We also investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of type 2 diabetes.

The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. We cannot exclude the possibility of recall bias in the assessments of diet based on the food frequency questionnaires.

1. Health expenditure on type 2 diabetes is increasing worldwide.
2. Epidemiological studies suggest that the intake of fruit and vegetables is beneficial in delaying or preventing the development of type 2 diabetes, though results from cohort studies are controversial.
3. Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.
4. Dose-response analyses indicated a 6% lower risk of type 2 diabetes per 1 serving/day increment of fruit intake and 13% lower risk of type 2 diabetes per 0.2 serving/day increment of green leafy vegetables intake.
5. Further evidence from preferably randomized controlled studies should explore what kind of fruit or green leafy vegetables can reduce the risk of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030,[1] with serious consequences for health care expenditure.[2] It has been estimated that the global health expenditure on diabetes is at least $ 376 billion in 2010 and will be $ 490 billion in 2030,[3] this creates a major public health burden. The prevention of T2D is thus clearly an important public health priority. In recent decades, concern has mounted regarding the premature mortality and morbidity associated with T2D, with growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D.[4] Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.[5]

To minimize the risk of dietary factors and reduce the incidence of T2D, a World Health Organization recommended the public to consume more than 400 g or five portions of combined fruit and vegetables per day for the prevention of T2D.[6] Nevertheless, in the Japan Public Health Center-based Prospective (JPHC) Study,[7] after a mean follow-up over five years, participants with the intake of fruit and vegetables may not be appreciably associated with the risk of T2D. Vegetables, especially green leafy vegetables (GLV), have been suggested to explain an apparent beneficial effect on T2D. In addition, several meta-analyses of observational studies have found that an increase in daily intake of GLV could significantly reduce the risk of T2D.[5,8,9] These studies were restricted by heterogeneous with respect to sample size. Additionally, recent studies involving relationship between the intake of fruit and vegetables and risk of T2D have been published from then on.[5,7,10,11] Furthermore, whether any dose-response relation exists between the intake of fruit and vegetables and risk of T2D is unknown. Therefore, we systematically reviewed and meta-analysed available studies to quantify the associations between dietary intake of fruit and vegetables and incidence of T2D based on identified prospective cohort studies. We pooled risk estimates for the highest versus lowest category of intake to examine the overall association. We also conducted a dose-response analysis for the trend estimation.

Methods

Search strategy

We carried out a systematic search of PubMed (Medline) and Embase through February 2014 for prospective cohort studies examining the association between the intake of fruit and vegetables and risk of T2D. The following key words were used in our search strategies: (“fruits” OR “vegetables” OR “citrus”) AND (“Type 2 diabetes” OR “non-insulin-dependent diabetes mellitus” OR “Diabetes Mellitus, Type 2” OR “NIDDM” OR “prediabetes” OR “impaired glucose tolerance” OR “impaired fasting glucose” OR “glucose” OR “hyperglycaemia” OR “insulin”) AND (“Follow-up studies” OR “prospective studies” OR “cohort studies” OR “longitudinal studies”). We restricted the search to human studies. No language restrictions were imposed. In addition, we scrutinized possible eligible references from relevant original papers and review articles to identify potential publications. We followed standard criteria for the performing and reporting of the meta-analyses of observational studies.[12]

Study selection

Citations selected from the initial search were subsequently screened for eligibility. Studies were included in this meta-analysis if they satisfied the following criteria: (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design); (3) the exposure of interest was the intake of fruits, vegetables, or fruit and vegetables combined; (4) the outcome was T2D; and (5) reported multivariate-adjusted risk estimates for the association between the fruit, vegetables, or fruit and vegetables combined, assessed as dietary intake, and T2D. Additionally, we excluded animal studies and letters without sufficient data. If data were reported more than once, we included the study with the longest follow-up time.

Validity assessment

Two authors (ML and YF) independently assessed all studies for quality using a modified scoring system, which allowed a total score from 0 to 6 points (6 reflecting the highest quality) on the basis of MOOSE,[12] QUATSO,[13] and STROBE.[14] The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).[9]

Data extraction

Data were carried out independently by two other authors (XZ and WH) using a standard electronic sheets and cross-check to reach a consensus. For each study, the following information was abstracted: name of the first author, publication year, study population, geographical location, sex, age range, sample size (number of T2D cases, number of non-T2D cases, and number of participants), duration of follow-up, methods used to assess fruit and vegetables intake and ascertain T2D cases, highest and lowest of fruit and vegetables intake, and covariates adjusted for in the multivariable model. Study quality was evaluated by using the modified scoring system. All data were extracted from the published papers. If necessary, the primary authors were contacted to retrieve further information. For two studies that expressed data separately for men and women,[7,15] one study that included data from multiple cohorts,[11] we considered the analysis for each sex or cohort as an independent comparison and extracted data separately.

Statistical analysis

Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to be a valid approximation of relative risks, we converted these values in every study by taking their natural logarithms and calculating standard errors and corresponding 95% confidence intervals. Relative risks and their standard errors were pooled with the DerSimonian and Laird random effects model, which takes into account both within-study and between-study variabilities.[16] When some studies included in our meta-analysis used different measurement units (eg, grams per day or portions per day or servings per day),[5,10,15] we standardized fruit and vegetables intake into servings per day using a standard portion size of 106 g.[17] As different studies might use different exposure categories (thirds, quarters, or fifths),[7,11,15] we used the study specific relative risk for the highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the

meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker[18] and Orsini et al[19,20] was used to calculate study-specific slopes (linear trends) and 95% confidence intervals. The method requires the distributions of cases and person years for exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the median or mean intake was not provided. When the highest category was open ended, we assumed that the average of the category was set at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the distribution.[21] A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the fractional polynomials component is equal to zero. Heterogeneity among studies was evaluated using the chi-square test based on Cochran's Q test and I^2 statistic at $P<0.10$ level of significance,[16] and quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total variation in point estimates that is due to heterogeneity rather than chance.[22] We considered low, moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively. To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We also performed the Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.[23-25] All the statistical analyses were performed in Stata 12 (Stata Corp, College Station, TX). A threshold of P<0.1 was used to decide whether heterogeneity or publication bias was present.[24] In other ways, P values were 2-sided and P<0.05 was considered statistically significant.

Results

Literature search

Fig 1 shows the results of literature research and selection. We identified 308 articles from PubMed and 365 articles from Embase. After exclusion of duplicate records and studies that did not fulfill our inclusion criteria, 27 articles remained, and we further evaluated the full texts of these 27 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to lack of sufficient data for estimation of relative risks.[26-30] Five articles were excluded because no original data could be extracted (review, type 1 diabetes, or cross sectional studies).[31-35] Another four articles were excluded because we deemed irrelevant.[36-39] We also excluded three articles because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to warrant inclusion within the meta-analysis.[40-42] Finally, eleven articles met the inclusion criteria and were included in the meta-analysis.[5,7,10,11,15,43-47] Among these ten articles, Ford et al and Kurotani et al study examined male and female separately,[7,11] Cooper et al have two studies (study a:2012 and study b:2012) and Muraki et al report included data from two independent cohorts.[15] Thus, our meta-analysis included thirteen comparisons.

Study characteristics

Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted from the included studies, all ten articles were prospective cohort designs and participants who

were free of self reported diabetes at baseline.[5,7,10,11,15,43-47] In aggregate, the included studies consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10 articles, five cohorts were conducted primarily in the United States,[11,15,43,44,47] two articles were done in Asian countries (China and Japan)[7,46] and three cohorts were from European countries.[5,10,45] The number of participants ranged from 3704 in the EPIC-Norfolk study by Cooper et al[10] to 91 246 in the Nurses' Health Study II by Muraki et al.[11] Five studies included both male and female,[5,7,10,15,45] four studies included only female.[43, 44,46,47] One article by Muraki et al reported two independent cohorts, one cohorts included only female, and another only male.[11] The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and vegetables intake separately and combined.[5,7,10,43,44,47] two papers provided information on fruit and vegetables intake separately,[45,46] one paper provided only the combined data,[15] and another paper provided separate data on fruit.[11] Five papers also included separate data on the intake of GLV.[5,7,44-46] In most papers intake of fruit and vegetables was divided into fifths.[11,43-47] All studies provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most studies yielded a score of 3 or below (low quality).

Fruit intake and risk of T2D

11 comparisons from nine studies reported an association between fruit intake and risk of T2D, with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no heterogeneity among studies (P=0.477, I^2=0%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.533) and Egger regression tests (P=0.849) (see supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model, we found a mild curvilinear association (P=0.059 for non-linearity, fig 3). Dose-response analysis indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D (relative risk 0.94, 95% confidence interval 0.89 to 1.00, I^2=0%) (see supplemental fig A in appendix 2).

Vegetables intake and risk of T2D

Eight studies exported an association between vegetables intake and risk of T2D, with 20 933 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95% confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity (P=0.002, I^2=66.5%). However, no evidence of substantial publication bias was observed from the Begg (P=0.602) and Egger regression tests (P=0.176) (see supplemental table B in appendix 1). Among 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative risk 0.98, 95% confidence interval 0.89 to 1.08, I^2=45.8%) (see supplemental fig B in appendix 2). No publication bias was observed (P=0.117). We found no evidence of a curve linear association between vegetables intake and risk (P=0.671 for non-linearity, see supplemental fig C in appendix 2).

Fruit and vegetables intake and risk of T2D

Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and

vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies (P=0.141, I^2=34.6%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.348) and Egger regression tests (P=0.609) (see supplemental table B in appendix 1). Among 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and vegetables intake and risk of T2D. We did not find a significant curvilinear association (P=0.456 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval 0.86 to 1.07, I^2=47.6%) (see supplemental fig F in appendix 2).

GLV intake and risk of T2D

7 comparisons from six studies reported an association between GLV intake and risk of T2D, with 19 139 T2D outcomes and 251 235 participants. Overall, GLV intake was inversely associated with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant heterogeneity was detected among studies (P=0.496, I^2=0%). Additionally, we did not observe evidence of substantial publication bias (the Begg and Egger regression tests, P=0.133 and P=0.101, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only three comparisons were eligible for the trend estimation. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.036 for non-linearity, fig 6). Dose-response analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, I^2=20.9%) (see supplemental fig G in appendix 2). No publication bias was observed (P=0.282).

Subgroup analyses

To examine the stability of the primary results, we carried out subgroup analyses (table 1). The association between fruit, vegetables, or fruit and vegetables intake and risk of T2D were similar in subgroup analyses, which were separately defined study quality, length of follow-up, sex, location, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) affected the results. The summary estimates of relative risks from each category were pooled (see supplemental table B in appendix 1). We paid close attention to the highest versus lowest category. Almost all subgroups that analysed intake of GLV showed a benefit of consuming greater quantities (fig 5). Supplemental table B in appendix 1 also showed significant reductions in risk of T2D events for consumption of fruit, vegetables, or fruit and vegetables combined.

Discussion

In this meta-analysis dietary intake of fruit, vegetables, and GLV, but not fruit and vegetables combined, were associated with a lower risk of T2D. Dose-response analyses indicated a 6% lower risk of T2D per 1 serving/day increment of fruit intake and 13% lower risk of T2D per 0.2 serving/day increment of GLV intake, but no significant trend for vegetables or fruit and vegetables combined.

Results in relation to other studies

Over the past decades, extensive prospective studies have reported the association of fruit, vegetables, or fruit and vegetables combined with T2D risk.[5,7,10,11,15,43-47] However, the role of dietary factors in T2D is still controversial. Some of the studies failed to find the association between fruit intake or fruit and vegetables combined and risk of T2D.[8,43] However, Bazzano and

colleagues analysed data from 11 different U.S. states with 18 years of follow-up and found that consumption of fruit was associated with a lower hazard of diabetes, whereas no significant association for total fruit and vegetables consumption.[47] Similar to previous analysis in the Nurses' Health Study, the results from three prospective longitudinal cohort studies also supported an inverse association between fruit intake and risk of T2D.[11] But these studies have the potential for bias due to measurement error. In addition, two cohort studies have suggested an inverse association between total fruit and vegetables consumption and risk of T2D.[10,15]

A few large cohort studies have found an inverse association between vegetables consumption, especially GLV and risk of T2D.[10,44-46] These findings all agreed with two meta-analyses.[5,9] But another systematic review based on five cohort studies suggested that there was no protective association between vegetables intake and T2D.[8]

Several plausible biological mechanisms have been proposed to explain abovementioned association. Fruit and vegetables are rich in fibre,[48] which has been shown to improve insulin sensitivity and insulin secretion to overcome insulin resistance.[49] However, meta-analyses showed that fruit and vegetables fibre is inconsistently associated with the risk of T2D.[50] On the other hand, it may contribute to a decreased incidence of T2D through their low energy density and glycemic load, and high micronutrient content.[51] In particular, GLV are rich in bioactive phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant properties.[52-54] Antioxidants in fruit and vegetables have been hypothesized to improve insulin sensitivity and protect against diabetes in several supplementation trials.[55,56] In addition, it also might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis detected Mg intake to be inversely associated with the risk of T2D.[57] Taking this evidence into consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. The inverse association may be also mediated through weight gain or obesity which is an established risk factor for type 2 diabetes. Fruits are low in energy, which would promote the feeling of fullness and prevent over consumption of energy-dense foods, and resulting in weight loss.[54] Further investigation is warranted to understand the mechanisms involved in the proposed relation between fruit, vegetables, or GLV and risk of T2D.

Exploration of heterogeneity

Heterogeneity between studies was found, which did not alter much in the subgroup analyses. There are differences in types of vegetable consumed between Asian (such as China) and Non-Asian populations. Therefore, within the subgroup analysis we examined location as a possible source of heterogeneity. As traditional Chinese diets are high in vegetables (such as GLV and cruciferous vegetables), unsurprisingly, vegetables (including GLV) intake were greater in China than the US or Europe. We also examined study quality, length of follow-up, sex, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) as possible sources of heterogeneity, these did not show any significant heterogeneity between studies. Although the subgroup analysis could not explain the level of heterogeneity, in interpreting the results, several differences between the studies are worth discussing.

Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs) to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.[7,10,11,43,44,46,47] It is

less suitable for the assessment of absolute intake, which they tend to overestimate.[58,59] However, two studies collected data via a single 24 hour recall and dietary history interviews, respectively.[15,45] These measurements may underestimate true associations between fruit, vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition, calculations of daily consumption were differed (such as servings per week, servings per day, or grams per day). Although we standardized primary data using a standard portion size of 106g, conclusions should be interpreted with caution. Another possible explanation for the differences between the studies might be the classification of food groups. GLVs'criteria was inconsistent: three studies included spinach and lettuce; one included spinach and greens; others did not provide specific description. If they were included with an uniform definition of each groups, the associations might differ.

Strengths and limitations

Compared with the previous meta-analyses,[5,8,9] our study has several strengths. To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of T2D. In addition, to examine the shape of these possible associations, we investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of T2D. Therefore, the results should be more reliable.

In interpreting the results, several limitations of this meta-analysis should also be acknowledged. Firstly, although in the multivariable analysis we considered a multitude of lifestyle and dietary factors. The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. Second, we cannot exclude the possibility of recall bias in the assessments of diet based on the FFQs. However, the prospective study design and exclusion of participants with chronic diseases at baseline should minimize such bias. Third, the noticeable limitation of our study was the potential for bias due to inevitable measurement error, especially for individual with lower consumption levels. We attempted to reduce measurement error in adjusting for energy intake and using of cumulatively averaged intake levels. Fourth, because we had no source of information other than questionnaire for the identification of T2D, we might have underestimated the incidence of T2D. In addition, subclinical diseases at baseline might have distorted our risk estimate to some extent. Finally, the possible limitation is due to language bias. We attempted to minimize this bias by searching major electronic databases with no language restriction. However, several articles published in non-English languages might not appear in international journal databases, and could be omitted by our searches.[60]

Conclusions

In summary, our meta-analysis suggests that higher fruit or GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.

Contributors: ML and ZT conceived and designed the study. ML and YF searched the databases and checked them according to the eligible criteria and exclusion criteria. ZT helped develop search strategies. XZ and WH extract quantitative data. YF, XZ, and WH analyzed the data. ML wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper. ZT is the guarantor.

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Data sharing: No additional data available.

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Supplemental fig A. Forest plot of fruit intake and risk of type 2 diabetes.
(TIFF)

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Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

Variables	Fruit only			Vegetables only			Fruit and vegetables			Green leafy vegetables		
	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value
Location												
Non-Asia	8	0.93 (0.87 to 1.00)	0.049	6	0.96 (0.87 to 1.06)	0.397	7	0.94 (0.84 to 1.04)	0.223	4	0.89 (0.81 to 0.97)	0.012
Asia	3	0.96 (0.82 to 1.12)	0.584	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Quality												
High (≥4)	4	0.92 (0.86 to 1.00)	0.045	2	0.83 (0.52 to 1.33)	0.448	1	1.04 (0.87 to 1.25)	0.671	2	0.86 (0.76 to 0.98)	0.024
Low (<4)	7	0.94 (0.85 to 1.04)	0.240	7	0.93 (0.84 to 1.02)	0.109	8	0.93 (0.84 to 1.02)	0.138	5	0.86 (0.77 to 0.97)	0.010
Duration of follow-up (years)												
≥10	5	0.90 (0.83 to 0.97)	0.006	4	0.91 (0.79 to 1.05)	0.190	5	0.89 (0.77 to 1.02)	0.098	3	0.85 (0.75 to 0.97)	0.014
<10	6	0.98 (0.90 to 1.07)	0.654	5	0.89 (0.72 to 1.10)	0.296	4	1.03 (0.91 to 1.16)	0.674	4	0.87 (0.78 to 0.97)	0.013
Sex												
M and F	5	0.88 (0.79 to 0.98)	0.022	5	0.89 (0.81 to 0.97)	0.010	6	0.87 (0.77 to 0.98)	0.026	4	0.80 (0.69 to 0.92)	0.002
F only	5	0.95 (0.88 to 1.02)	0.168	4	0.94 (0.77 to 1.15)	0.544	3	1.02 (0.94 to 1.11)	0.610	3	0.89 (0.81 to 0.98)	0.014
M only	1	0.99 (0.82 to 1.19)	0.916	0	-	-	0	-	-	0	-	-
Fractions of distribution												
Thirds	1	0.91 (0.71 to 1.16)	0.451	1	0.94 (0.84 to 1.05)	0.277	1	0.90 (0.80 to 1.01)	0.076	1	0.84 (0.65 to 1.08)	0.170
Quarters	3	0.92 (0.81 to 1.04)	0.193	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Fifths	7	0.94 (0.87 to 1.02)	0.144	5	0.96 (0.84 to 1.09)	0.499	6	0.94 (0.82 to 1.08)	0.385	3	0.89 (0.78 to 1.01)	0.062
No of participants												
≥50000	3	0.91 (0.84 to 0.99)	0.032	2	0.83 (0.52 to 1.33)	0.448	1	1.01 (0.91 to 1.13)	0.858	2	0.86 (0.76 to 0.98)	0.024
<50000	8	0.95 (0.87 to 1.04)	0.237	7	0.93 (0.84 to 1.02)	0.109	8	0.92 (0.83 to 1.03)	0.146	5	0.86 (0.77 to 0.97)	0.010
No of cases												
≥1000	5	0.95 (0.88 to 1.03)	0.233	4	1.01 (0.94 to 1.08)	0.810	6	0.96 (0.86 to 1.07)	0.456	3	0.91 (0.84 to 0.98)	0.018
<1000	6	0.91 (0.82 to 1.00)	0.042	5	0.75 (0.66 to 0.85)	0.000	3	0.87 (0.73 to 1.04)	0.119	4	0.78 (0.68 to 0.89)	0.000

M=male, F=female, RR=relative risk.

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Abstract

Objective To clarify and quantify the potential dose-response association between the intake of fruit and vegetables and risk of type 2 diabetes.

Design Meta-analysis and systematic review of prospective cohort studies.

Data source Studies published before February 2014 identified through electronic searches using PubMed and Embase.

Eligibility criteria for selecting studies Prospective cohort studies with relative risks and 95% confidence intervals for type 2 diabetes according to the intake of fruit, vegetables, or fruit and vegetables.

Results A total of ten articles including thirteen comparisons with 24 013 cases of type 2 diabetes and 434 342 participants were included in the meta-analysis. Evidence of curve linear associations were seen between fruit and green leafy vegetables consumption and risk of type 2 diabetes (P=0.059 and P=0.036 for non-linearity, respectively). The summary relative risk of type 2 diabetes for an increase of 1 serving fruit consumed per day was 0.93 (95% confidence interval 0.88 to 0.99) without heterogeneity among studies (P=0.477, I^2=0%). For vegetables, the combined relative risk of type 2 diabetes for an increase of 1 serving consumed per day was 0.90 (95% confidence interval 0.80 to 1.01) with moderate heterogeneity among studies (P=0.002, I^2=66.5%). For green leafy vegetables, the summary relative risk of type 2 diabetes for an increase of 0.2 serving consumed per day was 0.87 (95% confidence interval 0.81 to 0.93) without heterogeneity among studies (P=0.496, I^2=0%). The combined estimates showed no significant benefits of increasing the consumption of fruit and vegetables combined.

Conclusions Higher fruit or green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.

Article summary

Strengths and limitations of this study

To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of type 2 diabetes. We also investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of type 2 diabetes.

The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. We cannot exclude the possibility of recall bias in the assessments of diet based on the food frequency questionnaires.

1. Health expenditure on type 2 diabetes is increasing worldwide.
2. Epidemiological studies suggest that the intake of fruit and vegetables is beneficial in delaying or preventing the development of type 2 diabetes, though results from cohort studies are controversial.
3. Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.
4. Dose-response analyses indicated a 6% lower risk of type 2 diabetes per 1 serving/day increment of fruit intake and 13% lower risk of type 2 diabetes per 0.2 serving/day increment of green leafy vegetables intake.
5. Further evidence from preferably randomized controlled studies should explore what kind of fruit or green leafy vegetables can reduce the risk of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030,[1] with serious consequences for health care expenditure.[2] It has been estimated that the global health expenditure on diabetes is at least $ 376 billion in 2010 and will be $ 490 billion in 2030,[3] this creates a major public health burden. The prevention of T2D is thus clearly an important public health priority. In recent decades, concern has mounted regarding the premature mortality and morbidity associated with T2D, with growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D.[4] Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.[5]

To minimize the risk of dietary factors and reduce the incidence of T2D, a World Health Organization recommended the public to consume more than 400 g or five portions of combined fruit and vegetables per day for the prevention of T2D.[6] Nevertheless, in the Japan Public Health Center-based Prospective (JPHC) Study,[7] after a mean follow-up over five years, participants with the intake of fruit and vegetables may not be appreciably associated with the risk of T2D. Vegetables, especially green leafy vegetables (GLV), have been suggested to explain an apparent beneficial effect on T2D. In addition, several meta-analyses of observational studies have found that an increase in daily intake of GLV could significantly reduce the risk of T2D.[5,8,9] These studies were restricted by heterogeneous with respect to sample size. Additionally, recent studies involving relationship between the intake of fruit and vegetables and risk of T2D have been published from then on.[5,7,10,11] Furthermore, whether any dose-response relation exists between the intake of fruit and vegetables and risk of T2D is unknown. Therefore, we systematically reviewed and meta-analysed available studies to quantify the associations between dietary intake of fruit and vegetables and incidence of T2D based on identified prospective cohort studies. We pooled risk estimates for the highest versus lowest category of intake to examine the overall association. We also conducted a dose-response analysis for the trend estimation.

Methods

Search strategy

We carried out a systematic search of PubMed (Medline) and Embase through February 2014 for prospective cohort studies examining the association between the intake of fruit and vegetables and risk of T2D. The following key words were used in our search strategies: ("fruits" OR "vegetables" OR "citrus") AND ("Type 2 diabetes" OR "non-insulin-dependent diabetes mellitus" OR "Diabetes Mellitus, Type 2" OR "NIDDM" OR "prediabetes" OR "impaired glucose tolerance" OR "impaired fasting glucose" OR "glucose" OR "hyperglycaemia" OR "insulin") AND ("Follow-up studies" OR "prospective studies" OR "cohort studies" OR "longitudinal studies"). We restricted the search to human studies. No language restrictions were imposed. In addition, we scrutinized possible eligible references from relevant original papers and review articles to identify potential publications. We followed standard criteria for the performing and reporting of the meta-analyses of observational studies.[12]

Study selection

Citations selected from the initial search were subsequently screened for eligibility. Studies were included in this meta-analysis if they satisfied the following criteria: (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design); (3) the exposure of interest was the intake of fruits, vegetables, or fruit and vegetables combined; (4) the outcome was T2D; and (5) reported multivariate-adjusted risk estimates for the association between the fruit, vegetables, or fruit and vegetables combined, assessed as dietary intake, and T2D. Additionally, we excluded animal studies and letters without sufficient data. If data were reported more than once, we included the study with the longest follow-up time.

Validity assessment

Two authors (ML and YF) independently assessed all studies for quality using a modified scoring system, which allowed a total score from 0 to 6 points (6 reflecting the highest quality) on the basis of MOOSE,[12] QUATSO,[13] and STROBE.[14] The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).[9]

Data extraction

Data were carried out independently by two other authors (XZ and WH) using a standard electronic sheets and cross-check to reach a consensus. For each study, the following information was abstracted: name of the first author, publication year, study population, geographical location, sex, age range, sample size (number of T2D cases, number of non-T2D cases, and number of participants), duration of follow-up, methods used to assess fruit and vegetables intake and ascertain T2D cases, highest and lowest of fruit and vegetables intake, and covariates adjusted for in the multivariable model. Study quality was evaluated by using the modified scoring system. All data were extracted from the published papers. If necessary, the primary authors were contacted to retrieve further information. For two studies that expressed data separately for men and women,[7,15] one study that included data from multiple cohorts,[11] we considered the analysis for each sex or cohort as an independent comparison and extracted data separately.

Statistical analysis

Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to be a valid approximation of relative risks, we converted these values in every study by taking their natural logarithms and calculating standard errors and corresponding 95% confidence intervals. Relative risks and their standard errors were pooled with the DerSimonian and Laird random effects model, which takes into account both within-study and between-study variabilities.[16] When some studies included in our meta-analysis used different measurement units (eg, grams per day or portions per day or servings per day),[5,10,15] we standardized fruit and vegetables intake into servings per day using a standard portion size of 106 g.[17] As different studies might use different exposure categories (thirds, quarters, or fifths),[7,11,15] we used the study specific relative risk for the highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the

meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker[18] and Orsini et al[19,20] was used to calculate study-specific slopes (linear trends) and 95% confidence intervals. The method requires the distributions of cases and person years for exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the median or mean intake was not provided. When the highest category was open ended, we assumed that the average of the category was set at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the distribution.[21] A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the fractional polynomials component is equal to zero. Heterogeneity among studies was evaluated using the chi-square test based on Cochran's Q test and I^2 statistic at $P<0.10$ level of significance,[16] and quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total variation in point estimates that is due to heterogeneity rather than chance.[22] We considered low, moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively. To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We also performed the Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.[23-25] All the statistical analyses were performed in Stata 12 (Stata Corp, College Station, TX). A threshold of P<0.1 was used to decide whether heterogeneity or publication bias was present.[24] In other ways, P values were 2-sided and P<0.05 was considered statistically significant.

Results

Literature search

Fig 1 shows the results of literature research and selection. We identified 308 articles from PubMed and 365 articles from Embase. After exclusion of duplicate records and studies that did not fulfill our inclusion criteria, 27 articles remained, and we further evaluated the full texts of these 27 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to lack of sufficient data for estimation of relative risks.[26-30] Five articles were excluded because no original data could be extracted (review, type 1 diabetes, or cross sectional studies).[31-35] Another four articles were excluded because we deemed irrelevant.[36-39] We also excluded three articles because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to warrant inclusion within the meta-analysis.[40-42] Finally, eleven articles met the inclusion criteria and were included in the meta-analysis.[5,7,10,11,15,43-47] Among these ten articles, Ford et al and Kurotani et al study examined male and female separately,[7,11] Cooper et al have two studies (study a:2012 and study b:2012) and Muraki et al report included data from two independent cohorts.[15] Thus, our meta-analysis included thirteen comparisons.

Study characteristics

Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted from the included studies, all ten articles were prospective cohort designs and participants who

were free of self reported diabetes at baseline.[5,7,10,11,15,43-47] In aggregate, the included studies consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10 articles, five cohorts were conducted primarily in the United States,[11,15,43,44,47] two articles were done in Asian countries (China and Japan)[7,46] and three cohorts were from European countries.[5,10,45] The number of participants ranged from 3704 in the EPIC-Norfolk study by Cooper et al[10] to 91 246 in the Nurses' Health Study II by Muraki et al.[11] Five studies included both male and female,[5,7,10,15,45] four studies included only female.[43, 44,46,47] One article by Muraki et al reported two independent cohorts, one cohorts included only female, and another only male.[11] The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and vegetables intake separately and combined.[5,7,10,43,44,47] two papers provided information on fruit and vegetables intake separately,[45,46] one paper provided only the combined data,[15] and another paper provided separate data on fruit.[11] Five papers also included separate data on the intake of GLV.[5,7,44-46] In most papers intake of fruit and vegetables was divided into fifths.[11,43-47] All studies provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most studies yielded a score of 3 or below (low quality).

Fruit intake and risk of T2D

11 comparisons from nine studies reported an association between fruit intake and risk of T2D, with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no heterogeneity among studies (P=0.477, I^2=0%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.533) and Egger regression tests (P=0.849) (see supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model, we found a mild curvilinear association (P=0.059 for non-linearity, fig 3). Dose-response analysis indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D (relative risk 0.94, 95% confidence interval 0.89 to 1.00, I^2=0%) (see supplemental fig A in appendix 2).

Vegetables intake and risk of T2D

Eight studies exported an association between vegetables intake and risk of T2D, with 20 933 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95% confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity (P=0.002, I^2=66.5%). However, no evidence of substantial publication bias was observed from the Begg (P=0.602) and Egger regression tests (P=0.176) (see supplemental table B in appendix 1). Among 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative risk 0.98, 95% confidence interval 0.89 to 1.08, I^2=45.8%) (see supplemental fig B in appendix 2). No publication bias was observed (P=0.117). We found no evidence of a curve linear association between vegetables intake and risk (P=0.671 for non-linearity, see supplemental fig C in appendix 2).

Fruit and vegetables intake and risk of T2D

Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and

vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies (P=0.141, I^2=34.6%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.348) and Egger regression tests (P=0.609) (see supplemental table B in appendix 1). Among 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and vegetables intake and risk of T2D. We did not find a significant curvilinear association (P=0.456 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval 0.86 to 1.07, I^2=47.6%) (see supplemental fig F in appendix 2).

GLV intake and risk of T2D

7 comparisons from six studies reported an association between GLV intake and risk of T2D, with 19 139 T2D outcomes and 251 235 participants. Overall, GLV intake was inversely associated with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant heterogeneity was detected among studies (P=0.496, I^2=0%). Additionally, we did not observe evidence of substantial publication bias (the Begg and Egger regression tests, P=0.133 and P=0.101, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only three comparisons were eligible for the trend estimation. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.036 for non-linearity, fig 6). Dose-response analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, I^2=20.9%) (see supplemental fig G in appendix 2). No publication bias was observed (P=0.282).

Subgroup analyses

To examine the stability of the primary results, we carried out subgroup analyses (table 1). The association between fruit, vegetables, or fruit and vegetables intake and risk of T2D were similar in subgroup analyses, which were separately defined study quality, length of follow-up, sex, location, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) affected the results. The summary estimates of relative risks from each category were pooled (see supplemental table B in appendix 1). We paid close attention to the highest versus lowest category. Almost all subgroups that analysed intake of GLV showed a benefit of consuming greater quantities (fig 5). Supplemental table B in appendix 1 also showed significant reductions in risk of T2D events for consumption of fruit, vegetables, or fruit and vegetables combined.

Discussion

In this meta-analysis dietary intake of fruit, vegetables, and GLV, but not fruit and vegetables combined, were associated with a lower risk of T2D. Dose-response analyses indicated a 6% lower risk of T2D per 1 serving/day increment of fruit intake and 13% lower risk of T2D per 0.2 serving/day increment of GLV intake, but no significant trend for vegetables or fruit and vegetables combined.

Results in relation to other studies

Over the past decades, extensive prospective studies have reported the association of fruit, vegetables, or fruit and vegetables combined with T2D risk.[5,7,10,11,15,43-47] However, the role of dietary factors in T2D is still controversial. Some of the studies failed to find the association between fruit intake or fruit and vegetables combined and risk of T2D.[8,43] However, Bazzano and

colleagues analysed data from 11 different U.S. states with 18 years of follow-up and found that consumption of fruit was associated with a lower hazard of diabetes, whereas no significant association for total fruit and vegetables consumption.[47] Similar to previous analysis in the Nurses' Health Study, the results from three prospective longitudinal cohort studies also supported an inverse association between fruit intake and risk of T2D.[11] But these studies have the potential for bias due to measurement error. In addition, two cohort studies have suggested an inverse association between total fruit and vegetables consumption and risk of T2D.[10,15]

A few large cohort studies have found an inverse association between vegetables consumption, especially GLV and risk of T2D.[10,44-46] These findings all agreed with two meta-analyses.[5,9] But another systematic review based on five cohort studies suggested that there was no protective association between vegetables intake and T2D.[8]

Several plausible biological mechanisms have been proposed to explain abovementioned association. Fruit and vegetables are rich in fibre,[48] which has been shown to improve insulin sensitivity and insulin secretion to overcome insulin resistance.[49] However, meta-analyses showed that fruit and vegetables fibre is inconsistently associated with the risk of T2D.[50] On the other hand, it may contribute to a decreased incidence of T2D through their low energy density and glycemic load, and high micronutrient content.[51] In particular, GLV are rich in bioactive phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant properties.[52-54] Antioxidants in fruit and vegetables have been hypothesized to improve insulin sensitivity and protect against diabetes in several supplementation trials.[55,56] In addition, it also might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis detected Mg intake to be inversely associated with the risk of T2D.[57] Taking this evidence into consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. The inverse association may be also mediated through weight gain or obesity which is an established risk factor for type 2 diabetes. Fruits are low in energy, which would promote the feeling of fullness and prevent over consumption of energy-dense foods, and resulting in weight loss.[54] Further investigation is warranted to understand the mechanisms involved in the proposed relation between fruit, vegetables, or GLV and risk of T2D.

Exploration of heterogeneity

Heterogeneity between studies was found, which did not alter much in the subgroup analyses. There are differences in types of vegetable consumed between Asian (such as China) and Non-Asian populations. Therefore, within the subgroup analysis we examined location as a possible source of heterogeneity. As traditional Chinese diets are high in vegetables (such as GLV and cruciferous vegetables), unsurprisingly, vegetables (including GLV) intake were greater in China than the US or Europe. We also examined study quality, length of follow-up, sex, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) as possible sources of heterogeneity, these did not show any significant heterogeneity between studies. Although the subgroup analysis could not explain the level of heterogeneity, in interpreting the results, several differences between the studies are worth discussing.

Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs) to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.[7,10,11,43,44,46,47] It is

less suitable for the assessment of absolute intake, which they tend to overestimate.[58,59] However, two studies collected data via a single 24 hour recall and dietary history interviews, respectively.[15,45] These measurements may underestimate true associations between fruit, vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition, calculations of daily consumption were differed (such as servings per week, servings per day, or grams per day). Although we standardized primary data using a standard portion size of 106g, conclusions should be interpreted with caution. Another possible explanation for the differences between the studies might be the classification of food groups. GLVs'criteria was inconsistent: three studies included spinach and lettuce; one included spinach and greens; others did not provide specific description. If they were included with an uniform definition of each groups, the associations might differ.

Strengths and limitations

Compared with the previous meta-analyses,[5,8,9] our study has several strengths. To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of T2D. In addition, to examine the shape of these possible associations, we investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of T2D. Therefore, the results should be more reliable.

In interpreting the results, several limitations of this meta-analysis should also be acknowledged. Firstly, although in the multivariable analysis we considered a multitude of lifestyle and dietary factors. The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. Second, we cannot exclude the possibility of recall bias in the assessments of diet based on the FFQs. However, the prospective study design and exclusion of participants with chronic diseases at baseline should minimize such bias. Third, the noticeable limitation of our study was the potential for bias due to inevitable measurement error, especially for individual with lower consumption levels. We attempted to reduce measurement error in adjusting for energy intake and using of cumulatively averaged intake levels. Fourth, because we had no source of information other than questionnaire for the identification of T2D, we might have underestimated the incidence of T2D. In addition, subclinical diseases at baseline might have distorted our risk estimate to some extent. Finally, the possible limitation is due to language bias. We attempted to minimize this bias by searching major electronic databases with no language restriction. However, several articles published in non-English languages might not appear in international journal databases, and could be omitted by our searches.[60]

Conclusions

In summary, our meta-analysis suggests that higher fruit or GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.

Contributors: ML and ZT conceived and designed the study. ML and YF searched the databases and checked them according to the eligible criteria and exclusion criteria. ZT helped develop search strategies. XZ and WH extract quantitative data. YF, XZ, and WH analyzed the data. ML wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper. ZT is the guarantor.

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Data sharing: No additional data available.

Figures Information

Figure 1. Process of literature search and study selection.
(TIFF)

Figure 2. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and risk of type 2 diabetes.
(TIFF)

Figure 3. Dose-response analyses of fruit intake and risk of type 2 diabetes.
(TIFF)

Figure 4. Random effects analysis of fully adjusted studies for highest versus lowest intake of vegetables and risk of type 2 diabetes.
(TIFF)

Figure 5. Random effects analysis of fully adjusted studies for highest versus lowest intake of green leafy vegetables and risk of type 2 diabetes.
(TIFF)

Figure 6. Dose-response analyses of green leafy vegetables intake and risk of type 2 diabetes.
(TIFF)

Appendix figure information

Supplemental fig A. Forest plot of fruit intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig B. Forest plot of vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig C. Dose-response analyses of vegetables intake and risk of type 2 diabetes. (TIFF)

Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and vegetables and risk of type 2 diabetes.
(TIFF)

Supplemental fig E. Dose-response analyses of fruit and vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes. (TIFF)

Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes. (TIFF)

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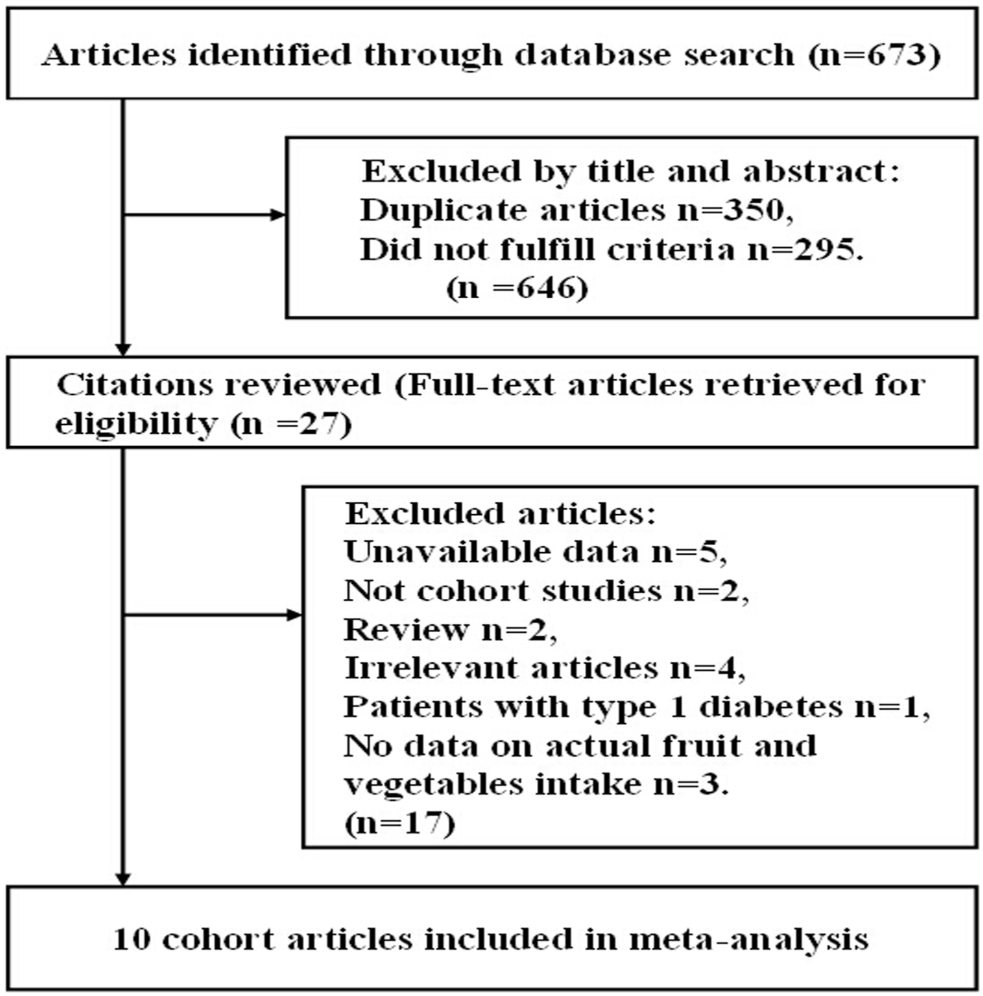
Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

Variables	Fruit only			Vegetables only			Fruit and vegetables			Green leafy vegetables		
	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value
Location												
Non-Asia	8	0.93 (0.87 to 1.00)	0.049	6	0.96 (0.87 to 1.06)	0.397	7	0.94 (0.84 to 1.04)	0.223	4	0.89 (0.81 to 0.97)	0.012
Asia	3	0.96 (0.82 to 1.12)	0.584	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Quality												
High (≥4)	4	0.92 (0.86 to 1.00)	0.045	2	0.83 (0.52 to 1.33)	0.448	1	1.04 (0.87 to 1.25)	0.671	2	0.86 (0.76 to 0.98)	0.024
Low (<4)	7	0.94 (0.85 to 1.04)	0.240	7	0.93 (0.84 to 1.02)	0.109	8	0.93 (0.84 to 1.02)	0.138	5	0.86 (0.77 to 0.97)	0.010
Duration of follow-up (years)												
≥10	5	0.90 (0.83 to 0.97)	0.006	4	0.91 (0.79 to 1.05)	0.190	5	0.89 (0.77 to 1.02)	0.098	3	0.85 (0.75 to 0.97)	0.014
<10	6	0.98 (0.90 to 1.07)	0.654	5	0.89 (0.72 to 1.10)	0.296	4	1.03 (0.91 to 1.16)	0.674	4	0.87 (0.78 to 0.97)	0.013
Sex												
M and F	5	0.88 (0.79 to 0.98)	0.022	5	0.89 (0.81 to 0.97)	0.010	6	0.87 (0.77 to 0.98)	0.026	4	0.80 (0.69 to 0.92)	0.002
F only	5	0.95 (0.88 to 1.02)	0.168	4	0.94 (0.77 to 1.15)	0.544	3	1.02 (0.94 to 1.11)	0.610	3	0.89 (0.81 to 0.98)	0.014
M only	1	0.99 (0.82 to 1.19)	0.916	0	-	-	0	-	-	0	-	-
Fractions of distribution												
Thirds	1	0.91 (0.71 to 1.16)	0.451	1	0.94 (0.84 to 1.05)	0.277	1	0.90 (0.80 to 1.01)	0.076	1	0.84 (0.65 to 1.08)	0.170
Quarters	3	0.92 (0.81 to 1.04)	0.193	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Fifths	7	0.94 (0.87 to 1.02)	0.144	5	0.96 (0.84 to 1.09)	0.499	6	0.94 (0.82 to 1.08)	0.385	3	0.89 (0.78 to 1.01)	0.062
No of participants												
≥50000	3	0.91 (0.84 to 0.99)	0.032	2	0.83 (0.52 to 1.33)	0.448	1	1.01 (0.91 to 1.13)	0.858	2	0.86 (0.76 to 0.98)	0.024
<50000	8	0.95 (0.87 to 1.04)	0.237	7	0.93 (0.84 to 1.02)	0.109	8	0.92 (0.83 to 1.03)	0.146	5	0.86 (0.77 to 0.97)	0.010
No of cases												
≥1000	5	0.95 (0.88 to 1.03)	0.233	4	1.01 (0.94 to 1.08)	0.810	6	0.96 (0.86 to 1.07)	0.456	3	0.91 (0.84 to 0.98)	0.018
<1000	6	0.91 (0.82 to 1.00)	0.042	5	0.75 (0.66 to 0.85)	0.000	3	0.87 (0.73 to 1.04)	0.119	4	0.78 (0.68 to 0.89)	0.000

M=male, F=female, RR=relative risk.



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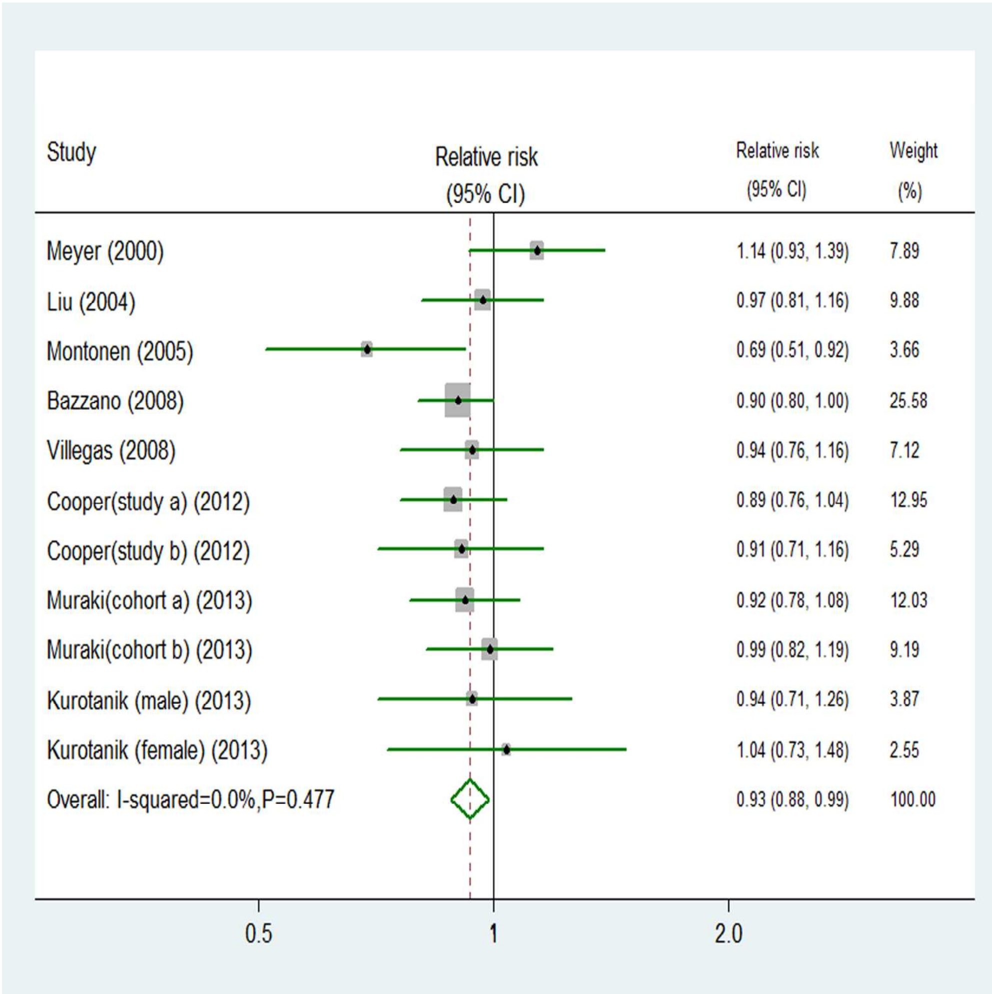

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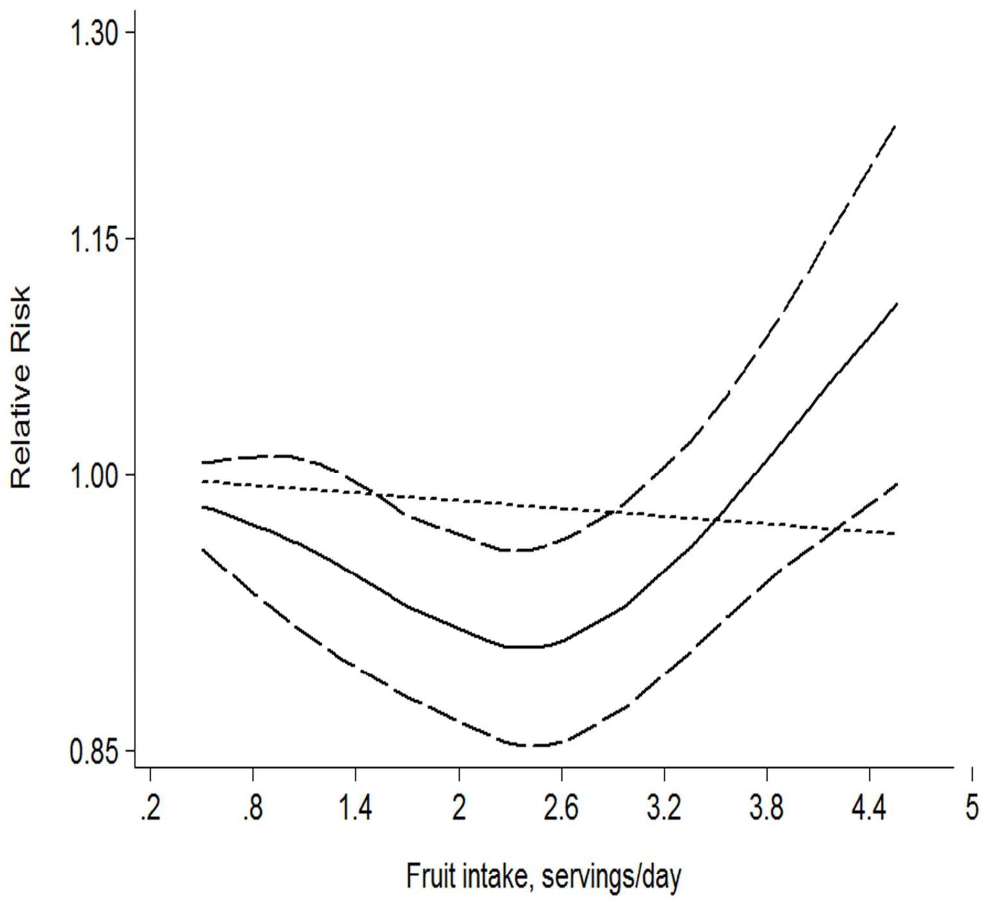

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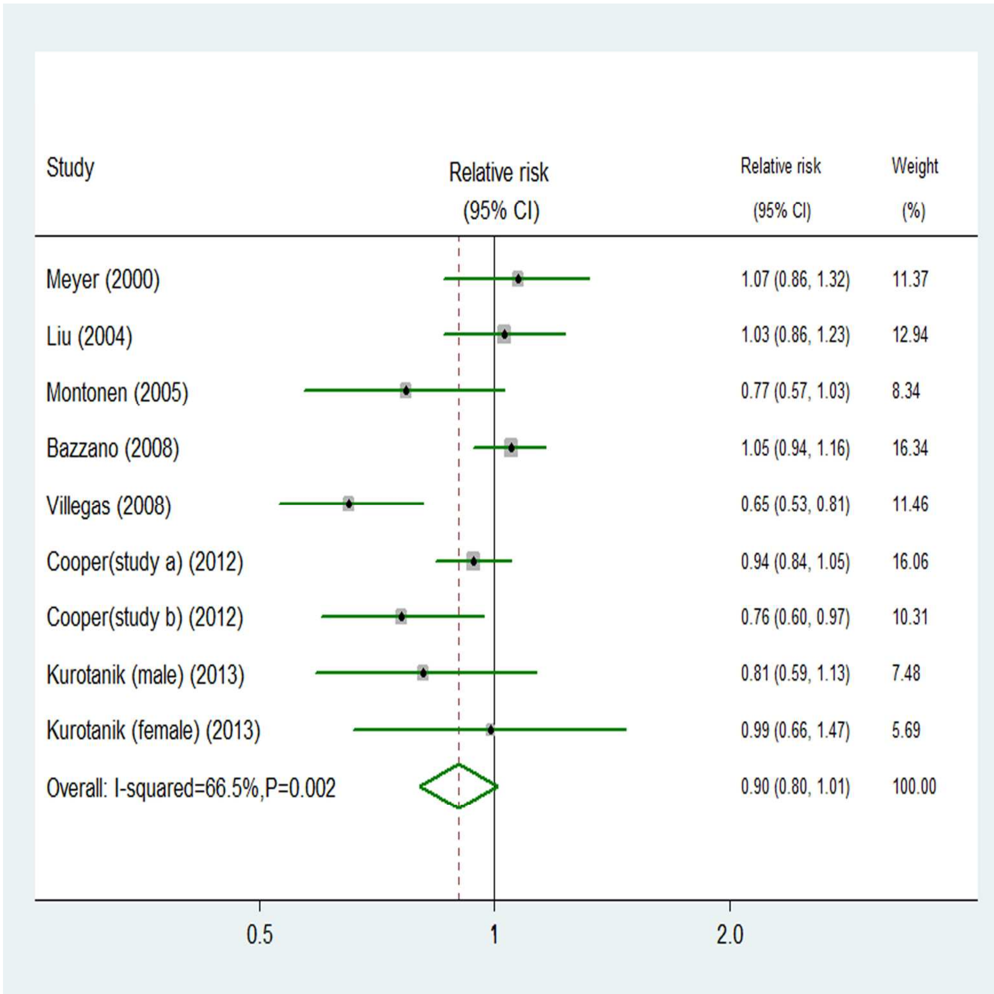

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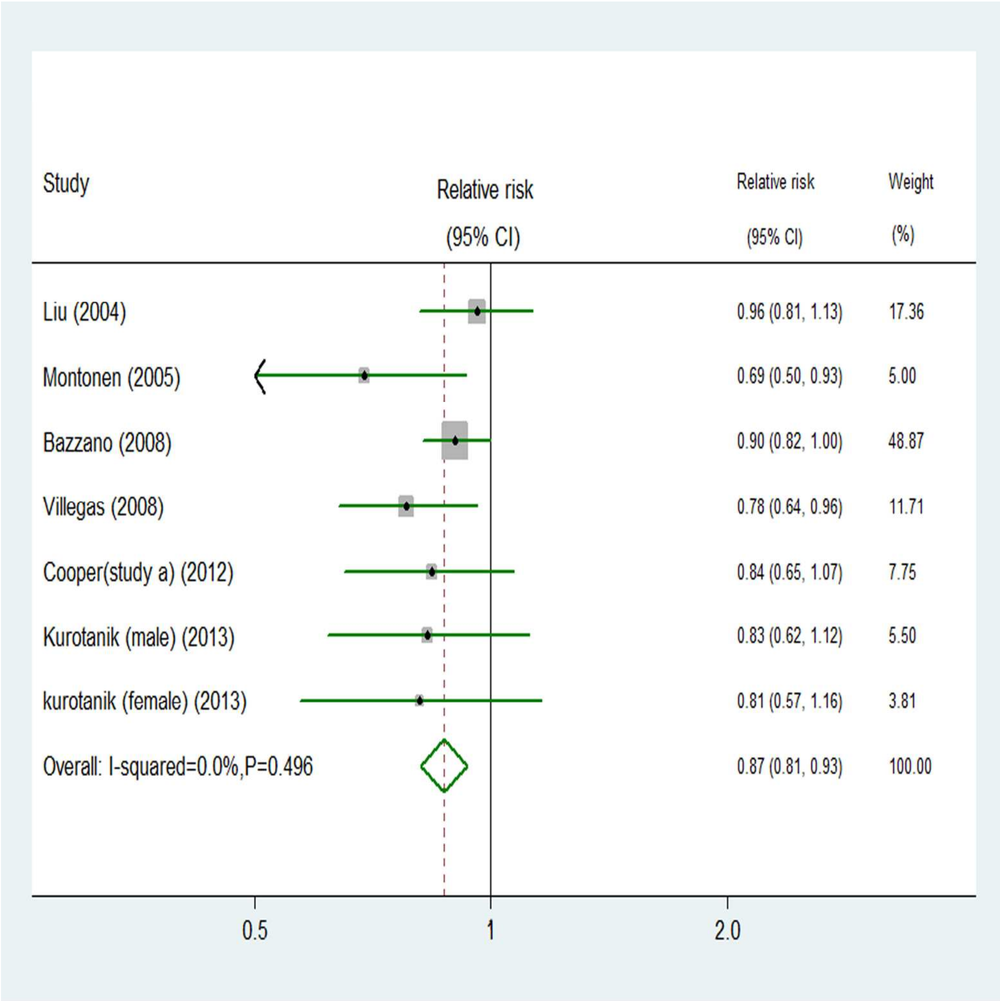

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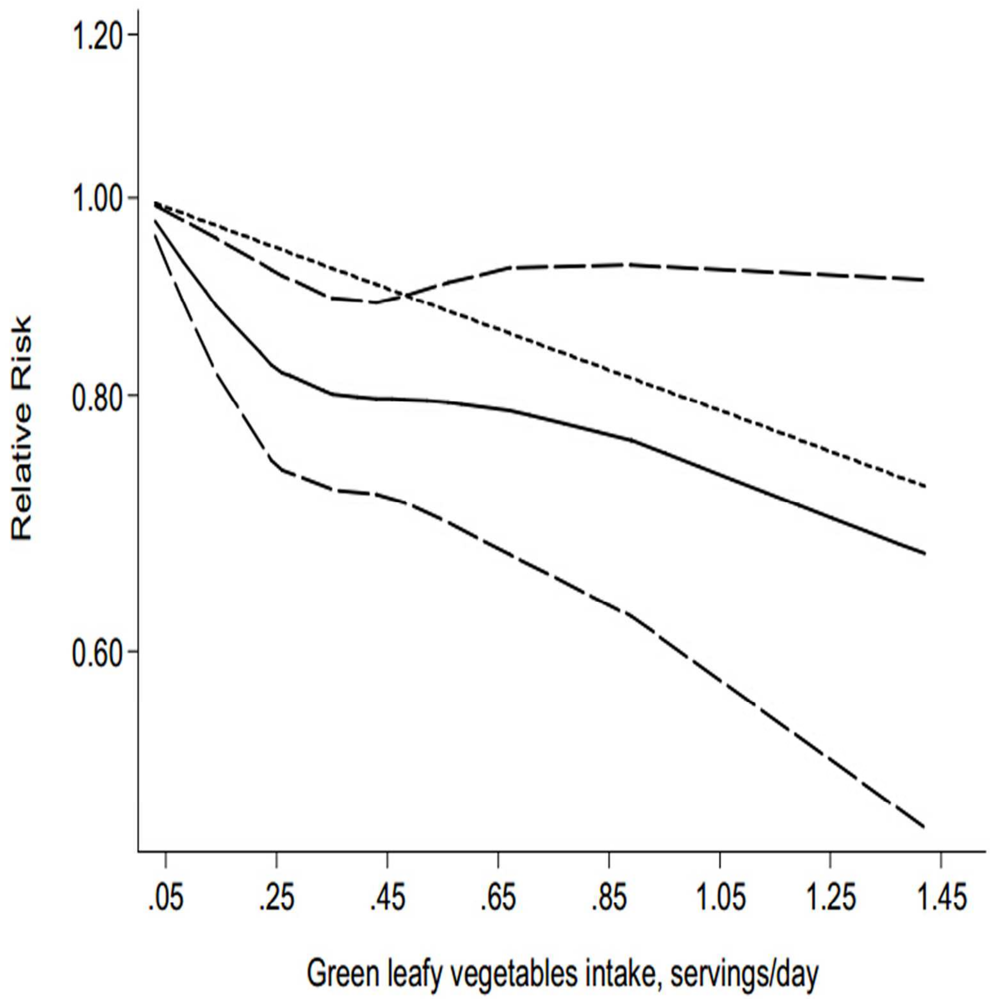

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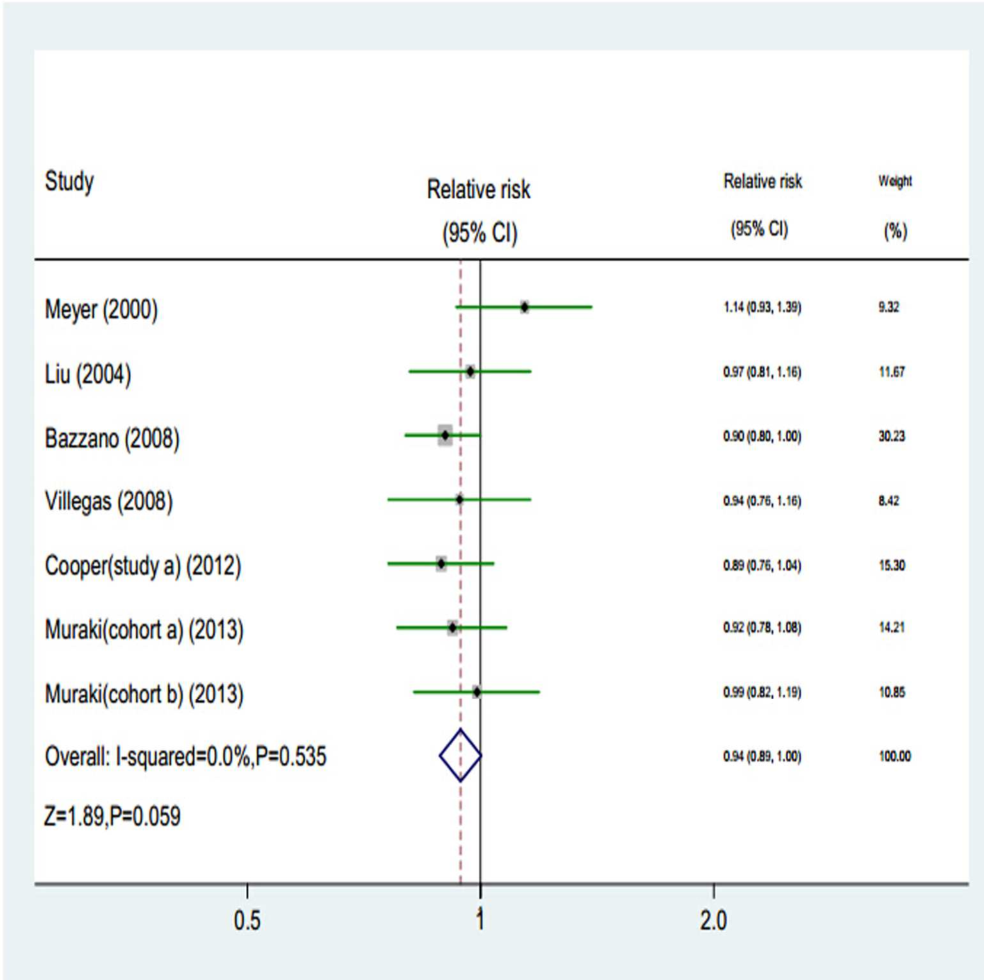

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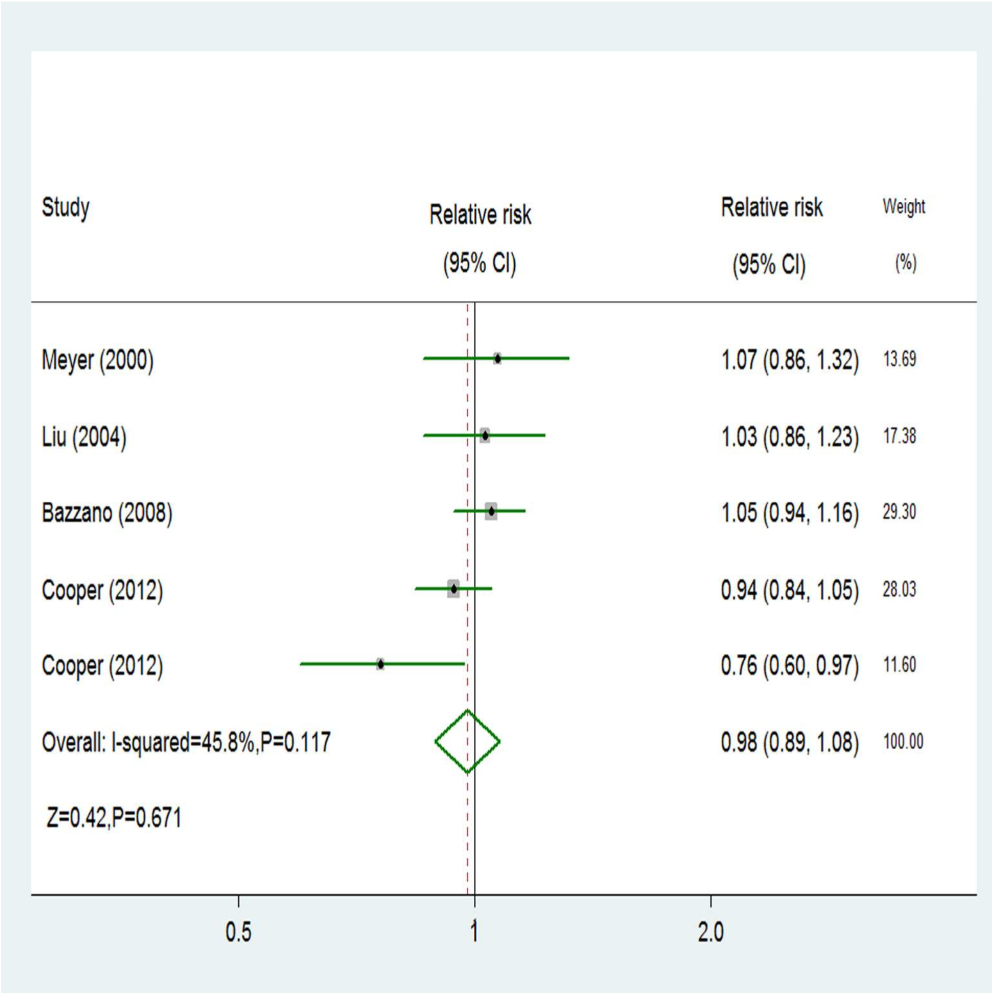

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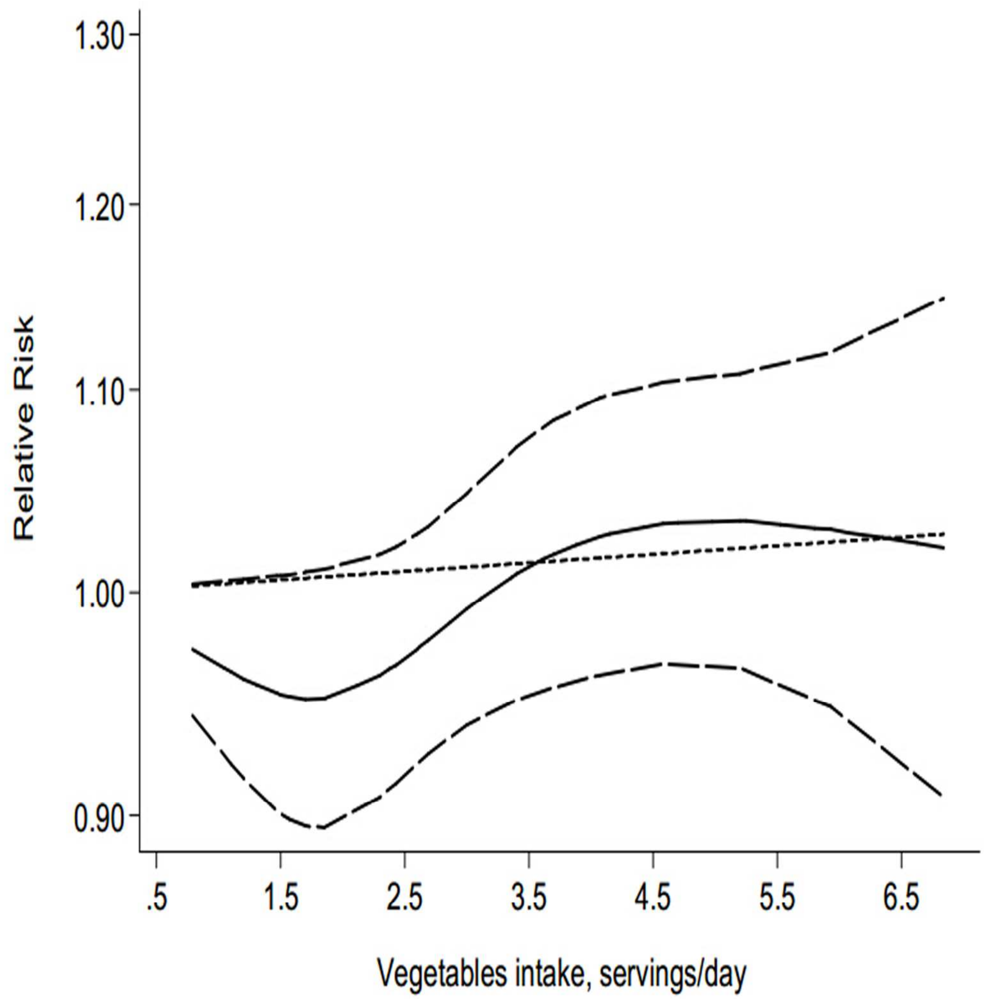

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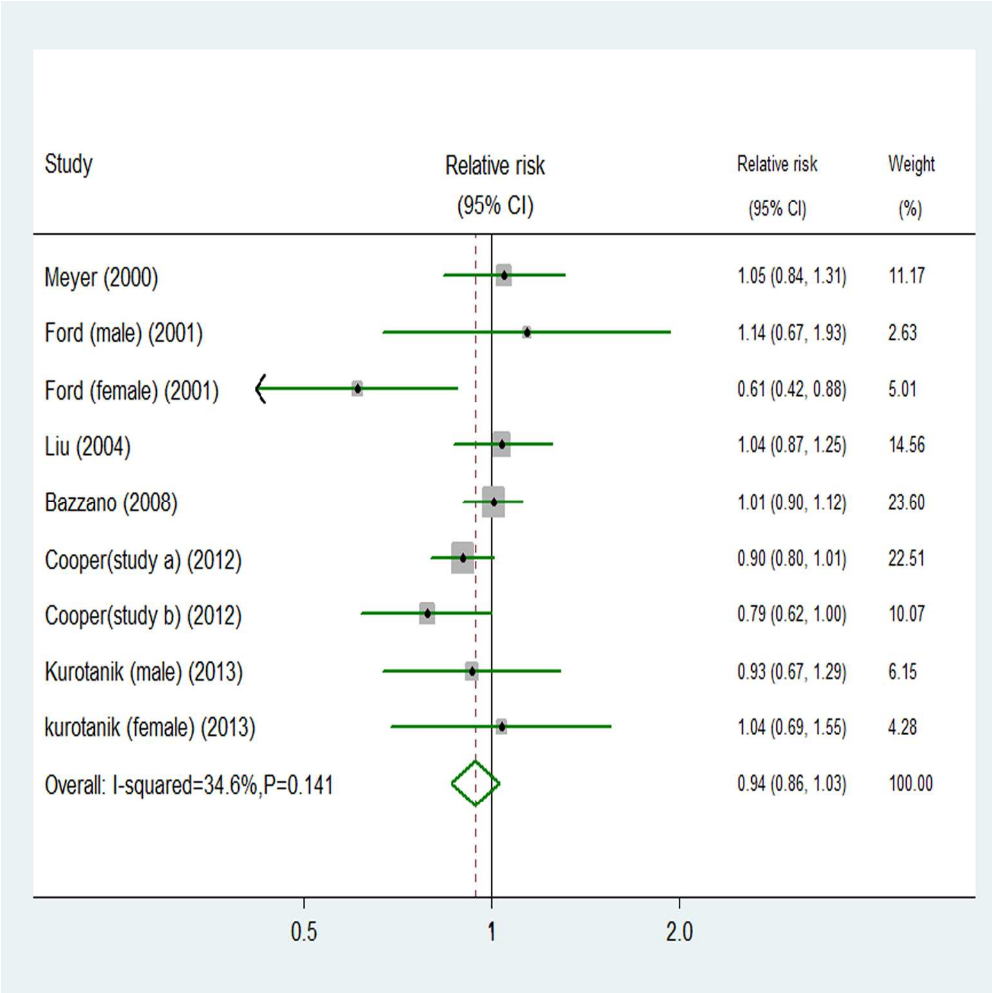

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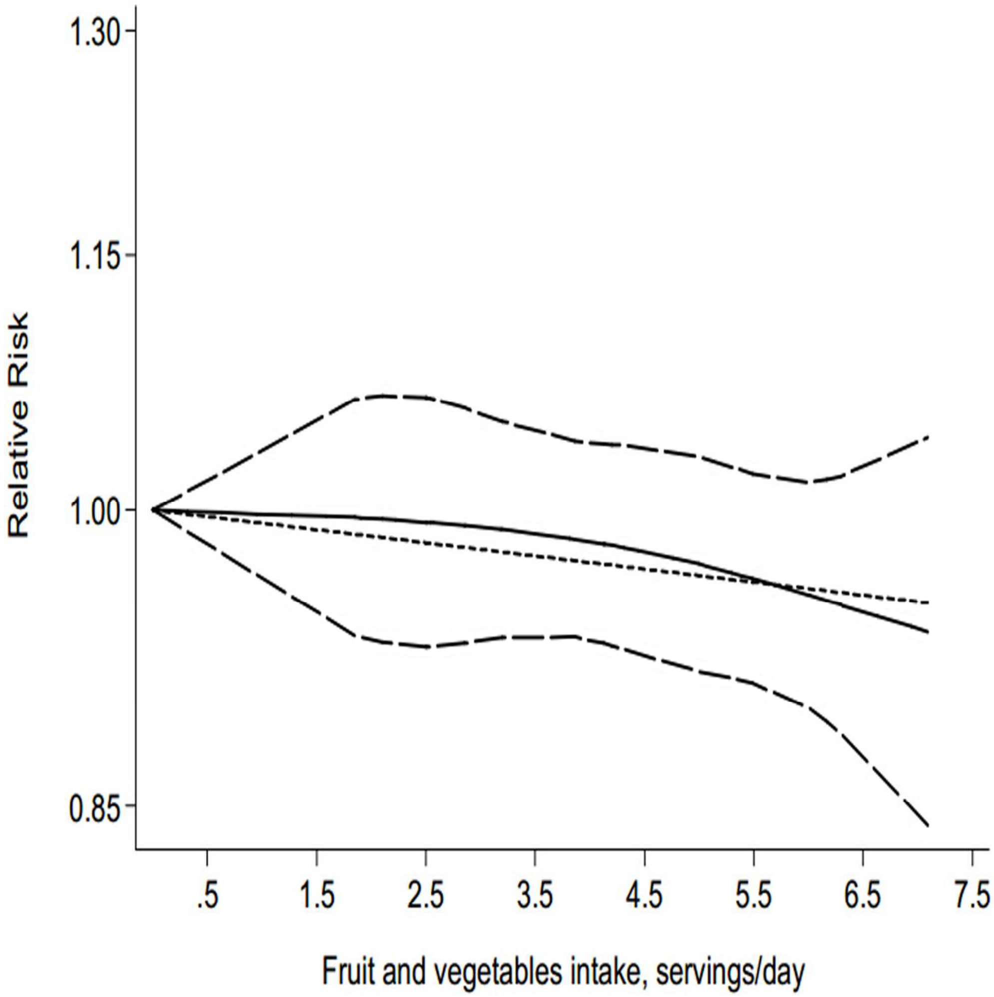

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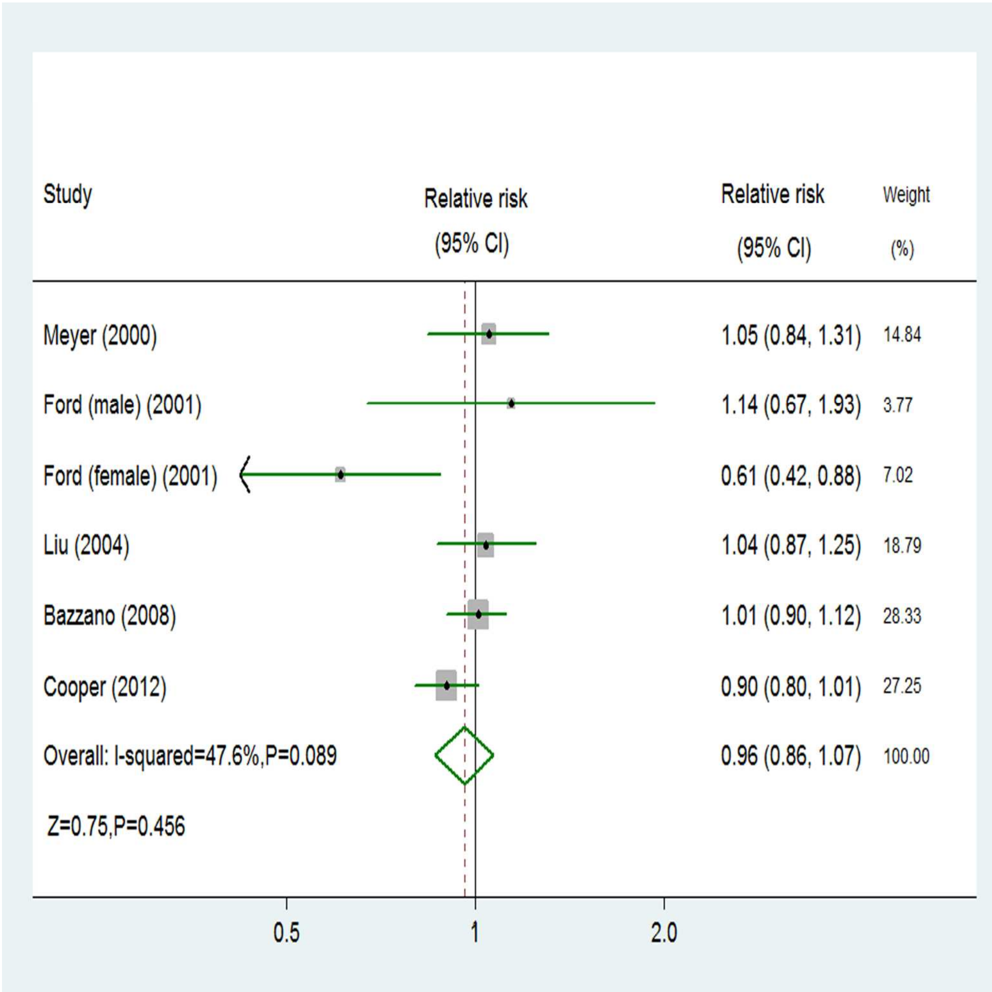

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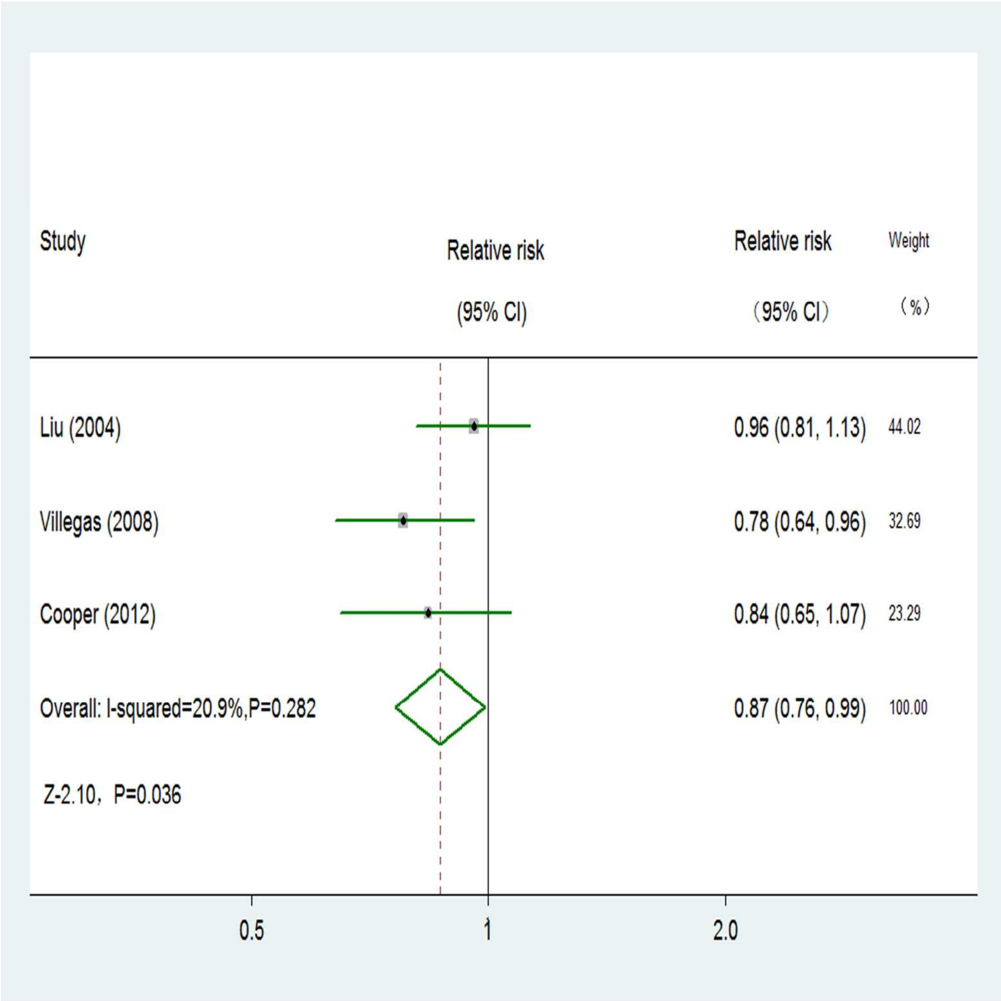

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Table A. Characteristics of included studies of fruit and vegetables intake in relation to incident type 2 diabetes

First author	Country/ cohort	Age (years) /Sex	No of total/follow -up(years)	No of cases /non-cases	Assessment of type 2 diabetes	Measure of intake	Highest/lowest intakes as servings/day	Adjustments	Quality score
Meyer et al 2000,[43]	USA/lowa Women's Health Study	55-69/F	35988/6	1141/34847	Based on self reported	127 item FFQ, Calculated servings/day for fruit, vegetables, and combined. Data divided into fifths	Fruit: 3.36/0.57. Vegetables: 5.93/1.57. Fruit and vegetables: 8.86/2.57	Age, BMI, total energy intake, WHR, education, smoking, alcohol intake, physical activity	2
Ford et al 2001,[15]	USA/NHANES I	25-74/M and F	9665/20	1018/8647	Confirmed by self report or hospital records or death certificate	24 hour recall. Calculated servings/week for fruit and vegetables combined. Data divided into thirds	Fruit and vegetables: ≥5/0	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004,[44]	USA/Women's Health Study	≥45/F	38018/8.8	1614/36404	Based on self reported	131 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce. Data divided into fifths	Fruit: 3.91/0.62. Vegetables: 6.84/1.47. Fruit and vegetables: 10.16/2.54. Green leafy vegetables: 1.42/0.14	Age, BMI, smoking, total calories, alcohol, exercise, history of hypertension/high cholesterol, family history of diabetes	3
Montonen et al 2005,[45]	Finland/Finnish	40-69/M and F	4304/23	383/3921	Confirmed via social	Dietary history interview. Calculated g/day for fruit	Fruit: ＞1.47/＜0.31. Vegetables:	Age, BMI, sex, smoking, energy intake,	3

	Mobile Clinic Health Examination Survey				insurance register	and vegetables separately. Data divided into fifths	> 1.23/ < 0.4. Green leafy vegetables: > 0.4/<0.1	family history of diabetes, geographic area	
Bazzano et al 2008,[47]	USA/Nurses' Health Study	30-55/F	71346/18	4529/66817	Confirmed if met WHO criteria (before 1997) or ADA criteria (after 1998)	61 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce. Data divided into fifths	Fruit: 2.5/0.5. Vegetables: 5.2/1.5. Fruit and Vegetables: 7.5/2.1	Age, BMI, physical activity, smoking, alcohol, hormone therapy, family history of diabetes, total energy intake	4
Villegas et al 2008,[46]	China/Shanghai Women's Health Study	40-70/F	64191/4.6	896/63295	Confirmed by ADA criteria	77 item FFQ. Calculated g/day for fruit and vegetables separately. Defined green leafy vegetables as greens/Chinese greens/spinach. Data divided into fifths	Fruit: 4.56/0.82. Vegetables: 4.04/1.15. Green leafy vegetables: 1.28/0.26	Age, BMI, WHR, education, smoking, alcohol, hypertension, disease history, hormone use, occupational history, physical activity, income, daily energy intake	4
Cooper et al (study a) 2012,[5]	8countries/ EPIC-Inter Act study	40-79/M and F	24939/11	10821/14118	Based on self reported	Country specific dietary questionnaires. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as	Fruit: 5.39/0.75. Vegetables: 3.94/0.88. Fruit and Vegetables: 8.71/2.13. Green	Age, BMI, sex, education, centre, physical activity, smoking, total energy intake, alcohol	2

						chard/endive/lettuce/borage /watercress/beet leaves/spinach. Data divided into quarters	leafy vegetables: 5.93/0.05		
Cooper et al (study 2012,[10] b)	England/EPIC-Norfolk	40-79/M and F	3704/11	653/3051	Based on self reported	117 item FFQ. Calculated servings/day for fruit, vegetables and combined. Data divided into thirds	Fruit: 3.4/0.6. Vegetables: 2.6/1.1. Fruit and Vegetables: 5.7/2.1	Age, BMI, sex, waist circumference, education, TDI, occupational social class, smoking, physical activity, family history of diabetes, energy intake, season	2
Muraki et al (cohort 2013,[11] a)	USA/Nurses' Health Study II	24-44/F	91246/8	741/90505	Confirmed by ADA criteria (after 1998)	133 item FFQ. Calculated servings/week for fruit. Data divided into fifths	Fruit: ≥3/＜0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
Muraki et al (cohort 2013,[11] b)	USA/Health Professionals Follow-up Study	40-75/M	42504/12	1321/41183	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: ≥3/＜0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use,	4

								total energy intake	
Kurotanik et al 2013,[7]	Japan/JPHC Study	40-69/M and F	48437/5	896/47541	Based on self reported	147 item FFQ. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/Chinese chives/garland chrysanthemums/cbingensai/leaf mustard/mugwort/chard/komatsuna. Data divided into quarters	M Fruit: 3.42/0.34. Vegetables: 3.35/0.71. Fruit and Vegetables: 6.48/1.38. Green leafy vegetables: 0.45/0.04. F Fruit: 4.60/0.7. Vegetables: 3.84/0.94. Fruit and Vegetables: 8.1/1.98. Green leafy vegetables: 0.54/0.07	Age, BMI, public health centre area, smoking, alcohol, leisure-time activity, history of hypertension, coffee, family history of diabetes, Mg intake, Ca intake, energy intake	3

FFQ=food frequency questionnaire, BMI=body mass index, SBP=systolic blood pressure, TDI=townsend deprivation index, WHR=weight:height ratio, ADA=American Diabetes Association, WHO=World Health Organization, M=male, F=female, study a= the EPIC-InterAct study, study b= the EPIC-Norfolk study, cohort a= the Nurses' Health Study II study, cohort b= the Health Professionals Follow-up Study. The analysis included 13 cohorts among the ten articles, where Ford et al and Kurotani et al study examined male and female separately, Cooper et al have two studies in 2012 and Muraki et al report included data from two independent cohorts.

Table B. Meta-analysis of intake of fruit and vegetables and risk of type 2 diabetes (highest versus lowest category)

Variables	No of comparisons	Cases/ total	Test of association	Test of heterogeneity	Analysis of publication bias
			Pooled RR (95% CI), P value	Heterogeneity (I^2, %), P value	Begg's test, Egger's test (P value)
Fruit only	11	22995/424677	0.93 (0.88 to 0.99), 0.015	0, 0.477	0.533, 0.849
Vegetables only	9	20933/290927	0.90 (0.80 to 1.01), 0.068	66.5, 0.002	0.602, 0.176
Fruit and vegetables	9	20672/232097	0.94 (0.86 to 1.03), 0.202	34.6, 0.141	0.348, 0.609
Green leafy vegetables	7	19139/251235	0.87 (0.81 to 0.93), 0.000	0, 0.496	0.133, 0.101

MOOSE Checklist

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030, with serious consequences for health care expenditure. The prevention of T2D is thus clearly an important public health priority. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D. Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.
√	Hypothesis statement	Fruit and vegetable intake decrease risk of type 2 diabetes mellitus.
√	Description of study outcomes	Type 2 diabetes mellitus.
√	Type of exposure or intervention used	Fruit, vegetables, or green leafy vegetables
√	Type of study designs used	We included (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design).
√	Study population	We placed no restriction.
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the two investigators XZ and WH are indicated in the author list.
√	Search strategy, including time period included in the synthesis and keywords	PubMed from 1965 –February 2014 EMBASE from 1974 –February 2014 Keywords: (“fruits” OR “vegetables” OR “citrus”) AND (“Type 2 diabetes” OR “non-insulin-dependent diabetes mellitus” OR “Diabetes Mellitus, Type 2” OR “NIDDM” OR “prediabetes” OR “impaired glucose tolerance” OR “impaired fasting glucose” OR “glucose” OR “hyperglycaemia” OR “insulin”) AND (“Follow-up studies” OR “prospective studies” OR “cohort studies” OR “longitudinal studies”).
√	Databases and registries searched	PubMed and EMBASE
√	Search software used, name and version, including special features	We did not employ a search software. EndNote was used to merge retrieved citations and eliminate duplications
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for

		additional references,
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the process of literature search and study selection. The citation list is available upon request
√	Method of addressing articles published in languages other than English	We placed no restrictions on language; local scientists fluent in the original language of the article were contacted for translation
√	Method of handling abstracts and unpublished studies	We had contacted a few authors for unpublished studies on the association.
√	Description of any contact with authors	We contacted authors who had conducted multivariate analysis with diabetes as a covariate, but had not reported relative risk for fruit, vegetables, or green leafy vegetables.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Assessment of confounding	Restricted the analysis to age-adjusted estimates only. Conducted sensitivity analyses by eliminating studies that had not adjusted for possible confounders.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Quality was assessed with a total score from 0 to 6 points. The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).
√	Assessment of heterogeneity	Heterogeneity of the studies were explored within two types of study designs using Cochrane's Q test of heterogeneity and I^2 statistic that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	Description of methods of meta-analyses, sensitivity analyses, subgroup analyses and assessment of publication bias are detailed in the methods.

√	Provision of appropriate tables and graphics	We included 1 flow chart, several summary tables and figures.
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 2, 4, 5 and D
√	Table giving descriptive information for each study included	Table A
√	Results of sensitivity testing	Table 1
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	Subgroup analyses indicate heterogeneity in strengths of the association due to most common biases in cohort studies.
√	Justification for exclusion	We excluded studies that had not adjusted for or were standardized by age, a potential confounder, and used different exposure or outcome assessment for the comparison groups.
√	Assessment of quality of included studies	We discussed the results of the subgroup analyses, and potential reasons for the observed heterogeneity.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	We discussed that potential unmeasured confounders such as other chronic diseases may have caused residual confounding, but the measured factors that are correlated with such confounders would have mitigated the bias. We noted that the variations in the strengths of association may be due to true population differences, or to differences in quality of studies.
√	Generalization of the conclusions	Our meta-analysis suggests that higher fruit or vegetables, particularly GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D.
√	Guidelines for future research	We recommend future preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.
√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.

BMJ Open

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Abstract

Objective To clarify and quantify the potential dose-response association between the intake of fruit and vegetables and risk of type 2 diabetes.

Design Meta-analysis and systematic review of prospective cohort studies.

Data source Studies published before February 2014 identified through electronic searches using PubMed and Embase.

Eligibility criteria for selecting studies Prospective cohort studies with relative risks and 95% confidence intervals for type 2 diabetes according to the intake of fruit, vegetables, or fruit and vegetables.

Results A total of ten articles including thirteen comparisons with 24 013 cases of type 2 diabetes and 434 342 participants were included in the meta-analysis. Evidence of curve linear associations were seen between fruit and green leafy vegetables consumption and risk of type 2 diabetes (P=0.059 and P=0.036 for non-linearity, respectively). The summary relative risk of type 2 diabetes for an increase of 1 serving fruit consumed per day was 0.93 (95% confidence interval 0.88 to 0.99) without heterogeneity among studies (P=0.477, I^2=0%). For vegetables, the combined relative risk of type 2 diabetes for an increase of 1 serving consumed per day was 0.90 (95% confidence interval 0.80 to 1.01) with moderate heterogeneity among studies (P=0.002, I^2=66.5%). For green leafy vegetables, the summary relative risk of type 2 diabetes for an increase of 0.2 serving consumed per day was 0.87 (95% confidence interval 0.81 to 0.93) without heterogeneity among studies (P=0.496, I^2=0%). The combined estimates showed no significant benefits of increasing the consumption of fruit and vegetables combined.

Conclusions Higher fruit or green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.

Article summary

Strengths and limitations of this study

To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of type 2 diabetes. We also investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of type 2 diabetes.

The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. We cannot exclude the possibility of recall bias in the assessments of diet based on the food frequency questionnaires.

1. Health expenditure on type 2 diabetes is increasing worldwide.
2. Epidemiological studies suggest that the intake of fruit and vegetables is beneficial in delaying or preventing the development of type 2 diabetes, though results from cohort studies are controversial.
3. Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.
4. Dose-response analyses indicated a 6% lower risk of type 2 diabetes per 1 serving/day increment of fruit intake and 13% lower risk of type 2 diabetes per 0.2 serving/day increment of green leafy vegetables intake.
5. Further evidence from preferably randomized controlled studies should explore what kind of fruit or green leafy vegetables can reduce the risk of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030,[1] with serious consequences for health care expenditure.[2] It has been estimated that the global health expenditure on diabetes is at least $ 376 billion in 2010 and will be $ 490 billion in 2030,[3] this creates a major public health burden. The prevention of T2D is thus clearly an important public health priority. In recent decades, concern has mounted regarding the premature mortality and morbidity associated with T2D, with growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D.[4] Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.[5]

To minimize the risk of dietary factors and reduce the incidence of T2D, a World Health Organization recommended the public to consume more than 400 g or five portions of combined fruit and vegetables per day for the prevention of T2D.[6] Nevertheless, in the Japan Public Health Center-based Prospective (JPHC) Study,[7] after a mean follow-up over five years, participants with the intake of fruit and vegetables may not be appreciably associated with the risk of T2D. Vegetables, especially green leafy vegetables (GLV), have been suggested to explain an apparent beneficial effect on T2D. In addition, several meta-analyses of observational studies have found that an increase in daily intake of GLV could significantly reduce the risk of T2D.[5,8,9] These studies were restricted by heterogeneous with respect to sample size. Additionally, recent studies involving relationship between the intake of fruit and vegetables and risk of T2D have been published from then on.[5,7,10,11] Furthermore, whether any dose-response relation exists between the intake of fruit and vegetables and risk of T2D is unknown. Therefore, we systematically reviewed and meta-analysed available studies to quantify the associations between dietary intake of fruit and vegetables and incidence of T2D based on identified prospective cohort studies. We pooled risk estimates for the highest versus lowest category of intake to examine the overall association. We also conducted a dose-response analysis for the trend estimation.

Methods

Search strategy

We carried out a systematic search of PubMed (Medline) and Embase through February 2014 for prospective cohort studies examining the association between the intake of fruit and vegetables and risk of T2D. The following key words were used in our search strategies: (“fruits” OR “vegetables” OR “citrus”) AND (“Type 2 diabetes” OR “non-insulin-dependent diabetes mellitus” OR “Diabetes Mellitus, Type 2” OR “NIDDM” OR “prediabetes” OR “impaired glucose tolerance” OR “impaired fasting glucose” OR “glucose” OR “hyperglycaemia” OR “insulin”) AND (“Follow-up studies” OR “prospective studies” OR “cohort studies” OR “longitudinal studies”). We restricted the search to human studies. No language restrictions were imposed. In addition, we scrutinized possible eligible references from relevant original papers and review articles to identify potential publications. We followed standard criteria for the performing and reporting of the meta-analyses of observational studies.[12]

Study selection

Citations selected from the initial search were subsequently screened for eligibility. Studies were included in this meta-analysis if they satisfied the following criteria: (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design); (3) the exposure of interest was the intake of fruits, vegetables, or fruit and vegetables combined; (4) the outcome was T2D; and (5) reported multivariate-adjusted risk estimates for the association between the fruit, vegetables, or fruit and vegetables combined, assessed as dietary intake, and T2D. Additionally, we excluded animal studies and letters without sufficient data. If data were reported more than once, we included the study with the longest follow-up time.

Validity assessment

Two authors (ML and YF) independently assessed all studies for quality using a modified scoring system, which allowed a total score from 0 to 6 points (6 reflecting the highest quality) on the basis of MOOSE,[12] QUATSO,[13] and STROBE.[14] The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).[9]

Data extraction

Data were carried out independently by two other authors (XZ and WH) using a standard electronic sheets and cross-check to reach a consensus. For each study, the following information was abstracted: name of the first author, publication year, study population, geographical location, sex, age range, sample size (number of T2D cases, number of non-T2D cases, and number of participants), duration of follow-up, methods used to assess fruit and vegetables intake and ascertain T2D cases, highest and lowest of fruit and vegetables intake, and covariates adjusted for in the multivariable model. Study quality was evaluated by using the modified scoring system. All data were extracted from the published papers. If necessary, the primary authors were contacted to retrieve further information. For two studies that expressed data separately for men and women,[7,15] one study that included data from multiple cohorts,[11] we considered the analysis for each sex or cohort as an independent comparison and extracted data separately.

Statistical analysis

Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to be a valid approximation of relative risks, we converted these values in every study by taking their natural logarithms and calculating standard errors and corresponding 95% confidence intervals. Relative risks and their standard errors were pooled with the DerSimonian and Laird random effects model, which takes into account both within-study and between-study variabilities.[16] When some studies included in our meta-analysis used different measurement units (eg, grams per day or portions per day or servings per day),[5,10,15] we standardized fruit and vegetables intake into servings per day using a standard portion size of 106 g.[17] As different studies might use different exposure categories (thirds, quarters, or fifths),[7,11,15] we used the study specific relative risk for the highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the

meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker[18] and Orsini et al[19,20] was used to calculate study-specific slopes (linear trends) and 95% confidence intervals. The method requires the distributions of cases and person years for exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the median or mean intake was not provided. When the highest category was open ended, we assumed that the average of the category was set at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the distribution.[21] A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the fractional polynomials component is equal to zero. Heterogeneity among studies was evaluated using the chi-square test based on Cochran's Q test and I^2 statistic at $P < 0.10$ level of significance,[16] and quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total variation in point estimates that is due to heterogeneity rather than chance.[22] We considered low, moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively. To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We also performed the Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.[23-25] All the statistical analyses were performed in Stata 12 (Stata Corp, College Station, TX). A threshold of P<0.1 was used to decide whether heterogeneity or publication bias was present.[24] In other ways, P values were 2-sided and P<0.05 was considered statistically significant.

Results

Literature search

Fig 1 shows the results of literature research and selection. We identified 308 articles from PubMed and 365 articles from Embase. After exclusion of duplicate records and studies that did not fulfill our inclusion criteria, 27 articles remained, and we further evaluated the full texts of these 27 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to lack of sufficient data for estimation of relative risks.[26-30] Five articles were excluded because no original data could be extracted (review, type 1 diabetes, or cross sectional studies).[31-35] Another four articles were excluded because we deemed irrelevant.[36-39] We also excluded three articles because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to warrant inclusion within the meta-analysis.[40-42] Finally, eleven articles met the inclusion criteria and were included in the meta-analysis.[5,7,10,11,15,43-47] Among these ten articles, Ford et al and Kurotani et al study examined male and female separately,[7,11] Cooper et al have two studies (study a:2012 and study b:2012) and Muraki et al report included data from two independent cohorts.[15] Thus, our meta-analysis included thirteen comparisons.

Study characteristics

Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted from the included studies, all ten articles were prospective cohort designs and participants who

were free of self reported diabetes at baseline.[5,7,10,11,15,43-47] In aggregate, the included studies consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10 articles, five cohorts were conducted primarily in the United States,[11,15,43,44,47] two articles were done in Asian countries (China and Japan)[7,46] and three cohorts were from European countries.[5,10,45] The number of participants ranged from 3704 in the EPIC-Norfolk study by Cooper et al[10] to 91 246 in the Nurses' Health Study II by Muraki et al.[11] Five studies included both male and female,[5,7,10,15,45] four studies included only female.[43, 44,46,47] One article by Muraki et al reported two independent cohorts, one cohorts included only female, and another only male.[11] The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and vegetables intake separately and combined.[5,7,10,43,44,47] two papers provided information on fruit and vegetables intake separately,[45,46] one paper provided only the combined data,[15] and another paper provided separate data on fruit.[11] Five papers also included separate data on the intake of GLV.[5,7,44-46] In most papers intake of fruit and vegetables was divided into fifths.[11,43-47] All studies provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most studies yielded a score of 3 or below (low quality).

Fruit intake and risk of T2D

11 comparisons from nine studies reported an association between fruit intake and risk of T2D, with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no heterogeneity among studies (P=0.477, I^2=0%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.533) and Egger regression tests (P=0.849) (see supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model, we found a mild curvilinear association (P=0.059 for non-linearity, fig 3). Dose-response analysis indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D (relative risk 0.94, 95% confidence interval 0.89 to 1.00, I^2=0%) (see supplemental fig A in appendix 2).

Vegetables intake and risk of T2D

Eight studies exported an association between vegetables intake and risk of T2D, with 20 933 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95% confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity (P=0.002, I^2=66.5%). However, no evidence of substantial publication bias was observed from the Begg (P=0.602) and Egger regression tests (P=0.176) (see supplemental table B in appendix 1). Among 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative risk 0.98, 95% confidence interval 0.89 to 1.08, I^2=45.8%) (see supplemental fig B in appendix 2). No publication bias was observed (P=0.117). We found no evidence of a curve linear association between vegetables intake and risk (P=0.671 for non-linearity, see supplemental fig C in appendix 2).

Fruit and vegetables intake and risk of T2D

Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and

vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies (P=0.141, I^2=34.6%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.348) and Egger regression tests (P=0.609) (see supplemental table B in appendix 1). Among 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and vegetables intake and risk of T2D. We did not find a significant curvilinear association (P=0.456 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval 0.86 to 1.07, I^2=47.6%) (see supplemental fig F in appendix 2).

GLV intake and risk of T2D

7 comparisons from six studies reported an association between GLV intake and risk of T2D, with 19 139 T2D outcomes and 251 235 participants. Overall, GLV intake was inversely associated with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant heterogeneity was detected among studies (P=0.496, I^2=0%). Additionally, we did not observe evidence of substantial publication bias (the Begg and Egger regression tests, P=0.133 and P=0.101, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only three comparisons were eligible for the trend estimation. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.036 for non-linearity, fig 6). Dose-response analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, I^2=20.9%) (see supplemental fig G in appendix 2). No publication bias was observed (P=0.282).

Subgroup analyses

To examine the stability of the primary results, we carried out subgroup analyses (table 1). The association between fruit, vegetables, or fruit and vegetables intake and risk of T2D were similar in subgroup analyses, which were separately defined study quality, length of follow-up, sex, location, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) affected the results. The summary estimates of relative risks from each category were pooled (see supplemental table B in appendix 1). We paid close attention to the highest versus lowest category. Almost all subgroups that analysed intake of GLV showed a benefit of consuming greater quantities (fig 5). Supplemental table B in appendix 1 also showed significant reductions in risk of T2D events for consumption of fruit, vegetables, or fruit and vegetables combined.

Discussion

In this meta-analysis dietary intake of fruit, vegetables, and GLV, but not fruit and vegetables combined, were associated with a lower risk of T2D. Dose-response analyses indicated a 6% lower risk of T2D per 1 serving/day increment of fruit intake and 13% lower risk of T2D per 0.2 serving/day increment of GLV intake, but no significant trend for vegetables or fruit and vegetables combined.

Results in relation to other studies

Over the past decades, extensive prospective studies have reported the association of fruit, vegetables, or fruit and vegetables combined with T2D risk.[5,7,10,11,15,43-47] However, the role of dietary factors in T2D is still controversial. Some of the studies failed to find the association between fruit intake or fruit and vegetables combined and risk of T2D.[8,43] However, Bazzano and

colleagues analysed data from 11 different U.S. states with 18 years of follow-up and found that consumption of fruit was associated with a lower hazard of diabetes, whereas no significant association for total fruit and vegetables consumption.[47] Similar to previous analysis in the Nurses' Health Study, the results from three prospective longitudinal cohort studies also supported an inverse association between fruit intake and risk of T2D.[11] But these studies have the potential for bias due to measurement error. In addition, two cohort studies have suggested an inverse association between total fruit and vegetables consumption and risk of T2D.[10,15]

A few large cohort studies have found an inverse association between vegetables consumption, especially GLV and risk of T2D.[10,44-46] These findings all agreed with two meta-analyses.[5,9] But another systematic review based on five cohort studies suggested that there was no protective association between vegetables intake and T2D.[8]

Several plausible biological mechanisms have been proposed to explain abovementioned association. Fruit and vegetables are rich in fibre,[48] which has been shown to improve insulin sensitivity and insulin secretion to overcome insulin resistance.[49] However, meta-analyses showed that fruit and vegetables fibre is inconsistently associated with the risk of T2D.[50] On the other hand, it may contribute to a decreased incidence of T2D through their low energy density and glycemic load, and high micronutrient content.[51] In particular, GLV are rich in bioactive phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant properties.[52-54] Antioxidants in fruit and vegetables have been hypothesized to improve insulin sensitivity and protect against diabetes in several supplementation trials.[55,56] In addition, it also might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis detected Mg intake to be inversely associated with the risk of T2D.[57] Taking this evidence into consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. The inverse association may be also mediated through weight gain or obesity which is an established risk factor for type 2 diabetes. Fruits are low in energy, which would promote the feeling of fullness and prevent over consumption of energy-dense foods, and resulting in weight loss.[54] Further investigation is warranted to understand the mechanisms involved in the proposed relation between fruit, vegetables, or GLV and risk of T2D.

Exploration of heterogeneity

Heterogeneity between studies was found, which did not alter much in the subgroup analyses. There are differences in types of vegetable consumed between Asian (such as China) and Non-Asian populations. Therefore, within the subgroup analysis we examined location as a possible source of heterogeneity. As traditional Chinese diets are high in vegetables (such as GLV and cruciferous vegetables), unsurprisingly, vegetables (including GLV) intake were greater in China than the US or Europe. We also examined study quality, length of follow-up, sex, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) as possible sources of heterogeneity, these did not show any significant heterogeneity between studies. Although the subgroup analysis could not explain the level of heterogeneity, in interpreting the results, several differences between the studies are worth discussing.

Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs) to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.[7,10,11,43,44,46,47] It is

less suitable for the assessment of absolute intake, which they tend to overestimate.[58,59] However, two studies collected data via a single 24 hour recall and dietary history interviews, respectively.[15,45] These measurements may underestimate true associations between fruit, vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition, calculations of daily consumption were differed (such as servings per week, servings per day, or grams per day). Although we standardized primary data using a standard portion size of 106g, conclusions should be interpreted with caution. Another possible explanation for the differences between the studies might be the classification of food groups. GLVs'criteria was inconsistent: three studies included spinach and lettuce; one included spinach and greens; others did not provide specific description. If they were included with an uniform definition of each groups, the associations might differ.

Strengths and limitations

Compared with the previous meta-analyses,[5,8,9] our study has several strengths. To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of T2D. In addition, to examine the shape of these possible associations, we investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of T2D. Therefore, the results should be more reliable.

In interpreting the results, several limitations of this meta-analysis should also be acknowledged. Firstly, although in the multivariable analysis we considered a multitude of lifestyle and dietary factors. The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. Second, we cannot exclude the possibility of recall bias in the assessments of diet based on the FFQs. However, the prospective study design and exclusion of participants with chronic diseases at baseline should minimize such bias. Third, the noticeable limitation of our study was the potential for bias due to inevitable measurement error, especially for individual with lower consumption levels. We attempted to reduce measurement error in adjusting for energy intake and using of cumulatively averaged intake levels. Fourth, because we had no source of information other than questionnaire for the identification of T2D, we might have underestimated the incidence of T2D. In addition, subclinical diseases at baseline might have distorted our risk estimate to some extent. Finally, the possible limitation is due to language bias. We attempted to minimize this bias by searching major electronic databases with no language restriction. However, several articles published in non-English languages might not appear in international journal databases, and could be omitted by our searches.[60]

Conclusions

In summary, our meta-analysis suggests that higher fruit or GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.

Contributors: ML and ZT conceived and designed the study. ML and YF searched the databases and checked them according to the eligible criteria and exclusion criteria. ZT helped develop search strategies. XZ and WH extract quantitative data. YF, XZ, and WH analyzed the data. ML wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper. ZT is the guarantor.

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Ethical approval: Not required.

Data sharing: No additional data available.

Figures Information

Figure 1. Process of literature search and study selection.
(TIFF)

Figure 2. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and risk of type 2 diabetes.
(TIFF)

Figure 3. Dose-response analyses of fruit intake and risk of type 2 diabetes.
(TIFF)

Figure 4. Random effects analysis of fully adjusted studies for highest versus lowest intake of vegetables and risk of type 2 diabetes.
(TIFF)

Figure 5. Random effects analysis of fully adjusted studies for highest versus lowest intake of green leafy vegetables and risk of type 2 diabetes.
(TIFF)

Figure 6. Dose-response analyses of green leafy vegetables intake and risk of type 2 diabetes.
(TIFF)

Appendix figure information

Supplemental fig A. Forest plot of fruit intake and risk of type 2 diabetes.

(TIFF)

Supplemental fig B. Forest plot of vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig C. Dose-response analyses of vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and vegetables and risk of type 2 diabetes.
(TIFF)

Supplemental fig E. Dose-response analyses of fruit and vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.
(TIFF)

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Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

Variables	Fruit only			Vegetables only			Fruit and vegetables			Green leafy vegetables		
	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value
Location												
Non-Asia	8	0.93 (0.87 to 1.00)	0.049	6	0.96 (0.87 to 1.06)	0.397	7	0.94 (0.84 to 1.04)	0.223	4	0.89 (0.81 to 0.97)	0.012
Asia	3	0.96 (0.82 to 1.12)	0.584	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Quality												
High (≥4)	4	0.92 (0.86 to 1.00)	0.045	2	0.83 (0.52 to 1.33)	0.448	1	1.04 (0.87 to 1.25)	0.671	2	0.86 (0.76 to 0.98)	0.024
Low (<4)	7	0.94 (0.85 to 1.04)	0.240	7	0.93 (0.84 to 1.02)	0.109	8	0.93 (0.84 to 1.02)	0.138	5	0.86 (0.77 to 0.97)	0.010
Duration of follow-up (years)												
≥10	5	0.90 (0.83 to 0.97)	0.006	4	0.91 (0.79 to 1.05)	0.190	5	0.89 (0.77 to 1.02)	0.098	3	0.85 (0.75 to 0.97)	0.014
<10	6	0.98 (0.90 to 1.07)	0.654	5	0.89 (0.72 to 1.10)	0.296	4	1.03 (0.91 to 1.16)	0.674	4	0.87 (0.78 to 0.97)	0.013
Sex												
M and F	5	0.88 (0.79 to 0.98)	0.022	5	0.89 (0.81 to 0.97)	0.010	6	0.87 (0.77 to 0.98)	0.026	4	0.80 (0.69 to 0.92)	0.002
F only	5	0.95 (0.88 to 1.02)	0.168	4	0.94 (0.77 to 1.15)	0.544	3	1.02 (0.94 to 1.11)	0.610	3	0.89 (0.81 to 0.98)	0.014
M only	1	0.99 (0.82 to 1.19)	0.916	0	-	-	0	-	-	0	-	-
Fractions of distribution												
Thirds	1	0.91 (0.71 to 1.16)	0.451	1	0.94 (0.84 to 1.05)	0.277	1	0.90 (0.80 to 1.01)	0.076	1	0.84 (0.65 to 1.08)	0.170
Quarters	3	0.92 (0.81 to 1.04)	0.193	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Fifths	7	0.94 (0.87 to 1.02)	0.144	5	0.96 (0.84 to 1.09)	0.499	6	0.94 (0.82 to 1.08)	0.385	3	0.89 (0.78 to 1.01)	0.062
No of participants												
≥50000	3	0.91 (0.84 to 0.99)	0.032	2	0.83 (0.52 to 1.33)	0.448	1	1.01 (0.91 to 1.13)	0.858	2	0.86 (0.76 to 0.98)	0.024
<50000	8	0.95 (0.87 to 1.04)	0.237	7	0.93 (0.84 to 1.02)	0.109	8	0.92 (0.83 to 1.03)	0.146	5	0.86 (0.77 to 0.97)	0.010
No of cases												
≥1000	5	0.95 (0.88 to 1.03)	0.233	4	1.01 (0.94 to 1.08)	0.810	6	0.96 (0.86 to 1.07)	0.456	3	0.91 (0.84 to 0.98)	0.018
<1000	6	0.91 (0.82 to 1.00)	0.042	5	0.75 (0.66 to 0.85)	0.000	3	0.87 (0.73 to 1.04)	0.119	4	0.78 (0.68 to 0.89)	0.000

M=male, F=female, RR=relative risk.

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Abstract

Objective To clarify and quantify the potential dose-response association between the intake of fruit and vegetables and risk of type 2 diabetes.

Design Meta-analysis and systematic review of prospective cohort studies.

Data source Studies published before February 2014 identified through electronic searches using PubMed and Embase.

Eligibility criteria for selecting studies Prospective cohort studies with relative risks and 95% confidence intervals for type 2 diabetes according to the intake of fruit, vegetables, or fruit and vegetables.

Results A total of ten articles including thirteen comparisons with 24 013 cases of type 2 diabetes and 434 342 participants were included in the meta-analysis. Evidence of curve linear associations were seen between fruit and green leafy vegetables consumption and risk of type 2 diabetes (P=0.059 and P=0.036 for non-linearity, respectively). The summary relative risk of type 2 diabetes for an increase of 1 serving fruit consumed per day was 0.93 (95% confidence interval 0.88 to 0.99) without heterogeneity among studies (P=0.477, I^2=0%). For vegetables, the combined relative risk of type 2 diabetes for an increase of 1 serving consumed per day was 0.90 (95% confidence interval 0.80 to 1.01) with moderate heterogeneity among studies (P=0.002, I^2=66.5%). For green leafy vegetables, the summary relative risk of type 2 diabetes for an increase of 0.2 serving consumed per day was 0.87 (95% confidence interval 0.81 to 0.93) without heterogeneity among studies (P=0.496, I^2=0%). The combined estimates showed no significant benefits of increasing the consumption of fruit and vegetables combined.

Conclusions Higher fruit or green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.

Article summary

Strengths and limitations of this study

To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of type 2 diabetes. We also investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of type 2 diabetes.

The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. We cannot exclude the possibility of recall bias in the assessments of diet based on the food frequency questionnaires.

1. Health expenditure on type 2 diabetes is increasing worldwide.
2. Epidemiological studies suggest that the intake of fruit and vegetables is beneficial in delaying or preventing the development of type 2 diabetes, though results from cohort studies are controversial.
3. Higher fruit or vegetables, particularly green leafy vegetables intake is associated with a significantly reduced risk of type 2 diabetes.
4. Dose-response analyses indicated a 6% lower risk of type 2 diabetes per 1 serving/day increment of fruit intake and 13% lower risk of type 2 diabetes per 0.2 serving/day increment of green leafy vegetables intake.
5. Further evidence from preferably randomized controlled studies should explore what kind of fruit or green leafy vegetables can reduce the risk of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030,[1] with serious consequences for health care expenditure.[2] It has been estimated that the global health expenditure on diabetes is at least $ 376 billion in 2010 and will be $ 490 billion in 2030,[3] this creates a major public health burden. The prevention of T2D is thus clearly an important public health priority. In recent decades, concern has mounted regarding the premature mortality and morbidity associated with T2D, with growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D.[4] Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.[5]

To minimize the risk of dietary factors and reduce the incidence of T2D, a World Health Organization recommended the public to consume more than 400 g or five portions of combined fruit and vegetables per day for the prevention of T2D.[6] Nevertheless, in the Japan Public Health Center-based Prospective (JPHC) Study,[7] after a mean follow-up over five years, participants with the intake of fruit and vegetables may not be appreciably associated with the risk of T2D. Vegetables, especially green leafy vegetables (GLV), have been suggested to explain an apparent beneficial effect on T2D. In addition, several meta-analyses of observational studies have found that an increase in daily intake of GLV could significantly reduce the risk of T2D.[5,8,9] These studies were restricted by heterogeneous with respect to sample size. Additionally, recent studies involving relationship between the intake of fruit and vegetables and risk of T2D have been published from then on.[5,7,10,11] Furthermore, whether any dose-response relation exists between the intake of fruit and vegetables and risk of T2D is unknown. Therefore, we systematically reviewed and meta-analysed available studies to quantify the associations between dietary intake of fruit and vegetables and incidence of T2D based on identified prospective cohort studies. We pooled risk estimates for the highest versus lowest category of intake to examine the overall association. We also conducted a dose-response analysis for the trend estimation.

Methods

Search strategy

We carried out a systematic search of PubMed (Medline) and Embase through February 2014 for prospective cohort studies examining the association between the intake of fruit and vegetables and risk of T2D. The following key words were used in our search strategies: (“fruits” OR “vegetables” OR “citrus”) AND (“Type 2 diabetes” OR “non-insulin-dependent diabetes mellitus” OR “Diabetes Mellitus, Type 2” OR “NIDDM” OR “prediabetes” OR “impaired glucose tolerance” OR “impaired fasting glucose” OR “glucose” OR “hyperglycaemia” OR “insulin”) AND (“Follow-up studies” OR “prospective studies” OR “cohort studies” OR “longitudinal studies”). We restricted the search to human studies. No language restrictions were imposed. In addition, we scrutinized possible eligible references from relevant original papers and review articles to identify potential publications. We followed standard criteria for the performing and reporting of the meta-analyses of observational studies.[12]

Study selection

Citations selected from the initial search were subsequently screened for eligibility. Studies were included in this meta-analysis if they satisfied the following criteria: (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design); (3) the exposure of interest was the intake of fruits, vegetables, or fruit and vegetables combined; (4) the outcome was T2D; and (5) reported multivariate-adjusted risk estimates for the association between the fruit, vegetables, or fruit and vegetables combined, assessed as dietary intake, and T2D. Additionally, we excluded animal studies and letters without sufficient data. If data were reported more than once, we included the study with the longest follow-up time.

Validity assessment

Two authors (ML and YF) independently assessed all studies for quality using a modified scoring system, which allowed a total score from 0 to 6 points (6 reflecting the highest quality) on the basis of MOOSE,[12] QUATSO,[13] and STROBE.[14] The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity). We have adapted Carter et al's scoring system.[9]

Data extraction

Data were carried out independently by two other authors (XZ and WH) using a standard electronic sheets and cross-check to reach a consensus. For each study, the following information was abstracted: name of the first author, publication year, study population, geographical location, sex, age range, sample size (number of T2D cases, number of non-T2D cases, and number of participants), duration of follow-up, methods used to assess fruit and vegetables intake and ascertain T2D cases, highest and lowest of fruit and vegetables intake, and covariates adjusted for in the multivariable model. Study quality was evaluated by using the modified scoring system. All data were extracted from the published papers. If necessary, the primary authors were contacted to retrieve further information. For two studies that expressed data separately for men and women,[7,15] one study that included data from multiple cohorts,[11] we considered the analysis for each sex or cohort as an independent comparison and extracted data separately.

Statistical analysis

Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to be a valid approximation of relative risks, we converted these values in every study by taking their natural logarithms and calculating standard errors and corresponding 95% confidence intervals. Relative risks and their standard errors were pooled with the DerSimonian and Laird random effects model, which takes into account both within-study and between-study variabilities.[16] When some studies included in our meta-analysis used different measurement units (eg, grams per day or portions per day or servings per day),[5,10,15] we standardized fruit and vegetables intake into servings per day using a standard portion size of 106 g.[17] As different studies might use different exposure categories (thirds, quarters, or fifths),[7,11,15] we used the study specific relative risk for the highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker[18] and Orsini et al[19,20] was used to calculate study-specific slopes (linear trends) and 95% confidence intervals. The method requires the distributions of cases and person years for exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the median or mean intake was not available.[21] When the highest category was open ended, we assumed that the average of the category was set at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the distribution.[22] A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the fractional polynomials component is equal to zero. Heterogeneity among studies was evaluated using the chi-square test based on Cochran's Q test and I^2 statistic at $P<0.10$ level of significance,[16] and quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total variation in point estimates that is due to heterogeneity rather than chance.[23] We considered low, moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively. To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We performed the Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.[24-26] Funnel plots were also used to assess the publication bias. All the statistical analyses were performed in Stata 12 (Stata Corp, College Station, TX). A threshold of P<0.1 was used to decide whether heterogeneity or publication bias was present.[24] In other ways, P values were 2-sided and P<0.05 was considered statistically significant.

Results

Literature search

In total, the search strategy retrieved 673 unique articles (308 articles from PubMed and 365 articles from Embase) (Fig 1). After exclusion of duplicate records and studies that did not fulfill our inclusion criteria, 27 articles remained, and we further evaluated the full texts of these 27 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to lack of sufficient data for estimation of relative risks.[27-31] Five articles were excluded because no original data could be extracted (review, type 1 diabetes, or cross sectional studies).[32-36] Another four articles were excluded because we deemed irrelevant.[37-40] We also excluded three articles because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to warrant inclusion within the meta-analysis.[41-43] In addition, three articles with the same participants involved Nurses Health Study (Colditz et al 1992, Bazzano et al 2008, and Muraki et al 2013). According to the study selection criteria, we included the study by Bazzano et al, which followed for 18 years.[44] Finally, eleven articles met the inclusion criteria and were included in the meta-analysis.[5,7,10,11,15,44-48] Among these ten articles, Ford et al and Kurotani et al study examined male and female separately,[7,11] Cooper et al have two studies (study a:2012 and study b:2012) and Muraki et al report included data from two independent cohorts.[15] Thus, our meta-analysis included thirteen comparisons.

Study characteristics

Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted from the included studies, all ten articles were prospective cohort designs and participants who were free of self reported diabetes at baseline.[5,7,10,11,15,44-48] In total, the included studies consisted of 434 342 participants. Of these participants, we identified 24 013 cases of T2D occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10 articles, five cohorts were conducted primarily in the United States,[11,15,44-46] two articles were done in Asian countries (China and Japan)[7,48] and three cohorts were from European countries.[5,10,47] The number of participants ranged from 3704 in the EPIC-Norfolk study by Cooper et al[10] to 91 246 in the Nurses' Health Study II by Muraki et al.[11] Five studies included both male and female,[5,7,10,15,47] four studies included only female.[44-46,48] One article by Muraki et al reported two independent cohorts, one cohorts included only female, and another only male.[11] The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and vegetables intake separately and combined.[5,7,10,44-46] two papers provided information on fruit and vegetables intake separately,[47,48] one paper provided only the combined data,[15] and another paper provided separate data on fruit.[11] Five papers also included separate data on the intake of GLV.[5,7,46-48] In most papers intake of fruit and vegetables was divided into fifths.[11,44-48] All studies provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most studies yielded a score of 3 or below (low quality).

Fruit intake and risk of T2D

11 comparisons from nine studies reported an association between fruit intake and risk of T2D, with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no heterogeneity among studies (P=0.477, I^2=0%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.533) and Egger regression tests (P=0.849) (see supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model, we found a mild curvilinear association (P=0.059 for non-linearity, fig 3). Dose-response analysis indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D (relative risk 0.94, 95% confidence interval 0.89 to 1.00, I^2=0%) (see supplemental fig A in appendix 2).

Vegetables intake and risk of T2D

Eight studies exported an association between vegetables intake and risk of T2D, with 20 933 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95% confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity (P=0.002, I^2=66.5%). However, no evidence of substantial publication bias was observed from the Begg (P=0.602) and Egger regression tests (P=0.176) (see supplemental table B in appendix 1). Among 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative risk 0.98, 95% confidence interval 0.89 to 1.08, I^2=45.8%) (see supplemental fig B in appendix 2). No publication bias was observed (P=0.117). We found no evidence of a curve linear association between vegetables intake and risk (P=0.671 for non-linearity, see supplemental fig C in appendix 2).

Fruit and vegetables intake and risk of T2D

Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies (P=0.141, I^2=34.6%). Additionally, no evidence of substantial publication bias was observed from the Begg (P=0.348) and Egger regression tests (P=0.609) (see supplemental table B in appendix 1). Among 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and vegetables intake and risk of T2D. We did not find a significant curvilinear association (P=0.456 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval 0.86 to 1.07, I^2=47.6%) (see supplemental fig F in appendix 2).

GLV intake and risk of T2D

7 comparisons from six studies reported an association between GLV intake and risk of T2D, with 19 139 T2D outcomes and 251 235 participants. Overall, GLV intake was inversely associated with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant heterogeneity was detected among studies (P=0.496, I^2=0%). Additionally, we did not observe evidence of substantial publication bias (the Begg and Egger regression tests, P=0.133 and P=0.101, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only three comparisons were eligible for the trend estimation. Using a restricted cubic splines model, we found a significant curvilinear association (P=0.036 for non-linearity, fig 6). Dose-response analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, I^2=20.9%) (see supplemental fig G in appendix 2). No publication bias was observed (P=0.282).

Subgroup analyses

To examine the stability of the primary results, we carried out subgroup analyses (table 1). The association between fruit, vegetables, or fruit and vegetables intake and risk of T2D were similar in subgroup analyses, which were separately defined study quality, length of follow-up, sex, location, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) affected the results. The summary estimates of relative risks from each category were pooled (see supplemental table B in appendix 1). We paid close attention to the highest versus lowest category. Almost all subgroups that analysed intake of GLV showed a benefit of consuming greater quantities (fig 5). Supplemental table B in appendix 1 also showed significant reductions in risk of T2D events for consumption of fruit, vegetables, or fruit and vegetables combined.

Discussion

In this meta-analysis dietary intake of fruit, vegetables, and GLV, but not fruit and vegetables combined, were associated with a lower risk of T2D. Dose-response analyses indicated a 6% lower risk of T2D per 1 serving/day increment of fruit intake and 13% lower risk of T2D per 0.2 serving/day increment of GLV intake, but no significant trend for vegetables or fruit and vegetables combined.

Results in relation to other studies

Over the past decades, extensive prospective studies have reported the association of fruit, vegetables, or fruit and vegetables combined with T2D risk.[5,7,10,11,15,44-48] However, the role of dietary factors in T2D is still controversial. Some of the studies failed to find the association between fruit intake or fruit and vegetables combined and risk of T2D.[8,45] However, Bazzano and colleagues analysed data from 11 different U.S. states with 18 years of follow-up and found that consumption of fruit was associated with a lower hazard of diabetes, whereas no significant association for total fruit and vegetables consumption.[44] Similar to previous analysis in the Nurses' Health Study, the results from three prospective longitudinal cohort studies also supported an inverse association between fruit intake and risk of T2D.[11] But these studies have the potential for bias due to measurement error. In addition, two cohort studies have suggested an inverse association between total fruit and vegetables consumption and risk of T2D.[10,15]

A few large cohort studies have found an inverse association between vegetables consumption, especially GLV and risk of T2D.[10,46-48] These findings all agreed with two meta-analyses.[5,9] But another systematic review based on five cohort studies suggested that there was no protective association between vegetables intake and T2D.[8]

Several plausible biological mechanisms have been proposed to explain abovementioned association. Fruit and vegetables are rich in fibre,[49] which has been shown to improve insulin sensitivity and insulin secretion to overcome insulin resistance.[50] However, meta-analyses showed that fruit and vegetables fibre is inconsistently associated with the risk of T2D.[51] On the other hand, it may contribute to a decreased incidence of T2D through their low energy density and glycemic load, and high micronutrient content.[52] In particular, GLV are rich in bioactive phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant properties.[53-55] Antioxidants in fruit and vegetables have been hypothesized to improve insulin sensitivity and protect against diabetes in several supplementation trials.[56,57] In addition, it also might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis detected Mg intake to be inversely associated with the risk of T2D.[58] Taking this evidence into consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. The inverse association may be also mediated through weight gain or obesity which is an established risk factor for type 2 diabetes. Fruits are low in energy, which would promote the feeling of fullness and prevent over consumption of energy-dense foods, and resulting in weight loss.[55] Further investigation is warranted to understand the mechanisms involved in the proposed relation between fruit, vegetables, or GLV and risk of T2D.

Exploration of heterogeneity

Heterogeneity between studies was found, which did not alter much in the subgroup analyses. There are differences in types of vegetable consumed between Asian (such as China) and Non-Asian populations. Therefore, within the subgroup analysis we examined location as a possible source of heterogeneity. As traditional Chinese diets are high in vegetables (such as GLV and cruciferous vegetables), unsurprisingly, vegetables (including GLV) intake were greater in China than the US or Europe. We also examined study quality, length of follow-up, sex, number of cases or participants, and whether the different ways in which authors had grouped intake (thirds, quarters, or fifths) as possible sources of heterogeneity, these did not show any significant heterogeneity between studies. Although the subgroup analysis could not explain the level of heterogeneity, in interpreting the results, several differences between the studies are worth discussing.

Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs) to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.[7,10,11,44-46,48] It is less suitable for the assessment of absolute intake, which they tend to overestimate.[59,60] However, two studies collected data via a single 24 hour recall and dietary history interviews, respectively.[15,47] These measurements may underestimate true associations between fruit, vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition, calculations of daily consumption were differed (such as servings per week, servings per day, or grams per day). Although we standardized primary data using a standard portion size of 106g, conclusions should be interpreted with caution. Another possible explanation for the differences between the studies might be the classification of food groups. GLVs'criteria was inconsistent: three studies included spinach and lettuce; one included spinach and greens; others did not provide specific description. If they were included with an uniform definition of each groups, the associations might differ.

Strengths and limitations

Compared with the previous meta-analyses,[5,8,9] our study has several strengths. To our knowledge, this is the largest systematic review and meta-analysis on the intake of fruit and vegetables and risk of T2D. In addition, to examine the shape of these possible associations, we investigated a dose-response relation between fruit, vegetables, fruit and vegetables combined consumption and risk of T2D. Therefore, the results should be more reliable.

In interpreting the results, several limitations of this meta-analysis should also be acknowledged. Firstly, although in the multivariable analysis we considered a multitude of lifestyle and dietary factors. The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged. Second, the possibility of recall bias in the measurements of dietary habits based on the FFQs cannot be ruled out. However, the strict inclusion and exclusion criteria should minimize such bias. Third, the noticeable limitation of our study was the potential for bias due to inevitable measurement error, especially for individual with lower consumption levels. We attempted to reduce measurement error in adjusting for energy intake and using of cumulatively averaged intake levels. Fourth, because we had no source of information other than questionnaire for the identification of T2D, we might have underestimated the incidence of T2D. In addition, subclinical diseases at baseline might have distorted our risk estimate to some extent. Finally, the possible limitation is due to language bias. We attempted to minimize this bias by searching major electronic databases with no language restriction. However, several articles published in non-English languages might not appear in international journal databases, and could be omitted by our searches.[61]

Conclusions

In summary, our meta-analysis suggests that higher fruit or GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.

Contributors: ML and ZT conceived and designed the study. ML and YF searched the databases and checked them according to the eligible criteria and exclusion criteria. ZT helped develop search strategies. XZ and WH extract quantitative data. YF, XZ, and WH analyzed the data. ML wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper. ZT is the guarantor.

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Ethical approval: Not required.

Data sharing: No additional data available.

Figures Information

Figure 1. Process of literature search and study selection.
(TIFF)

Figure 2. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and risk of type 2 diabetes.
(TIFF)

Figure 3. Dose-response analyses of fruit intake and risk of type 2 diabetes.
(TIFF)

Figure 4. Random effects analysis of fully adjusted studies for highest versus lowest intake of vegetables and risk of type 2 diabetes.
(TIFF)

Figure 5. Random effects analysis of fully adjusted studies for highest versus lowest intake of green leafy vegetables and risk of type 2 diabetes.
(TIFF)

Figure 6. Dose-response analyses of green leafy vegetables intake and risk of type 2 diabetes.
(TIFF)

Appendix figure information

Supplemental fig A. Forest plot of fruit intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig B. Forest plot of vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig C. Dose-response analyses of vegetables intake and risk of type 2 diabetes.

(TIFF)

Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus lowest intake of fruit and vegetables and risk of type 2 diabetes.
(TIFF)

Supplemental fig E. Dose-response analyses of fruit and vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.
(TIFF)

Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.
(TIFF)

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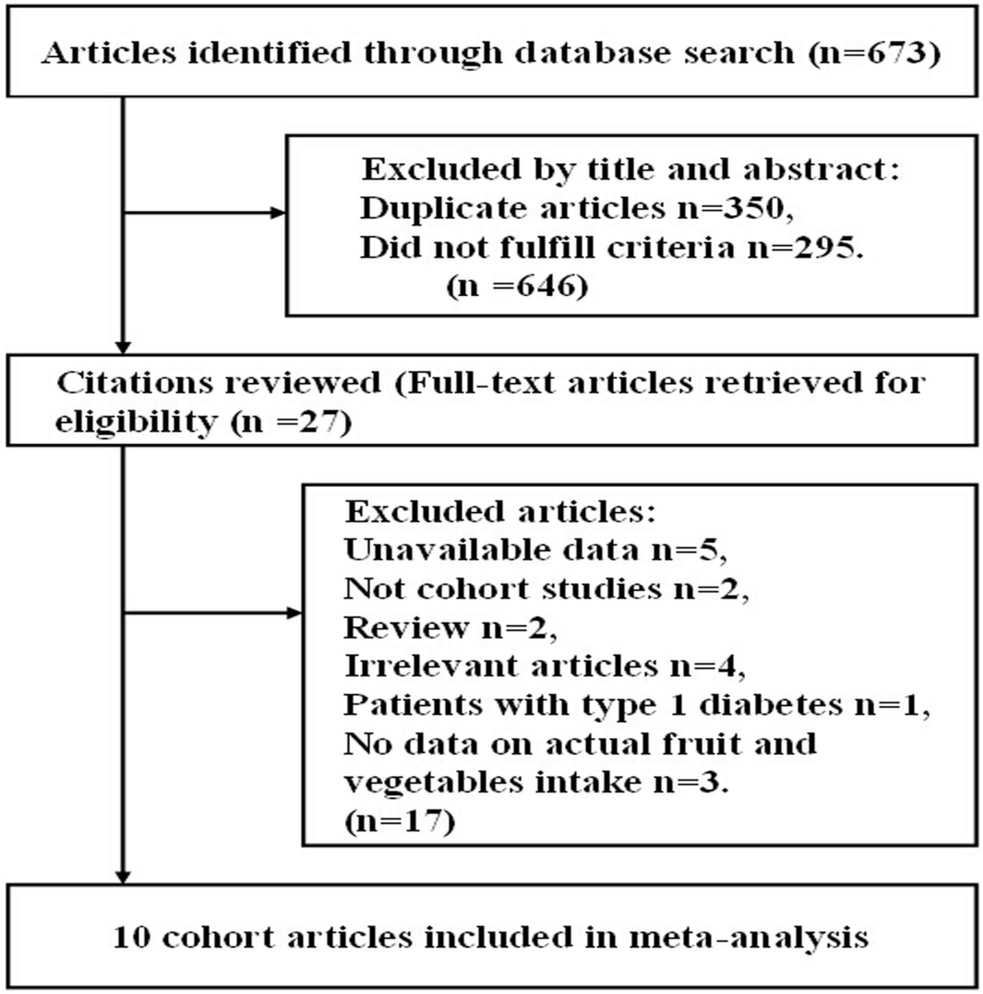
Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

Variables	Fruit only			Vegetables only			Fruit and vegetables			Green leafy vegetables		
	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value	No	Pooled RR (95% CI)	P value
Location												
Non-Asia	8	0.93 (0.87 to 1.00)	0.049	6	0.96 (0.87 to 1.06)	0.397	7	0.94 (0.84 to 1.04)	0.223	4	0.89 (0.81 to 0.97)	0.012
Asia	3	0.96 (0.82 to 1.12)	0.584	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Quality												
High (≥4)	4	0.92 (0.86 to 1.00)	0.045	2	0.83 (0.52 to 1.33)	0.448	1	1.04 (0.87 to 1.25)	0.671	2	0.86 (0.76 to 0.98)	0.024
Low (<4)	7	0.94 (0.85 to 1.04)	0.240	7	0.93 (0.84 to 1.02)	0.109	8	0.93 (0.84 to 1.02)	0.138	5	0.86 (0.77 to 0.97)	0.010
Duration of follow-up (years)												
≥10	5	0.90 (0.83 to 0.97)	0.006	4	0.91 (0.79 to 1.05)	0.190	5	0.89 (0.77 to 1.02)	0.098	3	0.85 (0.75 to 0.97)	0.014
<10	6	0.98 (0.90 to 1.07)	0.654	5	0.89 (0.72 to 1.10)	0.296	4	1.03 (0.91 to 1.16)	0.674	4	0.87 (0.78 to 0.97)	0.013
Sex												
M and F	5	0.88 (0.79 to 0.98)	0.022	5	0.89 (0.81 to 0.97)	0.010	6	0.87 (0.77 to 0.98)	0.026	4	0.80 (0.69 to 0.92)	0.002
F only	5	0.95 (0.88 to 1.02)	0.168	4	0.94 (0.77 to 1.15)	0.544	3	1.02 (0.94 to 1.11)	0.610	3	0.89 (0.81 to 0.98)	0.014
M only	1	0.99 (0.82 to 1.19)	0.916	0	-	-	0	-	-	0	-	-
Fractions of distribution												
Thirds	1	0.91 (0.71 to 1.16)	0.451	1	0.94 (0.84 to 1.05)	0.277	1	0.90 (0.80 to 1.01)	0.076	1	0.84 (0.65 to 1.08)	0.170
Quarters	3	0.92 (0.81 to 1.04)	0.193	3	0.77 (0.60 to 0.98)	0.032	2	0.97 (0.75 to 1.25)	0.827	3	0.80 (0.69 to 0.93)	0.004
Fifths	7	0.94 (0.87 to 1.02)	0.144	5	0.96 (0.84 to 1.09)	0.499	6	0.94 (0.82 to 1.08)	0.385	3	0.89 (0.78 to 1.01)	0.062
No of participants												
≥50000	3	0.91 (0.84 to 0.99)	0.032	2	0.83 (0.52 to 1.33)	0.448	1	1.01 (0.91 to 1.13)	0.858	2	0.86 (0.76 to 0.98)	0.024
<50000	8	0.95 (0.87 to 1.04)	0.237	7	0.93 (0.84 to 1.02)	0.109	8	0.92 (0.83 to 1.03)	0.146	5	0.86 (0.77 to 0.97)	0.010
No of cases												
≥1000	5	0.95 (0.88 to 1.03)	0.233	4	1.01 (0.94 to 1.08)	0.810	6	0.96 (0.86 to 1.07)	0.456	3	0.91 (0.84 to 0.98)	0.018
<1000	6	0.91 (0.82 to 1.00)	0.042	5	0.75 (0.66 to 0.85)	0.000	3	0.87 (0.73 to 1.04)	0.119	4	0.78 (0.68 to 0.89)	0.000

M=male, F=female, RR=relative risk.



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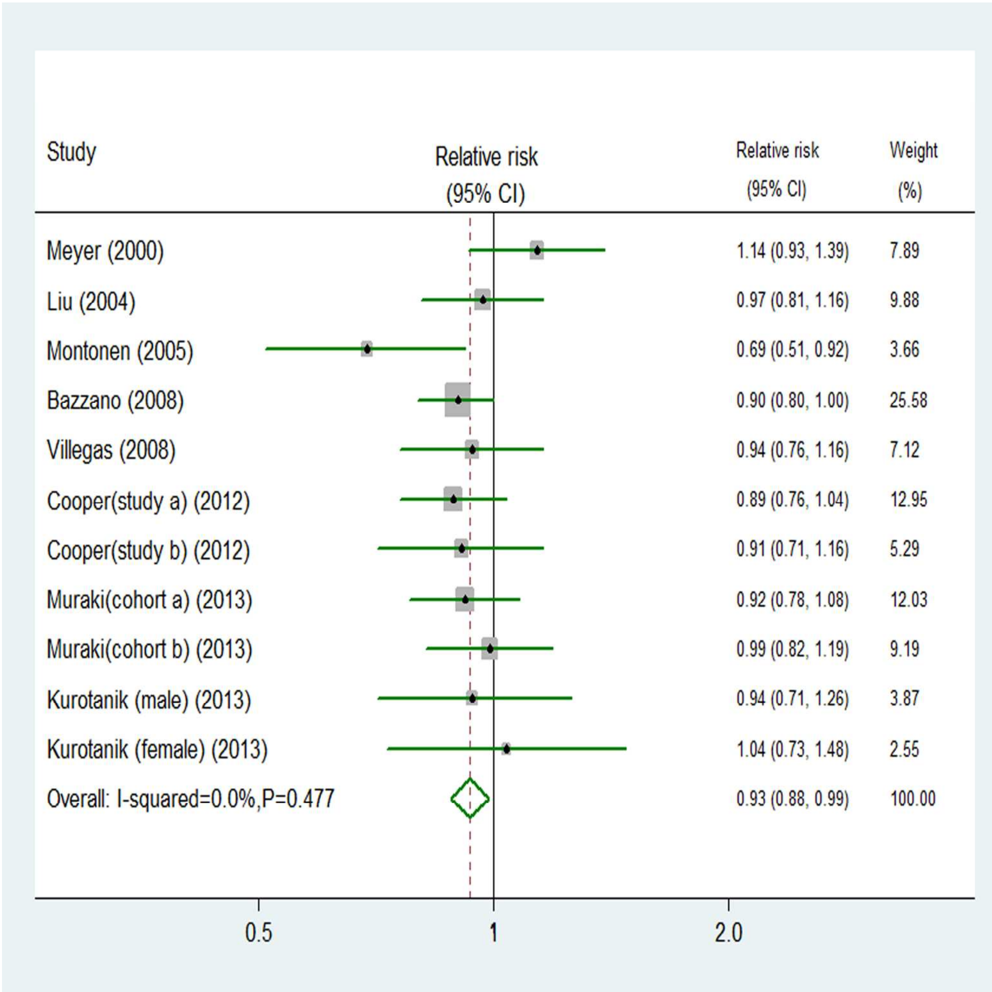

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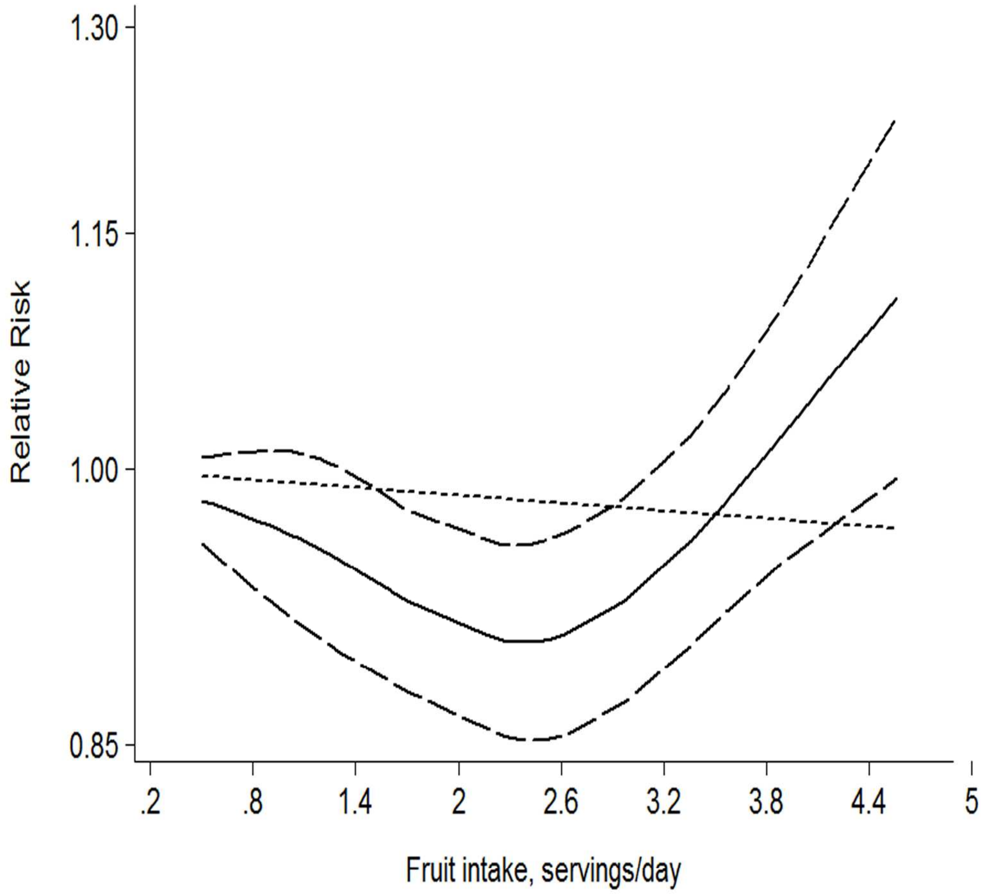

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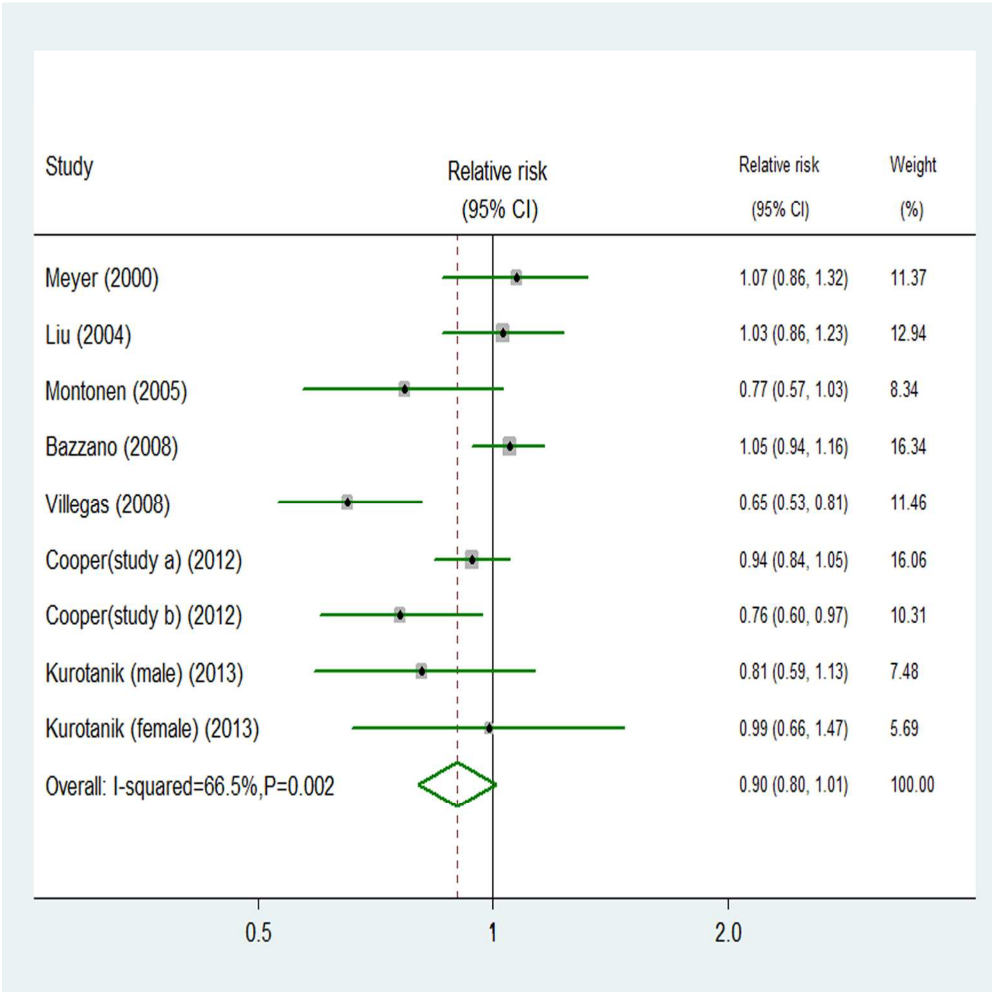

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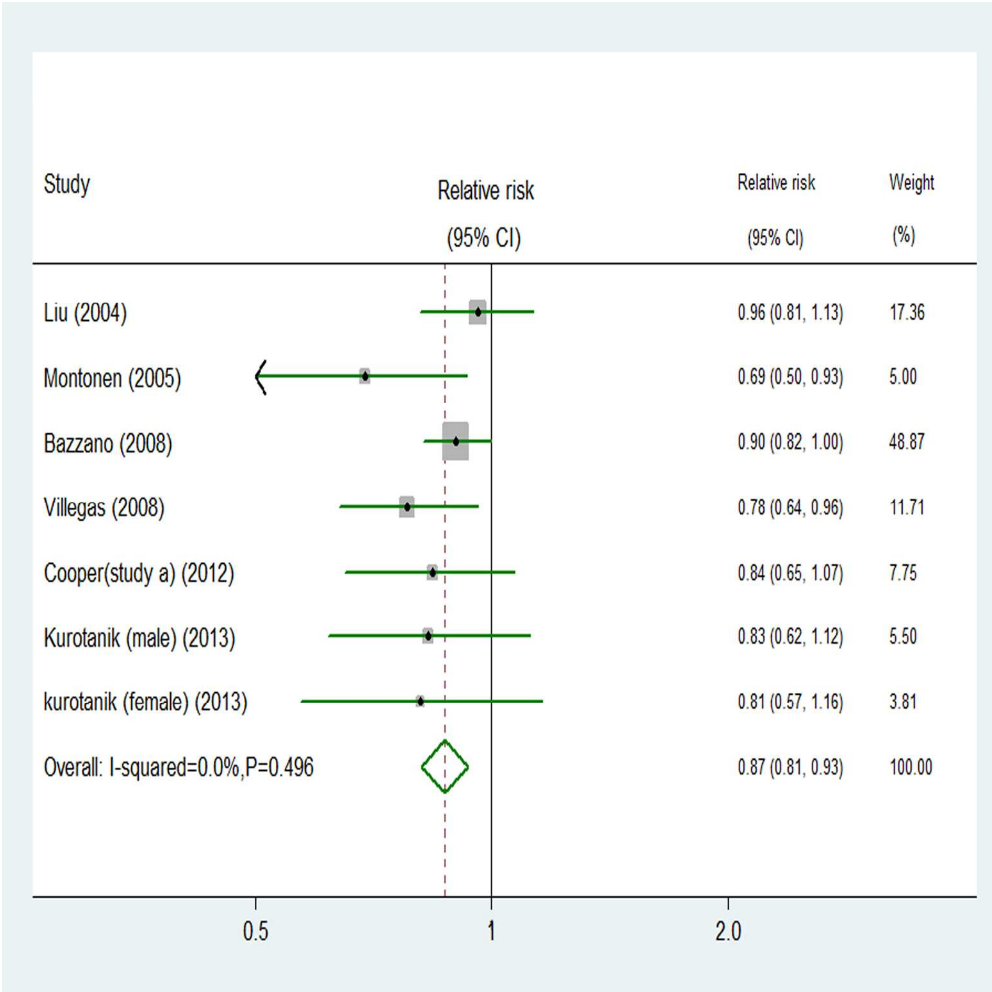

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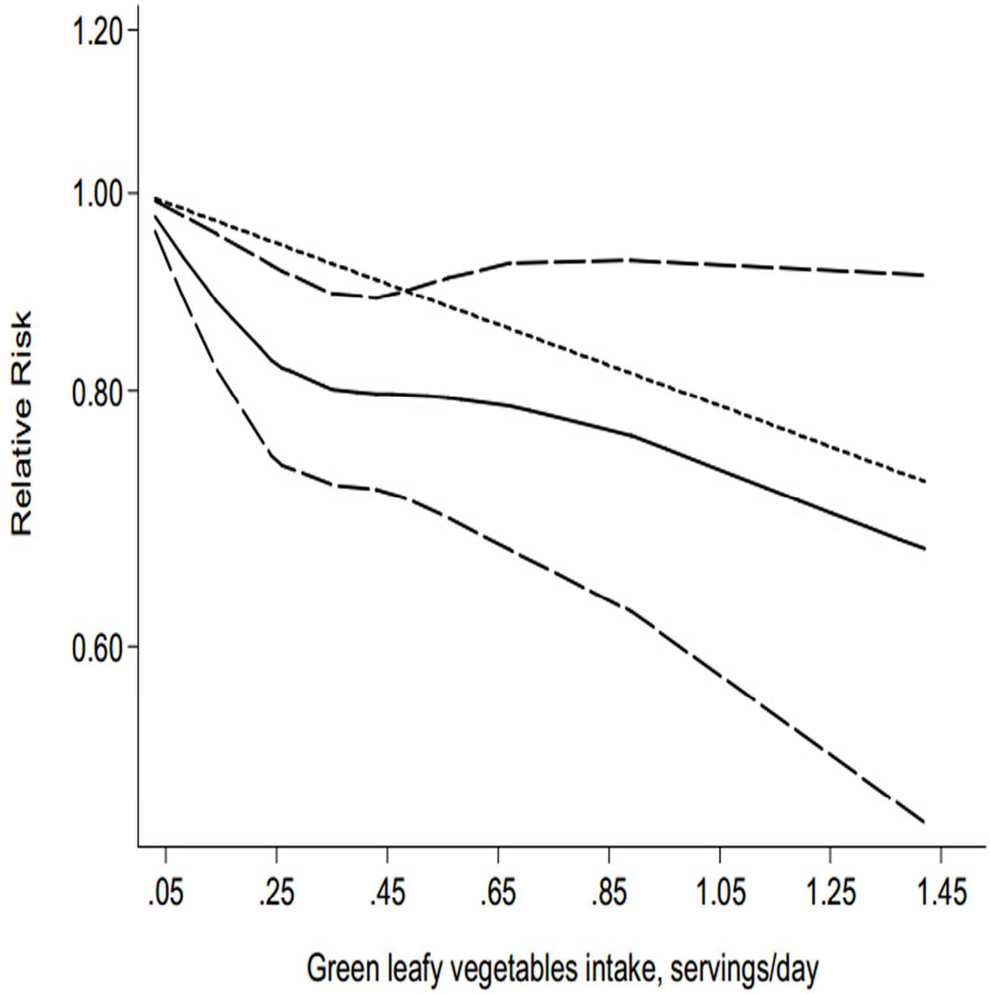

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Table A. Characteristics of included studies of fruit and vegetables intake in relation to incident type 2 diabetes

First author	Country/ cohort	Age (years) /Sex	No of total/follow -up(years)	No of cases /non-cases	Assessment of type 2 diabetes	Measure of intake	Highest/lowest intakes as servings/day	Adjustments	Quality score
Meyer et al 2000,[44]	USA/Iowa Women's Health Study	55-69/F	35988/6	1141/34847	Based on self reported	127 item FFQ, Calculated servings/day for fruit, vegetables, and combined. Data divided into fifths	Fruit: 3.36/0.57. Vegetables: 5.93/1.57. Fruit and vegetables: 8.86/2.57	Age, BMI, total energy intake, WHR, education, smoking, alcohol intake, physical activity	2
Ford et al 2001,[15]	USA/NHANES I	25-74/M and F	9665/20	1018/8647	Confirmed by self report or hospital records or death certificate	24 hour recall. Calculated servings/week for fruit and vegetables combined. Data divided into thirds	Fruit and vegetables: ≥5/0	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004,[45]	USA/Women's Health Study	≥45/F	38018/8.8	1614/36404	Based on self reported	131 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce. Data divided into fifths	Fruit: 3.91/0.62. Vegetables: 6.84/1.47. Fruit and vegetables: 10.16/2.54. Green leafy vegetables: 1.42/0.14	Age, BMI, smoking, total calories, alcohol, exercise, history of hypertension/high cholesterol, family history of diabetes	3
Montonen et al 2005,[46]	Finland/Finnish	40-69/M and F	4304/23	383/3921	Confirmed via social	Dietary history interview. Calculated g/day for fruit	Fruit: ＞1.47/＜0.31. Vegetables:	Age, BMI, sex, smoking, energy intake,	3

	Mobile Clinic Health Examination Survey				insurance register	and vegetables separately. Data divided into fifths	> 1.23/ < 0.4. Green leafy vegetables: > 0.4/<0.1	family history of diabetes, geographic area	
Bazzano et al 2008,[43]	USA/Nurses' Health Study	30-55/F	71346/18	4529/66817	Confirmed if met WHO criteria (before 1997) or ADA criteria (after 1998)	61 item FFQ. Calculated servings/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/kale/lettuce. Data divided into fifths	Fruit: 2.5/0.5. Vegetables: 5.2/1.5. Fruit and Vegetables: 7.5/2.1	Age, BMI, physical activity, smoking, alcohol, hormone therapy, family history of diabetes, total energy intake	4
Villegas et al 2008,[47]	China/Shanghai Women's Health Study	40-70/F	64191/4.6	896/63295	Confirmed by ADA criteria	77 item FFQ. Calculated g/day for fruit and vegetables separately. Defined green leafy vegetables as greens/Chinese greens/spinach. Data divided into fifths	Fruit: 4.56/0.82. Vegetables: 4.04/1.15. Green leafy vegetables: 1.28/0.26	Age, BMI, WHR, education, smoking, alcohol, hypertension, disease history, hormone use, occupational history, physical activity, income, daily energy intake	4
Cooper et al (study a) 2012,[5]	8countries/ EPIC-Inter Act study	40-79/M and F	24939/11	10821/14118	Based on self reported	Country specific dietary questionnaires. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as	Fruit: 5.39/0.75. Vegetables: 3.94/0.88. Fruit and Vegetables: 8.71/2.13. Green	Age, BMI, sex, education, centre, physical activity, smoking, total energy intake, alcohol	2

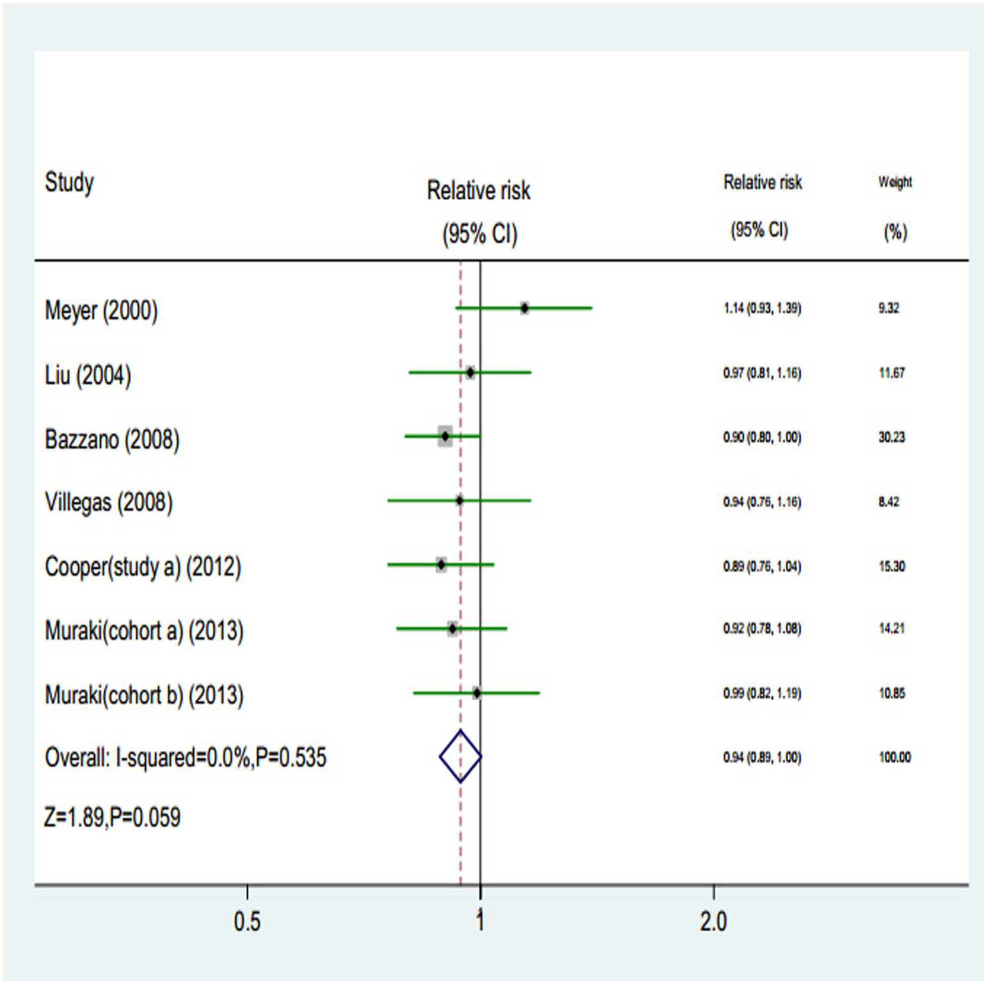
						chard/endive/lettuce/borage /watercress/beet leaves/spinach. Data divided into quarters	leafy vegetables: 5.93/0.05		
Cooper et al (study b) 2012,[10]	England/EPIC-Norfolk	40-79/M and F	3704/11	653/3051	Based on self reported	117 item FFQ. Calculated servings/day for fruit, vegetables and combined. Data divided into thirds	Fruit: 3.4/0.6. Vegetables: 2.6/1.1. Fruit and Vegetables: 5.7/2.1	Age, BMI, sex, waist circumference, education, TDI, occupational social class, smoking, physical activity, family history of diabetes, energy intake, season	2
Muraki et al (cohort a) 2013,[11]	USA/Nurses' Health Study II	24-44/F	91246/8	741/90505	Confirmed by ADA criteria (after 1998)	133 item FFQ. Calculated servings/week for fruit. Data divided into fifths	Fruit: ≥3/＜0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
Muraki et al (cohort b) 2013,[11]	USA/Health Professionals Follow-up Study	40-75/M	42504/12	1321/41183	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: ≥3/＜0.57	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use,	4

								total energy intake	
Kurotanik et al 2013,[7]	Japan/JPHC Study	40-69/M and F	48437/5	896/47541	Based on self reported	147 item FFQ. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/Chinese chives/garland chrysanthemums/cbingensai/leaf mustard/mugwort/chard/komatsuna. Data divided into quarters	M Fruit: 3.42/0.34. Vegetables: 3.35/0.71. Fruit and Vegetables: 6.48/1.38. Green leafy vegetables: 0.45/0.04. F Fruit: 4.60/0.7. Vegetables: 3.84/0.94. Fruit and Vegetables: 8.1/1.98. Green leafy vegetables: 0.54/0.07	Age, BMI, public health centre area, smoking, alcohol, leisure-time activity, history of hypertension, coffee, family history of diabetes, Mg intake, Ca intake, energy intake	3

FFQ=food frequency questionnaire, BMI=body mass index, SBP=systolic blood pressure, TDI=townsend deprivation index, WHR=weight:height ratio, ADA=American Diabetes Association, WHO=World Health Organization, M=male, F=female, study a= the EPIC-InterAct study, study b= the EPIC-Norfolk study, cohort a= the Nurses' Health Study II study, cohort b= the Health Professionals Follow-up Study. The analysis included 13 cohorts among the ten articles, where Ford et al and Kurotani et al study examined male and female separately, Cooper et al have two studies in 2012 and Muraki et al report included data from two independent cohorts.

Table B. Meta-analysis of intake of fruit and vegetables and risk of type 2 diabetes (highest versus lowest category)

Variables	No of comparisons	Cases/ total	Test of association	Test of heterogeneity	Analysis of publication bias
			Pooled RR (95% CI), P value	Heterogeneity (I^2, %), P value	Begg's test, Egger's test (P value)
Fruit only	11	22995/424677	0.93 (0.88 to 0.99), 0.015	0, 0.477	0.533, 0.849
Vegetables only	9	20933/290927	0.90 (0.80 to 1.01), 0.068	66.5, 0.002	0.602, 0.176
Fruit and vegetables	9	20672/232097	0.94 (0.86 to 1.03), 0.202	34.6, 0.141	0.348, 0.609
Green leafy vegetables	7	19139/251235	0.87 (0.81 to 0.93), 0.000	0, 0.496	0.133, 0.101



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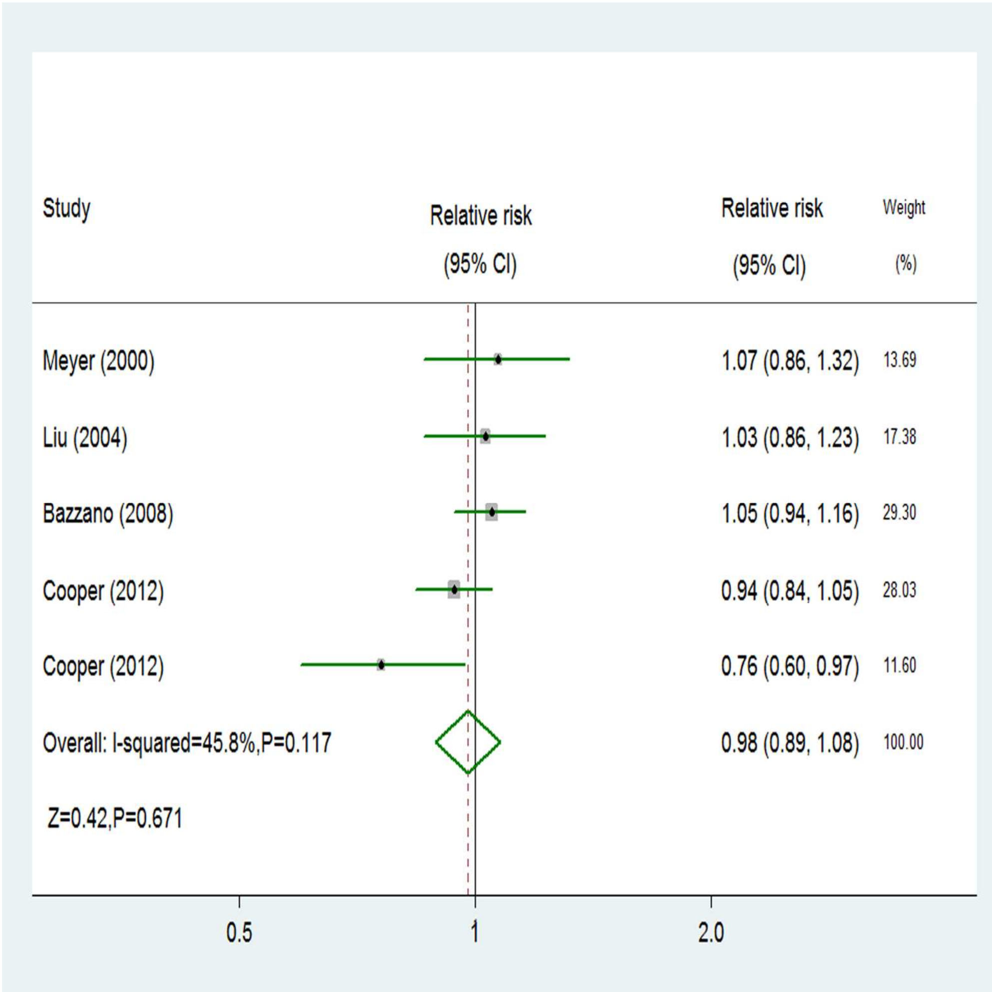

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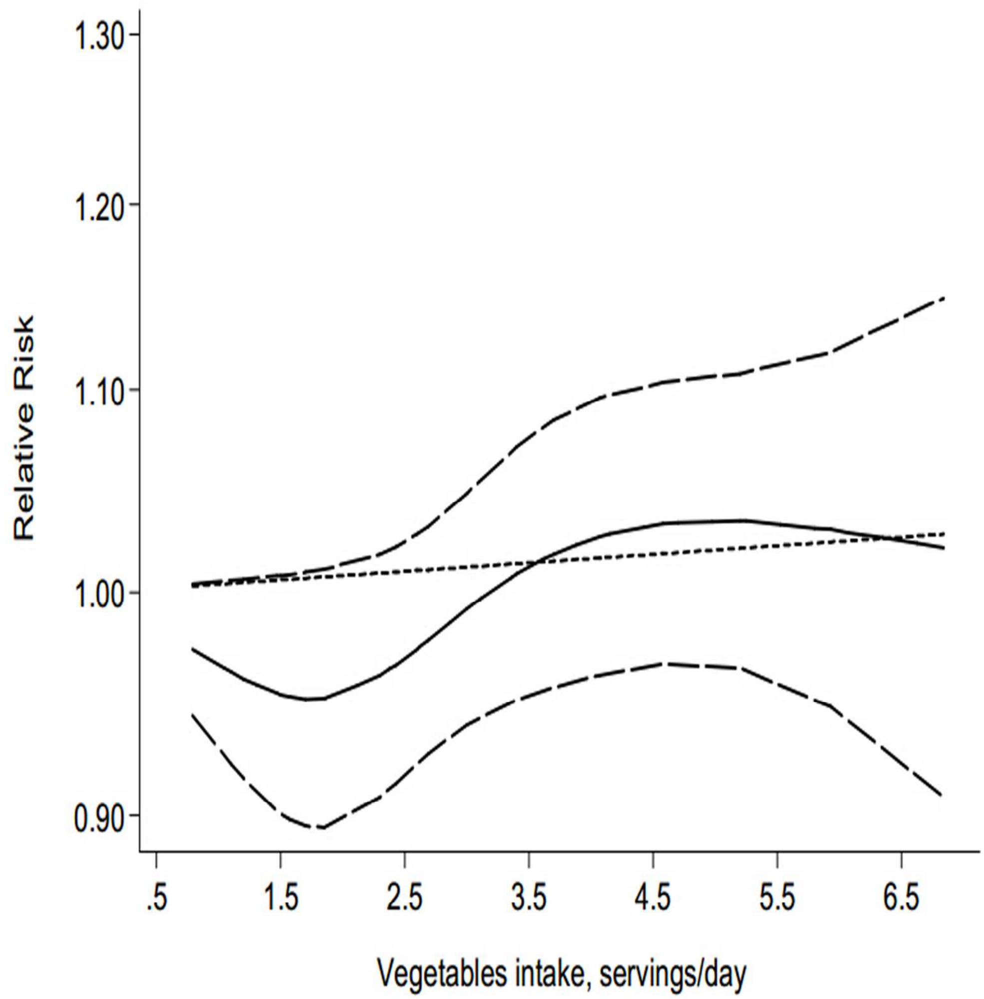

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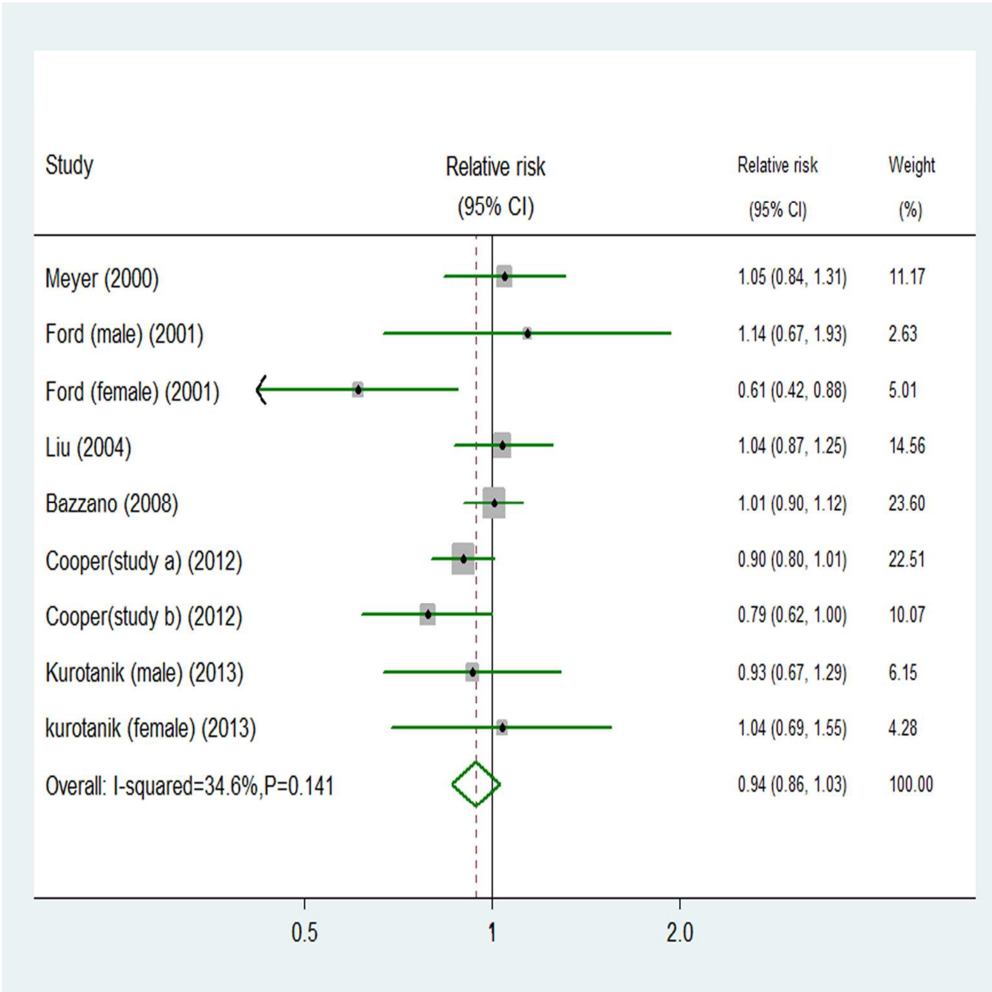

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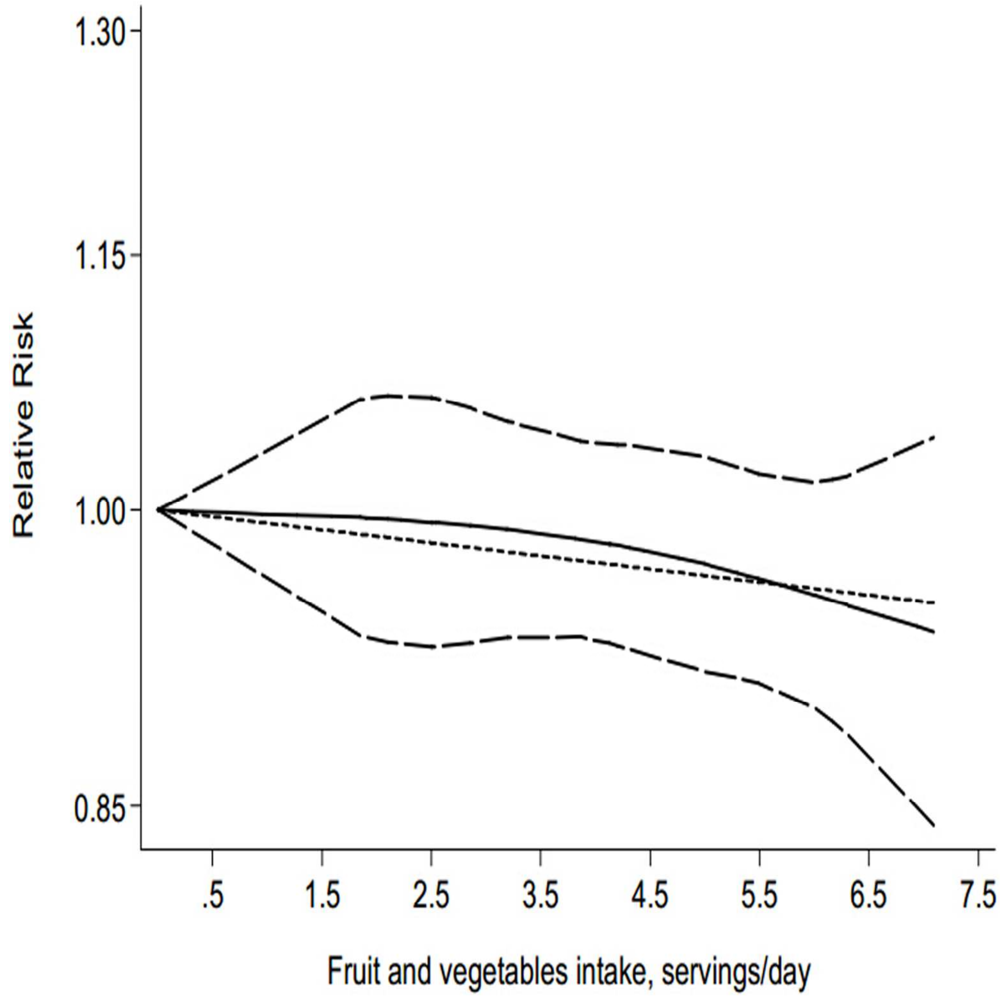

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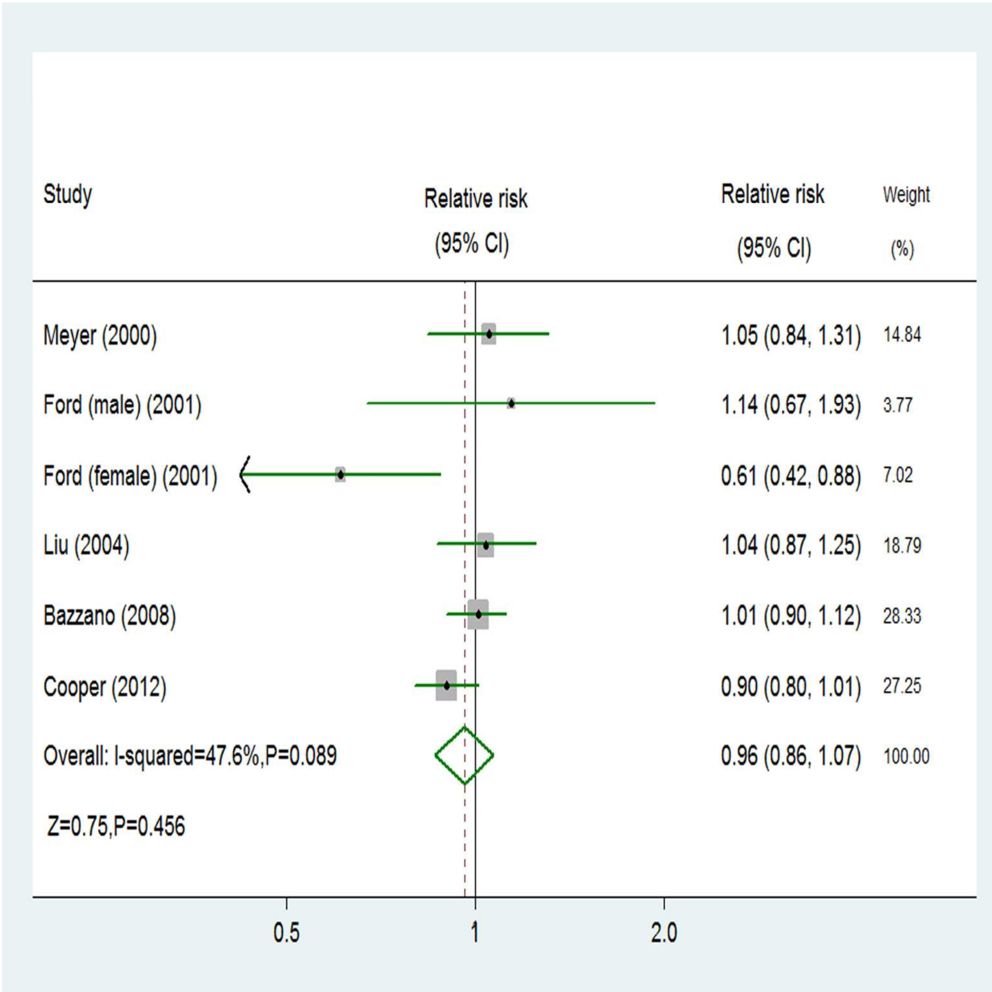

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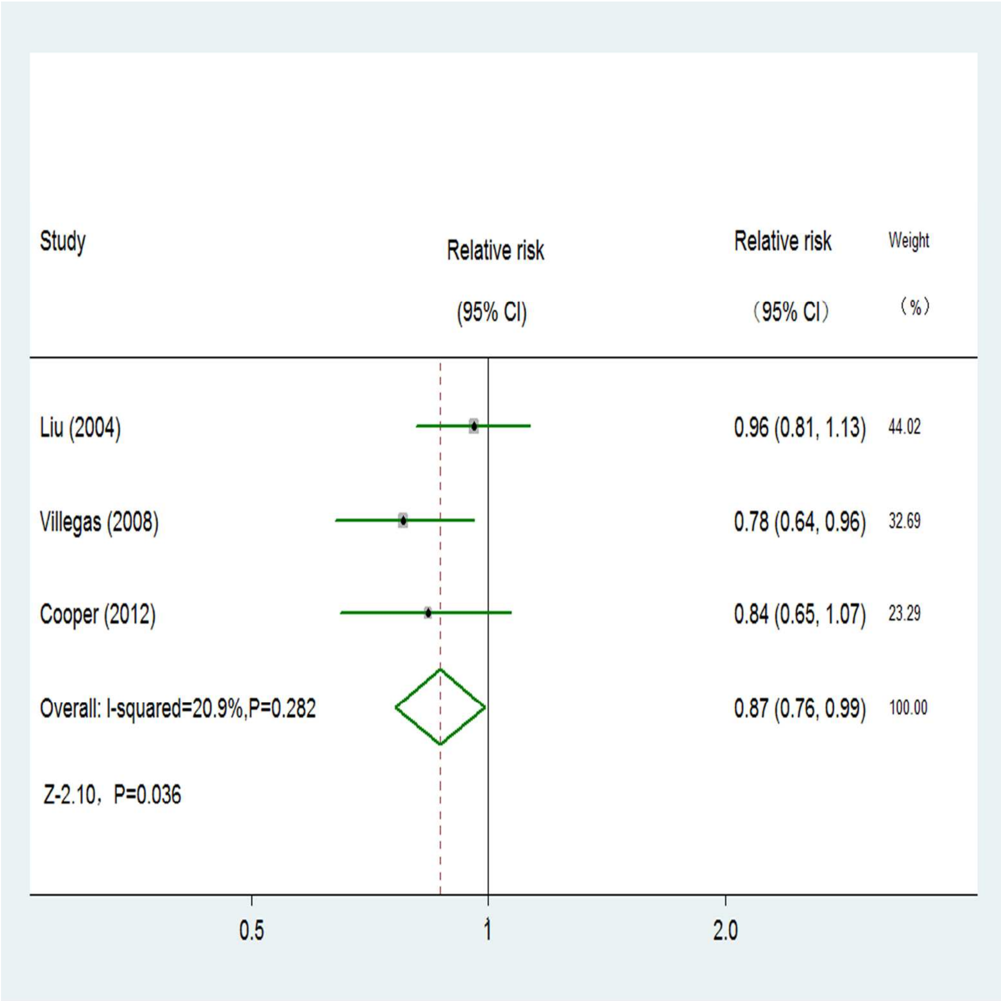

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MOOSE Checklist

Fruit and vegetable intake and risk of type 2 diabetes mellitus: meta-analysis of prospective cohort studies

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Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Type 2 diabetes (T2D) is one of the most common noncommunicable diseases which is expected to affect in excess of 439 million adults worldwide by 2030, with serious consequences for health care expenditure. The prevention of T2D is thus clearly an important public health priority. Among the known risk factors for T2D, dietary factors have aroused particular attention. Lifestyle intervention trials that include dietary modification have been shown to be effective in delaying or preventing the development of T2D. Although the effect of individual components or interactions between nutrients is still largely unknown, fruit and vegetables intake may explain some of this beneficial effect.
√	Hypothesis statement	Fruit and vegetable intake decrease risk of type 2 diabetes mellitus.
√	Description of study outcomes	Type 2 diabetes mellitus.
√	Type of exposure or intervention used	Fruit, vegetables, or green leafy vegetables
√	Type of study designs used	We included (1) original studies (eg, not review articles, meeting abstracts, editorials, or commentaries); (2) prospective design (eg, not cross sectional design, case-control design).
√	Study population	We placed no restriction.
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the two investigators XZ and WH are indicated in the author list.
√	Search strategy, including time period included in the synthesis and keywords	PubMed from 1965 –February 2014 EMBASE from 1974 –February 2014 Keywords: ("fruits" OR "vegetables" OR "citrus") AND ("Type 2 diabetes" OR "non-insulin-dependent diabetes mellitus" OR "Diabetes Mellitus, Type 2" OR "NIDDM" OR "prediabetes" OR "impaired glucose tolerance" OR "impaired fasting glucose" OR "glucose" OR "hyperglycaemia" OR "insulin") AND ("Follow-up studies" OR "prospective studies" OR "cohort studies" OR "longitudinal studies").
√	Databases and registries searched	PubMed and EMBASE
√	Search software used, name and version, including special features	We did not employ a search software. EndNote was used to merge retrieved citations and eliminate duplications
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for

		additional references,
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the process of literature search and study selection. The citation list is available upon request
√	Method of addressing articles published in languages other than English	We placed no restrictions on language; local scientists fluent in the original language of the article were contacted for translation
√	Method of handling abstracts and unpublished studies	We had contacted a few authors for unpublished studies on the association.
√	Description of any contact with authors	We contacted authors who had conducted multivariate analysis with diabetes as a covariate, but had not reported relative risk for fruit, vegetables, or green leafy vegetables.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Assessment of confounding	Restricted the analysis to age-adjusted estimates only. Conducted sensitivity analyses by eliminating studies that had not adjusted for possible confounders.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Quality was assessed with a total score from 0 to 6 points. The system was created to account for study eligibility (1 point for appropriate inclusion and exclusion criteria), outcome (1 point if diagnosis of T2D was based on accepted clinical criteria, and not solely based on self-report), exposure (1 point if fruit and vegetables consumption were assessed with a validated tool, and 1 point if fruit and vegetables consumption were appropriately categorized), statistical analysis (1 point was given if adjustment included a few variables such as age, sex, body mass index, and family history of T2D, these being proven risk factors for T2D). Another point was given for any other factors were adjusted (such as alcohol, education, and physical activity).
√	Assessment of heterogeneity	Heterogeneity of the studies were explored within two types of study designs using Cochrane's Q test of heterogeneity and I^2 statistic that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	Description of methods of meta-analyses, sensitivity analyses, subgroup analyses and assessment of publication bias are detailed in the methods.

√	Provision of appropriate tables and graphics	We included 1 flow chart, several summary tables and figures.
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 2, 4, 5 and D
√	Table giving descriptive information for each study included	Table A
√	Results of sensitivity testing	Table 1
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	Subgroup analyses indicate heterogeneity in strengths of the association due to most common biases in cohort studies.
√	Justification for exclusion	We excluded studies that had not adjusted for or were standardized by age, a potential confounder, and used different exposure or outcome assessment for the comparison groups.
√	Assessment of quality of included studies	We discussed the results of the subgroup analyses, and potential reasons for the observed heterogeneity.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	We discussed that potential unmeasured confounders such as other chronic diseases may have caused residual confounding, but the measured factors that are correlated with such confounders would have mitigated the bias. We noted that the variations in the strengths of association may be due to true population differences, or to differences in quality of studies.
√	Generalization of the conclusions	Our meta-analysis suggests that higher fruit or vegetables, particularly GLV intake is associated with a significantly reduced risk of T2D. In addition, the dose-response relations also indicate that relatively high fruit or GLV may still decrease risk of T2D.
√	Guidelines for future research	We recommend future preferably randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of T2D.
√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.