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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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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



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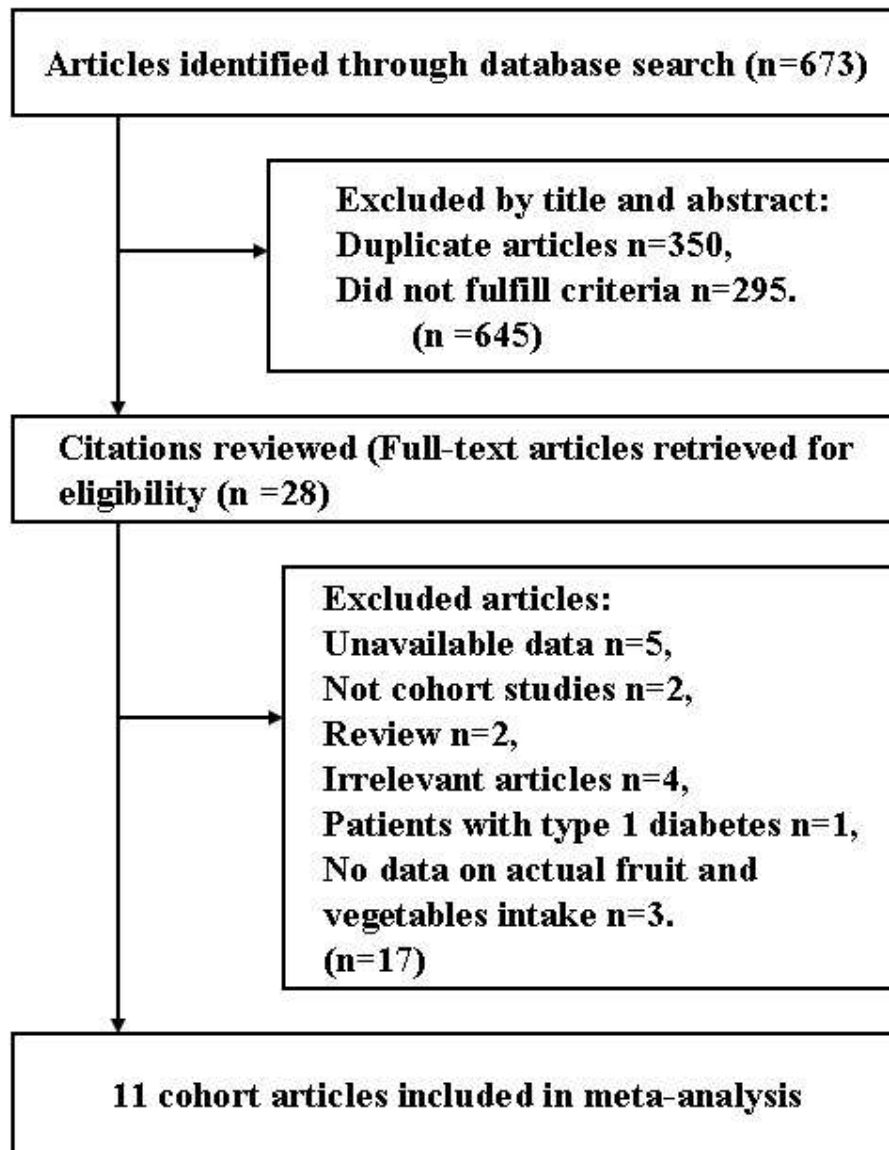
Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity 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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42 **Fig 1** Process of literature search and study selection

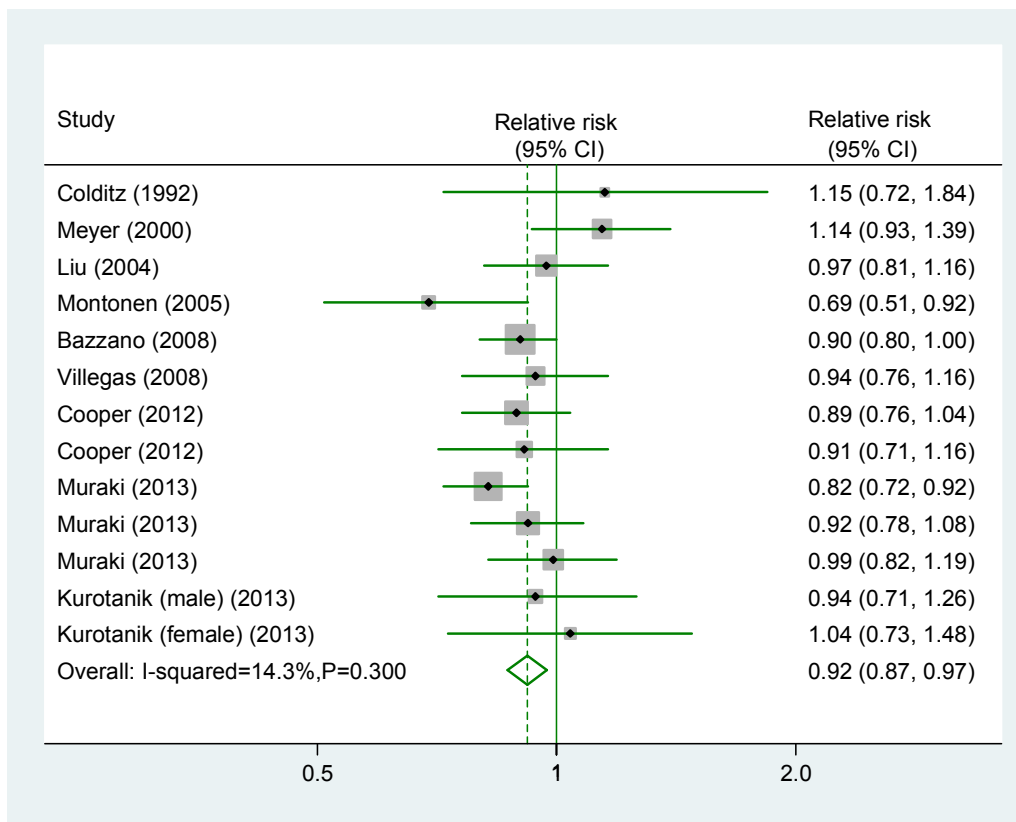


Fig 2 Random effects analysis of fully adjusted studies for the association between fruit intake and risk of type 2 diabetes

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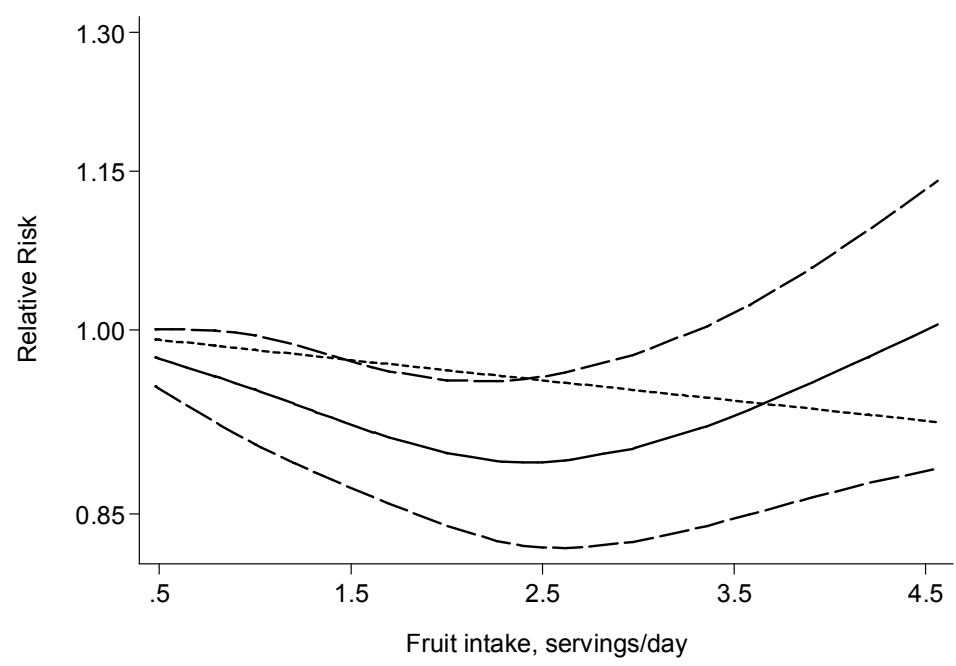


Fig 3 Dose-response analyses of fruit intake and risk of type 2 diabetes

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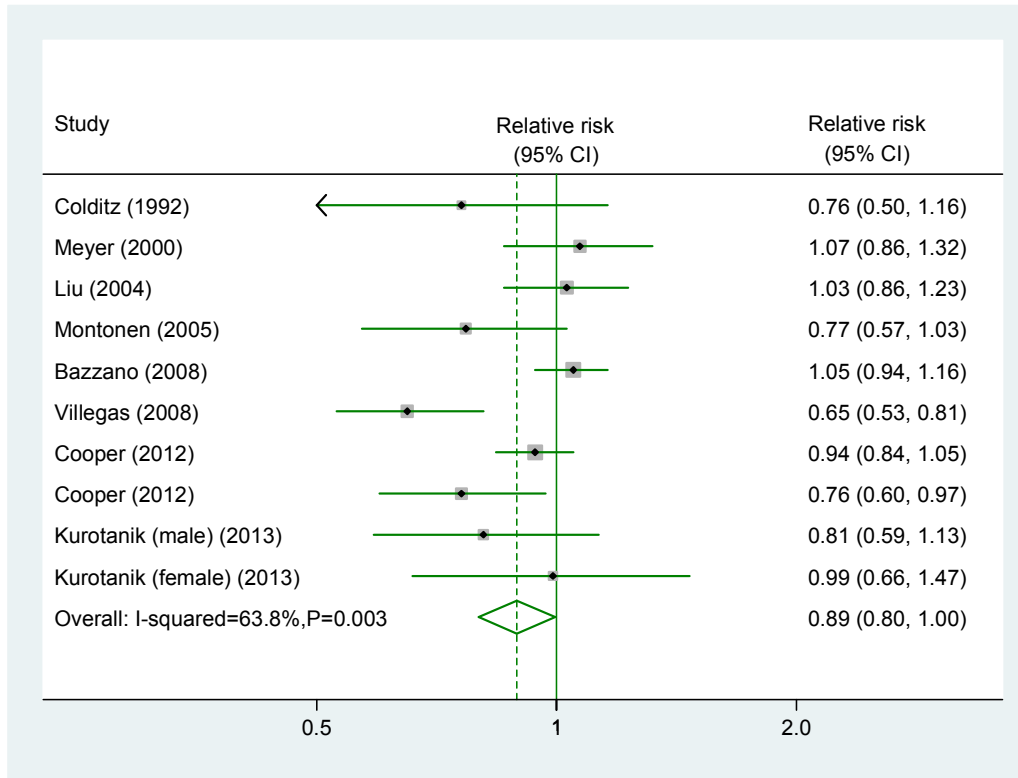
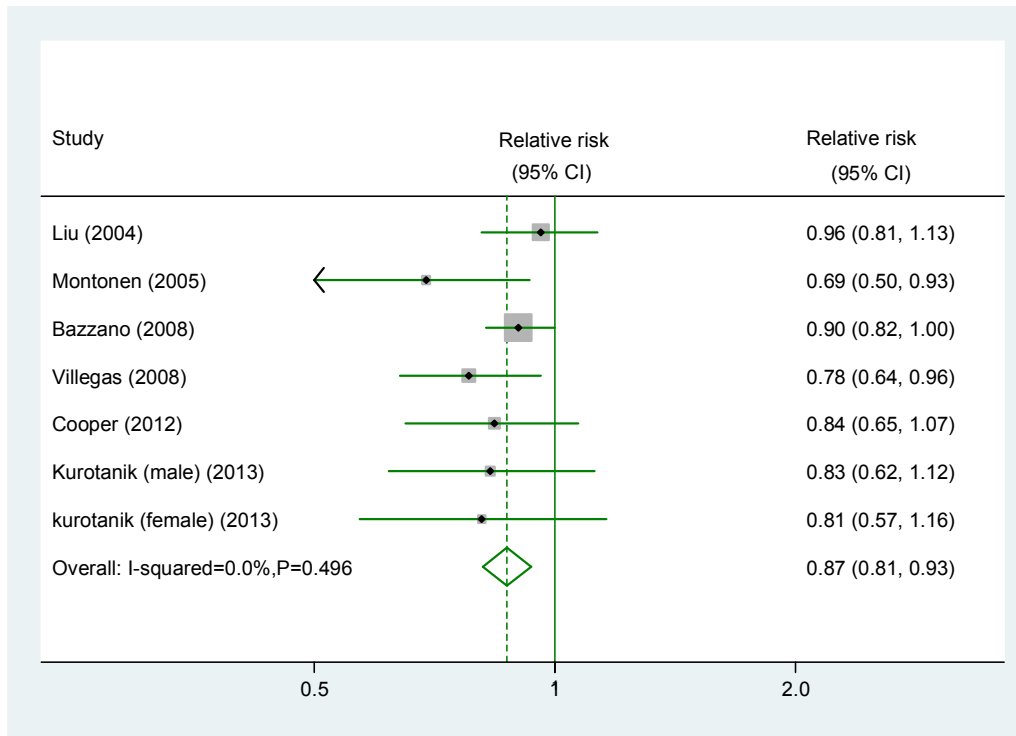


Fig 4 Random effects analysis of fully adjusted studies for the association between vegetables intake and risk of type 2 diabetes



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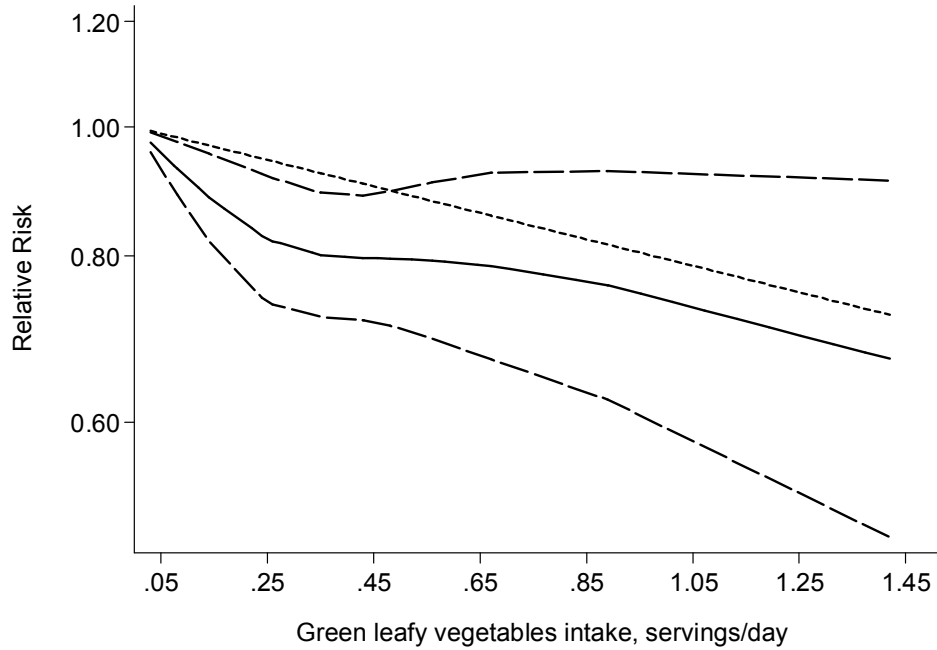


Fig 6 Dose-response analyses of green leafy vegetables intake and risk of type 2 diabetes

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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
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: $\geq 3 / < 0.6$. Vegetables: $\geq 2.9 / < 1.2$	Age, BMI, alcohol, family history of diabetes, prior weight change, time period	2
Meyer et al 2000, ⁴⁴	USA/Iowa Women's Health Study	55-69/F	35988/6	1141/3484 7	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: $\geq 5 / 0$	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004, ⁴⁵	USA/Women's Health Study	$\geq 45 / F$	38018/8.8	1614/3640 4	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	
10	Montonen et al 2005, ⁴⁶	Finland/Finland 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
19	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
28	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

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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

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

Fruit: $\geq 3 / < 0.57$

Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake

Age, BMI, ethnicity,

Cooper et al 2012, ⁵	8countries/ EPIC-Inter Act study	40-79/M and F	24939/11	10821/141	18	Based on self reported
Cooper et al 2012, ¹⁰	England/E PIC-Norfolk	40-79/M and F	3704/11	653/3051		Based on self reported
Muraki et al 2013, ¹¹	USA/Nurses' Health Study	30-55/F	69554/14	2699/6685	5	Confirmed by self report or medical records or death certificate
Muraki et al	USA/Nurse	24-44/F	91246/8	741/90505		Confirmed

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	1321/4118	3	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: $\geq 3 / < 0.57$	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/cbingensa i/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

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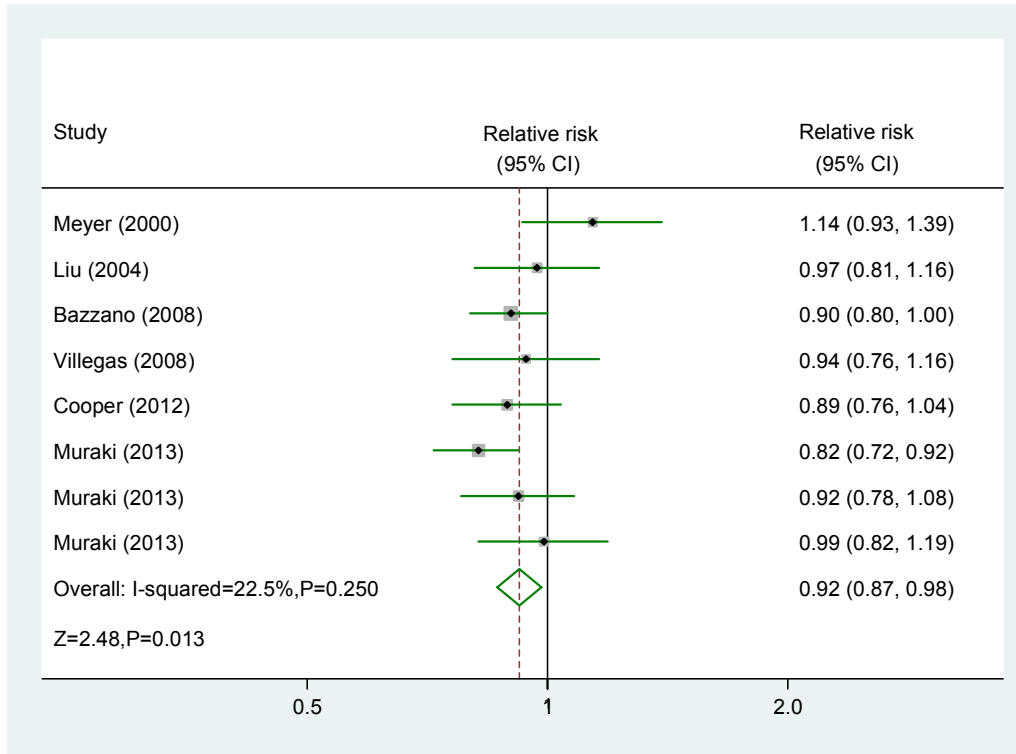
leafy vegetables:
0.54/0.07

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.

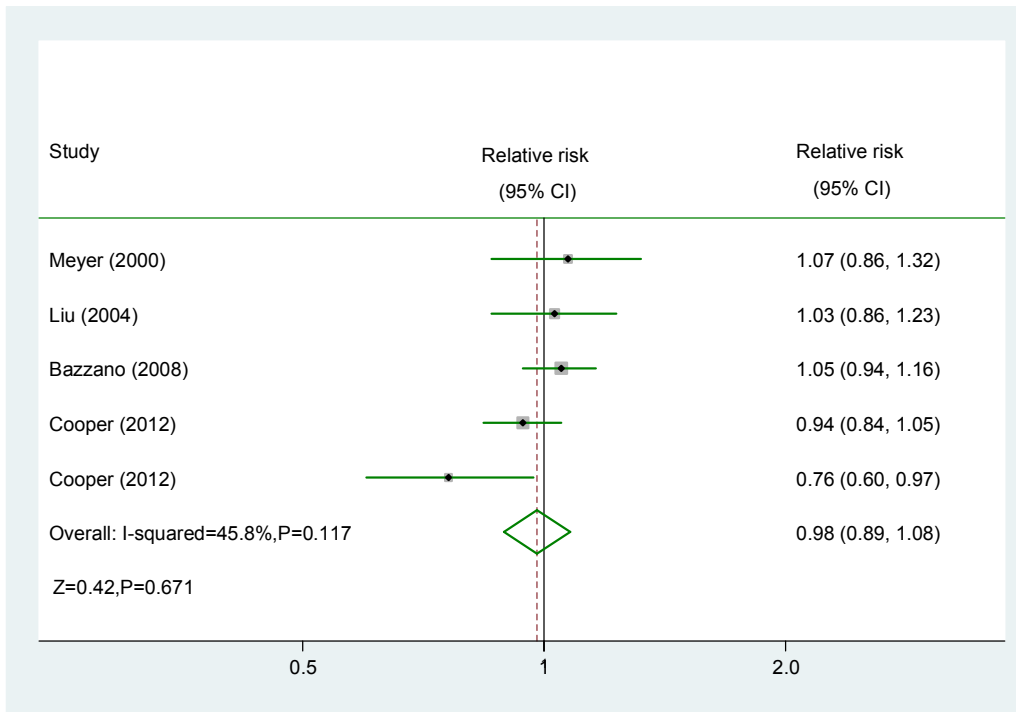
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Table B. Meta-analysis of intake of fruit and vegetables and risk of type 2 diabetes (highest versus lowest category)

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



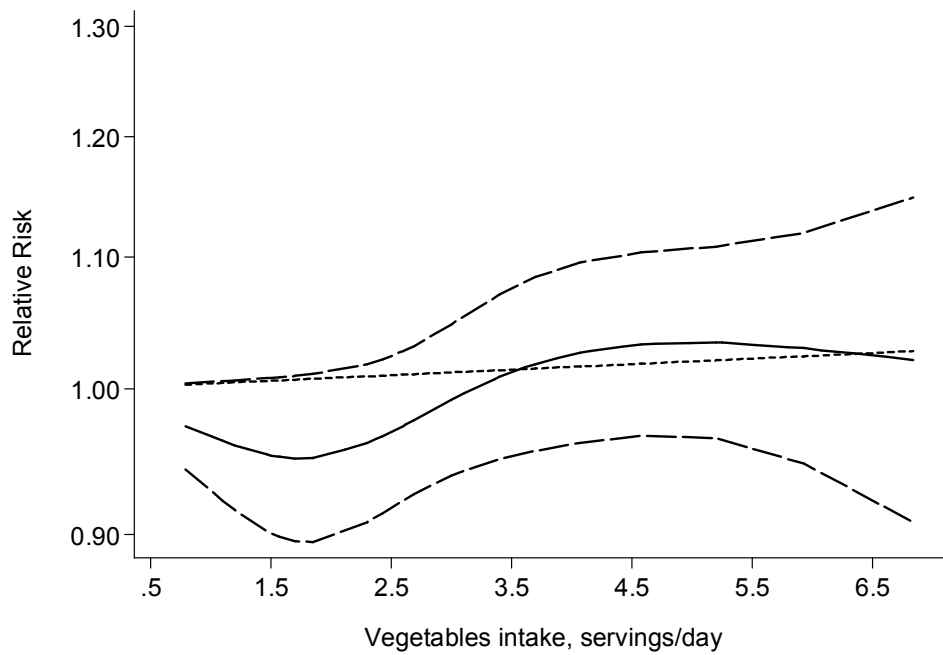
28 **Supplemental fig A** Random effects analysis of fully adjusted studies for the
29 association between fruit intake and risk of type 2 diabetes
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Supplemental fig B Random effects analysis of fully adjusted studies for the association between vegetables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

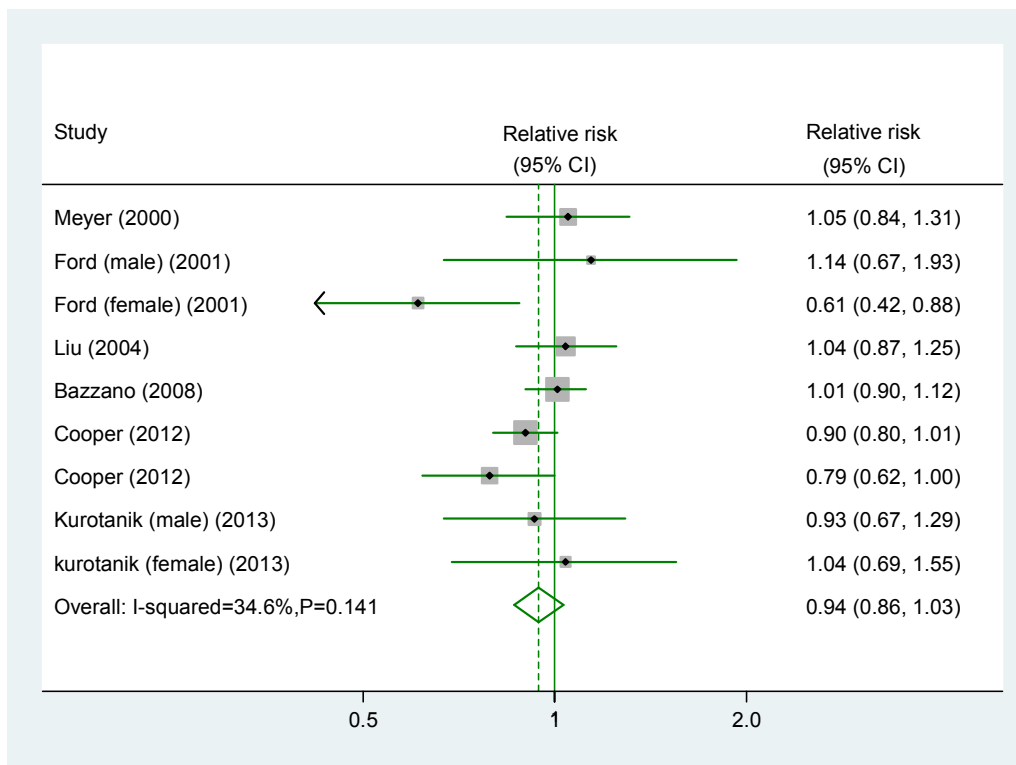
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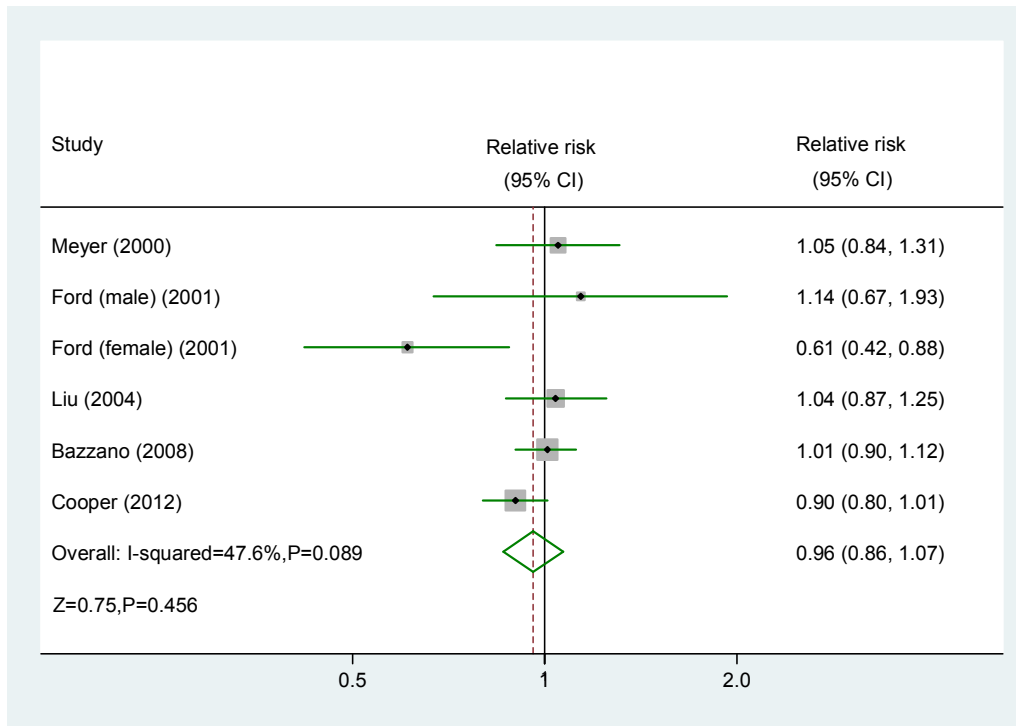
Supplemental fig C Dose-response analyses of vegetables intake and risk of type 2 diabetes

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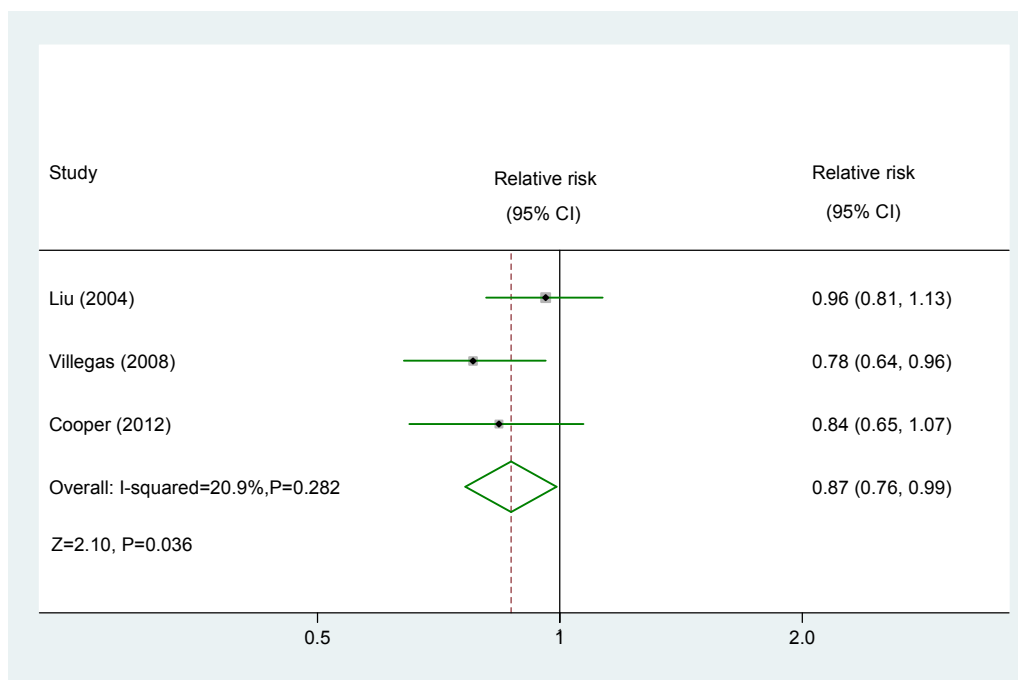


Supplemental fig D Random effects analysis of fully adjusted studies for the association between fruit and vegetables intake and risk of type 2 diabetes

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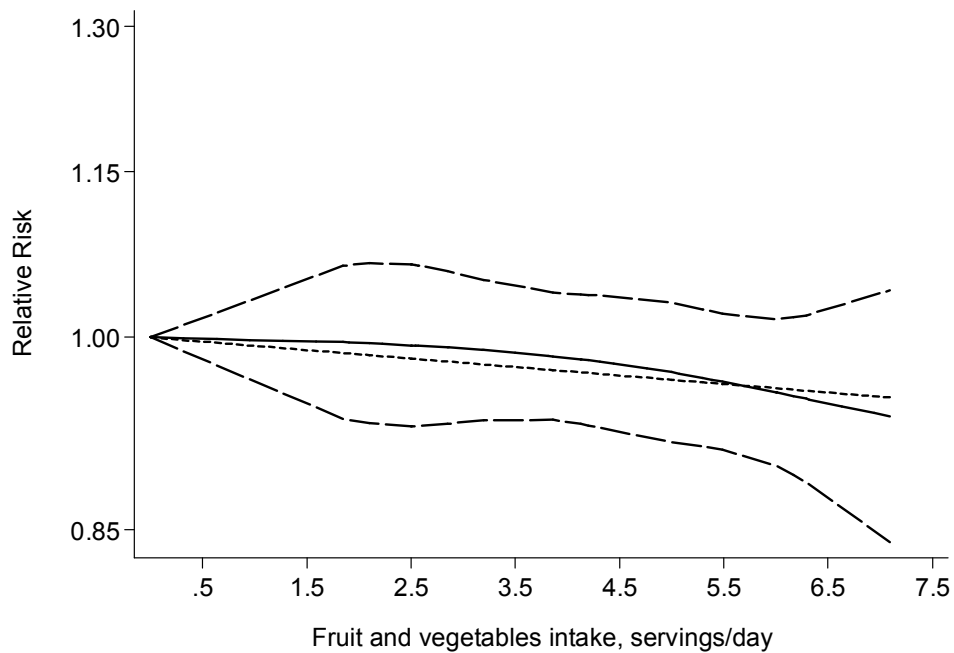
Supplemental fig F Random effects analysis of fully adjusted studies for the association between fruit and vegetables intake and risk of type 2 diabetes



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

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

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4 **Fruit and vegetable intake and risk of type 2 diabetes mellitus:**
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6 **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

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

12 **Validity assessment**

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

24 **Data extraction**

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

37 **Statistical analysis**

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39 Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and
40 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to
41 be a valid approximation of relative risks, we converted these values in every study by taking their
42 natural logarithms and calculating standard errors and corresponding 95% confidence intervals.
43 Relative risks and their standard errors were pooled with the DerSimonian and Laird random
44 effects model, which takes into account both within-study and between-study variabilities.¹⁶ When
45 some studies included in our meta-analysis used different measurement units (eg, grams per day or
46 portions per day or servings per day),^{5,10,15} we standardized fruit and vegetables intake into
47 servings per day using a standard portion size of 106 g.¹⁷ As different studies might use different
48 exposure categories (thirds, quarters, or fifths),^{7,11,15} we used the study specific relative risk for the
49 highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the
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3 meta-analysis. We carried out a dose-response analysis for the trend estimation using the method
4 that described by Greenland and Longnecker¹⁸ and Orsini et al.^{19,20} This analysis used data from
5 the relative risks and 95% confidence intervals, distributions of cases and person years for
6 exposure categories, and median/mean of fruit, vegetables, or fruit and vegetables intake levels for
7 each comparison group. We assigned the midpoint of the upper and lower boundaries of each
8 comparison group to determine mean fruit, vegetables, or fruit and vegetables intake levels if the
9 median or mean intake was not provided. When the highest category was open ended, we assumed
10 that the average of the category was set at 1.5 times the lower boundary. Additionally, we first
11 created restricted cubic splines with 4 knots at percentiles 5%, 35%, 65%, and 95% of the
12 distribution.²¹ A P value for nonlinearity was calculated by testing the null hypothesis that the
13 coefficient of the second spline is equal to zero. Heterogeneity among studies was evaluated using
14 the chi-square test based on Cochran's Q test and I^2 statistic at $P < 0.10$ level of significance,¹⁶ and
15 quantification of heterogeneity was made by the I^2 metric, which describes the percentage of total
16 variation in point estimates that is due to heterogeneity rather than chance.²² We considered low,
17 moderate, and high degrees of heterogeneity to be I^2 values of 25%, 50%, and 75%, respectively.
18 To explore possible explanations for heterogeneity and to test the robustness of the association, we
19 conducted subgroup analyses based on the location (Asia v Non-Asia), the quality of the study
20 (high quality (≥ 4) v lower quality (< 4), length of follow-up (≥ 10 years v < 10 years), sex (male
21 and female included v female only v male only), fractions of intake (thirds, quarters, or fifths),
22 number of participants (≥ 50000 v < 50000), and number of cases (≥ 1000 v < 1000). We also
23 performed the Begg rank correlation test and Egger's regression test to visualize a possible
24 asymmetry.²³⁻²⁵ All the statistical analyses were performed in Stata 12 (Stata Corp, College Station,
25 TX). A threshold of $P < 0.1$ was used to decide whether heterogeneity or publication bias was
26 present.²⁴ In other ways, P values were 2-sided and $P < 0.05$ was considered statistically
27 significant.

36 Results

37 Literature search

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

54 Study characteristics

55 Supplemental tables A and B in appendix 1 show the characteristics and main outcomes extracted
56 from the included studies, all eleven articles were prospective cohort designs and participants who
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3 were free of self reported diabetes at baseline.^{5,7,10,11,15,43-48} In aggregate, the included studies
4 consisted of 588 256 participants. Among the participants, we documented 27 414 cases of T2D
5 occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 11
6 articles, six cohorts were conducted primarily in the United States,^{11,15,43-45,48} two articles were
7 done in Asian countries (China and Japan)^{7,47} and three cohorts were from European
8 countries.^{5,10,46} The number of participants ranged from 3704 in the EPIC-Norfolk study by
9 Cooper et al¹⁰ to 84 360 in the Nurses Health Study by Colditz et al.⁴³ Five studies included both
10 male and female,^{5,7,10,15,46} five studies included only female.^{43-45,47,48} One article by Muraki et al
11 reported three independent cohorts, two cohorts included only female, and one cohort only male.¹¹
12 The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and
13 vegetables intake separately and combined.^{5,7,10,44,45,48} Three papers provided information on fruit
14 and vegetables intake separately,^{43,46,47} one paper provided only the combined data,¹⁵ and another
15 paper provided separate data on fruit.¹¹ Five papers also included separate data on the intake of
16 GLV.^{5,7,45-47} In most papers intake of fruit and vegetables was divided into fifths.^{11,43-48} All studies
17 provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most
18 studies yielded a score of 3 or below (low quality).

19 **Fruit intake and risk of T2D**

20 13 comparisons from ten studies reported an association between fruit intake and risk of T2D,
21 with 26 396 T2D outcomes and 578 591 participants. Overall, fruit intake was inversely associated
22 with risk (relative risk 0.92, 95% confidence interval 0.87 to 0.97) (fig 2). We saw no
23 heterogeneity among studies ($P=0.3$, $I^2=14.3\%$). Additionally, no evidence of substantial
24 publication bias was observed from the Begg ($P=0.127$) and Egger regression tests ($P=0.266$) (see
25 supplemental table B in appendix 1). Among 13 comparisons, eight comparisons were eligible for
26 the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model,
27 we found a significant curvilinear association ($P=0.013$ for non-linearity, fig 3). Dose-response
28 analysis indicated that a 1 serving/day increment of fruit intake was associated with 8% lower risk
29 of T2D (relative risk 0.92, 95% confidence interval 0.87 to 0.98, $I^2=22.5\%$) (see supplemental fig
30 A in appendix 2).

31 **Vegetables intake and risk of T2D**

32 Nine studies reported an association between vegetables intake and risk of T2D, with 21 635 T2D
33 outcomes and 37 5287 participants. Using a random effects model summarizing all 10
34 comparisons, we found significant association between vegetables intake and risk (relative risk
35 0.89, 95% confidence interval 0.80 to 1.00) (fig 4). There was moderate study heterogeneity
36 ($P=0.003$, $I^2=63.8\%$). However, no evidence of substantial publication bias was observed from the
37 Begg ($P=0.474$) and Egger regression tests ($P=0.122$) (see supplemental table B in appendix 1).
38 Among 10 comparisons, five comparisons were eligible for the trend estimation. Dose-response
39 analysis found no association with risk of T2D per 1 serving/day increment of vegetables intake
40 (relative risk 0.98, 95% confidence interval 0.89 to 1.08, $I^2=45.8\%$) (see supplemental fig B in
41 appendix 2). No publication bias was observed ($P=0.117$). We found no evidence of a curve linear
42 association between vegetables intake and risk ($P=0.671$ for non-linearity, see supplemental fig C
43 in appendix 2).

44 **Fruit and vegetables intake and risk of T2D**

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

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3 consumption of fruit was associated with a lower hazard of diabetes, whereas no significant
4 association for total fruit and vegetables consumption.⁴⁸ Similar to previous analysis in the Nurses'
5 Health Study, the results from three prospective longitudinal cohort studies also supported an
6 inverse association between fruit intake and risk of T2D.¹¹ But these studies have the potential for
7 bias due to measurement error. In addition, two cohort studies have suggested an inverse
8 association between total fruit and vegetables consumption and risk of T2D.^{10,15}

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

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

30 31 32 **Exploration of heterogeneity**

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

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

10 11 **Strengths and limitations**

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

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

35 36 37 38 39 40 41 42 43 **Conclusions**

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

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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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4 **Fruit and vegetable intake and risk of type 2 diabetes mellitus:**
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6 **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 Longnecker¹⁸ 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

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3 were free of self reported diabetes at baseline.^{5,7,10,11,15,43-47} In aggregate, the included studies
4 consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D
5 occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10
6 articles, five cohorts were conducted primarily in the United States,^{11,15,43,44,47} two articles were
7 done in Asian countries (China and Japan)^{7,46} and three cohorts were from European
8 countries.^{5,10,45} The number of participants ranged from 3704 in the EPIC-Norfolk study by
9 Cooper et al¹⁰ to 91 246 in the Nurses' Health Study II by Muraki et al.¹¹ Five studies included
10 both male and female,^{5,7,10,15,45} four studies included only female.^{43,44,46,47} One article by Muraki et
11 al reported two independent cohorts, one cohorts included only female, and another only male.¹¹
12 The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and
13 vegetables intake separately and combined.^{5,7,10,43,44,47} two papers provided information on fruit
14 and vegetables intake separately,^{45,46} one paper provided only the combined data,¹⁵ and another
15 paper provided separate data on fruit.¹¹ Five papers also included separate data on the intake of
16 GLV.^{5,7,44-46} In most papers intake of fruit and vegetables was divided into fifths.^{11,43-47} All studies
17 provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most
18 studies yielded a score of 3 or below (low quality).

19 **Fruit intake and risk of T2D**

20 11 comparisons from nine studies reported an association between fruit intake and risk of T2D,
21 with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely
22 associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no
23 heterogeneity among studies ($P=0.477$, $I^2=0\%$). Additionally, no evidence of substantial
24 publication bias was observed from the Begg ($P=0.533$) and Egger regression tests ($P=0.849$) (see
25 supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for
26 the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model,
27 we found a mild curvilinear association ($P=0.059$ for non-linearity, fig 3). Dose-response analysis
28 indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D
29 (relative risk 0.94, 95% confidence interval 0.89 to 1.00, $I^2=0\%$) (see supplemental fig A in
30 appendix 2).

31 **Vegetables intake and risk of T2D**

32 Eight studies reported an association between vegetables intake and risk of T2D, with 20 933
33 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9
34 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95%
35 confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity ($P=0.002$,
36 $I^2=66.5\%$). However, no evidence of substantial publication bias was observed from the Begg
37 ($P=0.602$) and Egger regression tests ($P=0.176$) (see supplemental table B in appendix 1). Among
38 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis
39 found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative
40 risk 0.98, 95% confidence interval 0.89 to 1.08, $I^2=45.8\%$) (see supplemental fig B in appendix 2).
41 No publication bias was observed ($P=0.117$). We found no evidence of a curve linear association
42 between vegetables intake and risk ($P=0.671$ for non-linearity, see supplemental fig C in appendix
43 2).

44 **Fruit and vegetables intake and risk of T2D**

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

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

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

15
16 Several plausible biological mechanisms have been proposed to explain abovementioned
17 association. Fruit and vegetables are rich in fibre,⁴⁸ which has been shown to improve insulin
18 sensitivity and insulin secretion to overcome insulin resistance.⁴⁹ However, meta-analyses showed
19 that fruit and vegetables fibre is inconsistently associated with the risk of T2D.⁵⁰ On the other
20 hand, it may contribute to a decreased incidence of T2D through their low energy density and
21 glycemic load, and high micronutrient content.⁵¹ In particular, GLV are rich in bioactive
22 phytochemicals (such as vitamin C and carotenoids), which are known for their antioxidant
23 properties.⁵²⁻⁵⁴ Antioxidants in fruit and vegetables have been hypothesized to improve insulin
24 sensitivity and protect against diabetes in several supplementation trials.^{55,56} In addition, it also
25 might reduce the risk of T2D due to the supply of magnesium (Mg), a recent meta-analysis
26 detected Mg intake to be inversely associated with the risk of T2D.⁵⁷ Taking this evidence into
27 consideration, it appears that the beneficial effects of vegetables, particularly GLV consumption
28 on the risk of T2D can be mainly explained by antioxidant vitamins and magnesium. The inverse
29 association may be also mediated through weight gain or obesity which is an established risk
30 factor for type 2 diabetes. Fruits are low in energy, which would promote the feeling of fullness
31 and prevent over consumption of energy-dense foods, and resulting in weight loss.⁵⁴ Further
32 investigation is warranted to understand the mechanisms involved in the proposed relation
33 between fruit, vegetables, or GLV and risk of T2D.

34 **Exploration of heterogeneity**

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

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48 Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed
49 between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs)
50 to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.^{7,10,11,43,44,46,47} It is
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3 less suitable for the assessment of absolute intake, which they tend to overestimate.^{58,59} However,
4 two studies collected data via a single 24 hour recall and dietary history interviews,
5 respectively.^{15,45} These measurements may underestimate true associations between fruit,
6 vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition,
7 calculations of daily consumption were differed (such as servings per week, servings per day, or
8 grams per day). Although we standardized primary data using a standard portion size of 106g,
9 conclusions should be interpreted with caution. Another possible explanation for the differences
10 between the studies might be the classification of food groups. GLVs' criteria was inconsistent:
11 three studies included spinach and lettuce; one included spinach and greens; others did not provide
12 specific description. If they were included with an uniform definition of each groups, the
13 associations might differ.

14 **Strengths and limitations**

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

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

36 **Conclusions**

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

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

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

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

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

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Appendix figure information

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

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

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4 **Supplemental fig C. Dose-response analyses of vegetables intake and risk of type 2 diabetes.**
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8 **Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus**
9 **lowest intake of fruit and vegetables and risk of type 2 diabetes.**
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13 **Supplemental fig E. Dose-response analyses of fruit and vegetables intake and risk of type 2**
14 **diabetes.**
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18 **Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.**
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22 **Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.**
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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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4 **Fruit and vegetable intake and risk of type 2 diabetes mellitus:**
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6 **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

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

14 **Validity assessment**

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

28 **Data extraction**

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

44 **Statistical analysis**

45 Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and
46 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to
47 be a valid approximation of relative risks, we converted these values in every study by taking their
48 natural logarithms and calculating standard errors and corresponding 95% confidence intervals.
49 Relative risks and their standard errors were pooled with the DerSimonian and Laird random
50 effects model, which takes into account both within-study and between-study variabilities.¹⁶ When
51 some studies included in our meta-analysis used different measurement units (eg, grams per day or
52 portions per day or servings per day),^{5,10,15} we standardized fruit and vegetables intake into
53 servings per day using a standard portion size of 106 g.¹⁷ **As different studies might use different
54 exposure categories (thirds, quarters, or fifths),^{7,11,15} we used the study specific relative risk for the
55 highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the
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meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker¹⁸ 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

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3 were free of self reported diabetes at baseline.^{5,7,10,11,15,43-47} In aggregate, the included studies
4 consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D
5 occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10
6 articles, five cohorts were conducted primarily in the United States,^{11,15,43,44,47} two articles were
7 done in Asian countries (China and Japan)^{7,46} and three cohorts were from European
8 countries.^{5,10,45} The number of participants ranged from 3704 in the EPIC-Norfolk study by
9 Cooper et al¹⁰ to 91 246 in the Nurses' Health Study II by Muraki et al.¹¹ Five studies included
10 both male and female,^{5,7,10,15,45} four studies included only female.^{43,44,46,47} One article by Muraki et
11 al reported two independent cohorts, one cohorts included only female, and another only male.¹¹
12 The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and
13 vegetables intake separately and combined.^{5,7,10,43,44,47} two papers provided information on fruit
14 and vegetables intake separately,^{45,46} one paper provided only the combined data,¹⁵ and another
15 paper provided separate data on fruit.¹¹ Five papers also included separate data on the intake of
16 GLV.^{5,7,44-46} In most papers intake of fruit and vegetables was divided into fifths.^{11,43-47} All studies
17 provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most
18 studies yielded a score of 3 or below (low quality).

19 **Fruit intake and risk of T2D**

20 11 comparisons from nine studies reported an association between fruit intake and risk of T2D,
21 with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely
22 associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no
23 heterogeneity among studies ($P=0.477$, $I^2=0\%$). Additionally, no evidence of substantial
24 publication bias was observed from the Begg ($P=0.533$) and Egger regression tests ($P=0.849$) (see
25 supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for
26 the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model,
27 we found a mild curvilinear association ($P=0.059$ for non-linearity, fig 3). Dose-response analysis
28 indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D
29 (relative risk 0.94, 95% confidence interval 0.89 to 1.00, $I^2=0\%$) (see supplemental fig A in
30 appendix 2).

31 **Vegetables intake and risk of T2D**

32 Eight studies reported an association between vegetables intake and risk of T2D, with 20 933
33 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9
34 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95%
35 confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity ($P=0.002$,
36 $I^2=66.5\%$). However, no evidence of substantial publication bias was observed from the Begg
37 ($P=0.602$) and Egger regression tests ($P=0.176$) (see supplemental table B in appendix 1). Among
38 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis
39 found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative
40 risk 0.98, 95% confidence interval 0.89 to 1.08, $I^2=45.8\%$) (see supplemental fig B in appendix 2).
41 No publication bias was observed ($P=0.117$). We found no evidence of a curve linear association
42 between vegetables intake and risk ($P=0.671$ for non-linearity, see supplemental fig C in appendix
43 2).

44 **Fruit and vegetables intake and risk of T2D**

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

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

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

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

34 35 36 37 38 39 **Exploration of heterogeneity**

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

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52 Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed
53 between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs)
54 to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.^{7,10,11,43,44,46,47} It is
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3 less suitable for the assessment of absolute intake, which they tend to overestimate.^{58,59} However,
4 two studies collected data via a single 24 hour recall and dietary history interviews,
5 respectively.^{15,45} These measurements may underestimate true associations between fruit,
6 vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition,
7 calculations of daily consumption were differed (such as servings per week, servings per day, or
8 grams per day). Although we standardized primary data using a standard portion size of 106g,
9 conclusions should be interpreted with caution. Another possible explanation for the differences
10 between the studies might be the classification of food groups. GLVs' criteria was inconsistent:
11 three studies included spinach and lettuce; one included spinach and greens; others did not provide
12 specific description. If they were included with an uniform definition of each groups, the
13 associations might differ.

14 **Strengths and limitations**

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

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

36 **Conclusions**

37 In summary, our meta-analysis suggests that higher fruit or GLV intake is associated with a
38 significantly reduced risk of T2D. In addition, the dose-response relations also indicate that
39 relatively high fruit or GLV may still decrease risk of T2D. Further evidence from preferably
40 randomized controlled studies should explore what kind of fruit or GLV can reduce the risk of
41 T2D.
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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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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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.

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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.

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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.

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4 **Supplemental fig C. Dose-response analyses of vegetables intake and risk of type 2 diabetes.**
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8 **Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus**
9 **lowest intake of fruit and vegetables and risk of type 2 diabetes.**
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12 **Supplemental fig E. Dose-response analyses of fruit and vegetables intake and risk of type 2**
13 **diabetes.**
14 **(TIFF)**
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16 **Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.**
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19 **Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.**
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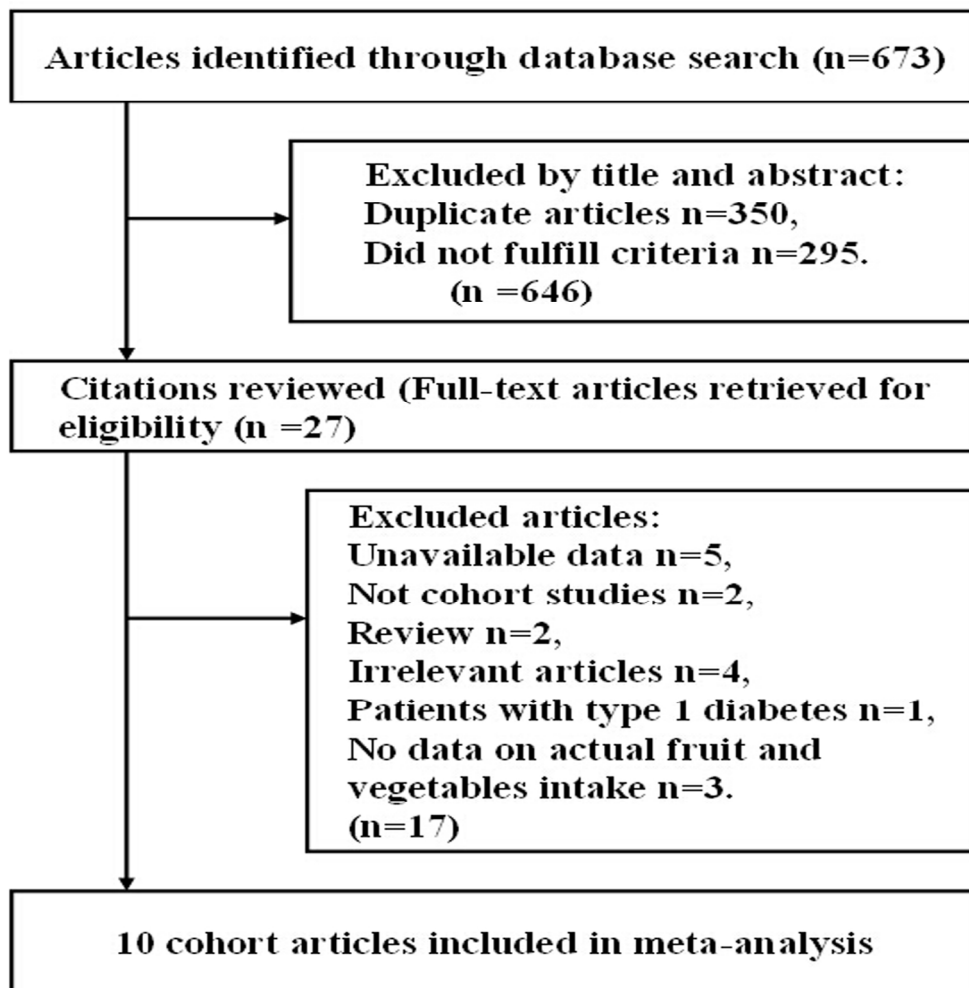
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Table 1. Subgroup analyses to investigate differences between studies included in meta-analysis (highest versus lowest category)

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

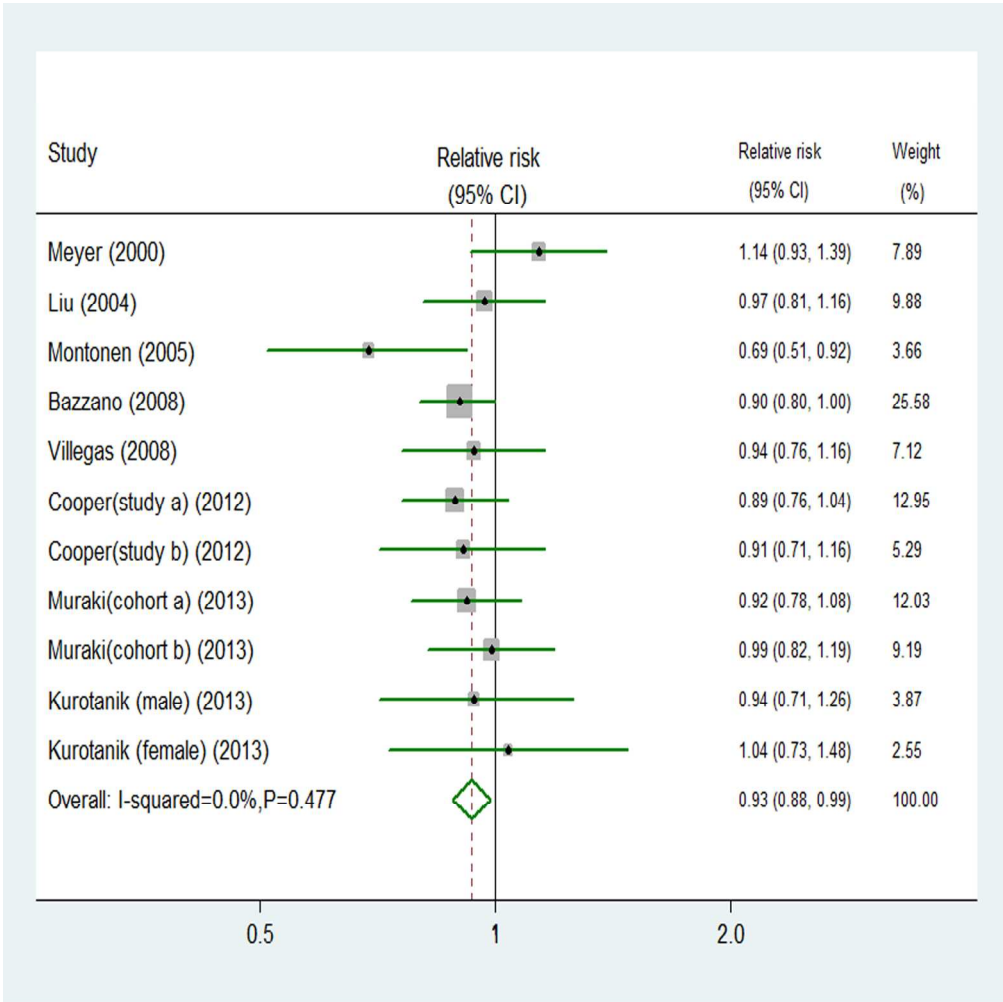
M=male, F=female, RR=relative risk.



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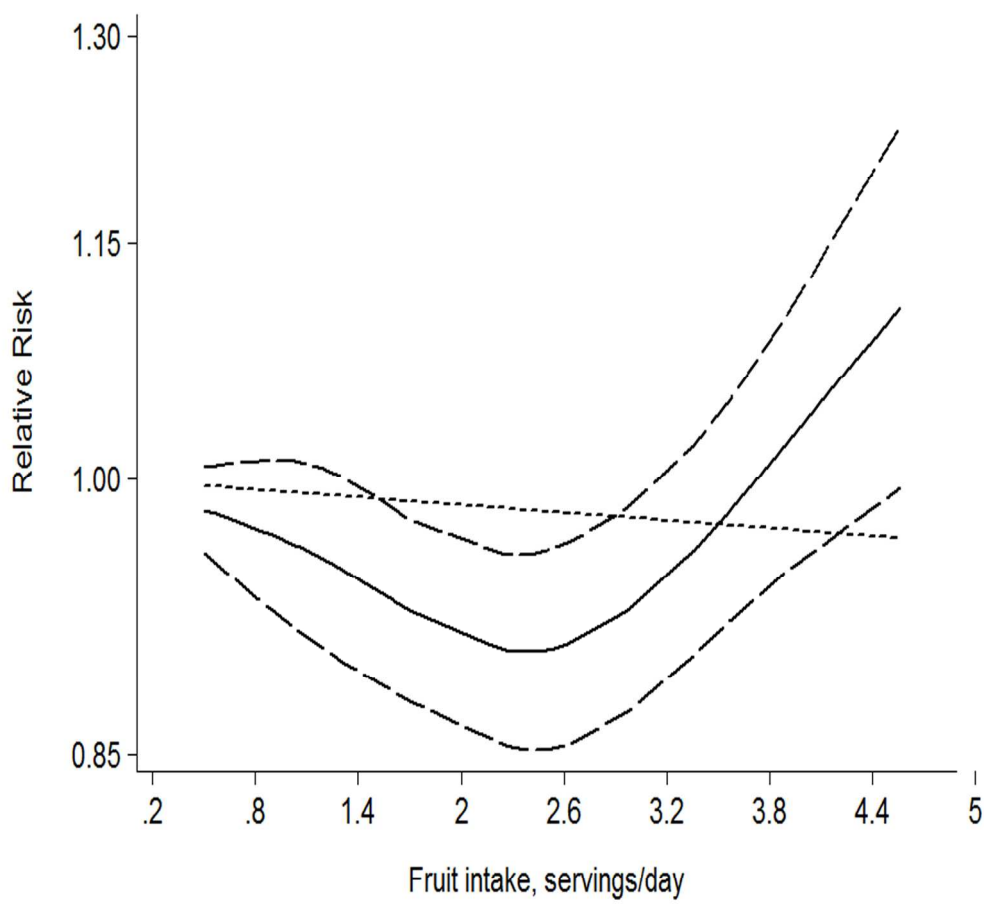
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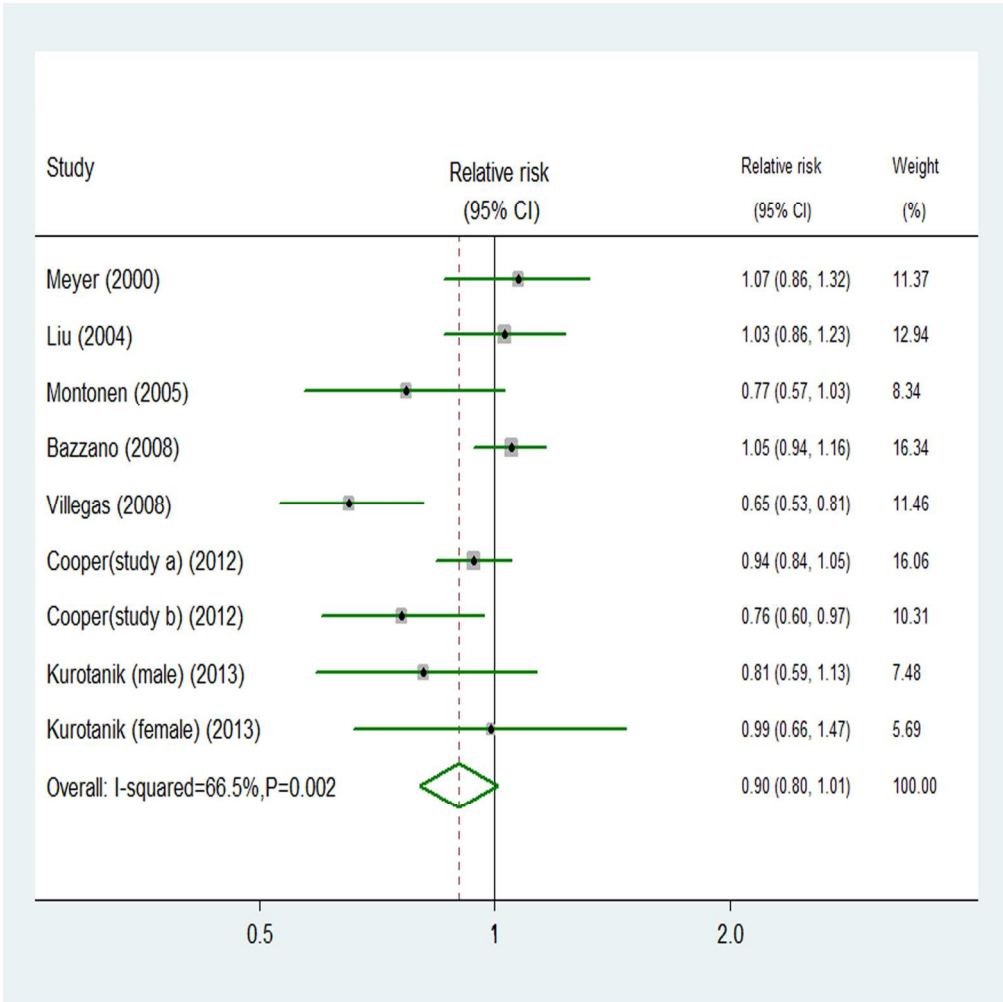
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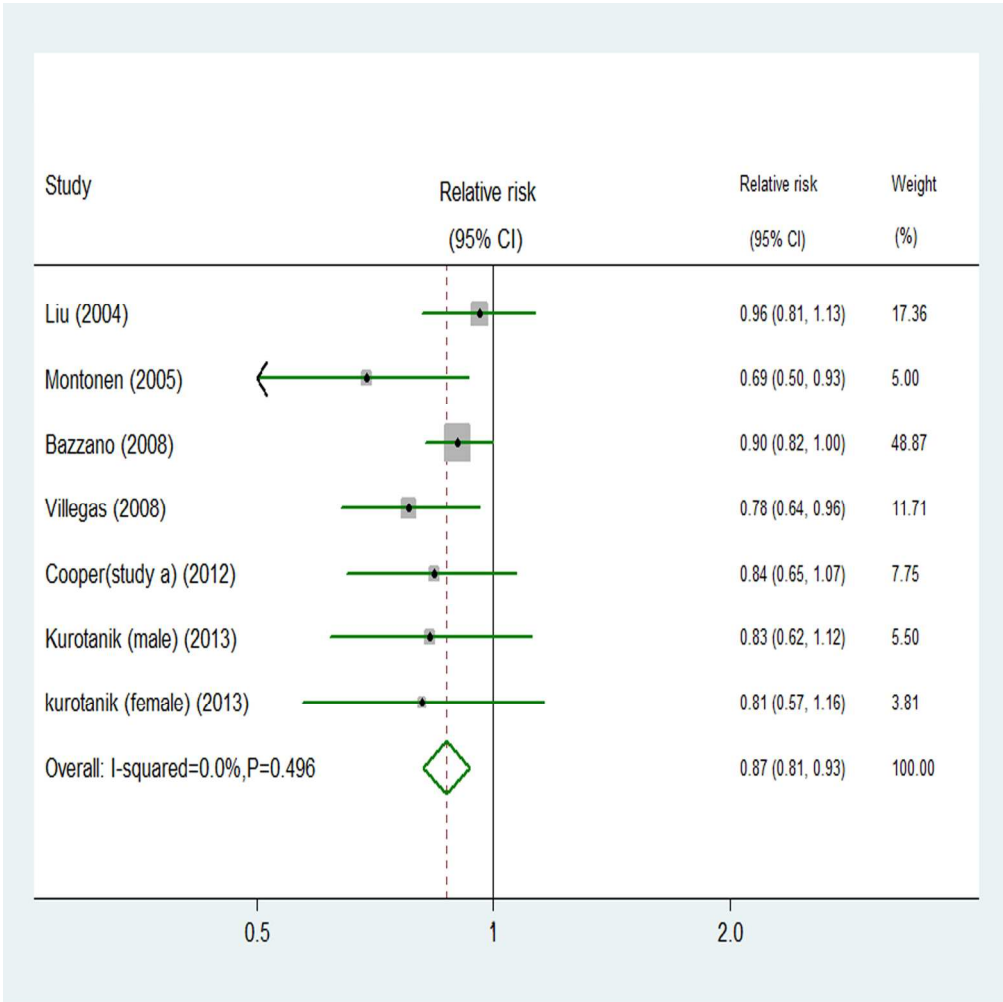
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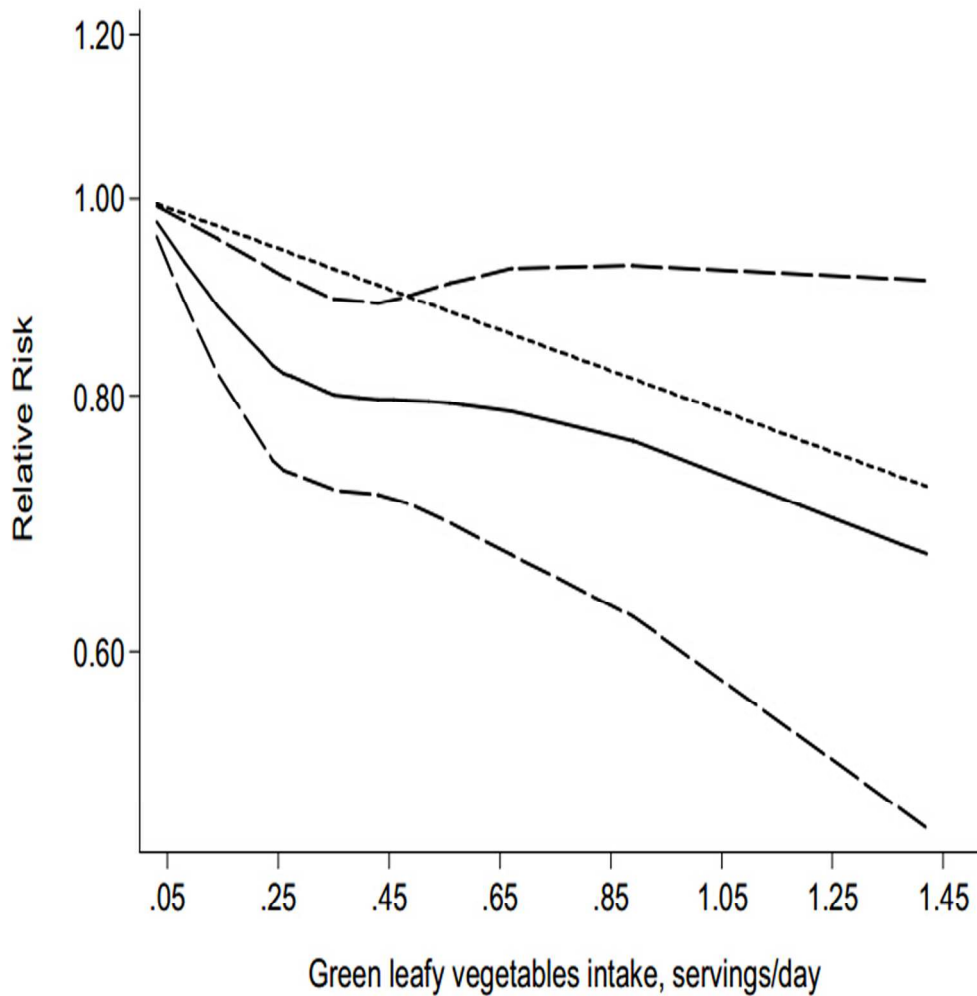


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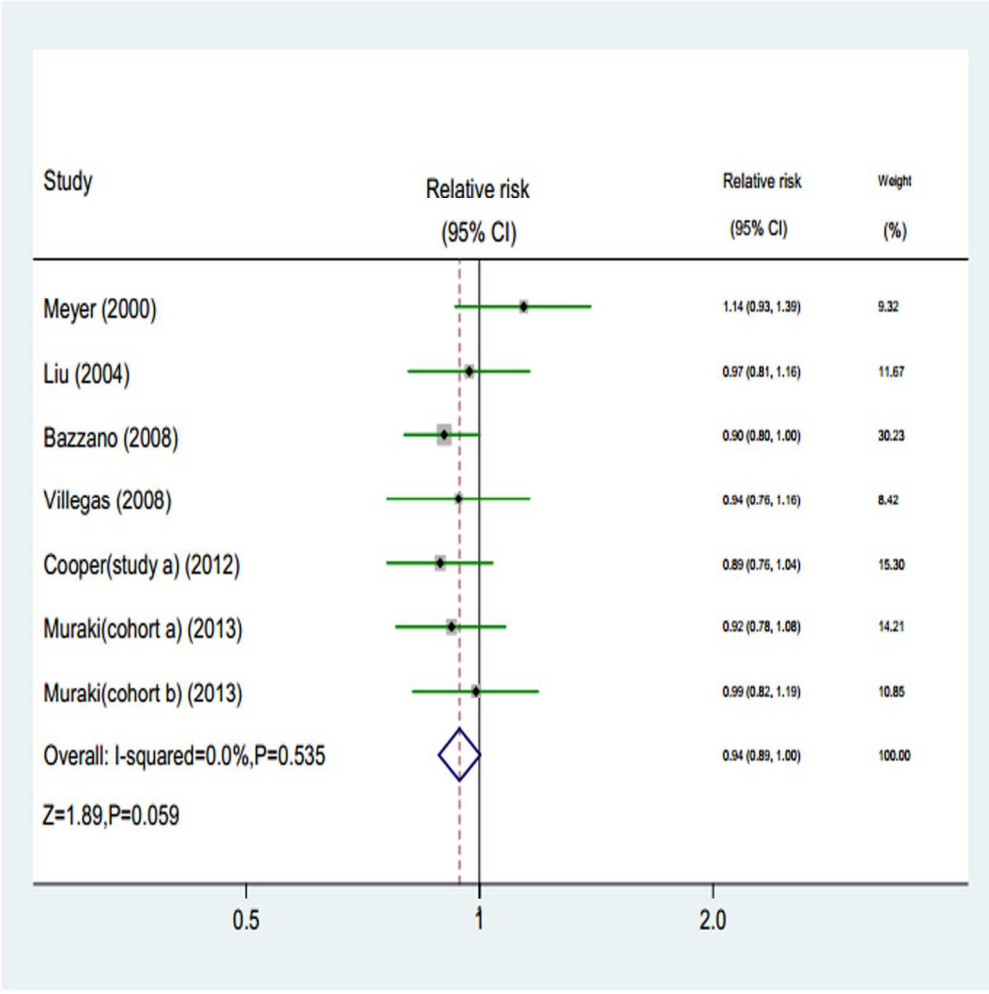




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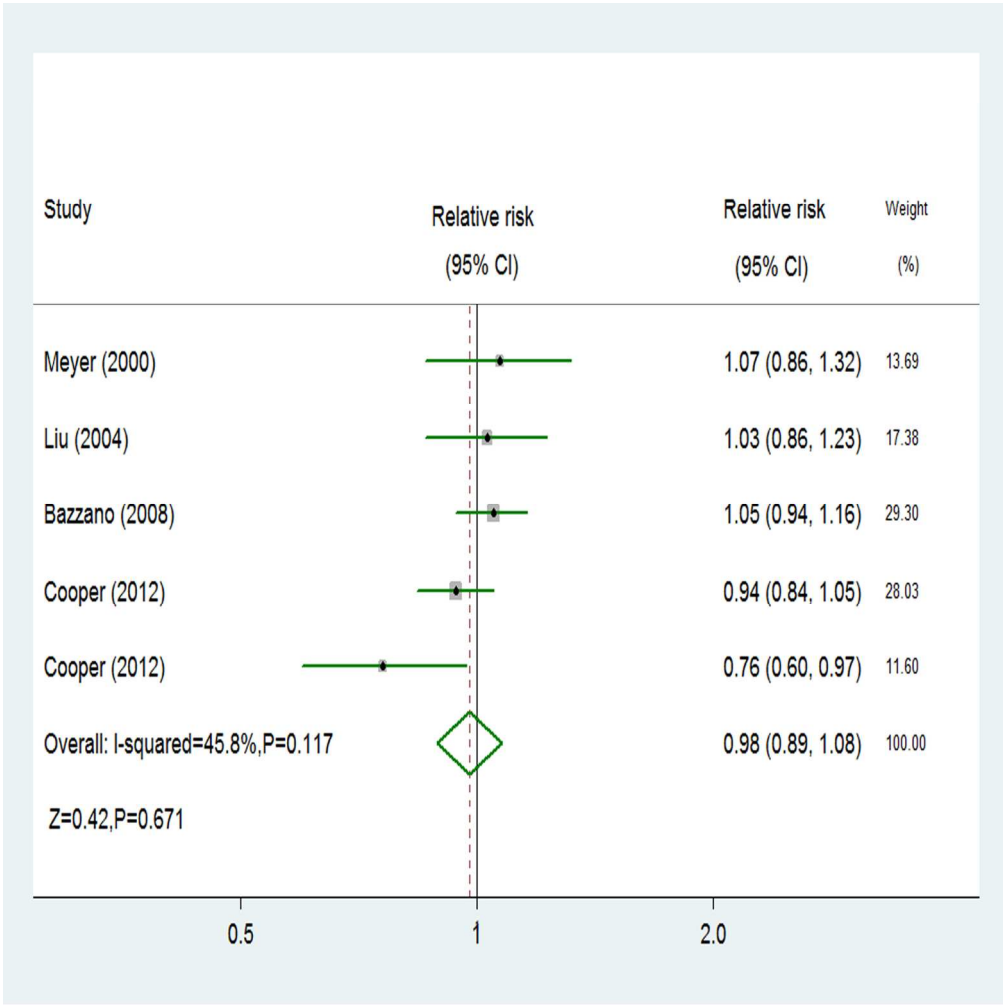
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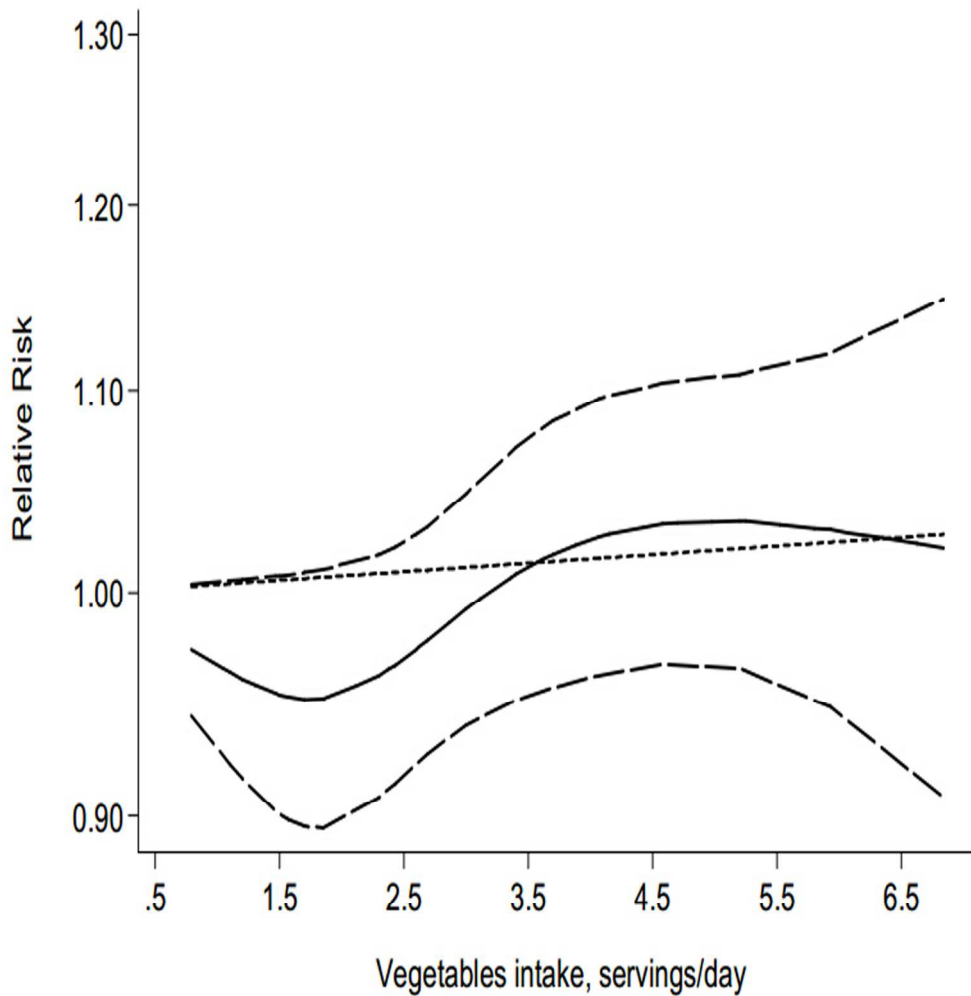
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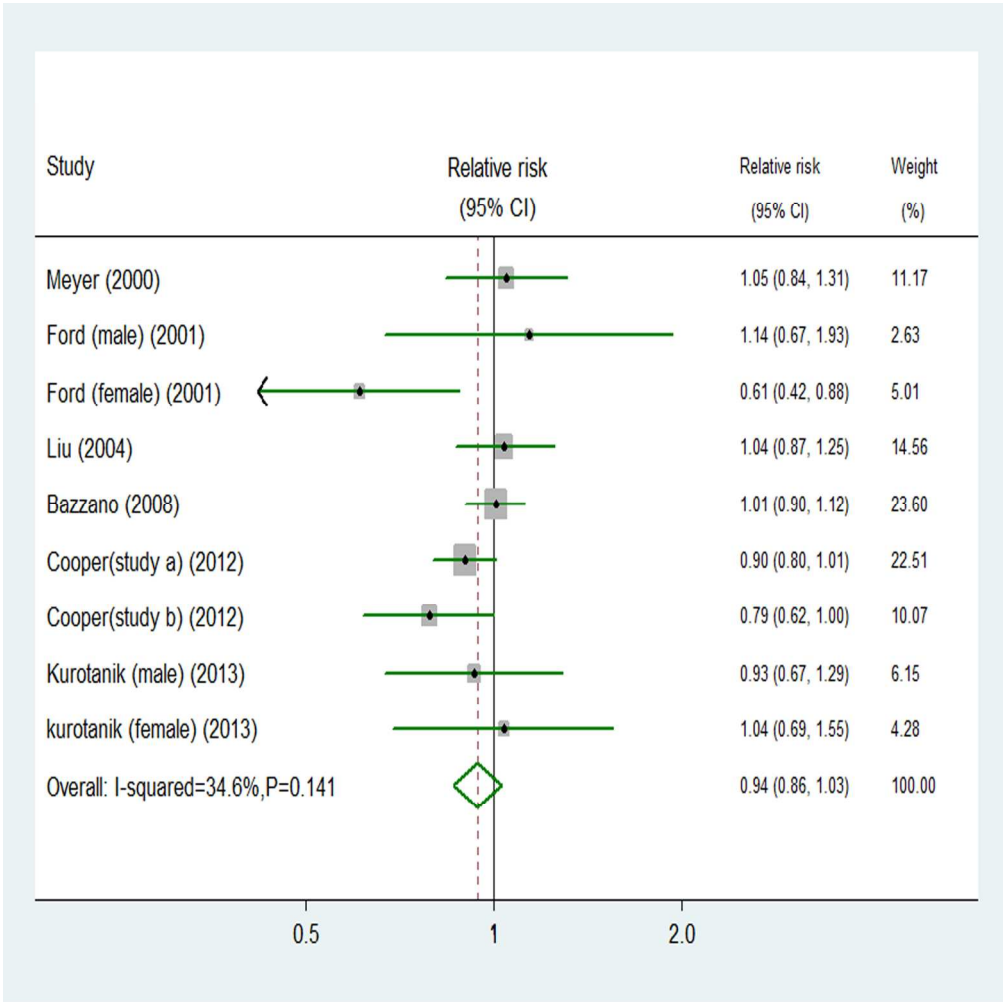
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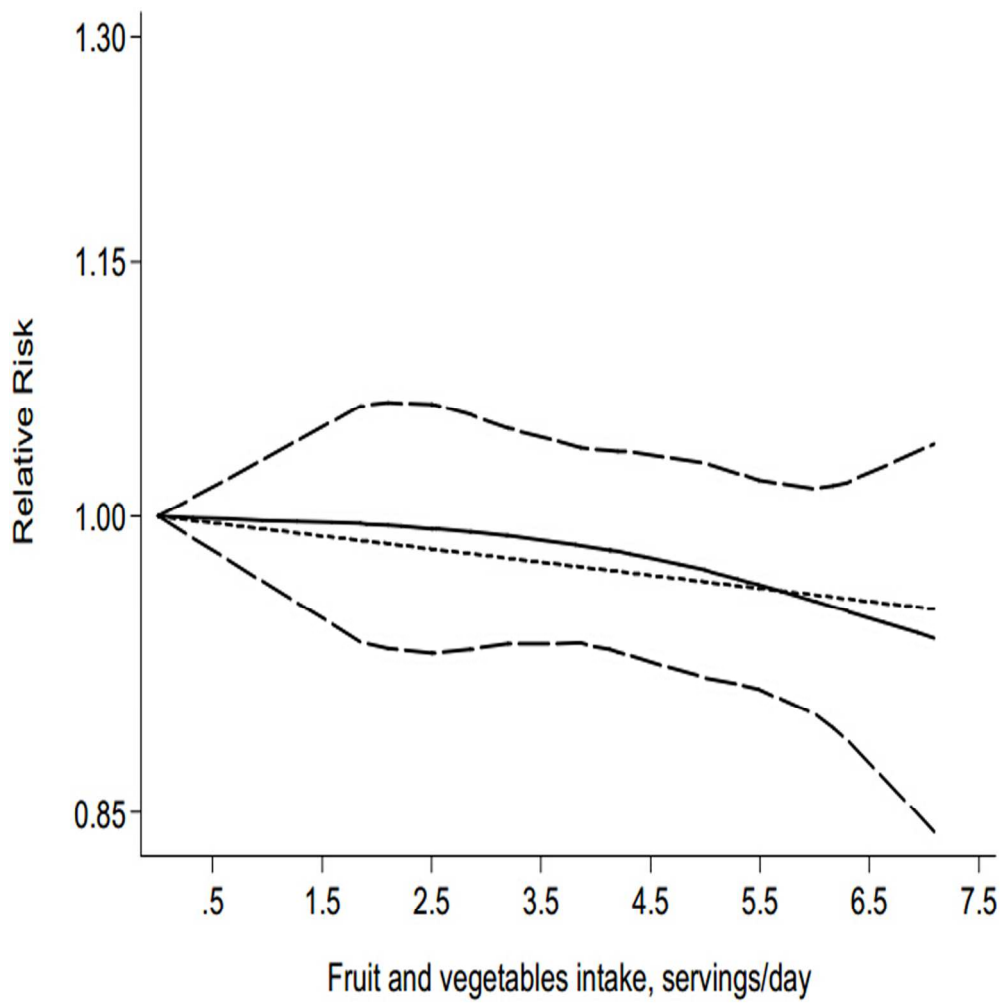
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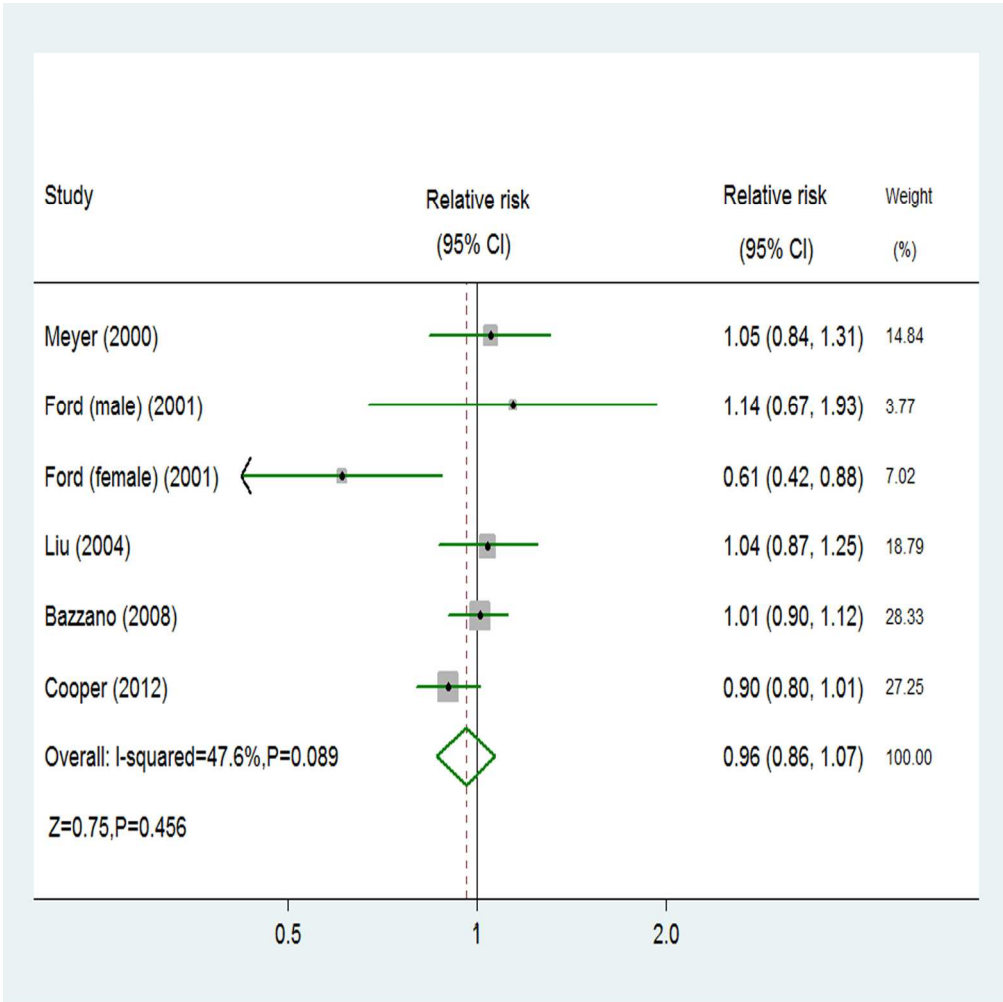
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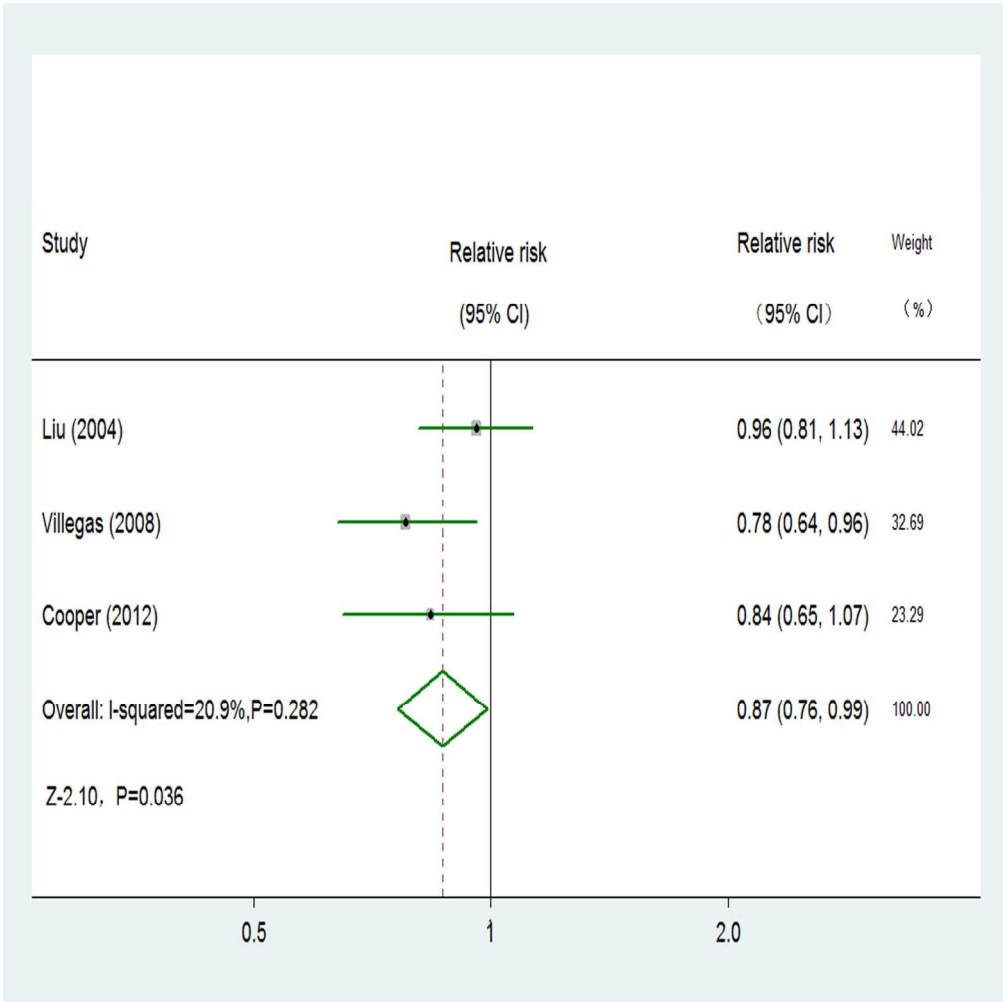
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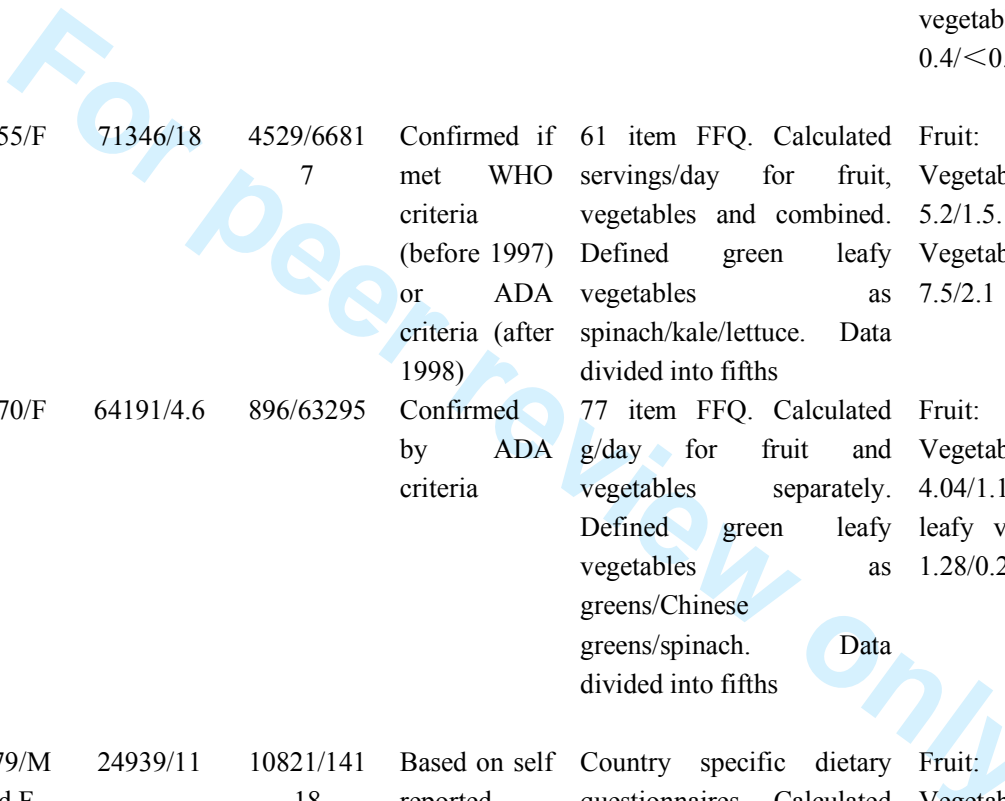
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Table A. Characteristics of included studies of fruit and vegetables intake in relation to incident type 2 diabetes

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/Iowa Women's Health Study	55-69/F	35988/6	1141/3484 7	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/NHA NES 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: $\geq 5/0$	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004, ⁴⁴	USA/Wom en's Health Study	$\geq 45/F$	38018/8.8	1614/3640 4	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/Fin nish	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

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		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 7	4529/6681 7	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	10821/141 18	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		
11	Cooper et al	England/England	40-79/M	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
12	(study	b) PIC-Norfolk	and F							
13	2012, ¹⁰									
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23	Muraki et al	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: $\geq 3 / < 0.57$	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
24	(cohort	a)								
25	2013, ¹¹									
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32	Muraki et al	USA/Health Professionals Follow-up Study	40-75/M	42504/12	1321/4118	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: $\geq 3 / < 0.57$	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use,	4
33	(cohort	b)								
34	2013, ¹¹									
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Kurotanik et al 2013, ⁷	Japan/JPH C 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/cbingensa i/leaf mustard/mugwort/chard/ko matsuna. 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	total energy intake 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
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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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Email: zytang07016@sina.com

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

Journal:	<i>BMJ Open</i>
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Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, Quality in health care < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Diabetes & endocrinology < INTERNAL MEDICINE

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4 **Fruit and vegetable intake and risk of type 2 diabetes mellitus:**
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6 **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

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

14 **Validity assessment**

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

28 **Data extraction**

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

44 **Statistical analysis**

45 Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and
46 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to
47 be a valid approximation of relative risks, we converted these values in every study by taking their
48 natural logarithms and calculating standard errors and corresponding 95% confidence intervals.
49 Relative risks and their standard errors were pooled with the DerSimonian and Laird random
50 effects model, which takes into account both within-study and between-study variabilities.¹⁶ When
51 some studies included in our meta-analysis used different measurement units (eg, grams per day or
52 portions per day or servings per day),^{5,10,15} we standardized fruit and vegetables intake into
53 servings per day using a standard portion size of 106 g.¹⁷ As different studies might use different
54 exposure categories (thirds, quarters, or fifths),^{7,11,15} we used the study specific relative risk for the
55 highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the
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meta-analysis. For the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker¹⁸ 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

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3 were free of self reported diabetes at baseline.^{5,7,10,11,15,43-47} In aggregate, the included studies
4 consisted of 434 342 participants. Among the participants, we documented 24 013 cases of T2D
5 occurred during follow-up periods ranging from 4.6 to 23 years (median of 11 years). Among 10
6 articles, five cohorts were conducted primarily in the United States,^{11,15,43,44,47} two articles were
7 done in Asian countries (China and Japan)^{7,46} and three cohorts were from European
8 countries.^{5,10,45} The number of participants ranged from 3704 in the EPIC-Norfolk study by
9 Cooper et al¹⁰ to 91 246 in the Nurses' Health Study II by Muraki et al.¹¹ Five studies included
10 both male and female,^{5,7,10,15,45} four studies included only female.^{43,44,46,47} One article by Muraki et
11 al reported two independent cohorts, one cohorts included only female, and another only male.¹¹
12 The age of participants ranged from 24 to 79 years. Six papers provided information on fruit and
13 vegetables intake separately and combined.^{5,7,10,43,44,47} two papers provided information on fruit
14 and vegetables intake separately,^{45,46} one paper provided only the combined data,¹⁵ and another
15 paper provided separate data on fruit.¹¹ Five papers also included separate data on the intake of
16 GLV.^{5,7,44-46} In most papers intake of fruit and vegetables was divided into fifths.^{11,43-47} All studies
17 provided adjusted risk estimates, results of study quality assessment (score 0-6) showed that most
18 studies yielded a score of 3 or below (low quality).

19 **Fruit intake and risk of T2D**

20 11 comparisons from nine studies reported an association between fruit intake and risk of T2D,
21 with 22 995 T2D outcomes and 424 677 participants. Overall, fruit intake was inversely
22 associated with risk (relative risk 0.93, 95% confidence interval 0.88 to 0.99) (fig 2). We saw no
23 heterogeneity among studies ($P=0.477$, $I^2=0\%$). Additionally, no evidence of substantial
24 publication bias was observed from the Begg ($P=0.533$) and Egger regression tests ($P=0.849$) (see
25 supplemental table B in appendix 1). Among 11 comparisons, seven comparisons were eligible for
26 the dose-response analysis of fruit intake and risk of T2D. Using a restricted cubic splines model,
27 we found a mild curvilinear association ($P=0.059$ for non-linearity, fig 3). Dose-response analysis
28 indicated that a 1 serving/day increment of fruit intake was associated with 6% lower risk of T2D
29 (relative risk 0.94, 95% confidence interval 0.89 to 1.00, $I^2=0\%$) (see supplemental fig A in
30 appendix 2).

31 **Vegetables intake and risk of T2D**

32 Eight studies reported an association between vegetables intake and risk of T2D, with 20 933
33 T2D outcomes and 290 927 participants. Using a random effects model summarizing all 9
34 comparisons, we found no association between vegetables intake and risk (relative risk 0.90, 95%
35 confidence interval 0.80 to 1.01) (fig 4). There was moderate study heterogeneity ($P=0.002$,
36 $I^2=66.5\%$). However, no evidence of substantial publication bias was observed from the Begg
37 ($P=0.602$) and Egger regression tests ($P=0.176$) (see supplemental table B in appendix 1). Among
38 9 comparisons, five comparisons were eligible for the trend estimation. Dose-response analysis
39 found no association with risk of T2D per 1 serving/day increment of vegetables intake (relative
40 risk 0.98, 95% confidence interval 0.89 to 1.08, $I^2=45.8\%$) (see supplemental fig B in appendix 2).
41 No publication bias was observed ($P=0.117$). We found no evidence of a curve linear association
42 between vegetables intake and risk ($P=0.671$ for non-linearity, see supplemental fig C in appendix
43 2).

44 **Fruit and vegetables intake and risk of T2D**

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

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

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

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

34 **Exploration of heterogeneity**

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

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48 Assessment methods of fruit, vegetables, or fruit and vegetables combined consumption differed
49 between the studies. Most epidemiological studies used the food frequency questionnaires (FFQs)
50 to assess quantity of fruit, vegetables, or fruit and vegetables combined intake.^{7,10,11,43,44,46,47} It is
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3 less suitable for the assessment of absolute intake, which they tend to overestimate.^{58,59} However,
4 two studies collected data via a single 24 hour recall and dietary history interviews,
5 respectively.^{15,45} These measurements may underestimate true associations between fruit,
6 vegetables, or fruit and vegetables combined consumption and risk of T2D. In addition,
7 calculations of daily consumption were differed (such as servings per week, servings per day, or
8 grams per day). Although we standardized primary data using a standard portion size of 106g,
9 conclusions should be interpreted with caution. Another possible explanation for the differences
10 between the studies might be the classification of food groups. GLVs' criteria was inconsistent:
11 three studies included spinach and lettuce; one included spinach and greens; others did not provide
12 specific description. If they were included with an uniform definition of each groups, the
13 associations might differ.

14 **Strengths and limitations**

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

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

36 **Conclusions**

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

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Data sharing: No additional data available.

27 28 **Figures Information**

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Figure 1. Process of literature search and study selection.

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

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

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

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

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

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53 54 **Appendix figure information**

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

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5 **Supplemental fig B. Forest plot of vegetables intake and risk of type 2 diabetes.**

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

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12 **Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus**
13 **lowest intake of fruit and vegetables and risk of type 2 diabetes.**

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

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22 **Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.**

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26 **Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.**

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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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4 **Fruit and vegetable intake and risk of type 2 diabetes mellitus:**
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6 **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

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

14 **Validity assessment**

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

29 **Data extraction**

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

45 **Statistical analysis**

46 Within each study, we used multivariate-adjusted outcome data (expressed as relative risks and
47 95% confidence intervals) for risk estimates. For the present analyses we assumed hazard ratios to
48 be a valid approximation of relative risks, we converted these values in every study by taking their
49 natural logarithms and calculating standard errors and corresponding 95% confidence intervals.
50 Relative risks and their standard errors were pooled with the DerSimonian and Laird random
51 effects model, which takes into account both within-study and between-study variabilities.¹⁶ When
52 some studies included in our meta-analysis used different measurement units (eg, grams per day or
53 portions per day or servings per day),^{5,10,15} we standardized fruit and vegetables intake into
54 servings per day using a standard portion size of 106 g.¹⁷ As different studies might use different
55 exposure categories (thirds, quarters, or fifths),^{7,11,15} we used the study specific relative risk for the
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3 highest versus lowest category of fruit, vegetables, or fruit and vegetables intake for the
4 meta-analysis. For the dose-response analysis, the generalized least square for trend estimation
5 method described by Greenland and Longnecker¹⁸ and Orsini et al^{19,20} was used to calculate
6 study-specific slopes (linear trends) and 95% confidence intervals. The method requires the
7 distributions of cases and person years for exposure categories, and median/mean of fruit,
8 vegetables, or fruit and vegetables intake levels for each comparison group. **We assigned the
9 midpoint of the upper and lower boundaries of each comparison group to determine mean fruit,
10 vegetables, or fruit and vegetables intake levels if the median or mean intake was not available.**²¹
11 When the highest category was open ended, we assumed that the average of the category was set
12 at 1.5 times the lower boundary. Additionally, we first created restricted cubic splines with 4 knots
13 at percentiles 5%, 35%, 65%, and 95% of the distribution.²² A P value for nonlinearity was
14 calculated by testing the null hypothesis that the coefficient of the fractional polynomials
15 component is equal to zero. Heterogeneity among studies was evaluated using the chi-square test
16 based on Cochran's Q test and I² statistic at P<0.10 level of significance,¹⁶ and quantification of
17 heterogeneity was made by the I² metric, which describes the percentage of total variation in point
18 estimates that is due to heterogeneity rather than chance.²³ We considered low, moderate, and high
19 degrees of heterogeneity to be I² values of 25%, 50%, and 75%, respectively. To explore possible
20 explanations for heterogeneity and to test the robustness of the association, we conducted
21 subgroup analyses based on the location (Asia v Non-Asia), the quality of the study (high quality
22 (≥4) v lower quality (<4), length of follow-up (≥10 years v <10 years), sex (male and female
23 included v female only v male only), fractions of intake (thirds, quarters, or fifths), number of
24 participants (≥50000 v <50000), and number of cases (≥1000 v <1000). We performed the
25 Begg rank correlation test and Egger's regression test to visualize a possible asymmetry.²⁴⁻²⁶
26 Funnel plots were also used to assess the publication bias. All the statistical analyses were
27 performed in Stata 12 (Stata Corp, College Station, TX). A threshold of P<0.1 was used to decide
28 whether heterogeneity or publication bias was present.²⁴ In other ways, P values were 2-sided and
29 P<0.05 was considered statistically significant.

37 Results

38 Literature search

39 **In total, the search strategy retrieved 673 unique articles (308 articles from PubMed and 365**
40 **articles from Embase) (Fig 1).** After exclusion of duplicate records and studies that did not fulfill
41 our inclusion criteria, 27 articles remained, and we further evaluated the full texts of these 27
42 publications. Of these, we excluded 17 studies as follows. Five articles were excluded owing to
43 lack of sufficient data for estimation of relative risks.²⁷⁻³¹ Five articles were excluded because no
44 original data could be extracted (review, type 1 diabetes, or cross sectional studies).³²⁻³⁶ Another
45 four articles were excluded because we deemed irrelevant.³⁷⁻⁴⁰ We also excluded three articles
46 because they did not give enough details on fruits, vegetables, or fruit and vegetables intake to
47 warrant inclusion within the meta-analysis.⁴¹⁻⁴³ In addition, three articles with the same
48 participants involved Nurses Health Study (Colditz et al 1992, Bazzano et al 2008, and Muraki et
49 al 2013). According to the study selection criteria, we included the study by Bazzano et al, which
50 followed for 18 years.⁴⁴ Finally, eleven articles met the inclusion criteria and were included in the
51 meta-analysis.^{5,7,10,11,15,44-48} Among these ten articles, Ford et al and Kurotani et al study examined
52 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 al¹⁰ 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 reported 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

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3 between vegetables intake and risk ($P=0.671$ for non-linearity, see supplemental fig C in appendix
4 2).

5 **Fruit and vegetables intake and risk of T2D**

6 Information on fruit and vegetables intake and T2D were available in 9 comparisons from seven
7 prospective studies, totalling 20 672 T2D outcomes and 232 097 participants. Overall, fruit and
8 vegetables intake was not associated with risk (relative risk 0.94, 95% confidence interval 0.86 to
9 1.03) (see supplemental fig D in appendix 2). We saw no heterogeneity among studies ($P=0.141$,
10 $I^2=34.6\%$). Additionally, no evidence of substantial publication bias was observed from the Begg
11 ($P=0.348$) and Egger regression tests ($P=0.609$) (see supplemental table B in appendix 1). Among
12 9 comparisons, six comparisons were eligible for the dose-response analysis of fruit and
13 vegetables intake and risk of T2D. We did not find a significant curvilinear association ($P=0.456$
14 for non-linearity, see supplemental fig E in appendix 2). Dose-response analysis indicated that a 1
15 serving/day increment of fruit and vegetables intake (relative risk 0.96, 95% confidence interval
16 0.86 to 1.07, $I^2=47.6\%$) (see supplemental fig F in appendix 2).

17 **GLV intake and risk of T2D**

18 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
20 with risk (relative risk 0.87, 95% confidence interval 0.81 to 0.93) (fig 5). No significant
21 heterogeneity was detected among studies ($P=0.496$, $I^2=0\%$). Additionally, we did not observe
22 evidence of substantial publication bias (the Begg and Egger regression tests, $P=0.133$ and
23 $P=0.101$, respectively) (see supplemental table B in appendix 1). Among 7 comparisons, only
24 three comparisons were eligible for the trend estimation. Using a restricted cubic splines model,
25 we found a significant curvilinear association ($P=0.036$ for non-linearity, fig 6). Dose-response
26 analysis indicated that a 0.2 serving/day increment of GLV intake was associated with 13% lower
27 risk of T2D (relative risk 0.87, 95% confidence interval 0.76 to 0.99, $I^2=20.9\%$) (see supplemental
28 fig G in appendix 2). No publication bias was observed ($P=0.282$).

29 **Subgroup analyses**

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

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

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3 heterogeneity, in interpreting the results, several differences between the studies are worth
4 discussing.

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

22 **Strengths and limitations**

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

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

49 **Conclusions**

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

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

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

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

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

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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.

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

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6 **Supplemental fig D. Random effects analysis of fully adjusted studies for highest versus**
7 **lowest intake of fruit and vegetables and risk of type 2 diabetes.**

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

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16 **Supplemental fig F. Forest plot of fruit and vegetables intake and risk of type 2 diabetes.**

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20 **Supplemental fig G. Forest plot of green leafy vegetables intake and risk of type 2 diabetes.**

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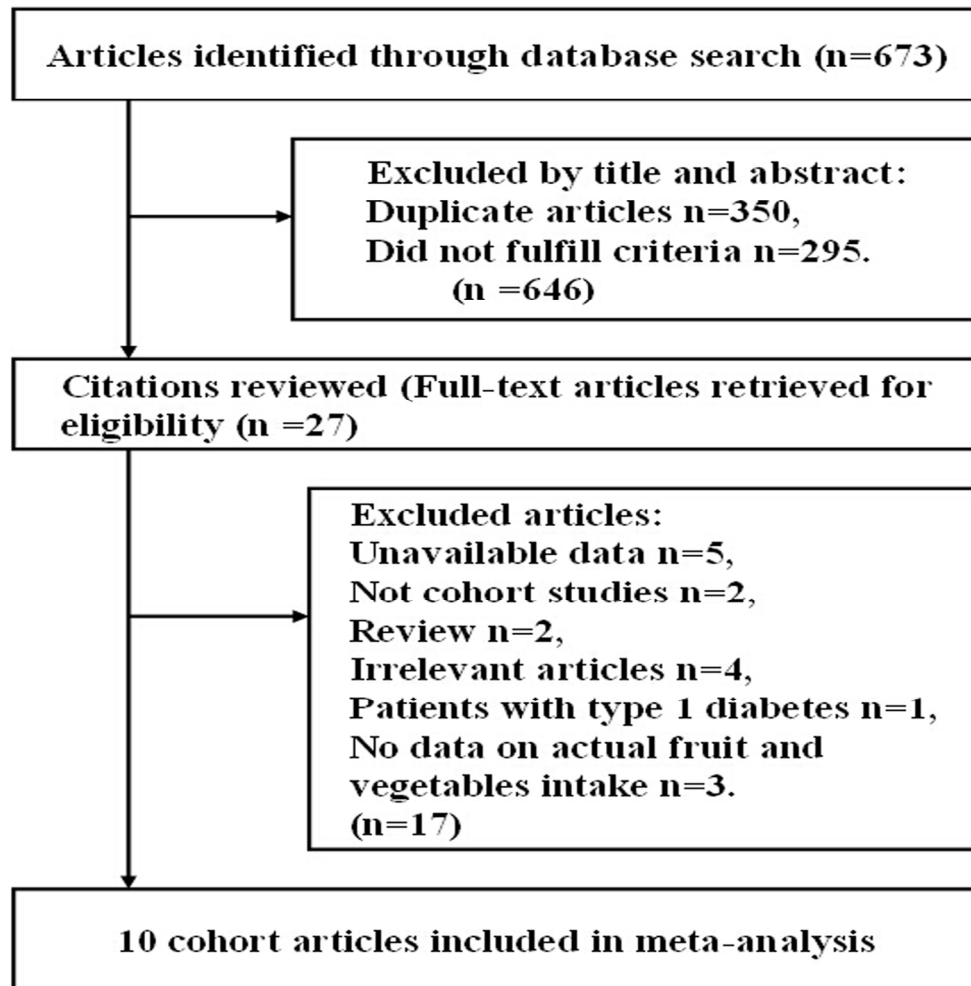
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60 61 Li M, Hou W, Zhang X, Hu L, Tang Z. Hyperuricemia and risk of stroke: a systematic review
and meta-analysis of prospective studies. *Atherosclerosis* 2014;232:265-70.

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

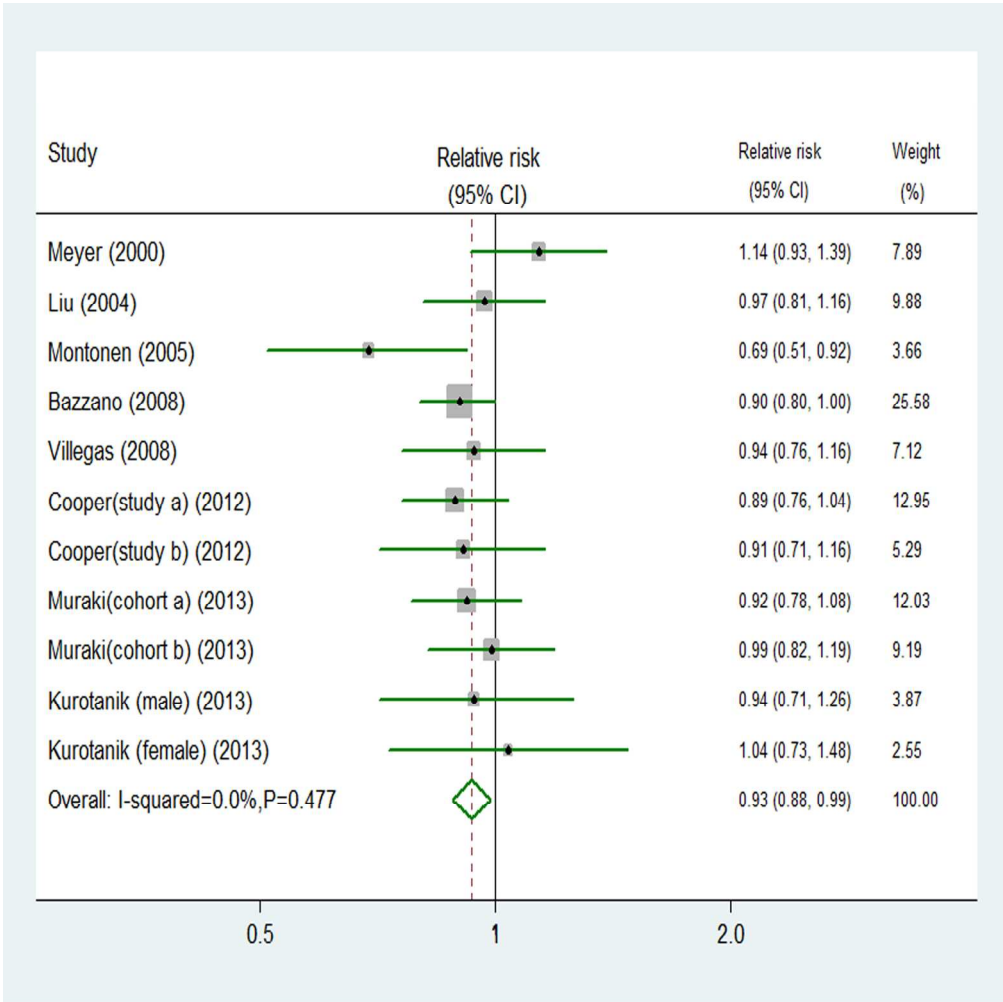
M=male, F=female, RR=relative risk.



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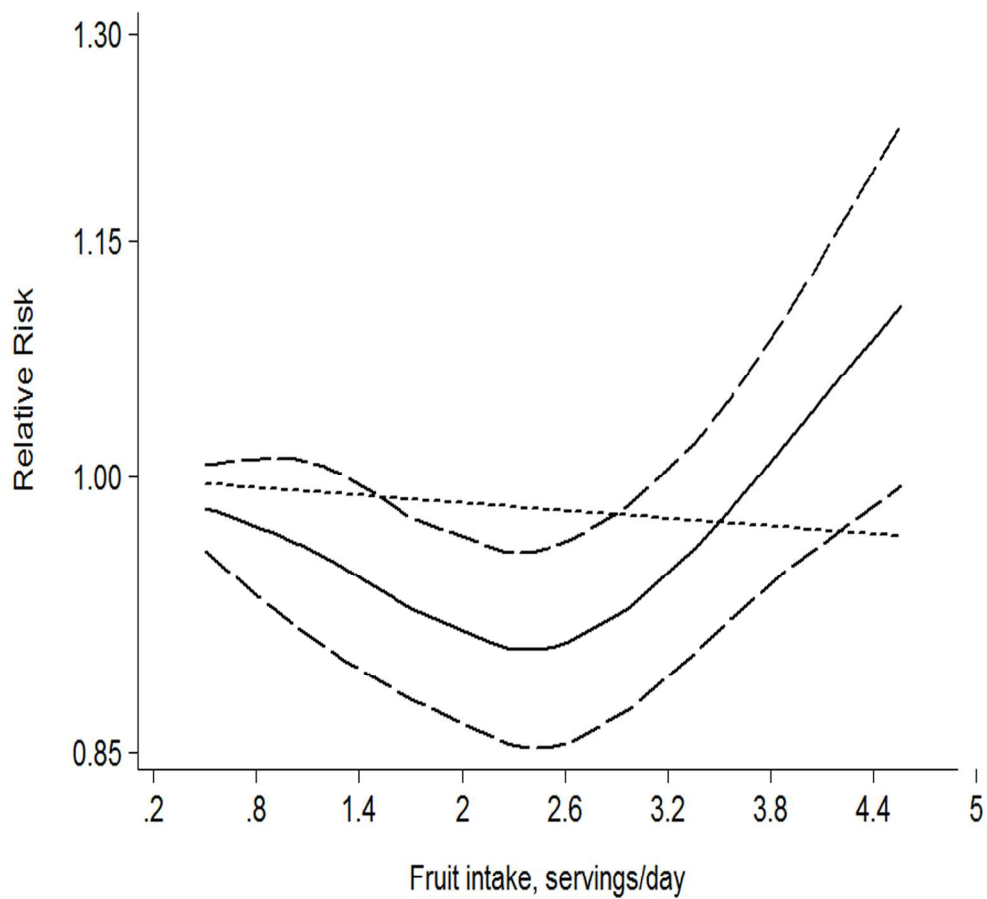
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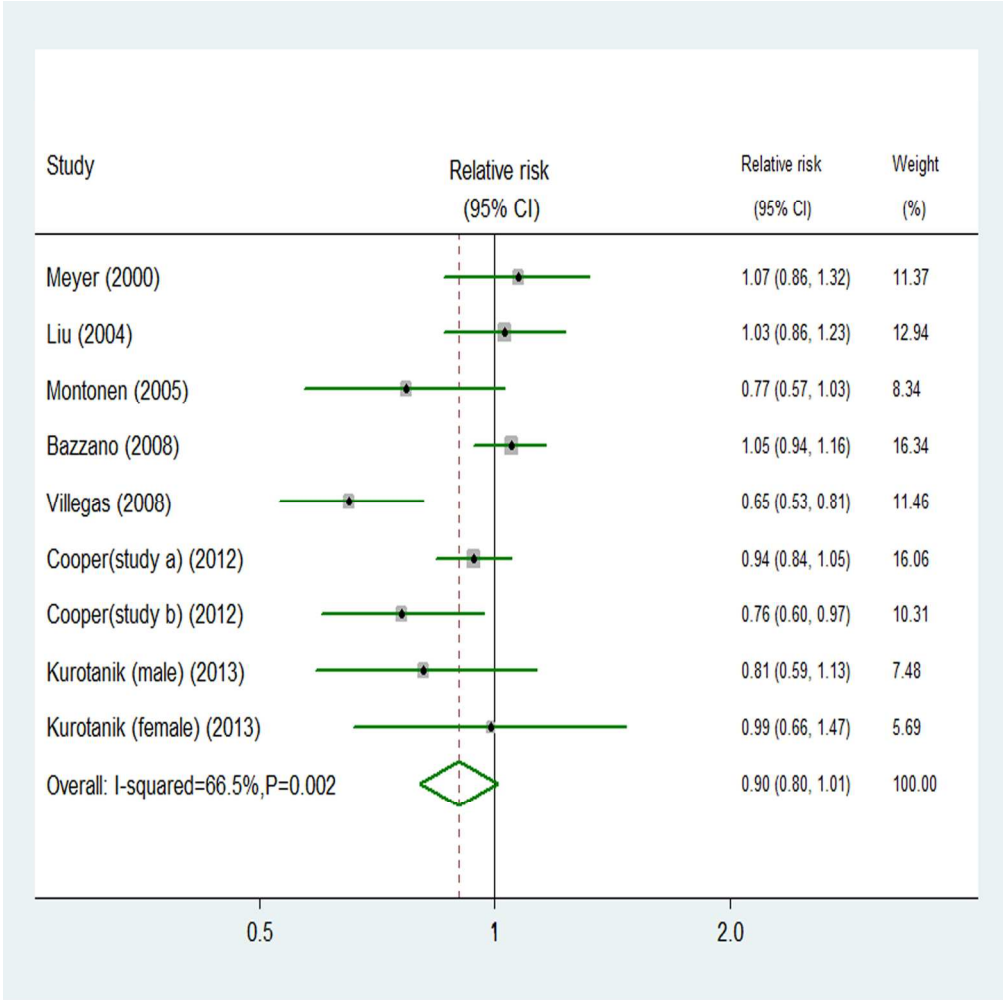




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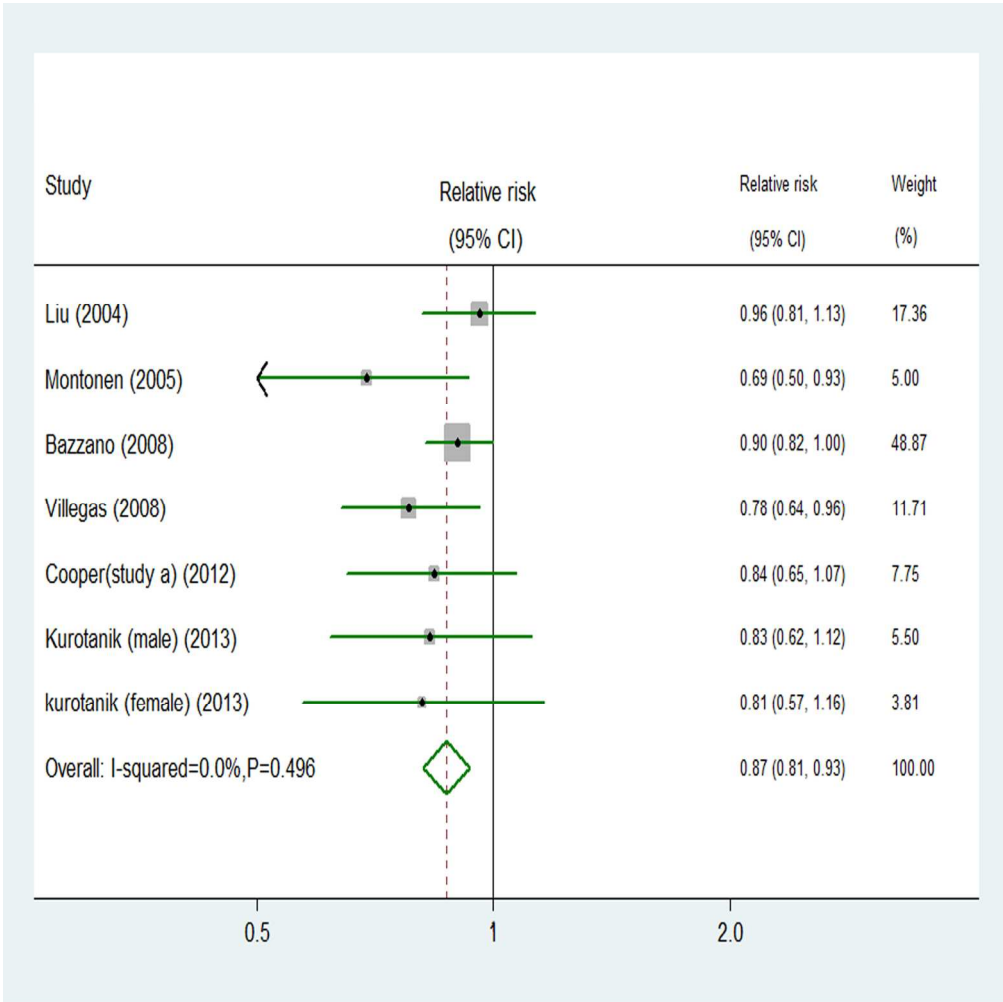
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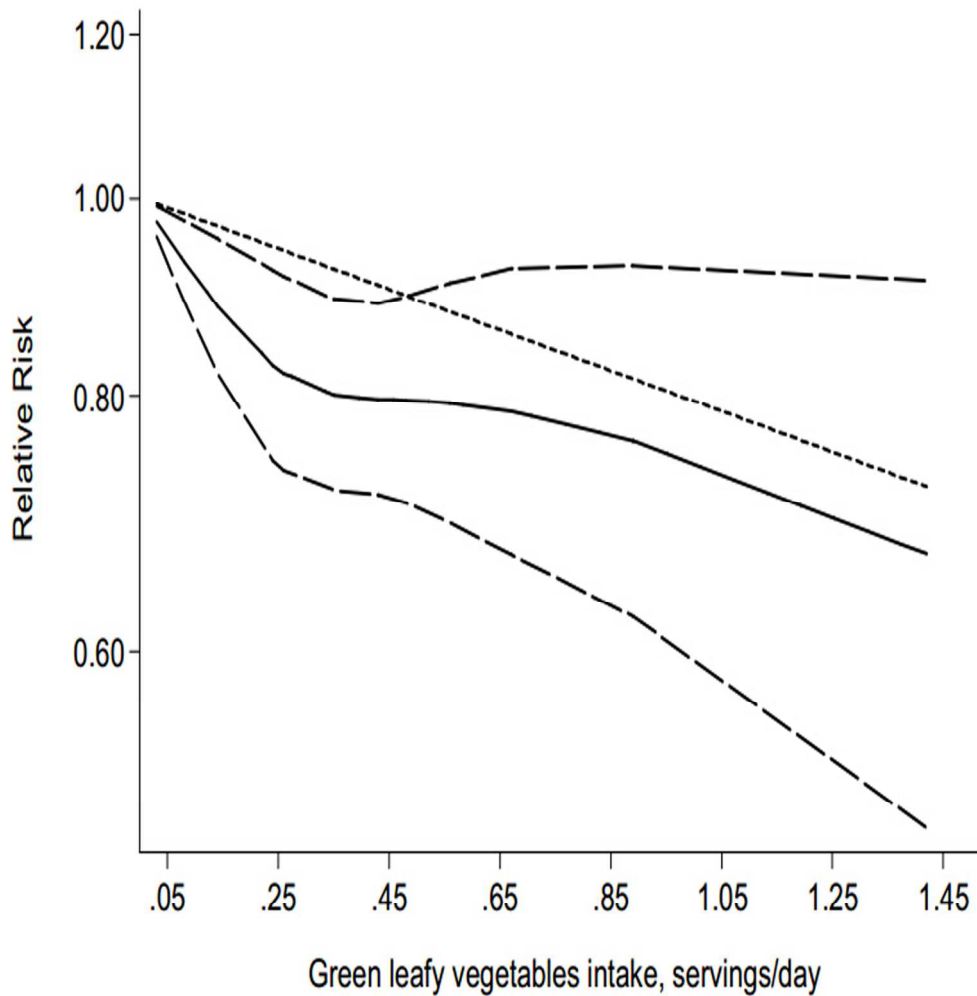
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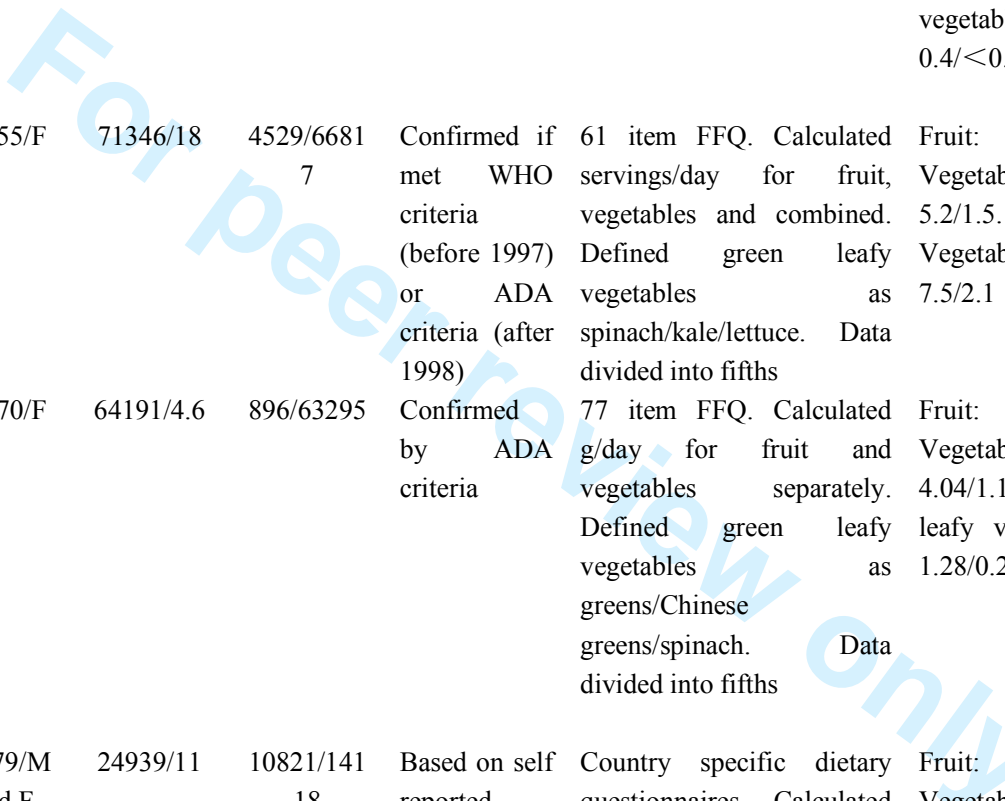
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Table A. Characteristics of included studies of fruit and vegetables intake in relation to incident type 2 diabetes

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	1141/3484 7	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/NHA NES 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: $\geq 5/0$	Age, BMI, smoking, SBP, cholesterol, antihypertensive medication, exercise, alcohol, education, ethnicity	1
Liu et al 2004, ⁴⁵	USA/Wom en's Health Study	$\geq 45/F$	38018/8.8	1614/3640 4	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/Fin nish	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

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insurance and vegetables separately. > 1.23/ < 0.4. family history of register Data divided into fifths Green leafy diabetes, geographic vegetables: > area 0.4/<0.1

Bazzano et al 2008, ⁴³	USA/Nurses' Health Study	30-55/F	71346/187	4529/6681	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		
11	Cooper et al	England/England	40-79/M	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
12	(study	b) PIC-Norfolk	and F							
13	2012, ¹⁰									
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23	Muraki et al	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: $\geq 3 / < 0.57$	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use, total energy intake	4
24	(cohort	a)								
25	2013, ¹¹									
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32	Muraki et al	USA/Health Professionals Follow-up Study	40-75/M	42504/12	1321/4118	Confirmed by WHO criteria (before 1997)	131 item FFQ. Calculated servings/month or servings/week for fruit. Data divided into fifths	Fruit: $\geq 3 / < 0.57$	Age, BMI, ethnicity, smoking, multivitamin use, physical activity, family history of diabetes, hormone use, oral contraceptive use,	4
33	(cohort	b)								
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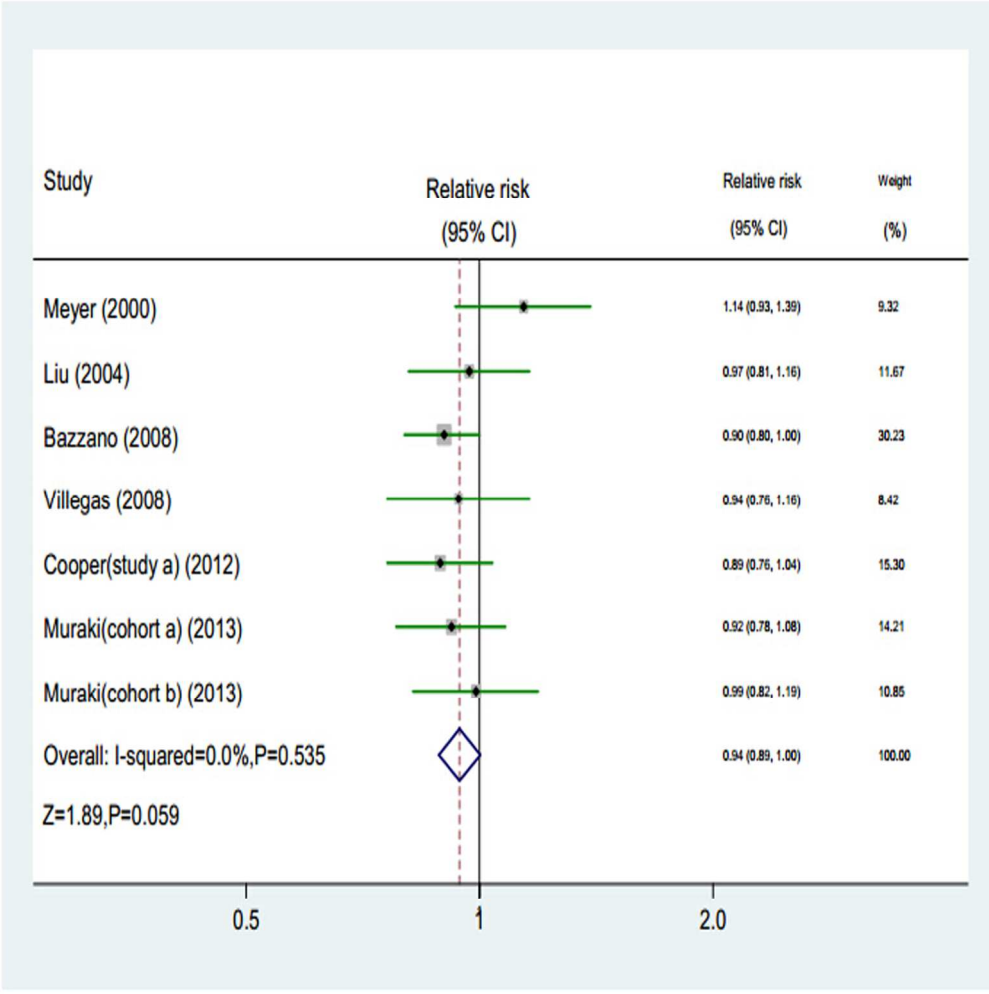
Kurotanik et al 2013, ⁷	Japan/JPH C Study	40-69/M and F	48437/5	896/47541	Based on self reported	147 item FFQ. Calculated g/day for fruit, vegetables and combined. Defined green leafy vegetables as spinach/Chinese chives/garland chrysanthemums/cbingensa i/leaf mustard/mugwort/chard/ko matsuna. Data divided 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	total energy intake 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
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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

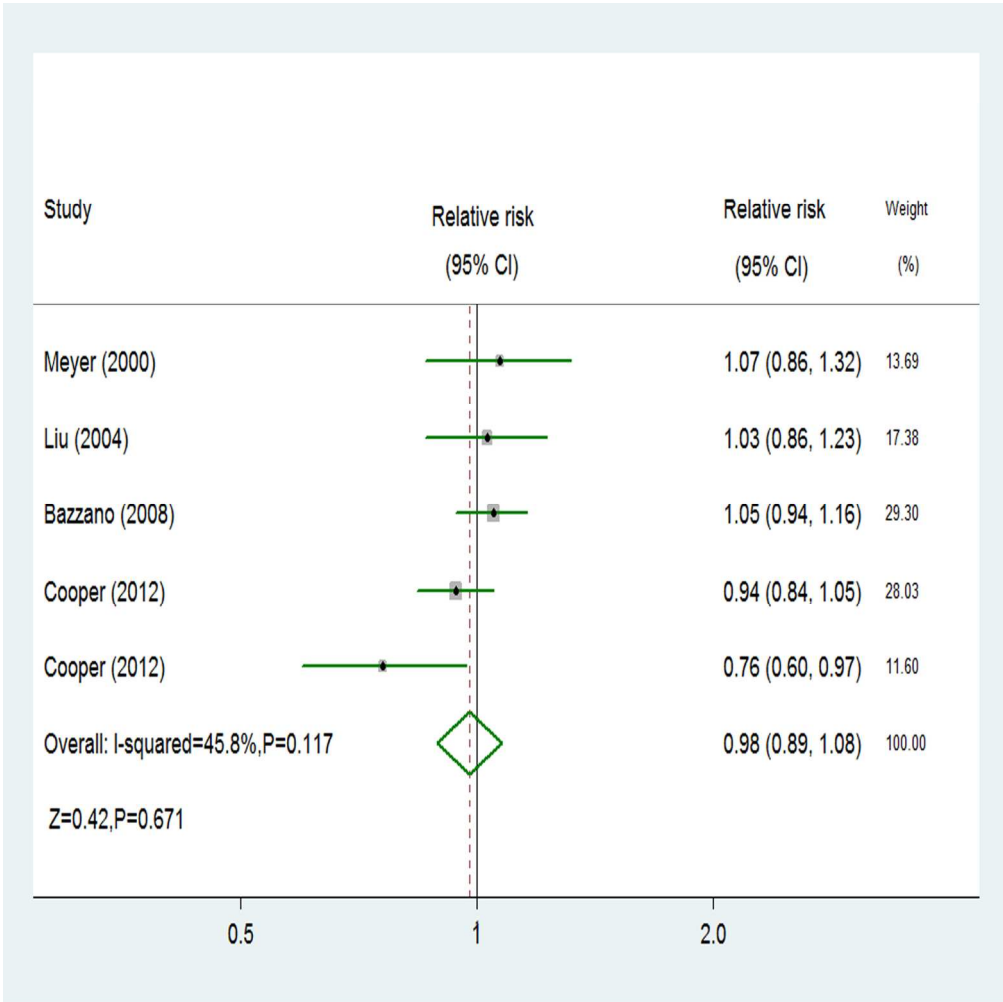
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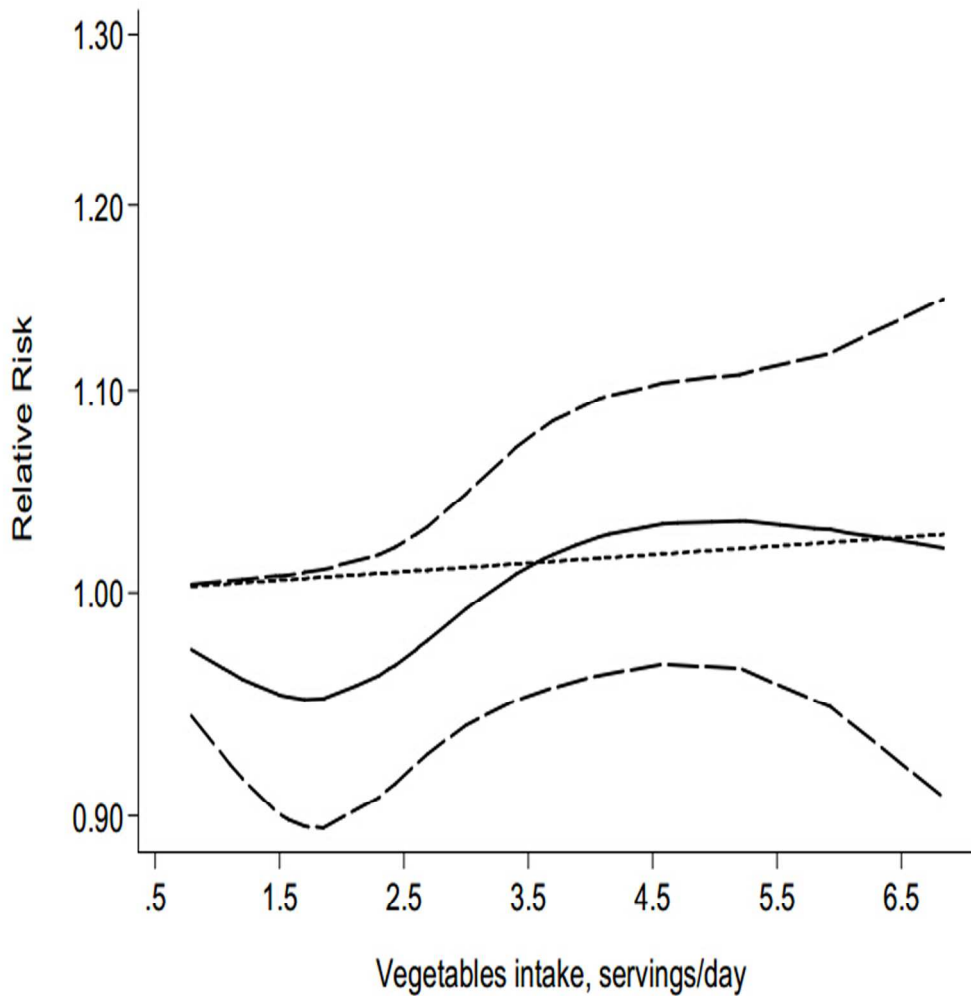
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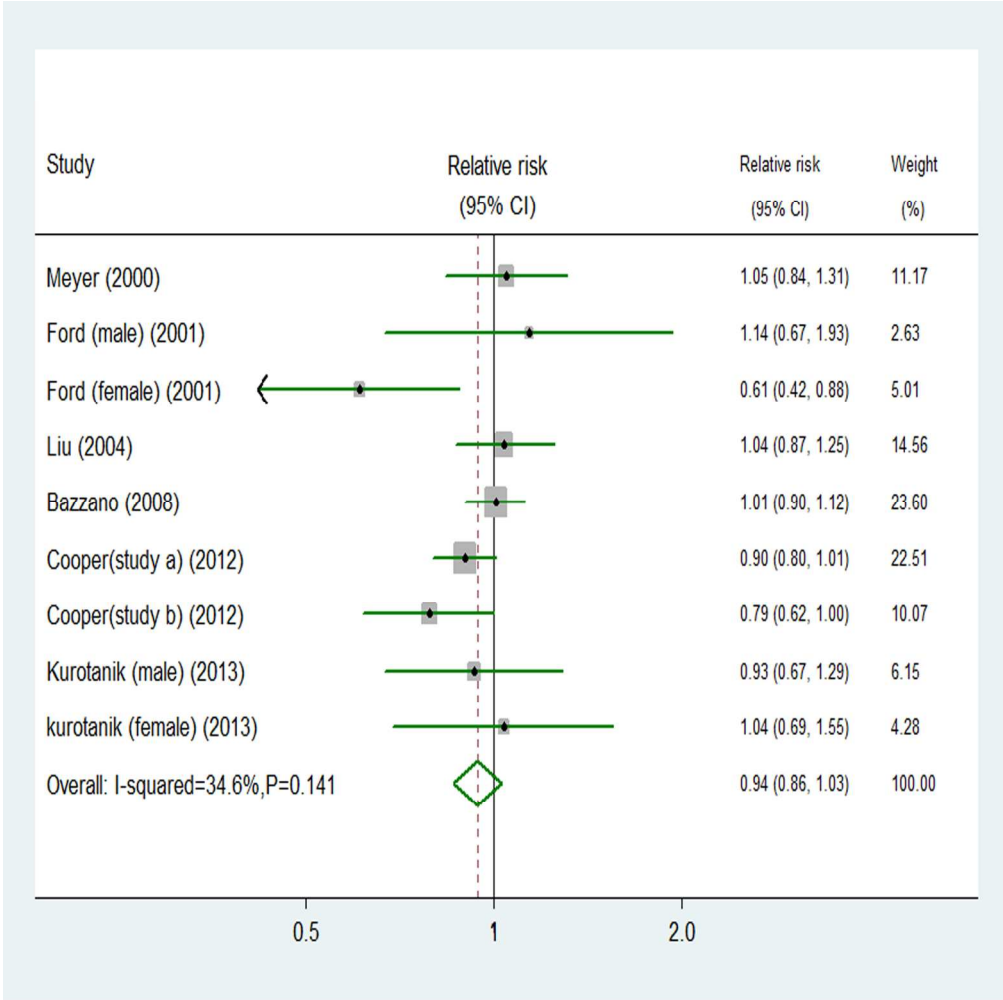
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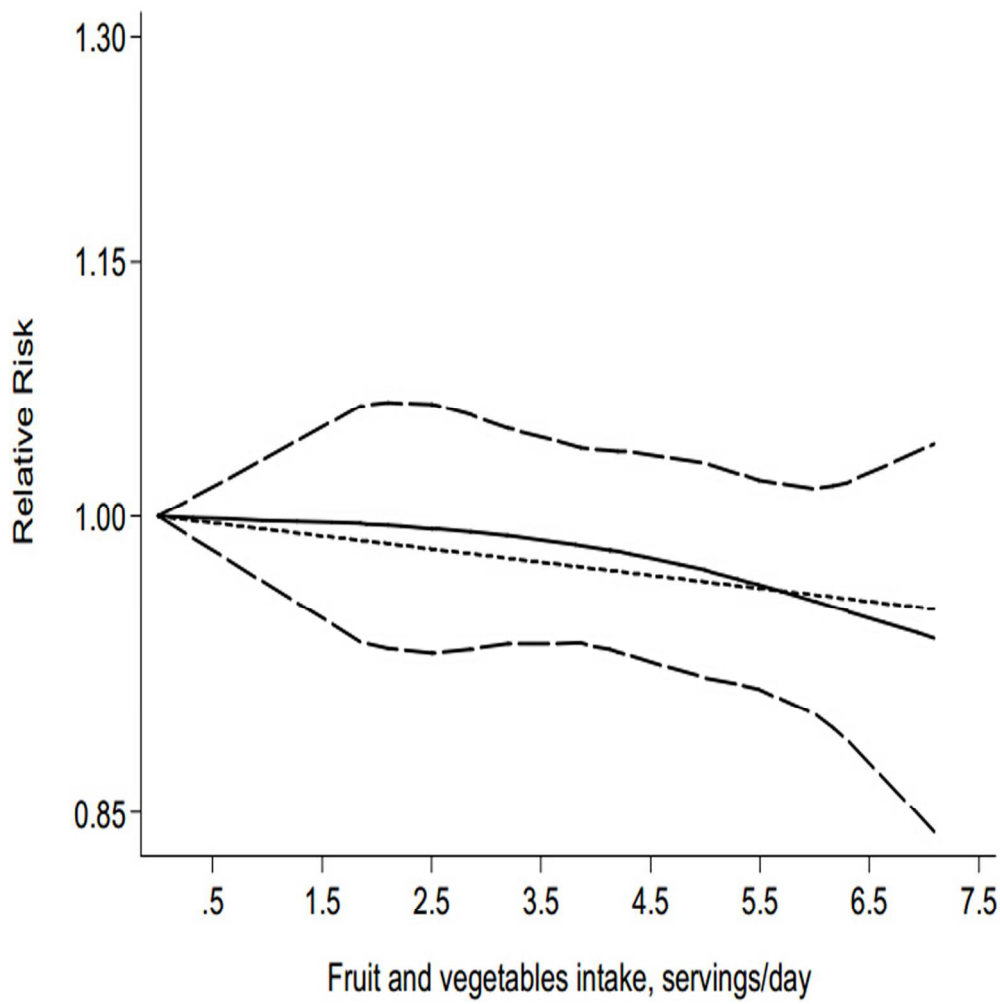
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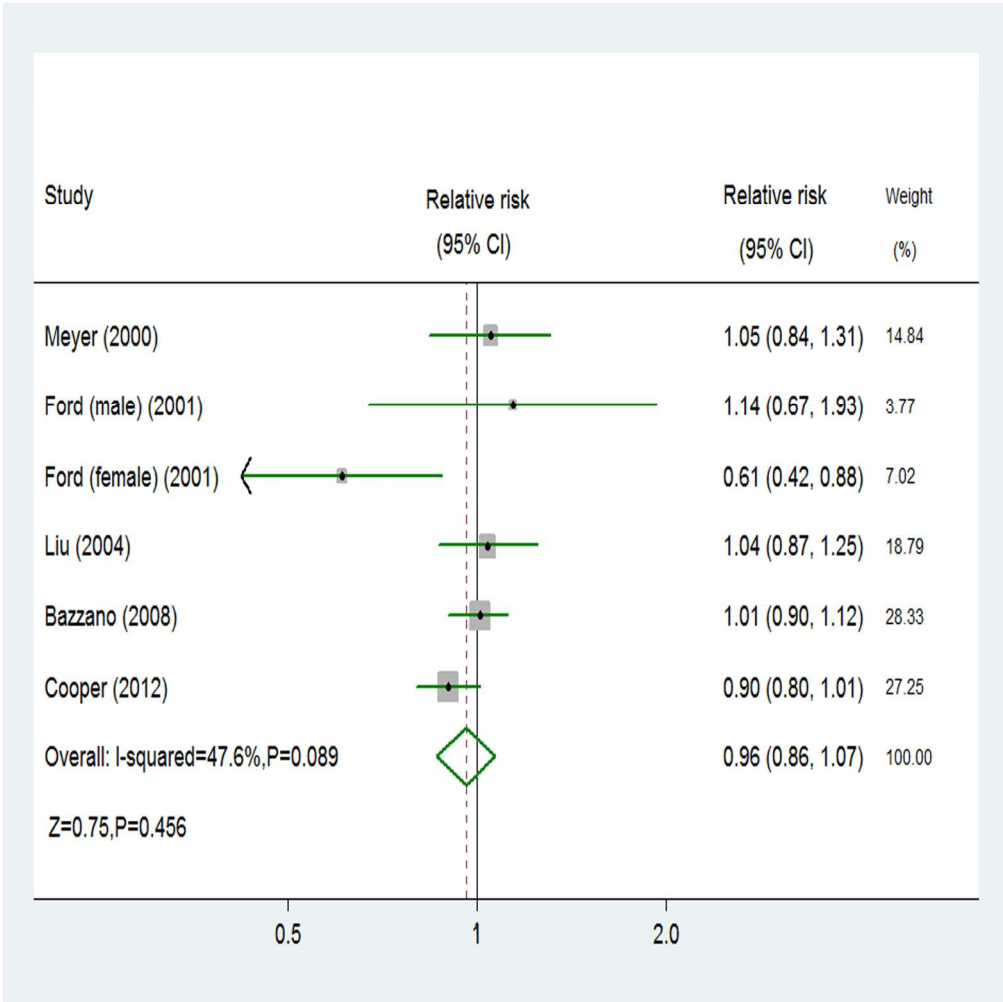
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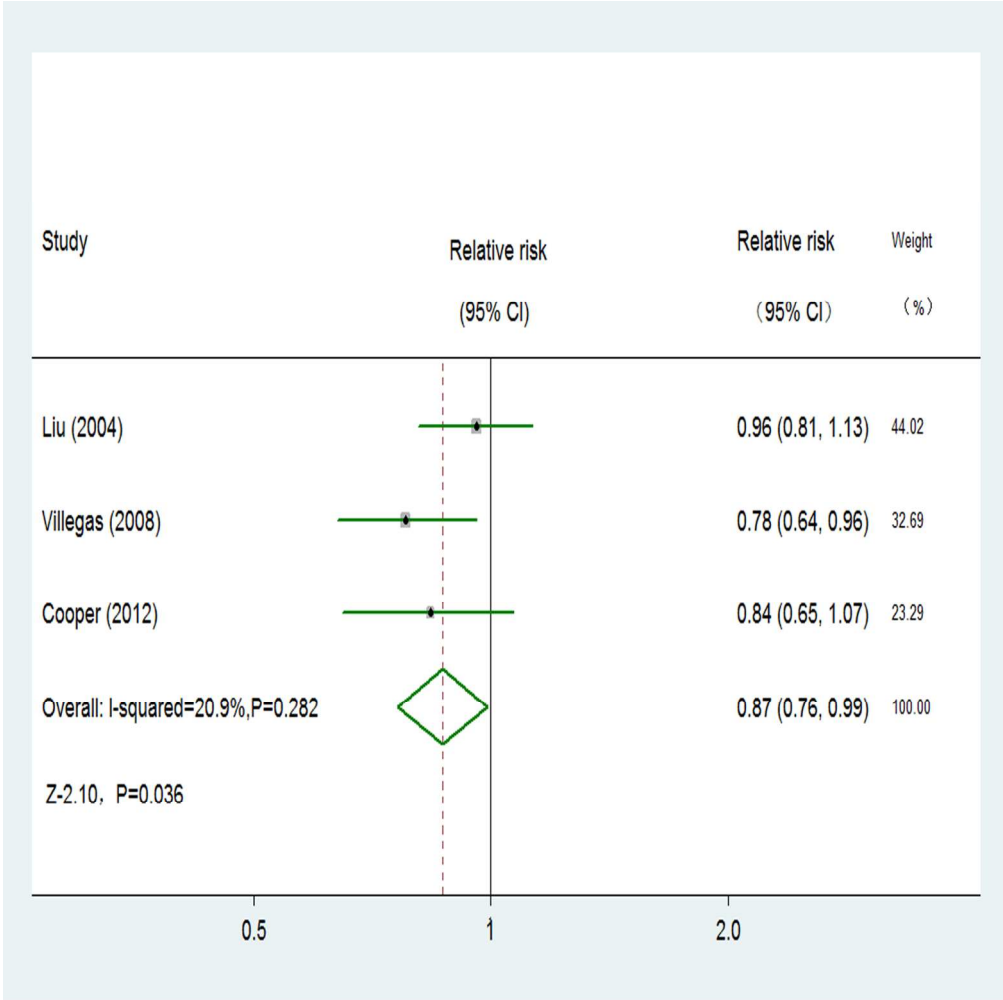
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MOOSE Checklist

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

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Wenshang Hou *master student*¹, Zhenyu Tang *associate professor*¹

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of China

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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.

1	√	Provision of appropriate tables and graphics	We included 1 flow chart, several summary tables and figures.
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6		Reporting of results should include	
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8	√	Graph summarizing individual study estimates and overall estimate	Figure 2, 4, 5 and D
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12	√	Table giving descriptive information for each study included	Table A
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15	√	Results of sensitivity testing	Table 1
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18	√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
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21		Reporting of discussion should include	
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24	√	Quantitative assessment of bias	Subgroup analyses indicate heterogeneity in strengths of the association due to most common biases in cohort studies.
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27	√	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.
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32	√	Assessment of quality of included studies	We discussed the results of the subgroup analyses, and potential reasons for the observed heterogeneity.
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34		Reporting of conclusions should include	
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37	√	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.
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45	√	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.
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51	√	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.
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55	√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.
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