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Fruits and Vegetables Intake and Risk of Bladder Cancer

A PRISMA-Compliant Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies

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Abstract: Clinical practice recommends eating ≥2.5 cups of fruits and vegetables (FVs) each day for cancer prevention, in which the evidence from epidemiological studies for the association between FVs intake and bladder cancer (BC) prevention is inconsistent.

We searched the PubMed, Embase, and Willy online Library for relevant studies published up to September 27, 2014. Prospective cohort studies investigated FVs intake, and the risk of BC with ≥3 categories of exposure was included. A dose-response meta-analysis was carried out to evaluate the association between FVs intake and risk of BC.

Fourteen cohorts with 17 studies including 9447 cases were identified. No evidence of nonlinear association was examined between FVs intake and risk of BC. The summarized relevant risk (RR) of every 0.2 serving increment a day was 1.00 (95%CI: 0.99, 1.00; $P=0.17$; $I^2=41.7\%$; $n=14$) for total fruits; 0.99 (95%CI: 0.96, 1.01; $P=0.28$; $I^2=37.0\%$; $n=13$) for total vegetables; and 0.99 (95%CI: 0.97, 1.01; $P=0.24$; $I^2=57.5\%$; $n=8$) for both FVs. In further analysis, we observed inverse association between every 0.2 serving increment of green leafy vegetables intake a day and risk of BC (RR = 0.98, 95%CI: 0.96, 0.99; $I^2=0.0\%$; $P<0.01$; Power = 0.76; $n=6$), but neither for cruciferous vegetables (RR = 0.97, 95%CI: 0.93, 1.01; $P=0.19$; $I^2=55.8\%$; $n=8$) nor for citrus (RR = 1.00, 95%CI: 1.00, 1.00; $P=0.83$; $I^2=0.0\%$; $n=7$). Subgroup analysis showed consistent results.

Little evidence supports a beneficial effect for total fruits, vegetables, both FVs, and citrus intake against bladder cancer. Green leafy vegetables may help prevent bladder cancer.

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Abbreviations: BC = bladder cancer, CI = confidence intervals, FVs = fruits and vegetables, RCS = restricted cubic splines, RRs = relevant risks.

INTRODUCTION

Bladder cancer (BC) is the most common malignancy in the urinary tract.[1] The incidence rate (age standardized) is about 9 per 10,000 for men and 2.2 for women worldwide.[2] According to the newest data of International Agency for Research on Cancer, BC has reached to the 9th most common cancer all over the world (the 6th in men and the 19th in women).[2]

As the base layer of the food pyramid, fruits and vegetables (FVs) contain many vitamins, fibers, minerals, and other bioactive compounds may be beneficial for cancer prevention.[3] Previous randomized controlled trials have suggested improved immune function, enhanced antioxidant status, and reduced oxidative DNA damage for people with high FVs diet.[4–6] Evidence from meta-analysis also showed reduced risk of specified cancers (such as renal cancer) in the high FVs intake population.[7,8] The American Cancer Society recommended eating ≥2.5 cups of vegetables and fruits each day for cancer prevention.[9] However, whether FVs intake can help BC prevention is unknown.

Recently, 2 meta-analyses of observational studies concluded that intake of FVs were associated with reduced risk of BC.[10,11] Another meta-analysis[12] based on cohort studies detected no associations between FVs intake and risk of BC. However, neither the observational-study-based nor the cohort-study-based meta-analysis performed a sufficient literature search. Moreover, the result of observational-study-based meta-analysis may be confused by the recall bias of case–controls studies,[13] and the methodology of the cohort-study-based meta-analysis showed some limitations, such as lack of interactions and sensitivity analysis. Thus, the relationship between FVs intake and BC, to data, still remains controversy.

We designed a more rigorous systematic review and dose-response meta-analysis based on prospective cohort studies to investigate the association between FVs intake and risk of BC.

MATERIALS AND METHODS

Our meta-analysis was designed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA Compliant) statement.[14] There are no ethical issues involved in our study for our data were based on published studies.

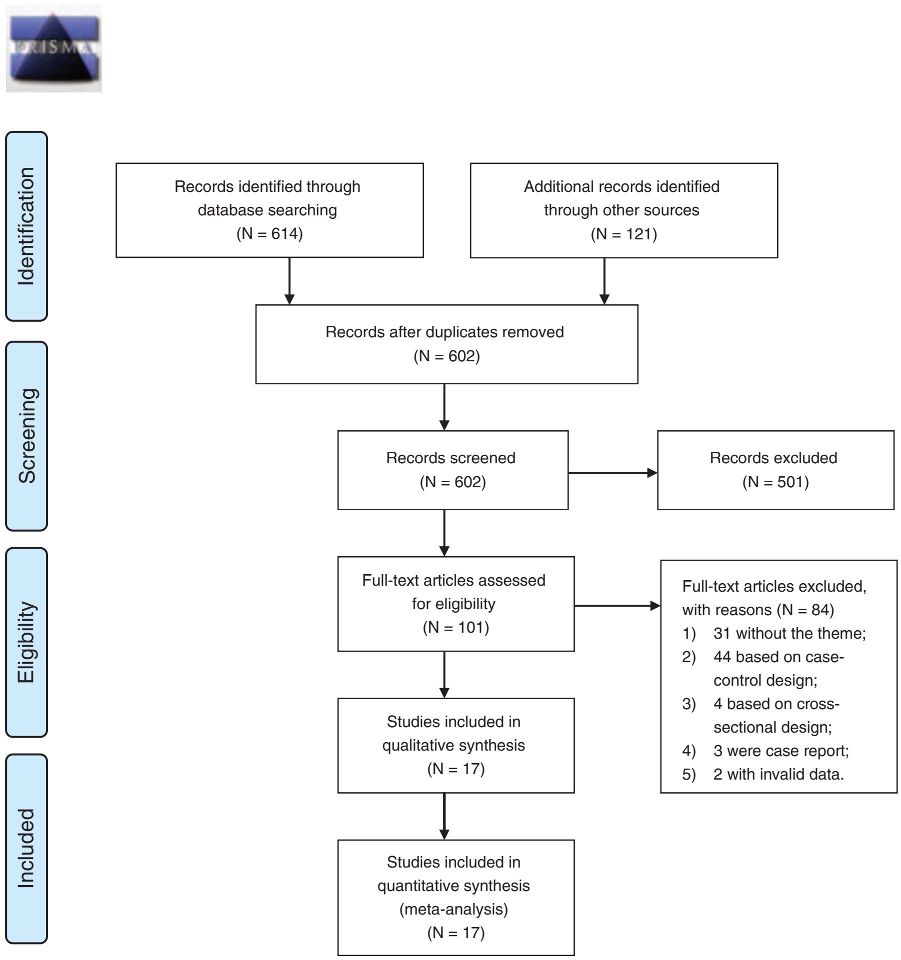

FIGURE 1. The flow diagram of the literature inclusion.

Literature Search

We searched the PubMed, Embase, and Willy online Library for relevant studies published up to September 27, 2014. The free text words "bladder neoplasm," "bladder tumor," "bladder cancer," "bladder carcinoma," "urothelium carcinoma," "transitional cell carcinoma" and "fruit," "vegetable," "cruciferae," "citrus" were used for the search without any restriction (see Table, Supplemental Digital Content 1, http://links.lww.com/MD/A258, which demonstrates the search details). We also checked the reference list of related studies, reviews or meta-analyses.

Eligibility Criteria

Publications that based on prospective cohort, case–cohort, or nested case–control design were only considered in present meta-analysis. The interested exposure was any type of FVs that with $\geq$3 quantitative exposure levels, for dose-response meta-analysis with restricted cubic splines (RCS) required $\geq$3 categories.[15,16] Given that there were various kinds of FVs, we only focused on those investigated by $\geq$6 cohorts. For outcomes, primary BC or urothelial (transitional cell) carcinoma were permitted. Because a small number of urothelial carcinoma (<10%) was not originating in bladder,[17] sensitivity analysis was used to see if this influences the results. Moreover, studies should report the case/noncase numbers, serving size, relative risk, and relevant 95% confidence intervals (CI) in each category. If not reported, such information should be calculated by the raw data or obtained by the authors. Grey literatures or meeting abstracts were not included.

We totally identified 3 types of exposure including total fruits (n = 14), vegetables (n = 13), both FVs (n = 8), and 3 subtypes of exposure including citrus (n = 7), green leafy vegetables (n = 6), and cruciferous vegetables (n = 8) that met our criteria.

Data Collection and Items

We used the relevant risks (RRs) to measure the association between FVs and BC. A standardized data collection sheet was designed before the extraction. Two reviewers, then, separately extracted the basic information (first author's name, publication year, country, populations, age distribution at entry, and follow-up years), interested data (type of FVs, numbers of cases and noncases or person-years, serving size, adjusted, or crude RRs with 95% CI in each category), and adjusted variables. When different types of adjusted RRs were presented, we extracted the one that controlled for the most confounders.[18] Crude RRs were only extracted when no other one were given.[18] If multiple measurements of FVs intake were reported, such as gram,

TABLE 1. Main Characteristic of 17 Included Cohort Studies

Author	Population	Follow-up (y)	Age at Entry, (y)	Men, Rate	Csmoker, rate	Case, n (CIR*)	Main Exposure	Outcomes (Cancer Site)	Diagnose	Main Findings (Highest vs. Lowest)
Büchner et al,[39] 2009	Europeans	8.7	51	30.0%	22.0%	1015 (6.8)	Fruit, vegetables, and both	Bladder cancer	84% Were histologically verified	Fruit: 1.04 (0.82, 1.31) Vegetable: 0.90 (0.70, 1.16) Both: 0.93 (0.72, 1.19)
Chyou et al,[40] 1993	American men of Japanese ancestry	22	Not report	100.0%	43.7%	96 (5.5)	Fruit, fried vegetables	Bladder (83), renal pelvis (8), ureter (5) cancer	100% Were tissue confirmed	Fruit: 0.63(0.37, 1.08) Vegetable: 2.56 (0.63, 10.4) –
George et al,[41] 2009	AARP members from 6 US states	6.9	50 to 71	59.6%	15.7%	1664 (5.0)	Fruit, vegetables	Bladder cancer	90% Were certified by the Central Cancer Registries	Fruit: 1.52 (1.00, 2.33)^{F} Fruit: 0.90 (0.75, 1.08)^{M} Vegetable: 1.07 (0.71, 1.60)^{F} Vegetable: 0.92 (0.77, 1.09)^{M}
Grant et al,[42] 2012	Atomic bomb survivors of Japan	10 year for fruit, vegetable	52.5	40.7%	55.7%	573 (2.0)	Fruit, vegetables	Urothelial carcinoma	Tumor registries	Fruit: 0.81 (0.58, 1.14) Vegetable: 0.75 (0.51, 1.10) –
Holick et al,[43] 2005	Registered US female nurses	20	30 to 55	0%	28.8%	237 (1.4)	Fruit, vegetables, and both	Bladder cancer	87% Were medical record verified	Fruit: 0.95 (0.62, 1.46) Vegetable: 1.29 (0.87, 1.91) Both: 1.08 (0.70, 1.65)
Larsson et al,[44] 2008	Resident in central Sweden	9.4	39 to 86	55.3%	24.2%	485 (6.3)	Fruit, vegetables, and both	Bladder cancer	National Swedish Cancer Register	Fruit: 0.93 (0.69, 1.25) Vegetable: 0.89 (0.67, 1.19) Both: 0.80 (0.60, 1.05)
Li et al,[45] 2010	Residents in Nagasaki of Japan	9	40 to 79	47.6%	24.2%	3398 (105.1)	Fruit (citrus)	Bladder cancer	Medical history	Fruit: 0.93 (0.69, 1.25) Vegetable: 0.89 (0.67, 1.19) Both: 0.80 (0.60, 1.05)
Michaud et al,[46] 1999	Health professionals	9.1	40 to 75	100.0%	About 20%	252 (5.8)	Fruit, vegetables, and both	Bladder cancer	84.5% Confirmed by medical records	Fruit: 1.12 (0.70, 1.78) Vegetable: 0.72 (0.47, 1.09) Both: 0.75 (0.49, 1.14)
Michaud et al,[47] 2002	Male smokers in southwestern Finland	11	50 to 69	100.0%	100.0%	344 (11.5)	Fruit, vegetables, and both	Bladder cancer	Finnish Cancer Registry and medical records	Fruit: 1.10 (0.77, 1.57) Vegetable: 1.16 (0.82, 1.63) Both: 1.16 (0.82, 1.63)
Mills et al,[48] 1991	Adventist in California	6	25 or over	40.2%	3.3%	52 (2.7)	Real fruit juice, cooked green vegetables	Bladder cancer	Histologically confirmed (100%)	Fruit: 0.31(0.09, 1.00) Vegetable: 0.54(0.26, 1.14) –

Author	Population	Follow-up (y)	Age at Entry, (y)	Men, Rate	Csmoker, rate	Case, n (CIR*)	Main Exposure	Outcomes (Cancer Site)	Diagnose	Main Findings (Highest vs. Lowest)
Nagano et al,[49] 2000	Atomic-bomb survivors	13	52.8 for men, 56.8 for women	38.0%	31.1%	114 (2.5)	Fruit, vegetables	Bladder cancer	Tumor registry information	Fruit: 0.62 (0.39, 0.99) Vegetable: 0.54 (0.30, 0.94) –
Park et al,[50] 2013	Resident in Hawaii and California	12.5	59.9	0%	16.1%	582 (2.5)	Fruit, vegetables, and both	Invasive bladder cancer	Cancer registry information	Fruit: 0.98 (0.60, 1.60)^{F} Fruit: 0.89 (0.66, 1.19)^{M} Vegetable: 0.49 (0.29, 0.83)^{F} Vegetable: 0.89 (0.66, 1.19)^{M} Both: 0.35 (0.22, 0.56)^{F} Both: 0.87 (0.64, 1.17)^{M}
Ros et al,[51] 2012	Europeans	8.9	51	30.0%	23.0%	947 (2.3)	Fruit, vegetables, and both	Urothelial carcinoma of the bladder.	Pathology reports	Fruit: 1.00 (0.82, 1.21) Vegetable: 0.88 (0.72, 1.08) –
Sakauchi et al,[52] 2005	Japanese	9	40 to 79	42.0%	<35.5%	123 (2.5)	Fruits other than oranges, green leafy vegetables	Bladder (104), renal pelvis (12), ureter (7) cancer	Cancer registry information	Fruit: 0.56 (0.28, 1.11) Vegetable: 0.74 (0.41, 1.36) –
Shibata et al,[53] 1992	Residents form retirement community	9	65 to 84	34.5%	9% For male, 13% for female	71 (10.1)	Fruit, vegetables, and both	Bladder cancer	From local hospitals	Fruit: 0.56 (0.28, 1.11) Vegetable: 1.1 (0.64, 1.90) Both: 0.85 (0.46, 1.56)
Zeegers et al,[54] 2001	Netherlander	6.3	55 to 69	48.2%	27.9%	619 (8.1)	Fruit, vegetables, and both	Urinary urothelial, ureters, renal pelvis, or urethra cancer	Microscopically confirmed	Fruit: 0.74 (0.53, 1.04) Vegetable: 0.91 (0.65, 1.27) Both: 0.98 (0.60, 1.61)
Iso and Kubota,[55] 2007	Japanese	About 11	40 to 79	42.3%	Not mention	91 (0.7)	Fried vegetable, citrus	Urothelial carcinoma	Not mention	Vegetable: 1.33 (0.41–4.33)^{F} Vegetable: 2.02 (0.92–4.41)^{M} –

Csmoker indicates current smokers, CIR the crude incidence rate of per 10,000 person-years; F the female, and M indicates the male.
AARP = American Association of Retired Persons.

TABLE 2. The Quality of Included Articles (17)

Author	Country	Study Describe	Adjusted Item	Quality Score
Büchner et al,[39] 2009	10 European countries	European Prospective Investigation into Cancer and Nutrition study	Adjusted for smoking, vegetable consumption, energy intake from fat, and nonfat sources.	8
Chyou et al,[40] 1993	America	Honolulu Heart Program's study	Age and smoking adjusted.	6
George et al,[41] 2009	America	National Institutes of Health–AARP Diet and Health Study	Age, smoking, energy intake, BMI, alcohol, physical activity, education, race, marital status, family history, menopausal hormone therapy, and vegetable intake.	6
Grant et al,[42] 2012	Japan	The Life Span Study	Crude	4
Holick et al,[43] 2005	America	Nurses' Health Study	Age, pack-years of cigarette smoking, current smoking, and total caloric intake.	6
Larsson et al,[44] 2008	Sweden	The Swedish Mammography Cohort Study	Age, sex, education, smoking status (never, past, and current), and pack-years of smoking, and total energy intake.	7
Li et al,[45] 2010	Japan	The Ohsaki National Health Insurance Cohort Study	Age, sex, job, education, BMI, exercise, smoking, alcohol, hypertension, diabetes, family history, energy, food, vegetables, fruits, and tea.	6
Michaud et al,[46] 1999	America	The Health Professionals Follow-up Study	Age, pack-years of cigarette smoking, current smoking status, geographic region, total fluid intake, and caloric intake.	6
Michaud et al,[47] 2002	Finland	The Alpha-Tocopherol, β-Carotene Cancer Prevention Study	Age, duration of smoking, smoking dose, total energy, and trial interventions (α-tocopherol and β-carotene supplements).	5
Mills et al,[48] 1991	America	Adventist Hearth Study in California	Age, sex, and smoking adjusted relative risk.	4
Nagano et al,[49] 2000	Japan	Life Span Study in Japan	Age, gender, radiation dose, smoking status, education level, BMI, and calendar time.	6
Park et al,[50] 2013	America	The Multiethnic Cohort Study in Hawaii	Family history, employment, smoking status, and quitting.	7
Ros et al,[51] 2012	10 European countries	European Prospective Investigation into Cancer and Nutrition Study	Adjusted for smoking status, duration and intensity of smoking, and energy intake.	8
Sakauchi et al,[52] 2005	Japan	The Japan Collaborative Cohort Study	Sex, age, and smoking index.	5
Shibata et al,[53] 1992	America	The Leisure World Study	Age, smoking.	4
Zeegers et al,[54] 2001	Netherlands	Netherlands cohort study	Age, sex, number of cigarettes per day, years of cigarette smoking, and total vegetable consumption or total fruit consumption.	6
Iso and Kubota,[55] 2007	Japan	The Japan Collaborative Cohort Study	Age and area of study.	3

Article [42] and [49] was the same cohort, article [39] and [51] was the same cohort, and article [52] and [55] was the same cohort. AARP = American Association of Retired Persons, BMI = body mass index.

serving, times, or cups, we used serving as a common scale. We assumed 68.1 g vegetables or 127.3 g fruits or 97.7 g (the mean value of FVs) FVs as one standard serving.[19] Given that multiple publications may lead to reporting bias, we used the data of the study with the largest sample size or with higher quality.[20] A third parity author checked the data.

We used the Newcastle-Ottawa Scale checklist for the assessment of the study quality.[21] The check list contains 9 items for cohort studies with every item accounts for 1 point. We assumed low quality studies as with a score ≤ 4.[22,23]

Statistics Analysis

We conducted our dose-response meta-analysis by the methods of Greenland and Longnecker and Orsini et al.[24,25] That is, an RCS function, with the log relative risk as independent variable and the exposure level as dependent variable, was used to fit the potential trend.[26,27] The linear regression model was also nested within this function.[28] Three knots at fixed 10th, 50th, and 90th percentiles of the exposure distribution were modeled.[27] We assumed the coefficient of the second spline equal to zero to examine the probability of a nonlinearity relationship.[27] Generalized least-square method was used to estimate the parameters,[25] and then, the coefficients in each study were combined in a weighted random-effect model. We assigned the median values or middle point of each category to the corresponding relative risk for each study.[29] For open-ended categories, we assumed the range to be the same as the adjacent interval.[30] The method of Bekkering et al[31] was used to evaluate the missing data if there were incomplete report. For studies reported the data by subsets (eg, men and women), we combined the corresponding RRs of the subsets in a fixed-effect model before pooling them into overall analysis.[31]

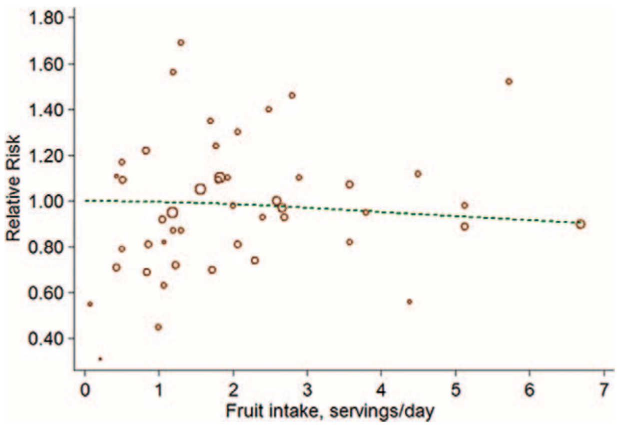

FIGURE 2. The forest plot of nonlinear association between total fruit intake and risk of bladder cancer. The hollow circles represent the relative ratios in each study weighted with inverse variance, and the green dash line is the nonlinear trend fitted by restricted cubic splines function.

We conducted subgroup analysis on geographical location, length of follow-up ($\geq$10 years and <10 years), primary unit of measurement, assumed for data or not, and controlled for energy or not to investigate the potential discrepancy among each subgroup. Since subgroup analysis may result in credibility lose,[32] we reported our subgroup analysis following the Guidelines for Interpreting Subgroup Analysis.[33] The interaction test was used to compare the two or more results among subgroups.[34] The statistical power of positive results was evaluated by the method of Hedges and Pigott.[35]

We used Egger regression test to detect the potential small study effect in above analyses that with $\geq$10 cohorts.[36–38] If evidence of asymmetry was detected, we used both fixed- and random-effect trim and fill method for an adjusted meta-analysis to examine whether the bias influence the results.[38] Sensitivity analysis was used to test whether the results were robust. All analyses were conducted by Stata/SE12.0 (Stata Corp, College Station, TX, USA). A 2-side test with $\alpha = 0.05$ as significant level.

RESULTS

Figure 1 shows the detailed process of the literature inclusion. We first selected 19 studies[39–57] that of interest. Two large cohort studies,[56,57] one did not report valid data and another only reported the data of daily versus no FVs intake, were also excluded. We have contacted the authors for details, but got a reply that relevant data are no longer available.

Of the remaining 17 studies, the studies from references[39,49,55], were identified as the same cohort to articles,[51,42,53] respectively. Finally, 14 cohorts met our eligibility criteria.

We assessed the quality of all the 17 studies. The scores range from 3 to 8, with a mean quality score was 5.7. Four studies[42,48,53,55] were assessed as low quality (score $\leq$4).

Apart from the duplicate articles, there were 9447 cases and 1 664 036 participants identified in our meta-analysis with a follow-up ranged from 6 to 20 years. The participants were distributed in European, American, and Asian. Tables 1 and 2 show the main characteristics of the included studies.

Fruit Intake and Risk of Bladder Cancer

Fourteen cohorts including 17 studies[39–55] investigated the association between total fruit intake and risk of BC. There were no evidence for a nonlinear association between them ($P = 0.66$ for nonlinearity test, Figure 2). We then used a linear regression model. The summarized RR of every 0.2 serving increment of total fruit intake a day was 0.99 (95%CI: 0.99, 1.00; $P = 0.17$; $I^2 = 43.1\%$; Figure 3).

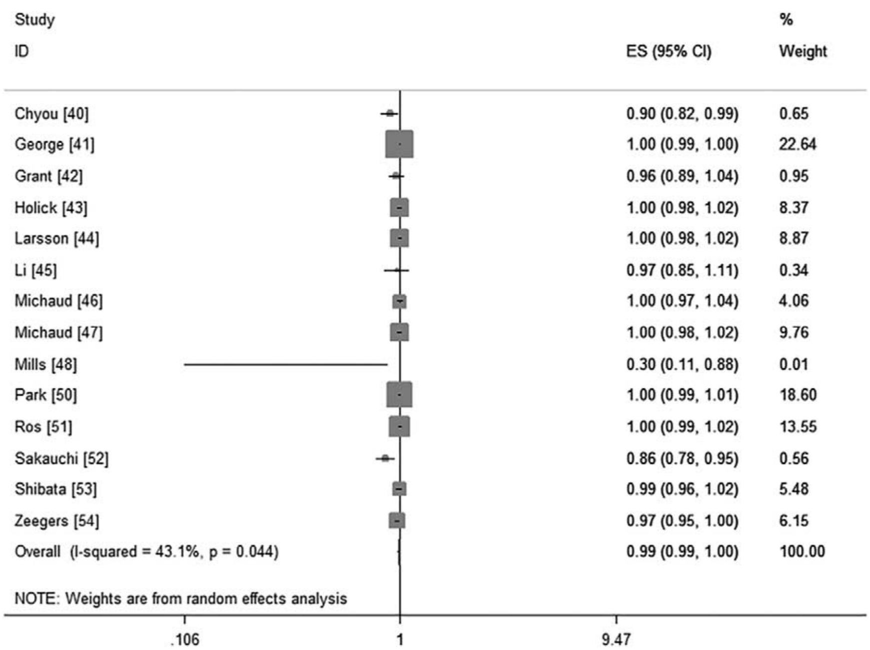

FIGURE 3. The forest plot of linear trend between total fruit intake and risk of bladder cancer, with the dose scale was every 0.2 serving increment.

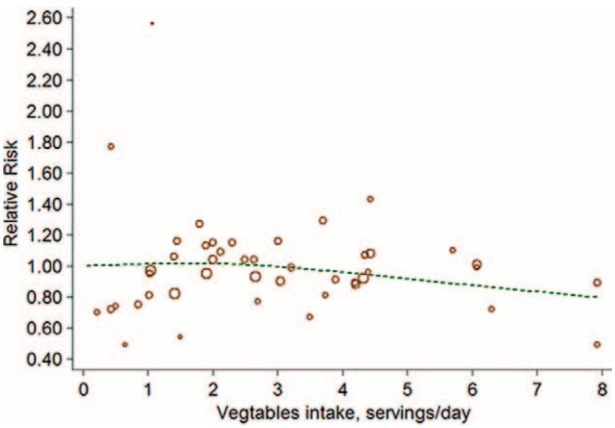

FIGURE 4. The forest plot of nonlinear association between total vegetables intake and risk of bladder cancer. The hollow circles represent the relative ratios in each study weighted with inverse variance, and the green dash line is the nonlinear trend fitted by restricted cubic splines function.

Vegetable Intake and Risk of Bladder Cancer

Thirteen cohorts including 16 studies[39–44,46–55] investigated the association between vegetables intake and risk of BC. Little evidence supported a nonlinear association between them (P for nonlinearity test was 0.20; Figure 4). The linear model, then, reached a pooled RR was 1.00 (95%CI: 0.99, 1.00; $P = 0.28$; $I^2 = 28.1\%$; Figure 5) for every 0.2 serving increment of total vegetables intake a day.

Both FVs Intake and Risk of Bladder Cancer

There were 8 cohorts[39,43,44,46,47,50,53,54] investigated the association of both FVs intake on risk of BC. The combined RR was 0.99 (95%CI: 0.97, 1.01; $P = 0.24$; $I^2 = 57.5\%$; Figure 6) of every 0.2 serving increment of FVs intake a day. No evidence of nonlinearity association was examined (P for nonlinearity test was 0.25; Figure 7).

Citrus, Cruciferous Vegetables, Green Leafy Vegetables and Risk of BC

Among the studies, there were seven[43,44,45,51,52,54,55] cohorts reported citrus, eight[43,44,46,47,50,51,52,54] reported cruciferous, six[45,46,48,49,50,52] reported green leafy vegetables intake and risk of BC. Because several studies[51,52,55] only reported linear association between citrus, cruciferous and risk of BC, we only pooled the linear trend (0.2 serving increment a day) for them.

For green leafy vegetables, no evidence of a nonlinear association was detected ($P = 0.11$), the pooled RRs of linear association (0.2 serving increment a day) were 0.98 (95%CI: 0.96, 0.99; $P < 0.01$; $I^2 = 0.0\%$; Power = 0.76; see Figure S1, Supplemental Digital Content 2, http://links.lww.com/MD/A258, which demonstrates the forest plot of results). For citrus, the summarized RR was 1.00 (95%CI: 1.00, 1.00; $P = 0.83$; $I^2 = 0.0\%$); for cruciferous vegetables, the pooled RR was 0.97 (95%CI: 0.93, 1.01; $P = 0.19$; $I^2 = 55.8\%$), respectively (see Figures S2 and S3, Supplemental Digital Content 2, http://links.lww.com/MD/A258, which demonstrate the forest plot of the results of citrus and cruciferous, respectively).

Subgroup Analysis and Sensitivity Analysis

Table 3 and Table S1 (see Table S1, Supplemental Digital Content 3, http://links.lww.com/MD/A258, which demonstrates the results of subgroup analysis of citrus, cruciferous, and green leafy vegetables) present the results of subgroup analysis. There were no substantial changes in each subgroup analysis.

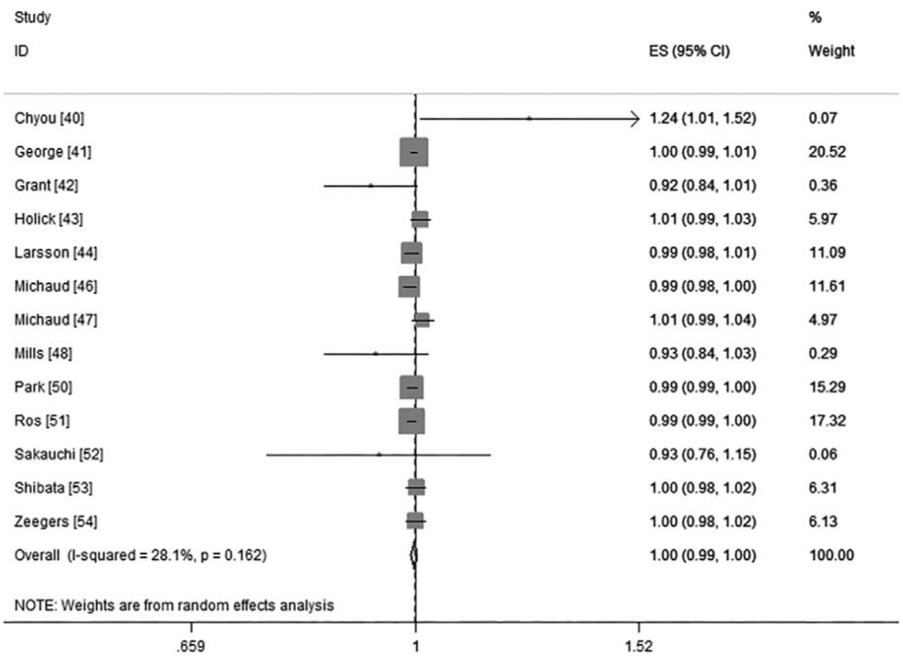

FIGURE 5. The forest plot of linear trend between total vegetables intake and risk of bladder cancer, with the dose scale was every 0.2 serving increment.

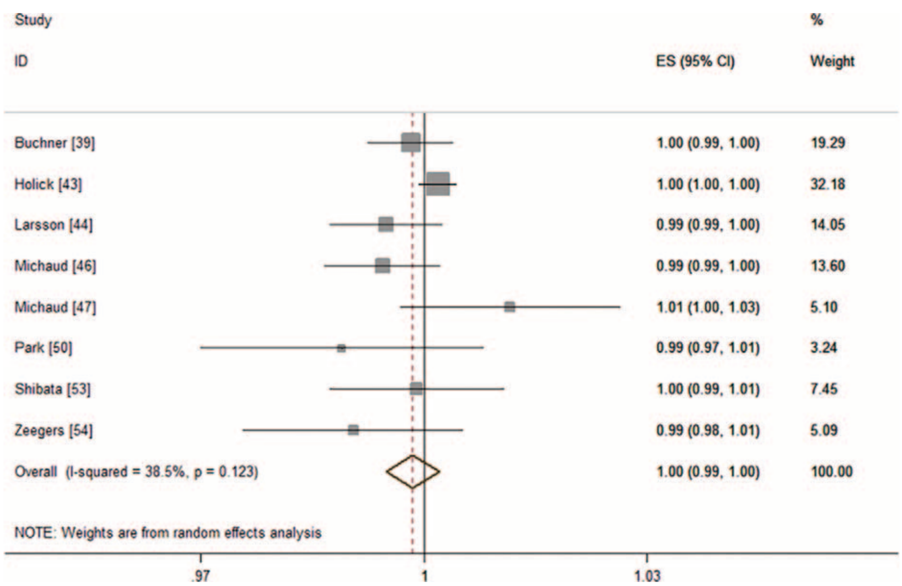

FIGURE 6. The forest plot of linear trend between both FVs intake and risk of bladder cancer, with the dose scale was every 0.2 serving increment.

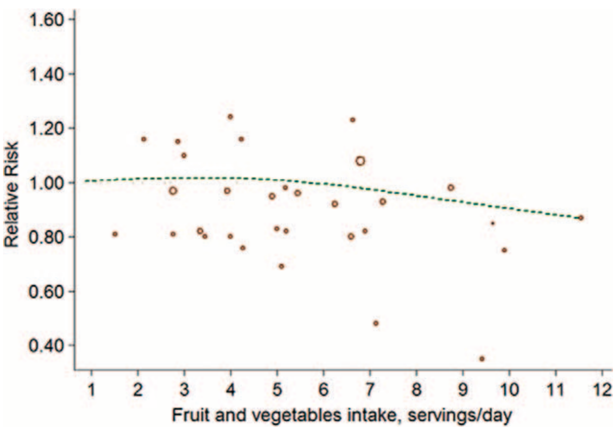

FIGURE 7. The forest plot of nonlinear association between both FVs intake and risk of bladder cancer. The hollow circles represent the relative ratios in each study weighted with inverse variance, and the green dash line is the nonlinear trend fitted by restricted cubic splines function.

The interaction test showed no obvious discrepancy between subgroups.

We conducted sensitivity analysis by omitting those studies with special population (such as Adventist), special exposure (such as fried vegetables, high smoking rate [>40%]), or low quality each time on a random-effect model to detect whether these confounders influence our results or not. Sensitivity analysis was also used to test the influence of individual studies on the overall results. After the omitting, for total fruits, vegetables, and both FVs intake, the summarized RRs of remaining studies kept consistency with before (Table 4). But for cruciferous intake, study[47] influenced the result obviously. For green leafy vegetables intake, study[50] influenced the result obviously (see Table S2, Supplemental Digital Content 3, http://links.lww.com/MD/A258, which demonstrates the results of sensitivity analyses of citrus, cruciferous, and green leafy vegetables).

Publication Bias

No evidence of publication bias was found in the analysis of vegetables intake ($P = 0.93$). However, we observed obvious asymmetry of the plot in fruit intake and risk of BC ($P < 0.01$). We then used the trim and fill method for an adjusted meta-analysis. Both fixed- and random-effect model showed stable results ($RR_{fixed} = 1.00$, 95%CI: 0.99, 1.00, $P = 0.14$; $RR_{random} = 1.00$, 95%CI: 0.99, 1.00, $P = 0.17$). (See Figures S4 and S5, Supplemental Digital Content 2, http://links.lww.com/MD/A258, which demonstrate the filled funnel plot for fruit intake and vegetables by trill and fill method.)

DISCUSSION

In present dose-response meta-analysis, we confirmed no associations between total fruits intake, vegetables intake, both FVs intake and risk of BC. We also found no obvious association between citrus, cruciferous vegetables intake and risk of BC. However, we observed inverse association between green leafy vegetables intake and risk of BC. That is, per 0.2 serving increment of daily green leafy vegetables intake is associated with 2% decrease of BC risk.

The results for total fruits, vegetables, and both FVs were credible. It is similar to another meta-analysis of cohort studies,[12] although studies in the meta-analysis were insufficiently included. Our subgroup analysis and sensitivity analysis also showed consistent results, which supported the conclusions.

The results of cruciferous vegetables and green leafy vegetables should be treated with caution. In our meta-analysis, we analyzed some subtypes of fruits or vegetables and the risk of BC. We observed unstable results in cruciferous vegetables and green leafy vegetables when conducting

TABLE 4. Sensitivity Analysis

	Fruit (14)			Vegetables (14)			Both (8)		
Sensitivity Analysis	**RR (95%CI)**	**I^2 (%)**	***P***	**RR (95%CI)**	**I^2 (%)**	***P***	**RR (95%CI)**	**I^2 (%)**	***P***
Primary results	1.00 (0.99, 1.00)	43.1	0.17	1.00 (0.99, 1.00)	28.1	0.28	1.00 (0.99, 1.00)	38.5	0.42
Omitting item									
Adventist[48]	1.00 (0.99, 1.00)	33.5	0.18	1.00 (0.99, 1.00)	25.5	0.28	–	–	–
High smoking rate[40,42,47]	1.00 (0.99, 1.00)	42.3	0.24	1.00 (0.99, 1.00)	0.0	0.06	1.00 (0.99, 1.00)	33.4	0.27
Did not adjust for smoking[42]	1.00 (0.99, 1.00)	45.8	0.20	1.00 (0.99, 1.00)	19.0	0.24	–	–	–
Atomic bomb survivors[42]	1.00 (0.99, 1.00)	45.8	0.20	1.00 (0.99, 1.00)	19.0	0.24	–	–	–
100% females[43,50]	0.99 (0.98, 1.00)	51.8	0.14	1.00 (0.99, 1.00)	31.9	0.29	1.00 (0.99, 1.00)	8.9	0.15
100% males[40,46,47]	1.00 (0.99, 1.00)	44.6	0.23	1.00 (0.99, 1.00)	0.0	0.12	1.00 (1.00, 1.00)	28.3	0.51
Urothelial cancer[40,42,51,52,54]	1.00 (0.99, 1.00)	0.0	0.29	1.00 (0.99, 1.00)	15.7	0.33	1.00 (1.00, 1.00)	38.2	0.58
Low quality score[42,48,53,55]	1.00 (0.99, 1.00)	41.8	0.23	1.00 (0.99, 1.00)	20.8	0.23	1.00 (0.99, 1.00)	47.1	0.41
Processed fruit or vegetable[40,48]	1.00 (0.99, 1.00)	17.4	0.22	1.00 (0.99, 1.00)	4.1	0.12	–	–	–
Citrus[45]	1.00 (0.99, 1.00)	47.1	0.18	–	–	–	–	–	–

RRs assigned to per 0.2 servings increment per day.

sensitivity analysis. Study[47,50] influenced the results of cruciferous and green leafy vegetables, respectively. Interestingly, the cases in reference[47] were all males while in reference[50] were all females. This suggested that, there were some differences between male and female of the prevention effect of some specific vegetables. But we have no sufficient, available data for further subgroup analysis by sex in our included studies. Another possibility may be that different stage or grade of BC may influence the results. We found that, in the study,[50] the outcome was invasive BC. But there were no

TABLE 3. Results of Subgroup Analysis of Total Fruits, Total Vegetables, and Both of Them (per 0.2 Serving Increment per Day)

	Fruit (14)			Vegetables (13)			Both (8)		
Subgroup analysis	**RRs (95%CI)**	**I^2 (%)**	***P***	**RRs (95%CI)**	**I^2 (%)**	***P***	**RRs (95%CI)**	**I^2 (%)**	***P***
Geographical location									
American	1.00 (0.99, 1.00)	38.7	0.38	1.00 (0.99, 1.00)	39.2	0.49	1.00 (0.99, 1.00)	37.6	0.66
European	1.00 (0.98, 1.01)	21.0	0.53	1.00 (0.99, 1.00)	9.9	0.41	1.00 (0.99, 1.00)	38.3	0.51
Asian	0.93 (0.86, 1.01)	44.9	0.07	0.92 (0.85, 1.00)	0.0	>0.05	–	–	–
Interaction test	–	32.1	0.23	–	42.9	0.17	–	0.0	0.80
Primary unit									
Serving or cup	1.00 (0.99, 1.00)	0.0	0.34	1.00 (0.99, 1.00)	0.0	0.28	1.00 (0.99, 1.00)	46.0	0.54
Gram	1.00 (0.99, 1.01)	20.1	0.47	1.00 (0.99, 1.00)	8.9	0.33	1.00 (0.99, 1.01)	41.4	0.65
Time	0.92 (0.85, 0.99)	51.6	0.02	0.97 (0.88, 1.08)	57.9	0.60	–	–	–
Interaction test	–	58.8	0.09	–	0.0	0.89	–	0.0	0.83
Length of follow-up									
Less than 10 years	0.99 (0.98, 1.01)	59.5	0.34	1.00 (0.99, 1.00)	0.0	0.16	1.00 (0.99, 1.00)	0.0	0.13
10 years or over	1.00 (0.99, 1.01)	9.9	0.42	1.00 (0.99, 1.01)	62.2	0.96	1.00 (0.99, 1.01)	54.9	0.97
Interaction test	–	0.0	0.63	–	0.0	0.67	–	63.8	0.10
Data assumption									
Yes	0.99 (0.99, 1.00)	46.4	0.24	1.00 (0.99, 1.00)	24.7	0.36	1.00 (0.99, 1.01)	23.7	0.90
No	0.99 (0.97, 1.01)	48.5	0.45	1.00 (0.99, 1.01)	39.8	0.61	1.00 (0.99, 1.00)	58.6	0.33
Interaction test	–	0.0	0.78	–	0.0	1.00	–	0.0	0.49
Energy controlled									
Yes	1.00 (0.99, 1.00)	0.0	0.52	1.00 (0.99, 1.00)	8.3	0.29	1.00 (0.99, 1.01)	48.4	0.82
No	0.98 (0.97, 1.00)	62.3	0.07	1.00 (0.99, 1.01)	40.1	0.54	1.00 (0.99, 1.00)	39.4	0.41
Interaction test	–	64.2	0.10	–	0.0	1.00	–	0.0	0.82

RRs = relevant risks.

sufficient evidence to verify it since other studies did not subgroup the results by cancer stage or grade.

To our knowledge, this was the first meta-analysis that found green leafy vegetables were associated with reduced risk of BC. Although sensitivity analysis tested unstable result, we have evaluated the statistic power of it and it showed a reasonable amount of power ($P = 0.76$). We recommend a high green leafy vegetables diet instead of other types of FVs for BC prevention.

Green leafy vegetables contain high concentrations of vitamins such as β-carotene, ascorbic acid, and folic acid.[58] These bioactivators are beneficial for immune function, antioxidant status and can protect DNA from oxidative damage[4–6] which may help prevent BC. But it is hard to explain why total fruits or vegetables are not associated with reduced BC risk. Research has found that orange fruit is more effective than dark-green leafy vegetables in increasing serum concentrations of β-carotene.[59] Further studies were needed.

We detected moderate heterogeneity in our meta-analysis. We found parts of the source of heterogeneity by sensitivity analysis. According to the results of sensitivity analysis, the outcomes, sex, processed fruits or vegetables (such as juice, cooked vegetables), and smoking status consist of the main source of heterogeneity. When omitting the studies with these characteristics, the heterogeneity reduced to a low level.

In our meta-analysis, we detected obvious asymmetry in publication bias analysis of fruit intake and risk of BC. However, our further trim and fill method showed no substantial changes of the results in both fixed- and random-effect model, which suggested that the asymmetry may not be caused by publication bias.

There were some limitations in present meta-analysis. First, smoking is the main risk factor for BC.[1] We observed a borderline statistical significant result ($P = 0.06$) in vegetables when omitting the population with high current smoking rate (>40%), which suggested that smoking may influence our results. Some studies have reported the association between smoker and nonsmoker intake of fruits or vegetables and risk of BC; but we did not pool the results for there were limited studies ($n \leq 5$). The influence of smoking on our results should be treated with caution. Second, there did have selection bias in our dose-response meta-analysis—we excluded 2 cohort studies[56,57] with the data were not available that may influence our results. Third, as for analyses that with <10 studies, we did not test the publication bias, the influence of publication bias on the results should be noted. Fourth, we did not test the non-linearity association between citrus, cruciferous vegetables and risk of BC since there were several studies only reported the linear association, which may lead to reporting bias. Moreover, the cases of included studies were only distributed in European, America, and Asian; so, the results of our meta-analysis may suit better for these areas.

CONCLUSIONS

Current published evidence suggests no association between fruits intake, vegetables intake, both FVs intake and risk of BC. No evidence support citrus is benefit for BC prevention. The effect of cruciferous vegetables on BC prevention is inconsistent. Green leafy vegetables may be associated with reduced risk of BC. Studies should provide more detailed data for further analysis.

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