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Increased Intake of Vegetables, but not Fruit, Reduces Risk for Hepatocellular Carcinoma: A Meta-analysis

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Specific author contributions: Yang Y. conceived and drafted the study; Yang Y. Zhang D., and Feng N. collected all data; Yang Y. and Zhang D. analyzed and interpreted data, and drafted the manuscript; Chen G., Liu J., Chen G. and Zhu Y. critically revised the manuscript. All authors commented on drafts of the paper and approved the final manuscript.

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Conflicts of interest

The authors disclose no conflicts.

Abstract

BACKGROUND & AIMS: The anti-cancer effects of vegetables and fruit have been investigated extensively, but the association between vegetable and fruit consumption and risk of hepatocellular carcinoma (HCC) has not been quantified. We performed a meta-analysis of observational studies to clarify the association.

METHODS: We identified eligible studies, published from 1956 through May 31, 2014, by searching PubMed, Web of Science and EMBASE. Random-effects models were used to calculate summary relative risks (RRs), and dose-response analyses were conducted to quantify associations. Heterogeneity among studies was evaluated using Cochran's Q and I2 statistics.

RESULTS: A total of 19 studies involving 1,290,045 participants and 3912 cases of HCC were included in the meta-analysis. The summary RR for HCC was 0.72 for individuals with high intake vs low intake of vegetables (95% confidence interval [CI], 0.63–0.83) and 0.92 with a daily increase in vegetable intake (100 g/day) (95% CI, 0.88−0.95). Subgroup analyses showed that this inverse association did not change regardless of history of hepatitis, alcohol drinking, smoking, or energy intake. The summary RR for HCC among individuals with high vs low intake of fruit was 0.93 (95% CI, 0.80−1.09), and 0.99 with a daily increase in fruit intake (100 g/day) (95% CI, 0.94–1.05).

CONCLUSIONS: Based on a meta-analysis, increased intake of vegetables, but not fruit, is associated with lower risk for HCC. The risk of HCC decreases by 8% for every 100 g/day increase in vegetable intake. The findings should be confirmed by future studies with validated questionnaires and strict control of confounders.

KEYWORDS: Nutrition; Hepatocellular carcinoma; Cancer Prevention; Epidemiology

Introduction

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancer (PLC), and ranks as one of the most common malignancies worldwide. The incidence of HCC varies by different region, but is particularly high in East Asia (about 50% HCC cases occur in China) and South Africa[1]. The main causes for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol consumption, and exposure to aflatoxin B1. The problem of food- and water-borne carcinogens overall (e.g., pesticides or pollution) may deserve more concern. Other risk factors for HCC include fatty liver disease, obesity, diabetes mellitus, and smoking. HCC incidence has risen in Western countries in recent years, partly due to the prevalence of obesity and diabetes[2].

There is growing evidence that dietary factors play a role in the development of cancer. For people who do not use tobacco or alcohol, their dietary choices, physical activity levels and associated weight control are important modifiable determinants of cancer risk. Healthy eating is considered effective at preventing cancer; however, there is no definition for "healthy eating" to date[3]. As an essential component of diet, the protective role of vegetables and fruit in cancer prevention is still inconclusive and controversial. Epidemiological studies have indicated that vegetable or fruit intake might be associated with lower risk of certain types of cancer, such as colorectal cancer[4], breast cancer[5], oesophageal carcinoma[6] and non-Hodgkin's lymphoma[7].

There has been growing interest in the relationship between vegetable and fruit intake and risk of HCC since the 1990s, when Negri *et al*. reported that high consumption of vegetables and fruit was inversely associated with risk of upper digestive cancers, including HCC from a case-control study[8]. Subsequently, many other observational studies examined the relationship between vegetable and fruit consumption and risk of

HCC, but the results have been conflicting and inconsistent. Therefore, we carried out a comprehensive meta-analysis to quantitatively assess the association between vegetable and fruit intake and HCC risk.

Material and Methods

Literature search

A literature search of related studies was conducted in the databases of PubMed (Medline), Web of Science and EMBASE from 1956 to May 31, 2014, using the following key words or MeSH terms: (“fruit” or “vegetables” or “diet” or “nutrition” or “lifestyle”) combined with (“hepatocellular carcinoma” or “liver cancer” or “hepatoma” or “liver tumor”). In addition, the reference lists of retrieved articles were also checked to find additional relevant studies. Our search was restricted to full-length articles published in English.

Selection criteria

To be included in the meta-analysis, studies had to meet the following criteria: (1) a cohort or case–control design was used; (2) the exposure of interest was the consumption of vegetables and/or fruit; (3) the outcome was HCC (or primary liver cancer) incidence or mortality; and (4) the odds ratios (OR) or relative risks (RR) with corresponding 95% CIs were provided or could be calculated from the data presented in the manuscripts. Animal studies, reviews, letters, editorials, commentaries, abstracts, unpublished studies, and duplicate studies were excluded. If one study was reported repeatedly, the publication with the longest follow-up time was used in the meta-analysis.

Data extraction and quality assessment

The following information was extracted from each included study: the first author’s name, publication year, location, design, number of cases, cohort size or number of controls, dietary assessment, HCC diagnosis method, comparisons, RRs with corresponding 95% CIs for the highest compared with lowest intake, and adjusted variables. For each study, the risk estimates adjusted for the largest number of variables were extracted.

The Newcastle-Ottawa scale was employed to assess the quality of individual studies[9]. In brief, a maximum of 9 points was assigned to each study: 4 for selection, 2 for comparability, and 3 for outcomes. A final score > 6 was regarded as high quality. Two authors (Yang Y and Zhang D) independently extracted the data and gave each study a score. Differences were solved by discussion to achieve consensus, and confirmed by another author (Feng N).

Statistical methods

Relative risks and their corresponding 95% CIs were extracted from each study, and then log-transformed for the meta-analysis. Given the discrepancies of exposure categories among original studies, RRs comparing the highest with the lowest categories of vegetable and fruit intake were used to obtain a summary estimate. The random-effects model was adopted to pool relative risks, considering the heterogeneity among studies[10], which was further assessed by using the Q and I^2 statistics, with I^2>50% representing substantial heterogeneity[11].

Dose-response meta-analysis was performed by the method described by Greenland and Orsini *et al* (two stage GLST in Stata)[12, 13]. Only studies that reported RRs, with their corresponding 95% CIs, for at least 3 quantitative categories were included. For each category, the median or mean intake of vegetables and fruit was assigned to each corresponding RR. The results were presented per 100 g/day increment in dose-response analysis. A restricted cubic spline model with three knots was used to evaluate the potential nonlinear association between vegetable and fruit intake and HCC risk. The difference between the nonlinear and linear models was investigated by a likelihood ratio test[14].

Subgroup analyses stratified by potential confounding factors and meta-regression analyses were carried out to explore the sources of heterogeneity. Moreover, sensitivity analysis was performed by removing one study each time and examining the influence of a specific study on the pooled results. Publication bias was evaluated using a funnel plot and the Egger test, with $p<0.1$ indicating significant publication bias[15]. The "trim and fill" method was employed to correct publication bias[16]. All statistical analyses were performed using STATA12.0 (StataCorp, College Station, TX, USA). All tests were two-sided, with $p<0.05$ considered statistically significant.

Results

Search results and study characteristics

Nineteen studies were identified that met the inclusion criteria, including 10 cohort studies and 9 case-control studies. A flowchart of literature search is presented in Figure 1. The characteristics of each study are summarized in Table 1. Of the 19 studies, 6 were from Europe, 12 from Asia, and 1 from the USA. After combining all the studies, a total of 1,290,045 participants and 3,912 HCC cases were involved in our meta-analysis.

As shown in Table 1, the quality scores ranged from 4 to 9, with an average of 6.68. The median scores were 5.67 for case-control studies and 7.60 for cohort studies. It seemed that early studies had lower quality; however, the correlation between quality score and publication year was not statistically significant (r=0.31, p=0.20).

Of the 9 case-control-studies, 5 were hospital-based.[8,17,20,21,23] All the cohort studies lasted for more than 5 years, and some of them followed subjects more than 10 years[24,25,26,29]. In one Japanese cohort study, the study population was atomic-bomb survivors in Hiroshima and Nagasaki[24]. It should be noted that the exposure of interest was specified as "cruciferous vegetables" [33]or "green-leafy vegetables"[25] in some studies in the vegetable group, and the fruit in the Ohsaki cohort study were referred to as "citrus" [32]. In most studies, the RR estimates were adjusted for age (n = 14), hepatitis (n = 11), alcohol drinking (n = 12), smoking (n = 10), body mass index (n = 7), and energy intake (n=5). However, for one case-control study from Serbia, univariate logistic regression method was used and therefore, only crude ORs were reported [31].

Vegetables

Seventeen studies investigated the association between vegetable intake and HCC risk, including 9 cohort studies and 8 case-control studies. The summary RR for the highest compared with lowest analysis was 0.70 (95% CI: 0.56-0.87) by the random-effects model, with a high heterogeneity (I^2=79.1%, $p_{\text{heterogeneity}}$<0.001; Figure 2). The RRs were 0.76 (95% CI: 0.48-1.20) for case-control studies and 0.66 (95% CI: 0.51-0.86) for cohort studies.

Nine studies were eligible for the dose-response analysis. The results showed that the summary RR per 100g/day increase in vegetable intake was 0.92 (95%CI: 0.88-0.96), with an evident heterogeneity (I^2= 86.3%, $p_{\text{heterogeneity}}$<0.001). A significant curvilinear relationship was observed between vegetables intake and HCC risk ($p_{\text{non-linearity}}$<0.01). The downward trend was most obvious when vegetable intake increased up to about 100 g/d (Figure 4A).

Subgroup analyses were carried out to examine the stability of the pooled RRs. The results indicated that the inverse association was unchanged by most confounders, and the inverse association was slightly strengthened in high-quality or cohort studies. Nonetheless, in some subgroup analyses, the association became insignificant due to a small sample size or low quality (Table 2).

Meta-regression analysis indicated that ‘publication year’ was the only factor which associated with the heterogeneity significantly (*p* = 0.028), explaining 29.54% of the heterogeneity among all studies. Other confounders, such as design, location, quality score and types of FFQ were not associated with the heterogeneity. Moreover, we performed a sensitivity analysis by omitting one study each time. The recalculated RRs did not change significantly, with a range from 0.67 (95% CI: 0.53-0.84) to 0.77 (95% CI: 0.64-0.92). Some asymmetry was observed in the funnel plot, with *p* values of 0.27 for Begg’s test and 0.06 for Egger’s test, indicating the existence of slight publication bias. However, the results remained unaltered after the trim and fill analysis, suggesting no substantial influence of publication bias.

Fruits

Six cohort studies and 6 case-control studies were included in the analysis for the highest compared with lowest fruit intake and HCC risk. A moderate heterogeneity (I^2= 46.8%, *p* = 0.037) was found among all the studies. Random-effects pooled analysis suggested that high fruit intake was not associated with HCC risk (RR: 0.93; 95% CI: 0.80-1.09) (Figure 3).

Eight studies were included in the dose-response analysis. The summary RR per 100g/day increase in fruit intake was 0.99 (95%CI: 0.94-1.05), with a high heterogeneity (I^2= 72.2%, $p_{\text{heterogeneity}}$<0.01). There was no evidence of a potential nonlinear association between fruit intake and HCC risk ($p_{\text{non-linearity}}$ = 0.74) (Figure 4B).

Subgroup analysis showed that there was no relationship between fruit intake and HCC risk in cohort studies; however, the association was inversely significant in case-control studies. After stratifying by geographic location, the RR was 1.06 (95%CI: 0.92-1.23) for studies conducted in Asian countries and 0.76 (95%CI: 0.61-0.94) for studies from USA and European countries. The inverse association also existed in studies using an invalidated FFQ (RR=0.76, 95%CI: 0.58-0.99). No differences were observed when stratified by study quality, case numbers or whether other confounding factors were adjusted for in the models.

In the meta-regression analysis, we found that design (p=0.04), location (p=0.02), and diet assessment methods (p = 0.04) were significant sources of heterogeneity. The three confounders combined could explain 81.40% of heterogeneity in a multivariate model. The stability of the association between fruit intake and HCC risk was confirmed by the sensitivity analysis. No significant publication bias was detected, either from Egger's test (p = 0.70) or from Begg's test (p = 0.30).

Vegetables and fruits

Only two studies reported RRs for the association between total vegetable and fruit intake and HCC risk. One was a case-control study conducted in Italy, which reported an OR of 0.46 (95%CI: 0.32-0.67)[20]. The other was a cohort study from Japan, which found there was no significant relationship between total vegetable and fruit intake and HCC risk[29]. Considering the small sample sizes and high heterogeneity, as well as the mixed effects of fruit and vegetable intake, we did not carry out the pooled analysis.

Discussion

By carrying out a quantitative analysis for the first time, our study found that increased intake of vegetables, but not fruit, was associated with reduced HCC risk. Results of this study indicated that a daily increase of 100 grams of vegetables (100 g/day) was associated with an 8% lower risk of HCC. The present meta-analysis was based on a large sample size and a long time span, which thus enhanced the statistical power to detect possible associations. We also conducted subgroup analyses and meta-regression analyses to identify potential sources of heterogeneity.

In general, both vegetables and fruit are low in fat and calories, and rich in vitamins, minerals and dietary fiber, which have been found to be inversely associated with cardiovascular disease, diabetes, and stroke[35-37]. However, vegetables and fruit vary widely in nutritional components, with fruit containing more calories and antioxidants such as vitamin C. Vegetables not only provide dietary fiber, and vitamins A and E, but they are also good sources of phytochemicals (e.g., isothiocyanates, glucosinolates, indole-3-carbinol, and flavonoids), which possess anti-tumor activities. Intake of cruciferous vegetables has been found to reduce the risk of colorectal cancer[38], lung cancer[39] and gastric cancer[40]. It was also recently reported that vitamin E, widely existing in vegetables, could decrease HCC risk significantly, while vitamin C and multivitamin supplements were associated with higher risk of HCC in males[41]. In fact, some studies suggested that too much antioxidant supplementation seemed to increase overall mortality from cancers[42]. We had similar results with a recent cohort study of Health Survey in England, which found that vegetables might have a more protective benefit than fruit in the general population[43]. Based on the above information, the different effects of vegetables and fruit might be caused by differences in their nutrient compositions.

There are several limitations in our meta-analysis. First, the diet assessment methods and exposure ranges were different across the original studies, which led to incomparability of results to some extent. Nevertheless, relative risks for the highest compared with lowest category of vegetable or fruit intake were adopted and pooled, and the results were verified by dose-response analyses. Moreover, measurement errors were inevitable in the observational studies, the most obvious of which were due to the employment of invalidated FFQs, and selection and recall bias. There were indeed significant differences between studies that did or did not use a validated FFQ regarding the association between fruit intake and HCC risk.

The second limitation was the inadequate adjustment for confounders. However, no significant differences were found between studies whether they were adjusted for important confounders (e.g., HBV/HCV status, smoking, alcohol, and dietary energy intake) or not. For example, the observed RR for vegetable intake and HCC in the 10 studies that controlled for smoking (RR = 0.81; 95%CI: 0.74-0.90) was comparable to the results of 7 studies that did not control for smoking (RR = 0.76; 95%CI: 0.57-1.02). Moreover, when analyzing the studies that adjusted for important confounders in the vegetable group, the association was found to be even stronger, which highly supported the reliability of our analysis. Yet, there may be other uncontrolled confounders that weaken the association between vegetable and fruit intake and HCC risk. For instance, higher consumption of vegetables and fruit is often linked with other healthy lifestyles, such as more exercise, lower prevalence of overweight/obesity, and less frequent smoking or drinking[30].

One obvious example was the association between fruit intake and HCC risk in non-Asian population. Our analysis showed that there was a significantly negative association; however, the association was fragile and could not bear closer analysis. There were only 6 studies concerning fruit intake in non-Asian populations, 5 of which were case-control studies with relatively lower quality, and 2 studies were published before the year 2000. These older, low quality studies are more likely to be biased. In fact, the only one cohort study (AARP Diet and Health Study from the USA), reported that there was no significant association between fruit intake and HCC risk[30]. There is another plausible explanation: both dietary patterns and the etiology of HCC are distinct between Western and Asian countries[44] — the health benefit of fruit intake might be caused by other healthy lifestyles correlated with it.

The limitations described above contributed to the high heterogeneity observed across the studies. We employed meta-regression analyses to explore the sources of the heterogeneity and conducted subgroup analyses to avoid the influence of confounders. Last but not least, publication bias is always an important issue in the meta-analyses. Our literature was limited to English language publications, and studies with null results appear not to be published. In fact, the presence of publication bias was detected with respect to the association between vegetable intake and HCC risk; however, results remained unaltered after the trim and fill analysis, suggesting that the publication bias was negligible.

To demonstrate that increasing consumption of vegetables might be beneficial for liver cancer prevention at the population level, we estimated the number needed to treat (NNT) to prevent one death from HCC. Because of the presence of heterogeneity among studies and varying incidence of HCC across different regions worldwide, the NNTs that follow should be interpreted with caution. We estimated that approximately 125,000 people would need to increase their vegetable intake by 100 g/day to avoid one death from HCC over 9.3 years (NNT). This estimate was based on the RR per 100 g/day increment in vegetable intake with HCC risk, combined with the global HCC mortality rate (more than 600,000 deaths each year)[1], as well as the average number of follow-up years in the original studies (9.3 years). For the general population, vegetable consumption appears to have little effect on preventing HCC. However, if the analysis were restricted to the

Asian population, the NNT would be much smaller. In particular, the NNT to prevent one case of HCC among Asians with chronic HBV-associated cirrhosis, would be 338 (using an estimated incidence rate of HCC of 3.7 per 100 person-years) [45]. Hence, increasing vegetable intake is still a recommendable option, especially after considering the protective effect of vegetable intake on cardiovascular diseases and overall mortality[43].

In consideration of the increasing trend of HCC incidence and its high fatality, prevention of HCC is an urgent need. Attempts at present to prevent HCC mainly include control of HBC/HCV infection, reduction of alcohol consumption, smoking cessation and reducing the prevalence of obesity and diabetes[46]. It is of great significance to find novel strategies to prevent HCC, dietary and nutritional factors have presented an attractive prospect. In addition to vegetables, recent epidemiological studies have suggested that higher consumption of coffee, fish and white meat is helpful to prevent HCC[47,48].

Of note, since the effects of dietary factors interact with each other, a balanced diet with proper nutrition (which includes higher intake of vegetables, fish, white meat, and coffee, and reduced intake of fat, red meat, and alcohol) is more recommended overall, especially for those patients with liver diseases[49]. Turati *et al*. recently reported that a Mediterranean diet significantly decreased the risk of HCC, which provides a new paradigm for future studies[50]. As a matter of course, public health strategies must target cost effective measures; incantatory recommendations cannot help. While studies of dietary factors and ensuing consumer education can help people make informed decisions about the foods they choose to eat, the short- and long-term feasibility of population-wide changes in diet is dependent upon substantial policy change.

In summary, this meta-analysis showed that intake of vegetables, but not fruit, is associated with lower risk of HCC. Caution is needed in interpreting the association, which might be due to measurement errors, uncontrolled confounders or publication bias. The findings should be confirmed by future studies with validated questionnaires and strict control of confounders.

Figure legends

Figure 1 Flow chart of study selection.

Figure 2 Forest plot of vegetable intake and HCC risk for high vs low consumption.

Figure 3 Forest plot of fruit intake and HCC risk for high vs low consumption.

Figure 4 Non-linear association between vegetable and fruit intake and HCC risk.

Table 1 Characteristics of studies included in the meta-analysis on vegetable and fruit intake and HCC risk.

Table 2 Subgroup analyses of fruit and vegetable intake and HCC risk, High vs Low Intake.

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(Author names in bold designate shared co-first authors)

Table 1 Characteristics of studies included in the meta-analysis on the vegetable and fruit intake and HCC risk.

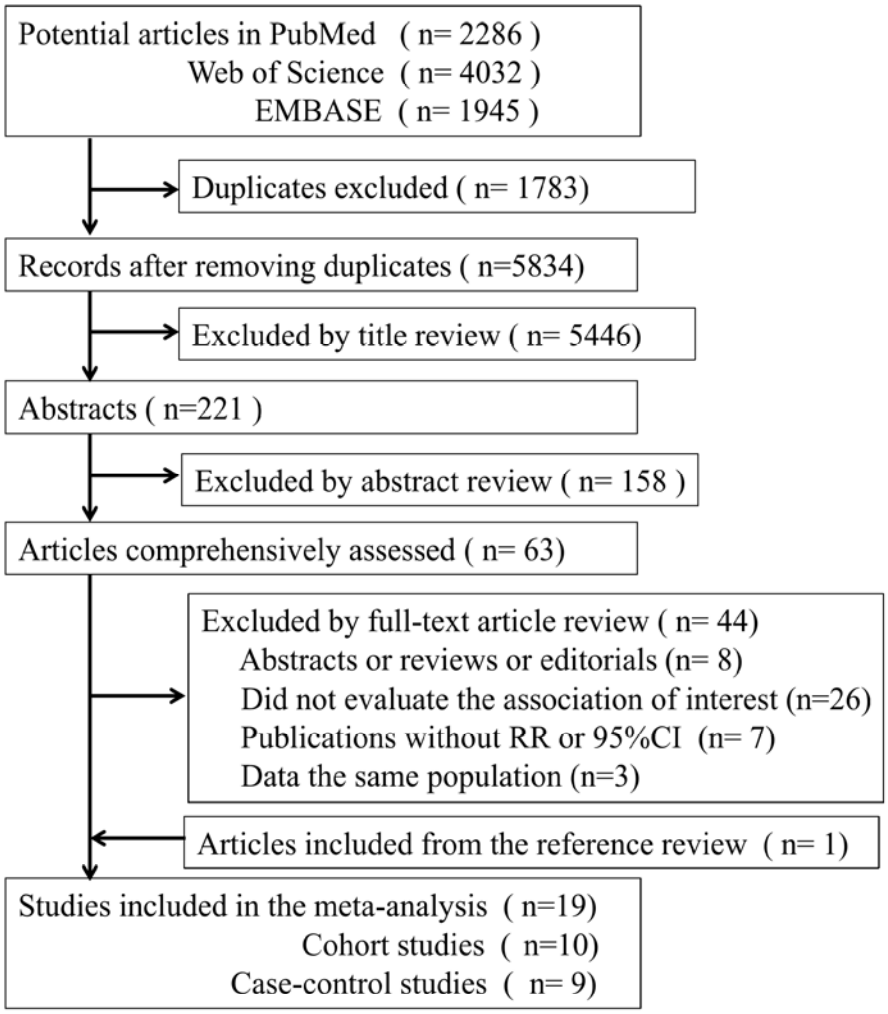
Author Year[Ref]	Duration	Design	Location	Cases	Cohort size /Controls	Dietary assessment	Diagnosis method	Exposure	Comparisons	RR(95%CI) (Highest to lowest)	Adjusted variables	Overall quality
P Srivatanakul 1991[17]	unreported	Case-control	Thailand	65	65	Questionnaire	Histology	Vegetables	≥2 vs <2 serv/d	0.20(0.04-1.0)	HbsAg, alcohol drinking, Shrimp paste consumption, peanut consumption, betel-nut chewing	5
Negri E 1991[8]	1983-1990	Case-control	Italy	258	6147	Questionnaire	Histology	Vegetables Fruits	Tertile 3 vs Tertile 1 Tertile 3 vs Tertile 1	0.30 (0.20-0.40) 0.60 (0.40-0.90）	Agc, area of residence, education, smoking and sex	4
Chen CJ 1993[18]	1983-1991	Cohort	China	60	13737	Questionnaire	Registry	Vegetables	>6 vs ≤ 2 serv /wk	0.22 (0.08-0.63)	HbsAg carrier status, Cigarette smoking, Alcohol intake	9
Yu MW 1995[19]	1984-1990	Cohort	China	50	8436	Questionnaire	Histology	Vegetables	≥6 vs <6 serv /wk	0.36 (0.17-0.77)	Age, HBsAg carrier status, habitual alcohol drinking, and past history of liver diseases	7
Braga C 1997[20]	1984-1993	Case-control	Italy	320	1408	Questionnaire	Histology	Fruits and vegetables	High vs low	0.46 (0.32-0.67)	Sex, age, area of residence, smoking status, education, alcoholic beverage consumption, oral contraceptive use, hepatitis, diabetes, liver cirrhosis	7
H Kuper 2000[21]	1995-1998	Case-control	Greece	97	128	FFQ-120 items	Histology	Vegetables Fruits	Quintile 5 vs Quintile 1 Quintile 5 vs Quintile 1	1.21 (0.80-1.82) 1.00 (0.71-1.41）	Age, gender, schooling, infection with HBV and/or HCV, alcohol consumption, tobacco smoking, total energy intake	6
Yu 2002[22]	1995-1997	Case-control	China	248	248	Questionnaire	Histology	Vegetables Fruits	≥3 vs < 3 serv/wk ≥2 vs < 2 serv/mo	1.35 (0.55-3.31) 0.73 (0.37-1.46)	HBsAg, History of intravenous injection, Average income	6
Huang YS 2003[23]	1999-2001	Case-control	China	185	185	FFQ-NA	Histology	Vegetables Fruits	≥1 vs <1 serv/d ≥1 vs <1 serv/d	1.24 (0.80 -1.93) 1.21 (0.74-1.98)	Age, Gender, Chronic viral hepatitis, Child-Pugh classification, Family history of HCC, Smoking, Habitual alcohol drinking, Red meat intake ,White meat intake ,Salted food intake	5
Sauvaget C 2003[24]	1980-1998	Cohort	Japan	555	38540	FFQ-22 items	Registry	Vegetables Fruits	High vs Low High vs Low	0.75 (0.60-0.95) 0.96 (0.78-1.19)	Sex, age, radiation dose, city, BMI, smoking status, alcohol habits, and education level	7
Kurozawa 2004[25]	1988-1999	Cohort	Japan	401	110688	Questionnaire	Registry	Vegetables Fruits	Daily vs ≤ 1- 2 serv/wk Daily vs ≤ 1- 2 serv/wk	0.90 (0.56-1.45) 1.14 (0.75-1.72)	Gender, age, history of liver diseases	7
TM Pham 2006[26]	1986-1999	Cohort	Japan	73	6049	Questionnaire	Registry	Vegetables	Daily vs Weekly intake	0.35 (0.19-0.68)	Age; body mass index; smoking habit; alcohol consumption; coffee drinking; history of transfusion; history of hepatitis; history of cirrhosis; history of diabetes; study area	8
Talamini 2006[27]	1999-2002	Case-control	Italy	185	412	FFQ-63 items	Histology	Vegetables Fruits	Quartile 4 vs Quartile 1 Quartile 4 vs Quartile 1	0.72 (0.31-1.64) 0.48 (0.22-1.05)	Gender, age, center, education, place of birth, drinking habits, maximal lifetime alcohol intake, hepatitis viruses and total energy intake	7
Yun 2008[28]	1996-2002	Cohort	Korea	169	444963	Questionnaire	Registry	Vegetables	Vegetables vs Meat	1.07 (0.92 -1.25)	Age, dietary preference, LPA, smoking status, alcohol drinking, body mass index, employment and fasting blood sugar	6
N Kurahashi 2009[29]	1993-2005	Cohort	Japan	101	235811	FFQ-52 items	Registry	Vegetables Fruits Vegetables and fruits	High vs Low High vs Low High vs Low	0.61 (0.36 -1.03) 1.45 (0.85 -2.48) 1.14 (0.70 -1.86)	Age, area, sex, HCV, HBsAg, smoking status, alcohol consumption, body mass index, history of diabetes mellitus and intake of coffee, genistein	8

SM George 2009[30]	1995-2003	Cohort	USA	394	483338	FFQ-124 items	Registry	Vegetables Fruits	Quintile 5 vs Quintile1 Quintile 5 vs Quintile1	0.71 (0.51 - 0.99) 0.91 (0.65 - 1.26)	Age, smoking, energy intake , BMI, alcohol, physical activity, education race, marital status, family history, menopausal hormone therapy.	7
M Kanazir 2010[31]	2004-2007	Case-control	Serbia	45	90	Questionnaire	Histology	Vegetables Fruits	Weekly vs Rarely Weekly vs Rarely	0.94 (0.65-1.34) 0.69 (0.54- 0.88)	Univariate logistic regression	5
Li WQ 2010[32]	1995-2003	Cohort	Japan	254	42470	FFQ-40 items	Registry	Fruits (Citrus)	Daily vs ≤ 2 serv/wk	1.26 (0.79–2.20)	Age, sex, job status, education, exercise,, smoking, alcohol drinking, hypertension, diabetes mellitus and gastric ulcer, family history of cancer, daily total energy intake, consumption of other food	8
C Bosetti 2012[33]	1991-2009	Case-control	Italy/ Switzerland	185	304	FFQ-78 items	Histology	Vegetables	≥1 vs <1 serv/d	0.72 (0.47–1.11)	Sex, age, study center, year of interview, education, alcohol drinking, tobacco smoking, body mass index, and total energy intake	6
W Zhang 2013[34]	1997-2006	Cohort	China	267	132837	FFQ-81items	Registry	Vegetables Fruits	Quartile 4 vs Quartile 1 Quartile 4 vs Quartile 1	0.73 (0.49 -1.07) 1.13 (0.78 -1.64)	Sex, age, body mass index, total energy intake, family income level, education level, family history of liver cancer in first-degree relatives, chronic viral hepatitis , diabetes, vitamin C and E and multivitamin supplement use,	9

Abbreviations: FFQ: food frequency questionnaire; NA: not available.

Table 2 Subgroup analyses of fruit and vegetable intake and HCC risk, High vs Low Intake

	Vegetables					Fruits				
Sub-groups	**N**	**RR**	***p*-het**	**I^2**	***p*-interaction**	**N**	**RR**	***p*-het**	**I^2**	***p*-interaction**
All studies	17	0.70(0.56-0.87)	<0.01	79.10%		12	0.93(0.80-1.08)	0.04	46.80%	
Design										
Cohort	9	0.66(0.51-0.86)	<0.01	75.00%		6	1.04(0.91-1.20)	0.10	0.00%	
Case-control	8	0.76(0.48-1.20)	<0.01	83.60%	0.49	6	0.78(0.61-0.98)	0.61	45.20%	**0.04**
Location										
Asian	11	0.70(0.54-0.92）	<0.01	73.40%		7	1.06(0.92-1.23)	0.15	41.40%	
Non-Asian	6	0.70(0.46-1.08）	<0.01	84.50%	0.94	5	0.76(0.61-0.94)	0.64	0.00%	**0.02**
Quality										
>6	9	0.68(0.59-0.78)	0.09	42.40%		7	1.03(0.87-1.20)	0.30	16.90%	
<=6	8	0.81(0.55-1.20)	<0.01	86.70%	0.23	5	0.81(0.63-1.03)	0.10	47.90%	0.11
Type of FFQ										
Validated	8	0.80(0.70-0.92)	0.19	30.20%		8	1.02(0.89-1.17)	0.39	5.70%	
Not validated	9	0.55(0.35-0.88)	<0.01	88.00%	0.18	4	0.76(0.58-0.99)	0.14	45.50%	**0.04**
Number of cases										
>100	11	0.51(0.29-0.90)	<0.01	79.00%		10	0.97(0.82-1.15))	0.11	37.90%	
<100	6	0.76(0.59-0.97)	<0.01	80.70%	0.57	2	0.81(0.57-1.17)	0.08	66.50%	0.37
Adjustment for confounders										
Hepatitis										
Yes	11	0.68(0.49-0.94)	<0.01	66.70%		7	1.05(0.87-1.28)	0.32	14.50%	
No	6	0.71(0.50-1.0)	<0.01	89.20%	0.92	5	0.84(0.68-1.04)	0.05	57.00%	0.15
BMI										
Yes	7	0.73(0.58-0.92)	<0.01	71.50%		4	1.01(0.87-1.18)	0.44	0.00%	
No	10	0.66(0.43-1.02)	<0.01	82.70%	0.90	8	0.86(0.69-1.08)	0.04	52.90%	0.25
Alcohol										
Yes	12	0.71(0.56-0.90)	<0.01	73.00%		7	1.01(0.86-1.18)	0.32	14.90%	
No	5	0.72(0.43-1.22)	<0.01	85.60%	0.90	5	0.83(0.63-1.09)	0.05	58.00%	0.22
Smoking										
Yes	10	0.81(0.74-0.90)	0.14	38.20%		7	0.98(0.82-1.17)	0.15	36.90%	
No	7	0.76(0.57-1.02)	<0.01	86.50%	0.77	5	0.84(0.63-1.13)	0.06	56.10%	0.40
Energy intake										
Yes	5	0.80(0.66-0.97)	0.30	18.30%		5	0.99(0.80-1.21)	0.02	18.20%	
No	12	0.64(0.47-0.87)	<0.01	84.60%	0.43	7	0.90(0.72-1.13)	0.30	58.90%	0.65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Web of Science (n= 4032)
EMBASE (n= 1945)
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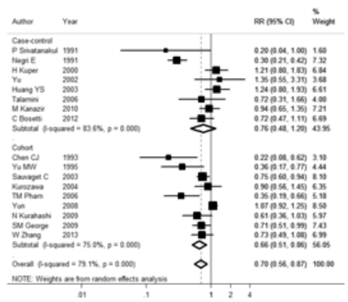
Abstracts (n=221)
Excluded by abstract review (n= 158)
Articles comprehensively assessed (n= 63)
Excluded by full-text article review (n= 44)
Abstracts or reviews or editorials (n= 8)
Did not evaluate the association of interest (n=26)
Publications without RR or 95%CI (n= 7)
Data the same population (n=3)
Articles included from the reference review (n= 1)
Studies included in the meta-analysis (n=19)
Cohort studies (n=10)
Case-control studies (n= 9)



Author
Year
RR (95% CI)
% Weight
Case-control
P Srivatanakul 1991 0.20 (0.04, 1.00) 1.60
Negri E 1991 0.30 (0.21, 0.42) 7.32
H Kuper 2000 1.21 (0.80, 1.83) 6.84
Yu 2002 1.35 (0.55, 3.31) 3.68
Huang YS 2003 1.24 (0.80, 1.93) 6.61
Talamini 2006 0.72 (0.31, 1.66) 4.00
M Kanazir 2010 0.94 (0.65, 1.35) 7.21
C Bosetti 2012 0.72 (0.47, 1.11) 6.69
Subtotal (I-squared = 83.6%, p = 0.000) 0.76 (0.48, 1.20) 43.95
Cohort
Chen CJ 1993 0.22 (0.08, 0.62) 3.10
Yu MW 1995 0.36 (0.17, 0.77) 4.44
Sauvaget C 2003 0.75 (0.60, 0.94) 8.10
Kurozawa 2004 0.90 (0.56, 1.45) 6.35
TM Pham 2006 0.35 (0.19, 0.66) 5.18
Yun 2008 1.07 (0.92, 1.25) 8.50
N Kurahashi 2009 0.61 (0.36, 1.03) 5.97
SM George 2009 0.71 (0.51, 0.99) 7.43
W Zhang 2013 0.73 (0.49, 1.08) 6.99
Subtotal (I-squared = 75.0%, p = 0.000) 0.66 (0.51, 0.86) 56.05
Overall (I-squared = 79.1%, p = 0.000) 0.70 (0.56, 0.87) 100.00
NOTE: Weights are from random effects analysis
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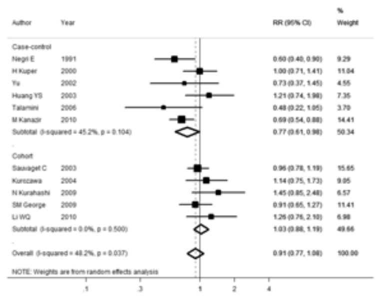
1
2
4


Author
Year
RR (95% CI)
%
Weight
Case-control
Negri E 1991 0.60 (0.40, 0.90) 9.29
H Kuper 2000 1.00 (0.71, 1.41) 11.04
Yu 2002 0.73 (0.37, 1.45) 4.55
Huang YS 2003 1.21 (0.74, 1.98) 7.35
Talamini 2006 0.48 (0.22, 1.05) 3.70
M Kanazir 2010 0.69 (0.54, 0.88) 14.41
Subtotal (I-squared = 45.2%, p = 0.104) 0.77 (0.61, 0.98) 50.34
Cohort
Sauvaget C 2003 0.96 (0.78, 1.19) 15.65
Kurozawa 2004 1.14 (0.75, 1.73) 9.05
N Kurahashi 2009 1.45 (0.85, 2.48) 6.57
SM George 2009 0.91 (0.65, 1.27) 11.41
Li WQ 2010 1.26 (0.76, 2.10) 6.98
Subtotal (I-squared = 0.0%, p = 0.500) 1.03 (0.88, 1.19) 49.66
Overall (I-squared = 48.2%, p = 0.037) 0.91 (0.77, 1.08) 100.00
NOTE: Weights are from random effects analysis
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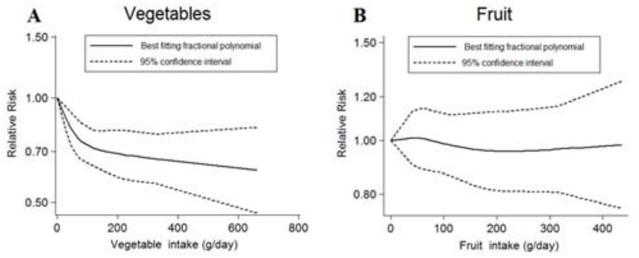
.3
1
2
4


A
Vegetables
Best fitting fractional polynomial
95% confidence interval
Relative Risk
1.50
1.00
0.70
0.50
0
200
400
600
800
Vegetable intake (g/day)
B
Fruit
Best fitting fractional polynomial
95% confidence interval
Relative Risk
1.50
1.20
1.00
0.80
0
100
200
300
400
Fruit intake (g/day)