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Cirmi, S., Navarra, M., Woodside, J. V., & Cantwell, M. M. (2018). Citrus fruits intake and oral cancer risk: A systematic review and meta-analysis. *Pharmacological Research*, 133, 187-194.
<https://doi.org/10.1016/j.phrs.2018.05.008>

Published in:
Pharmacological Research

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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***Citrus* fruits intake and oral cancer risk: a systematic review and meta-analysis**

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Declarations of interest

none

Abbreviations

MeSH, Medical Subject Heading; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; OR, odds ratio; CI, confidence interval; BMI, body mass index; DNA, Deoxyribonucleic Acid; FFQ, food frequency questionnaire.

ABSTRACT

Objective: To quantify the relationship between *Citrus* intake and risk of cancer of the oral cavity and pharynx.

Design: Systematic review and meta-analysis.

Data sources: Ovid MEDLINE, EMBASE, and Web of Science were searched until September 2017. Search terms included *Citrus*, *Citrus aurantifolia*, *Citrus sinensis*, *Citrus paradisi*, *Citrus* fruits, *Citrus* fruits extract, *Citrus* oil, fruits, oral cancer, mouth cancer, mouth neoplasm.

Study selection: The selection of studies and the systematic review were carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A pre-defined inclusion checklist resulted in the inclusion of articles which were (i) published in peer-reviewed scientific journals; (ii) English language; (iii) and included a measure of *Citrus* fruit intake and risk of oral and pharyngeal cancer. Studies were excluded if (i) preparations derived from other fruits were used, (ii) *Citrus* intake was combined with intake of other fruits; (iii) *in vitro* or animal models were used. We also excluded reviews, systematic reviews, meta-analyses, letters, personal opinions, conference abstracts and book chapters.

Data extraction: Three reviewers independently performed the extraction of data from studies included.

Results: Seventeen studies met our inclusion criteria and were included in the final review. Pooled analyses showed that those with the highest *Citrus* fruit intake compared to the lowest intake had a 50% reduction in risk of oral cavity and pharyngeal cancer (OR 0.50; 95% CI 0.43-0.59).

Conclusion: The studies included in this review and meta-analysis showed an inverse association between *Citrus* fruit intake and oral cancer.

Key word: *Citrus* fruits, oral cancer, cancer, pharynx, alternative and complementary medicine

INTRODUCTION

Oral cancer accounts for over 2% of the overall burden of cancer globally, with an incidence of 300,000 cases in 2012 (1) and it is the 7th major cause of death from cancer in Europe (2).

Patients with oral cancer have a poor prognosis despite advances in treatment as they continue to present with late stage disease. Indeed, the 5-year survival rate for all oral and pharyngeal cancers is only 47% for men and 55% for women (3).

Reducing the incidence of this disease through prevention is vital due to the high morbidity rates. Discovery of new drugs or novel therapeutic approaches, designed to prevent the development of this cancer, remains an important goal for scientists. Despite continuous development of synthetic drugs which are the basis of pharmaceutical care, the plant kingdom still remains an attractive source of novel anti-cancer drugs. Indeed, a large amount of anti-cancer drugs originate from natural sources (4). Moreover, it has been demonstrated that regular consumption of fruits and vegetables may play an important role in reducing cancer risk (5-8). It is estimated that a high dietary intake of vegetables and fruits (>400 g/day) could prevent at least 20% of all cancer cases (9, 10). However, other studies have cast some doubts over whether their intake is really producing these cancer risk reductions (5, 11). For instance, the results from a large European prospective cohort suggested that higher a consumption of fruit and vegetables was not associated with decreased risk of pancreatic cancer (12).

In the last decade, numerous epidemiological studies have provided evidence to suggest that regular intake of *Citrus* fruits may be associated with a reduced risk of all-cancers, although significant results were obtained only for prostate and pancreatic cancers (13). Moreover, Foschi and collaborators (14) have indicated that *Citrus* fruits have a protective role against

cancers of the digestive and upper respiratory tract, as well as Gonzalez and co-workers who have observed an inverse dose response relationship between total *Citrus* fruits intake and gastric cancer risk (15). Furthermore, Bae and co-workers have provided evidence for the protective effects of a high *Citrus* fruit intake on risk of both gastric (16) and pancreatic cancer (17) but no association with prostate cancer risk (18). More recently, meta-analyses have highlighted an inverse association between *Citrus* fruit intake and risk of the breast (19), bladder (20-22) and esophagus (23) cancer. In contrast, a recent systematic literature review of prospective studies showed only a marginally significant decrease in risk of esophageal cancer and a non-significant inverse association for gastric cardia cancer (24).

Due to the conflicting evidence regarding the effects of *Citrus* fruit intake on cancer, the aim of this systematic review and meta-analysis was to quantify the relationship between *Citrus* fruit intake and cancer of the oral cavity and pharynx.

METHODS

Search Strategy

The bibliographic databases Ovid MEDLINE (1946-present), EMBASE (1974-present), and Web of Science (1970-present) were searched independently by two reviewers (SC and MN) for literature related to *Citrus* fruit intake and risk of cancer of the oral cavity and pharynx. In order to retrieve all relevant papers, no limit was placed on search time frame. The last search was performed on September 25th, 2017.

The search strategy identified studies that contained at least one keyword or Medical Subject Heading (MeSH) term from each of the following exposures: (i) *Citrus*, *Citrus aurantifolia*, *Citrus sinensis*, *Citrus paradisi*, *Citrus* fruits, *Citrus* fruits extract, *Citrus* oil, fruits; or any of these terms combined (ii) oral cancer, mouth cancer, mouth neoplasm. The search strategy also incorporated limits to studies conducted on humans and in English language. An

example of full electronic search strategy for EMBASE is provided in **Table 1**. Citations, titles and abstracts were exported into Endnote X5.

The study selection and systematic review were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement following an a priori-established protocol registered with PROSPERO (CRD42017081718) (25).

The reviewers (SC, MN and MMC) initially screened titles and abstracts to remove obviously irrelevant articles and screened full text articles independently to identify studies for inclusion in the systematic review. Discrepancies were resolved by discussion. Reference lists of included articles were also searched for other relevant studies. The following exclusion criteria were applied: (i) studies using preparations derived from other fruits, (ii) studies in which intake was combined with a combination of other fruits; (iii) studies carried out *in vitro* or in animal models. We also excluded reviews, systematic reviews, meta-analyses, letters, personal opinions, conference abstracts and book chapters. All reviewers had previous experience with systematic reviews.

Data extraction

A standardized data collection protocol was used for gathering data: apart from results, study authors, publication year, residence of participants, age and gender distribution, study design, number of cases and controls, dietary assessment method used to measure *Citrus* fruit intake and outcome examined, details of the adjustment for confounders, and other variables were recorded. Corresponding authors were contacted for extra study details to enable evaluation and/or analysis if these were not reported in the articles.

Statistical analysis

Meta-analyses were conducted by comparing cancer risk in the highest reported category of *Citrus* fruit intake with that in the lowest reported category. Adjusted odds ratios (ORs) and 95% CIs were combined and weighted to produce pooled ORs using a random-effects model, to account for heterogeneity between studies. The extent of heterogeneity in the pooled estimates was investigated using the chi-squared test and the I^2 statistic: larger I^2 values indicate greater heterogeneity (26). Separate analyses were conducted for case-control and cohort studies.

Risk of publication and selection bias was evaluated by checking for asymmetry in the funnel plots of the study OR against the standard error of the logarithm of the OR (27). Statistical analysis was conducted using Stata 14.2 (StataCorp LP, Texas, USA).

RESULTS

The flowchart for study selection is shown in **Figure 1**. Following initial screening of 481 titles (397 after removal of duplicates) we identified 41 full text articles for review, of which 17 were included that examined the relationship between *Citrus* fruit intake and risk of cancer of the oral cavity and pharynx.

Articles excluded because they were conference abstracts or book chapters were n=16.

Records excluded after title and abstract screen were n=340. Articles excluded after full text review were n=24: one because it was a review, one because it was a commentary, twenty-one because the Authors tested the effects of fruits intake not specifying if they were *Citrus* fruits. The paper of Grideley and co-workers (1990) was excluded because the Authors did not report the confidence interval values (28).

The characteristic of studies investigating *Citrus* fruit intake and the risk of oral cavity and pharynx cancers are shown in **Tables 2 and 3**. Most of the included studies (n=15) originated from hospital-based case-control studies (29-43), only two studies are cohort studies (44, 45)

and all included studies utilized food frequency questionnaires to measure dietary intake (Tables 2 and 3). Most studies adjusted for age, sex, smoking status, alcohol use and education level; some also accounted for energy intake and body mass index (BMI) (Table 4). Adjustment for other confounders varied between studies (Table 4).

Figure 2 shows the results for all studies comparing the risk of oral cavity and pharyngeal cancer in those with the highest versus the lowest intake of *Citrus* fruit. The pooled risk estimate using the random-effects model showed a 50% statistically significant reduction in risk of oral cavity and pharyngeal cancers associated with *Citrus* fruit intake (OR 0.50; 95% CI 0.43-0.59).

There was moderate heterogeneity between the studies $I^2=43.9\%$; $P=0.024$, but this was not statistically significant. No clear evidence of publication bias was found on visual inspection of the funnel plot (**Figure 3**).

The protective effect of *Citrus* fruit was much stronger in case-control studies than in cohort studies only. In particular, the pooled risk of case-control studies showed a 53% statistically significant reduction in risk of oral cavity and pharyngeal cancers associated with *Citrus* fruit intake (OR 0.47; 95% CI 0.40-0.55) (**Figure 4**) while the reduction of risk in cohort studies was 27% (OR 0.73; 95% CI 0.55-0.96) (**Figure 5**). The heterogeneity was reduced in stratified analysis by study design ($I^2=36.3\%$; $P=0.073$ and $I^2=0\%$; $P=0.806$ for case-control and cohort studies, respectively) (Figures 4 and 5).

DISCUSSION

We investigated the association between *Citrus* fruit intake and the risk of oral cancer using a meta-analysis of existing epidemiologic studies. To the best of our knowledge, this is the first systematic review and meta-analysis of oral cancer and *Citrus* fruit intake. The major finding of the study suggests that dietary intake of *Citrus* fruits plays an important protective role in

the prevention of oral cancer with a pooled OR 0.50 (95% CI 0.43-0.59) for those in the highest category of intake. A higher *Citrus* fruit intake has also been shown to be inversely associated with cancer at other site such as stomach, pancreas, breast, bladder and oesophagus (15, 17, 19, 20, 23). Only 8 papers (47%), among all taken into account, display a dose-response relationship (30, 32, 33, 37, 39-41, 43).

The main limitation of this meta-analysis is that almost all the studies were hospital-based case-control studies; it is likely therefore that *Citrus* fruit intake will have been influenced by recall bias and it is not possible to demonstrate a temporal relationship between *Citrus* fruit intake and oral cancer risk. In addition, the use hospital-based controls may have affected the results of individual studies, as they are unlikely to be representative of healthy controls.

A random-effects model was used to pool the results from individual studies to account for heterogeneity between the studies included. In addition, although all the selected studies had collected information on factors considered to be potential confounders, such as demographic characteristics, smoking, alcohol, BMI and energy intake, the factors adjusted for the individual analyses of *Citrus* fruit intake varied.

There are numerous biologically plausible mechanisms whereby *Citrus* fruit intake may affect cancer risk. For example, *Citrus* fruits are rich in vitamin C that has been investigated since the early 1980's in epidemiological studies on upper aero-digestive tract cancer, with the most of the studies demonstrating inverse associations (46, 47). Among possible mechanisms of anti-cancer action, vitamin C has been hypothesized to counteract inflammation and subsequent oxidative damage to DNA, which play a role in the initiation and progression of cancer. Moreover, due to its pro-oxidant capacity, vitamin C can act as cancer cells killer (48, 49). Furthermore, this nutrient may act synergistically with other biological antioxidants and radical scavengers in quenching different elements of a radical

cascade. This might also support the strongest evidence in favour of fruit and vegetables, as compared to that focusing on nutrients (50, 51).

In addition to high vitamin C content, *Citrus* fruits contain a wide range of bioactive compounds, such as flavonoids, carotenoids, and limonoids. The flavonoids from *Citrus* fruits have been shown to have a protective effect against oxidative stress, inflammation, infection, cardiovascular disease, neurodegenerative disease and some types of cancer (52-56). It is likely that *Citrus* fruits reduce cancer risk by inhibition of oxidative stress and oxidative damage and by interfering with the initiation, promotion, and progression of cancer (57). It is inconclusive whether the antitumor effect of polyphenols is related to their antioxidant effects, however flavonoids which have superior free radical scavenging properties are more effective antioxidants than vitamin C, vitamin E, and carotenoids (58).

In the last two decades, numerous pre-clinical studies have documented the anti-proliferative activity of flavonoids, which has been largely linked to their abilities to interact with specific intracellular signalling pathways, in addition to their well-known antioxidant capacity (57, 59-64). In this field, recently, we have reported the anti-cancer activity of *Citrus bergamia* (bergamot) juice (BJ) in different *in vitro* (65, 66) and *in vivo* (67) models, and have proposed that its flavonoid content is responsible for this action (68). We have also shown the anti-proliferative effects of a flavonoid-rich extract from mandarin juice on human anaplastic thyroid carcinoma cells (69) and the pro-apoptotic activity of essential oils extracted from the peel of bergamot fruits (70). However, the majority of experimental studies aimed to evaluate the anti-cancer effect of *Citrus* juices have been performed *in vivo* (71-74).

In conclusion, on the basis of current epidemiologic evidence, we found that the consumption of *Citrus* fruits was associated with a reduced risk of oral cancer. Prospective studies, which are less prone to recall and selection bias, are needed to confirm this result. Data from *in vitro* and *in vivo* studies suggest that particular subtypes of *Citrus* fruit may affect cancer risk

differently and so further epidemiological research is required to examine these relationships in humans. This would provide the data necessary to develop a novel therapeutic approach, using *Citrus* fruits to prevent the development of oral cancer perhaps in high-risk groups such as smokers and heavy drinkers of alcohol.

Conflict of interest statement

The authors declare that the research was carried out in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Author contributions:

SC: performed the systematic literature search, data extraction, statistical analysis and drafting the paper;

MN: conceived the study, performed the systematic literature search, data extraction, and critically revised the paper;

JVW: critically revised the paper;

MMC: conceived the study, performed data extraction and critically revised the paper.

All authors read and approved the final manuscript.

Sources of Support

A fellowship from the “Prof. Antonio Imbesi” Foundation, Messina, Italy to S. Cirmi is gratefully acknowledged.

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Search strategy for EMBASE	
Limit applied: “English” and “Human”	
1. <i>Citrus</i>	6. 1 OR 2 OR 3 OR 4 OR 5
2. <i>Citrus</i> fruits	7. oral cancer
3. <i>Citrus</i> fruits extract	8. mouth cancer
4. <i>Citrus</i> oil	9. 7 OR 8
5. fruits	10. 6 AND 9

Table 1. Full electronic search strategy for EMBASE

reference	country	study design	n° of cases	n° of controls	study population	method of dietary assessment	<i>Citrus</i> exposure categories
Franco et al., 1989	Brazil	hospital based case-control	232	464	M/F	interview using 20-item FFQ	tertiles
Zheng et al., 1992*	China	population based case-control	39	269	M	interview using 41-item FFQ	tertiles
Zheng et al., 1992**	China	population based case-control	21	145	F	interview using 41-item FFQ	tertiles
Zheng et al., 1993	China	hospital based case-control	407	400	M/F	interview using 63-item FFQ	tertiles
Levi et al., 1998	Vaud	hospital based case-control	156	284	M/F	interview using 79-item FFQ	tertiles
Franceschi et al., 1999	Italy	hospital based case-control	598	1491	M/F	interview using 78-item FFQ	quintiles
Tavani et al., 2001	Italy	hospital based case-control	132	148	M/F	interview using 25-item FFQ	tertiles
Garrote et al., 2001	Cuba	hospital based case-control	200	200	M/F	interview using 25-item FFQ	tertiles
Nishimoto et al., 2002	Brazil	hospital based case-control	784	1564	M/F	interview using FFQ	tertiles
Lissowska et al., 2003	Poland	hospital based case-control	122	124	M/F	interview using 25-item FFQ	tertiles
Rajkumar et al., 2003	India	hospital based case-control	591	582	M/F	interview using FFQ	tertiles

Sanchez et al., 2003	Spain	hospital based case-control	375	375	M/F	interview using 25-item FFQ	tertiles
De Stefani et al., 2005	Uruguay	hospital based case-control	230	460	M	interview using 64-item FFQ	quartiles
Kreimer et al., 2006	Italy, Spain, Poland, Northern Ireland, India, Cuba, Canada, Australia and Sudan	hospital based case-control	1670	1732	M/F	interview using FFQ	quartiles
Toporcov et al., 2012	Brazil	hospital based case-control	296	296	M/F	interview using FFQ	quartiles
Bravi et al., 2013	Italy, Switzerland	hospital based case-control	768	2078	M/F	interview using 78-item FFQ	quintiles

Table 2. Characteristic of case-control studies included in the meta-analysis assessing the relationship between *Citrus* intake and the risk of oral cavity and pharynx cancers. Abbreviations: M, male; F, female; FFQ food frequency questionnaire; *male; **female

reference	country	study design	study population	method of dietary assessment	<i>Citrus</i> exposure categories
Boeing et al., 2006	Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, United Kingdom	cohort	M/F	interview using 300/350-item FFQ	quintiles
Maasland et al., 2015	Netherlands	cohort	M/F	interview using 150-item FFQ	quartiles

Table 3. Characteristic of cohort studies included in the meta-analysis assessing the relationship between *Citrus* intake and the risk of oral cavity and pharynx cancers. Abbreviations: M, male; F, female; FFQ food frequency questionnaire

Reference	Adjusted counfounders									
	Age	Sex	Ethnicity	Energy intake	BMI	Smoking status	Alcohol use	Chewing habit	Education level	Urban/rural status
Franco et al., 1989	✓	✓							✓	
Zheng et al., 1992*	✓									
Zheng et al., 1992**	✓									
Zheng et al., 1993	✓	✓				✓	✓		✓	
Levi et al., 1998	✓	✓		✓		✓	✓		✓	
Franceschi et al., 1999	✓	✓		✓		✓	✓		✓	✓
Tavani et al., 2001	✓	✓				✓	✓		✓	
Garrote et al., 2001	✓	✓				✓	✓		✓	✓
Nishimoto et al., 2002			✓			✓	✓		✓	✓
Lissowska et al., 2003	✓	✓				✓	✓			✓
Rajkumar et al., 2003	✓	✓				✓	✓	✓	✓	✓
Sanchez et al., 2003	✓	✓				✓	✓		✓	✓
De Stefani et al., 2005	✓			✓	✓	✓	✓		✓	✓
Kreimer et al., 2006	✓	✓	✓		✓	✓	✓	✓	✓	
Boeing et al., 2006	✓	✓		✓	✓	✓	✓		✓	
Toporcov et al., 2012	✓			✓		✓	✓			
Bravi et al., 2013	✓	✓	✓	✓	✓	✓	✓		✓	
Maaslan et al., 2015	✓	✓				✓	✓			

Table 4. Characteristic of studies included in the meta-analysis. Abbreviations: BMI, body mass index

Figure 1. PRISMA flow chart showing the process of literature search and study selection.

Figure 2. Meta-analysis of risk of oral cancer in the highest versus lowest category of *Citrus* intake. °Test for heterogeneity: $\chi^2=30.30$, $df=17$, $P=0.024$; $I^2=43.9\%$. *man; **woman

Figure 3. Funnel plot of studies evaluating the association between *Citrus* fruit intake and risk of cancer of the oral cavity and pharynx. Dotted lines indicate the 95% pseudo-confidence intervals. SE=standard error; OR=odds ratio.

Figure 4. Meta-analysis of risk of oral cancer in the highest versus lowest category of *Citrus* intake (case-control studies). °Test for heterogeneity: $\chi^2=23.54$, $df=15$, $P=0.073$; $I^2=36.3\%$. *man; **woman

Figure 5. Meta-analysis of risk of oral cancer in the highest versus lowest category of *Citrus* intake (cohort studies). °Test for heterogeneity: $\chi^2=0.06$, $df=1$, $P=0.806$; $I^2=0\%$