**ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ**

**ΙΑΤΡΙΚΗ ΣΧΟΛΗ**

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**ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ**

# «ΚΛΙΝΙΚΕΣ ΜΕΛΕΤΕΣ: ΣΧΕΔΙΑΣΜΟΣ ΚΑΙ ΕΚΤΕΛΕΣΗ»

**MSc: "Clinical Trials: Design andConduct"**

**Διευθυντής και Επιστημονικός Υπεύθυνος**

**Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ**

# "Διατροφικές παράμετροι και καρκίνος της ουροδόχου κύστης: συστηματική ανασκόπηση και μετά ανάλυση προοπτικών μελετών"

# "Dietary parameters and bladder cancer: a systematic review and meta-analysis of prospective cohort studies"

**Όνομα: ΔΗΜΗΤΡΑ ΞΕΝΟΥ**

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**Επάγγελμα/ή Ιδιότητα: Φαρμακοποιός, MSc**

**Επιβλέπουσα καθηγήτρια: Κα Θεοδώρα Ψαλτοπούλου**

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## ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

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## Abstract

We examined the association between fruit/vegetable consumption and bladder cancer (BC) risk in a systematic review and meta-analysis of prospective cohort studies, stratifying results by gender, smoking status and geographical region. Eligible studies were sought in MEDLINE and EMBASE up to April 20, 2020. Random-effects (Der Simonian-Laird) models were implemented for the calculation of pooled relative risks (RRs) and 95% confidence intervals (CI). Fifteen eligible studies were identified (1,855,277 subjects, 125,029 BC cases). Vegetable consumption (pooled RR=0.95, 95% CI:0.87-1.04, n=10) as well as combined fruit/vegetable consumption was not associated with BC risk. Regarding fruit intake, the overall protective trend did not reach significance (pooled RR=0.91, 95%CI: 0.81-1.02, n=11); we found however a significant association in East Asians. A trend towards a protective association with citrus fruit consumption was also noted (pooled RR=0.83, 95%CI: 0.69-1.01, n=6), once again with a significant effect in East Asians. Moreover, no association was found regarding the subgroups of leafy vegetables, dark green vegetables, and berries. Single study arms pointed to a reduced BC risk in never smoking males consuming cruciferous vegetables and East Asians consuming yellow vegetables. In conclusion, our study reveals possible protective effects; larger studies are needed to investigate the emerging trends.

**Key words:** fruits; citrus; yellow vegetables; cruciferous; bladder cancer; meta-analysis; meta-regression

## A. Introduction

### 1. Bladder cancerτ

### 1.1. Epidemiology

Bladder Cancer (BC) is the ninth most common cancer worldwide(1,2), most commonly diagnosed malignancy concerning males and the eleventh one, when it comes to both genders(3). Gender represents a major risk factor for BC; according to the GLOBOCAN database, about 430,000 new BC cases and 165,000 deaths due to BC have occurred worldwide in 2012, with 75% of total cases occurring in men(3). Antoni et al. (2017)(4)in their review about recent incidence and mortality trends, observed that incidence rates varied according to sex and Human Development Index (HDI); the highest rates have been found in men from very high HDI countries, 16.7 per 100 000, and the lowest in women from low and medium HDI countries. Overall, the lowest incidence and mortality rates were found in Central and South America, Sub-Saharan Africa, and South East Asia(4,5).

Regarding the age-standardized incidence rate (per 100,000 person/years) for men is 9.0 in global scale and 19.1 in European Union while for women it drops to 2.2 and 4.0 respectively(5,6).European reports show that Belgium holds the highest age-standardized incidence rate (31 in men and 6.2 in women) and Finland holds the lowest (18.1 in men and 4.3 in women)(5,7). The variety in incidence rates may partly be attributed to different risk factors across the countries, different practices in diagnosis and detection of tumor and different methodology, whether for example the different national registries include or not the UBC stage Ta or carcinoma in situ(6,8).

As far as prevalence is concerned, according to GLOBOCAN, BC prevalence is among the highest for all urologic malignancies(5) while mortality is mainly estimated by progression rates of high-risk non–muscle-invasive BC and by cure rates of muscle-invasive BC(5). Non-muscle invasive bladder cancer has higher prevalence because the progression rates are low and allow a longer patient survival while muscle-invasive bladder cancer, is characterized by high progression rates and thus the higher incidence rates (6).

According to Mahdavihar et al. (2012) (9), 165,084 bladder death cases occurred in the world, with the highest number of death cases being seen in countries such as China (26,820 cases), the United States (16,468 cases), India (9,523 cases), Japan (7,630 cases), and Russia (6,843 cases,) respectively. Regarding women most of the death cases were observed in China (6,762 cases), the United States (4,662 cases), Japan (2,462 cases), Germany (1,861 cases), and India (1,859 cases), respectively(9).

However, the incidence and mortality have decreased in many Western countries (North America, and West and Northern Europe) in contrast to other European countries (South, Central, and East Europe) and developing countries in Asia which show increase in rates(10). This trend can be explained due to differences in risk factors for bladder cancer (tobacco epidemic, changes in coding practices, the prevalence of Schistosoma Haematobium, especially in Africa, and occupational exposures)(10).

## 1.2 Aetiology

Substantial knowledge exists concerning the aetiology of bladder cancer. Epidemiological studies have identified carcinogens which are associated with most cases of bladder cancer risk while genetic effects might play a direct role in the initiation and progression of the disease(11). However, there are several risk factors reported in many studies which may have a different impact on the incidence and pathophysiology of BC: tobacco smoking, genetic susceptibility, occupational risk, dietary factors, environmental pollution, gender along with race and socioeconomic status, medical conditions.

### 1.2.1. Tobacco smoking

Tobacco smoking is recognized as the most important risk factor for BC and is accounts for more than 50% of tumors (10,12,13). It has been reported that 90% of cases with bladder cancer are linked to smoking history (14) with past smokers showing two times higher possibility to develop bladder cancer comparing to the control group (HR=2.22, 95% CL:2.03–2.44) while current tobacco smokers showing four times (HR=4.1, 95% CI:3.7–4.5)(12). Regarding pathophysiologic pathways of the association between tobacco smoking and BC, Burger et al. (2013) (6) suggested that aromatic amines such as b-naphthylamine, and polycyclic aromatic hydrocarbons contained in the smoke, are excreted by the renal and therefore exert a carcinogenic effect on the entire urinary system.

Moreover, it has been observed that there are differences in incidence rates between genders due to hormonal differences and different historical smoking patterns (6).In most Western communities, the prevalence of smoking among men was much higher than among women in 1950s whereas an opposite trend was observed in the second half of the previous century(15) leading to a decrease in the mortality rate (10). The opposite trend was observed in Eastern Europe where the increase in the incidence and mortality is due to the increase in the prevalence of cigarette smoking(10).

According to Burger et al.(2013) (6), there are some parameters which could explain the reducing prevalence of BC. Firstly, smoking populations are changing; it has been observed that higher-educated and more health-oriented persons are more prompt to quit smoking habits. Another changing parameter is the composition of tobacco products as well as the change of the type of smoking as it has been reported that the risk in smokers of black tobacco is higher than the risk in smokers of blond tobacco due to the higher concentrations of N-nitrosamine and 2-napthylamine(6). Samanic et al.(2006) (16) found that there is a significant decreased risk with increasing time for smokers who quitted smoking blond tobacco, while there was no such trend in smokers who quitted smoking black tobacco. Moreover, there is no association between low-tar cigarettes and a lower risk of developing bladder cancer (13). Regarding the smoking type, the risk associated with electronic cigarettes has not adequately been proved despite the fact that carcinogens have been identified in urine while it seems that smoking type influences BC risk as the into the chest inhalation increases risk compared with the into the mouth inhalation(12,13,16).

In MIBC (Muscle Invasive Bladder Cancer), the incidence of BC is directly related to the duration of smoking and the number of cigarettes smoked per day(17). A meta-analysis in 216 observational studies examining the link between cigarette smoking and cancer published between 1961 and 2003, demonstrated a significant association for both current and former smokers (18). Last but not least, according to Brennan et al. (2000) (17), an immediate decrease in the risk of BC was observed in those who stopped smoking with a reduction of about 40% within one to four years of quitting smoking and 60% after 25 years of quitting.

### 1.2.2. Occupational exposure

The second most important risk factor for BC, accounting for about 10% of all cases constitutes the occupational exposure to aromatic amines (benzidine, 4-aminobiphenyl, 2-naphthylamine, 4-chloro-o-toluidine), polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons, polycyclic aromatic hydrocarbons and chlorinated hydrocarbons (6). Occupational environment where dyes, rubbers, textiles, paints, leathers, and chemicals are used are associated with cases accounted for 20-25% of all BC cases(19). Past meta-analysis has shown that the risk of BC due to occupational exposure to carcinogenic aromatic amines increases after ten years or more of exposure(20,21). A case–control study by Samanic et al. (2006) (16) suggested that there is an increased risk in bladder cancer in men employed as machine operators in the printing industry whereas no significant associations were reported between occupation and bladder cancer risk among women after smoking duration adjustment(HR: 5.4; 96% CI, 1.6–17.7). Moreover, in a population based case–control study, Koutros et al. (2011) reported no relation between hair dye use and bladder cancer risk in women. However he suggested a gene-related association between N-acetylo-transferase 2 (NAT2) and bladder cancer risk in exclusive users of permanent hair dyes(22). More specifically, they proved that those who had NAT2 slow acetylation phenotype performed an increased risk compared to never users who had the NAT2 rapid acetylation phenotype (HR: 7.3, 95% Cl:1.6–32.6) (22). However, a population-based case–control study by Ros et al. (2012)(23), found no relation between personal hair dye use and UBC incidence (HR: 0.87, 95% CI: 0.65–1.18).

### 1.2.3. Genetic Susceptibility

Martin et al. (2018)(24)in a large scale population study in relatives showed, that genetic susceptibility factors and family association may influence the incidence of BC via their impact on susceptibility to other risk factors such as tobacco smoking. Such genetic factors are the genetic slow acetylator N-acetyltransferase 2 (NAT2) variants and glutathione S-transferase mu 1 (GSTM1)–null genotypes (25-27). The first one may indirectly lead to BCs via the bio-activation and detoxification of tobacco carcinogens by N-acetyl transferase enzymes (NAT1, NAT2) and this fact may explain why the slow NAT2 acetylator genotype is associated with an increased risk of bladder cancer in smokers (HR: 1.31; 95% CI, 1.01–1.70)(25,26). Regarding GSTM1-null genotypes, similarly with NAT2, are related to reduced detoxification

function, increased susceptibility to cytogenetic damage, and increased risk of cancer(28,29). Previous meta-analyses have indicated an association of GSTM1 and GSTT1 deletion polymorphisms with increased bladder cancer risk (30,31). Kiemeney et al. (2010) (32), recently reported data from a large genome-wide association study demonstrating a sequence variant on 4p16.3 which is not only associated with BC but it is also located close to the growth factor receptor 3 (FGFR3) and it is often mutated in low-grade in non-invasive UBC. Three large genome-wide association studies demonstrated eight common sequence variants associated with UBC located at 8q24.21, 3q28, 8q24.3, 4p16.3, 22q13.1, 19q12, 2q37.1, and 5p15.33(33).These genetic polymorphisms may influence disease progression and introduce variability in the way different people react to the risk factors previously mentioned.

### 1.2.4. Environmental pollution

It has been reported that arsenic in drinking water is considered to be one of the risk factors of BC(34,35). There are several examples which prove that the arsenic pollution if water has increases the bladder cancer mortality. For example, arsenic pollution of drinking water in Bangladesh caused the lifetime mortality risk from BC to double(36).In Chile, was observed that BC mortality was significantly higher in affected regions (HR: 3.6; 95% CI, 3.0–4.7) even more than 20 years after the cessation of such pollution(36). Baris et al. (2016) (37) in their study, reported a significant association between low-to-moderate levels of arsenic in drinking water and bladder cancer risk in New England due to the high concentration of arsenic in dug wells which were extensively used to collect water.

Aristolochic acid (AA) is a nitrophenanthrene carboxylic acid which is naturally produced by Aristolochia plants of the genus Aristolochia and it was used in traditional Chinese medicine (38). There are several studies (39-41) reporting that aristolochic acid induces highly distinctive mutation signatures in the genomes of upper urinary tract urothelial cell carcinoma, associating it with increased risk of urinary tract cancer. Moreover, Lai et al (2010), conducted a population-based case-control study in Taiwan suggesting that there is an increased risk of urinary tract cancer in people who ingested more than 60 grams of the Chinese herb Mu-tong in a dose response manner; however the risk was independent to the aristolochic exposure.

### 1.2.5. Gender

With regards to gender, women have a lower UBC incidence and a higher mortality rate than men(42). Patafio et al (2015) in their population- based study showed evidence that all patients treated with the same therapy, showed no differences, between males and females, in overall survival (OS), mortality and outcomes. A possible reason for the females' worse survival may be the delay in the diagnosis that women experienced as the differential diagnosis in women includes diseases that are more prevalent than BC (43). However, opposing to these findings, the meta-analysis by Liu et al. (2015), reported that females had a worse survival outcome comparing to males, after radical cystectomy (HR=1.20, 95% CI: 1.09-1.32) (44).

Furthermore, a large prospective cohort study examining the association between post-menopausal status and BC risk, found an increase in bladder risk even after adjustment for smoking status maybe because of the differences in oestrogen and androgen levels between men and women (45-47).

### 1.2.6. Medical conditions

Medical conditions may lead to bladder cancer tumor genesis through either directly or as a side effect of treatment; directly via chronic urinary retention and upper tract dilation increasing urothelial exposure to carcinogens and indirectly via carcinogenesis associated with chronic inflammation or schistosomiasis (6). Chronic urinary infections, urinary calculi and chronic irritation or inflammation of the urothelium have been positively associated with BC in meta-analysis by Yu et al. (2018) (48). Schistosomiasis, a chronic cystitis caused by a parasitic trematode, is the second most common parasitic infection after malaria, in some parts of northern Africa, Asia, South America, and the Caribbean and it is related indirectly to bladder cancer because it can progress to squamous cell carcinoma (SCC) (49). However, better control of the disease is decreasing the incidence of SCC of the bladder in endemic zones such as Egypt (50,51).

As far as metabolic disorders are concerned, in a large prospective study (52)pooling six cohorts from Norway, Sweden, authors found that metabolic disorders such as elevated blood pressure and triglycerides, were associated with increased risk of BC among men, whereas high BMI was associated with decreased BC risk. Regarding Diabetes Melitus (DM), Xu et al (2017) (53) reported a possible association with BC or cancer mortality risk in men while studies examining the association between its

therapeutic categories (thiazolidinediones (pioglitazone and rosiglitazone) are oral hypoglycaemic drugs)and BC have leaded to inconsistent results. However in a recent meta-analysis of observational studies, Mehtala et al. (2019) (54) suggested that pioglitazone use was significantly associated with an increased risk of BC which was furtherassociated with higher dose and longer duration of treatment.

Concerning the therapeutic treatments of medical conditions, some of them are associated with bladder cancer risk. Abern et al. (2013) (55) found an increased age-standardized incidence rate of UBC following external-beam radiotherapy for prostate cancer (HR: 1.70; 95% CI, 1.57–1.86) while Chrouser, K. et al. (2008) (56), found that increased rates of secondary bladder malignancies have been reported after external-beam radiotherapy (EBRT) for gynaecological malignancies. Similarly, Neider et al. (2008), in a population-based cohort study, confirms the increased bladder cancer incidence after radical prostatectomy (RP), EBRT, brachytherapy, and EBRT-brachytherapy (standardized incidence ratios 0.99, 1.42, 1.10, and 1.39) (57). However, Zelefskyet al. (2012), proposed that modern type of radiotherapy for prostate cancer such as intensity-modulated radiotherapy, may have lower rates of bladder malignancies (58).

### 1.2.7. Dietary factors

There is growing evidence that dietary factors have some linkage with many types of cancer, including UBS. Firstly, fluid intake has been involved in BC because depending on the type of fluid, it may reduce the exposure of urothelial tissue to carcinogens (e.g. arsenic, disinfection by- products)(6). Among the food categories having been accused, meat has been suggested to increase risk while consumption of vegetables and fruits has been suggested to be beneficial (59). Michaud et al. (2007)in his case–control study found that subjects consuming greater amounts of fluids had a lower UBC risk comparing to those who consumed smaller amounts of fluids per day (HR= 0.47, 95% CI: 0.33–0.66)(60).

Moreover, chlorination of drinking water and subsequent levels of trihalomethanes have been considered as possible carcinogenic factors (61).Concerning the latter ones, Villanueva et al. (2006),in their analysis of pooled case–control studies, found that UBC risk has higher when participants exposed to trihalomethanes and this is related to consumption of tap water independently from chlorination (62).

Zamora-Ros et al.(2014) (63) suggested an inverse association between dietary intake of flavonoids and lignans and the risk of aggressive BC tumors. Mediterranean diet plays also a possible protective role against bladder cancer risk because it includes a high consumption of fruits, vegetables, non-saturated fat (olive oil) and moderate consumption of protein, resulting in a possible decreased of BC risk (HR= 0.85, 95% CI: 0.77, 0.93](64,65). European Prospective Investigation into Cancer and Nutrition (EPIC), an on-going multi-centre cohort study, designed to examine the association between diet, lifestyle, environmental factors and cancer, found no links between BC and fluid intake, red meat, vegetable and fruit consumption(66). Furthermore, the intake of red meat was not associated with UBC after a mean follow-up of 9 years whereas in combined fruits and vegetables group , while comparing the highest tertile with the lowest tertile, a marginal significant association has been suggested (HR= 1.30, 95% CI: 1.00–1.69) (59). Ros et al.(2012) found no inverse association between total fluid intake and BC risk in approximately 250 000 individuals after a mean follow-up of 9 years (23).

Moreover, Villanueva et al. (2006)(62)recently examined the association between coffee consumption and UBC incidence in a case–control study and reported a modest increase in risk among coffee drinkers confounded by smoking. Concerning alcohol consumption, the latest meta-analysis by Vartolomei et al. (2019) (67), suggested a non-significant association between moderate and heavy alcohol consumption and bladder cancer risk. However, heavy consumption of alcohol might increase the risk of BCa in males and in some specific populations(67).

The role of vitamins and minerals in UBC risk has been analyzed et al.(2011) (68) in a prospective series of approximately 80 000 persons (VITamins) with a mean follow-up of 6 years and reported that none of the vitamin, mineral or anti-inflammatory supplements was significantly associated with urothelial carcinoma risk.. There was also no association of intake of selenium and vitamin E and UBC incidence in a secondary analysis of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a prospective placebo-controlled study randomizing patients with placebo, vitamin E, selenium, or combined vitamin E and selenium(69). However, it seems that Vitamin C and Vitamin A constitute important anti-carcinogenic and anti-oxidant nutrients associated with bladder cancer prevention as they are involved in the deactivation of reactive free radicals and repair oxidant-induced injury(70,71).

## 2. Fruits and vegetables

Among the dietary factors which have been associated with bladder cancer prevention, are fruits and vegetables due to the fact that they are rich in vitamins, minerals and other bioactive compounds. Epidemiological data strongly support a protective effect of increased fruits and vegetables consumption against epithelial cancers, mainly of the respiratory and digestive tract(72,73). Current accumulative data also suggested that fruits and vegetables may have a protective effect against cardiovascular disease and prevent from stroke (74-76). Moreover, it was suggested that a diet rich in fruits and vegetables would reduce cancers of the mouth, pharynx, esophagus, lung, stomach, colon and rectum while evidence of probable risk reduction was found for cancers of the larynx, pancreas, breast, and bladder(72,77).

Regarding the per day adequate consumption of fruits and vegetables, there is a wide range of detected intakes and it is difficult to estimate the exact effect of a very high intake of fruits and vegetables on cancer risk(78).

However, scientific guidelines suggested 400g per day while it was observed that people in the lower quartile who ate the least amount of fruits and vegetables showed a two-font relative risk of cancer compared to those who ate the most amount (72). Similarly, in lung cancer, after smoking adjustment, a significant inverse association was observed between the increased consumption of fruits and vegetables an additional 20%-33% reduced lung cancer (72). In the Nurses Health Study the upper quintiles of fruit and vegetable intake were 4.5 and 6.2 servings/day, respectively, while in the Health Professionals Follow-up Study were 4.3 and 5.4 serving/day(79).

Among the kind of vegetables which have been reported for their possible protective role against cancer, constitute the allium vegetables, cruciferous, green leafy and yellow-orange vegetables (77). Allium vegetables (garlic, onion, leeks, and scallions)have been found to be protective for stomach and colorectal cancers because they show antibacterial activity by inhibiting thebacterial conversion of nitrate to nitrite and thus eliminating it from forming carcinogenic nitrosamines (80). Cruciferous vegetables (broccoli, Brussel sprouts, cauliflower and cabbage) contain organosulfur compounds which are involved in the detoxification of carcinogens (81). Green leafy vegetables are rich in lutein and act protectively either by preventing the DNA from free radicals damage (82). There also have high concentration of folate

acid and indirectly prevent from chromosomal damageas folate acid deficiency leads to low DNA methylation (83). Yellow-orange vegetables ( carrots, sweet potatoes, summer squash and pumpkins) are of rich Vitamin A, a beta-carotene metabolite, which plays an important role in epithelial cells differentiation and protects from free radical damage (84).

Last but not least, among fruit categories, citrus fruits hold a point of interest because of their high concentration in Vitamin C. The latter one, is involved in antioxidant pathways due to its ability to reduce the formation of nitrosamine , increase collagen production protecting in this way DNA and membranes from oxidative injury (85,86)

## B. Materials and methods

### *1.1 Search algorithm and eligibility of studies*

This systematic review and meta-analysis was conducted in line with the principles of the Preferred Reporting Items of the Systemic Review and Meta-analyses (PRISMA) guidelines(87).The following databases were examined: MEDLINE and EMBASE. The search of eligible studies was restricted to English language and up to April 20, 2020. The algorithm used for literature searching was: (vegetables OR vegetable OR fruits OR fruit) AND (urothelial OR bladder) AND (neoplasms OR neoplasm OR cancer OR cancers OR carcinoma OR carcinomas OR tumor OR tumors OR neoplasia) AND (prospective OR prospectively OR follow-up OR “followed up” OR cohort OR cohorts OR longitudinal). Studies were reviewed by title, abstract with the most relevant being reviewed fully. Eligible studies were independently identified by two authors (D.X. and L.T.); disagreements were resolved after consensus with a third author (T.N.S.).

Only prospective cohort studies examining the association between fruit or vegetable consumption and risk of BC were considered eligible, while case-control studies, comments and conference abstracts were excluded. Reference lists of included studies as well as previous meta-analyses were screened with the “snowball” procedure to retrieve further eligible studies. In case of overlapping study populations, only the study with the larger sample for the same outcome was retained.

### *1.2 Data extraction*

Two authors (D.X.. and L.T.) extracted data from all eligible studies by working independently on a structured excel sheet which included descriptive and numeric information regarding studies. More specifically, the sheet included data about the first author, publication year, article title, PMID, comparison examined, cohort's size, cases in cohort, follow-up duration, study period, region, continent, males' percentage in cohort, inclusion and exclusion criteria, definition of BC, definition of nutritional exposure, definition of fruit and vegetable subgroup, adjusting factors as well as type of effect measured such as relative risks (RR) and hazard ratios (HR). The effect with the maximum adjustment was extracted together with the respective confidence interval (CI). In case of disagreements, consensus with a third author (T.N.S.) followed.

### *1.3 Statistical analysis: meta-analysis*

In this meta-analysis we defined three main exposures: vegetables, fruits, fruits and vegetables combined and six subgroup exposures: leafy vegetables, cruciferous vegetables, yellow vegetables, dark green vegetables, citrus fruits and berries (**Suppl. Table 3**). Comparisons were made among all subjects and subgroups (males, females, study arms evaluating both sexes jointly) contrasting the highest versus lowest consumption category. Moreover, subgroup analysis was performed according to geographical region (Europe, East Asia, Multi-regional, USA) and smoking status (any smoking status, current smokers, past smokers, never smokers). Pooled effect estimates were calculated with random effects models (Der Simonian-Laird) and heterogeneity between studies was assessed with Q-statistic (Cochran) and I-squared(88). Detailed forest plots present the results.

### *1.4 Assessment of risk of bias*

In order to examine the representativeness of participants, comparability of cohorts and assessment of outcome on the basis of study design, the Newcastle-Ottawa scale was used as a tool of assessment(89). In order to evaluate the outcome in terms of the adequacy of the duration of the follow-up period, the cut-off value was *a priori* set to 5 years. As far as the adequacy of completeness is concerned, a response rate of 85%

was set. Two authors (D.X. and L.T.), independently, rated the studies and disagreements reached consensus after consultation with a third author (T.N.S.). Finally, publication bias was assessed via Egger's statistical test in analyses synthesizing ten or more study arms. Statistical analysis was performed using STATA/SE version 13 (Stata Corp, College Station, TX,USA).

# 2. Results

### *2.1 Selection of studies*.

Overall, 79 abstracts from MEDLINE and 132 from EMBASE were identified by the algorithm search and screened.192 studies were excluded overall, due to duplicates and irrelevance with the title. The remaining19 studies were reviewed fully and assessed for data extraction. Three studies(59,92,93)were excluded due to duplicates; specifically, Ohno et al. (2001)(92)was excluded due to overlap with Sakauchi et al. (2005)(94), Bradbury et al. (2014)(93)was excluded overlapping with the findings of Ros et al. (2012)(95) and Buchner et al. (2011)(59)was excluded due to interpreting the same data of Buchner et al. (2009)(66)via diet diversity scores.The study of D.L. Preston (2007), et al.(96) was excluded due to reporting reasons.

Two more studies (97,98)were evaluated for inclusion through reference screening from the included studies ("snowball procedure"); however, both of them had to be ultimately excluded. Specifically, Mills PK et al.(1991)(98), was excluded because of the narrow dietary category examining only "cooked green vegetables" and the study by Li et al. (2010)(97)was also excluded because the data given about citrus consumption were confounded, coming from many sources (miso soup, soybean products, total meat, total fish etc.).

Moreover, from the study of Buchner et al. (2009)(66) only the analyses examining joint consumption of fruits and vegetables were retained, as well as those on vegetables stratified by smoking status and gender; the analyses on overall fruits or overall vegetables were excluded, as they overlapped with the larger studies by Jochems et al. (2020)(99) and Ros et al. (2012)(95),respectively. From the study by Zeegers et al. (2001)(100)reporting on the Netherlands Cohort study, the analyses on fruits and citrus fruits were excluded, due to overlap with the larger, recent study of

Jochems et al. (2020)(99). Notably, three studies on urothelial carcinomas (94,95,100) were deemed eligible in this systematic review and meta-analysis, as 90-95% of urothelial carcinoma cases are located in the bladder (101).

Finally, 15 studies were eligible for inclusion (125,029 cases of BC in a total cohort of 1,855,277 subjects). Characteristics of included studies and definition of vegetable/fruit subgroups are shown in **Supplemental Tables 1 and 2,** respectively**.**

### 2.2 Vegetables

The synthesis of nine studies(95,100,102-108)examining 10 study arms, reported no overall association between total vegetable intake and BC risk (pooled RR= 0.95, 95% Cl: 0.87-1.04), as shown in **Figure 1**. No significant publication bias was detected (p=0.419, Egger's test).

No association was found in males, females and both sexes (when evaluated jointly, (**Supplemental Figures 2a-2n**). After smoking status stratification, a non-significant trend towardsan inverse association between vegetable intake and risk of BC was observed in all subjects of *any smoking status* (pooled RR=0.92, 95% Cl: 0.84-1.02, p=0.11) (**Supplemental Figure 2d**) and a borderline trend when both sexes were evaluated jointly(pooled RR= 0.86, 95% Cl: 0.74-1.00, p=0.05, **Supplemental Figure 2g**). No association was detected between vegetable intake and BC risk in all analyses concerning current, never and past smokers. Relevant results have been summarized in **Table 1.**

### 2.3 Fruits

The overall protective trend did not reach significance in the synthesis of ten eligible studies(99,102-110) (eleven study arms)(pooled RR=0.91, 95%CI: 0.81-1.02, p=0.11), whereas the subgroup analysis in East Asians showed a statistically significant protective effect of fruit intake against BC risk (pooled RR= 0.74, 95% Cl: 0.56-0.97, **Figure 2a**).No significant publication bias was detected in the overall analysis (p=0.803, Egger's test).

Subgroup analyses are shown in **Supplemental Figures 3a-3n**and **Table 2**. Regarding *any smoking status* analysis, the synthesis of nine eligible studies(99,102,103,105-110) (10 study arms) pointed to a marginally inverse association (pooled RR= 0.89,95% Cl: 0.79-1.01, p=0.11, **Supplemental Figure 3d**),

once again with a significant effect in East Asians. Subgroup analyses in males and females did not yield significant associations (**Supplemental Figures 3e, 3f**). Similarly, null associations were found in the small pool of studies examining exclusively current, past and never smokers (**Supplemental Figures 3h-3n**).

### 2.4 Fruits and vegetables combined

The synthesis of seven eligible studies(66,100,103,104,106-108) yielded a null association between combined fruit/vegetable intake and BC risk (pooled RR= 0.93,95% Cl: 0.81-1.07, **Figure 2b**). Non-significant associations were similarly documented in males, females, both sexes jointly assessed, (**Supplemental Figures 4a-4d**), current, past and never smokers (**Supplemental Figures 4h-4n**). All results have been resumed in **Table 3**.

### 2.5 Cruciferous vegetables

Pooled analysis of the five eligible studies(103,104,106,107,111)(six study arms), concerning the association between cruciferous vegetables and the BC risk in all subjects, did not reveal a significant association (pooled RR=0.86, 95% Cl: 0.68-1.08, (**Figure 4**).

Detailed results have been summarized in **Table 4**and **Supplemental Figures 5a-5m**. Accordingly, no association was observed in males, females and in study arms of joint assessment. After smoking status stratification, Michaud et al. (1999)(107) indicated a protective effect of cruciferous vegetables against BC confined to never smoking males (RR= 0.26, 95% Cl: 0.10-0.66, **Supplemental Figure5m**).

### 2.6 Yellow vegetables

All results regarding yellow vegetables have been summarized in **Table 5** and **Supplemental Figures 6a-6c**. Although the overall analysis indicated no significant association (**Figure 5**), the study by Nagano et al. (2000)(109) showed that the consumption of yellow vegetables had a protective effect in BC in East Asians (RR=0.54, 95%CI: 0.31-0.96, **Supplemental Figure 6c**).No studies addressed separately current, past and never smokers.

### 2.7 Dark green vegetables

All results concerning dark green vegetables have been summarized in **Supplemental Table 3** and **Supplemental Figures 7a-7c.** A non-significant association was detected in the overall synthesis of three study arms (pooled RR=0.84, 95% Cl: 0.66-1.06), (**Supplemental Figure 7a**). Similarly, no associations were found subgroup analyses by gender; once again, no studies addressed separately current, past and never smokers.

**2.8 Leafy vegetables**

The synthesis of five eligible studies(94,95,100,103,107) in the overall analysis resulted in a non-significant association between consumption of leafy vegetables and BC risk (pooled RR=0.91, 95% Cl: 0.80-1.04) (**Supplemental Figure 8a**,**Supplemental Table 4**). No associations were found in the analyses stratified by gender(**Supplemental Figures 8b-8c**) and smoking status (**Supplemental Figure 8d-8h**).

**2.9 Citrus fruits**

In the overall analysis, the synthesis of five eligible studies(94,99,103,106,111) (six study arms), resulted in a marginally protective association (pooled RR= 0.83,95% Cl: 0.69-1.01, p=0.061, **Figure 3**). No associations were found in subgroup analyses by gender (**Supplemental Figures9a-9c**) or smoking (**Supplemental Figures 9d-9i**).All results regarding citrus fruits consumption and BC risk are depicted in **Table 6**.

**2.10 Berries**

No significant associations were revealed between consumption of berries and BC incidence, overall or at the analyses by gender or smoking status (**Supplemental Table 5**,**Supplemental Figures10a-10n).**

***3. Risk of bias and evaluation of the quality of studies***

The evaluation of quality of studies has been performed with Newcastle-Ottawa scale along with justification per domain and study, as presented on **Supplemental Tables 6a** and **6b,** respectively. The quality of the prospective cohort studies was mainly compromised by the non-representativeness because some studies(104,106-108,110) were based on specific populations along with the ascertainment of nutritional

exposure which was mainly based on self-reporting questionnaires. However, all studies were characterized by a long enough follow-up period along with an adequate response rate of at least 85%.

# 4. Discussion

This systematic review and meta-analysis highlighted potentially protective effects of fruit and especially citrus fruit consumption in East Asians, although the respective trends pooling all ethnicities did not reach statistical significance. No associations with vegetable consumption was noted; however, single study arms in relevant subgroup analyses pointed to potential protective effects in never smoking males consuming cruciferous vegetables and East Asians consuming yellow vegetables.

Our meta-analysis came to an agreement with the previous meta-analyses (112-114)regarding the non-significant inverse association between vegetables and bladder cancer risk; a larger number of eligible studies could provide additional precision in effect estimates.

Similarly to our findings, previous meta-analyses(112-114) reported no inverse association between combined fruit/vegetable intake and bladder cancer risk. In accordance with the remark by Vieira et al. (2015)(113) high heterogeneity was noted in the relevant analysis on females, as the study by Park et al. (2013)(111) found an inverse association in females but not in males.

Concerning fruit intake, a previous meta-analysis by Liu et al. (2015)(112)suggested a protective effect against bladder cancer risk in both case-control and cohort studies while the meta-analyses of Vieira et al.(2014)(113) and Xu et al.(2015)(114) found no inverse association. Our results highlighted the consumption of fruits playing a protective role against bladder cancer East Asians(102,109). We detected especially a protective effect of citrus fruits; a previous meta-analysis by Liang et al. (115) similarly highlighted an inverse association regarding the comparison between the highest and the lowest citrus fruit intake in case–control studies but not in cohort studies (RR= 0.96, 95% CI: 0.87–1.07). Vieira et al. (2014)(113) conducted a further meta-analysis and their finding was similar to Liang et al (2014)(115). Regarding mechanisms, it has been suggested that vitamin C, a basic nutrient of citrus fruits, can

have anti-cancer actions in the bladder by various pathways, including a malignancy-inhibiting shift in the transcriptome along with an increase 5-hydroxymethylcytosine levels (116). The explanation of the association being particularly evident in East Asians remains elusive; further studies are needed to validate this pattern, as it was based on two studies in the case of fruits (102,109) or one study regarding citrus fruits(94).

This meta-analysis suggests a non-significant effect of leafy vegetables and berries in bladder cancer risk. Regarding green leafy vegetables the previous meta-analysis by Xu et al. (114)found that they were associated with reduced bladder cancer risk in a dose-response meta-analysis for every 0.2 serving increment. This difference should however be evaluated in caution because of the high variability in the classification of the kind of vegetables and fruits included in each subgroup. To our knowledge, there were only three studies stemming from two studies (108,111) which examined this association between dark green vegetables and bladder cancer risk, with no significant pooled results. As far as berries are concerned, unfortunately the paucity of eligible studies constitutes a burden to reach firm conclusions.

This systematic review detected a protective effect of cruciferous vegetables in males, never smokers, against bladder cancer risk. However the finding should be considered with caution, as it has derived from only one study(107).Liu, et al. (2012)(112)included both case-control and cohort studies in their meta-analyses on cruciferous vegetable consumption and suggested a moderately reduced bladder cancer risk in case-control, but not in cohort studies. It has been found that cruciferous vegetables contain glucosinolates, which are hydrolyzed to isothiocyanates in the body leading to suppression of carcinogen activation and induction of detoxification through enzymes such as glutathione S-transferase and NAD(P)H:quinone reductase (117). The observed confinement of cruciferous vegetables' positive effect into never smokers, supported by a single study (107),should be confirmed by additional studies.

A possible protective association between yellow vegetable consumption and decreased risk of bladder cancer in East Asians also emerged in this systematic review, based on the study by Nagano et al. (2000)(109). Yellow vegetables, especially carrots, owe their benefits to vitamin A, carotenoids (carotenes, cryptoxanthins and xanthophylls), vitamin C, which have anti-carcinogenic and anti-oxidant actions preventing oxidative DNA damage, deactivating reactive free radicals and repairing oxidant-induced injury (70,71,118). Carotenoids can also reduce cell

proliferation and transformation, as well as enhance homeostasis and cell-cell communication increasing gap junctional communication between cells (119).At the epidemiological level, the beneficial role of carotenoids in bladder cancer risk has been confirmed by the latest meta-analysis(120), reporting an inverse association between circulating concentrations of α-carotene, b-carotene, lutein, zeaxanthin and bladder cancer risk. Vitamin A plays an important role in cell differentiation of bladder, while vitamin C helps against the formation of N-nitroso compounds, which are potential human carcinogens and constitute basic components of tobacco(72). Further studies are therefore needed regarding yellow vegetables in bladder cancer.

This meta-analysis has several strengths. It included only prospective cohort studies avoiding the recall bias. In addition, the selected studies had a long follow-up and many subgroups which allowed to numerous subgroup analyses, documenting original associations by smoking status, gender and geographical regions. Among the limitations of this meta-analysis is the variability of definitions in the classification of vegetable subgroups, as well as the small number of eligible studies (less than 10) concerning berries, leafy vegetables, citrus fruits, cruciferous vegetables, dark green vegetables and yellow vegetables. The findings were based on self-report questionnaires, less reliable than interviews and thus more vulnerable to risk of bias. Also the assessment of dietary intake varied significantly across eligible studies; other studies used servings/day, others used grams per caloric intake (g/kcal) per day, cup equivalents per caloric intake and times per week, precluding any reliable transformation for a potential dose-response meta-analysis.

In conclusion, this systematic review and meta-analysis suggests a possible protective role of fruits and citrus fruits in bladder cancer risk, especially in East Asians. It also indicates a possible protective effect of cruciferous vegetables in never smokers yellow vegetables. However, the data were extracted from self-reporting questionnaires with an innate reporting bias. The complexity of the classification of fruits and vegetables in subgroups, inherently affects the results. A larger number of new studies may provide further insight into the role of fruits and vegetables in bladder cancer prevention.

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