A comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: Level of evidence and limitations

Giuseppe Grosso[1,2], Justyna Godos[1], Rosa Lamuela-Raventos[3,4], Sumantra Ray[2,5], Agnieszka Micek[6], Andrzej Pajak[6], Salvatore Sciacca[1], Nicolantonio D'Orazio[7], Daniele Del Rio[2,8] and Fabio Galvano[9]*

[1] Integrated Cancer Registry of Catania-Messina-Siracusa-Enna, Azienda Ospedaliero-Universitaria Policlinico-Vittorio Emanuele, Catania, Italy
[2] NNEdPro Global Centre for Nutrition and Health, St John's Innovation Centre, Cambridge, UK
[3] Biomedical Research Center Network on Obesity and Nutrition (CIBERobn) Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, Madrid, Spain
[4] Nutrition and Food Science Department–XaRTA, INSA, University of Barcelona, Barcelona, Spain
[5] Medical Research Council (MRC) Human Nutrition Research Unit, Cambridge, UK
[6] Department of Epidemiology and Population Studies, Jagiellonian University Medical College, Krakow, Poland
[7] Department of Medical and Oral Sciences and Biotechnologies, University of Chieti, Chieti, Italy
[8] Department of Food Science, University of Parma, Parma, Italy
[9] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

Scope: To summarize available evidence on the association between dietary flavonoid as well as lignan intal observational studies.

Methods and results: A systematic search on electronic databases of all English language case– control and prospective studies published up to June 2016 was performed. Risk ratios (RRs) and 95% confidence intervals were calculated by random-effects model separately by study design. Heterogeneity and publication bias were tested. Out of the 143 studies included, meta-analyses of prospective studies showed isoflavones significantly associated with decreased risk of lung and stomach cancers and nearly significant breast and colorectal cancers; total flavonoids showed nonsignificant decreased risk of breast cancer. Meta-analyses of case–control studies showed: total and/or individual classes of flavonoids associated with upper aero-digestive tract, colorectal, breast, and lung cancers; isoflavones with ovarian, breast, and colorectal cancers, endometrial and lung cancers.

Conclusions: Most evidence reported in previous meta-analyses was driven by case–control studies. Overall results may be promising but are inconclusive. Further prospective cohorts assessing dietary polyphenol exposure and studies using other methods to evaluate exposure (i.e. markers of consumption, metabolism, excretion) are needed to confirm and determine consumption levels required to achieve health benefits.

Keywords:
Cancer / Dietary polyphenols / Flavonoids / Lignans / Meta-analysis /Observational studies

Correspondence**:** Daniele Del Rio

E-mail: daniele.delrio@unipr.it
*Additional corresponding author: Giuseppe Grosso
E-mail:
giuseppe.grosso@studium.unict.it

Abbreviations:CIs, confidence intervals; **HRs**, hazard ratios; **F-KB-**, nuclear factor kappa-light-chain-enhancer of activated B cells; **RR**, ris

1 Introduction

There is growing evidence suggesting that biologically active plant compounds may be responsible for the observed benefits of plant-based dietary patterns on risk of chronic diseases, including cancer [1]. According to current scientific literature, regular consumption of diets rich in vegetables, fruits, herbs, seeds, and plant-derived beverages would contribute to increased intake in phenolic compounds and phytoestrogens [2].

Several reviews and meta-analyses of observational studies have been performed to summarize evidence on the relation between polyphenols (including flavonoids and phytoestro- gens) and cancer risk. Briefly, current evidence can be sum-marized as follows: (i) dietary flavonoids (total or individual subclasses) are associated with decreased risk of breast [3], ovarian [4], esophageal [5], gastric [6], and lung cancer [7]; (ii) dietary isoflavones intake is associated with decreased risk of breast [8], prostate [9], and colorectal cancer [10]; (iii) lignan ex- posure is associated with decreased risk of postmenopausal breast cancer risk [11, 12]; (iv) higher intake of anthocyani- dins and flavan-3-ols is associated with increased prostate cancer risk [13]. However, it is worthy to specify that conclu-sions often differed between research groups due to different methodology in interpreting the findings. For instance, in the meta-analyses on flavonoids and breast [3] and ovarian

[4] cancer risk, some studies conducted on the same cohorts were included in the same analysis, thus overlapping results. Other meta-analyses meta-analyzed risk estimates for dietary intake of flavonoids labeled as "total," which in several studies referred to the sum of the individual flavonoids investigated limited to the compounds considered in each study [5, 6, 10]. Finally, some previous meta-analyses merged together risk estimates from several sources (i.e. dietary, plasma, and urine lignans), which provide qualitative, rather than quantitative information [11]. Regarding the methodological choice on grouping existing studies, almost the totality of previous sum-mary of evidence calculated risk estimates from case–control and prospective studies together. It has been previously ar- gued that case–control studies may be affected by recall and selection bias, resulting in either an underestimate or overes- timate of the risk estimates and more heterogeneous results across studies [14]. Especially in nutritional epidemiology, evidence judgment from organization and committee panels may place more emphasis on prospective cohort studies over case–control studies [15]. Thus, it is preferred to consider prospective studies with adequate characteristics to demon- strate stronger evidence, while case–control studies rather to confirm consistency of the findings.

Research is ongoing and new studies have been published since last summaries of evidence. Moreover, summary of re- sults on the majority of individual polyphenols is generally lacking. In order to provide a comprehensive evaluation of ex- isting literature with consistent methodology in production and interpretation of results, the aim of the present study was to systematically review and meta-analyze findings from observational studies on dietary total, subclasses, and indi- vidual flavonoid and lignan intake and cancer risk.

1 Methods

The design, analysis, and reporting of this study are compli-ant with the Meta-analysis of Observational Studies in Epi- demiology and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

1.1 Study selection

A systematic search on PubMed (http://www.ncbi.nlm. nih.gov/pubmed/) and EMBASE (http://www.embase.com/) databases of all English language studies published up to June 2016 was performed. The search terms and strategy used for the study selection is shown in Supporting Infor- mation

Table 1. Inclusion criteria for the systematic review were: (i) had a prospective or case–control design; (ii) evalu-ated the association between dietary total/classes/individual flavonoids/lignans intake and cancer. Inclusion criterion forthe meta-analysis was assessment and report odds ratios or hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) for cancer for each category of exposure; ex-clusion criteria were: (i) lack of sufficient statistics; (ii) re- ported unreliable amount of total flavonoids. Regarding thislast point, the doses for total flavonoids have been reviewed in order to test the comparability between studies, as older in- vestigations reported as "total flavonoids" only a minor partof compounds (i.e. the sum of the flavonoid classes inves- tigated in the paper). According to recent estimation of di- etary polyphenols [16, 17], we considered unreliable a total amount of total flavonoids <150 mg/day and, accordingly, we excluded from the analysis those studies reporting such amounts. Reference lists of included manuscripts were ex- amined for any additional studies not previously identified. If more than one article using the same cohort was published, only the study including the entire cohort or with the longest follow-up was included. The selection process was indepen-dently performed by two authors (G.G. and J.G.) and retrieved articles examined.

1.2 Data extraction and study quality

Data were abstracted from each identified study by using a standardized extraction form. The following information was collected: (i) first author name; (ii) year of publication; (iii) study cohort name; (iv) country; (v) number of participants; (vi) sex of participants; (vii) age range of the study popula- tion at baseline; (viii) dietary food source; (ix) follow-up pe-riod; (x) endpoints and cases; (xi) distributions of cases and person-years, HRs, and 95% CIs for all categories of expo- sure; (xii) covariates used in adjustments. This process was independently performed by two authors (G.G. and J.G.) anddiscrepancies were discussed and resolved by consensus.

The quality of each study was assessed according to the Newcastle-Ottawa Quality Assessment Scale [18], which consists of three variables of quality as follows: selection (4 points), comparability (2 points), and outcome (3 points)for a total score of 9 points (9 representing the highest quality). Studies scoring 7–9 points, 3–6 points, and 0–3 points were identified as high, medium, and low quality, respectively.

1.3 Statistical analysis

The summary analyses were conducted for case–control andprospective studies separately, as mixed results do not pro- vide adequate evidence according to the Joint WHO/FAO Expert Consultation criteria for evidence in nutrition [19]. The analyses were performed for total flavonoid intake as well as for individual classes and compounds. When a studyreported more than one dataset for an analysis, we consid- ered the most comprehensive of subgroups for potential ad-ditional analyses (i.e. by gender, menopausal status, receptor status, and smoking status). HRs/odds ratios with 95% CI for all categories of exposure were extracted for the analy- sis. Random-effects models were used to calculate relative risks with 95% CIs for the highest versus lowest categories of exposure. We used the risk estimate from the most fully adjusted models in the analysis of the RR. Heterogeneity wasassessed by using the Q test and I^2 statistic. The level of significance for the Q test was defined as $p < 0.10$. The I^2 statistic represented the amount of total variation that could be attributed to heterogeneity. I^2 values >50% indicated sig-nificant heterogeneity. A sensitivity analysis by exclusion ofone study at the time was performed to assess the stabilityof results and potential sources of heterogeneity. When pos-sible, additional sensitivity analyses were performed amongprospective studies to check for potential source of hetero- geneity by grouping according to gender, geographical area, and adjustment for smoking status, BMI, physical activity, family history of cancer, education, dietary factors, and otherpolyphenols. For specific cancer sites, further subgroup anal- yses were

conducted by menopausal and receptor status (for breast cancer) and smoking status (for lung cancer). Publica-tion bias was evaluated by an assessment of funnel plots for potential symmetry.

2 Results

2.1 Study characteristics

The process of identification and study selection is summa- rized in Fig. 1. Among the initial 1721 articles screened on the basis of title and abstract, 281 articles were screened by reading full-texts. One hundred and ten articles did not meet inclusion criteria and 26 were excluded from the quan-titative analysis (Fig. 1). A complete list of studies excluded from the quantitative analysis and respective reasons is pre-sented in Supporting Information Table 2. A total number of 143 studies were considered for the present meta-analysis (Fig. 1). The complete list of included studies and refer- ences is presented in Supporting Information. Background characteristics of the included studies are presented in Supporting Information Table 3. The overall quality of the studies was medium-high (data not shown). Most of the studies included individuals of 40–70 years age range. The investigated cohorts were often characterized by social (i.e. health care workers, postgraduate students) or clinical (i.e. postmenopausal women, individuals at high cardiovascular risk) peculiarities, which should be considered when apply- ing retrieved results to the general population. All studies included covariates that may influence cancer risk, such as age, gender (when not analyzed separately), BMI, educa- tion, physical activity, and smoking status, but only a mi-nority further adjusted for dietary variables (Supporting In- formation Table 3). The ascertainment of the exposure was conducted mostly by self-administered food frequency ques-tionnaires, despite in some studies dietary habits were evalu-ated by personal interview (Supporting Information Table 3). The exposure retrieved included the following compounds (or group of compounds): total flavonoids, flavonols, quercetin, kaempferol, myricetin, flavones, apigenin, luteolin, flavan- 3-ols, proanthocyanidins, catechins, flavanones, hesperidin, naringenin, anthocyanins, isoflavones, genistein, daidzein, glycitein, formononetin, biochanin A, lignans, secoisolari- ciresinol, matairesinol, lariciresinol, pinoresinol, and coume-strol. The main results obtained labeled according to the level of evidence are presented in Table 1.

2.2 Dietary polyphenol intake and female cancersrisk

A total of 16 prospective and 23 case–control studies were considered to perform the analyses on breast cancer risk (Supporting Information Figs. 1 and 2). Despite most of the analyses on main flavonoid classes included three or more studies, no significant results were found for any of the compound investigated among prospective studies (Sup-porting Information Table 4). Limited number of datasets was available for individual flavonoid intake and presented null results. However, an association with nonsignificant decreased risk of breast cancer was found for total flavonoid (RR = 0.96, 95% CI: 0.89, 1.04; I^2 = 0%, $p_{\text{heterogeneity}}$ = 0.99), flavonol (RR = 0.96, 95% CI: 0.90, 1.03; I^2 = 0%, $p_{\text{heterogeneity}}$ = 0.64), flavanol (RR = 0.96, 95% CI: 0.89, 1.03; I^2 = 0%, $p_{\text{heterogeneity}}$ = 0.90), and proanthocyanidins intake (RR = 0.94, 95% CI: 0.87, 1.01; I^2 = 0%, $p_{\text{heterogeneity}}$ = 0.69) with no evidence of heterogeneity or asymmetry of the funnel plot (Supporting Information Fig. 3). Summary risk estimates on flavanols and flavones were significant among case–control studies (Supporting Information Table 4) but showed small asymmetry of funnel plots (Supporting Information Fig. 3). In contrast, the association between flavanones intake and increased risk of cancer nearly reached statistical significance among prospective studies (Supporting Information Table 4). Regarding isoflavones, there was an adequate number of prospective studies suggesting an association with a de- creased, yet not significant, risk of breast cancer (RR = 0.90, 95% CI: 0.81, 1.01; I^2

= 60%, $p_{heterogeneity}$ = 0.002), while case–control studies showed significant results for either total isoflavones and individual compounds genistein, daidzein, glycitein, as well as for lignans (Supporting Information Table 4); however, all analyses were affected by significant heterogeneity and asymmetry of funnel plots (Supporting Information Fig. 3). Subgroup analyses on isoflavones were conducted grouping prospective studies by potential confounders, revealing that a significant decreased risk of breast cancer was observed among Asian cohorts and in studies adjusting for physical activity and education (Supporting Information Table 5). Analyses by menopausal status were performed for most of the main flavonoid classes, yet quite limited besides the isoflavone class and resulted in no particular lower risk estimates in any of the subgroups with the exception of proanthocyanidins in postmenopausal women (Supporting Information Table 6). Also regarding receptor status the analyses were limited to few studies as well, and resulted in decreased risk of estrogen and progesterone positive breast cancer for higher intakes of isoflavones and lignans in case–control studies, but results were confirmed in prospective ones only for the latter polyphenol group (Supporting Information Table 7). Out of the five nonoverlapping prospective and seven case–control studies on ovarian cancer (Supporting Information Fig. 4), the few conducted on flavonoids reported significant associations with various subclasses (flavonols and flavanones) and individual compounds (kaempferol and luteolin; Supporting Information Table 8). Null results were found for isoflavones among prospective studies while significant decreased summary risk estimates were retrieved among case–control studies, confirmed also in some individual isoflavones investigated (genistein, daidzein, glycitein, formononetin; Supporting Information Table 8) with no evidence of publication bias (Supporting Information Fig. 5). Only few studies were conducted on lignans and no significant results were found.

Three prospective and five case–control studies were conducted on endometrial cancer (Supporting Information Fig. 6). Significant association with lower cancer risk was reported in the prospective study on genistein and daidzein (but provided by limited number of studies), and significant risk estimates were retrieved among case–control studies on isoflavones and endometrial cancer (Supporting Information Table 9) with no evidence of publication bias (Supporting Information Fig. 7). No other significant results were found. Overall, none of the analyses provided convincing evidence of probable relation between the compounds considered and female cancers (Table 1). According to number of studies analyzed, strength of the association, and study design, some evidence of a possible relation between mainly isoflavones and some flavonoids classes and female cancers has been found; as well, we found suggestive no association
with lignan intake (Table 1).

2.3 Dietary polyphenol intake and upper aero-digestive and respiratory tract cancer risk

Two prospective studies and ten case–control studies were conducted on upper aero-digestive tract cancers (including larynx, esophagus, and pharynx; Supporting Information Fig. 8). The meta-analysis of results from case–control studies resulted in an association with decreased cancer risk for total flavonoids and subclasses flavonols, flavones, flavanols, flavanones, and anthocyanins (Supporting Information Table 10) with no evidence of heterogeneity and asymmetry in the funnel plot (with exception for flavonols; Supporting Information Fig. 9). Results on isoflavones neared significance among case–control studies but were not confirmed in the prospective one. Results on lignans were significant but relied on limited number of case–control studies.

Out of eight prospective and seven case–control studies on lung cancer (Supporting Information Fig. 10), only isoflavones were associated with significant decreased risk of cancer in both prospective (RR = 0.91, 95% CI: 0.84, 0.87; I^2 = 0%, $p_{heterogeneity}$ = 0.45) and case–control studies with no evidence of heterogeneity

and asymmetry of the funnel plot (Supporting Information Table 11 and Supporting Information Fig. 11). The analysis by smoking status showed that isoflavones were associated with decreased risk of lung cancer among never smokers (five datasets from four studies, RR = 0.64, 95% CI: 0.51, 0.79; I^2 = 0%, $p_{heterogeneity}$ = 0.76) but not among former/current smokers (four datasets from three studies, RR = 1.03, 95% CI: 0.86, 1.24; I^2 = 0%, $p_{heterogeneity}$ = 0.99). Subgroup analysis by potential confounders showed significant associations with isoflavones intake in prospective studies conducted on Asian women; however, adjustment for physical activity and family history of cancer resulted in weak- ening of results (Supporting Information Table 12). Other significant findings were found for kaempferol and individ- ual studies on quercetin, flavones, flavanones, and catechins intake, but mostly among case–control studies. No studies were conducted on individual isoflavones and only one case–control study on lignans resulted in decreased risk of cancer (Supporting Information Table 11).

Overall, the strongest evidence available was on isoflavones and lung cancer risk while the relation with total and some classes of flavonoids was only suggestive (Table 1).

2.4 Dietary polyphenol intake and digestive track cancers risk

Six prospective and six case–control studies were conducted on gastric cancer (Supporting Information Fig. 12). No sig- nificant findings were found among prospective studies (yet limited to one study in most of the analyses) with exception of isoflavones, which resulted in decreased risk of cancer with no evidence of heterogeneity and asymmetry of the funnel plot (Supporting Information Table 13 and Supporting Informa- tion Fig. 13). Also among case–control studies analyses were limited to few studies showing lower risk of cancer for in- dividual flavonols quercetin and kaempferol, proanthocyani- dins, and neared significance for anthocyanins (Supporting Information Table 13). No further significant results were found for lignans, although one study reported lower risk of stomach cancer for higher intake of secoisolariciresinol (Supporting Information Table 13).

A total of 11 prospective and nine case–control stud- ies were conducted to test the association between dietary polyphenol intake and colorectal cancer (Supporting Infor- mation Fig. 14). No significant findings on flavonoids were retrieved analyzing results from prospective studies, while several associations were found among case–control stud- ies, including flavonols (and individual compound quercetin), subclasses of flavanols proanthocyanidins and catechins, and anthocyanins (Supporting Information Table 14) with no ev- idence of publication bias (Supporting Information Fig. 15). Nonsignificant decreased risk of colorectal cancer was found associated with isoflavones among prospective studies (RR = 0.94, 95% CI: 0.83, 1.07; I^2 = 15%, $p_{heterogeneity}$ = 0.31), while association was significant among case–control stud- ies for total isoflavones and individual subclasses daidzein and glycitein (Supporting Information Table 14). Analysis by cancer localization (colon/rectum) was limited to individ- ual prospective studies and let to not significant results (data not shown). Subgroup analysis by potential confounders re- vealed no relevant results (Supporting Information Table 15). No prospective studies investigated the association between lignan intake and colorectal cancer while the case–control studies showed a significant summary lower risk (Support- ing Information Table 14).

Two prospective and two case–control studies were con- ducted on liver cancer mainly resulting in null findings (Sup- porting Information Fig. 16) with the exception of flavones, which showed higher intakes associated with lower risk of cancer among case–control studies (Supporting Information Table 16) with no evidence of publication bias (Supporting Information Fig. 17). Similarly, six prospective and one case–control study on pancreatic cancer risk showed no significant findings (Supporting Information Figs. 18 and 19) except for results of one case–control study on proanthocyanidins (Sup- porting Information Table 17).

Out of all digestive tract cancers, only the relation between isoflavones intake and gastric cancer was supported by good evidence level, while most of the other was only suggestive of a possible relation with

flavonols, quercetin, flavones, flavan- 3-ols, and proanthocyanidins with colorectal but not other cancers (Table 1).

2.5 Dietary polyphenol intake and male cancers risk

Five prospective and ten case–control studies were con- ducted on dietary polyphenol intake and prostate cancer risk (Supporting Information Fig. 20). Despite limited due to low number of studies, a significant increased risk of cancer was retrieved among prospective studies on total flavonoids, fla- vanols, and anthocyanins (Supporting Information Table 18) with no evidence of publication bias (Supporting Information Fig. 21). Similar results were obtained in case–control studies (Supporting Information Table 18). Results on isoflavones were not significant in both prospective and case–control studies, but among the latter an association between higher genistein intake and lower risk of prostate cancer was found (Supporting Information Table 18).

Only one case–control study was conducted on testicular cancer showing no significant results (data not shown).

Studies examined provided overall possible evidence of an inverse relation between individual, but not total, isoflavones intake and prostate cancer risk, while a direct association between flavonols and increased cancer risk should be further investigated (Table 1).

2.6 Dietary polyphenol intake and other cancers risk

Four prospective and one case–control study on bladder can- cer risk allowed analyses only on isoflavones, showing how- ever no significant results (Supporting Information Figs. 22 and 23). Results of individual studies showed an association with decreased risk of bladder cancer for flavonols and lig- nans (Supporting Information Table 19). Three prospective and one case–control study have been conducted on renal can- cer showing no significant results (Supporting Information Table 20 and Supporting Information Fig. 24). In contrast, one prospective and two case–control studies on thyroid can- cer risk showed association with flavanols and isoflavones, respectively (Supporting Information Table 21 and Support- ing Information Fig. 25). Similarly, three prospective and one case–control study on lymphoma risk reported a sum- mary lower risk with higher intake of proanthocyanidins and total flavonoids, flavonols, proanthocyanidins, and an- thocyanins (Supporting Information Table 22 and Support- ing Information Fig. 26). Finally, one case–control study was conducted on nervous system (gliomas) and reported lower odds of cancer occurrence for higher intakes of individual isoflavones daidzein and individual lignans matairesinol, sec- oisolariciresinol, and coumestrol (data not shown). In gen- eral, data available are not sufficient to provide any level of evidence (Table 1).

3 Discussion

In this study, we provided a comprehensive review of exist- ing observational studies on flavonoid and lignan intake and cancer risk. Contrary to current scientific literature, results from the present meta-analysis showed only small evidence of the association between dietary polyphenol intake and cancer risk. When taking into account only prospective studies, sig- nificant results from analyses including three or more stud- ies were limited to isoflavones and lung and stomach cancer risk. Among results based on prospective studies that neared significance, only isoflavones and decreased risk of breast and colorectal cancers could be supported in future studies whether the trends will be confirmed. Moreover, analyses on total flavonoids (as well as flavonols and proanthocyanidins) neared significance for breast cancer (actually only partially

supported by case–control studies). By reviewing the cate- gories of exposure for the aforementioned outcomes, on aver-age, isoflavones intake referred to about more than 45 mg/day among Asian populations and more than 1 mg/day among non-Asian populations; regarding total flavonoids, results re- ferred to an intake more than 500 mg/day, while flavonols and proanthocyanidins referred to more than 40 mg/day andmore than 245 mg/day, respectively. Most of evidence re- ported in previous meta-analyses was driven by case–controlstudies, which in our study showed the following results: total and/or individual classes of flavonoids were associated with lower odds of upper aero-digestive tract, colorectal, breast, and lung cancers; isoflavones and nearly all their subclasses with ovarian, breast, and colorectal cancers, and isoflavones with endometrial and lung cancers. Also results on individual polyphenol were limited mainly to case–control studies, yet derived by lower number of studies compared with those on subclasses, with the exception of phytoestrogens genistein, daidzein in relation to prostate, breast, and ovarian cancer risk, and flavonols quercetin and kaempferol in colorectal and lung cancer risk. Overall, results may be promising but are far from conclusive.

There are some common potential mechanisms of action through which flavonoids could exert protective effects to- ward cancer risk, namely by providing a direct inhibition of oxidative stress and oxidative damage and interfering with the initiation, promotion, and progression of cancer [20]. The antitumor effects of polyphenols have not been conclusively related to their antioxidant effects, but it has been suggested that the free radical scavenging properties of flavonoids may be related to beneficial effects on cancer risk, as flavonoids are more effective antioxidants than vitamin C, vitamin E, and carotenoids [21]. Moreover, in vitro and in vivo studies showed that flavonoids exert also antiproliferative, antiangio- genic effects, and antimetastatic effects by modulating ErbB receptors, hedgehog (HH)/GLI, and nuclear factor kappa- light-chain-enhancer of activated B cells (NF-KB) signaling transduction pathways related to cellular proliferation, dif- ferentiation, and apoptosis [22]. Besides the aforementioned mechanisms related to flavonoids, phytoestrogens may af- fect DNA mutagenesis, cell proliferation, tissue vascular- ization, decreased apoptosis, immune response, and other processes which can be modulated via estrogen receptor- dependent and receptor-independent mechanisms [23]. The antiangiogenic effects of certain phytoestrogens include sig- naling pathways for regulating the angiogenesis, such as vascular endothelial growth factor and its receptor, Ras/Raf- 1/MEK/ERK, PI3K/Akt, and ERK-NF-KB-cMyc-p21 [24]. Antimetastasis effects have been reported for several various cancer cells through epithelial–mesenchymal transition-related pathways, such as Notch-1 and TGF-beta signaling [25]. Fi- nally, newly elucidated anticarcinogenic mechanisms of ac- tion of phytoestrogens may include epigenetic modifications and topoisomerase inhibition [26]. Such mechanisms have been reported and widely described elsewhere for breast [27], lung [28], and colorectal cancers [29], thus supporting the bio- logical plausibility of the hypotheses suggested by the results of the present study. The main issue to be discussed is the substantial discordance between mechanistic experimental studies and evidence retrieved by human studies. There are mainly two hypotheses, either the anticarcinogenic effects of polyphenols observed in experimental studies do not play a relevant role in the etiology of cancers in humans or they do but observational studies fail to demonstrate it. In support of the latter hypothesis there may be several explanations. The first is linked to the common mistake of testing, in the framework of studies aimed at understanding the mecha- nisms of action of polyphenolic compounds in cancer cells, the molecules in the form they occur *in planta*. It has now been widely demonstrated that phenolics are extensively metabo- lized within the human body after ingestion, both at hepatic and at colonic level, after interaction with the gut microbiota [30]. If the parent compounds are able, in vitro, to elicit spe- cific anticarcinogenic pathways, this may not be true when it is their metabolites getting in contact with carcinogenic or precarcinogenic cells. In particular, it has been reported that the composition of the gut microflora may influence polyphe- nol absorption and production of specific colonic microbial catabolites that may in turn mediate their biological activities [31]. However, as the colonic microbiota has been reported to vary widely among individuals, this could represent a further component of the natural variability of the response of hu- mans toward the consumption of

dietary polyphenols. Moreover, also consumption of polyunsaturated fatty acids seems to regulate polyphenol absorption. Thus, potential effects of dietary polyphenol intake cannot be investigated easily by not taking into account the gut microbiome and its interaction with other key components of the diet. Another potential mechanistic issue depends on the exposure dose because the polyphenol concentrations used in in vitro and in vivo studies are difficult to attain through habitual dietary intake by humans [22]. Furthermore, also human intervention studies investigating the effect of flavonoid intake on intermediary or carcinogenesis biomarkers (i.e. inflammatory markers or DNA damage, respectively) used experimental doses that are normally not reached in habitual diets [32]. Thus, potential small existing effect may be affect by uncontrolled variability between individuals that is difficult to quantify.

As a result of our study, some other limitations and issue related to research on dietary polyphenol in observational studies should be addressed. Epidemiologic studies investigating the relation between polyphenol intake and health rely on the estimation of intake from the dietary components recalled by participants. This is affected by three kinds of bias, including (i) recall bias, either the dietary assessment method was a food frequency questionnaire or a 24-h dietary recall; (ii) polyphenol estimation related to food quality, such as plant variety, season of harvest, or food processing and cooking; (iii) polyphenol databases used, as more recent studies provided wider variety and more precise polyphenol content of a larger range of foods, leading to incomparable results with older ones. Another limitation that should be addressed in future studies is the potential co-linearity between polyphenols and with main food sources (which provide other compounds, such as vitamins and fiber, that may be responsible for the associations observed). Despite certain studies provided fully adjusted results for all polyphenol classes investigated and stratified analyses by key food sources, this issue is hard to be resolved by using this type of study design, and additional investigations involving, for instance, markers of consumption, should confirm findings retrieved by observational studies on dietary polyphenol consumption. Among other common limitations relative to prospective studies, reliability of data need to be considered, potentially affected by (i) single baseline assessment of dietary intake, with lack of specific information of intake over time (in prospective studies); (ii) limited number of cases in some studies weakening the statistical power of the analyses; (iii) lack of data on individual polyphenols, limiting the results for general classes of compounds.

In conclusion, despite promising trends, the present review of literature suggests the need for a more conservative evaluation of evidence in order to avoid exaggerated claims on effects of dietary polyphenols on cancer risk prior to these being adequately evidenced. The small amount of evidence that certain compounds may be related to lower risk of cancer cannot provide the backbone for dietary guidelines at the present time. There is some, albeit limited, suggestion of potential association with lower cancer risk of certain cancers (lung, stomach, breast, colorectal) that require further prospective and mechanistic studies to be confirmed and provide more robust evidence. Contrary to the conclusions provided by other meta-analyses, research on this topic is far from conclusive or even exhaustive. Further prospective studies on the aforementioned cancer sites are needed to increase the quantity and quality of available data and to confirm current potential trends toward decreased risk. Findings on potential associations between dietary polyphenols and cancer risk should ultimately be integrated with studies using other methods to evaluate exposure (i.e. markers of consumption, metabolism, excretion) to overcome the limitations emphasized in observational studies testing dietary intakes and provide better insights into which individual classes of polyphenol may have the most important roles and indication on the consumption levels required to achieve tangible and consistent health benefits.

G.G. and J.G. designed the study, performed the studysearch, built the databases, performed the analyses, and wrote the manuscript (equal contribution); R.L.-R. and S.R. provided expertise on methodology and insights concerning dietary polyphenols; A.M. provided expertise on statistical analysis; A.P. and S.S. provided expertise on epidemiological methodology and limitations to be taken into account; D.D.O. critically revised the

paper; D.D.R. and F.B. provided expertise on the mechanisms of action of polyphenols and provided support in writing the paper(equal contribution).

The authors have no conflicts of interest to disclose.

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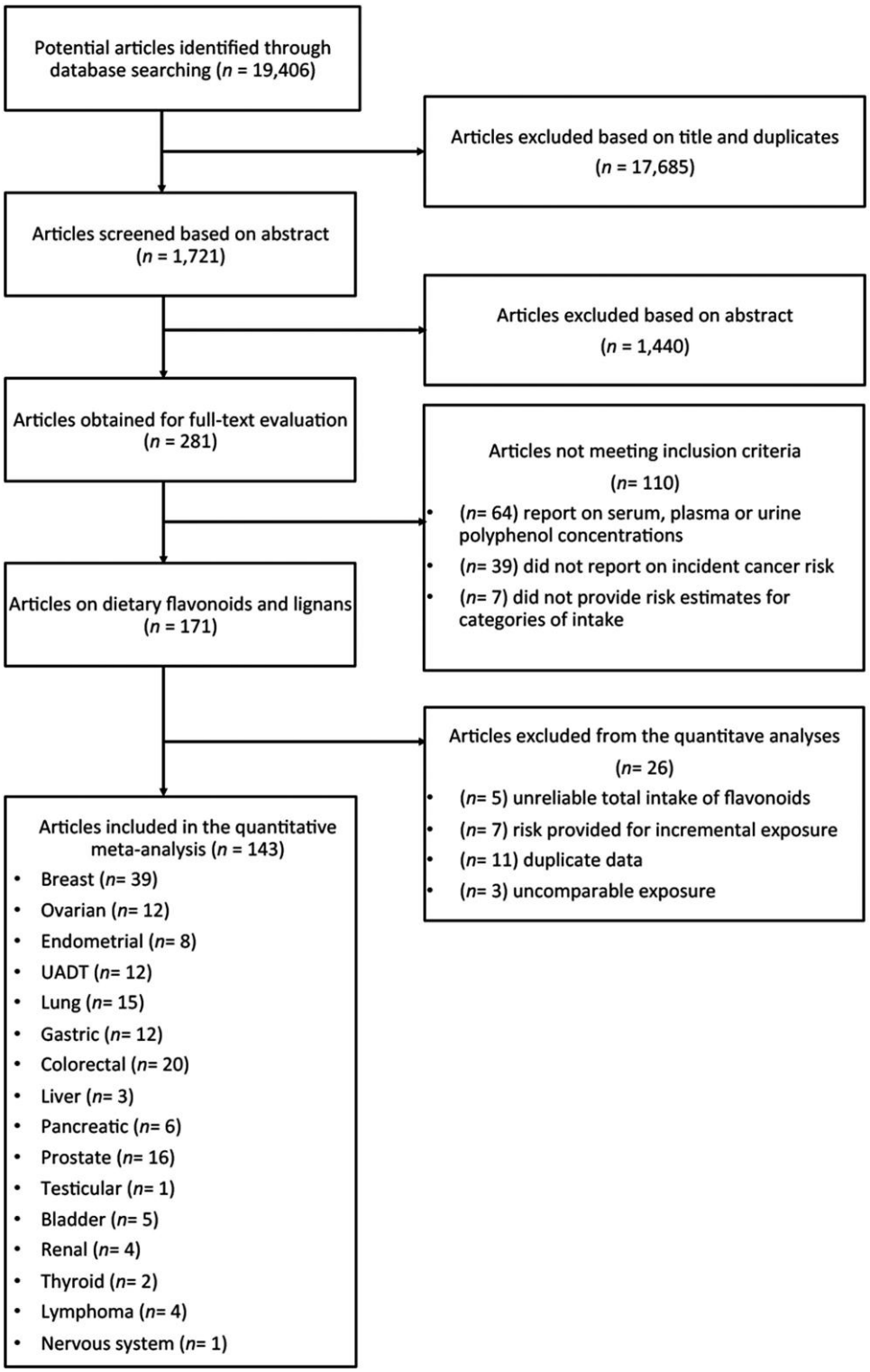

Figure 1. Flowchart indicating the results of the systematic re-view of relevant studies explor-ing association between dietarypolyphenol intake and risk of cancer.

Table 1. Summary results and level of evidence reached from overall analyses on dietary flavonoids and lignans intake and risk of cancer. Not listed compounds did not reach sufficient evidence

	No. of studies		No. of exposures		Main results		
Cancer type	Case-control	Prospective	Case-control	Prospective	Probable relation (medium/high evidence)	Suggestive/ possible relation (low evidence)	Suggestive no association
Female cancers							
Breast	23	16	18	24	None	Decreased risk associated with total flavonoids, flavonols, flavan-3-ols, isoflavones, genistein, and daidzein; increased risk associated with flavanones	Flavones, anthocyanins, total lignans, secoisolariciresinol, matairesinol, lariciresinol, and coumestrol
Ovarian	7	5	23	18	None	Decreased risk associated with isoflavones, genistein, daidzein, and glycitein	None
Endometrial	5	3	20	5	None	Decreased risk associated with isoflavones; increased risk associated with matairesinol	Genistein, daidzein, formononetin, lignans, and secoisolariciresinol
Upper aerodigestive and respiratory cancers							
UADT	10	2	14	9	None	Decreased risk associated with total flavonoids, flavonols, flavones, flavan-3-ols, flavanones, anthocyanins, and isoflavones	None
Lung	7	8	15	15	Decreased risk associated with isoflavones	Decreased risk associated with quercetin and kaempferol	None
Digestive tract cancers							
Gastric	6	6	14	15	Decreased risk associated with isoflavones	Decreased risk associated with anthocyanins	Flavonols, flavones, flavan-3-ols, and flavanones
Colorectal	9	11	18	15	None	Decreased risk associated with flavonols, quercetin, flavones, flavan-3-ols, and proanthocyanidins	Isoflavones

Table 1. Continued

	No. of studies		No. of exposures		Main results		
Cancer type	Case–control	Prospective	Case–control	Prospective	Probable relation (medium/high evidence)	Suggestive/ possible relation (low evidence)	Suggestive no association
Liver	2	2	9	11	None	None	None
Pancreatic	1	6	6	15	None	None	Flavonols, flavones, flavan-3-ols
Male cancers							
Prostate	10	5	20	17	None	Decreased risk associated with genistein and daidzein; increased risk associated with flavonols	Isoflavones
Testicular	1	0	4	NA	None	None	None
Other cancers							
Bladder	1	4	4	10	None	None	Isoflavones
Renal	1	3	7	5	None	None	None
Thyroid	2	1	7	9	None	None	None
Lymphomas	1	3	8	7	None	None	None

UADT, upper aero-digestive tract.