



Dietary flavonoids and respiratory diseases: a population-based multi-case–control study in Italian adults

Veronica Mattioli¹, Maria Elisabetta Zanolin¹, Lucia Cazzoletti¹, Roberto Bono², Isa Cerveri³, Marcello Ferrari⁴, Pietro Pirina⁵ and Vanessa Garcia-Larsen^{6,*}

¹Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy; ²Department of Public Health and Pediatrics, University of Turin, Turin, Italy; ³Division of Respiratory Diseases, Istituto di Ricovero e Cura a Carattere Scientifico 'San Matteo' Hospital Foundation, University of Pavia, Pavia, Italy; ⁴Unit of Respiratory Diseases, Department of Internal Medicine, University of Verona, Verona, Italy; ⁵Institute of Respiratory Diseases, University of Sassari, Sassari, Italy; ⁶Program in Human Nutrition, Department of International Health, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA

Submitted 14 June 2018: Final revision received 4 August 2019: Accepted 12 August 2019

Abstract

Objective: To analyse the associations between chronic respiratory diseases and intakes of total flavonoids and their major subclasses (flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, polymers and proanthocyanidins).

Design: Multi-case–control study.

Setting: The analysis was conducted in the frame of the Genes Environment Interaction in Respiratory Diseases (GEIRD) study. The European Prospective Investigation into Cancer and Nutrition FFQ was used to ascertain dietary intake. Multinomial regression models adjusting for age, sex, centre, BMI, smoking habit, alcohol intake, education, total energy intake, vitamin C intake and total fruit intake were used to examine the associations between dietary exposures and the relative risk ratio (RRR) of being a case.

Participants: Individuals (*n* 990) hierarchically defined as follows: cases with asthma (current, *n* 159; past, *n* 78), chronic bronchitis (*n* 47), rhinitis (allergic rhinitis, *n* 167; non-allergic rhinitis, *n* 142) and controls (*n* 97).

Results: An increase of 1 SD in flavanones was associated with a reduced risk of non-allergic rhinitis (adjusted RRR = 0.68, 95% CI 0.47, 0.97); a similar result was found comparing the highest *v.* lowest quartile of flavanones intake (adjusted RRR = 0.24, 95% CI 0.10, 0.59).

Conclusions: Flavonoids contained in fruits and vegetables, especially flavanones, might reduce the risk of non-allergic rhinitis. No associations were found between other flavonoids and the considered outcomes.

Keywords
Flavonoids
Asthma
Chronic bronchitis
Rhinitis

Chronic inflammation in the airways is a central feature in asthma and other respiratory diseases and is mostly driven by oxidative stress^(1,2). The imbalance between endogenous oxidant and antioxidant substances can be influenced by diet, in particular fruits and vegetables, which are rich in antioxidants. Epidemiological evidence suggests that a diet rich in these foods might have a beneficial effect on asthma and lung function in adults and children^(1,3–6).

Extensive experimental evidence shows that flavonoids, which are polyphenols synthesized by plants,

have antioxidant, anti-allergic and anti-inflammatory effects^(7–13). It is known that flavonoids can reduce the activation of the *cyclooxygenase* gene, which is involved in the inflammatory response^(14,15), and they might act as an inhibitor of lipoxygenase, whose products are involved in the pathogenesis of a series of inflammatory diseases, including rhinitis⁽¹⁶⁾. Lipoxygenase uses peroxy radical complexes for the metabolism of arachidonic acid and flavonoids might act as antioxidants by neutralizing the radicals⁽¹⁷⁾.

In spite of the extensive and promising experimental evidence suggesting a potential benefit of flavonoids on

*Corresponding author: Email vgl@jhu.edu

airways disease, epidemiological studies examining this association are still scant and limited to few flavonoid subclasses⁽¹⁸⁾. In Finnish adults, higher intakes of quercetin, naringenin and hesperetin were associated with a reduced risk of asthma⁽¹⁹⁾. In a case-control study conducted in London, dietary intake of three major flavonoids (catechins, flavonols and flavones) was not associated with asthma or chronic bronchitis⁽²⁰⁾.

GEIRD (Genes Environment Interaction in Respiratory Diseases), a population-based multi-case-control study, aimed to provide evidence on environmental exposures, history of disease, treatment, genetic information and measurements of markers of inflammation in individuals affected by asthma, allergic rhinitis and chronic obstructive pulmonary disease (COPD)⁽²¹⁾. In this population, we investigated the association of dietary intake of six major classes of flavonoids and risk of asthma, chronic bronchitis and rhinitis.

Materials and methods

Study design

GEIRD, a population-based multi-case-control study, is a two-stage project which involves seven Italian centres⁽²¹⁾. The first stage aimed to find probable cases and controls through a screening questionnaire on respiratory symptoms. The questionnaire was mailed to pre-existing random cohorts (the Italian Study on Asthma in Young Adults (ISAYA)⁽²²⁾, the Italian arm of the European Community Respiratory Health Survey (ECRHS-Italy)⁽²³⁾) and a new random sample from the general population (20–84 years of age, male/female = 1/1). The second stage aimed to ascertain the respiratory condition of the individuals through a clinical visit. Individuals invited to clinics were a random sample of those who reported signs suggestive of rhinitis and those free from any respiratory symptoms, and all individuals who reported signs suggestive of chronic bronchitis (CB), COPD or asthma. In this stage, each individual underwent a computer-assisted clinical interview, lung function tests, reversibility test, methacholine test and skin prick test (SPT), and dietary information was collected through a self-administered FFQ. All measurement protocols agreed with international guidelines (www.geird.org)⁽²¹⁾. In the present analysis, only the individuals with information on the FFQ recruited in the years from 2007 to 2010 in the centres of Pavia, Torino, Sassari and Verona were considered.

Lung function and allergological tests

All outcome variables were assessed following standard procedures that included previously validated spirometric tests. Individuals underwent spirometry for forced expiratory volume in the first second (FEV₁) and forced vital capacity (FVC), measured according to the American Thoracic Society reproducibility criteria⁽²⁴⁾. FEV₁ % predicted

and the lower limit of normal (LLN) for the FEV₁/FVC were calculated on the basis of Quanjer *et al.*'s equations⁽²⁵⁾. Individuals with a FEV₁/FVC \geq 70 % and \geq LLN underwent a methacholine challenge test, which followed a protocol described elsewhere⁽²⁶⁾. Individuals with a FEV₁/FVC < 70 % or <LLN underwent a bronchodilator challenge test and were invited (if eligible) to undergo a methacholine challenge test on a second occasion. Atopy was assessed by SPT to common allergens⁽²⁷⁾.

Dietary exposure assessment: flavonoid intake estimates

All exposure measurements were assessed following standard procedures that included previously validated questionnaires. Information on dietary intake was collected using the Italian version of the validated European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ, which was based on Italian dietary habits and developed in the frame of an international survey⁽²⁸⁾. The NAF (Nutritional Analysis of Food Frequency Questionnaires, National Cancer Institute, Milan, Italy) software⁽²⁹⁾ was used to compute the daily intakes of food items, energy, macro- and micronutrients. Nutrient data for specific foods were obtained from the food composition database for epidemiological studies in Italy⁽³⁰⁾. Information on flavonoid intake was derived from the foods contained in the FFQ (mostly plant-derived). Estimates for intakes of total flavonoids and seven major subclasses (flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, polymers and proanthocyanidins), expressed as aglycones (mg/d), were derived from sixty-five foods (see online supplementary material, Supplemental Table S1) using the US Department of Agriculture's updated and expanded flavonoid content of foods and proanthocyanidin databases^(31,32).

Overall, out of the 2749 individuals participating in the clinical stage of the survey, 1182 (43 %) filled in the FFQ. Forty-three individuals with incomplete FFQ (less than 80 % of the 434 questions and nested questions of the FFQ filled in) were excluded from the analyses. We estimated BMR using sex-specific equations for adults (\leq 60 years old)⁽³³⁾ and the elderly (>60 years old)⁽³⁴⁾. Twenty-four individuals were excluded as BMR could not be computed due to missing data. Individuals were also excluded if they had extreme values of total energy intake (EI), which might suggest an unrealistic response: we excluded those with a ratio of EI to expected BMR below the 0.5th sample centile or above the 99.5th sample centile. Eight individuals were excluded according to the cut-off points on the top (four persons) and bottom (four persons) 0.5th percentile of the distribution of EI: BMR. Ten individuals (five women and five men) with extremely low EI levels (<2510 kJ (<600 kcal) for women and 3347 kJ (<800 kcal) for men) were excluded from the analyses⁽³⁵⁾ (Fig. 1).

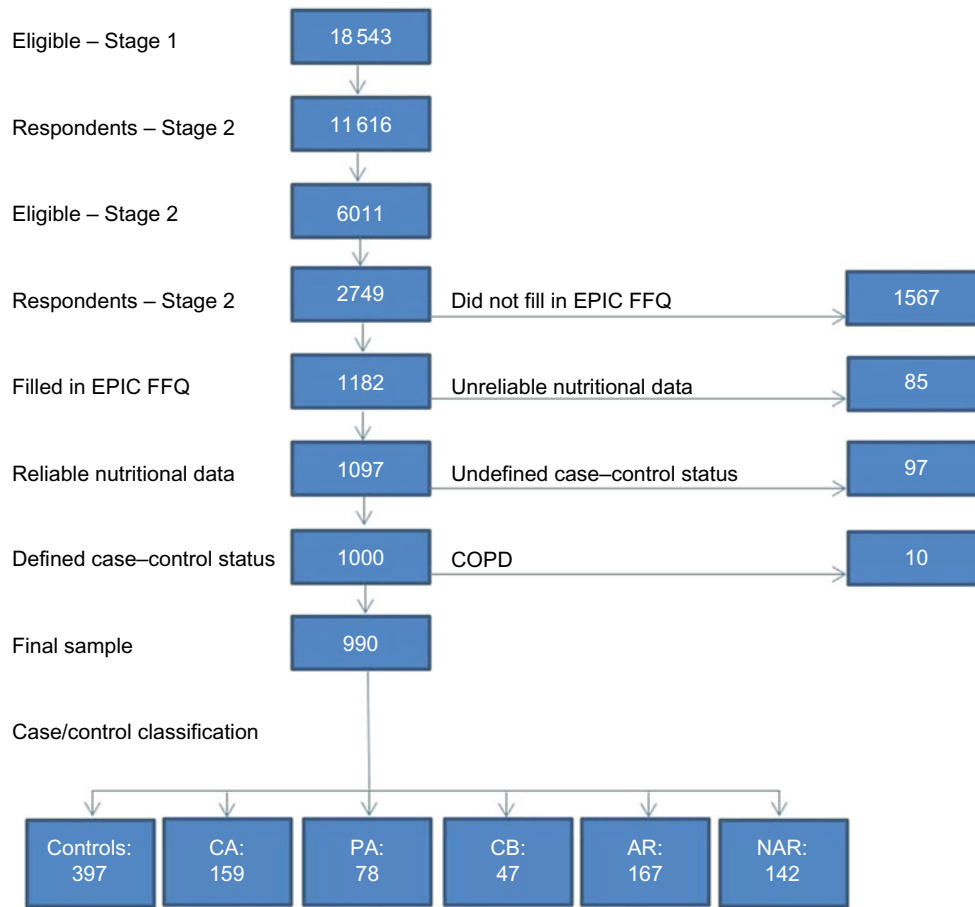


Fig. 1 (colour online) Flowchart of participant selection (EPIC, European Prospective Investigation into Cancer and Nutrition; COPD, chronic obstructive pulmonary disease; CA, current asthma; PA, past asthma; CB, chronic bronchitis; AR, allergic rhinitis; NAR, non-allergic rhinitis)

Identification of cases and controls in clinics

The sample for the current analysis included 1097 individuals (Fig. 1) with information on the clinical visit and on food and nutrient intakes, who were hierarchically classified as follows.

1. One hundred and fifty-nine cases of current asthma (CA):
 - a. he/she reported a history of asthma AND asthma-like symptoms/medicines in the last 12 months;
 - b. he/she reported a history of asthma OR asthma-like symptoms/medicines in the last 12 months plus one of the following conditions: (i) he/she had a positive methacholine challenge test with a provocative dose of methacholine causing a 20 % drop in FEV_1 (PD_{20}) of <1 mg; (ii) he/she had pre-bronchodilator $FEV_1/FEC < 70\%$ or $<LLN^{(25)}$ with a positive reversibility test (i.e. $FEV_1 > 12\%$ and >200 ml after the administration of 400 μ g salbutamol); (iii) he/she had pre-bronchodilator $FEV_1/FEC < 70\%$ or $<LLN$ with a post-bronchodilator $FEV_1/FVC > LLN$ and $>70\%$ and a post-bronchodilator $FEV_1 > 80\%$ predicted⁽²⁵⁾.
2. Seventy-eight cases of past asthma (PA): he/she reported a history of asthma but did not fulfil the criteria for CA.
3. Ten cases of COPD: post-bronchodilator $FEV_1/FEC < 70\%$ or $<LLN$ without asthma.
4. Forty-seven cases of CB: he/she was not a COPD or asthma case and he/she reported chronic cough or phlegm (>3 months/year for at least 2 years).
5. One hundred and sixty-seven cases of allergic rhinitis (AR) and 125 cases of non-allergic rhinitis (NAR): he/she had nasal allergies or nasal problems in the presence of animal(s), pollens and/or dust, plus a negative SPT (NAR) or a positive SPT to at least one allergen (AR).
6. Three hundred and ninety-seven controls: individuals without any nasal/respiratory symptoms/conditions reported in the clinical questionnaire neither in the clinic nor in the screening questionnaire, who were not cases and had both (i) pre-bronchodilator $FEV_1/FVC > LLN$ and $>70\%$; and (ii) $FEV_1 > 70\%$ predicted.

Ninety-seven individuals were unclassified due to missing data.

Individuals with COPD and unclassified individuals were excluded from the present analyses, therefore the final sample included 990 participants. Due to the hierarchical classification, cases of CA and PA could also present COPD/CB/AR/NAR; cases of CB could present AR/NAR.

Statistical analyses

Participant characteristics were summarized as percentages, or as medians and interquartile ranges (IQR), or means and standard deviations; flavonoid and food intakes were summarized as medians and IQR. The χ^2 test, Kruskal–Wallis test and Student's *t* test were used to test differences ($\alpha = 0.05$), where appropriate. The main exposures of interest (total flavonoids and each subclass) were considered as continuous variables and analysed for a variation of one standard deviation. Exposures were also categorized into quartiles based on the distribution of the exposure in controls. Quartiles allow the comparison of extreme groups, such as those with highest and lowest intakes of nutrients or foods, and are less sensitive to the effects of outliers than continuous variables. On the other hand, the estimates of measures of disease risk related to dietary exposures expressed as continuous variables make easier the comparison of evidence obtained in different studies.

To analyse the associations between each flavonoid class and case–control status, several multinomial logistic

regression models were fitted to the data, using a six-level variable (CA, PA, CB, AR, NAR, control) as outcome. Multivariable associations of exposures with case–control status were expressed by relative risk ratios (RRR; using control as the reference category) and their 95% confidence intervals. Analyses were adjusted for study sample/cohort (ISAYA, ECRHS-Italy, new random sample), centre (Verona, Pavia, Torino and Sassari), gender, age, BMI, education (low = completed before the age of 16 years; high = completed after the age of 16 years) as a proxy of socio-economic status, smoking habit (never smoker; past smoker, i.e. not smoking in the last month; current smoker), alcohol intake (g/d), total energy intake (kcal/d), vitamin C intake (mg/d) and total fruit intake (g/d). The analyses were performed using the statistical software package Stata version 14.2.

Results

Among the groups of cases and controls, we found no differences in terms of age (overall mean: 50.7 (SD 12.3) years), gender (overall proportion of males: 48.79%), BMI (overall median: 24.7 (IQR 22.3–27.7) kg/m²), physical activity and educational level (Table 1).

Comparing the characteristics of the individuals who fulfilled the EPIC FFQ (*n* 1182) and those who did not (*n* 1567), we found that mean age was significantly higher in the groups of controls, CA and AR among individuals who fulfilled the questionnaire (51.0 (SD 12.0),

Table 1 General characteristics of the participants: adults from the GEIRD (Genes Environment Interaction in Respiratory Diseases) population-based multi-case–control study in Pavia, Torino, Sassari and Verona, Italy, 2007–2010

	Controls (<i>n</i> 397)	CA (<i>n</i> 159)	PA (<i>n</i> 78)	CB (<i>n</i> 47)	AR (<i>n</i> 167)	NAR (<i>n</i> 142)	<i>P</i> value
Age at the clinical visit (years)							0.726
Mean	51.9	50.5	45.2	49.1	49.7	52.3	
SD	12.0	12.6	11.3	13.7	12.5	12.0	
Gender (% male)	48.36	49.69	44.87	55.32	52.69	44.37	0.625
Smoking habits (%)							0.013
Non-smoker	53.79	50.31	57.14	44.68	57.49	43.66	
Ex-smoker	32.58	27.67	27.27	21.28	26.35	34.51	
Current smoker	13.64	22.01	15.58	34.04	16.17	21.83	
Drinking habits (%)							0.018
Current drinker	35.10	47.17	37.18	57.45	40.72	41.13	
Abstainer	64.03	52.2	62.82	41.30	59.51	56.74	
Ex-drinker	1.53	0.63	0.00	2.17	1.23	2.13	
Total alcohol (%)							0.023
<5 g/d	6.63	14.47	10.26	10.87	5.52	13.48	
5–<15 g/d	17.60	16.98	17.95	19.57	15.95	13.48	
15–<30 g/d	6.12	10.06	6.41	13.04	13.50	10.64	
30–120 g/d	4.08	5.66	2.56	13.04	4.29	3.55	
BMI (kg/m ²)							0.692
Median	25.0	24.8	23.9	25.5	24.3	24.6	
IQR	22.4–27.7	22.0–27.6	21.9–27.0	22.1–28.6	22.6–27.2	22.2–28.0	
Physical activity (%)							0.35
Heavy	5.79	4.40	6.41	10.64	8.98	6.34	
Moderate	36.27	35.22	32.05	27.66	41.32	42.25	
Light	57.93	60.38	61.54	61.70	49.70	51.41	
Education level (% high)	75.95	75.95	87.18	70.21	78.44	68.79	0.056

CA, current asthma; PA, past asthma; CB, chronic bronchitis; AR, allergic rhinitis; NAR, non-allergic rhinitis; IQR, interquartile range.



50.5 (SD 12.6) and 49.7 (SD 12.5) years, respectively) compared with the individuals who did not (49.7 (SD 12.9), 45.2 (SD 11.8) and 46.9 (SD 12.7) years, respectively; see online supplementary material, Supplemental Table S2).

Among participants included in the analysis, we found that smoking habits, drinking habits and alcohol intake varied significantly across the groups ($P=0.013$, 0.018 and 0.023 , respectively), with the highest proportion of drinkers and current smokers in the group affected by CB (57.45 and 34.04%, respectively). There were no statistically significant differences in the intakes of total or subclasses of flavonoids across the case groups (overall median total flavonoids intake: 382.1 (IQR 248.8–529.8) mg) and in the intakes of foods rich in flavonoids (Table 2). Overall median fruit and vegetable intakes were 289.1 (IQR 191.9–425.5) g/d and 130.2 (IQR 86.7–194.5) g/d, respectively. There were no statistically significant differences in the intakes of total energy intake and vitamin C across the groups (overall median energy intake: 7988.5 (IQR 6299.0–10 037.0) kJ/d (1909.3 (IQR 1505.5–2398.9) kcal/d); overall median vitamin C intake: 117.6 (IQR 82.6–161.9) mg/d).

In the unadjusted continuous regression analyses, there were no significant associations between any of the flavonoid subclasses and the outcomes considered (Table 3). After adjustment, we found that an increase of 1 SD in intake of flavanones, equivalent to 26.1 mg/d, was associated with a reduced risk of NAR (RRR = 0.68, 95% CI 0.47, 0.97). Similarly, adjusted analyses exploring the exposures per quartile showed that those in the highest *v.* lowest quartile of flavanone intake had a reduced risk of NAR (RRR = 0.24, 95% CI 0.10, 0.59). We found no consistent evidence of an association between a high intake of other subclasses of flavonoids and the risk of any of the other outcomes.

Discussion

In this multi-case–control study of Italian adults from the general population, we found that having a higher intake of flavanones was associated with a reduced risk of NAR. The association was statistically significant when the groups were examined as the highest *v.* lowest quartile of intake and when the intake was analysed as a continuous exposure.

Flavonoids have been described to have anti-inflammatory and antioxidant properties^(7–13). Airway inflammation, which is exacerbated by oxidative stress⁽¹⁴⁾, is known to be related to the pathogenesis and worsening of pulmonary diseases^(2,36). In our study, we considered asthma, CB and rhinitis, diseases in which airway inflammation plays a key role⁽³⁷⁾. Depending on the severity of asthma, inflammation can affect the large and small airways⁽²⁾. Similarly, in CB, inflammation affects the lumen and the wall of the airways⁽³⁸⁾. Experimental studies show that flavonoids can counteract the oxidative stress and

inflammation caused by environmental insult to the airways. Quercetin, one of the most investigated flavonoids, has been shown to reduce superoxides and nitric oxide radicals, which are mediators of inflammation^(39,40). Flavonoids can inhibit the metabolism of arachidonic acid, which is involved in the production of reactive oxygen species^(10,41). Moreover, they seem to reduce the adhesion of inflammatory cells⁽¹⁴⁾.

In spite of the large body of experimental evidence supporting an association of flavonoid intake with respiratory diseases, epidemiological studies are still scant and have reported mixed results, which might partly be explained by the different types of flavonoids studied⁽⁴²⁾. Several population-based studies have suggested that higher intakes of foods rich in flavonoids (mainly fruits, vegetables, wine and tea) were associated with a lower risk of asthma^(1,43), although this association has not been confirmed in other studies⁽⁴⁴⁾. The few population-based studies that have examined the association of flavonoid intake and airways disease are limited and the evidence is inconclusive. Tabak *et al.* reported that a higher intake of catechins was associated with a lower risk of COPD and asthma in a population of Dutch adults⁽⁴⁵⁾. Garcia *et al.* found no evidence of an association of three subclasses of flavonoids with asthma or CB in a population-based case–control study in London⁽²⁰⁾. Moreover, a recent clinical trial on both adults and children with poor asthma control revealed no association between the supplementary use of soya isoflavone and lung function⁽⁴⁶⁾.

The recent hypothesis that microbiota might play a role to protect against inflammation-mediated airways diseases was investigated in a small sample of twenty-three allergic individuals⁽⁴⁷⁾. The study showed that a higher intake of a diet rich in phenolic compounds was associated with a more stable gut microbiota, which might contribute to the management of allergic conditions through a reduced inflammation. A recent cohort study reported that a higher intake of soya genistein was associated with improved lung function and asthma control in individuals with stable asthma⁽⁴⁸⁾. Evidence from human trials is also limited. A recent randomized controlled trial found no evidence that supplementation with soya genistein had any effect on outcomes of asthma or lung function in adults with unstable asthma⁽⁴⁶⁾, while in an intervention in forty-two asthmatic adults, a passion fruit puree extract rich in bioflavonoids reduced the severity of asthma symptoms⁽⁴⁹⁾.

In our study, we found limited evidence to suggest that flavonoids are associated with airways disease. The individuals in our population-based study had stable asthma, CB or AR. It might be possible that the beneficial effect of flavonoids occurs in cases of greater chronic airway inflammation, which is usually reduced in stable patients. We found a statistically significant negative association of flavanones with non-atopic rhinitis. The association with atopic rhinitis was also negative in both approaches used, but not statistically significant. Atopy in

Table 2 Distribution of the dietary flavonoid, food and nutrient intakes studied, according to case or control status, among adults from the GEIRD (Genes Environment Interaction in Respiratory Diseases) population-based multi-case–control study in Pavia, Torino, Sassari and Verona, Italy, 2007–2010

	Controls (n397)		CA (n159)		PA (n78)		CB (n47)		AR (n167)		NAR (n142)		P value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Flavonoids (mg/d)													
Total flavonoids	381.9	255.1–526.5	388.3	258.4–554.9	380.9	212.1–517.5	374.5	198.5–515.1	365.6	238.2–545.5	394.2	277.4–518.4	0.98
Flavanones	27.3	14.0–45.8	27.1	12.8–43.3	28.1	11.1–36.5	23.2	12.3–38.7	28.0	18.3–40.3	26.7	13.4–38.5	0.75
Anthocyanins	17.3	10.0–28.4	15.7	8.8–27.7	16.6	10.7–24.8	15.4	6.2–25.4	15.9	10.3–25.7	17.8	9.3–28.0	0.92
Flavan-3-ols	45.5	26.7–71.9	46.0	27.7–75.2	48.2	26.7–72.3	42.9	28.2–85.8	46.6	25.4–73.4	47.7	29.2–80.1	0.93
Flavonols	15.9	10.8–21.7	15.1	10.4–20.0	15.1	10.6–21.7	17.0	10.8–23.8	15.2	9.6–21.7	15.8	10.2–20.7	0.86
Flavones	1.9	1.1–2.9	2.0	1.2–3.2	1.9	1.3–2.8	1.9	1.2–2.7	1.9	1.0–2.8	1.9	1.0–3.0	0.94
Polymers	269.4	168.6–370.6	261.3	168.6–403.2	267.7	138.3–380.5	257.7	146.1–350.9	247.7	157.3–377.4	254.6	174.7–370.0	0.98
Proanthocyanidins	293.0	188.6–417.2	295.3	188.2–461.4	293.3	154.2–420.0	292.0	163.0–405.5	279.3	178.0–432.5	289.0	197.3–422.1	0.98
Foods (g/d)													
Fruits	302.6	203.0–433.0	288.4	193.3–424.1	275.35	168.8–425.7	211.4	142.6–398.6	290.2	200.1–401.6	280.75	188.6–429.0	0.22
Vegetables	129.0	79.8–195.1	118.6	81.4–190.9	150.95	105.2–232.0	152.1	101.1–196.3	128.7	89.4–182.5	131.05	91.1–195.2	0.18
Chocolate	2.7	0.0–8.6	2.9	0.7–12.8	2.9	0.7–11.4	2.9	1.3–10.3	3.7	0.7–8.6	3.7	0.7–11.4	0.12
Red wine	4.2	0.0–62.5	13.9	0.0–107.1	8.1	0.0–62.5	4.2	0.0–79.4	8.3	0.0–63.5	1.9	0.0–59.5	0.47
White wine	1.4	0.0–26.8	2.1	0.0–41.7	3.5	0.0–35.7	0.5	0.0–26.8	1.0	0.0–26.8	1.0	0.0–26.8	0.74
Tea	10.0	0.0–85.7	5.0	0.0–64.3	10.0	0.0–64.3	15.0	0.0–150	15.0	0.0–85.7	10.0	0.0–150	0.52
Nutrients													
Vitamin C (mg/d)	116.3	83.4–162.8	116.9	84.0–156.4	129.3	79.8–167.8	105.3	71.5–151.7	119.4	84.6–164.4	118.9	80.9–165.4	0.84
Total energy intake (kJ/d)	7849.6	6123.7–9989.3	7666.8	6353.4–9288.9	8986.4	6875.1–10732.8	8892.3	6377.3–11621.1	8217.4	6602.4–9699.3	7728.3	6278.9–10132.8	0.13
Total energy intake (kcal/d)	1876.1	1463.6–2387.5	1832.4	1518.5–2220.1	2147.8	1643.2–2565.2	2125.3	1524.2–2777.5	1964.0	1578.0–2318.2	1847.1	1500.7–2421.8	0.13

CA, current asthma; PA, past asthma; CB, chronic bronchitis; AR, allergic rhinitis; NAR, non-allergic rhinitis; IQR, interquartile range.

Table 3 Unadjusted and adjusted* relative risk ratios (RRR) and 95 % confidence intervals of being a respiratory disease case rather than a control (*n* 397), according to the intake of flavonoids†, among adults from the GEIRD (Genes Environment Interaction in Respiratory Diseases) population-based multi-case–control study in Pavia, Torino, Sassari and Verona, Italy, 2007–2010

Flavonoid intake (per mg/d increase of 1 sd)	CA (<i>n</i> 159)		PA (<i>n</i> 78)		CB (<i>n</i> 47)		AR (<i>n</i> 167)		NAR (<i>n</i> 142)	
	RRR	95 % CI	RRR	95 % CI	RRR	95 % CI	RRR	95 % CI	RRR	95 % CI
Total flavonoids (unadjusted)	0.98	0.81, 1.18	0.94	0.73, 1.21	0.94	0.69, 1.28	0.93	0.77, 1.12	0.99	0.82, 1.20
Total flavonoids (adjusted)	1.05	0.80, 1.37	0.92	0.65, 1.30	1.12	0.72, 1.73	0.94	0.72, 1.22	1.02	0.77, 1.34
Flavanones (unadjusted)	1.01	0.85, 1.20	0.80	0.60, 1.07	0.81	0.57, 1.16	0.98	0.82, 1.17	0.86	0.69, 1.06
Flavanones (adjusted)	1.09	0.81, 1.45	0.67	0.42, 1.08	0.97	0.52, 1.79	0.88	0.66, 1.19	0.68	0.47, 0.97
Anthocyanins (unadjusted)	0.93	0.77, 1.12	0.93	0.73, 1.19	0.92	0.68, 1.26	0.95	0.79, 1.13	0.96	0.79, 1.15
Anthocyanins (adjusted)	0.83	0.62, 1.11	1.14	0.76, 1.71	0.97	0.62, 1.53	0.93	0.70, 1.25	0.98	0.71, 1.35
Flavan-3-ols (unadjusted)	0.98	0.81, 1.19	0.98	0.76, 1.27	1.05	0.78, 1.41	0.93	0.76, 1.13	1.04	0.86, 1.26
Flavan-3-ols (adjusted)	0.98	0.79, 1.22	0.89	0.66, 1.20	1.08	0.76, 1.54	0.92	0.74, 1.15	1.06	0.86, 1.32
Flavonols (unadjusted)	0.88	0.72, 1.07	0.93	0.72, 1.20	1.20	0.92, 1.56	0.93	0.77, 1.12	0.97	0.80, 1.18
Flavonols (adjusted)	0.81	0.63, 1.06	0.98	0.71, 1.36	1.37	0.97, 1.94	0.88	0.69, 1.13	0.94	0.71, 1.21
Flavones (unadjusted)	1.04	0.87, 1.24	0.94	0.73, 1.21	1.14	0.86, 1.49	0.94	0.78, 1.13	0.96	0.79, 1.16
Flavones (adjusted)	0.93	0.71, 1.22	1.03	0.71, 1.51	1.00	0.69, 1.47	0.81	0.62, 1.07	0.95	0.71, 1.27
Polymers (unadjusted)	0.98	0.82, 1.18	0.96	0.75, 1.23	0.93	0.67, 1.27	0.93	0.77, 1.12	1.01	0.83, 1.22
Polymers (adjusted)	1.08	0.84, 1.39	0.97	0.70, 1.33	1.09	0.72, 1.66	0.98	0.76, 1.26	1.03	0.80, 1.34
Proanthocyanidins (unadjusted)	1.02	0.85, 1.22	0.96	0.75, 1.24	0.95	0.69, 1.30	0.95	0.79, 1.15	1.01	0.83, 1.22
Proanthocyanidins (adjusted)	1.11	0.88, 1.41	0.95	0.69, 1.29	1.05	0.70, 1.58	1.00	0.78, 1.26	1.03	0.81, 1.32

CA, current asthma; PA, past asthma; CB, chronic bronchitis; AR, allergic rhinitis; NAR, non-allergic rhinitis.

*The estimates were adjusted for age, gender, centre, study cohort, BMI, smoking habits, alcohol intake, educational level, total fruit intake, vitamin C intake and total energy intake.

†Significant results are shown in bold.

those affected by rhinitis is defined on the basis of an SPT, which measures the systemic presence of serum allergen-specific IgE in allergic individuals. However, it has also been found in individuals affected by non-atopic rhinitis that allergen-specific IgE can be found locally in the nasal mucosa, which is not detectable through an SPT⁽⁵⁰⁾. Flavonoids are thought to inhibit the formation of IgE^(9,18) and this might explain the positive effect found on individuals with non-atopic rhinitis in the present study.

Our study did not confirm an association of any of the flavonoid subclasses studied with CB, which is in line with the results reported in an earlier case–control study in the general population⁽²⁰⁾. Anthocyanin intake was associated with a reduced risk of CB, but this did not reach statistical significance. Although inflammation is a central feature in CB, it might be possible that avoidance of environmental pollutants and related environmental insult might be the best effective way to reduce the risk of this disease⁽³⁸⁾.

Some observational studies have analysed the association between flavonoids and atopic rhinitis. The extract of French maritime bark, which is rich in flavonoids, appeared to reduce eye and nasal symptoms, but the results were limited by the small sample of participants⁽⁵¹⁾. In another clinical trial, a preventive effect of enzymatically modified isoquercitrin was found on ocular symptoms, but not on nasal symptoms⁽⁵²⁾.

Our study has several strengths. We considered several chronic respiratory diseases, giving a wide view of the possible role of flavonoids in different pulmonary health conditions. Case and control status was assessed in a two-stage process; first by a screening questionnaire on

symptoms and use of medicines, then through a clinical visit⁽²¹⁾. The EPIC FFQ was validated through comparison with multiple interviews and the analysis of nitrogen in urine samples⁽²⁸⁾. We analysed the associations between flavonoid intakes and chronic respiratory diseases considering seven subclasses of flavonoids, computed using data from the US Department of Agriculture database, which provides updated information of flavonoid contents of foods.

We acknowledge some limitations to the study. Having five case groups and only one control group might have reduced the power to detect statistically significant differences between the participants. Moreover, we defined the potential confounders *a priori* based on pre-existing knowledge on their relationship with flavonoids and with the outcomes evaluated; however, residual confounding may still exist. The Italian version of the EPIC FFQ provides an extensive list of foods with high content of flavonoids, which allowed us to derive estimates for all major subclasses. However, the earlier validation study of the Italian version of the EPIC FFQ showed modest levels of agreement in the intakes of macronutrients and some foods, and has not been validated specifically for flavonoid intake⁽²⁸⁾. Finally, we used data from a single FFQ and assumed that adults maintained relatively stable dietary habits to investigate disease risk⁽⁵³⁾.

Overall, the results of the present population-based, multi-case–control study suggest that intake of flavanones is negatively associated with the risk of having NAR. No associations were found between other flavonoids and the considered outcomes.

Acknowledgements

Acknowledgements: The authors are indebted to all the individuals of the GEIRD study for their participation. **Financial support:** The GEIRD project was funded by the Cariverona Foundation; the Italian Ministry of Health; Chiesi Farmaceutici; and the Italian Medicines Agency (AIFA). The funders had no role in the design; in the collection, analysis and interpretation of the data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. **Conflict of interest:** The authors declare no conflict of interest. **Authorship:** V.G.-L., M.E.Z. and L.C. conceived and designed the study. L.C., M.E.Z., R.B., I.C., P.P. and M.F. contributed to the data collection. V.M. performed the statistical analysis. L.C., M.E.Z. and V.M. drafted the manuscript. L.C., M.E.Z., V.G.-L., A.G., V.M., P.P. and M.F. contributed to the interpretation of data, revised the paper critically for important intellectual content and approved the version to be published. **Ethics of human subject participation:** This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of Azienda Ospedaliera of Verona, as coordinating centre. Written informed consent was obtained from all subjects.

Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S1368980019003562>

References

- Hosseini B, Berthon BS, Wark P *et al.* (2017) Effects of fruit and vegetable consumption on risk of asthma, wheezing and immune responses: a systematic review and meta-analysis. *Nutrients* **9**, 341.
- MacNee W (2001) Oxidative stress and lung inflammation in airways disease. *Eur J Pharmacol* **429**, 195–207.
- Garcia-Larsen V, Del Giacco SR, Moreira A *et al.* (2016) Asthma and dietary intake: an overview of systematic reviews. *Allergy* **71**, 433–442.
- Seyedrezazadeh E, Moghaddam MP, Ansarin K *et al.* (2014) Fruit and vegetable intake and risk of wheezing and asthma: a systematic review and meta-analysis. *Nutr Rev* **72**, 411–428.
- Butland BK, Strachan DP & Anderson HR (1999) Fresh fruit intake and asthma symptoms in young British adults: confounding or effect modification by smoking? *Eur Respir J* **13**, 744–750.
- Uddenfeldt M, Janson C, Lampa E *et al.* (2010) High BMI is related to higher incidence of asthma, while a fish and fruit diet is related to a lower – results from a long-term follow-up study of three age groups in Sweden. *Respir Med* **104**, 972–980.
- Ferk F, Misik M, Nersesyan A *et al.* (2016) Impact of xanthohumol (a prenylated flavonoid from hops) on DNA stability and other health-related biochemical parameters: results of human intervention trials. *Mol Nutr Food Res* **60**, 773–786.
- Ross JA & Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* **22**, 19–34.
- Kimata M, Shichijo M, Miura T *et al.* (2000) Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. *Clin Exp Allergy* **30**, 501–508.
- Ferrandiz ML & Alcaraz MJ (1991) Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions* **32**, 283–288.
- Regal JF, Fraser DG, Weeks CE *et al.* (2000) Dietary phytoestrogens have anti-inflammatory activity in a guinea pig model of asthma. *Proc Soc Exp Biol Med* **223**, 372–378.
- Homma M, Minami M, Taniguchi C *et al.* (2000) Inhibitory effects of lignans and flavonoids in saiboku-to, a herbal medicine for bronchial asthma, on the release of leukotrienes from human polymorphonuclear leukocytes. *Planta Med* **66**, 88–91.
- Rice-Evans CA, Miller NJ & Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci* **2**, 152–159.
- Lago JH, Toledo-Arruda AC, Mernak M *et al.* (2014) Structure–activity association of flavonoids in lung diseases. *Molecules* **19**, 3570–3595.
- Smith WL, DeWitt DL & Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* **69**, 145–182.
- Ribeiro D, Freitas M, Tome SM *et al.* (2014) Inhibition of LOX by flavonoids: a structure–activity relationship study. *Eur J Med Chem* **72**, 137–145.
- Prochazkova D, Bousova I & Wilhelmova N (2011) Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* **82**, 513–523.
- Santana FP, Pinheiro NM, Mernak MI *et al.* (2016) Evidences of herbal medicine-derived natural products effects in inflammatory lung diseases. *Mediators Inflamm* **2016**, 2348968.
- Knekt P, Kumpulainen J, Jarvinen R *et al.* (2002) Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr* **76**, 560–568.
- Garcia V, Arts IC, Sterne JA *et al.* (2005) Dietary intake of flavonoids and asthma in adults. *Eur Respir J* **26**, 449–452.
- de Marco R, Accordini S, Antonicelli L *et al.* (2010) The gene–environment interactions in respiratory diseases (GEIRD) project. *Int Arch Allergy Immunol* **152**, 255–263.
- de Marco R, Poli A, Ferrari M *et al.* (2002) The impact of climate and traffic-related NO₂ on the prevalence of asthma and allergic rhinitis in Italy. *Clin Exp Allergy* **32**, 1405–1412.
- de Marco R, Verlato G, Zanolin E *et al.* (1994) Nonresponse bias in EC Respiratory Health Survey in Italy. *Eur Respir J* **7**, 2139–2145.
- American Thoracic Society (1995) Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* **152**, 1107–1136.
- Quanjer PH, Stanojevic S, Cole TJ *et al.* (2012) Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* **40**, 1324–1343.
- Chinn S, Burney P, Jarvis D *et al.* (1997) Variation in bronchial responsiveness in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* **10**, 2495–2501.
- European Academy of Allergology and Clinical Immunology (1993) Position paper: allergen standardization and skin tests. *Allergy* **48**, 48–82.
- Pisani P, Faggiano F, Krogh V *et al.* (1997) Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. *Int J Epidemiol* **26**, Suppl. 1, S152–S160.
- Pala V, Sieri S, Palli D *et al.* (2003) Diet in the Italian EPIC cohorts: presentation of data and methodological issues. *Tumori* **89**, 594–607.
- Salvini S (1997) A food composition database for epidemiological studies in Italy. *Cancer Lett* **114**, 299–300.



31. Bhagwat S, Gebhardt S, Haytowitz DB *et al.* (2013) *USDA Database for the Flavonoid Content of Selected Foods, Release 3.1*. Beltsville, MD: US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory.
32. Bhagwat S, Haytowitz D, Prior R *et al.* (2015) *USDA Database for Proanthocyanidin Content of Selected Foods, Release 2*. Beltsville, MD: US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory.
33. Harris JA & Benedict FG (1918) A biometric study of human basal metabolism. *Proc Natl Acad Sci U S A* **4**, 370–373.
34. Fredrix EW, Soeters PB, Deerenberg IM *et al.* (1990) Resting and sleeping energy expenditure in the elderly. *Eur J Clin Nutr* **44**, 741–747.
35. Slattery ML, Jacobs DR Jr, Dyer A *et al.* (1995) Dietary antioxidants and plasma lipids: the CARDIA Study. *J Am Coll Nutr* **14**, 635–642.
36. Rebuck AS & Chapman KR (1987) Asthma: 1. Pathophysiologic features and evaluation of severity. *CMAJ* **136**, 351–354.
37. Eifan AO & Durham SR (2016) Pathogenesis of rhinitis. *Clin Exp Allergy* **46**, 1139–1151.
38. Braman SS (2006) Chronic cough due to chronic bronchitis: ACCP evidence-based clinical practice guidelines. *Chest* **129**, 1 Suppl., 104S–115S.
39. Huk I, Brovkovych V, Nanobash Vili J *et al.* (1998) Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. *Br J Surg* **85**, 1080–1085.
40. van Acker SA, Tromp MN, Haenen GR *et al.* (1995) Flavonoids as scavengers of nitric oxide radical. *Biochem Biophys Res Commun* **214**, 755–759.
41. Middleton E Jr & Kandaswami C (1992) Effects of flavonoids on immune and inflammatory cell functions. *Biochem Pharmacol* **43**, 1167–1179.
42. Tanaka T & Takahashi R (2013) Flavonoids and asthma. *Nutrients* **5**, 2128–2143.
43. Shaheen SO, Sterne JA, Thompson RL *et al.* (2001) Dietary antioxidants and asthma in adults: population-based case-control study. *Am J Respir Crit Care Med* **164**, 1823–1828.
44. Garcia-Larsen V, Arthur R, Potts JF *et al.* (2017) Is fruit and vegetable intake associated with asthma or chronic rhinosinusitis in European adults? Results from the Global Allergy and Asthma Network of Excellence (GA(2)LEN) Survey. *Clin Transl Allergy* **7**, 3.
45. Tabak C, Arts IC, Smit HA *et al.* (2001) Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones: the MORGEN Study. *Am J Respir Crit Care Med* **164**, 61–64.
46. Smith LJ, Kalhan R, Wise RA *et al.* (2015) Effect of a soy isoflavone supplement on lung function and clinical outcomes in patients with poorly controlled asthma: a randomized clinical trial. *JAMA* **313**, 2033–2043.
47. Cuervo A, Hevia A, Lopez P *et al.* (2016) Phenolic compounds from red wine and coffee are associated with specific intestinal microorganisms in allergic subjects. *Food Funct* **7**, 104–109.
48. Bime C, Wei CY, Holbrook J *et al.* (2012) Association of dietary soy genistein intake with lung function and asthma control: a post-hoc analysis of patients enrolled in a prospective multicentre clinical trial. *Prim Care Respir J* **21**, 398–404.
49. Watson RR, Zibadi S, Rafatpanah H *et al.* (2008) Oral administration of the purple passion fruit peel extract reduces wheeze and cough and improves shortness of breath in adults with asthma. *Nutr Res* **28**, 166–171.
50. Hamizan AW, Rimmer J, Alvarado R *et al.* (2017) Positive allergen reaction in allergic and nonallergic rhinitis: a systematic review. *Int Forum Allergy Rhinol* **7**, 868–877.
51. Ross SM (2016) Allergic rhinitis: a proprietary extract of *Pinus pinaster* Aiton (pycnogenol) is found to improve the symptoms associated with allergic rhinitis. *Holist Nurs Pract* **30**, 301–304.
52. Hirano T, Kawai M, Arimitsu J *et al.* (2009) Preventative effect of a flavonoid, enzymatically modified isoquercitrin on ocular symptoms of Japanese cedar pollinosis. *Allergol Int* **58**, 373–382.
53. Macdonald HM, New SA & Reid DM (2005) Longitudinal changes in dietary intake in Scottish women around the menopause: changes in dietary pattern result in minor changes in nutrient intake. *Public Health Nutr* **8**, 409–416.