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Proanthocyanidins and other flavonoids in relation to endometrial cancer risk: a case–control study in Italy

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Background: Because of their antioxidant and antimutagenic properties, flavonoids may reduce cancer risk. Some flavonoids have antiestrogenic effects that can inhibit the growth and proliferation of endometrial cancer cells.

Methods: In order to examine the relation between dietary flavonoids and endometrial cancer, we analysed data from an Italian case–control study including 454 incident, histologically confirmed endometrial cancers and 908 hospital-based controls. Information was collected through a validated food-frequency questionnaire. We applied data on food and beverage composition to estimate the intake of flavanols, flavanones, flavonols, anthocyanidins, flavones, isoflavones, and proanthocyanidins. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from multiple logistic regression models conditioned on age and study centre and adjusted for major confounding factors.

Results: Women in the highest quartile category of proanthocyanidins with $\geqslant 3$ mers *vs* the first three quartile categories had an OR for endometrial cancer of 0.66 (95% CI = 0.48–0.89). For no other class of flavonoids, a significant overall association was found. There was a suggestion of an inverse association for flavanones and isoflavones among women with body mass index $<25\,\mathrm{kg\,m^{-2}}$, and, for flavanones, among parous or non-users of hormone-replacement therapy women.

Conclusion: High consumption of selected proanthocyanidins may reduce endometrial cancer risk.

The major recognised risk factors of endometrial cancer, such as overweight, obesity, earlier age at menarche, later age at menopause, and nulliparity, point to the unopposed oestrogen hypothesis (Parazzini *et al*, 1991; Levi *et al*, 1993; Kaaks *et al*, 2002; Rosato *et al*, 2011). However, other mechanisms, such as inflammation and oxidative stress, have also been proposed (Modugno *et al*, 2005; Fernandez-Sanchez *et al*, 2011). Diet may influence endometrial cancer independently from obesity, although the association with specific foods or food groups is still controversial (La Vecchia *et al*, 1986; Petridou *et al*, 2002; Adami *et al*, 2008; Biel *et al*, 2011). A modest inverse association with vegetable consumption, in particular with cruciferous vegetables, was reported in a few case–control studies (Tzonou *et al*, 1996; Bandera *et al*, 2007; Bravi *et al*, 2009). Data from cohort studies are scanty and tend not to consistently support a protective role of fruit or vegetable consumption on the risk of endometrial cancer

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(Terry *et al*, 1999; McCullough *et al*, 2007; Kabat *et al*, 2010). A favourable effect of plant food constituents on endometrial cancer is plausible from a biological standpoint, especially for phytoestrogens, including flavonoids, that have low oestrogenic activity and showed antioxidant and antimutagenic properties *in vitro* (Nijveldt *et al*, 2001). Flavonoids include over 5000 compounds with a similar structure, consisting of two phenolic benzene rings linked to heterocyclic pyre or pyrone. They are classified into six major classes (flavanols, flavanones, flavonols, anthocyanidins, flavones, and isoflavones) and a family of polymers of flavanols without added sugar, called proanthocyanidins. Besides their free radical scavenging and antioxidant properties, some flavones, flavanones and flavanols, and isoflavones also activate oestrogen-receptor-mediated signalling and can inhibit the growth and proliferation of endometrial cancer cells (Messina *et al*, 1994; Moutsatsou, 2007; Deming *et al*, 2008). A case–control study conducted in New Jersey found that an antioxidant index of phenolics was inversely related to endometrial cancer risk (Gifkins *et al*, 2012). In a previous investigation of that study, a decreased risk for endometrial cancer was reported for increased intakes of quercetin, a flavonol (Bandera *et al*, 2009b). No association emerged, however, between selected flavones and flavonols and endometrial cancer risk in the Women's Health Study (Wang *et al*, 2009). Isoflavones were inversely associated to the risk of endometrial cancer in one case–control study and one cohort study (Xu *et al*, 2004; Ollberding *et al*, 2012), whereas two other case–control studies reported null associations between isoflavones and endometrial cancer overall (Horn-Ross *et al*, 2003; Bandera *et al*, 2009b). To our knowledge, no study has investigated the relation between other flavonoids, including proanthocyanidins and endometrial cancer.

To further investigate this issue, we therefore analysed data from a case–control study conducted in Italy.

MATERIALS AND METHODS

We analysed data from a case–control study on endometrial cancer conducted between 1992 and 2006 in three Italian areas, including the greater Milan area, the provinces of Udine and Pordenone in northern Italy, and the urban area of Naples in southern Italy (Bravi *et al*, 2009). The study was approved by the local ethics committees.

Cases included 454 women (median age, 60 years; range, 18–79 years) with incident, histologically confirmed endometrial cancer (*International Classification of Diseases,* World Health Organization, 1997), admitted to major teaching and general hospitals of the study areas. Women with a first diagnosis of endometrial cancer and with no previous diagnosis of cancer at any site were eligible.

Controls included 908 women (median age, 61 years; range, 19–80 years) admitted to the same network of hospitals as cases for a wide spectrum of acute, non-neoplastic conditions. Women who had undergone hysterectomy, or those admitted for gynaecological or hormone-related conditions, or any medical condition related to long-term dietary changes were excluded. Controls were matched with cases by 5-year age group and study centre, with a case to control ratio of 1 : 2. Thirty-six percent of controls were admitted for traumas, 32% for other orthopaedic disorders, 9% for acute surgical conditions, and 23% for other illnesses, including eye, nose, ear, or skin disorders. Less than 5% of both cases and controls approached for the interview refused to participate.

Trained professionals concurrently interviewed cases and controls during their hospital stay using a structured questionnaire, including information on sociodemographic characteristics, anthropometric measures, selected lifestyle habits (including tobacco smoking and alcohol drinking), a problem-oriented medical history, a family history of cancer, menstrual and reproductive factors, and use of oral contraceptives (OCs) and menopause hormone-replacement therapy (HRT).

By design, cases and controls came from the same study centre and had similar age distribution. Cases reported more frequently than controls a body mass index (BMI) over 30 $kg\,m^{-2}$, a history of diabetes, an early age at menarche, a late age at menopause, low parity, and the use of HRT. They also reported less frequent use of OCs.

Information on patients' usual diet during the 2 years before cancer diagnosis or hospital admission was based on a food-frequency questionnaire (FFQ), which was tested for reproducibility for food items and specific nutrients and validated for nutrients (Franceschi *et al*, 1993, 1995; Decarli *et al*, 1996; Ferraroni *et al*, 1996). Patients were asked to indicate quantity and average weekly frequency of consumption for the period under investigation. The FFQ included 83 foods and food groups, as well as common Italian recipes and several types of alcoholic beverages. Intakes lower than once a week but at least once a month were coded as 0.5 per week. Questions on fat-intake pattern, as well as portion size, were used to fine tune the composition of recipes.

For each patient, we translated the frequency of consumption of each FFQ item into average daily intake of flavonoids, taking into account the portion size of each food item. We used food composition data in terms of the six major classes of flavonoids, which were published by the US Department of Agriculture (USDA) (U.S. Department of Agriculture, 2002; U.S. Department of Agriculture, 2003). For isoflavones, we further integrated these tables with other European data sources when available (Liggins *et al*, 2000a,b, 2002). Major flavonoids were epicathechin and catechin for flavanols, hesperetin and naringerin for flavanones, quercetin for flavonols, cyanidin and malvidin for anthocyanidins, apigenin and luteolin for flavones, and daidzein and genistein for isoflavones. In our control population, flavanols came mainly from tea, apples or pears and wine; flavanones from oranges and other citrus fruits; flavonols from apples or pears and various common vegetables; anthocyanidins from wine, strawberries, cherries, and onions; flavones from cooked vegetables and tea; and isoflavones from soya and bean soups. For proanthocyanidins, we used USDA data that were available according to their degree of polymerisation, that is, monomers, dimers, trimers, 4–6 mers, 7–10 mers, >10 mers (U.S. Department of Agriculture, 2004). Given the high correlation between some classes of proanthocyanidins, we further combined monomers and dimers, as well as polymers with three or more mers. The major sources of combined monomers and dimers of proanthocyanidins were wine, apples or pears, peaches or apricots or prunes, whereas major sources of proanthocyanidins with three or more mers were apples or pears, wine, vegetables or bean soups, chocolate, pulses, and grapes.

Energy intake was computed using an Italian food composition database (Salvini *et al*, 1998; Gnagnarella *et al*, 2004).

We computed 'energy-adjusted' flavonoid intakes using the residual method (Willett and Stampfer, 1986). The 'energy-adjusted' flavonoids were categorised into quartiles based on the controls distribution. The corresponding odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from separate multiple logistic regression models, conditioned on study centre and quinquennia of age, and adjusted for year of interview, years of education (<7, 7–11, ⩾12, categorically), BMI (quintiles, categorically), history of diabetes (yes/no), age at menarche (<12, 12–13, ⩾14 years, categorically), menopausal status/age at menopause (pre/perimenopausal, <50, 50–54, ⩾55, categorically), parity (0, 1, 2, ⩾3, categorically), OC use (never/ever), and HRT use (never/ever). We also computed test for trend across quartiles and estimated the continuous ORs for an increment equal to one SD. As the distributions of flavonoids were highly positively skewed and the associations were similar in the first three quartile

categories, separate quartiles provided no additional information, and we collapsed them as the reference category to improve the precision of the estimate. We, therefore, presented the ORs for the highest *vs* the first three combined quartile categories.

Only few foods – such as soy milk – contribute to the intake of isoflavones and are all very uncommon in the Italian diet. This explains the very low intakes of isoflavones and almost null correlation between isoflavones and total energy intake in our data (Bosetti *et al*, 2005). For this reason, the ORs for isoflavones were estimated entering into the models quartile categories based on the raw values of intake and total energy intake separately.

Stratified analyses were carried out according to age (<55, 55–69, $\geqslant 70$ years), BMI (<25, 25–<30, $\geqslant 30\,kg\,m^{-2}$), menopausal status (pre- and peri-/postmenopause), parity ($0/\geqslant 1$ birth), and HRT use (yes/no). To test for heterogeneity across strata, the likelihood ratio test of the models with and without interaction terms was used.

RESULTS

There was no significant trend in risk of endometrial cancer with increasing intake of flavonoids, but we found a threshold effect of proanthocyandins with three or more mers on the risk of endometrial cancer after the third quartile of intake. Table 1 presents the ORs and corresponding 95% CIs for patients in the highest quartile category of intake, as compared with those in the first three categories combined. Significant inverse associations were found for proanthocyanidins with three or more mers combined (OR = 0.66, 95% CI = 0.48–0.89), as well as for their components: 3 mers (OR = 0.73, 95% CI = 0.54–0.98), 4–6 mers (OR = 0.74, 95% CI = 0.55–1.01), 7–10 mers (0.70, 95% CI = 0.52–0.95), >10 mers (0.64, 95% CI = 0.47–0.87). Intakes in the highest category of other flavonoids, or total flavonoids, were not significantly related to endometrial cancer risk overall. There was a suggestion of an inverse relation for flavanones (OR = 0.78, 95% CI = 0.58–1.05) and isoflavones (OR = 0.84, 95% CI = 0.62–1.14), but both associations were not significant.

Table 2 shows the ORs of endometrial cancer for flavanones, isoflavones, proanthocyanidins $\geqslant 3$ mers, and total flavonoids in strata of selected covariates. For all these flavonoids, risk estimates were not significantly heterogeneous across the strata, with the exception of flavonones across strata of parity (*P* for heterogeneity in nulliparous *vs* parous women = 0.036). However, the inverse relations between flavonoids and endometrial cancer appeared to be stronger in strata characterised by lower levels of estrogens, that is, normal weight and lean, postmenopausal, parous, or never-HRT-user women. In particular, the ORs for endometrial cancer risk in women with a BMI $<25\,kg\,m^{-2}$ were 0.64 (95% CI = 0.39–1.04) for flavanones, 0.59 (95% CI = 0.34–1.02) for isoflavones, and 0.60 (95% CI = 0.35–1.01) for proanthocyanidins with three or more mers. There was also an inverse association between flavanones and endometrial cancer risk among parous women (OR = 0.70, 95% CI = 0.50–0.97) and in women who had never used HRT (OR = 0.70, 95% CI = 0.51–0.96).

DISCUSSION

In this multicentric Italian study, high intake of dietary proanthocyanidins with three or more mers was inversely associated with the risk of endometrial cancer, particularly in normal-weight women. For no other class of flavonoids there was a significant overall association. Flavanones and isoflavones appeared to be inversely related to endometrial cancer among normal-weight and lean women, and, only for flavanones, among parous or non-HRT-user women.

Phytoestrogens have been hypothesised to reduce the risk of hormone-related cancers, including endometrial cancer (Shahidi, 1997). Although almost all tests for heterogeneity were not significant, our data suggest that the protective associations between flavonoids and endometrial cancer were stronger in women with low levels of estrogens, supporting the hypothesis of a possible protection mechanism of flavonoids against endometrial cancer based on the regulation of estrogens (Bagchi *et al*, 2000; Moutsatsou, 2007).

This is the first study to investigate the relation between proanthocyanidins and endometrial cancer risk. The inverse association for proanthocyanidins with three or more mers may be related to their endocrine effects (Bagchi *et al*, 2000), although non-hormonal mechanisms may also be involved. Proanthocyanidins have antioxidant and antiangiogenesis effects and may influence signal transduction and inhibit the action of DNA topoisomerases (Bagchi *et al*, 2000; Cos *et al*, 2004; Jo *et al*, 2005; Wang *et al*, 2011). Although the bioavailability of higher molecular weight proanthocyanidins is lower, they are characterised by a higher gastric stability (Krook and Hagerman, 2012) and a higher potential scavenger activity (Hagerman *et al*, 1998).

In fact, bioavailability of proanthocyanidins (in monomeric, oligomeric, and polymeric forms of flavan-3-ols) is influenced by their degree of polymerisation; monomers are readily absorbed in the small intestine, whereas oligomers and polymers need to be biotransformed by the colonic microbiota because they are resistant to acid hydrolysis in the stomach (Krook and Hagerman, 2012). Therefore, phenolic metabolites, rather than the original high-molecular weight compounds found in foods, may be responsible for the health effects derived from proanthocyanidin consumption (Monagas *et al*, 2010), especially those with higher degree of polymerisation. In experimental studies, the microbial metabolites of proanthocyanidins still bearing a free phenolic acids showed protective effects against oxidative stress and obesity (Gonthier *et al*, 2003; Thom, 2007), the major risk factors for endometrial cancer. The anti-obesity activity of some phenolic acids may be, at least partly, connected with oestrogenic pathways (Zych *et al*, 2009). Moreover, one of the main extension units of proanthocyanidins, the (−)-Epigallocatechin-3-gallate, has been suggested to inhibit cellular proliferation by inhibiting ERK activation and inducing apoptosis via ROS generation and p38 activation in endometrial carcinoma cells (Manohar *et al*, 2013).

Proanthocyanidins may thus contribute to a favourable effect of vegetables on endometrial cancer risk, although other micronutrients and food components present in vegetables should also be considered (Jain *et al*, 2000; McCann *et al*, 2000; Xu *et al*, 2007; Pelucchi *et al*, 2008; Bandera *et al*, 2009a). Two studies have examined an overall antioxidant exposure rather than individual antioxidants (Cui *et al*, 2011; Gifkins *et al*, 2012), and, among the various antioxidant indices considered, only the one measuring phenolics was inversely related to endometrial cancer risk (Gifkins *et al*, 2012).

With reference to isoflavones, our results are compatible with previous evidence from the United States and China (Horn-Ross *et al*, 2003; Xu *et al*, 2004; Bandera *et al*, 2009b; Ollberding *et al*, 2012). In the Multiethnic Cohort study, including 489 women with incident endometrial cancer, a reduced risk was associated with total isoflavone intake (relative risk = 0.66 for the highest *vs* the lowest quintile category, 95% CI = 0.47–0.91; Ollberding *et al*, 2012). In a population-based case–control study conducted in China, including 832 cases and 846 controls, the ORs of endometrial cancer were 0.98–0.79, and 0.77 in successive quartile categories of isoflavones (*P* for trend 0.05; Xu *et al*, 2004). Another

Table 1. Odds ratios (ORs) and 95% confidence intervals (CIs) of 454 cases of endometrial cancer and 908 controls, contrasting the fourth quartile category to the first three quartile categories combined, for energy-adjusted intakes of flavonoids: Italy, 1992–2006

			Quartile category	
	Median[a]	75% percentile[a]	I–III	IV
Flavanols (mg per day)	36.6	69.1		
Cases:controls			350 : 681	104 : 227
OR[b] (95% CI)			1	0.99 (0.74–1.32)
Flavanones (mg per day)	32.3	54.7		
Cases:controls			362 : 681	92 : 227
OR[b] (95% CI)			1	0.78 (0.58–1.05)
Flavonols (mg per day)	18.7	24.7		
Cases:controls			328 : 681	126 : 227
OR[b] (95% CI)			1	1.11 (0.83–1.47)
Anthocyanidins (mg per day)	8.2	15.6		
Cases:controls			342 : 681	112 : 227
OR[b] (95% CI)			1	1.09 (0.82–1.45)
Flavones (mg per day)	0.5	0.6		
Cases:controls			346 : 681	108 : 227
OR[b] (95% CI)			1	0.91 (0.68–1.21)
Isoflavones (μg per day)[c]	42.4	58.2		
Cases:controls			350 : 681	104 : 227
OR[b] (95% CI)				0.84 (0.62–1.14)
Proanthocyanidins <3 mers (mg per day)	67.2	92.0		
Cases:controls			350 : 681	104 : 227
OR[b] (95% CI)			1	0.96 (0.72–1.29)
Monomers (mg per day)	28.7	40.6		
Cases:controls			344 : 681	110 : 227
OR[b] (95% CI)			1	1.01 (0.76–1.35)
Dimers (mg per day)	38.3	52.4		
Cases:controls			355 : 681	99 : 227
OR[b] (95% CI)			1	0.91 (0.68–1.22)
Proanthocyanidins $\geqslant 3$ mers (mg per day)	215.9	292.4		
Cases:controls			374 : 681	80 : 227
OR[b] (95% CI)			1	0.66 (0.48–0.89)
Trimers (mg per day)	16.9	23.2		
Cases:controls			364 : 681	90 : 227
OR[b] (95% CI)			1	0.73 (0.54–0.98)
4–6 mers (mg per day)	57.1	77.7		
Cases:controls			366 : 681	88 : 227
OR[b] (95% CI)			1	0.74 (0.55–1.01)
7–10 mers (mg per day)	45.5	62.9		
Cases:controls			372 : 681	82 : 227
OR[b] (95% CI)			1	0.70 (0.52–0.95)
>10 mers (mg)	96.8	130.4		
Cases:controls			372 : 681	82 : 227
OR[b] (95% CI)			1	0.64 (0.47–0.87)
Total flavonoids (mg per day)	408.7	527.0		
Cases:controls			365 : 681	89 : 227
OR[b] (95% CI)			1	0.82 (0.61–1.10)

[a]Median and third quartile of energy-adjusted flavonoids among controls plus the mean of flavonoid raw values (computed among controls). For isoflavones, median and third quartile of the raw values were presented.
[b]Estimated from multiple logistic regression models, conditioned on study centre and quinquennia of age, and adjusted for the year of interview, education, body mass index, history of diabetes, age at menarche, menopausal status/age at menopausal status, parity, oral contraceptive use, and hormone-replacement therapy use.
[c]The OR for isoflavones was estimated entering into the model the fourth quartile category based on the raw values of isoflavones and total energy intake separately.

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) of 454 cases of endometrial cancer and 908 controls, according to the fourth quartile category *vs* the first three quartile categories combined, for energy-adjusted intakes of flavonoids, in strata of various covariates: Italy, 1992–2006

		OR[a] (95% CI) IV *vs* I–III quartile category			
	Cases:controls	Flavanones	Isoflavones[b]	Proanthocyanidins ⩾3 mers	Total flavonoids
Age (years)					
<55	126 : 252	1.09 (0.63–1.89)	0.95 (0.52–1.75)	0.75 (0.40–1.42)	1.12 (0.64–1.95)
⩾55–<70	247 : 494	0.65 (0.43–0.99)	0.89 (0.58–1.38)	0.64 (0.42–0.98)	0.71 (0.47–1.09)
⩾70	81 : 162	0.82 (0.33–2.00)	0.49 (0.20–1.20)	0.68 (0.28–1.64)	0.78 (0.32–1.90)
BMI (kg m^{-2})					
<25	131 : 420	0.64 (0.39–1.04)	0.59 (0.34–1.02)	0.60 (0.35–1.01)	0.72 (0.44–1.17)
⩾25–<30	155 : 348	0.74 (0.43–1.25)	1.13 (0.68–1.87)	0.63 (0.38–1.07)	0.78 (0.47–1.29)
⩾30	168 : 140	0.91 (0.48–1.71)	1.13 (0.57–2.26)	0.91 (0.45–1.82)	1.18 (0.57–2.44)
Menopausal status					
Pre/peri	89 : 179	0.97 (0.48–1.99)	0.65 (0.32–1.32)	0.84 (0.38–1.86)	0.98 (0.48–1.98)
Post	365 : 729	0.78 (0.55–1.09)	0.87 (0.61–1.24)	0.63 (0.45–0.89)	0.72 (0.52–1.01)
Parity					
0	68 : 126	1.27[c] (0.58–2.78)	1.31 (0.52–3.29)	0.81 (0.34–1.92)	1.09 (0.51–2.32)
⩾1	386 : 782	0.70[c] (0.50–0.97)	0.86 (0.62–1.20)	0.68 (0.49–0.95)	0.77 (0.55–1.08)
HRT use					
Yes	49 : 78	2.77[c] (1.00–7.62)	0.39 (0.09–1.66)	1.04 (0.35–3.10)	1.17 (0.41–3.35)
No	405 : 830	0.70[c] (0.51–0.96)	0.88 (0.64–1.21)	0.62 (0.45–0.87)	0.79 (0.57–1.08)

[a]Estimated from multiple logistic regression models, conditioned on study centre and quinquennia of age, and adjusted for year of interview, education, body mass index (BMI), history of diabetes, age at menarche, menopausal status age at menopausal status, parity, oral contraceptive use, and hormone-replacement therapy use (HRT).
[b]The ORs for isoflavones were estimated entering into the models the fourth quartile category based on the raw values of isoflavones and total energy intake separately.
[c]*P* for heterogeneity for flavanones was 0.036 in strata of parity and 0.087 in strata of HRT use; *P* for heterogeneity was >0.1 in all other strata and flavonoids in the table.

population-based case–control study from New Jersey, including 424 cases and 398 controls, found an inverse association with isoflavones restricted to women with a BMI lower than 25 kg m^{-2} (OR = 0.50 for the highest *vs* the lowest tertile category, 95% CI = 0.25–0.98; Bandera *et al*, 2009b). Similarly, in our study, the association between isoflavones and endometrial cancer was of borderline significance in women with a BMI lower than 25 kg m^{-2} (OR = 0.59 for the highest *vs* the first three quartiles, 95% CI = 0.34–1.02). However, the test for heterogeneity was not significant for isoflavones in strata of BMI. Obese women have higher levels of oestrogens, and this may override any effect of phytoestrogens on endometrial cancer. Isoflavones interfere with the regulation of the menstrual cycle, and have been associated with increased length of the menstrual cycle and/or delayed menstruation in premenopausal women, and with reduced levels of pituitary luteinizing hormone, follicle stimulating hormone, and progesterone (Benassayag *et al*, 2002).

The low intake of soya or soya products – and consequently of isoflavones – in the Italian population makes results difficult to compare with other populations, especially the Asian ones. In those populations, the effects of isoflavone-rich foods on endometrial cancer have also been examined. In a case–control study conducted on a multiethnic population in the Hawaii, including 332 cases and 511 controls, a high consumption of tofu and other soy products was associated with a reduced risk of endometrial cancer (OR = 0.46 for the highest *vs* the lowest quartile category, 95% CI = 0.26–0.83, *P* for trend = 0.01; Goodman *et al*, 1997). In a previously mentioned study from China, the OR for the highest *vs* the lowest quartile of soya protein intake was 0.67, with a significant trend in risk (*P* for trend 0.01; Xu *et al*, 2004). Isoflavones and isoflavone-rich foods have also been linked to other hormone-related cancers including breast (Peterson *et al*, 2003; Bosetti *et al*, 2005; Trock *et al*, 2006; Duffy *et al*, 2007) and ovarian cancers (Zhang *et al*, 2004; Rossi *et al*, 2008).

Significant results obtained in subgroups of population, especially for flavanones, should be taken with caution, as in some strata statistical power might not be sufficient to detect significant associations. No previous study investigated the relation between flavanones and endometrial cancer risk, in spite of their estrogenic and antiestrogenic activity (Moutsatsou, 2007).

No association was found for other classes of flavonoids in our data, in agreement with a few previous studies (Bandera *et al*, 2009b; Wang *et al*, 2009), with the exception of quercetin (a flavonol) in the study conducted in New Jersey (OR = 0.65 for the highest *vs* the lowest quartile category; 95% CI = 0.41–1.01; *P* for trend 0.02; Bandera *et al*, 2009b).

With reference to possible sources of bias, dietary habits of hospital controls may differ from those of the general population (Breslow and Day, 1980). In this study, however, we excluded from the control group all diagnoses that might have involved any long-term changes in diet. The interview setting and catchment areas were the same for cases and controls, and the participation rate was almost complete. Limitations are related to the accuracy of the measurement of exposure to flavonoids. Variation of flavonoid intake may be affected by the variation of the food quantities in the recipes and the variability in plant flavonoid content attributable to several factors, such as sunlight and heat. Moreover, our questionnaire was not specifically designed to investigate flavonoids (Rossi *et al*, 2006). However, attention was paid to aspects that might influence flavonoid intakes including cooking method,

food preservation method, and country-specific types of foods. We took great care to consider Italian species of fruits and vegetables or to assign the flavonoid amount of the nearest comparable food, when food composition information was unavailable. Intake of some flavonoids, especially isoflavones, was very low in our population. This may explain the threshold effect of flavonoids in the absence of a clear trend in risk for lower levels. Other factors including the high inter- and intraindividual differences in phytoestrogen metabolism (due to a variety of factors ranging from the use of antibiotics, intestinal transit time, gut microflora to genetic polymorphisms (Duffy *et al*, 2007)) may have influenced our risk estimates.

Among the strengths of the study are the large sample size, and the use of a reproducible and valid FFQ (Franceschi *et al*, 1993; Decarli *et al*, 1996). Furthermore, we were able to adjust for major recognised risk factors for endometrial cancer, and the study has generated results on other endometrial cancer risk factors that were in line with other investigations (Parazzini *et al*, 1991; Zucchetto *et al*, 2009; Rosato *et al*, 2011), providing assurance that major biases were not operating.

In conclusion, our study suggests a role of proanthocyanidins in reducing endometrial cancer risk.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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