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Intake of specific flavonoids and risk of acute myocardial infarction in Italy

Alessandra Tavani[1,*], Luana Spertini[1], Cristina Bosetti[1], Maria Parpinel[2], Patrizia Gnagnarella[3], Francesca Bravi[1], Julie Peterson[4], Johanna Dwyer[5], Pagona Lagiou[6], Eva Negri[1] and Carlo La Vecchia[1,7]

[1]Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, I-20157 Milan, Italy: [2]Istituto di Igiene, Università di Udine, Udine, Italy: [3]Divisione di Epidemiologia e Biostatistica, Istituto Europeo di Oncologia, Milan, Italy: [4]Frances Stern Nutrition Center, Tufts–New England Medical Center, Boston, MA, USA: [5]Schools of Medicine, Nutrition and the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA: [6]Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece: [7]Istituto di Statistica Medica e Biometria, Università degli Studi di Milano, Milan, Italy

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Abstract

Objective: As intake of flavonoids has been associated with reduced risk of coronary heart disease but data on the relation with specific classes of flavonoids are scarce, we assessed the relation between dietary intake of specific classes of flavonoids and the risk of acute myocardial infarction (AMI) in an Italian population.

Design: Case–control study. Dietary information was collected by interviewers on a questionnaire tested for validity and reproducibility. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were obtained by multiple logistic regression models including terms for energy and alcohol intake, as well as sociodemographic factors, tobacco and other major recognised risk factors for AMI.

Setting: Milan, Italy, between 1995 and 2003.

Subjects: Cases were 760 patients, below age 79 years, with a first episode of non-fatal AMI, and controls were 682 patients admitted to hospital for acute conditions unrelated to diet.

Results: A reduced risk of AMI was found for increasing intake of anthocyanidins (OR = 0.45, 95% CI 0.26–0.78 for the highest vs. the lowest quintile, $P_{\text{trend}} = 0.003$) and flavonols (OR = 0.65, 95% CI 0.41–1.02, $P_{\text{trend}} = 0.02$). A tendency towards reduced risks, although not significant, was observed for flavan-3-ols (OR = 0.73, 95% CI 0.48–1.10) and total flavonoids (OR = 0.74, 95% CI 0.49–1.14). No meaningful heterogeneity was found between the sexes. No association emerged for other flavonoids, including isoflavones, flavanones and flavones.

Conclusions: High intake of anthocyanidins reduced the risk of AMI even after allowance for alcohol, fruit and vegetables, supporting a real inverse association between this class of flavonoids and AMI risk.

Keywords
Diet
Flavonoids
Myocardial infarction
Case–control studies
Risk factors

Flavonoids consist of over 4000 compounds present in food and beverages of plant origin, with antioxidant, anti-inflammatory, antithrombotic and endothelial protection activity[1,2]. These properties have led to the hypothesis that they are protective against cardiovascular disease[2,3].

Several epidemiological studies have considered the relation of total flavonoids or specific flavonoids with ischaemic heart diseases, but the results are not clear[4–14]. Data on specific classes of flavonoids using comprehensive databases and their effects on cardiovascular disease risk are few[15]. This is partly due to the lack, until recently, of a clear and complete classification and a reliable database on the flavonoid content of foods and beverages. A Greek case–control study carried out a systematic analysis of the effects of the six specific classes of flavonoids, and found an inverse association of flavan-3-ols with risk of coronary heart disease (CHD)[16].

To further assess the relation of flavonoid classes with acute myocardial infarction (AMI) risk, we computed the dietary intake of the six principal classes of flavonoids (i.e. isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones and flavonols)[17,18] from dietary information collected in a case–control study of non-fatal AMI conducted in Italy.

*Corresponding author: Email tavani@marionegri.it

Subjects and methods

Data derive from a case–control study of non-fatal AMI conducted in the greater Milan area, Italy, between 1995 and 2003[19]. Cases were 760 patients (580 men, 180 women; median age 61 years, range 19–79 years) with a first episode of non-fatal AMI, defined according to the World Health Organization criteria[20], admitted to a network of teaching and general hospitals in the area. Controls were 682 patients (439 men, 243 women; median age 59 years, range 16–79 years) from the same geographical area, admitted to the same hospitals for a wide spectrum of acute conditions not likely related to known AMI risk factors and diet. We excluded subjects with previous AMI or other major cardiovascular events. Among controls, 30% had traumas, 25% non-traumatic orthopaedic disorders, 18% acute surgical conditions, 18% eye, nose, throat or teeth disorders, and 9% miscellaneous other illnesses unrelated to diet. Less than 5% of the cases and controls approached refused to participate.

Interviews were conducted in hospital using a structured questionnaire, including information on socio-demographic factors, anthropometric variables, smoking, alcohol and coffee consumption, other lifestyle habits, physical activity, a problem-oriented medical history, and history of AMI in first-degree relatives. Cholesterol levels were obtained from clinical records.

Information on diet was based on a food-frequency questionnaire tested for reproducibility[21] and validity[22], which included questions on 78 foods or food groups and 15 questions aimed at assessing patterns of lipid intake and meal frequency. Energy and nutrient intakes were computed using an Italian food composition database[23]. Food and beverage content of total and six classes of flavonoids (isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones and flavonols) was computed using data from the US Department of Agriculture's provisional flavonoid food composition database[17,18], further integrated with other sources[24–26]. Major flavonoids included in these classes were: hesperidin and naringin for flavanones; epicatechin and catechin for flavan-3-ols; quercetin, myricetin and kaempferol for flavonols; apigenin and luteolin for flavones; cyanidin and malvidin for anthocyanidins; genistein and daidzein for isoflavones. In our population, oranges and other citrus fruits were the major sources of flavanones; tea, red wine, grapes and other fruits those of flavan-3-ols; various common vegetables and fruits were major sources of flavonols; vegetables, bean soups and tea those of flavones; red wine, red fruits onion and garlic were major sources of anthocyanidins; pulses those of isoflavones.

Data analysis

Odds ratios (OR) of AMI, and the corresponding 95% confidence intervals (CI), for subsequent quintiles of intake of various flavonoid classes were derived using unconditional multiple logistic regression models[27], including terms for sex, age, education, tobacco smoking, coffee, alcohol drinking, total energy intake, body mass index, physical activity, cholesterol levels, diabetes, history of hypertension and history of AMI in first-degree relatives. Flavonoids were also included in the models as continuous variables, with a measurement unit equal to one standard deviation of the distribution of all subjects.

Results

The distribution of cases of AMI and controls according to age, sex and other major covariates is shown in Table 1. Compared with controls, cases were more often smokers (OR = 2.25 for current smokers of 15 or more cigarettes per day, $P < 0.0001$) and heavy coffee drinkers (OR = 1.42), consumed less alcohol (OR = 0.72 for 2 or more drinks per day, $P < 0.01$), more often had a history of hypertension (OR = 1.58), diabetes (OR = 2.92), and AMI in first-degree relatives (OR = 2.17).

Table 2 gives the distribution of cases and controls and the corresponding OR of AMI according to dietary intake of total flavonoids and of the six major classes of flavonoids. The mean daily intake in controls was 134.0 mg for total flavonoids, 25.5 μg for isoflavones, 19.2 mg for anthocyanidins, 55.6 mg for flavan-3-ols, 38.2 mg for flavanones, 0.44 mg for flavones and 20.6 mg for flavonols. After allowance for major confounding factors, including energy and alcohol intake, we found a reduced risk of AMI for increasing intake of anthocyanidins (OR = 0.45 for the highest vs. the lowest quintile, $P_{trend} = 0.003$) and possibly flavonols (OR = 0.65, $P_{trend} = 0.02$). A tendency toward reduced risks, although not significant, was observed for flavan-3-ols (OR = 0.73) and total flavonoids (OR = 0.74). Other flavonoids, including isoflavones, flavanones and flavones, were not associated with AMI risk. After further adjustment for intake of vegetables and fruit, the OR for the highest quintile of intake compared with the lowest one was 0.89 (95% CI 0.56–1.40) for total flavonoids, 1.23 (95% CI 0.82–1.85) for isoflavones, 0.55 (95% CI 0.33–0.91) for anthocyanidins, 0.84 (95% CI 0.57–1.25) for flavan-3-ols, 1.26 (95% CI 0.70–2.29) for flavanones, 1.42 (95% CI 0.95–2.11) for flavones and 0.90 (95% CI 0.57–1.41) for flavonols. The OR for a continuous term computed for an increment of intake corresponding to one standard deviation was 0.97 for total flavonoids, 0.82 for anthocyanidins and 0.87 for flavonols among all subjects. The corresponding OR were 1.02, 0.81 and 0.86 in men and 0.85, 0.72 and 0.86 in women; the estimates were not heterogeneous between the two sexes.

Discussion

The present study adds epidemiological data on the relation between flavonoids and AMI. Reduced CHD

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Table 1 Distribution of 760 cases of acute myocardial infarction (AMI) and 682 controls, and corresponding odds ratios (OR) with 95% confidence intervals (CI), according to age and other selected variables, Milan, Italy, 1995–2003

	AMI		Controls		
	n	%	*n*	%	OR (95% CI)*/ χ^2 trend (*P*)
Age (years)					
< 50	140	10.4	168	24.6	
50–59	198	26.1	187	27.4	
60–69	293	38.6	224	32.8	
≥ 70	129	17.0	103	15.1	
Sex					
Men	580	76.3	439	64.4	
Women	180	23.7	243	35.6	
Education (years)†					
< 7	321	42.9	308	45.8	1.00‡
7–11	239	31.9	222	33.0	1.01 (0.79–1.29)
≥ 12	189	25.2	142	21.2	1.20 (0.91–1.59)
χ^2 trend (*P*)					1.38 (0.24)
Smoking habit					
Non smokers	425	55.9	476	69.8	1.00‡
Current smokers	335	44.1	206	30.2	1.82 (1.45–2.29)
< 15 (cigarettes/day)	77	10.1	76	11.1	1.17 (0.83–1.65)
≥ 15 (cigarettes/day)	258	34.0	130	19.1	2.25 (1.73–2.93)
χ^2 trend (*P*)					34.94 (< 0.0001)
Coffee consumption (cups/day)†					
< 3	402	53.0	422	61.9	1.00‡
≥ 3	357	47.0	260	38.1	1.42 (1.14–1.76)
Alcohol consumption (drinks/day)†					
< 1	305	40.2	268	39.3	1.00‡
1–2	113	14.9	106	15.5	0.83 (0.60–1.14)
> 2	341	44.9	308	45.2	0.72 (0.56–0.92)
χ^2 trend (*P*)					6.66 (< 0.01)
Body mass index ($kg\,m^{-2}$)†					
< 24.2	217	28.6	215	31.8	1.00‡
24.2–27.3	270	35.6	232	34.3	1.06 (0.81–1.38)
> 27.3	271	35.8	229	33.9	1.11 (0.85–1.44)
χ^2 trend (*P*)					0.59 (0.44)
History of hypertension					
No	519	68.3	513	75.2	1.00‡
Yes	241	31.7	169	24.8	1.58 (1.24–2.01)
History of diabetes					
No	650	85.5	644	94.4	1.00‡
Yes	110	14.5	38	5.6	2.92 (1.98–4.32)
Family history of AMI					
No	513	67.5	557	81.7	1.00‡
Yes	247	32.5	125	18.3	2.17 (1.69–2.79)

* Estimated by multiple logistic regression models including terms for age and sex.
† The sum does not add up to the total because of missing values.
‡ Reference category.

mortality with higher intake of total flavonoids was found in the Zutphen Elderly Study cohort[4], the Seven Countries Study cohort[5], a Finnish cohort[6] and the Iowa Womens' cohort of postmenopausal women[7], which also found protection with intake of kaempferol, a compound of the flavonol class. The ATBC (Alpha-Tocopherol Beta-Carotene) Cancer Prevention study reported no relation of consumption of flavonols and flavones combined with CHD mortality, but an inverse association with non-fatal AMI[8]. In contrast, the Male Health Professionals Study found no relation between non-fatal AMI and intake of flavonols and flavones combined, or with the individual flavonols quercetin, kaempferol and myricetin[9]. However, in the Rotterdam Study, the flavonols quercetin, kaempferol and myricetin were inversely related to risk of fatal AMI[10]. No relationship between flavonols and flavones with ischaemic heart disease mortality was found in the Caerphilly Study[11], whereas an inverse association was found in the Finnish Mobile Clinic Health Examination Survey with the flavonol quercetin[12]. In the Women's Health Study, flavonoid intake was not associated with risk of cardiovascular disease[13]. A meta-analysis of seven prospective cohorts of men and women, including more than 2000 fatal CHD events, found an overall reduced risk with dietary flavonol intake (risk ratio of 0.80 for the highest tertile of intake compared with the lowest one)[14].

The results of the present study indicate that intake of flavonoids is related to a reduced risk of non-fatal AMI, and that the strongest inverse association was observed for anthocyanidins. However, it is difficult to disentangle the effect of flavonoids from that of other dietary components, especially other antioxidants or other

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Table 2 Distribution of 760 cases of acute myocardial infarction (AMI) and 682 controls, and corresponding odds ratios (OR) with 95% confidence intervals (CI)*, according to quintile of intake of the major classes of flavonoids, Milan, Italy, 1995–2003

	Quintile						
	1 (low)	2	3	4	5 (high)	χ^2 trend (*P*)	Continuous†
Total flavonoids							
Cases	209	143	143	132	133		
Controls	137	135	138	135	137		
Upper cut-point (mg)	72.17	106.02	140.15	189.28	–		
OR	1.00‡	0.73	0.80	0.74	0.74	1.43 (0.23)	0.97
95% CI		0.49–1.07	0.53–1.20	0.49–1.12	0.49–1.14		0.85–1.11
Isoflavones							
Cases	164	177	108	159	152		
Controls	137	136	136	136	137		
Upper cut-point (μg)	14.59	19.78	24.42	32.91	–		
OR	1.00‡	1.19	0.73	0.91	0.90	1.04 (0.31)	0.96
95% CI		0.80–1.76	0.48–1.11	0.60–1.37	0.59–1.38		0.84–1.10
Anthocyanidins							
Cases	218	141	129	138	134		
Controls	136	136	137	137	136		
Upper cut-point (mg)	5.55	11.02	17.95	29.05	–		
OR	1.00‡	0.68	0.56	0.52	0.45	8.63 (0.003)	0.82
95% CI		0.46–1.02	0.35–0.87	0.32–0.84	0.26–0.78		0.68–0.98
Flavan-3-ols							
Cases	208	151	128	120	153		
Controls	136	137	136	136	137		
Upper cut-point (mg)	20.60	32.54	49.53	90.43	–		
OR	1.00‡	0.71	0.53	0.53	0.73	2.38 (0.12)	1.02
95% CI		0.48–1.06	0.35–0.82	0.34–0.84	0.48–1.10		0.90–1.16
Flavanones							
Cases	201	171	133	113	142		
Controls	136	129	145	135	137		
Upper cut-point (mg)	14.17	33.52	35.54	64.76	–		
OR	1.00‡	1.01	0.67	0.65	0.87	2.33 (0.13)	1.01
95% CI		0.69–1.47	0.45–0.99	0.44–0.97	0.58–1.29		0.89–1.16
Flavones							
Cases	174	136	165	123	162		
Controls	137	135	138	135	137		
Upper cut-point (mg)	0.27	0.35	0.46	0.59	–		
OR	1.00‡	0.91	1.05	0.85	1.17	0.33 (0.56)	1.07
95% CI		0.61–1.36	0.71–1.55	0.57–1.28	0.78–1.74		0.94–1.23
Flavonols							
Cases	173	163	176	125	123		
Controls	136	137	137	135	137		
Upper cut-point (mg)	12.14	15.57	19.44	25.00	–		
OR	1.00‡	0.98	1.02	0.66	0.65	5.60 (0.02)	0.87
95% CI		0.65–1.47	0.67–1.55	0.42–1.05	0.41–1.02		0.76–1.00

* Estimated by multiple logistic regression models including terms for sex, age, education, smoking, coffee, alcohol, total energy, body mass index, physical activity, cholesterol levels, history of hypertension, diabetes, and family history of AMI.
† Computed for an increment of intake corresponding to one standard deviation of the distribution of all subjects, i.e. 80.68 mg for total flavonoids, 25.39 μg for isoflavones, 19.59 mg for anthocyanidins, 57.44 mg for flavan-3-ols, 32.33 mg for flavanones, 0.25 mg for flavones and 13.10 mg for flavonols.
‡ Reference category.

potentially cardioprotective components of vegetables, fruit and wine. In our study the inverse association of flavonoids with AMI risk was weaker after allowance for vegetables, fruit and alcohol (mainly wine in the Italian population), suggesting that the favourable effect of flavonoids on AMI risk found in this study may partly depend on other substances contained in these foods and beverages. However, these may well represent over-adjustments, and the persistence of a protective effect of anthocyanidins also after adjustment for vegetables, fruit and alcohol supports a real inverse association between flavonoids and AMI risk. When alcohol was excluded from the multivariate model, a reduced risk was found for anthocyanidins (OR = 0.44 for the highest quintile of intake compared with the lowest), flavan-3-ols (OR = 0.67), flavonols (OR = 0.64), total flavonoids (OR = 0.58) and possibly for flavanones (OR = 0.75), while no association was found with intake of isoflavones and flavones.

Soy proteins containing isoflavones have been reported to reduce circulating total and low-density lipoprotein (LDL) cholesterol and to increase high-density lipoprotein (HDL) cholesterol, but changes were related to level and duration of intake[28]. The typical Western diet contains low quantities of soy products or soy derivatives in comparison with the Asian one[29]. In the Italian diet, the intake of

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isoflavones is among the lowest of Western countries[30] and the levels at which soy isoflavones are reported to be protective are not consumed by the majority of population[28]. Consequently our data are not very informative on this issue. The lack of association found for isoflavones is in agreement with the findings of the Dutch Prospect–EPIC cohort (European Prospective Investigation into Cancer and Nutrition)[31], conducted in another Western population with low intake of isoflavones.

Flavonoids also have been shown to reduce oxidation of LDL[32] and to increase cellular resistance to the deleterious effects of oxidised LDL[33]. They also reduce the release of cardiac mast cell mediators and decrease inflammation, which has been implicated in CHD[34,35]. Flavonoids also have favourable effects on endothelial function[36] and inhibit platelet aggregation[37].

In this study cases and controls were interviewed in the same hospitals, they came from the same geographical area, and their participation was similar and almost complete. We excluded from the comparison group patients admitted for chronic conditions or diseases related to known or potential risk factors for AMI, diet-related conditions and long-term modifications of diet. The food-frequency questionnaire was satisfactorily valid and reproducible[21,22], and there is no reason to assume different recall of intake of the major sources of flavonoids on the basis of disease status, since the possibility of a relationship between these compounds and AMI risk was unknown to the interviewers and probably to most subjects interviewed. A potential limitation of the study is the adaptability of the US flavonoid food composition database to the Italian diet. However, this is unlikely to have introduced spurious associations since the same food composition database was applied to the intakes of both cases and controls, and imprecise classification of exposure is likely to lead to an attenuation of any real association. Flavonoids may affect the risk of CHD, or death from CHD. Since the cases here were only AMI survivors, an inverse relation with mortality might have attenuated any real association. Furthermore, we cannot exclude that healthier lifestyle may play a role in the inverse association of flavonoid intake with AMI risk. However, the potential confounding of covariates associated to AMI risk in this study[38,39] was allowed for in the analysis.

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