RESEARCH ARTICLE

Mate Intake and Risk of Breast Cancer in Uruguay: a Case-Control Study

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Abstract

Regarding 'maté' intake (infusion of *Ilex paraguariensis* herb, a staple beverage in temperate South American regions), most epidemiologic studies showed positive associations with risk of some cancers, (e.g. upper aerodigestive tract), but evidence on breast cancer (BC) risk is limited to a previous multi-site study, which reported a non significant odds ratio [OR]=0.85, 95% confidence interval [95% CI] 0.67-1.09, p for trend=0.31) for the highest quartile of intake. The present study was conducted in order to further assess associations of 'mate' intake with BC risk. We combined two databases of women belonging to public and private healthcare hospitals. The sample included 572 BC incident cases and 889 controls interviewed with a specific questionnaire featured by socio-demographic, reproductive and lifestyle variables, and a food frequency questionnaire of 64 items, also analyzing 'mate' intake (consumer status, daily intake, age at start, age at quit, duration of habit, intensity of intake). ORs and their 95%CI were calculated through unconditional logistic regression, adjusting for relevant potential confounders. The highest quartile of 'mate' intake was inversely associated with BC risk (OR=0.40, 95%CI 0.26-0.57, p for trend <0.001). Stratified analyses also displayed strong significant inverse associations for 'mate' in frequent tea drinkers (OR=0.22), high energy intake (OR=0.23), high body mass index (OR=0.29) and in postmenopausal women (OR=0.36), among other results. As conclusions, we found evidence of a significant inverse association for 'mate' intake and BC risk.

Keywords: Breast cancer - *Ilex paraguariensis* - mate - tea - coffee - infusions.

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Introduction

Breast cancer (BC) is the leading malignancy among Uruguayan women, with a national age-adjusted incidence rate of 73.1 per 100.000 (Barrios et al., 2014), the highest one among South American registries (Ferlay et al., 2013). Furthermore, the capital city Montevideo showed in the past decade the highest incidence rate of the World (Parkin et al., 2002). Theoretically, this could be the outcome of unusually high prevalence of risk factors and a low prevalence of protective factors combined.

Several studies conducted in Uruguay have thoroughly analyzed the relationship between nutrition and BC (Ronco et al., 2010a; Ronco and De Stefani 2012; 2013a), from diet (De Stefani et al., 1997a,b; 1998a; Ronco et al., 1996; 1999; 2006; 2010b) as well as from anthropometry (Ronco et al., 2008; 2009; 2013b). Besides, international literature about hot drinks and BC is still inconsistent concerning possible associations (Gierach et al., 2012; Jiang et al., 2013; Wu et al., 2013; Gao et al., 2013; Nie et al., 2014; Bhoo-Pathy et al., 2015). On the other hand, local studies about 'mate' (a hot infusion made from the herb ilex paraguariensis, which is a staple nonalcoholic beverage in temperate South America) and its role on cancer were mainly focused on the upper aerodigestive sites (Vassallo et al., 1985; De Stefani et al., 1988; 1990; Castellague et al., 2000; Sewram et al., 2003; Ronco et al., 2004; Deneo-Pellegrini et al., 2013; Lubin et al., 2014), usually showing positive associations with these tumors.

Recently, our group published a multi-site case-control study focused on 'mate' drinking and the risk of several cancers in population admitted to the public hospitals healthcare system, which showed a non significant negative association for high 'mate' intake and BC risk (OR=0.85, 95% CI 0.67-1.09, p for trend=0.31) (De Stefani et al., 2011a). It was the first time that findings on this topic were reported.

In our opinion, the evidence linking intake of this hot infusion and BC is not enough, and in addition, the applied regression model did not take into account relevant variables to this cancer site, such as menstrual-reproductive history, body mass index and family history

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of BC. Considering that Uruguayans are the World's highest 'mate' consumers (9-10 kg/person/year of the herb and ca. 400 liters/person/year of infusion) (Comision Honoraria de Lucha Contra el Cancer, 1993), we believe that research on the proposed subject is scientifically rewarding. In order to clarify some of these aspects and using a detailed multi-topic questionnaire applied to a sample which includes women from both healthcare systems, we have conducted the present study.

Materials and Methods

Two case-control studies on BC were conducted in Montevideo (where 45% of inhabitants live) by our group: one of them was carried out during 1996-2004 in the major public hospitals (Oncology, Clinicas, Pasteur, Maciel) and the other one was performed in a private hospital (IMPASA, abbreviated name of former Instituto Medico de Prevision, Asistencia y Servicios Afines) in the years 1999-2001. The respective databases, having the same basic structure, allowed us to analyze in the present study a total sample of 1461 participants, 572 cases and 889 controls. We will briefly describe the selection criteria regarding participants from each healthcare system.

Public hospitals

During the study period a total number of 480 newly diagnosed and microscopically confirmed BC were considered eligible for the study. A number of 19 patients refused the interview, leaving a final total of 461 cases which were included in the study (response rate 96.0 %). In the same time period and in the same hospitals, 685 hospitalized patients having diseases not related with smoking, drinking and without recent changes in their diet were considered eligible for this study. Patients proceeded from any part of the country, even from rural areas. Of them, 18 patients refused the interview, leaving a final total of 667 controls (response rate 97.4%). Four

Table 1. Distribution of Cases and Controls

Variables	Categories	Controls	%	Cases	%	Total	%	Global p-value
Age groups	≤ 39	78	8.8	40	7.0	118	8.0	
	40-49	122	13.7	83	14.5	205	14.0	
	50-59	223	25.1	143	25.0	366	25.0	
	60-69	243	27.3	155	27.1	398	27.2	
	70-79	193	21.7	129	22.5	322	22.0	
	80-89	30	3.4	22	3.8	52	3.6	0.87
Health system	Public	667	75.0	461	80.6	1128	77.2	
	Private	222	25.0	111	19.4	333	22.8	0.01
Education years	≤ 6	551	62.0	359	62.8	910	62.3	
	07-12	223	25.1	142	24.8	365	25.0	
	≥ 13	115	12.9	71	12.4	186	12.7	0.94
Residence	Urban	805	90.5	498	87.1	1303	89.2	
	Rural	84	9.4	74	12.9	158	10.8	0.03
Body Mass Index (kg/m^2)	≤ 24.99	389	43.8	238	41.6	627	42.9	
	25.0-29.99	327	36.8	210	36.7	537	36.8	
	≥ 30.0	173	19.5	124	21.7	297	20.3	0.54
Fam.History of BC	No	811	91.2	450	78.7	1261	86.3	
	Yes	78	8.8	122	21.3	200	13.7	<0.001
Menopausal status	Pre	182	20.5	97	17.0	279	19.1	
	Post	707	79.5	475	83.0	1182	80.9	0.09
Age of menarche	≤ 11	207	23.3	138	24.1	345	23.6	
	12	273	30.7	145	25.3	418	28.6	
	13	175	19.7	136	23.8	311	21.3	
	≥ 14	234	26.3	153	26.7	387	26.5	0.09
Nº of live births	Nulliparous	111	12.5	104	18.2	215	14.7	
	1-2	394	44.3	252	44.1	646	44.2	
	≥ 3	384	43.2	216	37.8	600	41.1	0.006
Age at 1st live birth	≤ 20	281	36.1	150	32.0	431	34.6	
	21-26	304	39.1	173	37.0	477	38.3	
	≥ 27	193	24.8	145	31.0	338	27.1	0.054
Breastfeeding time (total months)	≤ 3	283	31.8	218	38.1	501	34.3	
	1-4	307	34.5	168	29.4	475	32.5	
	≥ 16	299	33.6	186	32.5	485	33.2	0.03
Total patients		889	100.0	572	100.0	1461	100.0	

trained social workers interviewed cases and controls in the hospitals shortly after admittance and no proxy interviews were conducted.

Private hospital

The chosen medical institution was representative of the pre-paid system in Montevideo, with ca. 15.000 female affiliates (mostly inhabitants at the capital city) and showing a crude rate of BC of 267/100.000 due to its ca. 40 incident cases/year. During the study period 116 histologically verified cases of BC were collected. In the same time period 223 healthy women with a normal control mammography (Birads 1) (Feig, 1999), performed no longer than one year before the interview, were selected as controls (2 controls per case). One control and two cases had rejected the interview and three other cases (0.9%) died during the study period, leading to a final number of 111 cases and 222 controls (response rates of 95.7% and 99.6% respectively). They were matched by age (± 5 years) and residence (Montevideo and surrounding neighbourhoods), they were not hospitalized at the moment of the interview nor diagnosed with cancer. All women had ages <85, in an attempt to reduce a possible recall bias. Both cases and controls were women undergoing routine mammography testing and belonged to mid-to-high socio-economic strata. All interviews were conducted in the hospital and performed face-to-face by a trained nurse, who was blinded concerning major risk factors.

Interviews and questionnaire

A structured questionnaire was applied to all participants. The questionnaire included the following sections: (1) socio-demographic variables; (2) a section on occupation based on job titles and the duration of each activity; (3) history of cancer in first- and second-degree relatives; (4) self-reported height and weight 5 years before the interview; (5) a tobacco smoking section (including age at starting, age of quitting, and average number of cigarettes smoked per day); (6) a history on alcohol drinking (including type of beverage, age at starting, age of quitting, and average amount of alcohol drunk per day); (7) a history of 'mate', tea and coffee drinking (age at starting, age of quitting, and average amount of the infusion drunk per day); (8) menstrual and reproductive events; and (9) a detailed food-frequency questionnaire (FFQ) on 64 items representative of the diet of the Uruguayan population, which asked about food consumption 5 years prior to diagnosis in cases and prior to the interview in controls. The FFQ was not validated, but was tested for reproducibility, having high correlations (Ronco et al., 2006). Furthermore, the FFQ allowed the total energy intake of each subject to be estimated. All dietary questions of our semi-quantitative questionnaire were open-ended.

Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression (Breslow and Day, 1980). Potential confounders were included in the multivariate analysis. All equations included terms for hospital, residence, age, education, age at menarche, body mass index, number of childbirths, menopausal status, family history of BC in first and second degree relatives, smoking status, alcohol intake, total energy intake, and intakes of red meat, total fruits, total vegetables and tea. Likelihood-ratio tests were performed in order to explore possible heterogeneities in

Table 2. Selected population features according to their healthcare system

Variable	Units	Public System mean ± SD*	Private System mean ± SD*	p-value
Age	years	59.2 ± 13.8	60.5 ± 9.9	0.12
Education	years	5.1 ± 3.0	12.4 ± 4.1	<0.001
Urban status	% of lifespan	81.3 ± 30.5	98.5 ± 6.3	<0.001
Height	cm	160.1 ± 6.2	162.6 ± 5.7	<0.001
Weight	Kg	69.9 ± 14.6	63.9 ± 8.8	<0.001
Body Mass Index	Kg/m^2	27.3 ± 5.5	24.2 ± 3.2	<0.001
Age at menarche	years	12.7 ± 1.6	12.3 ± 1.3	<0.001
Age at menopause	years	47.0 ± 5.5	48.5 ± 3.9	<0.001
Live births	number	3.4 ± 2.4	2.1 ± 0.9	<0.001
Age at 1st delivery	years	23.1 ± 5.7	25.0 ± 4.1	<0.001
Breastfeeding	months	27.8 ± 43.7	11.7 ± 8.4	<0.001
Red meat intake	servings/year	226.2 ± 149.7	202.6 ± 126.7	0.01
Chicken intake	servings/year	54.7 ± 46.5	102.0 ± 48.3	<0.001
Fish intake	servings/year	36.7 ± 35.3	80.4 ± 39.7	<0.001
Stew intake	servings/year	115.5 ± 94.7	40.8 ± 41.2	<0.001
Fruit intake	servings/year	378.3 ± 304.1	1297.9 ± 537.5	<0.001
Vegetables intake	servings/year	611.3 ± 423.8	1074.2 ± 458.5	<0.001
Coffee intake	cups/year	33.5 ± 106.8	440.1 ± 589.4	<0.001
Tea intake	cups/year	84.2 ± 189.8	421.0 ± 304.8	<0.001
Mate intake	liters/day	0.83 ± 0.71	0.73 ± 0.63	0.02

* SD = Standard Deviation

Table 3. Crude Odds Ratios (OR) of Selected Consumptions Linked to Lifestyle

Variable	Categories	Controls /cases	Global p-value	OR* (95% CI)	p-value for trend
Red meat	≤ 112	254/101			
(servings/year)	113-183	256/118			
	184-290	228/138			
	≥ 291	151/215	<0.001	3.58 (2.62-4.88)	<0.001
Fruits	≤ 218	207/159			
(units/year)	219-365	204/159			
	366-844	236/130			
	≥ 845	242/124	0.006	0.67 (0.49-0.90)	0.001
Vegetables	≤ 400	190/173			
(servings/year)	401-620	226/141			
	621-905	245/118			
	≥ 906	228/140	<0.001	0.67 (0.50-0.90)	0.003
Energy	≤ 1625	244/121			
(Kcal/day)	1626-1944	225/140			
	1945-2288	215/150			
	≥ 2289	205/161	0.02	1.58 (1.17-2.14)	0.002
Coffee	None	607/431			
(cups/year)	1-364	105/60			
	≥ 365	177/81	0.009	0.64 (0.48-0.86)	0.002
Tea	None	503/360			
(cups/year)	1-364	90/70			
	≥ 365	296/142	0.002	0.67 (0.53-0.85)	0.002
Alcohol	Non drinker	759/451			
Status	Ex –drinker	26/34			
	Curr.drinker	104/87	0.002	1.41 (1.03-1.91)	0.007
Smoking	Non smoker	659/409			
Status	Ex –smoker	59/54			
	Curr.smoker	171/109	0.14	1.03 (0.78-1.34)	0.57

*Crude OR (highest vs. lowest category)

the stratified analyses. All calculations were done with the software STATA (Release 10, StataCorp LP, College Station, TX, 2007).

Results

The distribution of cases and controls according to sociodemographic and reproductive factors is shown on Table 1. Although participants were not completely matched, the age distribution was adequate (p-value= 0.87). More cases proceeded from rural areas than controls (12.93 *vs*. 9.45 % respect.), but there were similarities regarding educational level (p= 0.94) and body mass index (p= 0.54). As expected, family history of BC, number of live births and breastfeeding time displayed significant differences and the age at first live birth was close to significance.

Table 2 compares the mean values with standard deviations of 20 selected items concerning the healthcare system of study participants. Unless age, all variables were significantly different. Women from the public system were less educated, lived a lesser part of their lifetime in urban areas and tended to be overweight. In addition, reproductive variables displayed a more protective profile. Conversely, dietary items showed the opposite except for the intake of stew. Regarding the studied hot infusions, high differences were found for coffee and tea intakes. Although the high prevalence of 'mate' intake in the whole population (82.6% of the sample, data not shown in this Table), its consumption was more intense among participants from the public system.

The comparison of selected items linked to lifestyle between cases and controls is shown on Table 3. Unless smoking status, the rest were significantly different. Crude ORs and their 95% CI indicate that intakes of red meat, total calories and alcohol were positively associated to BC risk. On the other hand, total fruits, total vegetables, coffee and tea intakes were negatively associated to BC risk.

Table 4 is focused on the particular features of 'mate' intake. Adjusted ORs and their 95% CI display a favorable situation for high exposure to this intake, compared to those categories of no/low intake. Current consumers showed a significant risk reduction of BC (OR=0.65, CI 0.47-0.90). A daily intake higher than 1 liter of the infusion was strongly protective (OR=0.40, CI 0.26-0.57, p-value for trend <0.001). Duration of the habit suggested the benefit of long-term 'mate' consumption (OR=0.66, CI 0.44-0.99, p-value for trend=0.04) and intensity of consumption (liters*years) also showed a strong inverse association for high consumers compared to non drinkers (OR=0.47, CI 0.32-0.68, p-value for trend <0.001). Only the estimates of daily intake remained unchanged when

Table 4. Crude and Adjusted* Odds Ratios (OR) and 95% Confidence Intervals (95% CI) for 'Mate' consumption

Variable	Categories	Controls/ Cases	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value for trend
Mate status	Non drinker	146/108	Ref.	Ref.	
	Ex-drinker	59/44	1.01 (0.63-1.60)	0.66 (0.39-1.11)	
	Current drinker	684/420	0.83 (0.63-1.09)	0.65 (0.47-0.90)	0.01
Amount (liters/day)	≤0.49	189/147	Ref.	Ref.	
	0.50-0.99	262/235	1.15 (0.87-1.52)	0.86 (0.62-1.17)	
	1.00	265/122	0.59 (0.44-0.80)	0.54 (0.39-0.76)	
	≥ 1.01	173/68	0.50 (0.35-0.72)	0.40 (0.27-0.60)	<0.001
Duration (years)	≤ 9	160/117	Ref.	Ref.	
	10--34	259/139	0.73 (0.54-1.01)	0.64 (0.44-0.93)	
	35-50	247/161	0.89 (0.65-1.22)	0.69 (0.48-0.98)	
	≥ 51	223/155	0.95 (0.69-1.30)	0.66 (0.44-0.99)	0.044
Intensity (liters*years)	No intake	146/108	Ref.	Ref.	
	0.10-24.5	240/166	0.92 (0.67-1.26)	0.74 (0.51-1.07)	
	24.6-44.2	248/173	0.93 (0.68-1.28)	0.68 (0.47-0.98)	
	≥ 44.3	255/125	0.66 (0.47-0.91)	0.50 (0.34-0.73)	<0.001

* Regression model included terms for: Health system (binary), residence (binary), age (categorical), education (continuous), age at menarche (categorical), menopausal status (binary), family history of breast cancer (binary), body mass index (categorical), number of live births (categorical), age at first delivery (continuous), breastfeeding months (categorical), smoking status (categorical), red meat intake (continuous), total fruit (categorical), total vegetables (categorical), dietary energy (categorical), tea intake (continuous) and alcohol intake (continuous).

Table 5. Odds Ratios (OR) of Mate Intake (Quartiles), Stratified for Categories of Selected Relevant Variables. Reference Category for Mate Intake is 0-0.49 Liters/Day

Variable	Categories	II 0.50-0.99 l/day	III 1.00 l/day	IV ≥1.01 l/day	p-value for trend	p-value Heterogeneity
Alcohol intake	Never	0.90 (0.63-1.28)	0.54 (0.38-0.78)	0.35 (0.23-0.56)	<0.001	
	Ever	0.87 (0.37-2.06)	0.61 (0.22-1.66)	0.75 (0.27-2.08)	0.33	0.33
Tea intake (cups/yr)	Never	1.13 (0.75-1.71)	0.84 (0.53-1.34)	0.56 (0.33-0.95)	0.01	
	≤ 364	0.79 (0.28-2.17)	0.57 (0.17-1.97)	0.33 (0.08-1.31)	0.10	
	≥ 365	0.63 (0.30-1.32)	0.29 (0.15-0.54)	0.22 (0.09-0.52)	<0.001	0.50
Red meat (serv/yr)	≤ 183	0.86 (0.54-1.37)	0.74 (0.46-1.19)	0.67 (0.37-1.20)	0.13	
	≥ 184	0.91 (0.57-1.45)	0.39 (0.24-0.65)	0.31 (0.18-0.55)	<0.001	0.21
Fruits+veget. (serv/yr)	≤ 1039	1.15 (0.73-1.79)	0.90 (0.52-1.55)	0.47 (0.25-0.88)	0.01	
	≥ 1040	0.68 (0.42-1.11)	0.40 (0.26-0.62)	0.35 (0.21-0.60)	<0.001	0.25
Energy (kcal/day)	≤ 1944	1.10 (0.70-1.74)	1.11 (0.68-1.83)	0.90 (0.49-1.65)	0.88	
	≥ 1945	0.71 (0.44-1.14)	0.30 (0.18-0.48)	0.23 (0.13-0.40)	<0.001	0.008
BMI status	NW	1.04 (0.63-1.72)	0.75 (0.45-1.24)	0.55 (0.30-1.02)	0.01	
	OW-OB	0.73 (0.48-1.12)	0.40 (0.25-0.64)	0.29 (0.17-0.51)	<0.001	0.17
FH of BC	No	0.87 (0.62-1.23)	0.53 (0.37-0.77)	0.45 (0.29-0.69)	<0.001	
	Yes	0.90 (0.32-2.54)	0.67 (0.25-1.82)	0.15 (0.04-0.63)	0.02	0.43
Menop.status	Pre-	1.09 (0.46-2.62)	0.56 (0.22-1.41)	0.71 (0.27-1.88)	0.23	
	Post-	0.85 (0.60-1.21)	0.59 (0.40-0.85)	0.36 (0.23-0.58)	<0.001	0.62
Health syst.	Public	0.98 (0.69-1.40)	0.69 (0.45-1.05)	0.49 (0.31-0.78)	<0.001	
	Private	0.59 (0.19-1.83)	0.32 (0.16-0.61)	0.26 (0.10-0.67)	<0.001	0.57

Regression model included terms for: Health system (binary), residence (binary), age (categorical), education (continuous), age at menarche (categorical), menopausal status (binary), family history of breast cancer (binary), body mass index (categorical), number of live births (categorical), age at first delivery (continuous), breastfeeding months (categorical), smoking status (categorical), red meat intake (continuous), total fruit (categorical), total vegetables (categorical), dietary energy (categorical), tea intake (continuous) and alcohol intake (g/day, continuous). Selected variables were excluded from the analyses when they were used for stratification; Abbreviations: BMI = Body Mass Index; NW = Normal weight (≤ 24.99 kg/m^2) ; OW-OB = Overweight-Obese (≥ 25.00 kg/m^2); FH of BC = Family history of breast cancer; Serv/yr = servings/year; Menop.status = Menopausal status

terms for 'mate' status, age at start and duration of habit were included in the multivariate model, whereas the other variables lost their association to BC risk (results not shown).

Finally, Table 5 shows the adjusted ORs for 'mate' intake, stratified for selected study variables. The inverse associations were stronger among high tea drinkers (OR=0.22), high energy intake (OR=0.23), overweight-obese women (OR=0.29), high red meat consumers (OR=0.31), high fruits and vegetables consumers (OR=0.35) and alcohol abstainers (OR=0.35). In addition, mainly postmenopausal participants seemed to be protected (OR=0.36). Although it was a small subset, women with family history of BC displayed stronger inverse associations compared to those without it (OR=0.15 *vs*. OR=0.45, respectively). Interestingly, the likelihood-ratio test revealed significant heterogeneity only for the strata of energy intake (p=0.008).

Discussion

We have found inverse associations of 'mate' intake and the risk of BC, considered from the viewpoint of consumer status (current consumers, OR=0.65, p=0.01), daily amount (>1 liter/day, OR=0.40, p<0.001), duration of habit (>50 years, OR=0.66, p=0.04) and intensity of intake (>44.3 liters*years, OR=0.50, p<0.001). In addition, only daily amount of 'mate' remained significantly associated when the other terms were included for adjustment in the regression model. These results differ from those ones emerging from the above quoted multi-site study (De Stefani et al., 2011a), where high intake of the beverage showed a negative and statistically not significant association to BC risk (OR=0.85 for high intake *vs*. no intake, 95%CI 0.67-1.09, p-value for trend=0.31). However, we think that the applied regression model probably explains in part those results. The former study had calculated the ORs using a polytomous multiple regression model including age, residence, education, smoking status, smoking cessation, smoking intensity, alcohol drinking and each 'mate' variable, having non 'mate' drinkers as reference category. On the other hand, for the present analysis we also took into account other covariates: hospital, family history of BC, body mass index, menstrual-reproductive variables (age at menarche, menopausal status, number of live births, age at first delivery, time of breastfeeding), total fruits, total vegetables, red meat, tea intake and total energy. These possible confounding variables are not a minor point.

The present study shows in Table 4 that crude risk for current 'mate' consumers is OR=0.83, 95% CI 0.63-1.09, having non drinkers as reference category. This is almost identical to the above quoted OR for high intake *vs*. non consumers reported in the multi-site study. In other words, the former ORs appeared similar to our crude results: it seems likely that the adjustment for those variables was not enough to reduce the influence of important confounding factors of BC risk. The former multivariate model was probably very useful regarding several cancer sites afflicted by smoking and drinking, but was not enough for a specific, thorough analysis of BC.

Our results also face the existing controversy regarding 'mate' effects on cancer. According to a monograph published by the International Agency for Research on Cancer (IARC), hot 'mate' drinking has been considered as a 2A agent, that is, a possible carcinogenic for humans (IARC, 1991). In fact, some studies found high levels of carcinogenic polycyclic aromatic hydrocarbons (PAH) (Gomes Zuin et al., 2005; Kamangar et al., 2008; Abnet, 2007; Golozar et al., 2012). A recent paper reported the presence of 8 PAHs in hot and cold 'mate' infusions (Thea et al., 2016). Among them, phenanthrene and benzo[a] pyrene (BaP) -considered as carcinogens to humans (IARC, 2010)- were found in both hot and cold 'mate', but the most recent paper also highlighted that none of the infusions exceeded the maximum level for BaP suggested by the World Health Organization for drinking water (700 ng/ml) (Thea et al., 2016). Besides, our group found dietary BaP intake as associated to an increase of BC risk (Ronco et al., 2011).

Regarding the links with BC, also Dimethylbenz[a] anthracene (DMBA) should be taken into account (Rengarajan et al., 2015), not only for causing BC in experimental rats, but also for being present in barbequed meat, tobacco smoke and overheated cooking oil, among other sources (Tiwari et al., 2014). DMBA is an important environmental contaminant that collaborates in generating several oxidative stress-mediated diseases, including cancer, but it has another relevant feature: it is a fat-soluble compound and because of this property it accumulates and persists in the adipose tissue of the mammary gland, therefore increasing the exposure of mammary epithelium to this chemical carcinogen (Ayyakkannu et al., 2014). Interestingly, DMBA, like BaP, is also an indirect-acting carcinogen, requiring metabolic activation to yield its ultimate carcinogenic form (Badal and Delgada, 2014), in particular an oxidation by CYP enzymes (Szaefer et al., 2014). Unlike the hot temperature of the infusion -accepted as a risk factor for cancers of the upper aerodigestive tract (De Stefani et al., 1988; 1990; Lubin et al., 2014)-, the quoted components could be partially responsible of the association of 'mate' with cancer in organs which have no direct contact with the beverage: lung (De Stefani et al., 1996), bladder (De Stefani et al., 1991; 2007), kidney (De Stefani et al., 1998b), prostate (De Stefani et al., 2011b) and also cervix uteri (De Stefani et al., 2011a).

On the other hand, nevertheless, hot 'mate' infusion appears within a long list of products which have priority to be reassessed by the IARC (IARC, 2014), since it has been extensively studied (Heck and de Mejia, 2007; Loria et al., 2009) and basic research has demonstrated the presence of several compounds which have antioxidant properties (polyphenols, flavonoids), among other ones as chlorogenic acids (Jaiswal et al., 2010) and methylxantines (caffeine, teobromin) (Heck and de Mejia, 2007; Bracesco et al., 2011). Moreover, 'mate' infusion has shown comparable oxygen radical scavenger activity as ascorbate, glutathione and cysteine (Coppes et al., 2014). Furthermore, a recent experimental study in mice showed preventive effects of 'mate' against mammary carcinogenesis induced by DMBA and dimethylhydrazine (Zapaterini et al., 2010). Despite this evidence, we must

recognize that some studies failed to report a decreased risk associated with this beverage (Heck and de Mejia, 2007; Loria et al., 2009; World Cancer Research Fund, 2007).

The protective effects of 'mate' described in the present study appear to be stronger among strata of tea drinkers and high plant foods eaters. According to recent reviews, although high polyphenols contents of different tea types have been described (Wang et al., 2015), lack of association tends to prevail in the literature regarding tea intake and the risk of BC (Gao et al., 2013; Wu et al., 2013). Nevertheless, potential benefits could be linked to an additional antioxidant load coming from different sources, and this was suggested regarding tea consumption, where the OR of high 'mate' drinkers goes from a general 0.40 (shown in Table 4) to 0.22 for the strata of highest tea drinkers (shown in Table 5), even though reference categories are not identical. Additional analyses considering non drinkers as reference category did not change the estimates for an intake higher than 1 liter/day (OR=0.40, 95% CI 0.26-0.62, results not shown in Tables). Furthermore, an OR=0.67 for high tea drinkers and an OR=0.40 for high 'mate' drinkers suggest that the estimated OR=0.22 for both high intakes combined could be an additive protection.

An inverse association was apparent mainly for postmenopausal women, although heterogeneity test was not significant between strata (p=0.62). Whether the difference is only a matter of time for the antioxidant effects or there is indeed a relation to hormonal status, is something we cannot disentangle at this moment due to its own complexity. In support to this statement, we must take into account that strong negative associations of 'mate' were also found in women with high dietary energy intake (heterogeneity test p<0.01) and high BMI: both are linked to higher oxidative stress, but the latter also represents more intense aromatization of androgens into active estrogens taking place within the adipose tissue, mainly in postmenopausal women. Consequently, obesity and certain dietary styles, among other factors, increase the BC risk by reduction of 2/16 α-hydroxyestrogens ratio (Muti et al., 2000; Ronco et al., 2010). Moreover, according to the above quoted recent studies on PAH, a potential antioxidant activity of 'mate' reducing or partially blocking the metabolic activation of BaP and DMBA could not be ruled out. In this sense, the potential carcinogenic compounds which have been found in 'mate' infusion could be counterbalanced and also overcome by its own antioxidant compounds.

Anyway, we believe that the evidence suggests a more protective effect along time, perhaps enhancing existing biological differences between BC in younger and in older women. It is not new that BC is different in both subgroups: we have already recognized that premenopausal women are in a disadvantageous situation from a dietary viewpoint compared to postmenopausal ones, mainly because of a relative lack of protective items (Ronco and De Stefani 2012; 2013; Ronco et al., 2012; 2013; 2015). Combining food and nutrients patterns, the latter communication (Ronco et al., 2015) reported lack of association for Carotenoids, Fruit-based, Prudent and Total fruits dietary patterns among premenopausal women, whereas these patterns showed a protective effect among postmenopausal ones. Something probably playing a role of common link among the quoted patterns is their antioxidant load. Since 'mate' intake was not taken into account in such study, the facts suggest us that regarding BC risk, those other dietary items with antioxidant capabilities apparently do not work either in favour of younger women.

As other case-control studies, our work has limitations and strengths. Among the limitations we recognize the lack of validation of the questionnaire, although the instrument was tested for reproducibility. Also, the control population displayed different profiles: hospitalized participants were recruited from the public system and non hospitalized ones from the private system. All of them shared a common condition, which was the absence of any cancer. However, the latter subgroup had also documented absence of any breast pathology. Therefore, having selected as controls women with normal mammograms and not only without cancer, if benign breast diseases had any association with the analyzed dietary items we reduced at least in part the possibility of biasing results due to this. Also to be mentioned as strength, the study population includes subsets of both existing healthcare systems, proceeding from the capital city as well as the rest of the country, and times of data collection were coincident. Although age matching was not perfect for the public hospitals subset, the whole resulting distribution was reasonable. Finally, a high participation was achieved (globally 97.1% of patients under the proposed age limits), reducing the likelihood of selection bias. Albeit it is not possible to avoid completely any bias, including recall bias, we think that results were not chance findings.

In conclusion, after analyzing a population sample comprised by women belonging to both healthcare systems existing in the country, we found evidence of a protective effect of 'mate' drinking on the risk of BC. This effect displayed linear trends for daily amount, duration of habit and intensity of intake, being in appearance mainly protective for postmenopausal women. The need of additional studies is evident, mainly to elucidate possible links to hormonal features which are currently taken into account at diagnostic stages and for therapeutical purposes, and this is a task for the close future.

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