RESEARCH ARTICLE

'Mate' Intake, Hormone-Based Risk Factors and Breast Cancer: a Case-Control Study

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Abstract

Previous reports on the inverse association between 'mate' intake (infusion of Ilex Paraguariensis herb) and breast cancer (BC) risk led us to consider two main roles for the infusion: as a substantial antioxidant contributor and as a hormone regulator, particularly through anti-aromatase capacities. Since menstrual-reproductive risk factors for BC reflect women's estrogenic exposure during the reproductive lifespan, and considering that 'mate' intake exerts putative stronger protection among high antioxidant contributors, we attempted to analyze interactions among the infusion, hormon-linked reproductive factors and BC risk, which have hitherto remained unexplored. We analyzed a database of 572 BC incident cases and 889 controls. Women were interviewed with a specific questionnaire featuring socio-demographic, lifestyle and reproductive variables (age at menarche, 1st live birth and menopause; number of live births; breastfeeding months), and a food frequency questionnaire, focusing on 'mate' intake (consumer status, daily intake, age at start, age at quit, duration of habit). Odds ratios (OR) and their 95% confidence Intervals were calculated through unconditional logistic regression, adjusting for relevant potential confounders. 'Mate' intake showed strong inverse associations with some high-risk hormone-related factors: early menarche, nulliparity, low breastfeeding, long reproductive years and high number of ovulatory cycles. Moreover, all subsets of high dietary energy demonstrated even stronger associations. In conclusion, regarding exposure to known hormone risk factors, we found strong inverse associations between high 'mate' intake and BC, which were greater among those consuming higher calorific diets. Our analyses support possible combined antioxidant and antiestrogenic effects for 'mate' infusions.

Keywords: Breast cancer- estrogens- Ilex paraguariensis- maté- reproductive life

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Introduction

Breast cancer (BC) is the leading malignancy among Uruguayan women, with an incidence rate of 73.1/105 (Barrios et al., 2014), the highest one in South America (Ferlay et al., 2013). 'Mate', a hot infusion made from the herb ilex paraguariensis, is a staple beverage in temperate South America. Hot 'mate' drinking was considered as a 2A carcinogenic for humans according to the IARC, due to the presence of polycyclic aromatic hydrocarbons (IARC, 2010). Furthermore, 'mate' infusion will be reassessed (IARC, 2014), since research revealed the presence of several compounds with antioxidant properties (polyphenols, flavonols) (Coppes et al., 2014; Bracesco et al., 2011).

Uruguayan studies analyzed thoroughly the nutritional epidemiology of BC (Ronco and De Stefani, 2010; 2012). We have recently reported significant reduced BC risks for high 'mate' intakes (Ronco et al., 2016a). Associations were stronger among high tea, fruits and vegetables consumers. Such results suggested us that: 1. Inactive procarcinogenic compounds of 'mate' infusion could be overcome by its own antioxidants. 2. Potential protection could be linked to an additional antioxidant load from different sources. We have later shown inverse associations of 'mate' despite the strata of several dietary antioxidants, albeit its protective effect was stronger in presence of high antioxidant intakes (Ronco et al., 2016b).

Leaves of Ilex paraguariensis are an excellent source of triterpenoid saponins (~10% of total dry weight), as oleanolic acid (OA) and ursolic acid (UA), which have chemopreventive properties: they upregulate the p53 cascade (Puangraphant et al., 2013). UA has multiple intracellular and extracellular targets that play role in apoptosis, metastasis, angiogenesis and inflammatory processes (Kashyap et al., 2016). Both OA and UA exert an aromatase-inhibitory activity and this common feature might explain in part an anti-tumour property (Kim et

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al., 2014). Due to the homology between the androgen androstenedione –the main aromatase substrate- and UA, configuration of the latter was seen as appropriate to recognize active site of enzyme and to block aromatisation (Gnoatto et al., 2008). Therefore, certain compounds can simultaneously display antioxidant and anti-estrogenic capabilities.

Previous analyses on 'mate' intake showed stronger inverse associations among higher caloric diets and for postmenopausal and overweight/obese women (Ronco et al., 2016 a,b). All aforementioned high strata represent at the same time, more intense androgen aromatisation taking place mainly within the adipose tissue, or higher levels of free estrogens, or both combined into a higher estrogen exposure, implying aromatisation as well as estrogen receptors. The reproductive lifespan involves also an estrogen exposure, which has not been yet analyzed regarding exposure to 'mate' intake and BC risk.

Considering that: a) Uruguay has the World's highest 'mate' consumption (9-10 kg/person/year of the herb and ~400 liters/person/year of infusion) (Comision Honoraria de Lucha Contra el Cancer, 1993); b) We have proposed two main roles for the infusion: as a substantial antioxidant contributor and as a hormonal regulator, particularly from its anti-aromatase capabilities (Ronco et al., 2016a); c) 'Mate' intake exerted a putative stronger protection among strata of high antioxidant contributors (Ronco et al., 2016b); and d) Traditional menstrual-reproductive risk factors for BC reflect women's estrogenic exposure during their reproductive lifespan, we decided to analyze possible interactions of the infusion with those reproductive variables and BC risk, a still unexplored issue.

Materials and Methods

Subjects and methods

Two case-control studies on BC were conducted in Montevideo (where 45% of inhabitants live) by our group: one was carried out during 1996-2004 in the major public hospitals of the city and the other one was performed in a private hospital during 1999-2001. Both databases had the same basic structure, allowing us to analyze a total sample of 1461 participants, 572 BC cases and 889 controls. Each one is briefly described as follows.

Public hospitals

Along the study period 480 newly diagnosed and microscopically confirmed BC cases were considered eligible for the study. Nineteen patients refused the interview, finally leaving 461 cases 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 dietary changes were considered eligible. Eighteen patients refused the interview, leaving 667 controls (response rate 97.4%). Four trained social workers interviewed cases and controls in the hospitals shortly after admittance; no proxy interviews were conducted.

Private hospital

The chosen medical institution belonged to the pre-paid system in Montevideo. In the study period 116 verified cases of BC were collected, and 223 healthy women with a normal mammography (Birads 1) (Sickles et al., 2013), performed no longer than one year before the interview, were selected as controls (2 controls per case). One control and two cases 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: 95.7% and 99.6% respectively). They were matched by age (± 5 years) and residence (Montevideo and surrounding neighbourhoods) and they were not hospitalized at the moment of the interview nor diagnosed with cancer. Women were <85 years old, they underwent routine mammography testing and belonged to mid-to-high socio-economic strata. 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

Participants received a structured questionnaire, including sections of: socio-demographic variables; occupation; BC history in 1º and 2º degree relatives; self-reported height and weight 5 years before the interview; tobacco smoking; history on alcohol drinking; history of 'mate', tea and coffee drinking (age at starting, age of quitting, and average daily amount of the infusion drunk); menstrual-reproductive events; and a detailed food frequency questionnaire (FFQ) on 64 items, representative of Uruguayan diet, which asked about food consumption 5 years prior to the interview. The FFQ was not validated, but was tested for reproducibility (Ronco et al., 2006), allowing the estimation of total energy for each subject. All dietary questions of our semi-quantitative questionnaire were open-ended. To calculate energy, we compiled an analysis program using servings/year and kilocalories of each food.

Assuming an average of 11 ovulatory cycles/year, 9 months for each pregnancy, absence of ovulations during breastfeeding, and taking oral contraceptives during 11 months/year, we designed the following formulas in order to calculate reproductive years and cycles:

Premenopausal women: Reproductive years = Age at diagnosis/interview – age at menarche.

Postmenopausal women: Reproductive years = Age at menopause – age at menarche.

Ovulatory cycles were estimated as follows

Cycles = (Reproductive years*11) – [(full-term pregnancies*9) + (breastfeeding months) + (oral contraception years*11)]

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. Most equations included terms for hospital, residence, age, education, age at menarche, body mass index (BMI), number of childbirths, menopausal status, family history of BC in 1º and 2º 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 the stratified analyses. All calculations were done with the software STATA (Release 10, StataCorp LP, College Station, TX, 2007).

Results

Distribution of cases and controls according to socio-demographic and lifestyle factors is shown on Table 1. Although participants were not completely matched, an adequate age distribution was achieved (p- value=0.87). More cases proceeded from rural areas than controls (12.93 vs. 9.45 % respect.), but there were similarities regarding education (p=0.94) and BMI (p=0.54). Cases showed higher energy, alcohol and red meat intake, whereas controls displayed higher intakes of plant foods. All linear trends were statistically significant.

Table 2 is focused on features of 'mate' intake. Adjusted ORs display a benefit for high exposure to 'mate', compared to the reference categories (no intake). Results showed certain similarities for both regression models: estimates and their trends tended to be slightly better, but always significant, when dietary and reproductive variables were included in the five analyses ('mate' status, daily amount, age at start, duration and intensity). The highest inverse association was found for high 'mate' intake (daily amount) (OR=0.38, trend<0.001).

Table 1. Distribution of Cases and Controls According to Selected Socio-Demographic and Lifestyle Variables.

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	
	7-12	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	≤ 24.99	389	43.8	238	41.6	627	42.9	
(kg/m2)	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
Red meat	≤ 112	254	28.6	101	17.7	355	24.3	
(servings/year)	113-183	256	28.8	118	20.6	374	25.6	
	184-290	228	25.6	138	24.1	366	25.1	
	≥ 291	151	17.0	215	37.6	366	25.1	<0.001
Vegetables	≤ 400	190	21.4	173	30.2	363	24.8	
(servings/year)	401-620	226	25.4	141	24.6	367	25.1	
	621-905	245	27.6	118	20.6	363	24.8	
	≥ 906	228	25.6	140	24.5	368	25.2	<0.001
Fruits	≤ 218	207	23.3	159	27.8	366	25.1	
(servings/year)	219-365	204	22.9	159	27.8	363	24.8	
	366-844	236	26.6	130	22.7	366	25.1	
	≥ 845	242	27.2	124	21.7	366	25.1	0.006
Energy (kcal/day)	≤ 1625	244	27.4	121	21.2	365	25.0	
	1626-1944	225	25.3	140	24.5	365	25.0	
	1945-2288	215	24.2	150	26.2	365	25.0	
	≥ 2289	205	23.1	161	28.1	366	25.1	0.02
Alcohol status	Non drinker	759	85.4	451	78.8	1,210	82.8	
	Ex –drinker	26	2.9	34	5.9	60	4.1	
	Curr.drinker	104	11.7	87	15.2	191	13.1	0.002
Total patients		889	100.0	572	100.0	1,461	100.0	

Table 2. Adjusted* Odds Ratios (OR) and 95% Confidence Intervals (95% CI) for 'Mate' Consumption. Regression Models with/without Dietary+Hormonal Variables

Variable	Categories	Controls/Cases	Adjusted* OR (95% CI)	Adjusted** OR (95% CI)
Mate status	Non drinker	146/108	Ref.	Ref.
	Ex –drinker	59/44	0.83 (0.51-1.35)	0.75 (0.46-1.25)
	Current drinker	684/420	0.75 (0.56-1.01)	0.65 (0.48-0.89)
	Trend		0.06	0.006
Amount	Non drinker	146/108	Ref.	Ref.
(liters/day)	0.01-0.99	262/235	0.97 (0.69-1.37)	0.87 (0.61-1.24)
	1.00	265/122	0.78 (0.57-1.06)	0.67 (0.48-0.93)
	≥ 1.01	173/68	0.46 (0.31-0.69)	0.38 (0.26-0.58)
	Trend		<0.001	<0.001
Age at start	Non drinker	146/108	Ref.	Ref.
	≤15	385/279	0.87 (0.63-1.21)	0.73 (0.52-1.03)
	≥16	358/185	0.69 (0.50-0.94)	0.63 (0.46-0.88)
	Trend		0.01	0.008
Duration	Non drinker	146/108	Ref.	Ref.
(years)	1-34	273/148	0.71 (0.50-1.01)	0.66 (0.46-0.95)
	35-50	247/161	0.82 (0.58-1.14)	0.71 (0.50-1.01)
	≥ 51	223/155	0.80 (0.54-1.17)	0.64 (0.43-0.96)
	Trend		0.25	0.03
Intensity	Non drinker	146/108	Ref.	Ref.
(liters*years)	0.1-24.0	238/165	0.90 (0.64-1.27)	0.82 (0.57-1.17)
	24.1-44.0	246/173	0.84 (0.61-1.18)	0.72 (0.51-1.02)
	≥ 44.1	259/126	0.56 (0.39-0.79)	0.47 (0.33-0.68)
	Trend		0.001	<0.001

* Regression model included terms for: Health system (binary), residence (binary), age (categorical), education (continuous), body mass index (categorical) and smoking status (categorical); ** Regression model included terms for: Health system (binary), residence (binary), age (categorical), education (continuous), age at menarche (categorical), menopausal status (binary), family history of BC (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 3 shows adjusted ORs for 'mate' intake, stratified for each one of the traditional risk factors. High 'mate' intakes displayed significant risk reductions at any level of almost every stratified variable. Compared to the basic estimate (OR=0.38), stronger inverse associations were found for positive history of BC (OR=0.12), early age at menarche (OR=0.35), nulliparity (OR=0.35), low breastfeeding (OR=0.34) and longer reproductive years (OR=0.27). Most of the strongest inverse associations are seen in the direction of the known risk factors. A high proportion of trends were significant (20/24 = 83.3%) plus an additional borderline, revealing consistency for 'mate' protective effect. The table also compares ORs of 'mate' intake stratified by low/high dietary calories. Most estimations showed consistently an improvement for the putative protective effect of high 'mate' intake with high dietary energy (≥1945 kcal). The global risk appears in first place (OR=0.24 vs. OR=0.68, high vs. low energy, respectively). Further comparisons repeat the facts: with early age at menarche (OR=0.10 vs. OR=0.83), nulliparity (OR=0.11 vs. OR=0.34), less breastfeeding (OR=0.18 vs. OR=0.59), oral contraception (OR=0.21 vs. OR=0.91), larger reproductive periods (OR=0.22 vs. OR=0.51) and higher number of ovulatory cycles (OR=0.26 vs. OR=0.78). Interestingly, the protective effect was stronger among postmenopausal women (OR=0.19 vs. OR=0.83), but not among premenopausal ones (OR=1.04 vs. OR=0.56).

Discussion

We found evidence of inverse associations between 'mate' drinking and BC risk, regarding reproductive risk factors. Whereas high 'mate' intake showed a significant OR=0.38 for the whole sample, most strata of high estrogen-related reproductive factors displayed the strongest inverse associations: earlier age at menarche, nulliparity, no breastfeeding, no oral contraception, longer reproductive lifespan, higher estimated number of ovulatory cycles and postmenopausal women.

Similar analyses, but performed according to dietary energy levels, remarked better protective effects among women having high caloric intake. This subset showed a significant OR=0.24. Again, most high estrogen-related reproductive factors displayed the strongest inverse associations: earlier age at menarche,

Table 3. Stratified Odds Ratios (OR) of BC for 'Mate' Intake by Traditional Risk Factors (Family History of BC and Menstrual-Reproductive Items), in Quartiles. Reference category (I) omitted. Adjusted OR of 'mate' intake (highest quartile, >1 liter/day), stratified for categories of dietary energy (low/high intake according to median values).

Variable	Category	II	III	IV	Trend	Low (<1945 kcal/day)	High (≥1945 kcal/day)
		OR (95% CI)	OR (95 % CI)	OR (95% CI)	(p)	OR (95% CI)	OR (95% CI)
Whole sample		0.87 (0.61-1.24)	0.67 (0.48-0.93)	0.38 (0.26-0.58)	<0.001	0.68 (0.37-1.26)	0.24 (0.13-0.44)
Menarche (age)	≤ 11	0.76 (0.33-1.71)	0.58 (0.28-1.19)	0.35 (0.14-0.86)	0.01	0.83 (0.22-3.10)	0.10 (0.02-0.47)
	12-13	0.79 (0.48-1.29)	0.77 (0.50-1.20)	0.47 (0.26-0.84)	0.02	0.97 (0.39-2.44)	0.26 (0.11-0.60)
	≥ 14	1.34 (0.65-2.76)	0.79 (0.39-1.59)	0.39 (0.17-0.89)	0.003	0.78 (0.18-3.40)	0.30 (0.10-0.92)
Nº of Live Births	Nullip.	0.52 (0.22-1.22)	0.35 (0.15-0.86)	0.35 (0.12-1.05)	0.02	0.34 (0.07-1.48)	0.11 (0.02-0.75)
	1-2	0.95 (0.55-1.65)	0.75 (0.47-1.21)	0.49 (0.27-0.89)	0.01	1.08 (0.42-2.76)	0.29 (0.12-0.69)
	≥ 3	1.29 (0.66-2.53)	1.12 (0.59-2.13)	0.48 (0.23-1.03)	0.01	0.91 (0.29-2.88)	0.40 (0.13-1.19)
Age at 1º birth	Nullip.	0.52 (0.22-1.22)	0.35 (0.15-0.86)	0.35 (0.12-1.05)	0.02	0.34 (0.07-1.48)	0.11 (0.02-0.75)
	≤ 23	0.92 (0.51-1.67)	0.77 (0.44-1.34)	0.35 (0.18-0.68)	0.001	1.50 (0.56-4.03)	0.12 (0.04-0.34)
	≥ 24	1.01 (0.56-1.82)	0.88 (0.52-1.46)	0.57 (0.30-1.10)	0.10	0.69 (0.23-2.09)	0.57 (0.23-1.39)
Breastfeeding	≤ 15	0.74 (0.49-1.14)	0.56 (0.38-0.81)	0.34 (0.21-0.55)	<0.001	0.62 (0.30-1.29)	0.20 (0.10-0.41)
	≥ 16	1.69 (0.77-3.71)	1.37 (0.65-2.89)	0.64 (0.27-1.53)	0.055	0.76 (0.19-3.06)	0.50 (0.14-1.82)
Oral contracept.	No	0.88 (0.58-1.32)	0.67 (0.46-0.97)	0.38 (0.23-0.62)	<0.001	0.60 (0.29-1.26)	0.29 (0.14-0.57)
	Yes	0.91 (0.42-1.95)	0.89 (0.45-1.74)	0.48 (0.21-1.08)	0.10	0.91 (0.28-2.94)	0.21 (0.06-0.78)
Reprod. lifespan	≤ 32	1.47 (0.80-2.71)	0.92 (0.51-1.64)	0.47 (0.23-0.99)	0.008	0.80 (0.25-2.54)	0.33 (0.10-1.03)
	33-37	0.64 (0.35-1.16)	0.80 (0.48-1.36)	0.53 (0.28-1.02)	0.17	0.76 (0.29-2.00)	0.25 (0.09-1.03)
	≥ 38	0.63 (0.34-1.34)	0.49 (0.26-0.92)	0.27 (0.12-0.61)	0.001	0.51 (0.13-2.03)	0.22 (0.07-0.70)
Ovulatory cycles	≤ 276	1.26 (0.64-2.49)	0.96 (0.50-1.84)	0.45 (0.21-0.98)	0.009	1.00 (0.30-3.35)	0.33 (0.10-1.03)
	277-362	1.05 (0.56-1.97)	0.89 (0.51-1.56)	0.46 (0.23-0.92)	0.03	0.76 (0.29-2.00)	0.25 (0.09-0.70)
	≥ 363	0.53 (0.29-0.97)	0.47 (0.28-0.81)	0.43 (0.21-0.89)	0.008	0.51 (0.13-2.03)	0.22 (0.07-0.70)
Menop.Status	Pre-	0.83 (0.32-2.10)	0.86 (0.36-2.03)	0.48 (0.18-1.26)	0.15	0.56 (0.11-2.86)	1.04 (0.24-4.55)
	Post	0.89 (0.61-1.30)	0.71 (0.50-1.01)	0.39 (0.25-0.62)	<0.001	0.83 (0.42-1.61)	0.19 (0.09-0.37)
F.H. of BC	No	0.95 (0.64-1.40)	0.69 (0.48-0.99)	0.50 (0.32-0.78)	<0.001	1.21 (0.64-2.32)	0.22 (0.12-0.41)
	Yes	0.53 (0.17-1.64)	0.69 (0.27-1.77)	0.12 (0.03-0.47)	0.03	0.02 (0.00-0.22)	0.35 (0.06-2.10)

Regression model, Adjusted for hospital (binary), residence (binary), age (categorized), menopausal status (binary), family history of BC in 1st and 2nd degree (categorized), Abbrev, F.H. of BC, family history of breast cancer; body mass index (categorized), dietary energy (categorized), smoking status (categorized), alcohol drinking frequency (continuous), total red meat (continuous) and vegetables, fruits, tea and coffee (all of these as continuous variables)

nulliparae, no breastfeeding, oral contraception, longer reproductive lifespan, higher number of ovulatory cycles and postmenopausal women. These facts suggest that the higher the exposure to estrogens derived from reproductive history is, the higher could be the protective effect of high 'mate' intake.

Epidemiologic data support the hypothesis of accumulative effects exerted by estrogen exposure through a woman's lifespan, which contributes to the risk increase for developing BC (Cepa et al., 2008). Most BC cases are postmenopausal; during postmenopause, estrogen production derives from adrenal and ovarian androgens conversion in peripheral tissues, a mechanism in which the aromatase enzymatic complex participates (Chumsri et al., 2011). It has two components: a flavoprotein, the enzyme NADPH-cytochrome p-450 reductase, and a specific form of the cytochrome p-450 enzymatic system, known as aromatase p-450. The latter has a heme group and a binding site for steroids, which are in charge of specific recognition and union of androgen substrates, in order to begin the aromatisation process through an oxidation reaction in the A ring of androstenedione. This process consumes oxygen and NADPH (Morris et al., 2011).

Overweight/obesity are recognized risk factors for developing hormone-dependent postmenopausal BC (Simone et al., 2016). Obesity causes chronic inflammation in humans and raises the levels of proinflammatory mediators within adipose tissue, which in turn stimulate the transcription of CYP450 aromatase gene, increasing its activity. Peripheral aromatase activity and plasma estrogen levels correlate with BMI in postmenopausal women. The existence of an obesity-inflammation-aromatase axis, also present in adipose tissue of mammary gland in obese women, has been established (Howe et al., 2013; Lyengar et al., 2015). High Cyclooxygenase-2 (COX-2) and aromatase mRNA levels found in invasive BC led researchers to postulate that COX-2 mediated inflammatory mechanism participates in the regulation of expression in specific promoter regions of aromatase CYP450 gene. Therefore, estradiol biosynthesis increased significantly and a higher BC risk could be assumed (Prosperi and Robertson, 2006). Studies conducted years ago demonstrated the existence of high levels of aromatase enzyme in neoplastic mammary tissue. Furthermore, experimental research on cell cultures and animals revealed that in situ estrogen biosynthesis can promote tumour growth in paracrine and autocrine ways. It was also shown that estrogen synthesis in mammary tumours could be suppressed through blocking the aromatase expression or through inhibiting its activity (Chen, 1998).

'Mate' components (chlorogenic acids, flavonoids, e.g.) were cited in our reports (Ronco et al., 2016a,b). At this point we consider of utmost importance to focus on the triterpenoid saponins ursolic acid (UA) and oleanolic acid (OA) as representative of the infusion's hormonal properties: Ilex paraguariensis herb can be considered an excellent source of UA (~10% of total dry weight) (Gnoatto et al., 2008). Both exhibited several biological and pharmacological properties (Yogeeswari and Sriram, 2005). UA has multiple intra- and extracellular targets playing a role in apoptosis, metastasis, angiogenesis and inflammation (Kashyap et al., 2016). Mate saponins have potent chemopreventive properties: they specifically upregulate the p53 cascade (Puangraphant et al., 2013). Anti-estrogenic activity of UA can also depend on its capabilities for inhibiting ERα expression (Kim et al., 2014).

UA's influence was demonstrated as capable of arresting proliferation of estrogen-dependent MCF-7 human BC cells, showing both cytostatic and cytotoxic activity (Es-Saady et al., 1995). Further research confirmed its capabilities against BC (Bishayee et al., 2011; Wang et al., 2012; Chakravarti et al., 2012; Tan et al., 2013). The inhibition of Nuclear factor-kappa B (NF-kB), a key link between inflammation and carcinogenesis, was shown for UA (Shishodia et al., 2003; Yoon and Liu, 2007), nevertheless, in vivo studies have not confirmed those findings (Bishayee et al., 2011). UA supresses COX-2 gene activation in mammary epithelium cells through inhibiting the transduction signaling way of proteinkinase-C. Therefore, when COX-2 transcription is inhibited, this might not participate any more regulating the expression of specific aromatase CYP19 P450 gene promoter regions, and somehow it would inhibit estrogen production derived from aromatisation (Nagendra et al., 2016).

According to updated literature (Okoh et al., 2011), an attempt of separating possible protective actions in antioxidant on one side and in hormonal on the other side seems to be nonsense. Estrogens undergo oxidative metabolism and physiologically achievable concentrations of hormones or their metabolites have been shown to generate reactive oxygen species (ROS) which are implicated in carcinogenic conversion and growth of cancer cells through induction of DNA synthesis, increased phosphorylation of kinases, and activated transcription factors (Roy et al., 2007). Besides, mitochondria are significant targets of estrogen (Felty et al., 2005a,b). Estrogen-induced oxidative DNA damage through ROS has been extensively reviewed (Cavalieri et al., 2000; Roy and Singh, 2004).

A high oxidative environment is closely linked to an inflammatory environment. Subclinical inflammation occurs in breast white adipose tissue in animal and human models of obesity. Breast inflammation, characterized by crown-like structures (CLS), consists of dead adipocytes surrounded by macrophages (Morris et al., 2011). Histologic inflammation, as determined by CLS, was paralleled by increased NF-kB binding activity and elevated levels of proinflammatory mediators and aromatase. Obesity determines an increase in lipolysis, deriving into higher levels of free fatty acids, which in turn trigger NF-kB activation in macrophages (Howe et al., 2013; Simone et al., 2016). Consequently, increased production of proinflammatory mediators [TNF-α, interleukin (IL)-1b, PGE2] induce aromatase in preadipocytes (Subbaramaiah et al., 2013). The involved obesity-inflammation-aromatase axis (Morris et al., 2011) is a probable contributor to the increased risk of postmenopausal ER+ BC and it could also contribute to the generally worse prognosis of obese patients (Subbaramaiah et al., 2013). We consider that several 'mate' components (e.g., phenols, saponins) associate

their individual effects as a multi-targeted oriented combination.

The evidence herewith presented gives a mechanistic background from the hormonal viewpoint, which could partially explain the relationship between the putative protective effects of 'mate' infusion reported in our case-control studies (Ronco et al., 2016a,b) and its high content of UA against BC, regarding aromatisation inhibition.

As other case-control studies, our work has limitations and strengths. Among limitations we recognize the lack of validation of the questionnaire, although the instrument was tested for reproducibility. It would have been desirable to have information about hormonal receptors and expression of Her2-neu growth factor. However, such data were unavailable since at the time of interviews they were not routinely requested by oncologists. Thus, we were not able to make deeper analyses in search for relationships between 'mate' intake and those hormonal items. Besides, control population displayed different profiles: hospitalized participants belonged to the public system and non hospitalized ones to the private system.

All of them shared a common condition: absence of any cancer. The latter subgroup had also documented absence of breast pathology. Therefore, having selected as controls women with normal mammograms and not only without cancer, we reduced at least in part the possibility of biasing results if benign breast diseases had any association with the analyzed dietary items. Also to be mentioned as strengths, the study population includes subsets proceeding from the whole country, and times of data collection were coincident. Although age matching was not perfect for the public hospitals subset, the distribution was reasonable. Finally, a high participation was achieved (~97% of patients), 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, we found evidence of inverse associations between 'mate' intake and BC risk, regarding reproductive risk factors. This fact suggests that the higher the exposure to estrogens derived from reproductive history is, the higher could be the protective effect. In addition, this association was stronger at a higher dietary energy intake and postmenopausal women displayed better results. Therefore, we propose that intense 'mate' intake could strongly display its anti-estrogenic, antioxidant and anti-inflammatory capabilities when a combined high estrogenic and high oxidant environment takes place. Further studies are needed in order to better know about 'mate' intake and its hormonal and red-ox associations.

Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

The original studies were conducted after approval by the Medical Head of all hospitals involved and a Bioethical Committee.

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