Research Article

Cancer Epidemiology, Biomarkers & Prevention

Urinary Concentrations of Polycyclic Aromatic Hydrocarbon Metabolites in *Maté* Drinkers in Rio Grande do Sul, Brazil

Antonio Barros Lopes[1,2], Marcela Metzdorf[1,2], Luiza Metzdorf[1,2], Marcos Paulo Ramalho Sousa[1,2], Caroline Kavalco[2,3], Arash Etemadi[4], Natalie R. Pritchett[4], Gwen Murphy[4], Antonia M. Calafat[5], Christian C. Abnet[4], Sanford M. Dawsey[4], and Renato Borges Fagundes[2,3]

Abstract

Background: Consumption of *maté*, an infusion of the herb *Ilex paraguariensis (yerba maté)*, is associated with increased risk of esophageal squamous cell carcinoma (ESCC), but the carcinogenic mechanism is unclear. Commercial brands of *yerba maté* contain high levels of carcinogenic polycyclic aromatic hydrocarbons (PAHs), which are acquired during the traditional drying process. The purpose of this study was to characterize exposure to PAHs in *maté* drinkers over a wide range of *maté* consumption.

Methods: We recruited 244 adults who answered a questionnaire and collected a fasting spot urine specimen. We quantified urinary concentrations of seven PAH metabolites and assessed associations between self-reported recent *maté* consumption and urinary PAH metabolites by multivariate regression.

Results: Recent *maté* consumption showed a significant dose–response association with 6 of 7 PAH metabolites in unadjusted models ($P_{trend} < 0.05$). After adjustment for creatinine and potential confounders, concentrations of 2-naphthol, 1-hydroxyphenanthrene, and the sum of 2- and 3-hydroxyphenanthrene remained significantly associated with recent *maté* intake. The sum of the urinary concentrations of the phenanthrene metabolites was similar or higher among *maté* drinkers who did not smoke than among smokers who did not drink *maté*.

Conclusions: Urinary concentrations of PAH metabolites were significantly associated with self-reported amounts of recent *maté* intake, and drinking *maté* increased urinary concentrations of some PAH metabolites as much as smoking cigarettes.

Impact: Drinking *maté* is a source of exposure to potentially carcinogenic PAHs, consistent with the hypothesis that the PAH content of *maté* may contribute to the increased risk of ESCC in *maté* drinkers. *Cancer Epidemiol Biomarkers Prev; 27(3); 331–7.* ©2017 AACR.

Introduction

Esophageal cancer is a highly lethal disease and was the sixth most common cause of cancer-related death in the world in 2012 (1). Esophageal squamous cell carcinoma (ESCC) is the most common histologic type of esophageal cancer worldwide, and it is by far the dominant type in low- and middle-income countries (2). This is also the case in southern Brazil (including Rio Grande do Sul state), Paraguay, Uruguay, and Argentina, where the habit of drinking hot *maté* has been implicated in the high incidence of this cancer (3, 4).

Maté is an aqueous infusion of the herb *Ilex paraguayensis* (also known as *yerba maté*) that is prepared in a gourd and is drunk hot through a metal straw, which delivers the liquid directly to oropharynx and esophagus. *Maté* is consumed in Rio Grande do Sul at temperatures that range between 63 and 69.5°C (5, 6), and multiple studies have reported a positive association between amounts of *maté* intake and risk of ESCC (4, 7–14).

The International Agency for Research on Cancer (IARC) has classified *maté* as "probably carcinogenic to humans" (group 2A) due to the consumption temperature (13). Furthermore, the infusion of *Ilex paraguariensis*, either hot or cold, has a high content of polycyclic aromatic hydrocarbons (PAHs; refs. 15–19). PAHs, a product of burning organic material, are classified by IARC as a group 1 carcinogen, that is, carcinogenic to humans (20). Thus, the high PAH content of *maté*, in addition to the hot temperature at which *maté* is consumed, may play a role in the risk of developing ESCC. There is also an association between *maté* consumption and PAH-associated cancers at other sites, such as the kidney, bladder, lung, and prostate, which have no exposure to thermal injury (21–23).

The traditional processing of *yerba maté* adds relatively high levels of PAHs to the final product (16–19). This process includes two drying steps: the first called "sapeco" is a rapid drying step where the leaves are exposed directly to fire or very high

[1]Gastroenterology Service, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. [2]Programa de Pós Graduação Ciências em Gastroenterologia e Hepatologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. [3]Departamento de Clínica Médica, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. [4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland. [5]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Antonio Barros Lopes, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2400, Porto Alegre, Rio Grande do Sul 90035-003, Brazil. Phone: 5551-9999-12021; Fax: 5551-3335-2822; E-mail: ablopes@hcpa.edu.br

doi: 10.1158/1055-9965.EPI-17-0773

temperatures (400–750°C) to reduce moisture and stop degradation. In the second drying stage, the leaves are placed in rotating cylinders heated by burning wood and exposed to lower temperatures (90–350°C) for 8 to 24 hours. Both steps increase the PAH content of the *yerba maté* (19). The avoidance of smoke exposure during those steps decreases the PAH content (18).

The aim of this study was to evaluate potential associations between amounts of *maté* consumption and exposure to PAHs (as measured by the urinary concentrations of PAH metabolites) among smokers and nonsmokers in Rio Grande do Sul, Brazil, a high-risk area for ESCC.

Materials and Methods

Participants

This was a cross-sectional study with a convenience sample of participants recruited from patients from gastrointestinal disease clinics at two sites, Hospital de Clinicas de Porto Alegre and Hospital Universitario de Santa Maria (Rio Grande do Sul, Brazil). We conducted the research following the Helsinki recommendations for medical research involving human subjects. The institutional review boards of both institutions approved the protocol, and all participants signed a written informed consent. The analysis of blinded specimens by the Centers for Disease Control and Prevention (CDC; Atlanta, GA) laboratory and the participation of NCI (Rockville, MD) and CDC staff in analyzing the blinded data were determined not to constitute engagement in human subjects' research.

We prospectively approached individuals between 30 and 70 years of age who were scheduled for upper gastrointestinal endoscopy and had no previous history of any cancer (except for nonmelanoma skin cancer). We aimed to enroll 250 subjects, equally divided among men and women, current smokers and nonsmokers, and five levels of *maté* consumption: none; 1 to 500 mL/day; 501 to 1,000 mL/day; 1,001 to 2,000 mL/day, and more than 2,000 mL/day. In each level of *maté* consumption, we planned to recruit 25 men (half current smokers) and 25 women (half current smokers). We interviewed the patients using a standard questionnaire and collected one fasting spot urine sample.

Questionnaire

All individuals were interviewed face-to-face using a standard questionnaire, which was administered by one of five trained interviewers. The inquiry included: (i) basic demographic variables; (ii) information on *maté* drinking (frequency of ingestion, self-reported typical amount in mL/day, amount ingested within the previous 3 days); (iii) tobacco smoking habits (current or past smoking, number of cigarettes/day, duration of smoking, and time since quitting); (iv) environmental exposure to smoke and passive smoke exposure (frequency and location – at work or home; the work or home environments were considered smoky if the answer to the questions "do you find your work smoky?" or "do you find your home smoky?" were "yes"); (v) barbecue eating and cooking habits (frequency of eating barbecue, grams of meat/meal, frequency of making barbecue, and the fuel used to grill); and (vi) alcohol consumption (kind of beverage, average amount in mL/week). Individuals were considered *maté* drinkers or smokers if they were regularly engaged in these habits within the last year. To improve the accuracy of *maté* consumption, we showed photographs of different sizes of gourds to the participants and asked them to choose which one they typically used. The same technique was used to optimize the estimates of meat consumption, using plastic models of 100 gram pieces of meat.

Quantification of urinary PAH metabolites

Participants arrived fasting for their endoscopy exams and collected urine in a sterile container before endoscopy and any preendoscopy drug administration. Ten milliliters of urine was frozen immediately at −80°C, and the rest was analyzed for creatinine and cotinine (see below). Excess urine from 13 current smokers was combined to make a quality control pool, which was analyzed blindly 12 times to evaluate coefficients of variation (CV) for each PAH metabolite.

All frozen urine samples were shipped on dry ice first to the National Cancer Institute and then to the Organic Analytical Toxicology Branch of the Division of Laboratory Sciences of the National Center for Environmental Health at the CDC. Quantification of the PAH metabolites was conducted by online solid phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as described previously (24). The following seven metabolites of PAHs were measured: 1-naphthol (CV = 10.33), 2-naphthol (CV = 12.59), 2-hydroxyfluorene (CV = 13.12), 3-hydroxyfluorene (CV = 13.34), 1-hydroxyphenanthrene (CV = 15.33), sum of 2- and 3-hydroxyphenanthrene ($\sum$2,3-hydroxyphenanthrene, CV = 14.47), and 1-hydroxypyrene (CV = 14.42). We also summed the urine concentrations of all measured metabolites of each parent PAH compound ($\sum$naphthols, $\sum$hydroxyfluorenes, $\sum$hydroxyphenanthrenes), so we could analyze these "parent compound" variables. Urine creatinine was measured by enzymatic colorimetry and expressed in mg/dL.

In addition to self-reported smoking, we measured urinary cotinine using Accutest NicAlert strips (Jant Pharmaceutical Corp.). This assay, which measures exposure to tobacco smoke within the past 48 hours, was performed shortly after urine collection, according to the manufacturer's instructions. Results ranged from 0 to 6 [0–2 = nonuser of/no exposure to tobacco products (cotinine = 1 – 100 ng/mL); 3–6 = user of/exposure to tobacco products (including exposure to second hand tobacco smoke; cotinine> 100 ng/mL)]. Two members of the research team scored the cotinine dipstick results. A third reviewer, unaware of the patient's smoking status, resolved disagreements by evaluating a photograph of the strip. A two-category cotinine-determined smoking status of current nonsmoker or current smoker (due to primary, secondhand, or environmental exposure to tobacco smoke) was used for all statistical analyses.

Statistical analysis

Urinary concentrations of PAH metabolites are short-term markers reflecting only the past 1 to 3 days of PAH exposure (25, 26). Thus, we categorized patients by their self-reported average daily *maté* intake within the past 3 days to evaluate the association of *maté* intake and the urinary PAH metabolites (the individual metabolites and the summed metabolites of each parent PAH compound). For categorical analyses, we divided the patients into quartiles based on this average recent *maté* intake: (i) *maté* quartile 1: 0 mL/day; (ii) *maté* quartile 2: 1 to 600 mL/day; (iii) *maté* quartile 3: 601 to 1,500 mL/day; (iv) *maté* quartile 4: >1,500 mL/day.

Table 1. Characteristics of the study participants in Rio Grande do Sul, Brazil

		Maté quartile[a]				
	Total	**Q1 0 mL**	**Q2 1–600 mL**	**Q3 601–1,500 mL**	**Q4 >1,500 mL**	***P***
Characteristics						
Total participants, no. (%)	244	79 (32)	44 (18)	70 (29)	51 (21)	—
Mean age, years (SD)	53 (10)	55 (10)	53 (11)	54 (10)	50 (9)	0.20[b]
Sex, *n* (%)						
Males	114 (47)	37 (47)	20 (45)	35 (50)	22 (43)	0.90[c]
Females	130 (53)	42 (53)	24 (55)	35 (50)	29 (57)	—
Reported current smoker, *n* (%)						
Yes	109 (45)	37 (47)	20 (45)	28 (40)	24 (47)	0.83[c]
No	135 (55)	42 (53)	24 (55)	42 (60)	27 (53)	—
Urine cotinine, *n* (%)						
Current smoker	116 (48)	37 (47)	21 (48)	30 (43)	28 (55)	0.63[c]
Nonsmoker	128 (52)	42 (53)	23 (52)	40 (57)	23 (45)	—
Current alcohol drinker, *n* (%)						
Yes	76 (31)	32 (41)	16 (36)	22 (31)	6 (12)	0.005[c]
No	168 (69)	47 (59)	28 (64)	48 (69)	45 (88)	—
Work smoky[d], *n* (%)						
Yes	18 (7)	4 (5)	2 (5)	6 (9)	6 (12)	0.44[c]
No	226 (93)	75 (95)	42 (95)	64 (91)	45 (88)	—
Home smoky[d], *n* (%)						
Yes	57 (23)	14 (18)	8 (18)	17 (24)	18 (35)	0.19[c]
No	187 (77)	65 (82)	36 (82)	53 (76)	33 (65)	—
Ever eat BBQ, *n* (%)						
Yes	230 (94)	72 (91)	41 (93)	67 (96)	50 (98)	0.37[c]
No	14 (6)	7 (9)	3 (7)	3 (4)	1 (2)	—
Days since eating BBQ, mean (SD)	17 (41)	28 (66)	13 (14)	9 (12)	15 (29)	0.04‡
Ever make barbecue, *n* (%)						
Yes	78 (32)	22 (28)	16 (36)	25 (36)	15 (29)	0.75†
No	150 (61)	50 (63)	25 (57)	41 (59)	34 (67)	—
None	16 (7)	7 (9)	3 (7)	4 (5)	2 (4)	—
Days since making BBQ, mean (SD)	25 (42)	38 (60)	27 (27)	15 (19)	21 (45)	0.03‡
Barbecue cooking fuel, *n* (%)						
Coal	192 (79)	69 (87)	33 (75)	51 (73)	39 (76)	0.14†[c]
Other/none	52 (21)	10 (13)	11 (25)	19 (27)	12 (24)	—

[a]*N* (column %) within characteristic, mean (SD) shown for continuous characteristics.
[b]*P* values were calculated by an ANOVA comparison of means test.
[c]*P* values were calculated by a two-sided Pearson χ^2 test.
[d]The work or home environments were considered smoky if the answer to the questions "do you find your work smoky?" or "do you find your home smoky?" were "yes."

Baseline and demographic characteristics of the study population were described by means and SDs or medians and interquartile ranges (IQR) and were compared across categories of *maté* intake by the χ^2 test (for categorical variables) or the one-way ANOVA (for continuous variables).

We calculated geometric means and 95% confidence intervals (95% CI) of the urinary concentrations of the PAH metabolites and the summed metabolite concentrations of the parent PAH compounds for each quartile of *maté* intake. Crude and multivariate associations between these concentrations and *maté* intake quartiles were examined in linear regression models. The final adjusted model included creatinine and all potentially confounding variables (age, sex), urine cotinine (current smoker/nonsmoker), a combined indoor smoke exposure variable (work and/or home smoky/both not smoky), fuel type (coal/other or none), days since last eating barbecue, days since last making barbecue, and log-creatinine). For completeness, we also examined crude and adjusted models of creatinine-corrected metabolite concentrations (ng/g creatinine), in which the adjusted models did not include creatinine. We also tested the model for interactions by age, sex, and days since eating barbecue, and days since making barbecue, but no significant associations were found. We constructed a jitter plot depicting the median and IQR of the creatinine-corrected PAHs for each of four categories combining recent *maté* drinking (yes/no) and cotinine-defined current cigarette smoking (yes/no) to demonstrate the effect of smoking and *maté* on the sum of all measured metabolites of each parent PAH compound. All analyses were performed using STATA 13.0 software (StataCorp). All *P* values were two-sided tests and $P < 0.05$ was considered statistically significant.

We compared the agreement between self-reported usual daily *maté* intake and average intake within the past 3 days, and the agreement between self-reported smoking status and that determined by the cotinine NicAlert test, using Cohen κ coefficient.

Results

We successfully enrolled 244 patients with a wide range of usual daily *maté* consumption (0–6,000 mL/day. We found it difficult to enroll male smokers who drank >1,000 mL/day. Table 1 summarizes the participants' characteristics by quartiles of average *maté* intake in the past 3 days. We found inverse associations between *maté* intake quartile and current alcohol drinking, days since eating barbecue, and days since making barbecue.

The geometric mean PAH urinary metabolite concentrations and their 95% CIs by quartile of *maté* intake in the past 3 days are

presented in Table 2. We found a significant association between recent *maté* consumption and six of the seven measured PAH metabolites in unadjusted models ($P_{trend} < 0.05$). There was also a significant association between recent *maté* consumption and the summed metabolites of 3 of the 4 parent PAH compounds. After adjustment for creatinine and potential confounders, such as age, sex, urine cotinine–determined smoking status, indoor smoke exposure, barbecue cooking fuel, and days since eating or making barbecue, significant associations remained with three of the measured PAH metabolites and the summed measured metabolites of phenanthrene. When we performed this analysis using creatinine-corrected urinary metabolite concentrations (ng/g creatinine), the results were similar (Supplementary Table S1).

When the sums of all creatinine-corrected metabolite concentrations of each parent PAH compound were compared by recent *maté* drinking and cotinine-determined smoking status (Fig. 1; Supplementary Table S2), we found a significant ($P \leq 0.01$) increase in concentrations of the biomarkers of fluorene, phenanthrene, and pyrene among *maté* drinkers, smokers, and patients who were both *maté* drinkers and smokers compared with patients with no recent exposure to either *maté* or tobacco smoke. In addition, *maté* drinkers who did not smoke had equivalent or higher concentrations of the sum of the urinary metabolites of phenanthrene (mean 732 ng/g, median 532 ng. g) than smokers who did not drink *maté* (mean 673 ng/g, median 543 ng/g).

There was good agreement between self-reported usual daily *maté* intake and average intake within the past 3 days (weighted κ test = 74.3%; Supplementary Table S3). But these measures presented an irregular pattern, especially among individuals in the lowest level of usual daily *maté* intake (1–500 mL/day). This group included some people who said that they usually drink *maté* only on weekends or only in occasional social settings. Thus, their usual (estimated average) *maté* intake might be in the range of 1 to 500 mL/day, but they did not consume any *maté* in the 3 days before urine collection. There was also excellent agreement between self-reported smoking status, and the smoking status determined by the cotinine NicAlert test (κ = 95.5%; Supplementary Table S4).

Discussion

Rio Grande do Sul has a high incidence of esophageal cancer, estimated by the National Institute of Cancer of Brazil to be 20.3 new cases/100,000 men and 6.6 new case/100,000 women in 2016 (27). ESCC is by far the predominant histologic type (28, 29), and its main risk factors are tobacco smoking, heavy alcohol consumption, barbecue eating/cooking, and *maté* drinking (7). *Maté* drinking, a widespread and deep-rooted habit in Rio Grande do Sul, usually starts at an early age. In previous studies, we have shown that commercial brands of *yerba maté* contain approximately 40 ng of benzo[a]pyrene (B[a]P)/gm of leaves, and half of this B[a]P gets into the *maté* infusion (17). A typical gourd of *maté*, containing 50 gm of leaves and drunk in the traditional way (refilling the gourd with water ∼10–12 times) delivers approximately 1,000 ng B[a]P to the person drinking it, which is the same amount of B[a]P that is in the smoke of 100 cigarettes. We have hypothesized that the high PAH content and the high consumption temperature of *maté* may both contribute to the increased risk of ESCC observed in *maté* drinkers (15, 17, 18).

Table 2. PAH metabolite concentrations [geometric mean (confidence interval)] by quartile of average daily *maté* intake in the past 3 days

		Maté quartile					
		Q1[a]	Q2	Q3	Q4		
Analyte	**Abbreviation**	**0 mL**	**1–600 mL**	**601–1,500 mL**	**>1,500 mL**	**Crude P_{trend}**	**Adjusted[b] P_{trend}**
PAH metabolite concentrations (ng/L) - All participants							
1-Naphthol	1-nap	7,217 (4,776–10,907)	8,428 (5,522–12,864)	6,771 (5,156–8,892)	11,045 (7,364–16,567)	0.08	0.30
2-Naphthol	2-nap	11,285 (8,885–14,333)	13,582 (10,614–17,380)	12,760 (10,373–15,695)	14,557 (10,564–20,058)	0.02	0.03
	Σ-napthols	21,488 (15,614–29,570)	24,983 (18,163–34,363)	20,525 (16,440–25,625)	28,118 (19,954–39,624)	0.10	0.19
2-Hydroxyfluorene	2-flu	660 (492–884)	816 (610–1,092)	761 (613–945)	890 (638–1,241)	0.02	0.11
3-Hydroxyfluorene	3-flu	339 (243–474)	421 (295–601)	385 (298–499)	502 (344–732)	0.02	0.39
	Σ-fluorenes	1,013 (747–1,374)	1,253 (917–1,711)	1,160 (924–1,458)	1,405 (994–1,988)	0.02	0.19
1-Hydroxyphenanthrene	1-phe	220 (178–272)	417 (334–519)	521 (447–606)	642 (504–817)	$P < 0.0001$	$P < 0.0001$
2-3-Hydroxyphenanthrene	2-3-phe	260 (209–324)	361 (287–454)	405 (354–463)	456 (355–585)	$P < 0.0001$	0.004
	Σ-phenanthrenes	489 (395–604)	789 (635–981)	936 (814–1,077)	1,108 (870–1,410)	$P < 0.0001$	0.001
1-Hydroxypyrene	1-pyr	284 (232–348)	361 (287–454)	359 (308–417)	400 (316–508)	0.001	0.06

[a]Q1 was the reference category for all models.
[b]Fully adjusted for age, sex, urine cotinine-determined smoking status (current smoker, nonsmoker), indoor smoke exposure (reported at work and/or at home), fuel type (coal vs. other or none), days since last eating barbecue, days since last making barbecue, and log-creatinine.

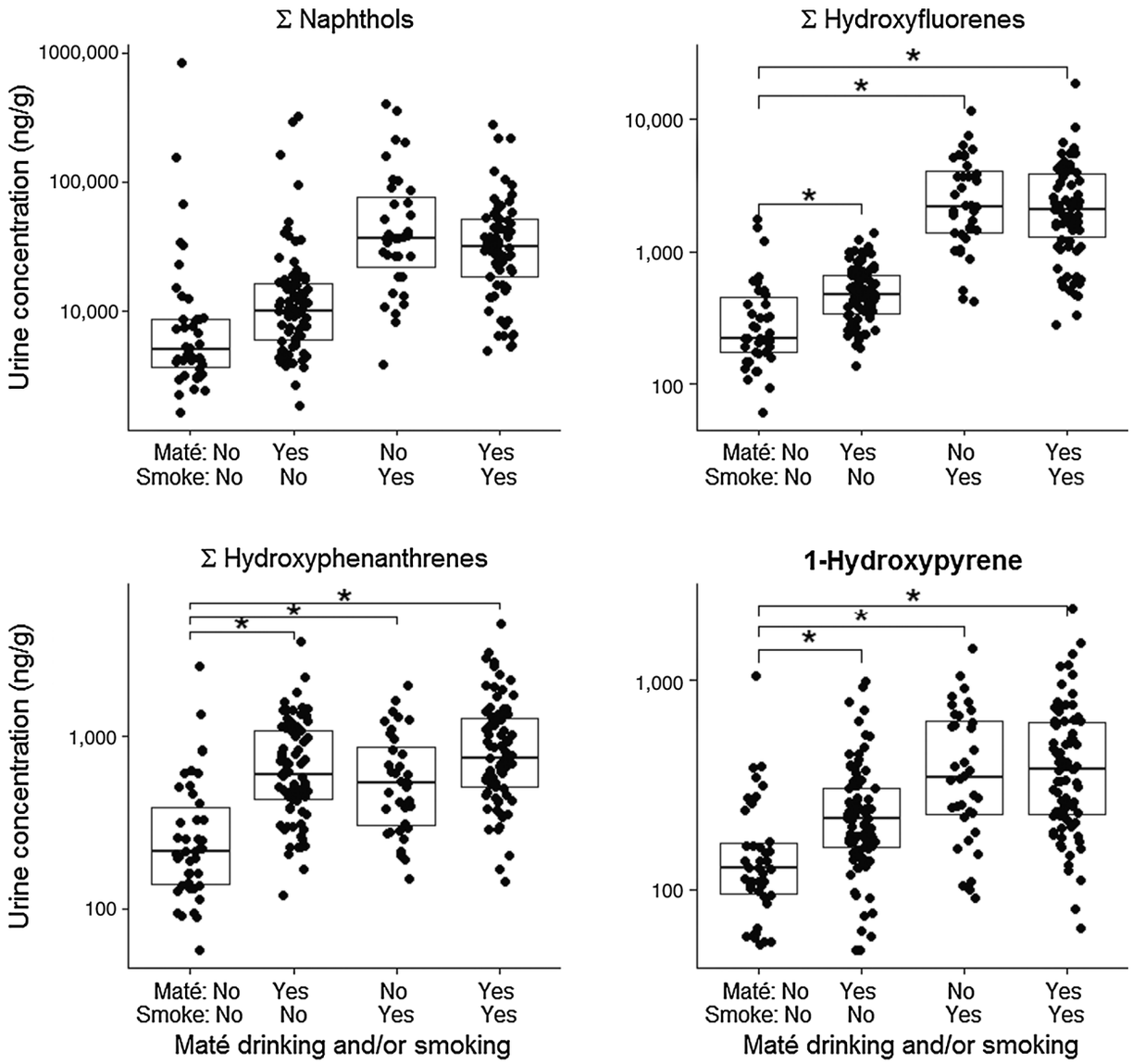

Figure 1.

Creatinine-corrected urine metabolite concentrations plotted by recent *maté* consumption and cotinine-determined smoking status (*$P < 0.01$); contains creatinine-corrected PAH urine metabolite concentrations plotted by recent *maté* consumption and cotinine-determined smoking status.

In the current study, we quantified the urinary concentrations of PAH metabolites in men and women, and smokers and nonsmokers, living in Rio Grande do Sul. Besides these characteristics, we enrolled the patients based on their levels of *maté* drinking, so we could assess their PAH exposure over a wide range of *maté* consumption.

Fagundes and colleagues previously detected 1-hydroxypyrene glucuronide in the urine of *maté* drinkers in Rio Grande do Sul (15). The differences between the current study and this previous one are that in the previous study the authors did not select the participants based on the amount of *maté* intake, they did not confirm the smoking status by measuring cotinine, and they measured only one urinary PAH metabolite, whereas we measured seven metabolites of four parent PAHs.

In our study, in unadjusted analyses, six of the seven measured PAH metabolites and the summed metabolites of three of four parent PAH compounds had significant dose–response associations with the amount of *maté* ingested in the past 3 days. After multivariate adjustment for creatinine and potential confounders, the associations of three of the seven measured PAH metabolites and the summed metabolites of phenanthrene remained significant. In addition, *maté* drinking increased the summed concentrations of the measured phenanthrene metabolites as much as cigarette smoking did.

The PAH content of commercial *yerba maté* is largely a contaminant acquired during the traditional drying of the *yerba maté* leaves, which involves many hours of contact with smoke (19), and avoiding smoke during this processing decreases the PAH content (18). This last study, by Golozar and colleagues, showed that a commercial brand of *yerba maté*, which was manufactured without exposure to smoke had 5.11 ng benzo[a]pyrene/gram of leaves, much lower than the 11.9 to 99.3 ng/g benzo[a]pyrene content of 11 other commercial brands of *yerba maté* processed in the traditional way (18). Thus, changes in commercial *yerba maté* manufacturing processes, along with public education about the beneficial effects of drinking *maté* at lower temperatures, can probably decrease the carcinogenic risk of *maté* and make it a safer and healthier drink. Moreover, a reduction of PAH content in commercial *yerba maté* may also have an impact on the risk of other PAH-associated neoplasms, such as kidney, bladder, lung, and prostate cancer.

Our study had a number of strengths. Our participants had a wide range of *maté* intake. We recorded both the usual daily consumption and the intake within the 3 days before the urine collection. We showed photographs of different size gourds to the participants to better estimate the amount of *maté* they consumed. Urinary cotinine was measured to minimize inaccuracy of self-reported data on tobacco smoking, and it showed excellent correlation with questionnaire data. Potential confounders of *maté*-related PAH exposure, such as exposure to current smoking, environmental smoke, and eating or cooking barbecue, were equally distributed among individuals in the four *maté* intake quartiles (Table 1), and they were included as adjusting variables in the regression analyses.

Our study also had some limitations. Our patients were enrolled in tertiary hospitals and, thus, may not reflect the general population of southern Brazil. Indeed, there are earlier reports of the average *maté* consumption in this region ranging from 1,200 to 1,800 mL/day (5, 6) and reports of it not being uncommon to find people who drink more than 2 L/day, but this was not our experience. Many people with digestive symptoms refrain from drinking *maté*, or reduce their intake, which may help explain why recruiting patients in gastrointestinal disease clinics in referral hospitals enrolled fewer patients with very high consumption. Recruiting participants exclusively in tertiary hospitals may also have affected generalizability with respect to smoking habits, as quitting smoking is highly encouraged in such hospitals. Our study relied solely on self-reported information on many habits, which could be subject to inaccuracy. Finally, we did not collect information on *maté* consumption temperature in the current study. Although it is true that the temperature of hot infusions may influence the risk of ESCC (3, 7, 8, 12, 30, 31), the PAH content in *maté* is not affected by the water temperature (17).

In conclusion, in this study, urinary concentrations of PAH metabolites were significantly associated with self-reported amount of recent *maté* intake, and drinking *maté* increased urinary concentrations of some PAH metabolites as much as smoking cigarettes. These results confirm that drinking *maté* is a source of exposure to potentially carcinogenic PAHs, consistent with the hypothesis that the PAH content of *maté* may contribute to the increased risk of ESCC in *maté* drinkers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the U.S. Department of Health and Human Services.

Authors' Contributions

Conception and design: A.B. Lopes, M. Metzdorf, L. Metzdorf, M.P.R. Sousa, C. Kavalco, A. Etemadi, C.C. Abnet, S.M. Dawsey, R.B. Fagundes
Development of methodology: A.B. Lopes, M. Metzdorf, L. Metzdorf, M.P.R. Sousa, C. Kavalco, A. Etemadi, R.B. Fagundes
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.B. Lopes, M. Metzdorf, L. Metzdorf, M.P.R. Sousa, C. Kavalco, A.M. Calafat, R.B. Fagundes
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Etemadi, N.R. Pritchett, G. Murphy, A.M. Calafat, C.C. Abnet, S.M. Dawsey, R.B. Fagundes
Writing, review, and/or revision of the manuscript: A.B. Lopes, M. Metzdorf, L. Metzdorf, M.P.R. Sousa, C. Kavalco, A. Etemadi, N.R. Pritchett, G. Murphy, A.M. Calafat, C.C. Abnet, S.M. Dawsey, R.B. Fagundes
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.B. Lopes, R.B. Fagundes
Study supervision: A.B. Lopes, C.C. Abnet, S.M. Dawsey, R.B. Fagundes

Acknowledgments

All funding for this study came from the Intramural Research Program of the NCI. We acknowledge Yuesong Wang, Kendra Hubbard, Lei Meng, Nikki Pittman, Debra Trinidad, and Xiaoyun Ye for their technical assistance measuring PAH biomarkers.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 25, 2017; revised October 21, 2017; accepted December 14, 2017; published OnlineFirst December 20, 2017.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. Gut 2015;64:381–7.
3. Islami F, Boffetta P, Ren JS, Pedoeim L, Khatib D, Kamangar F. High-temperature beverages and foods and esophageal cancer risk–a systematic review. Int J Cancer 2009;125:491–524.
4. Andrici J, Eslick GD. Mate consumption and the risk of esophageal squamous cell carcinoma: a meta-analysis. Dis Esophagus 2013;26:807–16.
5. de Barros SG, Ghisolfi ES, Luz LP, Barlem GG, Vidal RM, Wolff FH, et al. [High temperature "mate" infusion drinking in a population at risk for squamous cell carcinoma of the esophagus]. Arq Gastroenterol 2000;37:25–30.
6. Victora CG, Munoz N, Horta BL, Ramos EO. Patterns of mate drinking in a Brazilian city. Cancer Res 1990;50:7112–5.
7. Castellsague X, Munoz N, De Stefani E, Victora CG, Castelletto R, Rolon PA. Influence of mate drinking, hot beverages and diet on esophageal cancer risk in South America. Int J Cancer 2000;88:658–64.
8. De Stefani E, Boffetta P, Fagundes RB, Deneo-Pellegrini H, Ronco AL, Acosta G, et al. Nutrient patterns and risk of squamous cell carcinoma of the esophagus: a factor analysis in Uruguay. Anticancer Res 2008;28:2499–506.

9. De Stefani E, Deneo-Pellegrini H, Ronco AL, Boffetta P, Brennan P, Munoz N, et al. Food groups and risk of squamous cell carcinoma of the oesophagus: a case-control study in Uruguay. Br J Cancer 2003;89:1209–14.
10. Vassallo A, Correa P, De Stefani E, Cendan M, Zavala D, Chen V, et al. Esophageal cancer in Uruguay: a case-control study. J Natl Cancer Inst 1985;75:1005–9.
11. De Stefani E, Munoz N, Esteve J, Vasallo A, Victora CG, Teuchmann S. Mate drinking, alcohol, tobacco, diet, and esophageal cancer in Uruguay. Cancer Res 1990;50:426–31.
12. Castelletto R, Castellsague X, Munoz N, Iscovich J, Chopita N, Jmelnitsky A. Alcohol, tobacco, diet, mate drinking, and esophageal cancer in Argentina. Cancer Epidemiol Biomarkers Prev 1994;3:557–64.
13. Loomis D, Guyton K, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of drinking coffee, mate, and very hot beverages. Lancet Oncol 2016;17:877–8.
14. Lubin JH, De Stefani E, Abnet CC, Acosta G, Boffetta P, Victora C, et al. Mate drinking and esophageal squamous cell carcinoma in South America: pooled results from two large multicenter case-control studies. Cancer Epidemiol Biomarkers Prev 2014;23:107–16.
15. Fagundes RB, Abnet CC, Strickland PT, Kamangar F, Roth MJ, Taylor PR, et al. Higher urine 1-hydroxy pyrene glucuronide (1-OHPG) is associated with tobacco smoke exposure and drinking mate in healthy subjects from Rio Grande do Sul, Brazil. BMC Cancer 2006;6:139.
16. Garcia Londono VA, Reynoso M, Resnik S. Polycyclic aromatic hydrocarbons (PAHs) in yerba mate (*Ilex paraguariensis*) from the Argentinean market. Food Addit Contam Part B Surveill 2014;7:247–53.
17. Kamangar F, Schantz MM, Abnet CC, Fagundes RB, Dawsey SM. High levels of carcinogenic polycyclic aromatic hydrocarbons in mate drinks. Cancer Epidemiol Biomarkers Prev 2008;17:1262–8.
18. Golozar A, Fagundes RB, Etemadi A, Schantz MM, Kamangar F, Abnet CC, et al. Significant variation in the concentration of carcinogenic polycyclic aromatic hydrocarbons in yerba mate samples by brand, batch, and processing method. Environ Sci Technol 2012;46:13488–93.
19. Vieira MA, Maraschin M, Rovaris AA, Amboni RD, Pagliosa CM, Xavier JJ, et al. Occurrence of polycyclic aromatic hydrocarbons throughout the processing stages of erva-mate (*Ilex paraguariensis*). Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010;27:776–82.
20. Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Cogliano V. Carcinogenicity of polycyclic aromatic hydrocarbons. Lancet Oncol 2005; 6:931–2.
21. Bates MN, Hopenhayn C, Rey OA, Moore LE. Bladder cancer and mate consumption in Argentina: a case-control study. Cancer Lett 2007; 246:268–73.
22. Deneo-Pellegrini H, Ronco AL, De Stefani E, Boffetta P, Correa P, Mendilaharsu M, et al. Food groups and risk of prostate cancer: a case-control study in Uruguay. Cancer Causes Control 2012;23:1031–8.
23. Stefani ED, Moore M, Aune D, Deneo-Pellegrini H, Ronco AL, Boffetta P, et al. Mate consumption and risk of cancer: a multi-site case-control study in Uruguay. Asian Pac J Cancer Prev 2011;12:1089–93.
24. Wang Y, Meng L, Pittman EN, Etheredge A, Hubbard K, Trinidad DA, et al. Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 2017;409:931–7.
25. Buckley TJ, Lioy PJ. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br J Ind Med 1992;49:113–24.
26. Kang DH, Rothman N, Poirier MC, Greenberg A, Hsu CH, Schwartz BS, et al. Interindividual differences in the concentration of 1-hydroxypyrene-glucuronide in urine and polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells after charbroiled beef consumption. Carcinogenesis 1995;16:1079–85.
27. Nacional Institute of Cancer - Brazil. Estimated new cases of esophageal cancer in 2016. Available from: http://www.inca.gov.br/estimativa/2016/tabelaestados.asp?UF=RS.
28. de Barros SG, Vidal RM, Luz LP, Ghisolfi ES, Barlem GG, Komlos F, et al. [Prevalence of adenocarcinoma of the esophagus and esophagogastric junction in a 10 year period at a cancer referral center in southern Brazil]. Arq Gastroenterol 1999;36:32–6.
29. Fagundes RB, de Carli D, Xaubet RV, Cantarelli JC Jr. Unchanging pattern of prevalence of esophageal cancer, overall and by histological subtype, in the endoscopy service of the main referral hospital in the central region of Rio Grande do Sul State, in Southern Brazil. Dis Esophagus 2016;29:603–6.
30. Sewram V, De Stefani E, Brennan P, Boffetta P. Mate consumption and the risk of squamous cell esophageal cancer in uruguay. Cancer Epidemiol Biomarkers Prev 2003;12:508–13.
31. Islami F, Pourshams A, Nasrollahzadeh D, Kamangar F, Fahimi S, Shakeri R, et al. Tea drinking habits and oesophageal cancer in a high risk area in northern Iran: population based case-control study. BMJ 2009;338:b929.