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Dietary Acid Load and Bladder Cancer Risk: An Epidemiologic Case-Control Study

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Introduction: Dietary acid load contributes to metabolic acidosis, closely linked to cancer development through inflammation and cell transformation. There is very limited epidemiologic evidence; linking diet-dependent acid load and cancer risk. Since there are few published studies specifically on urinary pH and bladder cancer (BC) risk, we sought to explore this association in the present study.

Methods: A case-control study was performed in 765 patients (255 cases and 510 age-matched controls) through a multi-topic inquiry including a food frequency questionnaire. Food-derived nutrients were calculated from available databases. The dietary acid load was calculated based on two validated measures including potential renal acid load (PRAL) and net endogenous acid production (NEAP) scores. Odds ratios (OR) and their 95% confidence intervals were estimated by unconditional logistic regression adjusted for potential confounders.

Results: We found direct associations between dietary acid load and BC risk. Both acid load scores were significantly associated with an increased BC risk (OR=1.74 and OR=1.83 for PRAL and NEAP scores, respectively). Linear trends were found for both risk estimates.

Conclusions: A high dietary acid load may contribute to BC development. Both acid load scores were directly associated with animal-based foods (mainly meat) and inversely associated with the intake of plant-based foods. To our knowledge, this is the first epidemiologic case-control study analyzing associations of dietary acid load and BC risk in the Latin American population. Further research is warranted to confirm our findings.

INTRODUCTION

Bladder cancer (BC) is a highly prevalent disease associated with substantial morbidity and mortality [1]. With more than 573,000 new cases in 2020, BC is the 10th most prevalent cancer worldwide [2]. The highest burden of BC is currently found in the most developed countries [2-4]. Its incidence varies significantly between geographical regions with a higher incidence rate in Southern and Western Europe as well as in Northern America [2, 3]. The disease is more common in men than in women which have led to consider sex hormones and their receptors as potential risk factors [5]. Major risk factors include tobacco smoking, occupational exposure to certain chemicals (particularly aromatic amines), long-term drinking of arsenic-contaminated or chlorinated water, and infection with Schistosoma haematobium [2-4]. BC presents a substantial challenge to public health [4]; thus, understanding potential risk factors is of utmost importance to increase public awareness and improve disease prevention [6]. More recent studies have pointed out the potential role of modifiable risk factors; including physical activity and diet which may influence BC incidence and recurrence [6-8]. The role of dietary factors on BC risk is controversial. International experts recognized the epidemiologic evidence on diet and BC risk still as inconsistent [8-10]. However, some evidence suggests that a high intake of processed foods and red meats may increase BC risk [11]. In contrast, dietary patterns with high volumes of fruits and vegetables may provide beneficial e□ects on BC risk [12]. The average diet in Uruguay is meat-based, with the world's highest per capita beef intake [13]. A western diet, abundant in meat, fat, sugar, and with ~15 mg/day iron, might be epidemiologically linked to the increased development of tumors in humans [14]. Both heme (in animal foods) and non-heme (in plant foods, also in meat) dietary iron are mostly present as Fe3+ (oxidized state) [15]. Heme-iron contributes to 2/3 of the average individual iron intake in developed countries [14]. The contributory role of iron in cancers could be mediated by overproducing reactive oxygen species and free radicals through Fenton reaction (Fe2+ oxidized to Fe3+), participating in inflammation and DNA synthesis, and catalyzing the formation of lipid peroxides and nitroso-compounds [16]. Contemporary Western diets rich in red and processed meats, high-fat dairy products, and refined carbohydrates constitute a high dietary acid load [17]. Both in vitro and clinical studies suggested a potential link between a high dietary acid load and increased cancer risk [18]. From 2019 on, positive associations were found between dietary habits and various cancer types; including pancreatic [18], colorectal [19, 20], bronchopulmonary [21], mammary [22, 23], prostatic [24], and central nervous system cancer [25]. However, no association was found in recent papers on the kidney [26] and breast cancer [27]. In addition, a systematic review of dietary acid load, alkaline water, and cancer [28] also highlights a clear gap in knowledge and the need for further studies. To the best of our knowledge, there is only a single study that investigated dietary acid load and BC risk which did not show an association among a very small cohort of Caucasian male smokers [29]. To test whether there is an association between an acidogenic diet and BC, we performed a case-control study in a population from Montevideo, Uruguay.

METHODS

Selection of Cases and Controls

The methods have been previously described in detail [21, 26]. Within 8 years (1996-2004), all newly microscopically diagnosed cases of transitional cell carcinoma of the urinary bladder registered in the four major hospitals from Montevideo were considered eligible for this study. The public health system is centralized in Montevideo (the capital of Uruguay harboring more than half of the Uruguayan population) where more than 50% of total cancers are diagnosed [30]. The public system covers around 40% of the whole population, and the pre-paid (private) insurance system covers the remaining 60%. Two trained social laborers who worked at the hospitals and were unaware of the study objectives investigated two phases. First, they looked routinely for newly diagnosed cancer patients through medical records personnel. Second, they contacted eligible patients to be age- and sex-matched with cases. After obtaining oral informed consent that is the only requirement by our system, all the participants underwent an in-person interview in

the hospital. Each hospital director has allowed the project after receiving approval from the respective Ethical Committee. An auto-generated number was built to preserve anonymity; based on first, last name, and ID number. Two hundred sixty-one cases were identified, and six patients refused the interview; resulting in 255 cases for inclusion in the study (response rate 97.7%). In the same period and medical facilities, 527 potential controls afflicted with non-neoplastic diseases were considered eligible. After the exclusion of 17 patients who refused to participate in the study, a final number of 510 controls were included in the study (response rate 96.8%). These controls were admitted for diseases unrelated to alcohol disorders or tobacco smoking, and they had no history of recent dietary modifications. Controls were presented with the following conditions: eye disorders (132 patients, 25.7%), abdominal hernia (114, 22.4%), fractures (52, 10.2%), skin diseases (40, 7.8%), injuries and trauma (45, 8.9%), appendicitis (37, 7.2%), varicose veins (29, 5.7%), hydatid cyst (20, 4.0%), blood disorders (18, 3.5%), and other medical disorders (23, 4.5%). The study intended to obtain two matched controls per case. A value of 0.2 was assumed as the correlation for the exposure rates between cases and controls since prior data were not readily available; following Dupont's suggestions [31]. With a theoretical OR=1.8 for disease in exposed/unexposed individuals, we needed at least 201 cases with two matched controls per case to reject the null hypothesis with a power=0.80 and an α-error=0.05 [32]. Patients admitted to the public healthcare subsystem had low incomes and came from all over the country and had free access to most medical services, as established by Uruguayan law. The population's features represented a third-world country, different from the population subset admitted to the private health subsystem.

Interviews and Questionnaire

First of all, blinded trained social workers undertook routine screenings to identify potentially eligible patients with a recent diagnosis of BC. Then, potentially eligible individuals and controls were contacted by the interviewers. After consenting to our study, all subjects were finally interviewed face-to-face and proxy interviews were not accepted. The administered questionnaire included socio-demographic and anthropometric variables, history of tobacco smoking or alcohol drinking, occupational exposures, and cancer history in first or second-degree relatives. Additionally, it included a 64 items food frequency questionnaire (FFQ), representative of the Uruguayan diet with a focus on the last 5 years' food consumption habits. Experts accept this 5-year period [33] which has been already used by the research group in Uruguay since 1994 [34-36]. The FFQ was tested for reproducibility with good results [35]. Dietary questions were open-ended and local food composition tables [37] were used to estimate energy, water, and nutrient intake. Eight items evaluated smoking habit: smoking status (no smoker, ex-smoker, current smoker), amount (number of cigarettes/day), type (blond, mixed, black), rolling (manufactured, hand-rolled), age at start, age at quit, duration (age at quit – the age at the start), and intensity (pack-years, = the product of calculated packs of 20 units smoked per day × smoking duration in years). Patients who reported quitting within the same year of their interview were considered current smokers.

Dietary Assessment

The individual dietary energy/day was calculated through a compiled analysis program using the following formula:

Equation 1:

$$\text{Daily Energy} = \frac{(\text{number of servings per year}) \times \left(\frac{\text{calories of each food serving}}{100\text{g}}\right)}{365}$$

The program made the sum of all individual values to obtain the total energy value.

Regarding the daily intake of nutrients, the same procedure was applied, as follows:

Equation 2:

$$\text{Daily Nutrient} = \frac{(\text{number of servings per year}) \times \left(\frac{\text{nutrient content of each serving}}{100\text{g}}\right)}{365}$$

The nutrient content was in milligrams or grams per 100 g of serving, depending on each case.

Most average servings of solid foods are within 100-150 g. Since iron intake was highly correlated with dietary energy an iron density was calculated as daily mg of the mineral/kcal×1000. Since the cooking method and doneness of meats were not available

at the time of the study design, iron estimations were made irrespective of these data. Heme iron intake was estimated using the FFQ, considering its percentage of total iron in available foods according to the previous dietary studies [38-40]. The average daily heme iron intake was calculated by multiplying consumption frequency by total iron and accepted fractions. Non-heme iron intake was calculated by subtracting heme intake from total iron.

Another hallmark of the present study is the assessment of mate intake, a hot aqueous infusion made from the herb Ilex paraguariensis. Mate is a staple in temperate South America, and Uruguayans are the world's highest "mate" consumers: ~85% of the population has the habit (approx. 9-10 kg/person/year of the herb and approx. 400 liters/person/year of infusion) [41]. According to the International Agency for Research on Cancer (IARC) [42], hot "mate" drinking has been considered as a 2A agent (a possible carcinogenic for humans) because of the presence of polycyclic aromatic hydrocarbons (PAH) [43, 44]. Recently, iron, "mate", and water intakes were reported positively associated with BC risk. High "mate" intake derived an adjusted OR=2.81 (95%CI 1.77-4.36) [45].

Estimation of Dietary Acid Load

Two widely established formulas were used to calculate dietary acid load, according to the previously published data [46, 47]. Potential renal acid load (PRAL) of diet was calculated as follows:

Equation 3:

$$\text{PRAL}\left(\frac{\text{mEq}}{\text{day}}\right)=(0.49\times\text{total protein [g/day]})+(0.037\times\text{phosphorus}[\frac{\text{g}}{\text{day}}])-(0.021\times\text{potassium}[\frac{\text{mg}}{\text{day}}])-(0.026\times\text{magnesium}[\frac{\text{mg}}{\text{day}}])-(0.013\times\text{calcium}[(0.013\times\text{calcium}[\frac{\text{mg}}{\text{day}}])])$$

As mentioned above, this score included intestinal absorption rates for the following macro and micronutrients: protein, potassium, phosphate, magnesium, and calcium. Remer and Manz [47] validated PRAL scores versus urinary pH in healthy individuals with good results. Net endogenous acid production (NEAP) was calculated as follows:

Equation 4:

$$\text{NEAP}\left(\frac{\text{mEq}}{\text{day}}\right)=\left(\frac{54.5\times\text{protein}(\frac{\text{g}}{\text{day}})}{0.0256\times\text{potassium}(\frac{\text{mg}}{\text{day}})}\right)-10.2$$

The NEAP score considers sulfuric acid production due to protein metabolism and the rate of bicarbonate production after the metabolization of intestinally absorbed potassium salts of organic acids [46]. In previous studies, both scores were strongly correlated ($r=0.84$, $p<0.001$). A positive NEAP or PRAL score reflects an acid-forming potential, whereas negative scores indicate an alkaline-forming potential. Both scores were previously used to investigate the relationship between cancer risk and dietary acid load [20, 21, 23].

Statistical Analysis

In statistical analyses, the questionnaire variables were usually treated as continuous variables. Categorization was done for analysis purposes. Together with basic descriptive analyses (frequencies, mean values, and chi-square tests), we calculated odds ratios (ORs) and 95% confidence intervals (95% CI) by unconditional logistic regression [48]. Terms for potential observable confounders were included in the multivariate analyses. Most equations included age, urban/rural residence, education, body mass index (BMI), family history of cancer, smoking intensity, alcohol status, and intakes for total energy, fiber, heme iron, tea, food-derived water, and "mate". Since water intake might be associated with BC risk, and we have already shown its role among Uruguayan people [45], it was mandatory to include at least one variable related to it. Mate and tea infusions and a calculated food water intake were part of the employed regression models. No participants were excluded as outliers for any dietary component. Heterogeneities in the stratified analyses were explored through likelihood-ratio tests. The analyses were done using STATA software (Release 10, Stata Corp LP, College Station, TX, 2007).

RESULTS

Table 1 shows the baseline features of participants. The matching design was reflected in the lack of differences regarding age and sex. Although cases and controls had somewhat similar education levels and BMI, most cases belonged to rural areas (24.3% vs. 16.1%, respectively). Additionally, a family history of cancer was significantly higher among cases (40% vs. 24.3%, respectively, $P<0.001$). Smoking status and intensity were significantly higher among cases ($P=0.001$ and $P<0.001$, respectively).

Table 1: Baseline Characteristics of Cases and Controls

	Controls, No.(%) (n=510)	Cases, No.(%) (n=255) %	P Value
Age Groups			0.57
≤63	178 (34.9)	86 (33.7)	
64-71	171 (33.5)	79(31.0)	
≥72	161 (31.6)	90 (35.3)	
Sex			1.00
Men	450 (88.2)	225 (88.2)	
Women	60 (11.8)	30 (11.8)	
Education, y			0.47
≤3	234 (45.9)	113 (44.3)	
4-6	225 (44.1)	109 (42.8)	
≥7	51 (10.0)	33 (12.9)	
Urban/Rural Status			<0.01
Urban	428 (83.9)	193 (75.7)	
Rural	82 (16.1)	62 (24.3)	
Residence Regions			0.05
Montevideo	271 (53.1)	116 (45.5)	
Other Counties	239 (46.9)	139 (54.5)	
Body Mass Index (kg/m²)			0.85
≤24.99	237 (46.5)	123 (48.2)	
25.0-29.99	217 (42.5)	103 (40.4)	
≥30.0	56 (11.0)	29 (11.4)	
Family History of Cancer in 1st and 2nd-Degree Relatives			<0.001
No	386 (75.7)	153 (60.0)	
1	98 (19.2)	80 (31.4)	
>1	26 (5.1)	22 (8.6)	
Smoking Status			0.001
Never	164 (32.2)	49 (19.2)	
Ex-Smoker	123 (24.1)	80 (31.4)	
Current	223 (43.7)	126 (49.4)	
Smoking Intensity (pack-years)			<0.001
Non-Smoker	164 (32.2)	49 (19.2)	
0.1-39.9	189 (37.1)	90 (35.3)	
≥40	157 (30.8)	116 (45.5)	

The dietary features of the studied population with their crude ORs are presented in Table 2. Intakes of tea (OR=1.74 [1.12-2.69]), "mate" (OR=2.50 [1.66-3.75]), and food-derived water (OR=1.87 [1.29-2.72]) were positively and significantly associated with BC risk. Besides, total energy intake (OR=0.54 [0.37-0.78]) was inversely associated with BC risk. Finally, red meat, processed meat, plant foods, coffee, and alcohol had no significant associations with BC risk.

The dietary iron intake of participants is shown in Table 3. Accordingly, the categories created in tertiles that are made from the overall sample and the mean±SD energy-adjusted intakes (mg/1000 kcal/d) are compared between cases and controls. Total and animal-based iron showed no differences (P=0.51 and 0.24, respectively). On the other hand, differences for plant-based, heme, and non-heme iron were in borderline values (P values between 0.08 and 0.09).

Table 4 shows the outcomes yielded by the regression models (crude and adjusted) for exposure to acid load. Globally considered, the highest acid loads are associated with a doubled risk of BC; compared to the reference tertiles of both scores. These ORs were obtained only through the adjusted models, and the linear trends were also significant for both PRAL and NEAP scores.

Table 2: Dietary Features of Participants (n=765)[a]

	Controls, No.(%) (n=510)	Cases, No.(%) (n=255)	P Value	OR (95% CI)
Tea Status			0.01	1.74 (1.12-2.69)
Never	458 (89.8)	213 (83.5)		
Ever Drinker	52 (10.2)	42 (16.5)		
Mate Intake, liters/d			<0.001	2.50 (1.66-3.75)
≤0.99	166 (32.6)	49 (19.2)		
1.00	203 (39.8)	102 (40.0)		
≥1.01	141 (27.6)	104 (40.8)		
Coffee Status			0.28	1.27 (0.82-1.98)
Never	450 (88.2)	218 (85.5)		
Ever Drinker	60 (11.8)	37 (14.5)		
Alcohol Status			0.14	1.26 (0.93-1.71)
Never	237 (46.5)	104 (40.8)		
Ever Drinker	273 (53.5)	151 (59.2)		
Red Meat Intake, serve/y			0.49	0.82 (0.57-1.19)
≤313	167 (32.7)	89 (34.9)		
314-390	167 (32.7)	89 (34.9)		
≥391	176 (34.5)	77 (30.2)		
Processed Meat, serve/y			0.25	0.79 (0.55-1.14)
≤113	160 (31.4)	95 (37.2)		
114-259	178 (34.9)	79 (31.0)		
≥260	172 (33.7)	81 (31.8)		
Plant Foods, serve/y			0.26	1.32 (0.92-1.91)
≤367	176 (34.5)	79 (31.0)		
368-689	174 (34.1)	81 (31.8)		
≥690	160 (31.4)	95 (37.2)		
Water From Foods, ml/1000 kcal/d			0.004	1.87 (1.29-2.72)
≤319	188 (36.9)	69 (27.1)		
320-400	172 (33.7)	83 (32.5)		
≥401	150 (29.4)	103 (40.4)		
Energy, kcal/d			0.005	0.54 (0.37-0.78)
≤1881	154 (30.2)	101 (39.6)		
1882-2394	166 (32.6)	87 (34.1)		
≥2395	190 (37.2)	67 (26.3)		

[a] Distribution of cases and controls, crude odds ratios, and 95% confidence intervals.

Table 3: Comparison of Dietary Iron Intakes of Cases and Controls

	Tertiles of Intake, mg/1000 kcal/d			Controls, mean±SD[a]	Cases, mean±SD[a]	P Value
	Low	Middle	High			
Animal-Based	≤3.02	3.03-3.92	≥3.93	3.52±1.04	3.62±1.20	0.24
Plant-Based	≤3.87	3.88-4.75	≥4.76	4.49±1.28	4.32±1.26	0.08
Heme	≤1.71	1.72-2.26	≥2.27	1.99±0.66	2.09±0.79	0.08
Non-Heme	≤5.36	5.37-6.27	≥6.28	6.01±1.26	5.85±1.26	0.09
Total	≤7.35	7.36-8.44	≥8.44	8.01±1.38	7.94±1.46	0.51

[a] Mean energy-adjusted intakes (mg/1000 kcal/d)±standard deviation (SD)

Table 4: Crude and Adjusted Odds Ratios of Bladder Cancer for Dietary Acid Load Scores and Their 95% Confidence Intervals[a, b]

	Exposure Levels of Acid Load, OR (95% CI)			Continuous, OR (95% CI)	Trend (P Value)
	I	II	III		
PRAL	≤0.66[c]	0.67–9.04[c]	≥9.05[c]		
Crude	1.00 (—–)	1.50 (1.04–2.17)	1.03 (0.71–1.51)	1.01 (0.99-1.02)	0.37
Adjusted	1.00 (—–)	1.72[c] (1.12–2.62)	1.74[c] (1.08–2.82)	1.03[c] (1.01-1.05)	0.002[c]
NEAP	≤45.17[c]	45.18–61.40[c]	≥61.41[c]		
Crude	1.00 (—–)	1.63 (1.12–2.36)	1.33 (0.91–1.94)	1.01 (0.99-1.01)	0.17
Adjusted	1.00 (—–)	2.10[c] (1.38–3.20)	1.83[c] (1.15–2.89)	1.01[c] (1.00-1.02)	0.02[c]

[a] Abbreviations: NEAP, net endogenous acid production; PRAL, potential renal acid load
[b] P values for linear trend tests are also calculated.
[c] Data are statistically significant

Table 5: Pearson Correlation Coefficients (r) of Selected Study Variables[a, b]

	Cancer (n/y)	PRAL Score	NEAP Score	Food-Based Water	Total Water	Heme Iron	Non-Heme Iron
Cancer (n/y)	1.00						
PRAL Score	0.03	1.00					
NEAP Score	0.05	0.81[c]	1.00				
Food-Based Water	0.11[c]	-0.41[c]	-0.10[c]	1.00			
Total Water	0.13[c]	-0.40[c]	-0.18[c]	0.80[c]	1.00		
Heme Iron	0.06	0.49[c]	0.34[c]	-0.20[c]	-0.18[c]	1.00	
Non-Heme Iron	-0.06	-0.48[c]	-0.55[c]	0.15[c]	0.17[c]	-0.07	1.00
Smoking Intensity	0.20[c]	0.11[c]	0.09[c]	-0.07[c]	-0.04	0.07[c]	-0.08[c]

[a] Abbreviations: NEAP, net endogenous acid production; PRAL, potential renal acid load
[b] Water and iron variables are adjusted by dietary 1000kcal/day.
[c] Significant correlation (P<0.05)

Table 5 displays the Pearson's correlation coefficients among selected variables interacting with PRAL and NEAP scores. The food-based water adjusted by energy is based mainly on plant foods since animal-based foods include limited amounts of fluid (data not shown). Food-based water is negatively correlated with heme iron (from animal sources, r=-0.200). So, the negative correlations between PRAL and water (r=-0.415 and r=-0.402) and between NEAP and water (r=-0.104 and r=-0.176) reflect that the more water from plant foods patients receive, the less PRAL or NEAP will be. Finally, smoking intensity showed a positive correlation with PRAL and NEAP scores, food-derived water, and iron intake.

DISCUSSION

This study explored whether a high dietary acid load was associated with an increased risk of BC in a Uruguayan population. Our results demonstrated that higher acid load scores (both NEAP and PRAL) may significantly increase the risk of BC. The adjusted ORs and CIs (95% CI) were respectively, 1.74 (1.08-2.82, P(trend)=0.002) for PRAL, and 1.83 (1.08-2.82, P(trend)=0.02) for NEAP; comparing highest vs. lowest tertile of exposure. The association between a high dietary acid load and general cancer risk is a rapidly emerging area of current epidemiological interest. Various cancer types, including colorectum [19, 20], pancreas [18], lung [21], breast [22, 23], prostate [23], and brain tumors [25] have been recently associated with a high dietary acid load. Regarding the previously published studies performed in the Uruguayan population [19, 21, 23], most of the estimates shown in the highest tertiles/quartiles were within the 1.5 to 2.5 range, consistent with the outcomes achieved in the present study. Despite minor differences among these studies, the best regression models kept remarkable similarities between them. Besides, the only research that did not show significant risk associations was performed on kidney cancer [26] that demanded a rationale to explain such outcomes. The other cited case-control studies showed consistent results for the highest tertiles/quartiles [18, 20, 22, 25]. In addition, studies on

gliomas [25] and colorectum [20] yielded adjusted ORs for the highest vs. the lowest tertiles (OR=1.66 and OR=4.82, respectively). The cohort studies on American people applied a different methodology (Cox proportional hazard regression) and showed HR=1.20 and HR=1.73 for breast [22] and pancreatic [18] cancer risk, respectively.

The modern Western-type diet contains excessive animal products and high amounts of fat, protein, and sodium chloride, whereas it is deficient in fruits and vegetables [49, 50]. This shortage of fruits, vegetables, and legumes may not compensate for the high dietary acid load induced by meat, cheese, and other animal products [49]. The composition of the diet can strongly affect acid-base balance [49], and the PRAL and NEAP values in our examined sample indicate a low intake of plant foods and an excessive intake of animal-based foods in the examined Uruguayan population. Such a dietary pattern has been associated with an increased risk of BC [36]. A recent meta-analysis [7] found a protective effect of a Mediterranean pattern as opposed to the detrimental effects of a Western one. Di Maso et al. [51] reported related findings and highlighted that water obtained from vegetable sources was significantly associated with a reduced BC risk. The recently published BLEND trial results confirmed the negative association between high consumption of legumes and vegetables with the BC risk which is by other previous findings [52-54]. Dietary intake is an important environmental factor that may drive the development or maintenance of cancer [55]. In the current study, we found a positive correlation between smoking intensity and the dietary acid scores (PRAL and NEAP); however, after stratified analyses (data not shown), this was not reflected as an effect modification for higher smoking strata, compared to non-smokers. Nevertheless, two epidemiological studies which measured urinary pH reported controversial results: On the one hand, a Japanese case-control study in male smokers showed no apparent association with urinary acid pH and BC risk [56]. In addition, the authors did not specify if risk estimates were adjusted for potentially important confounding variables. On the other hand, a Spanish case-control study reported preliminary results, suggesting that individuals with consecutive urine pH under 6.0 had an increased disease risk [57]. Their results suggested that urine pH is primarily determined by diet and body surface area and may be an important modifier of smoking and BC risk, while our findings do not support these results. Nevertheless, the authors admitted that urinary pH may have been directly or indirectly influenced by the disease itself or its treatment, representing a limitation for their results. Similarly, we believe that there is a complex, multifactorial interplay between BC, urinary pH, dietary acid load, and smoking which raises the need for additional research to disentangle.

To the best of our knowledge, no translational studies found a direct mechanistic link between diet-induced acidosis and the development of BC. Nevertheless, there is accumulating evidence that a chronic (unnoticed) state of low-grade metabolic acidosis may lead to detrimental metabolic alterations and adverse clinical outcomes [49, 55, 58]. More specifically, a high dietary acid load may contribute to an increased risk of diabetes, hypertension, and obesity [59-61]. These three conditions are in turn associated with an increased risk of BC [62-64]. Higher concentrations of plasma carotenoids (exerting anti-inflammatory effects) may reduce the risk of urothelial cell carcinoma [65]. In contrast, pro-inflammatory dietary patterns were associated with an increased risk of BC [66]. Systemic inflammation in humans has been repeatedly associated with an increased intake of red and processed meats [67]. These meat types have, in turn, been associated with an increased BC risk [68, 69].

The present study has several limitations and strengths that warrant further discussion. First, selection bias is a common problem in epidemiological investigations, although we have tried to reduce this bias by selecting age-matched controls and cases. Moreover, we may not exclude a certain degree of recall bias that is common in case-control studies, whereas interviewer bias is less likely to have occurred. Cases were drawn from a large cohort (also investigating other cancers and their association with environmental factors), and the involved interviewers were unaware of the study's objectives. The sample size regarding the female subset was small which could be considered as a limitation for specific analyses. Although it would have been desirable to have larger numbers, BC cases among women were not as frequent as among men. Another limitation is the lack of urine

analyses. A high dietary acid load may influence urine pH values that could not be investigated due to missing data. Potential confounders such as occupational or home exposure to pollution (e.g., toxic chemicals) and smoking, were not assessed. Finally, the employed FFQ has not been validated due to external factors; yet it showed reproducibility satisfactorily in other studies [35].

As for the strengths, the interviews were done face-to-face by the same interviewers at the same hospitals. We performed data collection in the same period. Moreover, our sample is characterized by a low attrition rate and limiting potential selection bias. Another strength of our study is the dietary assessment, adequately representing the Uruguayan diet. Although data collection in the present report was performed more than a decade ago, a recent study by another Uruguayan team revealed that not much has changed concerning meat consumption [70].

In conclusion, both calculated NEAP and PRAL scores were found as directly and significantly associated with BC risk, in both cases supported by adjusted regression models. The associations of acid load scores are directly correlated with meat intake and inversely with plant foods intake. Our results suggest that an acidogenic dietary style featuring the studied population subset could contribute to the BC risk. This risk might involve a complex interplay among acid scores, iron intake, and water intake. To the best of our knowledge, the present study is the first Latin American epidemiologic case-control study analyzing associations of dietary acid load and BC risk. Further investigations are warranted to confirm our findings.

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None declared.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

Each hospital director has allowed the project after receiving approval from the respective Ethical Committee. An auto-generated number was built to preserve anonymity; based on first, last name, and ID number. In Uruguay, until year 2005 it was not necessary to comply with any other requirements.

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