World Cancer Research Journal WCRJ 2020; 7: e1685

DIETARY IRON, WATER INTAKE AND RISK OF URINARY BLADDER CANCER: A CASE-CONTROL STUDY

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Abstract – *Objective:* *Urinary bladder cancer (UBC) incidence and mortality in Uruguay show the highest rates in Latin America. Epidemiological research shows that iron and fluid intake have been inconsistently related to UBC risk regarding nutritional items. The present study was conducted to explore dietary iron and total water consumption on the incidence of UBC in the Uruguayan population since its intake of meat and "mate" infusion is the highest worldwide..*

Patients and Methods: *A case-control study was performed using a specific multi-topic questionnaire, including a food frequency questionnaire. The sample included 255 UBC incident cases and 510 controls (675 men and 90 women). Controls were matched by sex and age (± 5 years) to cases. Food-derived water was calculated from available databases. Dietary iron was calculated according to its heme or non-heme source, adjusted by energy. Odds Ratios (ORs) were calculated through unconditional logistic regression, adjusting for potential confounders. Animal/plant and heme/non-heme (H/NH) ratios were created for analysis purposes.*

Results: *Total iron, plant-based, and non-heme-iron intake were inversely associated with UBC risk (OR=0.69, 0.43, and 0.54, respectively, for 3rd vs. 1st tertile). Animal-based iron lacked risk association (OR=1.06). Heme-iron risk showed a significant linear trend (p=0.04). The Animal/Plant and H/NH iron ratios were directly associated (OR=2.10 and 2.33, respectively). High water intake displayed a risk increase (OR=2.33), even higher for rural residents than urban ones (OR=5.98* vs. *OR=1.97, respectively).*

Conclusions: *The present study gives evidence of a role for dietary iron and the intake of water in the UBC risk. Regarding iron intake, it showed different associations with UBC risk according to its source.*

KEYWORDS: *Bladder cancer, Chelation, chlorine, Heme, Ilex paraguariensis, Iron, Nonheme.*

INTRODUCTION

Urinary bladder cancer (UBC) is one of the most common malignancies and a significant threat to public health worldwide[1]. According to international statistics, Uruguay has the highest UBC age-adjusted incidence rate of Latin America for males (18.2/10^5 men) and the 5th place for females (2.5/10^5 women). Among men, Uruguayan incidence and mortality rates are in the highest quin-

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tile at a world level[2]. National figures show that UBC is the 4th more frequent cancer among males (after prostate, lung, and colorectum) and the 7th most frequent when both sexes are analyzed together[3], somewhat similar to societies with high human development index[1]. The most common risk factors for UBC include tobacco smoke, and occupational and environmental carcinogens [4].

Fluid intake has been inconsistently related to UBC risk[5,6]; also, infusions intake results remain controversial[7-13]. Nevertheless, "mate" drinking is a remarkable collective and individual source of water in temperate South America. "Mate" is the name of a hot aqueous infusion, made from the herb *Ilex paraguariensis* and it is a staple, non-alcoholic beverage. Still, Uruguayans are the world's highest "mate" consumers: ~85% of the population has the habit, which means 9-10 kg/person/year of the herb and ca. 400 liters/person/year of infusion[14]. Hot "mate" drinking has been considered as a 2A agent (a possible carcinogenic for humans) according to the International Agency for Research on Cancer (IARC)[13], because of the presence of polycyclic aromatic hydrocarbons (PAH)[15,16]. It must be remarked that inhabitants do not prepare the infusion with bottled water (whose content of arsenic and other metals is still unknown), but with tap water or from other sources.

A significant fraction of the water samples collected in Uruguay to estimate arsenic concentration are above the limit of 10 µg L−1 recommended by the WHO for drinking water[17]. Rural homes and small towns do not have a water supply from the state company in charge of[18]. In these zones, people drink water from their wells, most of which are improperly controlled, making up a significant health risk[19]. Inorganic arsenic compounds are classified by the IARC as Group 1 compounds (carcinogenic to humans)[20]. Also, hundreds of by-products –most of them trihalomethanes (THM)- are produced because of using chlorine to disinfect water for human consumption[21], as is done in Uruguay[22]. High levels of intake of THM in tap water were associated with increased UBC risk[23]. Chlorine compounds increase the corrosion of metallic distribution systems since they are strong oxidizers, releasing dissolved iron into the water[24]. Iron can be naturally present in freshwater not exceeding 50 mg/liter, and in soluble forms as ferrous ions (Fe2+) or complexed forms like the ferric ion (Fe3+ as Fe(OH)3)[25]; however, the WHO has set a guideline value of 0.3 mg/liter of iron in drinking water[26].

Regarding iron intake and UBC risk, the evidence is inconsistent, tending to null associations. A Spanish case-control study reported a slight decrease for iron, which disappeared after adjustment for saturated fat[27]. More recent studies do not support an effect of dietary iron on UBC risk[28-31]. Although iron -a component of red and processed meat- is essential for many biological processes, it is related to the carcinogenicity found in those foods[32]. The contributory role of iron in cancers could be mediated by overproduction of Reactive Oxygen Species and free radicals through Fenton reaction (Fe2+ oxidized to Fe3+) and participating in inflammation and DNA synthesis[33]. A Western diet -high in meat, fat, sugar, and with ~15 mg/day of iron, might be epidemiologically linked to the increased development of tumors in humans[34]. Both heme (in animal foods) and non-heme (in plant foods, also in meat) dietary iron are mostly present as Fe3+ (oxidized state) [33]. Heme-iron involves 2/3 of the average individual iron intake in developed countries[35]. The average Uruguayan diet is meat-based, with the world's highest per capita beef intake[36].

A series of epidemiological studies analyzed UBC among the Uruguayan population, focusing on foods[37], non-alcoholic beverages[38], dietary patterns[39], and meat and animal products[40]. We have recently evaluated dietary iron according to its sources and subtypes, and its risk associations with breast[41,42], lung[43], and colorectal [44] cancers, giving epidemiologic evidence of an animal/plant ratio and a heme/non-heme ratio of dietary iron in support of a direct association to the disease.

Taking into account: a) the high iron intake estimated for the population, related to the prevailing Western-like dietary pattern[40,45]; b) three items associated to water intake, as follows: b1) the recent findings of dangerous high arsenic levels in local aquifers[17,19,46]; b2) the disinfection by-products and derived iron compounds, which are present in drinking water[22]; and b3) a high intake of "mate" infusion and its PAH contents[13,16], we conducted a case-control study to analyze potential roles of dietary iron and water intake in the UBC risk, applying a similar methodology to previous studies[41-44]. To our knowledge, this is the first Latin American epidemiologic case-control study focusing on dietary iron sources, water intake, and UBC risk.

PATIENTS AND METHODS

Selection of Cases and Controls

As part of a multi-site epidemiologic research study (1996-2004), all newly diagnosed and microscopically confirmed cases of transitional cell carcinoma of the urinary bladder in the Uruguayan population were considered eligible for this study. These cases were drawn from the four major public hospitals of Montevideo and Uruguay (Clinicas University, Maciel, Pasteur, and Oncol-

ogy Institute), which catch a significant fraction of patients from the public system for diagnosis and/or treatment of cancer. The public health system is centralized in Montevideo, where less than 50% of the country's population lives, while more than 50% of total cancer cases are diagnosed[3].

Each hospital Director has authorized the project after receiving the approval from the respective Ethical Committee. In past years, only an oral consent was required from the patients, assuming the confidentiality about their data. An auto-generated number was built, based on initials (first and last name + ID number), in order to preserve the anonymity.

A total of 261 cases were approached for a possible interview and six patients refused, leaving 255 cases for inclusion in the study (response rate 97.7%). At the same time and the same hospitals, all patients afflicted with non-neoplastic conditions not related to smoking and alcohol drinking were considered eligible for the study as control subjects. A total of 527 potential controls were approached for a possible interview, and 17 patients refused, leaving a final total of 510 controls, which were included in the study (response rate 96.8%). These controls presented the following diseases: eye disorders (132 patients, 25.7%), abdominal hernia (114, 22.4%), fractures (52, 10.2%), injuries (45, 8.9%), skin diseases (40, 7.8%), acute appendicitis (37, 7.2%), varicose veins (29, 5.7%), hydatid cyst (20, 4.0%), blood disorders (18, 3.5%), prostate hypertrophy (14, 2.8%), and bone diseases (9, 1.7%). Patients admitted to public hospitals were low income people from all around the country who had free access to most medical services, mandatory law in Uruguay. According to the population's features, they were good representatives of a third world country, different from the population admitted at the private health subsystem.

Interviews and questionnaire

Two trained social workers, unaware of the study objectives, worked at the hospitals in two phases: First, they looked for newly diagnosed cancer patients, working with the collaboration of Medical Records personnel. Second, they contacted patients who were eligible to be matched by the age-frequencies of the cases. Patients' participation was voluntary and without a remuneration. After giving their consent to cooperate with the study, all participants were face-to-face interviewed in the hospitals. Proxy interviews were not accepted in our study. Participants answered a structured questionnaire that included socio-demographic variables, occupation, cancer history in relatives of 1st-2nd degree, and self-reported height and weight five years before the interview. A history of "mate," smoking and alcohol, tea and coffee drinking, and a food frequency questionnaire (FFQ) of 64 items, representative of the Uruguayan diet, focused on food consumption five years before the interview. The FFQ was not validated, even though it was tested for reproducibility [47], allowing individual energy estimation. All dietary questions were open-ended. Eight items evaluated smoking habit: smoking status (No smoker, Ex-, Current), amount (Nº of cigarettes/day), type (blond, mixed, black), rolling (manufactured, hand-rolled), age at start, age at quit, duration (age at quit - age at 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 as current smokers.

Dietary assessment

An analysis program was compiled to calculate energy, which made the sum of all individual values, each one obtained after multiplying the number of servings/year by the ratio calories of the serving/100 g of each, divided by 365 days. The same calculation applied for nutrients and iron: the program made the sum of all individual values, each obtained after multiplying the number of servings/year by the ratio milligrams (or grams) of the serving/100 g of each, divided by 365 days. Most typical or average servings of solid foods are within the range of 100-150 g. Since iron intake showed a high correlation with energy, an iron density was calculated as daily mg of the mineral/kcal*1000. Local tables of food composition were used for estimating energy, water, and nutrients [48]. Estimations of iron were made irrespective of the cooking method and doneness of meats since such accurate data were not available at the time of the study design. Heme iron intake was estimated using our FFQ and following previous dietary studies [41-44], taking into account its percentage of total iron in the following foods: 69% for beef, 39% for ham, bacon, mortadella, salami, hot dogs, saucisson and sausage, 26% for chicken, 21% for liver, and 26% for fish, eggs, and milk. The mean daily heme iron intake was calculated by multiplying consumption frequency by amount of total iron and the quoted percentages. Non-heme iron intake was calculated subtracting heme iron intake from total iron. For analysis purposes and based on the original iron variables (animal iron, plant iron, heme iron, non-heme iron), an Animal/Plant iron ratio (APIR), and a Heme/Non-Heme ratio (H/NH) were created.

Statistical analysis

In statistical analyses, the variables in the questionnaire were mostly treated as continuous variables. When necessary, they were categorized for analysis purposes. Apart from basic descriptive analyses (frequencies, mean values, chi-square tests), we calculated Odds Ratios (ORs) and 95% confidence intervals (95% CI) by unconditional logistic regression [49]. Terms for potential confounders were included in the multivariate analyses. Most equations included age, urban/rural residence, education, Body Mass Index (BMI), family history of cancer, smoking status, alcohol status, and intakes for total energy, red meat, processed meat, plant foods (vegetables + fruits + legumes), tea, "mate", and coffee. No participants were excluded as outliers for iron or other dietary components. Heterogeneities in the stratified analyses were explored through likelihood-ratio tests. All analyses were conducted using STATA software (Release 10, Stata Corp LP, College Station, TX, USA).

RESULTS

The baseline characteristics of cases and controls are shown in Table 1. Due to the matching design, no differences were expected to exist regarding age and sex. Although having both cases and controls somewhat similar education level and body mass index, cases tended to belong to rural areas more than controls (43.1% *vs.* 31.1%, respectively). A family history of cancer in first and 2nd-degree relatives was significantly higher among cases (<0.001 and 0.01, respectively). Cases were also significantly more smokers than controls (p=0.001), as well as they displayed higher intensity in the habit (p<0.001).

Features of food, nutrient, and energy consumption were analyzed and presented with their crude ORs in Table 2. Intakes of tea intake (OR=1.74), "mate" intake (OR=2.50), water from foods (OR=1.87), and eggs (OR=1.80) were positively, significantly associated with UBC risk. On the other hand, total energy intake (OR=0.54) was inversely and significantly associated with UBC risk. Finally, red meat, processed meat, plant foods, coffee, alcohol, polyunsaturated fatty acids, and cholesterol did not significantly associate with UBC risk.

Table 3 shows the dietary iron intake of participants. Firstly, the categories created in tertiles, made from the whole sample. Second, the mean crude and energy-adjusted intakes (mg/1000kcal/day) ± SD are presented, comparing cases and controls. Total iron showed no difference (p=0.18 and p=0.51, crude and energy-adjusted, respectively). Differences for plant-based, heme, non-

TABLE 1. Baseline characteristics of cases and controls.

Variables	*Categories*	*Controls (n=510)*	*%*	*Cases (n=255)*	*%*	*Global p-value*
Age groups	≤ 63	178	67.4	86	32.6	
	64-71	171	68.4	79	31.6	
	≥ 72	161	64.1	90	35.9	0.57
Sex	Men	450	66.7	225	33.3	
	Women	60	66.7	30	33.3	1.00
Education years	≤ 4	279	65.8	145	34.2	
	≥ 5	231	67.7	110	32.3	0.57
Urban/Rural status	Urban	428	68.9	193	31.1	
	Rural	82	56.7	62	43.1	0.01
Residence Regions	Montevideo	271	70.0	116	30.0	
	Other counties	239	63.2	139	36.8	0.05
Body Mass Index (kg/m^2)	≤ 24.99	237	65.8	123	34.2	
	25.0-29.99	217	67.8	103	32.2	
	≥ 30.0	56	65.9	29	34.1	0.85
FHC in 1st degree	No	396	70.7	164	29.3	
	Yes	114	55.6	91	44.4	<0.001
FHC in 2nd degree	No	495	67.6	237	32.4	
	Yes	15	45.5	18	54.5	0.01
Smoking status	Never	164	76.6	50	23.4	
	Ex smoker	123	60.6	80	39.4	
	Current	223	64.1	125	35.9	0.001
Smoking intensity (pack-years)	Non smoker	164	77.0	49	23.0	
	0.1-39.9	189	67.7	90	32.3	
	≥ 40	157	57.5	116	42.5	<0.001

Abbreviations: FHC = family history of cancer in 1st and 2nd degree relatives.

TABLE 2. Dietary features of participants (n=765). Distribution of cases and controls. Crude Odds Ratios and 95% Confidence intervals.

Variables	*Categories*	*Controls (n=510)*	*%*	*Cases (n=255)*	*%*	*Global p-value*	*OR*	*95% CI*
Tea status	Never	458	68.3	213	31.7			
	Ever drinker	52	55.3	42	44.7	0.01	1.74	1.12-2.69
'Mate' intake (liters/day)	≤ 0.99	166	77.2	49	22.8			
	1.00	203	66.6	102	33.4			
	≥ 1.01	141	57.5	104	42.5	<0.001	2.50	1.66-3.75
Coffee status	Never	451	67.4	218	32.6			
	Ever drinker	59	61.5	37	38.5	0.25	1.30	0.83-1.02
Alcohol status	Never	237	69.5	104	30.5			
	Ever drinker	273	64.4	151	35.6	0.14	1.26	0.93-1.71
Red meat intake (serv/year)	≤ 313	167	65.2	89	34.8			
	314-390	167	65.2	89	34.8			
	≥ 391	176	69.6	77	30.4	0.49	0.82	0.57-1.19
Processed meat (serv/year)	≤ 113	160	62.8	95	37.2			
	114-259	178	69.3	79	30.7			
	≥ 260	172	68.0	81	32.0	0.25	0.79	0.55-1.14
Eggs (units/year)	≤ 52	203	72.5	77	27.5			
	53-104	131	69.3	58	30.7			
	≥ 105	176	59.5	120	40.5	0.003	1.80	1.27-2.55
Plant foods (serv/year)	≤ 367	176	69.0	79	31.0			
	368-689	174	68.2	81	31.8			
	≥ 690	160	62.7	95	37.3	0.26	1.32	0.92-1.91
Cholesterol (mg/day)	≤ 379	172	66.7	86	33.3			
	380-523	179	71.0	73	29.0			
	≥ 524	159	62.3	96	37.7	0.12	1.21	0.84-1.73
PUFA (g/day)	≤ 8.73	162	63.8	92	36.2			
	8.74-12.25	168	65.6	88	34.4			
	≥ 12.26	180	70.6	75	29.4	0.24	0.73	0.51-1.06
Water from foods (ml/10^3 k/d)	≤319	188	73.2	69	26.8			
	320-400	172	67.4	83	32.6			
	≥401	150	59.3	103	40.7	0.004	1.87	1.29-2.72
Energy (kcal/day)	≤1881	154	60.4	101	39.6			
	1882-2394	166	65.6	87	34.4			
	≥ 2395	190	73.9	67	26.1	0.005	0.54	0.37-0.78

Abbreviations: PUFA = polyunsaturated fatty acids; serv = servings; ml/10^3 k/d = ml/1000 kcal/day.

heme iron, and APIR were close to statistical significance (*p*-values between 0.06 and 0.09). The only statistically significant difference was found for H/NH ratio (p=0.02).

Table 4 shows the Risk assessment of UBC concerning dietary iron and the 95% confidence intervals (95% CI): total, animal-based, plant-based, animal/plant (A/P) ratio, heme, non-heme, and H/NH ratio. *p*-values for linear trend tests were also calculated. Total iron intake tended to be inversely associated with UBC risk (adj. OR=0.69, pTrend=0.04), and this was supported by the inverse association of plant-based iron (adj. OR=0.43, pTrend=0.003), since animal-based iron was not associated (adj.OR=1.06, pTrend=0.22). The APIR was also significantly associated with UBC risk (adj.OR=2.10, pTrend = 0.007). Regarding iron types, heme iron displayed a non-significant risk estimate (adj.OR=1.13) with a significant trend (pTrend=0.04), and non-heme iron was inversely, strongly associated with UBC risk (adj.OR=0.54, pTrend=0.004). The H/NH ratio was also highly significant (adj.OR=2.33, pTrend=0.001). Except for animal and heme iron, the remaining iron variables experienced improvements in their risk estimations through the adjusted regression models.

Risk assessment of UBC concerning water intake in different locations is displayed in Table 5. The estimates tended to be similar for both adjusted regression models employed, with and without infusions. In the case of water from foods and beverages (milk, colas, fruit cocktail), the model using terms for infusions derived an OR for the 3rd *vs.* the 1st tertile, which was somewhat similar but with a significant trend (OR=1.92, pTrend<0.05). Regarding the total water, both regression models achieved similar risks (OR=2.34 and 2.33), including and excluding infusions, respectively). The stratified analyses by urban/rural status ex-

TABLE 3. Dietary iron intakes of participants. Left side: Tertiles of intake (mg/1000kcal/day). Right side: Mean energy-adjusted intakes (mg/1000 kcal/day) ± Standard Deviation (SD). Comparison of cases and controls. Not energy-adjusted iron intake is indicated as (crude) and expressed in mg/day.

	TERTILES OF INTAKE			*CONTROLS*	*CASES*	
Iron items	***Low***	***Mid***	***High***	***Mean ± SD***	***Mean ± SD***	***Diff. (p)***
Total (crude)	< 14.7	14.7 – 19.1	≥ 19.2	17.73 ± 5.61	17.11 ± 6.83	0.18
Total	< 7.36	7.36 – 8.44	≥ 8.45	8.01 ± 1.38	7.94 ± 1.46	0.51
Animal-based	< 3.03	3.03 – 3.92	≥ 3.93	3.52 ± 1.04	3.62 ± 1.20	0.24
Plant-based	< 3.88	3.88 – 4.75	≥ 4.76	4.49 ± 1.28	4.32 ± 1.26	0.08
APIR	< 0.64	0.64 – 0.97	≥ 0.98	0.87 ± 0.44	0.94 ± 0.55	0.06
Heme	< 1.72	1.72 – 2.26	≥ 2.27	1.99 ± 0.66	2.09 ± 0.79	0.08
Non-Heme	< 5.37	5.37 – 6.27	≥ 6.28	6.01 ± 1.26	5.85 ± 1.26	0.09
H/NH ratio	< 0.28	0.28 – 0.39	≥ 0.40	0.35 ± 0.14	0.37 ± 0.16	0.02

Abbreviations: APIR = Animal/Plant iron ratio; H/NH ratio = heme/non-heme iron ratio.

hibited consistency: ORs of water from food/beverages and total water tended to be higher among rural subjects than urban ones, independent of the regression model employed. Regarding water from foods/beverages, the estimated ORs were statistically nonsignificant, but estimates coming from total water were highly significant.

Table 6 shows the risk assessment of UBC for selected iron variables among participants. Besides, stratified analyses were done by "mate" intensity and water intake levels, whose sources were foods. H/NH ratio displayed its highest estimate in the high "mate" stratum (OR=3.29, pTrend= 0.02). A similar finding was observed

TABLE 4. Crude and Adjusted Odds Ratios (OR) of bladder cancer for dietary iron and their 95% confidence intervals (95% CI): total, animal-based, plant-based, animal/plant ratio (APIR), heme, non-heme, and heme/non-heme ratio (H/NH). p-values for linear trend tests were also calculated.

Tertiles of iron intake		***I***		***II***		***III***		
Iron types		***OR***	***95% CI***	***OR***	***95% CI***	***OR***	***95%CI***	***Trend (p)***
Total	**C**	1.00	---	0.69	0.48-1.01	0.96	0.67-1.37	0.51
	A	1.00	---	0.66	0.44-0.99	0.69	0.45-1.06	0.04
Animal	**C**	1.00	---	0.78	0.53-1.13	1.09	0.76-1.57	0.24
	A	1.00	---	0.76	0.48-1.22	1.06	0.61-1.84	0.22
Plant	**C**	1.00	---	0.83	0.58-1.19	0.61	0.42-0.88	0.08
	A	1.00	---	0.73	0.49-1.09	**0.43**	0.27-0.66	0.003
APIR	**C**	1.00	---	0.97	0.68-1.41	1.36	0.94-1.97	0.06
	A	1.00	---	1.20	0.77-1.87	**2.10**	1.26-3.52	0.007
Heme	**C**	1.00	---	0.69	0.47-1.00	1.17	0.82-1.68	0.08
	A	1.00	---	0.68	0.41-1.13	1.13	0.60-2.13	0.04
NonHeme	**C**	1.00	---	0.77	0.53-1.11	0.71	0.49-1.02	0.09
	A	1.00	---	0.70	0.47-1.05	**0.54**	0.35-0.82	0.004
H/NH ratio	**C**	1.00	---	0.87	0.60-1.28	1.42	0.98-2.04	0.02
	A	1.00	---	1.08	0.68-1.73	**2.33**	1.31-4.16	0.001

Regression model including terms for cancer (binary, as dependent variable), age (categorical), sex (binary), education years (categorical), urban/rural residence (categorical), family history of cancer in 1st and 2nd degree relatives (binary no/yes), body mass index (categorical), energy as kilocalories (categorical), smoking intensity (pack-years, continuous), alcohol status (categorical), total plant foods (vegetables+fruits+legumes)(continuous), tea intake (binary never/ever), "mate" intake intensity (liters*years, continuous), red meat (categorical), processed meat (categorical), and total heterocyclic amines (continuous) as independent variables.

Iron variables were calculated as mg/10^3 kcal/day = milligrams/1000 kilocalories per day.

Abbreviations: APIR = Animal/Plant iron ratio; H/NH ratio = Heme/Non-heme-iron ratio.

C=Crude OR A=Adjusted OR

Significant ORs appear in bold letter.

TABLE 5. Adjusted Odds Ratios (OR) of bladder cancer for water intake and their 95% confidence intervals (95% CI). Stratified analyses by Urban (n=621) or Rural (n=144) residence.

			Tertiles of water intake			
Water source	***Regr. Model***	***Urban/ rural***	***I***	***II***	***III***	
Foods and beverages			***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***
	I	**ALL**	1.00 ---	1.39 0.92-2.10	**1.87** 1.19-2.95	0.07
		Urban	1.00 ---	1.28 0.80-2.03	**1.70** 1.03-2.82	**0.04**
		Rural	1.00 ---	1.97 0.70-5.50	2.44 0.75-8.01	0.14
	II	**ALL**	1.00 ---	1.29 0.85-1.98	**1.92** 1.21-3.05	**0.049**
		Urban	1.00 ---	1.23 0.76-1.97	**1.80** 1.07-3.00	**0.03**
		Rural	1.00 ---	1.78 0.60-5.27	2.67 0.78-9.21	0.12
Total water			***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***
	I	**ALL**	1.00 ---	**1.84** 1.21-2.82	**2.34** 1.47-3.74	**0.03**
		Urban	1.00 ---	**1.76** 1.09-2.83	**2.08** 1.24-3.49	**0.006**
		Rural	1.00 ---	2.28 0.79-6.61	**4.08** 1.14-14.6	**0.03**
	II	**ALL**	1.00 ---	**1.73** 1.13-2.67	**2.33** 1.42-3.82	0.055
		Urban	1.00 ---	**1.64** 1.01-2.66	**1.97** 1.14-3.41	**0.02**
		Rural	1.00 ---	2.47 0.82-7.50	**5.98** 1.50-23.9	**0.01**

All regression models including terms for cancer (binary, as dependent variable), age (categorical), sex (binary), education years (categorical), urban/rural residence (categorical), family history of cancer in 1st and 2nd degree relatives (binary no/yes), body mass index (categorical), energy as kilocalories (categorical), smoking intensity (pack-years, continuous), alcohol status (categorical), total plant foods (vegetables+fruits+legumes) (continuous), red meat (categorical), processed meat (categorical), and total heterocyclic amines (continuous) as independent variables. Significant estimates in bold letter.

Model I = adjusted, excluding terms for infusions. Model II = adjusted, including terms for infusions: "mate" intake intensity (liters-years, continuous), tea intake (binary never/ever), coffee intake (binary never/ever).

Abbreviations: Regr. model = Regression model.

Significant ORs appear in bold letter.

for the highest APIR in the high "mate" stratum (OR=2.54, pTrend <0.05). The analyses performed by strata of water from foods showed similarities regarding the H/NH ratio: it displayed its highest estimate in the highest water stratum (OR=3.61, pTrend= 0.01). Nevertheless, the highest APIR showed a nonsignificant risk increase for high water intake (OR=1.59, pTrend = 0.32).

Finally, Figure 1 summarizes those ORs found for high intakes of iron, "mate" and water, just in global analyses and stratified ones. Eighteen estimates, derived from results already shown in previous Tables, were classified according to their values into inversely associated (significant OR<1), not associated, positively and moderately associated (significant OR>1 and <2.5), and positively and intensely associated (significant OR≥2.5). On the one hand, the individual iron variables (e.g., plant, non-heme, total, heme) tended to be inversely or not associated. On the other hand, the iron ratios, "mate" intake and water intake, tended to be highly, positively associated. Furthermore, these items displayed higher values for rural subjects. High "mate" intake was calculated as adjusted OR (=2.81, 95%CI 1.77-4.36) for analysis purposes (data shown in Table 2 is the *crude* OR=2.50).

DISCUSSION

According to our results, total iron, plant-based, and non-heme-iron intake were inversely associated with UBC risk (OR=0.69[not significant], 0.43 and 0.54, respectively, for 3rd *vs.* 1st tertile). Animal-based iron lacked risk association (OR=1.06), and heme-iron showed a significant linear trend (p=0.04), although its risk was not significant. The Animal/Plant and H/NH ratios were directly associated (OR=2.10 and 2.33, respectively), and both ratios displayed their highest estimates in the high "mate" stratum (OR=2.54 and 3.29, respectively). The H/NH ratio showed its highest estimate in the highest water stratum (OR=3.61).

TABLE 6. Adjusted Odds Ratios (OR) of bladder cancer for selected variables of iron intake and their 95% confidence intervals (95% CI). Stratified analyses by "mate" intensity and levels of water intake (source: foods and non-alcoholic beverages).

Tertiles of "Mate" intensity (liters-years)					
Iron Variables	***Low <45***	***Mid 45-67.9***	***High 68+***		***Continuous Odds Ratio***
H/NH	***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***	***OR 95% CI***
Low	1.00 ---	1.60 0.70-3.69	2.80 0.98-7.99	0.054	1.67 0.99-2.83
Mid	1.00 ---	0.80 0.34-1.88	**3.17** 1.05-9.55	0.052	1.71 0.99-2.94
High	1.00 ---	1.55 0.68-3.54	**3.29** 1.20-8.97	0.02	**1.84** 1.17-3.04
APIR	***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***	
Low	1.00 ---	0.91 0.40-2.06	1.87 0.72-4.82	0.19	1.37 0.85-2.21
Mid	1.00 ---	1.33 0.58-3.02	**3.13** 1.18-8.27	0.02	**1.78** 1.09-2.89
High	1.00 ---	1.89 0.89-4.04	**2.54** 1.02-6.30	0.046	**1.59** 1.01-2.49
Tertiles of water intake (ml/1000kcal/day)					
Iron Variables	***Low <320***	***Mid 320-400***	***High 401+***		***Continuous Odds Ratio***
H/NH	***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***	***OR 95% CI***
Low	1.00 ---	0.72 0.28-1.83	1.52 0.48-4.76	0.45	1.25 0.70-2.22
Mid	1.00 ---	0.99 0.43-2.27	2.17 0.78-6.08	0.12	1.51 0.90-2.52
High	1.00 ---	1.55 0.67-3.59	**3.61** 1.28-10.2	0.01	**1.92** 1.14-3.22
APIR	***OR 95% CI***	***OR 95% CI***	***OR 95% CI***	***Trend (p)***	
Low	1.00 ---	0.82 0.34-1.99	1.11 0.39-3.20	0.82	1.06 0.62-1.81
Mid	1.00 ---	1.09 0.50-2.40	2.40 0.96-6.01	0.06	1.55 0.98-2.46
High	1.00 ---	1.26 0.58-2.73	1.59 0.64-3.95	0.32	1.26 0.80-1.99

All regression models including terms for cancer (binary, as dependent variable), age (categorical), sex (binary), education years (categorical), urban/rural residence (categorical), family history of cancer in 1st and 2nd degree relatives (binary no/yes), body mass index (categorical), energy as kilocalories (categorical), smoking intensity (pack-years, continuous), alcohol status (categorical), total plant foods (vegetables+fruits+legumes)(continuous), red meat (categorical), processed meat (categorical), and total heterocyclic amines (continuous) as independent variables. Significant estimates in bold letter.

"Mate" intensity = liters-years.

Water intake = milliliters of water from foods+beverages/ 1000 kcal/day.

Significant ORs appear in bold letter.

Our findings are not entirely consistent with those from previous studies involving dietary iron and UBC risk[27-30]. Some similarities could be found regarding our animal-based or heme iron but having done an additional division according to source and subtype, we found that the relationship between these might shed some light on iron's role. On the one hand, our estimations of total iron for the studied population were very high (average ~17 mg/day), with an animal/plant ratio of ~0.9, which is also very high. The heme-iron derived from a high-meat dietary style is not biologically regulated and represents several health risks; however, we must emphasize the oxidative stress derived from the excess of this type of iron[34,50]. On the other hand, non-heme iron is the only hormonally and biochemically regulated in the human body. Heme-iron is absorbed ~30% and non-heme-iron ~10%, but the former is less stringently regulated[51].

Besides, non-heme iron has suggested a protective role regarding UBC risk, which we found in previous studies on other cancer sites [41-44]. We cannot assure their role for plant and non-heme iron, since it sounds reasonable to think that a general dietary profile makes the difference. Regardless, the regression model included terms for

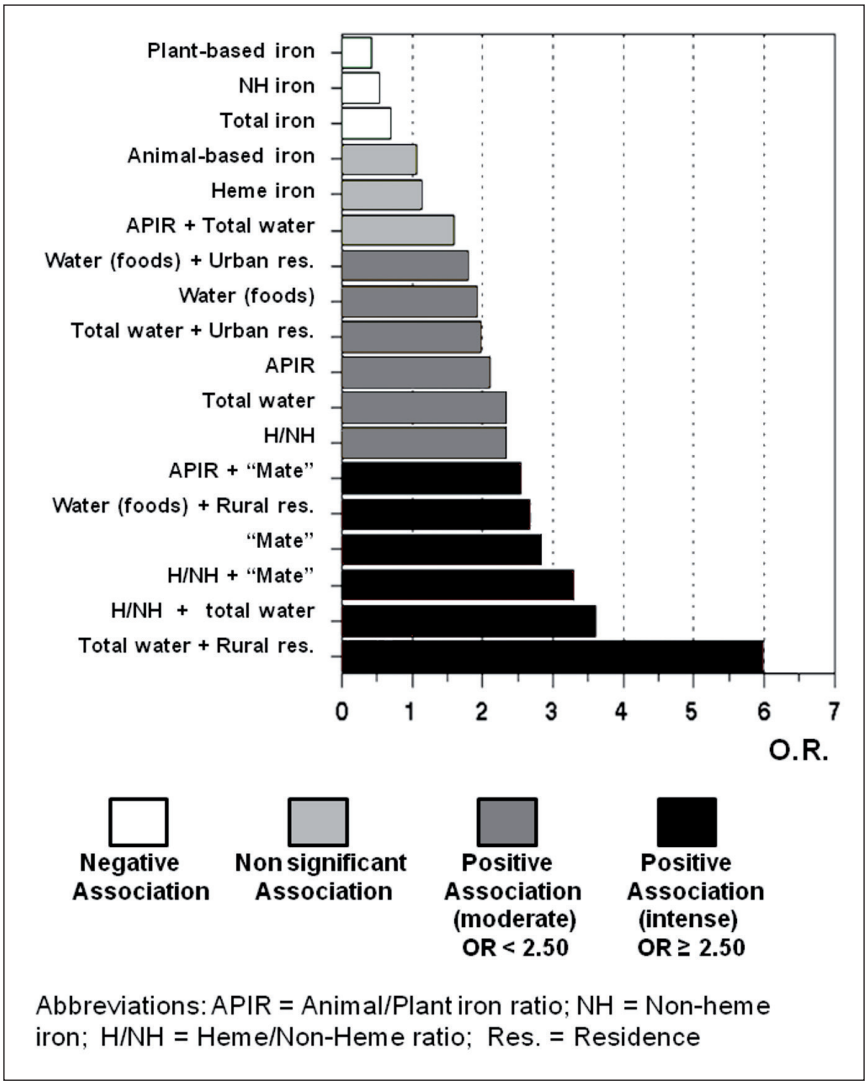

Fig. 1. Graphic expression of ORs corresponding to the highest tertiles of intakes reported in previous Tables.

vegetables, fruits, and legumes, as an attempt to separate the effects of the iron types mentioned above from its source foods. The H/NH ratio findings perhaps reflect the potential roles of each iron type and the need for not trespassing a regulation threshold. Therefore, we can accept some likelihood for real effects, despite possible confounding from other dietary components.

We have found that high water intake displayed a risk increase (OR=2.33) and ORs of water from food/beverages, and total water tended to be higher among rural subjects than urban ones. These estimations could reflect the fact that people living in small towns and rural homes drink water from their improperly controlled wells since they do not have a water supply from the national company[18]. Besides, "mate" intake -a remarkable water contributor among Uruguayan inhabitants- displayed positive associations with UBC risk, although it was not a new finding from the present study[52]. Our findings are consistent with other studies that reported a positive association between fluid intake and UBC[53,54]. One of these[53] is a meta-analysis of 26 studies that reported a risk increase among European and American males and over intakes of 2.000 ml/day or higher.

Regarding our study population (88% males), the similarity is to be taken into consideration. The inverse associations among Asian people were assigned to boiled water used in tea preparation [8], but we could not find something comparable in our study, since tea intake is not frequently consumed in Uruguay. The other meta-analysis [54] was done on 54 articles and found that each 500 ml/day increase in total fluid intake increased 3.3% the UBC, also stronger above 3000 ml/day. Some studies communicate no association [55], and past studies usually based their findings on beverages but not on water contents in solid foods [56-58]. Besides, another study reported the total fluid intake as protective, estimating a 7% risk reduction for every 240 ml of fluid increase[59].

When analyzing total fluid intake, studies include considerations about nitrate as a typical drinking contaminant arising mainly from agricultural sources such as nitrogen fertilizers and human waste, which is a precursor in the endogenous formation of N-nitroso compounds (possible bladder carcinogens)[60]. Nitrate and nitrite are also found at high levels in certain foods: we have already reported a bladder cancer risk increase (OR=1.86) for the highest tertile of nitrate intake[40]. A nitrite and nitrate role in the water intake cannot be ruled out in the present study.

There is considerable epidemiologic support for the benefits of consuming plants (mainly fruits and vegetables) rich in antioxidants, notably polyphenols, since most polyphenolic compounds (flavones, anthocyanidins, among others) have not only antioxidant properties, but they may also chelate iron[61]. High intakes of vegetables and fruits are believed to reduce the risk of urothelial cancer. Green leafy vegetables are typically high in dietary fiber, iron, calcium, and very high in phytochemicals and nutrients such as vitamin C, carotenoids, lutein, folate, magnesium as well as vitamin K[62]. For example, vitamin C could inhibit malignant phenotypes in UBC both *in vitro* and *in vivo*[63]. "Mate" infusion might be included in this combined category, according to recent research[64]. However, we have observed that its antioxidant and anticarcinogenic potential was expressed mainly in consumers of an antioxidant-rich diet[65]. Some carcinogens like Dimethylbenz[a]anthracene and PAH like Benzo(a)pyrene (BaP)[15] are present in barbequed meat, tobacco smoke, "mate" infusion and overheated cooking oil, among other sources[66]. They are indirect-acting carcinogens requiring metabolic activation to yield its ultimate carcinogenic form[67], particularly oxidation by CYP enzymes[68]. The quoted components could be partially responsible for the association of "mate" with cancer in organs with no direct contact with the beverage, such as the nephrourinary system[52,69,70], among other sites. Besides, inorganic arsenic, whose high level at the water consumed in Uruguay[17,19], might be present despite the "mate" consumer status, therefore adding its potential influence on a significant fraction of the local population.

The molecular etiology of arsenic carcinogenicity is unclear. However, there are several molecular mechanisms induced by arsenic exposure, such as oxidative stress induction, indirect genotoxic damage, epigenetic regulation of gene expression, and activation of critical proliferative and anti-apoptotic signaling pathways[46]. Therefore, it becomes an ideal partner for the activation of the well-known "mate" pro-carcinogens as BaP. Regarding the low socio-economic status of the interviewed people, we have proved that rural patients displayed higher UBC risks than the urban ones when their "mate" or total water intake increased. We consider it relevant to mention that in 2010, sanitary authorities lowered the maximum permitted arsenic concentration level in drinking water from 50 μg L−1 to the target value of 10 μg L−1 as recommended by the WHO guidelines[71]. Therefore, our study population, interviewed several years before (during 1996-2004), was theoretically exposed to the aforementioned high arsenic values.

The authors highlight the potential importance of water supply in the country. On the one hand, the capital city Montevideo (with almost half of the whole country population) receives drinking water from surface sources, which are lower in arsenic content but implying the risks of iron oxide (perhaps from some pipelines) and THM from disinfection. On the other hand, the rest of the country has a partial water intake coming from its wells, implying groundwater sources and higher arsenic levels: rural homes and small towns do not have a water supply from the state sanitation company. In our viewpoint, research is mandatory due to the potential links to cancer development in the local population, overcoming UBC risks.

During the years of data collection, women cases constituted a small sample to be separately analyzed. Therefore, comparisons with men were not possible to be done. They would have been useful regarding their biologically different iron level management during adulthood. Iron accumulation during lifespan poses an advantage for women because they can balance dietary iron excesses with their menses (periodical iron losses) during the reproductive years. Assuming that both sexes share similar dietary styles, different body iron levels can be expected close to age 50. The different UBC rates between men and women deserve then some considerations. Men have a substantially higher UBC risk, while women tend to have higher infection rates and more aggressive tumors[72]. Since the most expected and treated pathology is urinary tract infection, with high recurrence rates, women might be delayed in the diagnosis of UBC. This delay could lead to more advanced cancers[72], given that many bacterial enzymes can degrade the extracellular matrix, regardless of inflammation and generation of oxygen radicals[73]. Besides, Escherichia coli can increase the iron uptake when Enterococcus faecalis is present[74]. Therefore, frequent infections could partially remove iron from urine and/or from urothelium, leading to partial protection against local carcinogenesis. The existing hormonal differences related to cancers must be taken into account [75,76]. Androgens and estrogens have biologic

effects in UBC: preclinical evidence suggested a critical role of hormonal receptors in the development and progression of urothelial cancer [76].

As strengths of the study, cases and controls were directly interviewed by the same trained personnel in the same hospital settings, the analyzed population included subsets coming from the whole country, and times of data collection were coincident.

As for other hospital-based case-control studies, the present study may suffer from information, recall, and selection biases. Hospital patients may not be fully representative of the general population. Within-person variability over the study period may also be a source of information bias. Selection bias was limited by the nearly full participation of the identified cases and controls (rates ~97%), favored by the interview during the hospital stay. Dietary habits were quite stable in the Uruguayan population, and patients were asked to report any relevant dietary changes occurring during their life. Moreover, the bias in recalling dietary habits should be negligible in our study population, as the awareness of the dietary hypothesis for UBC is limited. Although the FFQ was not validated, it was satisfactorily reproducible [47]. The validation was projected to be done, but due to budgetary cuts suffered in the early 2000s -reflecting the most severe financial crisis in the story of Uruguay- it has never been performed later. Epidemiologic research on cancer in Uruguay continued with the remaining databases -like the one used for the present study- and without funds to update or improve them.

Another limitation was related to iron intake estimations. They might not have been as accurate as desirable because they were based on average serving sizes rather than actual food sizes. Iron supplements were not part of the FFQ. We cannot exclude confounders' role by other dietary factors, such as other constituents of animal foods, the effects of different cooking methods, and the iron contents in water. It deserves to be mentioned that since data collection was done before 2005, a potential effect was not expected in the participants of the current study: Wheat flour fortification with iron was established by a 2005 decree in ferrous sulphate 30 mg/kg.

Caution is needed in the interpretation of water intake and residence. Uruguay has experienced all along the 20th century a remarkable inner migration from rural to urban locations and residence changes within the same location. Consequently, the nature of their source of domestic water could have changed, and they may have drunk chlorinated and unchlorinated water with high-iron or low-iron content, and boiled water, bottled water, in different life periods. Besides, the exposure to water constituents by skin absorption or inhalation cannot be ruled out within these considerations.

CONCLUSIONS

The present study gives evidence to partially support a role for dietary iron and the intake of water in the risk of UBC. The typical dietary style, high in animal-derived iron and low in plant-derived antioxidant, anti-inflammatory and anti-carcinogenic compounds, might bring proper conditions for bladder carcinogenesis. Results suggest the convenience of reducing the animal iron sources while increasing the plant sources, to achieve a healthier iron status. A high-water intake displayed a risk increase, and risks of water from food/beverages, as well as total water, tended to be higher among rural subjects compared to urban ones. Also, "mate" infusion -a remarkable water source among Uruguayan inhabitants- displayed positive associations with UBC risk. The dietary iron associated with water iron and combined with own or added pro-carcinogenic compounds of water are probably increasing the UBC risk in the analyzed population.

Acknowledgment:
The authors wish to express their gratitude to Adriana Fonseca and Maria Gabriela Harris (Court Certified interpreters of Ohio, U.S.A.), for their English language review of the present manuscript.

Funding interests:
The authors have not received any funding for the present manuscript.

Conflict of interest:
The authors declare no conflict of interest.

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