DIETARY ACID LOAD AND COLORECTAL CANCER RISK: A CASE-CONTROL STUDY

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Abstract – Objective: *If the endogenous acid-base balance is not well regulated, dietary acid load contributes to metabolic acidosis, leading to inflammation and cancer metastasis. Nevertheless, there is still no epidemiologic evidence on the association between diet-dependent acid load and colorectal cancer risk. Therefore, we aim to explore its possible role therein.*

Materials and Methods: *A case-control study was performed on 611 colorectal cancer incident cases and 2394 age-frequency matched controls, using a specific multi-topic questionnaire, including a food frequency questionnaire. Food-derived nutrients were calculated from available databases. We assessed dietary acid load based on existing and validated measures as potential renal acid load (PRAL) score and net endogenous acid production (NEAP) score. Odds Ratios (ORs) were estimated by logistic regression, adjusting for potential confounders.*

Results: *We found direct associations between dietary acid load and colorectal cancer risk. The highest quartile of the PRAL score was significantly associated (OR=1.53, p_{trend} = 0.03). A positive family history of cancer and female sex derived even higher risks (OR=2.31 and OR=2.23, respectively). Nevertheless, no heterogeneities were found in these strata. The NEAP score tended to display similar associations.*

Conclusions: *PRAL and NEAP scores are directly associated with meat intake and inversely associated with plant-based foods intake. Results suggest that a low acid load dietary style may reduce colorectal cancer risk, which agrees with studies focused on food groups and dietary patterns. To our knowledge, the present one is the first reported epidemiologic study on dietary acid load and colorectal cancer risk.*

KEYWORDS: *Acid load, Colorectal cancer, Diet, Net endogenous acid production, Potential renal acid load.*

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent malignancy worldwide and was recently ranked as the second leading cause of cancer mortality[1]. Its incidence has been increasing mainly in countries with a mid-to-high human development index[2]. CRC is the most frequent malignancy in the Uruguayan population, taking into account both sexes combined[3]. The age-adjusted incidence and mortality rates locate Uruguayan men at the top of the list in America

and very high in the world's ranking[1]. Besides, mortality trends in Uruguay change annually in +0.3% among men but -0.5% among women over the last two decades[3].

The worldwide heterogeneity in CRC incidence strongly suggests environmental exposures' etiological involvement, mainly lifestyle and diet[4,5]

Among foods, processed and red meats are major risk factors for CRC[6,7]. Their implication in colorectal carcinogenesis is based on some of their own or added components as fats, heterocyclic amines (HCA), nitrosodimethylamine, and heme-iron[7,8] .

Although Uruguay is a developing country, its human development index is high[9], and its average diet is meat-based, with the world's highest per capita beef intake[10]. Meat, iron types, and their role in the CRC risk were thoroughly analyzed in Uruguayan studies along more than two decades[11-18].

An acidogenic diet characterized by high dietary intake of proteins and some minerals can influence the body's acid-base balance[19,20]. These estimates are mainly calculated through the potential renal acid load (PRAL)[19] and net endogenous acid production (NEAP)[20] formulas, which are validated and straightforward methods to estimate the dietary acid load from diet-composition data. Epidemiological studies have often assessed the association between dietary acid load and disease risk, mainly related to chronic non-communicable diseases[21]. Indeed, a prolonged diet-induced low-grade metabolic acidosis over the years may predispose to metabolic abnormalities, in particular, insulin resistance, diabetes, high serum triglycerides, and obesity[22], all of which are relevant to CRC risk increase[23]. Nevertheless, there is limited and inconsistent epidemiologic evidence on the association between diet-dependent acid load and cancer risk[24], mainly restricted to breast cancer incidence and recurrence risk[25-28].

Due to a lack of evidence on a possible role for dietary acid load in CRC risk, we decided to carry out the present study to explore it within a recognized high-risk Latin American country. Up to our knowledge, this is the first epidemiologic case-control study focused on dietary acid load and CRC risk.

MATERIALS AND METHODS

Selection of cases and controls

During the period 1992-2004, all incident and pathologically confirmed CRC cases were obtained from the major public hospitals in Montevideo, Uruguay (Clínicas, Maciel, Pasteur, and National Oncology Institute). Incident CRC cases were considered as those having a diagnosis within the past twelve months. Other tumor sites in the digestive tract were not taken into account. The quoted institutions catch a high fraction of patients from the public healthcare system to diagnose and/or treat cancer. The public health system centralizes its activity in Montevideo, where around 50% of total cancer cases are diagnosed[3]. Of 625 initial patients, 611 cases were included in the study after the refusal of 14 to be interviewed (response rate 97.7 %). The International Diseases Code for Oncology (3rd version)[29] was used to classify lesions at the colon (C18.0 to C18.9) or rectum (C19.9 and C20.9).

At the same time and hospitals, 2394 controls were considered as eligible for the study. They were obtained from the initial 2460 patients, after excluding 66 of them (2.7%), who refused the interview (response rate 97.3%). These controls, afflicted with non-neoplastic diseases not related to tobacco smoking or alcohol drinking and without recent dietary changes, had the following pathologies: skin diseases (357 patients, 14.9%), eye disorders (349, 14.6%), ear disorders (309,12.9%), abdominal hernia (258, 10.8%), fractures (184, 7.7%), hydatid cysts (151, 6.3%), lipoma (101, 4.2%), osteoarticular diseases (100, 4.2%), varicose veins (91, 3.8%), injuries (92, 3.9%), urinary stones (73, 3.1%), goiter (62, 2.6%), and other acute diseases (267, 11.1%).

Due to external factors, the analytical epidemiological research on cancer in Uruguay, located at the National Oncology Institute, was officially terminated in 2004. The activity continued with the remaining databases -like the one used for the present study- and without funds to update or improve them. The projected 4/1 ratio of controls/cases could not be reached; however, the final sample derived a 3.92/1 ratio. Indeed, 50 missing controls (2394 instead of 2444) represent only 2% under a perfect ratio and can be considered a negligible risk of bias.

Only low-income people coming from all around the country who had free access to most medical services, according to Uruguayan law, were included in the present study. Considering the population's features, they were good representatives of a third world country. Each hospital Director has authorized the project's development after receiving the approval from the respective Ethical Committee. In past years, only 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) to preserve anonymity.

Interviews and questionnaire

The interviewers were two social workers, blinded about the study objectives, who worked at the hospitals in two consecutive phases: 1) With the collaboration of Medical Records personnel, they looked for newly diagnosed cancer patients who were eligible to be matched according to the cases' age (± five years), sex, and urban/rural residence. 2) After giving their consent to cooperate with the study, all participants were face-to-face interviewed in the hospitals. The study design did not accept proxy interviews.

All participants were interviewed based on a structured questionnaire, which included the following sections: socio-demographic variables; a family history of cancer; self-reported height and weight five years before the interview; menstrual and reproductive events in female participants; tobacco smoking (average daily number of cigarettes); alcohol drinking (beverage type and amount); daily intake of "mate," tea, and coffee infusions, including the ages at starting and quitting for these habits.

Finally, a detailed semi-quantitative food–frequency questionnaire (FFQ) was designed, including 64 items representative of the Uruguayan diet and asking about food consumption five years before the interview. The FFQ, which allowed the estimation of individual total energy intake, was not validated but was tested for reproducibility, having good correlations [30]. All dietary questions were open-ended, and each amount was converted to times/year. To obtain nutritional information about foods, we used foreign tables from a neighboring country (Argentina) with similar habits [31].

Estimation of dietary acid load

We calculated diet-dependent acid load using two formulas that have been previously defined and utilized in other epidemiologic studies: potential renal acid load (PRAL)[19] and net endogenous acid production (NEAP)[20].

These measures were calculated as follows:
- PRAL (mEq/day) = (0.49 × total protein [g/day]) + (0.037 × phosphorus [mg/day]) − (0.021 × potassium[mg/day]) − (0.026 × magnesium [mg/day]) − (0.013 × calcium [mg/day])
- NEAP (mEq/day) = (54.5 × protein[g/day]) / (0.0256 × potassium [mg/day]) – 10.2

Estimation of iron and nutrients intake

The estimation of heme-iron intake was done by applying our FFQ and following previous studies[32-34], according to the following data: beef (69%); ham, bacon, mortadella, salami, hot dogs, saucisson, and sausage (39%); chicken, fish, eggs and milk (26%), and liver (21%). The mean daily heme-iron intake was calculated by multiplying consumption frequency by the total iron and the quoted percentages. Non-heme-iron intake was calculated by the subtraction of heme-iron from total iron[35]. Animal-based iron was calculated by adding estimations from all animal foods; plant-based iron derived from subtracting animal-based iron from total iron.

An analysis program was run to calculate energy and daily nutrients, making the sum of all individual values, each one obtained after multiplying the amount of servings/year by the ratio calories or nutrient content of the serving/100 g of each, divided by 365 days. Usually, servings of solid foods are around 100-150 g. Since iron intake is highly correlated with energy, an iron density was calculated as daily mg of iron/1000 kcal.

Statistical analysis

Mean values, frequencies, and percentages were used to describe the patients' features. Most questionnaire variables were initially continuous and were categorized for analysis purposes. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression[36]. Potential confounders were included in the multivariate analyses. Regression models included the following independent variables: age, sex, urban/rural residence, education, history of cancer in first-degree relatives, body mass index, smoking status, alcohol status, intake of "mate" and tea infusions, total energy, total fiber, carotenoids, flavonols, lignans, glutathione, vitamin C, vitamin E, animal-based iron, and total HCA. The best models employed continuous and categorized variables. Likelihood-ratio tests were performed to explore possible heterogeneities in the stratified analyses. All calculations were done with STATA software (Release 10, 2007; StataCorp LP, College Station, TX, USA).

RESULTS

Table 1 shows the distribution and comparison of general features between cases and controls. Albeit there was not a perfect matching, the distribution of age groups was adequate (p=0.42). Neither urban/rural status nor residence displayed significant differences (p=0.23 and p=0.30, respectively). Red meat intake and dietary energy showed highly significant differences (p<0.001). Whereas "mate" intake was highly prevalent (~86% ever consumers), tea and coffee were less frequently consumed. Finally, cases tended to be lesser smokers and alcohol drinkers.

TABLE 1. Selected socio-demographic characteristics, dietary features, and tumour localization of the population under study (n=3005). Distribution of cases and controls.

Variables	Categories	Controls		Cases		Global p-value
		(n=2394)	%	(n=611)	%	
Age groups	≤ 39	47	2.0	12	2.0	
	40-49	211	8.8	53	8.7	
	50-59	442	18.5	100	16.4	
	60-69	818	34.2	194	31.7	
	70-79	730	30.5	208	34.0	
	80-89	146	6.1	44	7.2	0.42
Sex	Men	1576	65.8	361	59.1	
	Women	818	34.2	250	40.9	0.002
Education years	≤ 3	1021	42.6	280	45.8	
	4-6	1067	44.6	269	44.0	
	≥ 7	306	12.8	62	10.2	0.14
Urban/Rural status	Urban	1940	81.0	508	83.1	
	Rural	454	19.0	103	16.9	0.23
Residence Regions	Montevideo	1259	52.6	307	50.3	
	Other counties	1135	47.4	304	49.7	0.30
Body Mass Index (kg/m²)	≤ 24.99	1108	46.3	287	47.0	
	25.0-29.99	944	39.4	252	41.2	
	≥ 30.0	342	14.3	72	11.8	0.26
FHC in siblings	No	2070	86.5	517	84.6	
	Yes	324	13.5	94	15.4	0.24
FHC in parents	No	2019	84.3	467	76.4	
	Yes	375	15.7	144	23.6	<0.001
Tea status	Never	1827	76.3	517	84.6	
	Ever drinker	567	23.7	94	15.4	<0.001
"Mate" status	Never	312	13.0	92	15.1	
	Ever drinker	2082	87.0	519	84.9	0.19
Coffee status	Never	1956	81.7	531	86.9	
	Ever drinker	438	17.3	80	13.1	0.002
Red meat intake (serv/year)	≤ 260	849	35.5	148	24.2	
	261-377	813	34.0	243	39.8	
	≥ 378	732	30.5	220	36.0	<0.001

Abbreviations: FHC = family history of cancer.

Table 2 compares the mean values of selected nutrients and bioactive substances. Cases showed higher intakes of specific components (energy, animal-based and heme iron, HCA, and animal glutathione). Conversely, controls' intake was higher for fiber, plant-based and non-heme iron, and all antioxidants except for the quoted animal glutathione). All differences were statistically significant. Regarding the comparison between sexes, men had higher intakes of energy, iron types, lignans, and animal glutathione. On the other hand, women showed higher intakes of fiber, HCA, carotenoids, flavonols, and vitamins C and E. Plant glutathione was also higher among women; however, without statistical significance.

The mean values of both acid load score, PRAL and NEAP, and their original components, are shown in Table 3. The comparison between cases and controls indicates that both scores are higher in cases than in controls, and the same occurs with the studied minerals. Regarding their food sources, whereas cancer cases tended to display higher intakes of the animal source than controls (except for calcium), those plant-based items did not show statistically significant differences between cases and controls.

Table 4 displays the ORs of CRC for exposure to acid load scores. Four regression models were employed, from a basic one to a more complex one, with the increasing use of variables with potential influence. Regarding both PRAL and NEAP scores, the highest estimates (OR=2.15 for PRAL and OR=1.79 for NEAP) were achieved applying the Model 2, including matching variables plus socio-demographic ones, nutritional ones (dietary energy, fiber, BMI), and habits (smoking, alcohol, "mate," tea). The addition of dietary antioxidants (Model 3) and further, of oxidants and carcinogens (Model 4), attenuated

TABLE 2. Mean daily values ± standard deviation of selected nutrients and bioactive substances adjusted by energy. Comparison between cases and controls, as well as between sexes.

Variable	Units	CONTROLS Mean ± SD	CASES Mean ± SD	Diff. (p-value)	MEN Mean ± SD	WOMEN Mean ± SD	Diff. (p-value)
Energy	Kcal	2170 ± 669	2404 ± 753	< 0.001	2279 ± 685	2106 ± 695	< 0.001
Total fibre	g/10^3 Kcal	7.60 ± 2.57	7.05 ± 2.60	< 0.001	7.28 ± 2.44	7.86 ± 2.79	< 0.001
Animal iron	mg/10^3 Kcal	2.69 ± 0.96	2.83 ± 1.00	0.001	2.76 ± 0.96	2.64 ± 0.98	0.001
Plant iron	mg/10^3 Kcal	4.49 ± 1.48	4.24 ± 1.39	< 0.001	4.66 ± 1.54	4.05 ± 1.22	<0.001
Heme iron	mg/10^3 Kcal	1.67 ± 0.67	1.75 ± 0.70	0.005	1.73 ± 0.66	1.61 ± 0.69	<0.001
NHeme iron	mg/10^3 Kcal	5.51 ± 1.40	5.32 ± 1.31	0.002	5.69 ± 145	5.08 ± 1.15	< 0.001
Total HCA	ng/10^3 Kcal	21.2 ± 14.2	25.7 ± 13.6	< 0.001	20.4 ± 14.6	25.4 ± 12.9	< 0.001
Carotenoids	µg/10^3 Kcal	5.54 ± 3.74	5.15 ± 3.50	0.02	4.85 ± 3.22	6.58 ± 4.20	< 0.001
Flavonols	mg/10^3 Kcal	1.68 ± 1.43	1.51 ± 1.37	0.007	1.59 ± 1.37	1.75 ± 1.49	0.003
Lignans	µg/10^3 Kcal	1434 ± 460	1369 ± 431	0.002	1496 ± 471	1284 ± 388	< 0.001
Animal gsh	mg/10^3 Kcal	8.32 ± 3.13	8.66 ± 3.25	0.02	8.57 ± 3.12	8.06 ± 3.19	< 0.001
Plant gsh	mg/10^3 Kcal	9.16 ± 3.82	8.31 ± 3.58	< 0.001	8.92 ± 3.79	9.11 ± 3.77	0.19
Vitamin C	mg/10^3 Kcal	49.7 ± 24.0	45.2 ± 22.5	< 0.001	46.7 ± 23.5	52.5 ± 23.8	< 0.001
Vitamin E	mg/10^3 Kcal	1.80 ± 0.49	1.74 ± 0.52	0.009	1.71 ± 0.47	1.92 ± 0.52	< 0.001

Abbreviations: g=grams; mg=milligrams; ng=nanograms; µg=micrograms; HCA=heterocyclic amines; gsh = glutathione.

the estimates of both acid load scores. Nevertheless, only PRAL remained finally significant (OR=1.53, 95%CI 1.02-2.31, p_{trend} = 0.03).

Finally, Table 5 displays the ORs of CRC for the exposure to each one of the studied scores, showing the results of selected stratified analyses. In all cases, the regression model employed was the final one (Model 4 in the previous Table 4). Albeit there was no heterogeneity between sexes, both PRAL and NEAP scores derived higher and significant ORs only among women than men (OR=2.31 *vs*. OR=1.40, women *vs*. men, respectively for PRAL, and OR=1.86 *vs*. OR=1.13, respectively for NEAP). *P*-values for trend were significant only among women in these comparisons. Besides, when the family history of cancer was present, only the PRAL score was directly and significantly associated with CRC risk (OR=2.23), but the NEAP score was not associated. Finally, concerning the anatomic subsites, the PRAL score showed a statistically significant direct risk association only for the rectum (OR=1.77) while

TABLE 3. Mean daily values ± standard deviation of the acid load scores and their components. Stratification of items according to their animal/plant original source. Comparison between cases and controls.

Variable	Units	CONTROLS Mean ± SD	CASES Mean ± SD	Diff. (p)
Total Proteins	g/d	53.6 ± 19.6	59.2 ± 20.8	<0.0001
Animal proteins	g/d	48.7 ± 18.9	54.2 ± 20.1	<0.0001
Plant proteins	g/d	4.9 ± 2.2	5.0 ± 2.6	0.34
Total Phosphorus	mg/d	776.5 ± 255.7	841.6 ± 275.6	<0.0001
Animal phosphorus	mg/d	461.7 ± 182.2	521.4 ± 192.6	<0.0001
Plant phosphorus	mg/d	314.9 ± 131.7	320.2 ± 151.0	0.39
Total Potassium	mg/d	1932.8 ± 657.7	2036.7 ± 735.2	<0.001
Animal potassium	mg/d	656.3 ± 265.5	745.7 ± 283.3	<0.0001
Plant potassium	mg/d	1276.5 ± 536.0	1291.0 ± 621.6	0.56
Total Magnesium	mg/d	180.9 ± 62.0	188.3 ± 69.7	0.01
Animal magnesium	mg/d	51.3 ± 20.2	58.2 ± 21.5	<0.0001
Plant magnesium	mg/d	129.6 ± 53.2	130.1 ± 61.1	0.84
Total Calcium	mg/d	616.3 ± 316.2	633.8 ± 323.1	0.23
Animal calcium	mg/d	465.7 ± 299.1	478.0 ± 302.4	0.36
Plant calcium	mg/d	150.7 ± 59.0	155.8 ± 67.5	0.06
PRAL score	mEq/d	1.70 ± 10.68	4.25 ± 12.04	<0.0001
NEAP score	mEq/d	50.59 ± 17.44	53.87 ± 17.21	<0.0001

Abbreviations: g=grams; mg=milligrams; mEq=milliequivalents

TABLE 4. Crude and Adjusted Odds Ratios (OR) of CRC for acid load scores (PRAL and NEAP).

	SCORE LEVELS								
	I		II		III		IV		
	OR	95% CI	OR	95%CI	OR	95%CI	OR	95% CI	Trend (p)
PRAL (mEq/d)	≤ -3.67		-3.66-2.69		2.70-8.62		≥ 8.63		
Model 1	1.00	---	1.13	0.86-1.49	**1.37**	1.05-1.79	**2.08**	1.60-2.69	<0.001
Model 2	1.00	---	1.28	0.96-1.69	**1.54**	1.16-2.04	**2.15**	1.16-2.04	<0.001
Model 3	1.00	---	1.23	0.91-1.66	**1.45**	1.05-2.01	**1.88**	1.29-2.76	<0.001
Model 4	1.00	---	1.14	0.83-1.55	1.28	0.91-1.79	**1.53**	1.02-2.31	0.03
NEAP (mEq/d)	≤ 39.1		39.2-49.6		49.7-61.5		≥ 61.6		
Model 1	1.00	---	1.24	0.95-1.62	**1.51**	1.16-1.96	**1.75**	1.25-2.26	<0.001
Model 2	1.00	---	1.26	0.96-1.67	**1.50**	1.13-1.99	**1.79**	1.32-2.43	<0.001
Model 3	1.00	---	1.15	0.86-1.54	1.30	0.96-1.77	**1.48**	1.04-2.11	0.02
Model 4	1.00	---	1.07	0.80-1.44	1.19	0.67-1.84	1.29	0.89-1.88	0.11

Regression models:
Model 1 = Adjusted by age (continuous), sex (binary), residence (urban/rural), education level (categorical, 3), and family history of cancer in first degree (binary, no/yes).
Model 2 = Model 1 + body mass index (continuous), smoking status (categorical, 3), alcohol status (categorical, 3), "mate" intensity (liters*years, continuous), tea intake (binary never/ ever) energy (continuous), and total fibre (continuous).
Model 3 = Model 2 + total carotenoids (continuous), lignans (continuous), flavonols (continuous), glutathione (continuous), vitamin C (continuous), and vitamin E (continuous).
Model 4 = Model 3 + animal-based iron/1000 kcal (continuous), and total heterocyclic amines (continuous).

it was not significantly associated with colon (OR=1.29). The NEAP associations tended to be weak or absent. The likelihood ratio test was negative for heterogeneity in all the analyzed strata.

TABLE 5. Adjusted Odds Ratios (ORs) of CRC for acid load scores (PRAL and NEAP), with global estimations and stratified analyses by sex, family history of cancer, and cancer subsite.

	SCORE LEVELS									
	I		II		III		IV		Trend (p)	Heter. (p)
	OR	95% CI	OR	95%CI	OR	95%CI	OR	95% CI		
PRAL (mEq/d)	≤ -3.67		-3.66 -2.69		2.70 -8.62		≥ 8.63			
Overall	1.00	---	1.14	0.83-1.55	1.28	0.91-1.79	**1.53**	1.02-2.31	0.03	
Men	1.00	---	1.37	0.89-2.12	1.36	0.85-2.17	1.40	0.80-2.45	0.38	
Women	1.00	---	1.11	0.69-1.77	1.49	0.89-2.50	**2.31**	1.22-4.39	0.008	0.40
FH No	1.00	---	1.23	0.84-1.79	1.30	0.86-1.97	1.55	0.94-2.56	0.10	
FH Yes	1.00	---	1.12	0.62-2.00	1.54	0.82-2.86	**2.23**	1.04-4.77	0.02	0.96
Colon	1.00	---	0.91	0.61-1.37	1.23	0.80-1.89	1.29	0.76-2.19	0.052	
Rectum	1.00	---	1.42	0.92-2.18	1.33	0.82-2.15	**1.77**	1.00-3.12	0.048	0.91
NEAP (mEq/d)	≤ 39.1		39.2 - 49.6		49.7 - 61.5		≥ 61.6			
Overall	1.00	---	1.08	0.80-1.44	1.20	0.87-1.65	1.30	0.90-1.89	0.11	
Men	1.00	---	1.14	0.77-1.71	1.07	0.69-1.65	1.13	0.68-1.88	0.90	
Women	1.00	---	1.06	0.67-1.68	1.58	0.97-2.58	**1.86**	1.04-3.33	0.01	0.61
FH No	1.00	---	1.15	0.80-1.65	1.21	0.82-1.80	1.52	0.96-2.41	<0.05	
FH Yes	1.00	---	0.99	0.58-1.69	1.39	0.79-2.44	1.10	0.57-2.14	0.69	0.21
Colon	1.00	---	1.08	0.74-1.59	1.18	0.78-1.79	1.37	0.85-2.24	0.20	
Rectum	1.00	---	1.06	0.70-1.59	1.18	0.76-1.83	1.22	0.73-2.04	0.24	0.99

Regression model included terms for:
Age (continuous), sex (binary), residence (urban/rural), education level (categorical, 3), family history of cancer in first degree (binary, no/yes), body mass index (continuous), smoking status (categorical, 3), alcohol status (categorical, 3), "mate" intensity (liters*years, continuous), tea intake (binary never/ ever) energy (continuous), total fibre (continuous), total carotenoids (continuous), lignans (continuous), flavonols (continuous), glutathione (continuous), vitamin C (continuous), vitamin E (continuous), animal-based iron/1000 kcal (continuous), and total heterocyclic amines (continuous).
Abbreviation: FH = family history of cancer.

DISCUSSION

In the present study, we have found direct associations between dietary acid load and CRC risk. These associations varied according to the employed regression models. Whereas the highest quartile of PRAL score was significantly associated (OR=1.53, 95% CI 1.02-2.31, p_{trend} 0.03), the highest NEAP score was not significant (OR=1.29, 95% CI 0.89-1.88, p_{trend} 0.11). These scores were obtained using the most demanding model, which included antioxidant substances (carotenoids, flavonols, glutathione, lignans, and vitamins C and E), pro-carcinogenic ones (heterocyclic amines), and a pro-oxidant one (animal-based iron). Excluding the latter, all the other estimations for high exposure to acid load were significant.

On the other hand, a positive family history of cancer and female sex derived even higher risks for the exposure to PRAL score (OR=2.31 and OR=2.23, respectively). Nevertheless, the analyses showed no heterogeneities in these strata. The NEAP score tended to display somewhat similar but not significant associations. These facts differ from what has been observed about inflammation, which is recognized as different for each sex[37]. Notwithstanding, the calculated acid load scores were found directly and significantly associated with CRC risk. Those scores are directly associated with meat intake and inversely associated with plant-based foods intake. Results suggest that the herewith studied Uruguayan population subset has an acidogenic dietary style, characterized by a low fruit and vegetable intake.

The existing evidence concerning acid load and cancer risk is only related to breast cancer, although these research works are not consistent enough[25,26,28]. Whereas there were positive associations for acid load with breast cancer risk among American and Puerto Rican women[25,26], the same association was not found in Iranian ones[28]. Several studies focused their interest on the influence of the acid load on the metabolic condition (hyperinsulinism and/or diabetes)[38,39] and the association with breast cancer recurrence and survival[26,27]. One of these works stated that acid load was identified as a novel dietary factor that may lead to inflammation and hyperglycemia[26].

It has been claimed that dietary acid load can contribute to metabolic acidosis. Metabolic acidosis is a condition characterized by a slight decrease in blood pH, and feeding is one of the main factors to produce such a situation. Actually, the excessive consumption of acid precursor foods (such as meat, cheese, and eggs, which are sources of phosphorus and proteins) leads to acid-base balance volubility. If this condition occurs in a prolonged, chronic way, low-grade metabolic acidosis can become

significant and predispose to metabolic imbalances[21,40,41]. In this respect, it is well known that metabolic acidosis can cause tissue damage, which can further initiate inflammation[26]. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Overproduced free radicals react with cell membrane fatty acids and proteins, impairing their function permanently. Also, free radicals can lead to mutation and DNA damage that can be a predisposing factor for cancer and age-related disorders[42].

Cancer patients have a reduced capacity to adjust the acid-base balance[43]. Therefore, a dietary acid load may contribute to inflammation in cancer patients. Many of the metabolic adaptations observed in cancer are recognized as similar to the same perturbations observed in diabetic patients[44]. Furthermore, an insulin sensitizer as metformin is commonly used to reduce hyperglycemia in diabetic patients. It has been associated with the reduction of cancer incidence[45] and the increase in cell survival[46]. Lactate derived from cancer cells suppresses T and NK cells function[47]; nevertheless, acidity has more than one source[48], since colonic tumors in human patients produced five times more CO_2 than lactate[49].

Besides, there is evidence that cellular phosphate burden from phosphate toxicity is a pathophysiological determinant of cancer cell growth: tumor cells express more phosphate cotransporters and store more inorganic phosphate than normal cells, and dysregulated phosphate homeostasis is associated with the genesis of various human tumors[50]. Although 550 mg/day of phosphorus is considered an adequate intake for adults in the general population, the average intake in European countries is estimated at 1000-1767 mg/day[51]. Dietary phosphorus has increased (perhaps doubled since 1990) over time[52], mainly due to phosphorus-containing additives in food manufacturing and processing[53]. Our study population sample showed an average intake of 790 mg/day (776 mg/day among controls), which is high, based on the quoted reference numbers.

Also, a diet incorporating foods enhanced with phosphorus can add an estimated additional 600–800 mg to the overall daily intake[54], as well as non-negligible amounts of sodium chloride (NaCl). Indeed, its intake is reported to be an independent predictor of plasma bicarbonate concentration. Assuming a causal relationship, NaCl may exert approximately 50-100% of the acidosis-producing effect of the dietary acid load and is, therefore, considered a predictor of diet-induced low-grade metabolic acidosis[55]. On the other hand, potassium, magnesium, and calcium are precursors of bases. Thus, in general, the main foods that release precursors of acids into the

bloodstream are mostly of animal origin (except for beans and nuts), and foods that are precursors of bases are mainly those of plant origin[21].

Another interesting issue is the possible relationship between iron and cancer since iron can engage in redox cycling and free radical formation, leading to the induction of genetic damage and accumulate mutations. In this respect, it has been shown that a high intake of dietary iron is associated with an increased risk for some cancers, particularly CRC [18,56]. Therefore, the increase of heme iron described in Table 2 for cases could also contribute to the production of reactive oxygen species, which could be in the base of cellular transformation. Table 3 shows that the components of acid load scores, discriminated by animal/plant source, somehow reflect what the statement mentioned above poses, stressing that a low acid load dietary style may reduce CRC risk. The latter agrees with studies focused on food groups and dietary patterns. However, further studies are needed to clarify these points.

As for the study's strengths, cases and controls were face-to-face, directly interviewed by the same trained personnel in the same hospital settings, and the studied population included subsets coming from the whole country and belonged to different socio-economic-cultural strata. Besides, times of data collection were coincident.

However, within-person variability over the study period may 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 relatively stable in the Uruguayan population, and patients were asked to report any relevant dietary changes occurring during their life. Furthermore, a recall bias related to dietary habits should be negligible in our study population, as the awareness of CRC's dietary hypothesis was very limited. Although the FFQ was not validated, it was satisfactorily reproducible [30]. Mineral estimations become one of the limitations of the present study since they were based on average serving sizes rather than actual food sizes. Besides, we could not exclude confounders' role by other dietary factors, such as other constituents of animal foods, the effects of different cooking methods, and the mineral contents in water.

CONCLUSIONS

In conclusion, the calculated acid load scores were found directly and significantly associated with CRC risk. Since those scores are directly asso-ciated with meat intake and inversely associated with plant-based foods intake, our results suggest that the herewith studied Uruguayan population subset had an acidogenic dietary style. It is worth to mention that there is no previous epidemiologic work on dietary acid load and CRC risk. Results are in agreement with studies focused on food groups and dietary patterns. Although the dietary acid load may increase CRC risk, further studies are needed to confirm these findings.

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CONFLICT OF INTEREST:
The authors declare no conflict of interest.

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