



# DIETARY ACID LOAD AND RISK OF GASTRIC CANCER: A CASE-CONTROL STUDY

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**Abstract – Objective:** *The dietary acid load can contribute to metabolic acidosis, which is closely linked to cancer development through mechanisms of inflammation and cell transformation. However, very limited epidemiologic evidence is linking diet-dependent acid load and cancer risk. Since no published studies focused on dietary acid load and gastric cancer (GC) risk, we explored this association in the present study.*

**Patients and Methods:** *A case-control study was performed in 1370 patients (274 cases and 1096 age-frequency, sex, and urban/rural residence 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: Potential Renal Acid Load (PRAL) score and Net Endogenous Acid Production (NEAP) score. Odds ratios (OR) and their 95% confidence intervals (95% CI) were estimated by unconditional logistic regression, adjusting for potential confounders.*

**Results:** *We found direct, significant associations between dietary acid load and GC risk: (OR=1.74, 95% CI 1.13-2.66) and (OR=1.90, 95% CI 1.26-2.84) for highest PRAL and NEAP, respectively. Both risk estimates also displayed linear trends. Both acid load scores were directly associated with animal-based foods (mainly meat) and inversely associated with the intake of plant-based foods.*

**Conclusions:** *A high dietary acid load may contribute to GC development. To the best of our knowledge, the present is the first epidemiologic case-control study analyzing associations of dietary acid load and GC risk in a Western population. Further research is warranted to confirm our findings.*

**KEYWORDS:** *Dietary acid load, Epidemiology, Gastric cancer, NEAP, PRAL.*





## INTRODUCTION

Gastric cancer (GC) is one of the most common causes of cancer deaths globally<sup>1</sup>. GC represents a clinically and biologically heterogeneous group of malignancies with an incidence varying substantially across different world regions and among various ethnic groups<sup>1,2</sup>. Recent studies indicated an up to 20-fold variation in risk between low-risk populations (particularly in North America and some African regions) and high-risk areas (which include Eastern Asia, Eastern Europe as well as Central and South America)<sup>2,3</sup>.

Despite continuous advances in the diagnosis and treatment of GC, the five-year survival rate remains poor in many countries<sup>2</sup>. Rates vary from approximately 10% to 30% in some European countries to about 90% in Japan<sup>3,4</sup>. Nowadays, it is estimated that more than 50% of the new GC cases occur in developing countries<sup>3</sup>. Notably, the mortality-to-incidence ratio for GC is higher than for many other cancers<sup>5</sup>. In light of the few treatment options available in many developing nations, reducing GC incidence seems of utmost importance to reducing mortality<sup>6</sup>. Thus, risk factor identification and management are essential<sup>7</sup>.

The etiology of GC is multifactorial and previously identified non-modifiable risk factors include age, gender, race/ethnicity, and family history<sup>1,2</sup>. Among the factors contributing to the development of GC, the Gram-negative bacterium *Helicobacter pylori* infection has been recognized in the last decades as a significant one and considered a Class I carcinogen<sup>8</sup>. It initiates a pathogenic cascade with chronic inflammation and is continued by the formation of chronic non-atrophic gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally gastric adenocarcinoma<sup>9</sup>.

Other modifiable risk factors include a high nitrate and salt intake<sup>2</sup>, a high intake of red and processed meats<sup>10</sup>, excessive alcohol consumption (particularly beer and liquor)<sup>11</sup>, and, finally, low consumption of vegetables, fruits, and legumes<sup>12,13</sup>. Recently, dietary habits became considered important factors modulating *H.pylori*-linked gastric diseases as GC<sup>14,15</sup>. In this regard, the infection with *H.pylori* may contribute to microbial dysbiosis, which can be induced by consuming unhealthy and unbalanced diets<sup>16</sup>. In addition, an excessive salt intake contributes to the formation of N-nitroso compounds that speed up the carcinogenesis process of GC, facilitated by the pathogenesis of *H. pylori* cytotoxic-associated gene A (CagA) protein<sup>8</sup>.

A recent Chinese study highlighted the importance of a potential additional risk factor that still

received little attention: the microenvironment of GC cells<sup>17</sup>. In an in-vitro study, Li et al<sup>17</sup> demonstrated that a microalkaline environment (as opposed to a microacidic one) promoted the apoptosis of GC cells and thus inhibited tumor growth. This appears of paramount importance, as human diets may, in fact, influence acid-base balance by providing acid or base precursors<sup>18</sup>. Recent studies emphasized that a high dietary acid load (DAL) may result in a low-grade metabolic acidosis state<sup>18</sup>, which has been closely linked to cancer development through low-grade inflammation and cell transformation<sup>19</sup>.

A series of studies investigated the role of DAL in several cancers and found mixed results. For example, some studies found positive associations for colorectal<sup>20,21</sup>, pancreas<sup>22</sup>, lung<sup>23</sup>, prostate<sup>24</sup>, bladder<sup>25</sup>, breast cancer<sup>26,27</sup>, head and neck<sup>28</sup>, esophagus<sup>29</sup>, and central nervous system<sup>30</sup>, whereas others found no associations for kidney and breast cancer<sup>31,32</sup>. Besides, a recent study based on the American NHANES data reported that DAL scores were consistently higher in cancer survivors compared with general population<sup>33</sup>. As the role of an increased DAL and cancer development remains controversial, we sought to illuminate potential associations in GC, which appears to be susceptible to an altered environmental pH<sup>17</sup>.

## PATIENTS AND METHODS

### *Cases and Controls selection*

This is a case-control study on environmental factors and the risk of cancer conducted between 1996 and 2004 in Montevideo, Uruguay. We described the methods elsewhere in detail<sup>21,23-25</sup>. All newly diagnosed cases of GC registered in the four major hospitals of Montevideo during that period were considered eligible for this study. Trained social workers (blinded with regard to research goals) performed routine screenings to identify potentially eligible participants. Potentially eligible individuals and controls were contacted by the interviewers and interviewed face-to-face after consenting to the study. We did not accept any form of proxy interviews. We identified 274 cases in total. At the same time and in the same institutions, 1096 controls afflicted with non-neoplastic diseases were considered eligible. Controls were admitted for conditions unrelated to tobacco usage or alcohol disorders. Controls with a recent history of a dietary modification were considered ineligible. Controls presented with the following conditions: eye disorders (260, 23.7%), abdominal

hernia (238 patients, 21.7%), injuries and trauma (138, 12.6%), nephrourological infections (98, 8.9%), skin diseases (88, 9.5%), appendicitis (72, 6.6%), varicose veins (60, 5.5%), hydatid cyst (47, 4.3%), blood disorders (45, 4.1%), and other medical disorders (39, 3.6%).

### Questionnaire

The administered questionnaire included socio-demographic and anthropometric variables, a detailed history of substance usage (including tobacco and alcohol), occupational exposures and, cancer history in 1st-2nd degree relatives. One key element of the questionnaire was a food frequency questionnaire (FFQ) with 64 items representative of the Uruguayan diet. This FFQ was tested for reproducibility with good results<sup>34</sup>. All dietary questions were open-ended. In addition, we used local tables of food composition to estimate total energy and nutrient intake.

### Dietary Acid Load Estimation

The methods used for DAL estimation can be found elsewhere in detail<sup>21,23-25,27-29</sup>. In brief, we used two common and validated formulas to calculate DAL<sup>35,36</sup>. Potential renal acid load (PRAL) of diet was calculated as follows:

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

This formula considers intestinal absorption rates protein, potassium, phosphate, magnesium, and calcium. The score has been validated vs. urinary pH in healthy individuals with good results<sup>35</sup>.

Net endogenous acid production (NEAP) was calculated as follows<sup>36</sup>:

$$\begin{aligned} \text{NEAP (mEq/day)} = & (54.5 \times \text{protein [g/day]}) / \\ & (0.0256 \times \text{potassium [mg/day]}) - 10.2 \end{aligned}$$

This score takes into account the sulphuric acid production due to protein metabolism and the rate of bicarbonate production subsequent to the metabolism of intestinally absorbed potassium salts of organic acids<sup>35</sup>. A positive NEAP or PRAL score reflects an acid-forming potential, whereas negative scores indicate an alkaline-forming potential<sup>36</sup>.

### Statistical Analysis

We used STATA software (Release 10, Stata Corp LP, College Station, TX, USA. 2007) for statistical analysis. Standard calculations indicate that including ~300 cases of a specific disease, with a control:case ratio of 4:1, will have a statistical power of 0.80 (at a significance level of 0.05) to detect an odds ratio (OR) of about 1.4 for a binary exposure with 20% population prevalence, or an OR of about 1.5 for top vs. bottom quartile categories of an exposure or risk factor. For the majority of analyses, we treated the questionnaire variables as continuous variables. Categorization was done for analysis purposes only. Basic descriptive analyses include frequencies for categorical variables and means (standard error in parenthesis) for continuous, normally-distributed variables. ORs and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression. Terms for potential observable confounders were included in the multivariate analyses. They included age, sex, residence, urban years, family history of cancer, education level, smoking status and intensity, energy, body mass index, animal and plant iron intake, and “mate” intake. No participants were excluded as outliers for any dietary component. Heterogeneities in the stratified analyses were explored through likelihood-ratio tests. Finally, 3-D graphic analyses were performed using STATISTICA software (Release 10, StatSoft Inc, Tulsa, OK, U.S.A. 2011) by applying the distance weighted least squares option.

### RESULTS

Table 1 shows the distribution of cases and controls according to selected variables. The study design yielded a distribution of age, sex, and residence (urban/rural status) with similar proportions. Education years were slightly less among cases. Besides, cases had a higher energy intake than controls and a higher smoking intensity than controls. No statistical differences were found concerning the family history of cancer rate, the alcohol status, and the “mate” intake.

Table 2 presents selected nutritional variables, which were analyzed as mean values  $\pm$  SD. Cancer cases had higher mean intakes of energy and all iron types. Conversely, they showed lower mean intakes of total fiber, carotenoids, vitamin C, and vitamin E.

Table 3 shows the mean values of both acid load scores (PRAL and NEAP) and their original components, and the latter expressed adjusted by 1000 kcal/day. Scores were significantly higher in



**TABLE 1.** Selected socio-demographic characteristics and habits of the population under study (n=1370). Distribution of cases and controls.

Variables	Categories	Controls (n=1096) %		Cases (n=274) %		p-value
Age groups	< 50	96	8.8	54	8.8	
	50-59	236	21.5	59	21.5	
	60-69	316	28.8	79	28.8	
	70-79	344	31.4	86	31.4	
	80-89	104	9.5	26	9.5	1.00
Urban/Rural status	Urban	913	83.3	227	82.9	
	Rural	183	16.7	47	17.1	0.86
Sex	Men	760	69.3	190	69.3	
	Women	336	30.7	84	30.7	1.00
Education years	<5	564	51.5	168	57.7	
	≥ 5	532	48.5	116	42.3	0.07
Body Mass Index (kg/m <sup>2</sup> )	<18.50	16	1.5	4	1.5	
	18.50-24.99	477	43.5	139	50.7	
	25.00-29.99	448	40.9	93	33.9	
	≥ 30.00	155	14.1	38	13.9	0.16
FH of cancer in 1 <sup>st</sup> & 2 <sup>nd</sup> degree	No	810	73.9	207	75.6	
	Yes	286	26.1	67	24.4	0.58
“Mate” intensity (liters-years)	Non drinkers	156	14.2	34	12.4	
	0.1-39.9	305	27.8	88	32.1	
	4-63.9	322	29.4	73	26.6	
	≥ 64.0	313	28.6	79	28.8	0.47
Dietary energy (kcal/day)	≤ 1347	402	36.7	55	20.1	
	1348-1741	364	33.2	93	33.9	
	≥ 1742	330	30.1	126	46.0	<0.001
Alcohol status	Never	558	50.9	133	48.5	
	Ex drinker	111	10.1	32	11.7	
	Current	427	39.0	109	39.8	0.67
Smoking status	Non smoker	439	40.0	94	34.3	
	Ever smoker	657	60.0	180	65.7	0.08
Smoking intensity (pack-years)	Non smoker	439	40.0	94	34.3	
	0.01-26.0	239	21.8	48	17.5	
	26.1-49.0	228	20.8	54	19.7	
	≥ 49.1	190	17.3	78	28.5	<0.001

**Abbreviations:** FH of Cancer = family history of cancer.

cases than in controls. Regarding protein and calcium intake, it was also higher but not significantly. However, the intake of phosphorus, potassium, and magnesium was significantly higher among controls than in cases. Regardless of statistical significance, all intakes derived from plant sources were also higher among controls.

Table 4 displays the adjusted ORs for both acid load scores. Even the basic regression models (using the matching variables plus urban years) derived significant estimates: OR=1.84, 95% CI 1.32-2.58,  $p_{\text{trend}} < 0.001$  for PRAL, and OR=1.78, 95% CI 1.27-2.48,  $p_{\text{trend}} = 0.002$  for NEAP. Besides,

the highest vs. lowest tertile of PRAL derived significant adjusted estimates (OR=1.74, 95% CI 1.13-2.66,  $p_{\text{trend}} < 0.001$ ). Similar results were found when analyzing the NEAP score: both risk and trend estimates were significant (OR=1.90, 95% CI 1.26-2.84,  $p_{\text{trend}} = 0.002$ ). These scores were obtained using the most demanding regression model, which included age, sex, residence, urban years, family history of cancer, education level, smoking status and intensity, energy, body mass index, animal and plant iron intake, and “mate” intake.

Table 5 shows the continuous ORs, their 95% CI of gastric cancer risk, and the p-trend values

**TABLE 2.** Mean daily values  $\pm$  standard deviation (SD) of selected nutrients and bioactive substances adjusted by energy. Comparison between cases and controls.

<i>Variable</i>	<i>Units</i>	<i>Controls Mean <math>\pm</math> SD</i>	<i>Cases Mean <math>\pm</math> SD</i>	<i>p-value</i>
Energy	Kcal	1533 $\pm$ 451	1697 $\pm$ 424	<0.0001
Total iron	mg/10 <sup>3</sup> Kcal	7.41 $\pm$ 1.45	7.71 $\pm$ 1.49	0.003
Animal iron	mg/10 <sup>3</sup> Kcal	2.85 $\pm$ 0.96	2.94 $\pm$ 0.99	0.15
Plant iron	mg/10 <sup>3</sup> Kcal	4.57 $\pm$ 1.49	4.76 $\pm$ 1.50	0.048
Heme iron	mg/10 <sup>3</sup> Kcal	1.68 $\pm$ 0.67	1.76 $\pm$ 0.68	0.08
NHeme iron	mg/10 <sup>3</sup> Kcal	5.74 $\pm$ 1.38	5.95 $\pm$ 1.40	0.02
Vitamin C	mg/10 <sup>3</sup> Kcal	91.5 $\pm$ 45.5	77.5 $\pm$ 37.7	<0.0001
Vitamin E	mg/10 <sup>3</sup> Kcal	2.69 $\pm$ 0.86	2.46 $\pm$ 0.87	0.0001
Carotenoids	mg/10 <sup>3</sup> Kcal	8.04 $\pm$ 4.97	6.62 $\pm$ 3.86	<0.0001
Total fibre	g/10 <sup>3</sup> Kcal	7.76 $\pm$ 3.02	6.68 $\pm$ 2.64	<0.0001

Abbreviations: g=grams; mg=milligrams; kcal=kilocalories; A/P=animal/plant; H/NH=heme/non-heme.

derived from stratified analyses of acid load scores performed to selected variables of interest. Both PRAL and NEAP scores displayed similarities to be remarked: significant ORs and linear trends were found only among men, in the absence of a family history of cancer, and with high intensity of smoking and “mate” drinking.

Figure 1 shows a 3-D graphic based on selected data of the studied population. It compares the features of the control subset with the cancer cases, analyzing the interrelationships of three variables employed in the regression model: the age (X-axis), the education level (Y-axis), and the PRAL score (Z-axis). Controls (left picture) display low

**TABLE 3.** Mean daily values  $\pm$  standard errors of the acid load scores and standard deviations of their components\*. Stratification of items according to their animal/plant original source. Comparison between cases and controls.

<i>Variable</i>	<i>Units</i>	<i>Controls Mean <math>\pm</math> SD</i>	<i>Cases Mean <math>\pm</math> SD</i>	<i>p-value</i>
<b>Total Proteins</b>	g/d	35.5 $\pm$ 7.1	34.9 $\pm$ 6.7	0.22
Animal proteins	g/d	32.2 $\pm$ 7.3	31.9 $\pm$ 7.0	0.54
Plant proteins	g/d	3.3 $\pm$ 1.4	3.0 $\pm$ 1.2	0.002
<b>Total Phosphorus</b>	mg/d	512.9 $\pm$ 59.3	503.2 $\pm$ 52.2	0.01
Animal phosphorus	mg/d	309.4 $\pm$ 74.1	308.1 $\pm$ 70.4	0.80
Plant phosphorus	mg/d	203.5 $\pm$ 57.4	195.1 $\pm$ 55.0	0.03
<b>Total Potassium</b>	mg/d	1285.1 $\pm$ 293.3	1190.8 $\pm$ 245.9	<0.001
Animal potassium	mg/d	435.5 $\pm$ 109.3	437.5 $\pm$ 106.5	0.79
Plant potassium	mg/d	849.5 $\pm$ 309.9	753.3 $\pm$ 272.0	<0.001
<b>Total Magnesium</b>	mg/d	119.1 $\pm$ 25.4	113.8 $\pm$ 22.8	0.002
Animal magnesium	mg/d	34.2 $\pm$ 8.1	34.3 $\pm$ 7.8	0.85
Plant magnesium	mg/d	84.9 $\pm$ 28.0	79.5 $\pm$ 25.9	0.004
<b>Total Calcium</b>	mg/d	386.2 $\pm$ 126.5	372.3 $\pm$ 116.2	0.10
Animal calcium	mg/d	229.4 $\pm$ 127.1	217.4 $\pm$ 116.9	0.15
Plant calcium	mg/d	156.7 $\pm$ 48.4	154.9 $\pm$ 46.7	0.57
		<b>Mean <math>\pm</math> SE</b>	<b>Mean <math>\pm</math> SE</b>	
<b>PRAL score</b>	mEq/d	2.69 $\pm$ 0.33	5.88 $\pm$ 0.71	<0.001
<b>NEAP score</b>	mEq/d	51.17 $\pm$ 0.52	54.74 $\pm$ 1.10	0.002

\*Mean values of minerals are presented in units/1000 kilocalories per day.



**TABLE 4.** Crude and Adjusted Odds Ratios (OR) of GC for acid load scores (PRAL and NEAP). p-values for their linear trends.

	I		II		III		Trend (p)
	OR	95% CI	OR	95% CI	OR	95% CI	
<b>PRAL (mEq/d)</b>	<b>≤ -0.72</b>		<b>-0.73</b>	<b>- 7.64</b>	<b>≥ 7.65</b>		
Model 1	1.00	---	1.32	0.81-1.52	<b>1.84</b>	1.32-2.58	<0.001
Model 2	1.00	---	1.26	0.88-1.79	<b>1.45</b>	1.02-2.07	0.004
Model 3	1.00	---	1.38	0.94-1.87	<b>1.74</b>	1.13-2.66	<0.001
<b>NEAP (mEq/d)</b>	<b>≤ 43.2</b>		<b>43.3 - 57.5</b>		<b>≥ 57.6</b>		
Model 1	1.00	---	1.38	0.98-1.94	<b>1.78</b>	1.27-2.48	0.002
Model 2	1.00	---	1.30	0.92-1.85	<b>1.55</b>	1.09-2.18	0.017
Model 3	1.00	---	<b>1.45</b>	1.00-2.10	<b>1.90</b>	1.26-2.84	0.002

Regression models:

**Model 1** = Adjusted by age (continuous), sex (male,female), residence (urban/rural), and urban years (continuous)

**Model 2** = Model 1 + family history of cancer in 1st and 2nd degree (binary No/Yes) + education (categorical, 3) + smoking status (categorical, 3) + smoking intensity (categorical, 4) + “mate” intensity (categorical, 4) + body mass index (continuous) + energy (categorical, 3)

**Model 3** = Model 2 + animal iron (continuous) + plant iron (continuous)

Iron variables = dietary iron/1000 kcal/day (in mg)

Animal or Plant iron, based on their dietary sources.

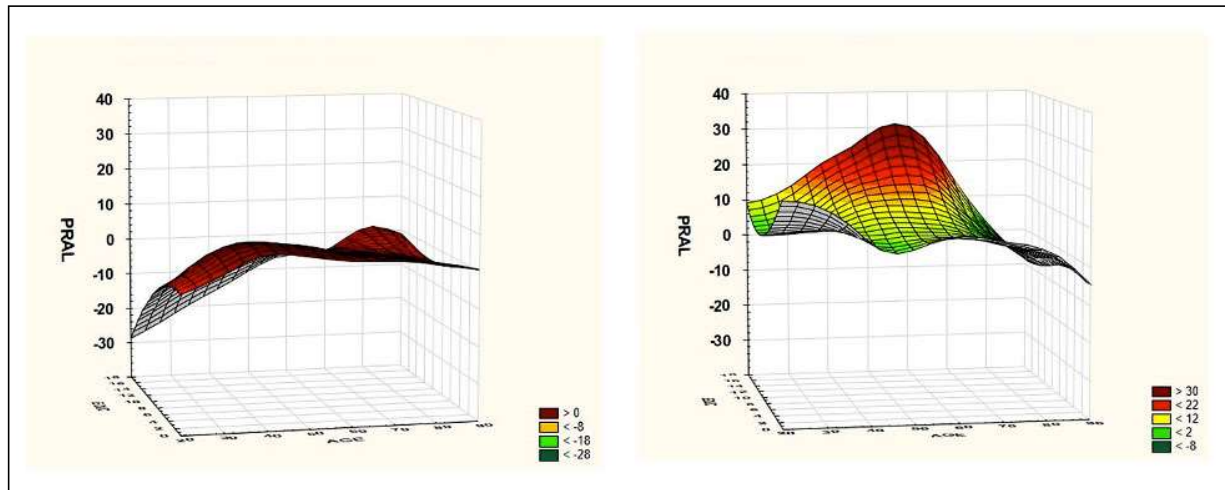
PRAL scores across all ages. Furthermore, there is a remarkable (alkaline load) trend in ages <50 and mostly among better educated patients. Regarding cases (right picture), there is a noteworthy high (acid load) area among cases under ages >70. The

comparison displays similarities for PRAL score in the aged population. Cases show two peaks of PRAL score: the first one, at young ages and low educational level; the second one and even higher, at middle ages and mid-to-high education levels.

**TABLE 5.** Continuous Odds Ratios (OR) and 95% Confidence Intervals of gastric cancer risk, derived from stratified analyses of acid load scores performed to selected variables of interest. p-values for their linear trends.

PRAL	Variable	Categories	OR	95% CI	Trend (p)
	Sex	Male	<b>1.036</b>	1.016 – 1.056	<0.0001
		Female	1.015	0.985 – 1.046	0.32
	FH of Cancer	No	<b>1.031</b>	1.011 – 1.051	0.002
		Yes	1.030	0.999 – 1.062	0.059
	Smoking intensity	≤26 Pack-years	1.019	0.997 – 1.041	0.10
		≥27 Pack-years	<b>1.042</b>	1.016 – 1.068	0.001
	“Mate” intensity	<40 Liters-years	1.021	0.998 – 1.044	0.08
		≥40 Liters-years	<b>1.038</b>	1.014 – 1.061	0.001
NEAP	Variable	Categories	OR	95% CI	Trend (p)
	Sex	Male	<b>1.017</b>	1.007 – 1.027	0.001
		Female	1.008	0.989 – 1.028	0.43
	FH of Cancer	No	<b>1.016</b>	1.005 – 1.028	0.004
		Yes	1.010	0.994 – 1.027	0.22
	Smoking intensity	≤26 Pack-years	1.006	0.993 – 1.019	0.38
		≥27 Pack-years	<b>1.023</b>	1.009 – 1.036	0.001
	“Mate” intensity	<40 Liters-years	1.009	0.995 – 1.023	0.20
		≥40 Liters-years	<b>1.020</b>	1.007 – 1.032	0.002

Significant estimates in bold letter. **Abbreviations:** FH of Cancer = Family History of Cancer.



**Fig. 1.** 3-D graphic comparison between cases and controls, based on data of PRAL score, ages and education level of the studied population (X-axis=age, Y-axis=education years, and Z-axis=PRAL score). Controls (left picture) display low PRAL scores along all the ages, being remarkable the low acid load among young-to-mid ages. Regarding cases (right picture), there is a remarkable high acid load area under ages  $\geq 70$ . Differences are stronger among mid-to-high educated people.

## DISCUSSION

This study explored whether a high DAL was associated with an increased risk for GC in an Uruguayan population. Our results demonstrate that higher acid load scores (both NEAP and PRAL) were significantly associated with GC risk. The ORs for the highest vs. lowest tertiles were OR=1.74, 95% CI 1.13-2.66,  $p$  trend <0.001 for PRAL, and OR=1.90, 95% CI 1.26-2.84,  $p$  trend=0.002 for NEAP, respectively. Besides, the estimates showed statistical significance only among men and within the strata of intense smokers and intense “mate” drinkers. The stratified analyses do not suggest any risk contribution for a positive family history of cancer. The graphic data displayed in Figure 1 reveals very different DAL between GC cases and controls, all across the ages between 20 and 70. The older subgroups suggest that they would share a dietary behavior, probably derived from medical recommendations to mitigate chronic diseases usually linked to aging. This fact might be reflected by a lower DAL, as a consequence of decreasing red and processed meats and at the same time increasing plant-based foods.

The association of a high DAL and cancer risk is a topical area of current epidemiological research and major interest to our group<sup>21,23-25,27-29</sup>. A high DAL has recently been associated with increased odds for many types of solid cancer<sup>21-30</sup> and, importantly, with cancer recurrence among breast cancer survivors, too<sup>37</sup>. As such, DAL appears to be an important novel risk factor in the field of general Oncology - a fact that apparently applies for GC, as well. Thus, our findings warrant a careful discussion in the context of previous studies and a succinct elucidation of potential biological mechanisms.

Although salt intake (NaCl) can enhance the carcinogenicity of *H.pylori*, as mentioned earlier in this text, its role should be emphasized as a pro-acidogenic element<sup>8</sup>. Indeed, the intake of non-negligible amounts of NaCl 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 DAL and is therefore considered a predictor of diet-induced low-grade metabolic acidosis<sup>38</sup>. Within this context, NaCl may produce inflammation of gastric cells through exogenous and endogenous ways at the same time.

Besides, salt-cured and preserved foods such as pickles are a relevant dietary source of carcinogens. Whereas pickles made with salt, consumed in Japan, constitute a risk factor, those made by adding vinegar and spices are prevalent in countries where GC has lower rates<sup>14</sup>. Again, the acidogenic properties of salt, and the alkalizing properties of vinegar and spices, should not be ruled out as playing their roles also by influencing the DAL. “Mate” intake was included in our analysis for better risk modeling. It is a hot aqueous infusion made from the herb *Ilex paraguariensis* and is a staple in temperate South America. 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)<sup>39</sup>. The International Agency for Research on Cancer (IARC)<sup>40</sup> considered hot “mate” drinking as a 2A agent (a possible carcinogenic for humans) because of the presence of polycyclic aromatic hydrocarbons<sup>41</sup>. In 2016, the IARC revised the “mate” classifica-



tion, changing it from group 2A to the low-risk group 3. However, this assignment was only for “not very hot” mate, based on insufficient animal and human evidence, establishing  $>65^{\circ}\text{C}$  as a lower limit for the “very hot” category<sup>42</sup>. The previous study had reported  $69.5^{\circ}\text{C}$  as the mean temperature for regional consumers<sup>40</sup>. A recent meta-analysis considered “mate” as a risk factor for upper aerodigestive cancers<sup>43</sup>. Different analyses showed it as a risk factor for GC among the Uruguayan population<sup>44-46</sup>. Therefore, we believe it was justified to include the infusion in our calculations since those consumers labeled as “hot mate” drinkers seem to have a high proportion of “very hot mate” consumers, according to IARC.

Cancer is increasingly seen as a “disease of metabolism,” and malignancies are highly associated with metabolic reprogramming<sup>47</sup>. A common feature of solid cancers is their generation of lactic acid (due to elevated rates of fermentative glycolysis)<sup>48</sup>, resulting in subsequent acidification of their surrounding microenvironment<sup>47</sup>. This acidic microenvironment, which is reinforced by local perfusion deficits<sup>49</sup>, has strongly influenced cancer progression<sup>50</sup>. Local acidosis has toxic effects on normal cells<sup>51</sup> while stimulating tumor invasion and metastasis<sup>52</sup>. In addition, an acid microenvironment inhibits immune cell surveillance (by promoting T-cell stasis)<sup>53,54</sup>. Most importantly, acidosis induces genomic instability and acts as an “evolutionary force” for aggressive clones of acid-adapted cells<sup>55</sup>.

The enhancement of various metabolic pathways (e.g., an increased drive of the pentose pathway with a subsequent increase in NADPH production countering reactive oxygen species and promoting cancer cell survival) is an additional factor worth mentioning<sup>53</sup>. While a detailed description of this pathway is beyond the scope of this epidemiological study, there is accumulating evidence that, altogether, these changes allow cancer cells to outcompete neighboring non-cancer cells<sup>56</sup>. As such, our epidemiologic findings are supported by various plausible biological mechanisms.

The use of adequate dietary styles could reduce the levels of *H. pylori* colonization or virulence, prevent or delay the development of GC, and be attractive from several perspectives, including cost, tolerability, and acceptability, as suggested by Raei et al<sup>15</sup>. From a translational perspective, our findings could also be significant. Various direct acidity targeting approaches exist (e.g., oral buffers, targeted (experimental) agents to raise tumor pH)<sup>47</sup>, and diet is probably the most accessible option to most populations<sup>57</sup>. Furthermore, spices like turmeric, nutmeg, cumin, e.g., as well

as cinnamon (inhibiting IL-8 secretion), exhibited promising anti-*H. pylori* activities in experimental studies and also have alkalizing properties<sup>14,58</sup>.

## Strengths and Limitations

The present analysis has multiple limitations and strengths that deserve mentioning. First, we tried to minimize selection bias by frequency matching controls and cases on age, sex, and urban/rural residence. Potential confounders, such as occupational or home exposure to pollution (e.g., toxic chemicals, heavy metals, etc.) and smoking, were not assessed. Yet, some of these occupational exposures are non-negligible risk factors for GC<sup>59,60</sup> that require consideration in future studies. Besides, no information about the presence of *H. pylori* was initially recorded, because our study was designed exclusively using a questionnaire and without any biological samples. Therefore, potential interactions between bacterial features and DAL was not feasible to analyze; it would have been desirable to perform such analyses, however, this is a pending task for future studies. Although our employed FFQ showed satisfactory reproducibility in other studies<sup>34</sup>, it has never been validated due to external factors. Yet, a recent study investigating dietary patterns in Uruguay revealed comparable results to our analysis<sup>61</sup>. As such, we believe that our nutritional assessment provides sufficient validity. The fact that all study interviews were done face-to-face by the same interviewers at the same hospitals is a major strength of this study. The same applies to the low attrition rate.

## CONCLUSIONS

In conclusion, both calculated PRAL and NEAP scores were found as directly and significantly associated with GC risk, in both cases supported by adjusted regression models. As reported in previous studies, DAL scores reflect direct associations with meat intake and inversely with the intake of plant foods. According to our results, an acidogenic dietary style could contribute to the GC risk. To the best of our knowledge, the present is the first epidemiologic case-control study analyzing DAL and GC risk associations in a Western population. Further investigations are needed to confirm our findings.

## ETHICAL APPROVAL:

Each hospital Director has allowed the project after receiving approval from the respective Ethical Committee.

**INFORMED CONSENT:**

In past years in Uruguay, up to 2009, it required only oral consent from the patients assuming their data confidentiality, and no specific code was formally requested for epidemiologic observational studies. However, an auto-generated number was built to preserve anonymity based on first and last name + ID number.

**AVAILABILITY OF DATA AND MATERIAL:**

The database is available under reasonable request.

**CONFLICT OF INTEREST:**

The authors (ALR, WML, JMC, BM, and MAS) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**AUTHORS CONTRIBUTIONS:**

A.L.R. participated in the original idea, design, data processing, statistical analyses, text redaction, graph design, and general supervision; W.M.L. collaborated in the text supervision, biochemical and molecular supervision, and final draft supervision; J.M.C. collaborated in tables design and the text supervision; B.M. collaborated in the text redaction and supervision; M.S. collaborated in the text redaction, draft supervision, language checking, and general supervision.

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