DIETARY ACID LOAD AND RISK OF CANCER: NEW INSIGHTS FROM A NATIONWIDE CASE-CONTROL STUDY

A.L. RONCO[1-3], W. MARTÍNEZ-LÓPEZ[4,5], J.M. CALDERÓN[3], G. MINTEGUI[6], M.A. STORZ[7]

[1]Unit of Oncology and Radiotherapy, Pereira Rossell Women's Hospital, Montevideo, Uruguay
[2]School of Medicine, CLAEH University, Prado and Salt Lake, Maldonado, Uruguay
[3]Biomedical Sciences Center, University of Montevideo, Montevideo, Uruguay
[4]Academic Unit on Radiation Protection, Faculty of Medicine, University of the Republic, Montevideo, Uruguay
[5]Genetics Department and Biodosimetry Service, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[6]Department of Endocrinology and Metabolism, School of Medicine, University of the Republic (UdelaR), Montevideo, Uruguay
[7]Department of Internal Medicine II, Center for Complementary Medicine, Freiburg Medical Center – Faculty of Medicine, University of Freiburg, Freiburg, Germany

Abstract – Objective: *Dietary acid load can contribute to metabolic acidosis, which is closely linked to cancer development through inflammation and cell transformation mechanisms. However, limited epidemiologic evidence is still linking diet-dependent acid load and cancer risk. Since we published nine studies specifically focusing on dietary acid load and the risk of cancer development, we decided to explore its potential role more deeply through the analysis of all databases combined.*

Materials and Methods: *A case-control study was performed on 13270 subjects (3736 cases and 9534 age-frequency and residence-matched controls) drawn from the major public hospitals in Uruguay. Participants were interviewed through a multi-topic questionnaire, including a food frequency questionnaire. Food-derived nutrients were calculated from available databases. The dietary acid load was calculated based on validated measures, including Potential Renal Acid Load and Net Endogenous Acid Production scores. Odds ratios (OR) were estimated by logistic regression, adjusting for potential confounders.*

Results: *We found significant and direct associations between dietary acid load and cancer risk (OR= 1.44 and OR= 1.64 for the highest scores). The estimated methionine intake was found also significantly and directly associated (OR= 1.97), while the plant fiber was significantly and inversely associated (OR= 0.49).*

Conclusions: *Results confirm that an acidogenic dietary style may increase the risk of cancer. Our findings suggest that both Met and plant fiber intakes might be independent factors influencing the risk linked to acid-base disbalance which turn into a metabolic stress, but acting in opposite directions. Furthermore, Met intake displayed comparable odds ratios as the scores themselves.*

KEYWORDS: *Dietary acid load, Cancer, Epidemiology, Methionine, Fiber, PRAL, NEAP.*

Corresponding Author: Alvaro L. Ronco, MD; e-mail: alv.ronco58@gmail.com

INTRODUCTION

Cancer is among the leading causes of death globally, and exposure to risk factors plays a crucial role in the biology and burden of numerous cancer types[1]. Most cancers are caused by a complex multifactorial etiology, including lifestyle, environmental and genetic factors[2,3]. In addition, prevention offers the most cost-effective long-term strategy for cancer control, and the World Health Organization suggested that up to 50% of cancers are preventable[4]. Through changes on hormonal, metabolic, and inflammatory effects, diet may play a pivotal role in cancer prevention strategies[5]. Whereas solid epidemiological associations exist between obesity and cancer[6], well-balanced, healthy diets abundant in plant foods are supposed to reduce cancer risk[7,8]. In recent years, a relatively new index to assess the overall quality of diet has gained increasing attention in this context: the Dietary Acid Load (DAL). It has been recently proposed that a higher DAL, representing a higher intake of (acidifying) animal products and a lower intake of vegetables and fruits, could lead to detrimental changes in acid-base balance[2]. An unfavorable acid-base disbalance with pH values at the lower end of the normal physiological range was shown to modulate molecular activity, including insulin growth factor and glucocorticoid activity, as well as altered adipocyte cytokine signaling. There are known consequences of these facts, expressed by a risk increase of chronic kidney disease[9], osteoporosis[10], non-fatty acid liver disease, among several other chronic diseases such as sarcopenia, and cardiovascular ones[11]. In addition, the aforementioned molecular disbalances may indirectly serve as intermediary or downstream effectors of carcinogenesis or tumor promotion[12]. Moreover, it has been demonstrated that low pH reduced the removal of DNA damage induced by Benzo[a]Pyrenes in pulmonary epithelial cells maintained in culture, leading to an accumulation of DNA damage[13]. Experimental and translational work suggests that an acidifying microenvironment favors cancer cells' survival and facilitates tumors' invasion and metastasis. In contrast, an alkalizing microenvironment has opposite effects[14,15]. Numerous epidemiological studies examined potential associations between cancer and DAL, and three independent meta-analyses confirmed these associations[2,16,17]. In this respect, Williamson et al[18] summarized the relevant molecular pathways that can be sensitive to acid-base equilibria in mammalian cells, their possible cellular effects, as well as the cascade of events that turn into an organism-level manifestation of the metabolic stress followed by disease. Despite these preliminary results, additional high-quality studies are warranted to determine further potential associations between DAL and risk and prognosis for specific cancers – particularly in large populations. In this respect, our research group has a strong interest in the role of DAL in health and disease and conducted numerous epidemiological analyses investigating the importance of DAL in various cancer types. This is a topic of current epidemiologic interest[19]. We merged herewith our nine (published) subsite-specific cancer databases[20-28] and conducted an extensive analysis with more than 13,000 participants.

Acidogenic foods are rich in sulfur-containing amino acids such as methionine (Met) and cysteine (Cys). Met is an essential amino acid in mammals. In addition to its role as a component of proteins, Met links to some important metabolic pathways that play vital roles in epigenetics (S-adenosyl-methionine), nuclear functions (polyamines), detoxification (glutathione), and cellular membranes (phospholipids). Furthermore, the Met cycle is intimately linked with folate metabolism and thus can indirectly modulate nucleotide biosynthesis[29].

Part of the earlier literature has focused on the effects of Met restriction because low Met diets appear to improve overall metabolic health by impacting several critical metabolic and nutrient-sensing pathways, improving glucose metabolism, reducing the accumulation of hepatic triglycerides, and favoring corrections in plasma biomarker levels as adiponectin, leptin, among others[30]. In addition, several biomarkers are usually affected when a high DAL is reported[31-33]. Therefore, it has become apparent that dietary Met and Cys reductions are required for maximum benefits. This is particularly relevant for translational consideration since Met and Cys are both common constituents in the diet[30].

Met metabolism has been connected to cancer on several levels[34]. However, since the human epidemiological evidence for Met intake and risk of diseases such as cancer is mixed[29], we decided to estimate its intake and analyze it in our study on cancer, given its relevant part as a component of the DAL scores. Regarding these latter, since the alkalizing effects rely mostly on fruits and vegetables and their contribution to fiber is considerable, we considered it timely to analyze the fiber intake within the context of our study. We paid particular consideration to the suggestions made by Wang et al[2] and included a large set of potential confounders as well as already known DAL measurements: potential renal acid load (PRAL) and net endogenous acid production (NEAP) scores to enhance our understanding of this topic of high epidemiological interest.

MATERIALS AND METHODS

Selection of cases and controls

As part of a multisite epidemiologic research (1993-2005), all newly diagnosed and confirmed cases of cancer, drawn from the four major public hospitals of Montevideo, Uruguay (Clinicas University Hospital, Maciel and Pasteur Public Hospitals, and the Oncology Institute), were considered eligible for this study. These institutions screen a significant fraction of patients from the public system to diagnose and/or treat cancer. In addition, the public health system is centralized in Montevideo, where ~50% of total incident cancer cases are diagnosed.

During the study period (1993-2005), 3736 cancer cases were identified and included in the already published original studies[20-28]. At the same period and in the same hospitals, all patients hospitalized for conditions unrelated to tobacco smoking or alcohol drinking and without recent changes in their diets were considered eligible for the study. A total of 9534 patients were included in the study, who have completed the questionnaire. They were frequency matched to cases on age (10-year groups), residence (urban/rural), and region (Montevideo/Other 18 counties). The corresponding control group was not hospitalized but had ambulatory consultation for medical reasons.

Trained social workers, unaware of the study objectives, worked at the hospitals in two phases: First, they screened routinely for newly diagnosed cancer patients, working with Medical Records personnel's collaboration. Second, they contacted patients who were eligible to be matched by the age-frequencies of the cases, as well as their urban/rural residence and their region (Montevideo/Other counties). After obtaining consent for the study, all participants underwent an in-person interview at the hospitals. Proxy interviews were not accepted in our research. Patients admitted to Public or University Hospitals were people with low incomes from all around the country, with free access to most medical services, which was mandatory by Uruguayan law. According to the population features the public health system served, they were considered good representatives of a third-world country population. Therefore, we have excluded no participants as outliers for any dietary component.

Questionnaire

Participants answered a structured questionnaire that included socio-demographic variables; occupation; cancer history in 1st-2nd degree relatives; self-reported height and weight five years before the interview; smoking and alcohol; a history of "mate," tea and coffee drinking; a food frequency questionnaire (FFQ) of 64 items, representative of the Uruguayan diet, focused on food consumption five years before the interview. Proxy interviews were not accepted. The FFQ was not validated, although it was tested for reproducibility[35], allowing the estimation of individual energy. All dietary questions were open-ended. Local tables of food composition were used for estimating energy and nutrients[36], and an external source was consulted to obtain information on Met in foods[37].

Regarding smoking habit, there were eight items considered: smoking status (Non-smoker, Ex-, Current); amount (N° of cigarettes/day); type (blond, mixed, black); rolling (manufactured, hand-rolled); age at start and at quitting; duration (age at stopping–the age at the start); and intensity (pack-years, = the product of calculated packs of 20 units smoked per day × smoking duration in years). Patients who reported quitting within the same year of their interview were considered current smokers.

Estimation of dietary acid load

We calculated diet-dependent DAL using formulas that have been previously defined and validated[38,39] and applied for our previous epidemiologic studies on DAL and cancer[20-28]: PRAL and NEAP. These measures were calculated as follows:

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

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

The PRAL score considers the intestinal absorption rates for protein, potassium, calcium, magnesium, and phosphate and has been validated against urine pH in healthy adults. The rate of sulfuric acid production from protein metabolism and bicarbonate generation from the metabolism of intestinally absorbed potassium salts of organic acids are prominent and become a highly variable component of the NEAP score, as developed by Frassetto et al[39]. These authors stated that "by considering both, the acidifying effect of protein and the alkalinizing effect of potassium (organic anions), NEAP can be predicted with confidence from the readily available contents of only two food nutrients". In addition, they found that PRAL and NEAP were highly correlated (r= 0.84, $p < 0.001$). Negative PRAL or reduced NEAP values reflect an alkaline-forming potential, whereas a positive or increased value reflects an acid-forming potential, respectively.

Estimation of nutrients intake

An analysis software was compiled to calculate energy as the sum of all individual values. Each one was obtained after multiplying the numbers of servings/year by the ratio of calories 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. This applies to Met and other nutrients[36,37]. Animal-based nutrients were calculated by adding estimations from all animal foods; plant-based nutrients were derived by subtracting the animal-based one from the total intake. For research purposes, we calculated a nutrient density expressed as daily milligrams or grams of the substance/kilocalories * 1000.

Statistical analysis

Most questionnaire variables were originally continuous; when necessary, they were categorized for analysis purposes. Categorization into quartiles was performed overall. Preliminary univariate analyses were performed to select variables to be further entered into the regression models. To make comparisons, selected interest variables were presented as mean values ± standard deviation (SD). We estimated Odds Ratios (ORs) and 95% confidence intervals (95% CI) for each interest variable to analyze the association between exposure levels of acid load scores and LC, which were calculated by unconditional logistic regression. Reported p-values were two-sided, and associations with p-values< 0.05 were considered statistically significant. The regression models were compared using a term for "energy" as a continuous and categori-

cal variable, searching for the best OR estimates. Potential observable confounders were included in the multivariate analyses.

Equations included terms for the following independent variables: age (categorical, 6), sex (binary), family history of cancer in 1st and 2nd-degree relatives (binary, no/yes), smoking status (categorical, 3), smoking intensity (pack-years, continuous), and intakes of energy (continuous), total fiber (categorical, 5), α-carotene (continuous), lycopene (continuous), calorie-adjusted total iron (continuous), and methionine (categorical, 5). Calculations were performed with STATA software (Release 10, Stata Corp., LP, College Station, TX, USA; 2007).

RESULTS

The distribution of the analyzed sample is summarized in Table 1: the cancer sites (cases) and the main health afflictions among controls. Besides, Table 1 shows the classification of cases according to anatomic sites and controls according to pathologies. A small subset of women (n= 333) belonged to the Prepaid Healthcare system, participating in the study on DAL and breast cancer[22].

Relevant socio-demographic features, selected habits, and a comparison between cases and controls (frequencies, mean ± standard deviation) of selected dietary items are shown in Table 2. The age distribution significantly differs due to the employed procedure of database merge. Other significant differences between cases and controls were found for almost all variables unless for sex, urban/rural status, as well as intakes of α-carotene and total iron.

TABLE 1. Distribution of the analyzed sample: number and relative frequency of the cancer sites (in cases) and main health afflictions (among controls).

Tumor sites in cancer cases	n	%	Health afflictions in controls	n	%
Lung	839	22.5	Abdominal hernia	1792	18.8
Breast	572	15.3	Eye diseases	1763	18.5
Prostate	323	8.6	Osteoarticular diseases	1725	18.1
Colon	319	8.5	Skin diseases	1010	10.6
Rectum	292	7.8	Traumatic injuries	925	9.7
Larynx	275	7.4	Appendicitis	610	6.4
Stomach	274	7.3	Varicose veins	534	5.6
Bladder	255	6.8	Other pathologies	505	5.3
Esophagus	185	4.9	Hydatic cyst	276	2.9
Pharynx	185	4.9	Not hospitalized	222	2.3
Kidney	114	3.0	Benign blood diseases	172	1.8
Oral cavity	103	2.7			
All cases	**3736**	**100.0**	**All controls**	**9534**	**100.0**

TABLE 2. Socio-demographic features, selected habits and comparison between cases and controls (frequency, mean ± standard deviation) of selected dietary items in the studied population.

Variables	Categories	Controls (n=9534) %		Cases (n=3736) %		Differences p-value
Age groups	< 40	215	2.3	89	2.4	
	40-49	787	8.2	355	9.5	
	50-59	1815	19.0	777	20.8	
	60-69	3245	34.0	1249	33.4	
	70-79	2870	30.1	1053	28.2	
	80-89	602	6.3	213	5.7	0.02
Status Urban/Rural	Urban	7696	80.7	2983	79.8	
	Rural	1838	19.3	753	20.2	0.25
Residence region	Montevideo	5060	53.1	1814	48.6	
	Other counties	4474	46.9	1922	51.4	<0.001
Sex	Men	7195	75.5	2763	74.0	
	Women	2339	24.5	973	26.0	0.07
Education years	<5	4990	52.3	2044	54.7	
	≥ 5	4544	47.7	1692	45.3	0.01
Body Mass Index (kg/m^2)	<18.50	164	1.7	60	1.6	
	18.50-24.99	4314	45.3	1935	51.8	
	25.00-29.99	3844	40.3	1307	35.0	
	≥ 30.00	1212	12.7	448	11.6	<0.001
Family history of cancer	No	7093	74.4	2559	68.5	
In 1st and 2nd degree	Yes	2441	25.6	1177	32.5	<0.001
Smoking Status	Not smoker	3194	33.5	977	26.2	
	Ex-smoker	2704	28.4	924	24.7	
	Current smoker	3636	38.1	1835	49.1	<0.001
Smoking intensity (pack-years)	Not smoker	3194	33.5	977	26.2	
	0.01-27.0	2442	25.6	606	16.1	
	27.1-53.0	2103	22.1	914	24.5	
	≥ 53.1	1795	18.8	1239	33.2	<0.001
Alcohol drinking	Not drinker	4128	43.3	1465	39.2	
	Ex drinker	1157	12.1	540	14.5	
	Current drinker	4249	44.6	1731	46.3	<0.001
"Mate'' status	Not drinker	1279	13.4	390	10.4	
	Ever drinker	8255	86.6	3346	89.6	<0.001
"Mate'' intensity (liters-years)	0.1-39	2747	28.8	1114	29.8	
	39.1-62.9	2773	29.1	1009	27.0	
	≥ 63.0	2735	28.7	1223	32.8	<0.001
Total iron	mg/10^3 Kcal/d	7.73 ± 1.40		7.82 ± 1.43		0.29
Animal iron	mg/10^3 Kcal/d	2.98 ± 0.96		3.27 ± 1.03		<0.0001
Plant iron	mg/10^3 Kcal/d	4.75 ± 1.55		4.54 ± 1.50		0.02
α-carotene	mg/d	1.54 ± 1.41		1.54 ± 1.45		0.87
Lycopene	mg/d	1.17 ± 0.79		1.10 ± 0.78		<0.0001
Total fiber	g/10^3 Kcal/d	5.16 ± 2.07		4.41 ± 2.65		<0.0001
Total methionine	g/d	2.68 ± 0.98		2.94 ± 1.05		<0.0001
Methionine	mg/kg body weight/d	38.1 ± 15.6		42.8 ± 17.3		<0.0001
Energy	Kcal/d	2256 ± 573		2499 ± 682		<0.0001

Abbreviations: g=grams; mg=milligrams; kcal=kilocalories; /d = per day.

On the other hand, Table 3 displays the methionine contents in representative foods of our FFQ, eleven animal sources, and nineteen plant sources, according to the American dietary reference[38]. The fractions used were adapted to those of the Uruguayan FFQ (in units or servings). Substantial differences exist between animal and plant sources.

TABLE 3. Methionine contents in selected, representative, analyzed foods, according to Ref. 31.

Food item – Animal source	Units	Content (g)	Food item – Plant source	Units	Content (g)
Beef, roasted, mid-size	S	0.957	Carrot, raw, mid-size	U	0.012
Lamb, roasted, mid-size	S	0.677	Tomato, raw, mid-size	U	0.007
Chicken, breast, skin	S	0.659	Lettuce, raw	S	0.013
Fish, white, mid-size	S	1.120	Onion, raw	S	0.010
Bacon	S	0.166	Chard, cooked	S	0.035
Sausage, pork	U	0.132	Spinach, cooked	S	0.099
Ham, cured	S	0.764	Potato, cooked	S	0.045
Cheese, Gruyere-type	S	0.429	Sweet potato, cooked	S	0.062
Butter, saltless	S	0.024	Pumpkin, cooked	S	0.020
Milk, whole	S	0.845	Cabbage, cooked	S	0.026
Egg, whole, boiled	U	0.210	Beans, kidney, cooked	S	0.261
			Lentils, cooked	S	0.152
			Oranges	U	0.010
			Apples	U	0.002
			Pears	U	0.040
			Grapes	S	0.016
			Peaches	U	0.014
			Bananas	U	0.011
			Bread, white, small	U	0.130

Abbreviations: S = serving; U = unit.

The correlations found between DAL scores and different fiber types are displayed in Table 4. All found correlations were statistically significant. The cereal fiber showed opposite associations: while it was inversely correlated to the NEAP score (same as the rest of fiber types), it was positively and significantly associated with the PRAL score. The other fiber types showed similar significant, inverse correlations with DAL scores.

The Odds Ratios for the PRAL and NEAP scores calculated in the previous Uruguayan studies are presented in Table 5. Depending on the sample size, some of those studies were categorized into tertiles (oral cavity, pharynx, larynx, esophagus, stomach, bladder, and prostate) or quartiles (breast, lung, colorectum, and kidney). Some cancers showed coincidence in the significance of high estimates for both scores (pharynx, larynx, esophagus, stomach, breast, bladder, and prostate). In addition, there were two tumor sites without a risk association (oral cavity and kidney), and the rest displayed significantly elevated ORs for one of the employed scores and nonsignificant estimates for the other score (lung, rectum, colorectum).

Table 6 compares the main PRAL and NEAP components (mean ± standard deviation) between cases and controls, including an adjusted calculation by energy for each item. Additionally, the table includes a stratification of items according to their animal or plant source. Unless three variables (plant phosphorus, plant calcium, and energy-adjusted calcium), all analyzed variables dis-

played significant differences between cases and controls. In general, the intake of alkalizing components was higher among controls, and those of acidifying ones were higher among cases. Regarding animal or plant sources, the latter tended to be more consumed by controls, and the former more consumed by cancer cases.

Table 7 shows the ORs of cancer for the exposure to PRAL and NEAP scores in the whole sample. Different regression models were employed: A basic one, only adjusting for sex and age, which yielded similar ORs for both scores (OR= 2.39 and OR= 2.37 for the highest quintiles of PRAL and NEAP, respectively). Then, three adjusted models, controlling for significantly associated variables, adding dietary fiber, and finally, methionine. The highest quintiles of PRAL and NEAP were also highly and significantly associated with the risk of cancer, even with the more complex

TABLE 4. Pearson correlation coefficients for dietary acid load scores and fiber types.

	PRAL	NEAP
Cereal fiber	.091 *	-.080 *
Vegetable fiber	-.403 *	-.379 *
Legume fiber	-.173 *	-.239 *
Fruit fiber	-.469 *	-.378 *
Vegetable+fruit+legume fiber	-.542 *	-.507 *
Total fiber	-.511 *	-.514 *

(*) = statistically significant ($p<0.05$).

TABLE 5. Odds Ratios for the PRAL and NEAP scores calculated in the previous Uruguayan studies.

PRAL										Trend
		I		II		III		IV		
Tumor site	Ref.	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	(p)
Oral cavity	26	1.00	---	0.84	0.46-1.55	1.29	0.69-2.43	---		0.49
Pharynx	26	1.00	---	1.16	0.71-1.91	**2.40**	**1.44-4.01**	---		**0.005**
Larynx	26	1.00	---	1.14	0.74-1.77	**2.22**	**1.42-3.47**	---		**<0.001**
Oro-Phar-larynx	26	1.00	---	1.11	0.79-1.57	**2.10**	**1.46-3.03**	---		**<0.001**
Lung	21	1.00	---	1.00	0.73-1.36	1.22	0.87-1.72	0.99	0.64-1.52	0.94
Esophagus	27	1.00	---	**1.74**	**1.09-2.77**	**2.28**	**1.44-3.61**	---		**<0.001**
Stomach	28	1.00	---	1.38	0.94-1.87	**1.74**	**1.13-2.66**	---		**<0.001**
Colon	20	1.00	---	0.91	0.61-1.37	1.23	0.80-1.89	1.29	0.76-2.19	**0.052**
Rectum	20	1.00	---	1.42	0.92-2.18	1.33	0.82-2.15	**1.77**	**1.00-3.12**	**0.048**
Colorectum	20	1.00	---	1.14	0.83-1.55	1.28	0.91-1.79	**1.53**	**1.02-2.31**	**0.03**
Breast	22	1.00	---	1.01	0.73-1.40	**1.76**	**1.28-2.42**	2.46	1.76-3.44	**<0.001**
Kidney	24	1.00	---	0.69	0.38-1.24	0.91	0.42-1.96	0.98	0.94-1.02	0.34
Bladder	25	1.00	---	**1.72**	**1.12-2.62**	**1.74**	**1.08-2.82**	---		**0.002**
Prostate	23	1.00	---	1.28	0.90-1.83	**1.52**	**1.01-2.28**	---		**0.01**

NEAP										Trend
		I		II		III		IV		
Tumor site	Ref.	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	(p)
Oral cavity	26	1.00	---	1.04	0.56-1.91	1.06	0.56-1.98	---		0.98
Pharynx	26	1.00	---	1.21	0.73-1.99	**2.28**	**1.40-3.71**	---		**0.006**
Larynx	26	1.00	---	1.37	0.89-2.11	**2.00**	**1.29-3.09**	---		**0.006**
Oro-Phar-larynx	26	1.00	---	1.27	0.89-1.79	**1.95**	**1.37-2.78**	---		**0.002**
Lung	21	1.00	---	1.09	0.81-1.49	**1.48**	**1.06-2.05**	2.22	1.52-3.22	**<0.001**
Esophagus	27	1.00	---	1.53	0.96-2.44	**2.17**	**1.38-3.41**	---		**<0.001**
Stomach	28	1.00	---	**1.45**	**1.00-2.10**	**1.90**	**1.26-2.84**	---		**0.002**
Colon	20	1.00	---	1.08	0.74-1.59	1.18	0.78-1.79	1.37	0.85-2.24	0.20
Rectum	20	1.00	---	1.06	0.70-1.59	1.18	0.76-1.83	1.22	0.73-2.04	0.24
Colorectum	20	1.00	---	1.07	0.80-1.44	1.19	0.67-1.84	1.29	0.89-1.88	0.11
Breast	22	1.00	---	0.98	0.72-1.35	**1.56**	**1.15-2.13**	1.78	1.30-2.42	**<0.001**
Kidney	24	1.00	---	0.91	0.49-1.68	1.59	0.77-3.31	1.00	0.98-1.02	0.92
Bladder	25	1.00	---	**2.10**	**1.38-3.20**	**1.83**	**1.15-2.89**	---		**0.02**
Prostate	23	1.00	---	**1.58**	**1.10-2.18**	**1.72**	**1.16-2.48**	---		**0.048**

models (OR= 1.44 and OR= 1.64 for PRAL and NEAP, respectively). Furthermore, all p-values for trend were highly significant.

Finally, given the obtained results with the aforementioned complex regression models, we decided to regress dietary fiber and methionine as our interest variables independently. The results of such analyses are displayed in Table 8. On the one hand, even including adjustments for PRAL and NEAP scores, fiber always showed significantly reduced ORs (OR= 0.60 and 0.66, respectively, p-trend < 0.001). On the other hand, methionine intake kept significantly increased risks for the highest quantiles, even when we adjusted for PRAL and NEAP scores (OR= 1.79 and 1.38, respectively). Again, the p-trend were also significant.

DISCUSSION

The analysis of the global database created by merging those of our previous studies[20-28] showed an expected increase in cancer risk, taking into account the individual outcomes of each publication. The highest quintiles of both DAL scores were highly and significantly associated with the risk of cancer even with the more complex regression models (OR= 1.44 and OR= 1.64 for PRAL and NEAP, respectively), and all p-values for trend were highly significant.

In the present analysis, we introduced two variables considered relevant for their potential roles related to cancer risk: the intakes of fiber and Met. The inclusion of dietary fiber is not new for the present

TABLE 6. Comparison of the main PRAL and NEAP components (mean ± standard deviation) between cases and controls, including an adjusted calculation by energy for each item. Stratification of items according to their animal or plant source.

Variable	Unit	Controls Mean ± SD	Cases Mean ± SD	Differ. (p-value)
Total Proteins	g/d	55.0 ± 19.3	60.3 ± 21.3	<0.001
Total Proteins adj.	g/10³ kcal/d	35.7 ± 7.3	36.9 ± 7.5	<0.001
Animal source	g/d	50.2 ± 18.6	55.7 ± 20.6	<0.001
Plant source	g/d	4.8 ± 2.2	4.6 ± 2.2	<0.001
Total Phosphorus	mg/d	792.5 ± 250.6	837.2 ± 272.1	<0.001
Total Phosphorus adj.	mg/10³ kcal/d	513.8 ± 61.1	511.0 ± 59.7	0.02
Animal source	mg/d	476.2 ± 179.1	521.0 ± 201.4	<0.001
Plant source	mg/d	316.3 ± 131.0	316.2 ± 137.0	0.96
Total Potassium	mg/d	1939.7 ± 635.5	1964.9 ± 677.3	0.04
Total Potassium adj.	mg/10³ kcal/d	1275.6 ± 287.8	1211.7 ± 259.8	<0.001
Animal source	mg/d	673.2 ± 261.6	744.0 ± 290.4	<0.001
Plant source	mg/d	1266.5 ± 517.5	1220.9 ± 545.3	<0.001
Total Magnesium	mg/d	181.6 ± 60.7	184.8 ± 64.3	0.006
Total Magnesium adj.	mg/10³ kcal/d	118.9 ± 25.4	113.7 ± 22.6	<0.001
Animal source	mg/d	52.7 ± 19.9	58.2 ± 22.3	<0.001
Plant source	mg/d	128.9 ± 52.2	126.7 ± 54.8	0.03
Total Calcium	mg/d	594.3 ± 254.5	622.4 ± 269.7	<0.001
Total Calcium adj.	mg/10³ kcal/d	387.4 ± 133.9	384.0 ± 137.4	0.19
Animal source	mg/d	351.0 ± 220.6	377.2 ± 238.6	<0.001
Plant source	mg/d	243.3 ± 97.9	245.2 ± 100.6	0.31
PRAL	mEq/d	3.10 ± 10.57	6.37 ± 11.29	<0.001
PRAL adj.	mEq/10³ kcal/d	1.59 ± 7.05	3.61 ± 6.64	<0.001
NEAP	mEq/d	51.88 ± 17.51	57.04 ± 17.81	<0.001
NEAP adj.	mEq/10³ kcal/d	36.39 ± 17.10	37.91 ± 17.40	<0.001

study; we have already included it in our studies on DAL and colorectum[20], lung[21], and kidney cancer[24], being part of the best regression models found. Recent research on nephrolithiasis showed that fiber was negatively correlated with PRAL (r = -0.246) and with NEAP (r = -0.399)[40]. In our study, dietary fiber is mostly inversely correlated with PRAL and NEAP scores (r ~ -.050), except for cereal fiber and PRAL, which showed a modest but significant positive correlation (r = 0.091).

Besides, it is recommended to ingest 20-35 g/day dietary fiber in healthy adults[41]. However, the control population in our study had a mean intake of 11.8 g/day (results not shown: Table 2 displays 5.16 g/ 103 Kcal/d of energy-adjusted fiber). This value is far below the lower limit of recommendations. Furthermore, aside from some hormonal regulations (estrogens, insulin growth factor), dietary fiber can inhibit histone deacetylases and related signaling pathways and may have anti-inflammatory effects, both through the production of short-chain fatty acids (SCFA) such as butyrate[42].

The consumption of hydrogen ions upon metabolization has alkalizing effects[31]. Most plant foods (unless ripened and processed grains) contain substantial amounts of organic anions, whereas they are scarce in animal-based foods[43]. The daily food supply of organic anions strongly depends on dietary patterns, ranging from 1 g/d in low plant consumers, 3-4 g/d in a diversified omnivorous diet, to > 5 g/d among vegetarians and vegans[43]. Another essential source of organic anions is SCFA (including butyrate, acetate, and propionate), which are the end-products of microbial fermentation, mainly in the distal part of the digestive tract[43]. SCFA production is closely dependent on nutritional factors, and fecal levels of those metabolites correlate positively with the consumption of vegetables, fruits, and legumes[44]. Significant increases in SCFA production were observed when omnivores consume a diet rich in fruits and vegetables[45]. Recent studies indicated that Met restriction (MR) considerably increased SCFAs by up- and down-regulating the abundance of specific bacteria[46,47]. Now is widely accepted that a plant-based vegan diet may increase SCFA production by modulation of the gut microbiota[48].

TABLE 7. Odds Ratios of cancer for the exposure to PRAL and NEAP scores in the whole sample. Comparisons among the employed regression models.

PRAL Cases/cont. (mEq/d)	I 554/2099 ≤ -3.97		II 640/2015 -3.96 – 1.83		III 722/1936 1.84 – 6.58		IV 800/1854 6.59 – 12.37		V 1020/1630 ≥ 12.38		Trend (p)
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Crude	1.00	---	1.20	1.06-1.37	1.41	1.24-1.60	1.63	1.44-1.85	2.39	2.12-2.71	<0.001
Model 1	1.00	---	1.21	1.05-1.38	1.41	1.23-1.62	1.60	1.39-1.84	2.20	1.91-2.54	<0.001
Model 2	1.00	---	1.12	0.97-1.29	1.22	1.05-1.42	1.30	1.11-1.52	1.65	1.38-1.97	<0.001
Model 3	1.00	---	1.09	0.94-1.25	1.14	0.98-1.33	1.17	0.99-1.38	1.44	1.20-1.73	<0.001

NEAP Cases/cont. (mEq/d)	I 539/2116 ≤ 38.53		II 631/2023 38.54 – 47.58		III 719/1934 47.59 – 55.89		IV 849/1802 55.90 – 67.01		V 998/1659 ≥ 67.02		Trend (p)
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Crude	1.00	---	1.22	1.08-1.39	1.46	1.28-1.66	1.84	1.63-2.09	2.37	2.09-2.68	<0.001
Model 1	1.00	---	1.16	1.01-1.32	1.41	1.23-1.62	1.83	1.59-2.10	2.35	2.04-2.70	<0.001
Model 2	1.00	---	1.11	0.97-1.28	1.31	1.14-1.51	1.60	1.38-1.86	1.93	1.63-2.28	<0.001
Model 3	1.00	---	1.06	0.92-1.22	1.21	1.04-1.41	1.43	1.20-1.69	1.64	1.34-2.01	<0.001

Crude model – Dependent variable: cancer (yes/no). Independent variables: age (categorized), sex (binary).
Model 1 – Dependent variable: cancer (yes/no). Independent variables: age (categorical, 6), sex (binary), family history of cancer in 1st and 2nd -degree relatives (binary, no/yes), smoking status (categorical, 3), smoking intensity (pack-years, continuous), and intakes of energy (continuous), α-carotene (continuous), lycopene (continuous), calorie-adjusted total iron (continuous), PRAL or NEAP (categorical, 5).
Model 2 – Model 1 + total fiber (categorical, 5)
Model 3 – Model 1 + total fiber (categorical, 5) + Met (categorical, 5).

TABLE 8. Odds Ratios of cancer for the exposure to plant fiber and methionine in the whole sample. Comparisons between the employed regression models.

Fiber Cases/cont. (g/d)	I 889/1765 ≤ 5.21		II 807/1848 5.22 – 7.27		III 734/1924 7.28 – 9.58		IV 654/1998 9.59 – 12.97		V 652/1999 ≥ 12.98		Trend (p)
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Crude	1.00	---	0.87	0.78-0.98	0.76	0.68-0.86	0.65	0.58-0.73	0.65	0.57-0.73	<0.001
Adjusted	1.00	---	0.83	0.73-0.94	0.67	0.59-0.76	0.54	0.47-0.62	0.49	0.42-0.57	<0.001
Adj.incl.PRAL	1.00	---	0.87	0.77-0.99	0.73	0.64-0.83	0.62	0.53-0.72	0.60	0.50-0.72	<0.001
Adj.incl.NEAP	1.00	---	0.90	0.80-1.02	0.77	0.67-0.89	0.68	0.58-0.79	0.66	0.55-0.80	<0.001

Methionine Cases/cont. (g/d)	I 559/2097 ≤ 1.90		II 688/1964 1.91 – 2.43		III 714/1942 2.44 – 2.89		IV 825/1829 2.90 – 3.52		V 950/1702 ≥ 3.53		Trend (p)
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Crude	1.00	---	1.34	1.18-1.52	1.40	1.24-1.59	1.72	1.52-1.95	2.14	1.89-2.42	<0.001
Adjusted	1.00	---	1.32	1.15-1.51	1.41	1.22-1.63	1.66	1.41-1.94	1.97	1.64-2.37	<0.001
Adj.incl.PRAL	1.00	---	1.30	1.13-1.49	1.36	1.17-1.57	1.56	1.33-1.84	1.79	1.48-2.17	<0.001
Adj.incl.NEAP	1.00	---	1.19	1.03-1.37	1.18	1.00-1.38	1.28	1.07-1.54	1.38	1.10-1.73	0.011

Crude model – Dependent variable: cancer (yes/no). Independent variables: age (categorical, 6), sex (binary).
Adjusted model – Dependent variable: cancer (yes/no). Independent variables: age (categorical, 6), sex (binary), family history of cancer in 1st and 2nd -degree relatives (binary, no/yes), smoking status (categorical, 3), smoking intensity (pack-years, continuous), and intakes of energy (continuous), α-carotene (continuous), lycopene (continuous), calorie-adjusted total iron (continuous) + total fiber (categorical, 5), and methionine (categorical, 5).
Adj.incl.PRAL – Adjusted model + PRAL score (categorical, 5).
Adj.incl.NEAP – Adjusted model + NEAP score (categorical, 5).

Besides, the emphasis analysis on Met intake is unique for this study. Met is an essential, sulfur-containing amino acid necessary for normal growth and development, which functions as an initiator of protein synthesis. It is found in higher levels of animal foods such as pork, beef, dairy products, and eggs compared to a plant-based diet. In humans, Met is obtained from both food and gut microbes and is also supplied by autophagy. Met is used not only for protein synthesis but also as a direct precursor for S-adenosylmethionine (SAM), the universal methyl donor for DNA and proteins, including histones[49].

In addition, Met metabolites contribute to many metabolic processes, including the synthesis of polyamine and nucleotide and glutathione production[50]. The average daily requirement of Met is 10.4 mg/kg body weight/day[51]. Both excessive and too low Met in the diet can cause adverse effects. Western diets contain Met at levels many times higher than dietary requirements: the harmful effects of this amino acid on lifespan have been strongly related to its disadvantageous ability to promote oxidative stress by several mechanisms, which might facilitate the aging process, among other health disruptions[52]. In this sense, according to our database and calculations displayed in Table 2, the studied population has a very high daily Met intake (~ 40 mg/kg body weight/day).

High levels of dietary Met mimicking those seen in the Western diet (1.95% per weight) were found to alter the intestinal microbiome in rodents. These changes were associated with damage to the gut epithelium, a loss of expression of tight junction proteins, and translocation of bacterial genetic material to the liver[53]. Since the Bacteroidetes/Firmicutes ratio is also correlated with alterations in gene-specific DNA methylation in blood[54], changes in Met intake may also have indirect effects on DNA and protein methylation through the synthesis of compounds by the gut microbiome, such as folate. It also highlights the interconnections between diet, gut microbiome, epigenetic regulation, and finally the human health[49]. Besides, modifications in the "one-carbon metabolism" due to the excess of Met may exacerbate the toxic potential of homocysteine and its metabolites, affecting the "methylation index", which in turn will change gene regulation[55].

Some of the facts mentioned above led researchers to explore the MR, first reported three decades ago, which has similar physiological effects as caloric restriction and is related to longevity and metabolic health[52]. A glucose restriction down-regulates Met biosynthesis and uptake[56]. The dietary MR seems to be related to reduced oxidative stress, induction of autophagy (more specifically mitophagy), and activation of the transsulfuration pathway. However, the mechanisms are very complex and still not well understood. Studies have shown that an 80% MR increases the glutathione content in the erythrocytes of rats via transsulfuration[52]. Currently, there is a high interest in the effects of MR in the fight against cancer, not only due to the cited mechanisms but also to the Met addiction in cancer cells, known as the Hoffman effect[34,57]. This phenomenon describes the inability of cancer cells to proliferate when Met is replaced with its metabolic precursor, homocysteine. In contrast, the proliferation of non-tumor cells is unaffected by these conditions. Dietary MR has been demonstrated to have antitumor activity against a broad spectrum of tumors *in vivo* and extend survival[50,58].

In this context, it is mandatory to mention the amino acid glycine. As recently reviewed by Storz[59], glycine can act as a functional Met antagonist since it can fulfil the role of a methyl group acceptor in a biochemical reaction catalyzed by the enzyme glycine n-methyltransferase –a key enzyme in the methyl group metabolism. It seems that the level of SAM must be regulated in response to developmental stages and metabolic changes, and glycine n-methyltransferase has been shown to play a significant role in such regulation in mammals[60]. In addition, plant proteins are higher in glycine than most animal proteins[59]. Interestingly, more than a half century ago, reduced circulating concentrations of glycine in obese humans were recognized[61], and recently the participating mechanisms were experimentally discovered[62].

Including dietary fiber in the regression models (Table 7, Model 2) represented a remarkable effect on both DAL scores estimates. Regarding PRAL, the highest OR fell from 2.20 to 1.64 (a reduction of 25 %), and regarding NEAP, the highest OR fell from 2.35 to 1.93 (a decrease of 18 %). After adding a term for Met in the regression models, the estimates were slightly reduced, but they still yielded significantly increased ORs for each DAL score. We observed significant results either for the highest quintiles of PRAL score (OR= 1.44, *p*-trend <.001) as well as for the NEAP score (OR= 1.64, *p*-trend <.001), when the regression models included an adjustment for fiber and Met. On the one hand, the putative protective effect of alkalizing foods (mainly plant-source foods) might be influenced by their fiber content. On the contrary, the putative risk effect of acidifying foods (mainly animal-source foods) might be influenced by the content of the principal sulfur amino acid, Met, contained in them.

To explore possible independent effects of fiber and Met, we have analyzed them, as it is shown in Table 7. Even employing the more demanding regression models allowed us to keep significant estimates for both variables. Results made us to think that the calculated ORs for PRAL and NEAP scores were only partially dependent on their Met content (for their direct risk association) or their fiber content (for their inverse risk association). Therefore, Met, the main sulfur amino acid in the diet, is partially responsible for the DAL. Nevertheless, the reviewed literature on cancer does not emphasize its excess's potential acidifying effect nor mentions a possible alkalizing impact of Met restriction[49,52-54]. In contrast, the leading roles described as being played by this amino acid are an antioxidant activity of its derivatives[49] and the participation in the one-carbon metabolism[53]. The latter encompasses both the folate and Met cycles and allows cells to generate one-carbon units (also referred to as methyl groups) and utilize them for the biosynthesis of critical anabolic precursors and methylation reactions.

Our study has several strengths and limitations that require a detailed discussion. As for the weaknesses, the study included a detailed but non-validated FFQ due to external factors. However, as reported earlier, the FFQ was shown to be satisfactorily reproducible in other studies[35]. Although our investigation dates back to the early 2000s, the general dietary habits were relatively stable in the Uruguayan population[63], and recent studies demonstrated the same heavily meat-based pattern. As such, our nutritional assessment still provides sufficient validity. We tried to minimize selection bias by frequency-matching techniques. We matched controls and cases on age and residence in each of the original studies that are part of the current database. The results of such matching yielded a not-perfect distribution of cases and controls in the global database. Besides, some epidemiological items, such as occupational, microbial, medicine prescription and supplementation, or home exposures, needed to be better captured in our questionnaire, and we recognize them as a limitation. Finally, it would have been helpful to have more significant numbers of women. Still, during the study years, several cancers were not high-ranked tumors regarding their incidence or mortality in the female sex. This now represents limited information for the desirable comparison between sexes. Mineral estimations became one of the limitations of the present study since they were based on average serving sizes rather than actual food sizes. Finally, 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.

As for the strengths, all the interviews with participants were done face-to-face (excluding proxy interviews) by the same interviewers at the same institutions to reduce potential selection bias. Besides, the times of data collection were coincident. 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. In addition, the selected population sample was comprehensive from the viewpoint of country areas: ca. 50 % proceeded from the Capital city, Montevideo, and ca. 50% from the rest of the country, from each one of the other eighteen political departments. The low attrition rate of identified cases and controls (rates ~3%), favored by the interview performed during the hospital stay, limited possible selection bias. Another strength is the exclusion of individuals that reported previous dietary modifications. Furthermore, a recall bias related to nutritional habits should be negligible in our study population, as the awareness of cancer's dietary hypothesis was inexistent or very limited during the years of data collection.

CONCLUSIONS

Results confirm that an acidogenic dietary style may increase the risk of cancers. Our findings suggest that the intakes of Met and plant fiber might be independent factors, both influencing the risk linked to acid-base balance and epigenetic actions, but in opposite directions. Furthermore, Met intake displayed comparable ORs to the DAL scores themselves. The DAL could play a role directly associated with Met in terms of cancer risk. However, further studies are warranted to confirm these findings.

ORCID ID:

Alvaro L. Ronco 0000-0002-6328-1482; Wilner Martínez 0000-0002-9384-4231; Juan M. Calderón 0000-0002-4083-3686; Gabriela Mintegui 0000-0002-9747-176X; Maximilian Storz 0000-0003-3277-0301.

REFERENCES

1. Tran KB, Lang JJ, Compton K, Xu R, Acheson AR, Henrikson HJ, GBD 2019 Cancer Risk Factors Collaborators. The global burden of cancer attributable to risk factors, 2010-19: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2022; 400: 563-591.
2. Wang R, Wen ZY, Liu FH, Wei YF, Xu HL, Sun ML, Zhao YH, Gong TT, Wang HH, Wu QJ. Association between dietary acid load and cancer risk and prognosis: An updated systematic review and meta-analysis of observational studies. Front Nutr 2022; 9: 891936.
3. Friedenreich CM, Ryder-Burbidge C, McNeil J. Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. Mol Oncol 2021; 15: 790-800.
4. World Health Organization. Preventing cancer [Internet]. Available from: https://www.who.int/activities/preventing- cancer. Accessed December 14, 2022.
5. Mittelman SD. The role of diet in cancer prevention and chemotherapy efficacy. Annu Rev Nutr 2020; 40: 273-297.
6. Basen-Engquist K, Chang M. Obesity and cancer risk: recent review and evidence. Curr Oncol Rep 2011; 13: 71-76.
7. Lanou AJ, Svenson B. Reduced cancer risk in vegetarians: an analysis of recent reports. Cancer Manage Res 2010; 3: 1-8.
8. Gupta N, Patel HD, Taylor J, Borin JF, Jacobsohn K, Kenfield SA, Eggener SE, Price C, Davuluri M, Byrne N, Bivalacqua TJ, Loeb S. Systematic review of the impact of a plant-based diet on prostate cancer incidence and outcomes. Prostate Cancer Prostatic Dis 2022; 25: 444-452.
9. Banerjee T, Crews DC, Wesson DE, Tilea AM, Saran R, Ríos-Burrows N, Williams DE, Powe NR. High dietary acid load predicts ESRD among adults with CKD. J Am Soc Nephrol 2015; 26: 1693-1700.
10. Cao JJ. High dietary protein intake and protein-related acid load on bone health. Curr Osteoporos Rep 2017; 15: 571-576.
11. Alves Carnauba R, Baptistella AB, Paschoal V, Hübscher GH. Diet-induced low-grade metabolic acidosis and clinical outcomes: a review. Nutrients 2017; 9: 538.
12. Robey IF. Examining the relationship between diet-induced acidosis and cancer. Nutr Metab (Lond) 2012; 9: 72.
13. Shi Q, Maas L, Veith C, Van Schooten FJ, Godschalk RW. Acidic cellular microenvironment modifies carcinogen-induced DNA damage and repair. Arch Toxicol 2017; 91: 2425-2441.
14. Wang JX, Choi SYC, Niu X, Kang N, Xue H, Killam J, Wang Y. Lactic acid and an acidic tumor microenvironment suppress anticancer immunity. Int J Mol Sci 2020; 21: 8363.
15. Wada H, Hamaguchi R, Narui R, Morikawa H. Meaning and significance of "Alkalization Therapy for Cancer". Front Oncol 2022; 12: 920843.
16. Keramati M, Kheirouri S, Musazadeh V, Alizadeh M. Association of high dietary acid load with the risk of cancer: a systematic review and meta-analysis of observational studies. Front Nutr 2022; 9: 816797.
17. Bahrami A, Khalesi S, Ghafouri-Taleghani F, Alibeyk S, Hajigholam-Saryazdi M, Haghighi S, Ehsan H. Dietary acid load and the risk of cancer: a systematic review and dose-response meta-analysis of observational studies. Eur J Cancer Prev 2022; 31: 577-584.
18. Williamson M, Moustaid-Moussa N, Gollahon L. The molecular effects of dietary acid load on metabolic disease (The cellular pasadoble: the fast-paced dance of pH regulation). Front Mol Med 2021; 1: 777088.
19. Storz MA, Ronco AL. Quantifying dietary acid load in U.S. cancer survivors: an exploratory study using NHANES data. BMC Nutr 2022; 8: 43.
20. Ronco AL, Martínez-López W, Calderón JM, Mendoza BA. Dietary acid load and colorectal cancer risk: a case-control study. WCRJ 2020; 7: e1750.
21. Ronco AL, Martínez-López W, Calderón JM, Golomar W. Dietary acid load and lung cancer risk: A case-control study in men. Cancer Treat Res Commun 2021; 28: 100382.
22. Ronco AL, Martínez-López W, Mendoza BA, Calderón JM. Epidemiologic evidence for association between a high dietary acid load and the breast cancer risk. Sci Med J 2021; 3: 166-176.
23. Ronco AL, Storz MA, Martínez-López W, Calderón JM, Golomar W. High dietary acid load is associated with prostate cancer risk: an epidemiological study. WCRJ 2021; 8; e2119.
24. Ronco AL, Storz MA, Martínez-López W, Calderón JM, Golomar W. Dietary acid load and risk of kidney cancer: an epidemiologic case-control study. WCRJ 2021; 8: e2096.
25. Ronco AL, Storz MA, Martínez-López W, Calderón JM, Golomar W. Dietary acid load and bladder cancer risk: an epidemiologic case-control study. Multidiscip Cancer Investig 2022; 6: 1-12.
26. Ronco AL, Martínez-López W, Calderón JM, Storz MA. Dietary acid load and risk of head and neck and oral cavity cancers: an epidemiologic study. Oral Sci Int 2022; 1-10.
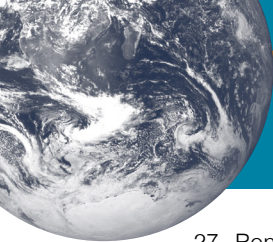
27. Ronco AL, Martínez-López W, Calderón JM, Storz MA. Dietary acid load and esophageal cancer risk: a case-control study. Thorac Cancer 2022; 1-8.

28. Ronco AL, Martínez-López W, Calderón JM, Mendoza B, Storz MA. Dietary acid load and risk of gastric cancer: a case-control study. WCRJ 2022; 9: e2403.

29. Elango R. Methionine nutrition and metabolism: insights from animal studies to inform human nutrition. J Nutr 2020; 150: 2518S-2523S.

30. Richie JP Jr, Sinha R, Dong Z, Nichenametla SN, Ables GP, Ciccarella A, Sinha I, Calcagnotto AM, Chinchilli VM, Reinhart L, Orentreich D. Dietary methionine and total sulfur amino acid restriction in healthy adults. J Nutr Health Aging 2023; 27: 111-123.

31. Osuna-Padilla IA, Leal-Escobar G, Garza-García CA, Rodríguez-Castellanos FE. Dietary Acid Load: mechanisms and evidence of its health repercussions. Nefrologia 2019; 39: 343-354.

32. Tariq A, Chen J, Yu B, Boerwinkle E, Coresh J, Grams ME, Rebholz CM. Metabolomics of dietary acid load and incident chronick kidney disease. J Renal Nutr 2022; 32: 292-300.

33. Smeha L, Scherlowski A, Franco YM, Gonzalez-Chica DA, Nunes EA. Dietary acid load is positively associated with insulin resistance: a population-based study. Clin Nutr 2022; 49: 341-347.

34. Kaiser P. Methionine dependence of cancer. Biomolecules 2020; 10: 568.

35. Ronco AL, De Stefani E, Boffetta P, Deneo-Pellegrini H, Acosta G, Mendilaharsu M. Food patterns and risk of breast cancer: A factor analysis study in Uruguay. Int J Cancer 2006; 19: 1672-1678.

36. Mazzei ME, Puchulu MR, Rochaix MA. Table of food chemical composition. Buenos Aires, Cenexa y Feiden Publishers, 2nd Ed., 1995. (in Spanish)

37. Food Data Central. United States Department of Agriculture. https:// fdc.nal.usda.gov/ fdc-app.html#/?-component=1215. Accessed December 1st, 2022.

38. Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. Am J Clin Nutr 1994; 59: 1356-1361.

39. Frassetto LA, Todd KM, Morris RC Jr, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 1998; 68: 576-583.

40. Betz MV, Penniston KL. Primary contributors to dietary acid load in patients with urolithiasis. J Renal Nutr 2023; 33: 53-58.

41. Pop C, Suharoschi R, Pop OL. Dietary fiber and prebiotic compounds in fruits and vegetables food waste. Sustainability 2021; 13: 7219.

42. He Y, Wang B, Wen L, Wang F, Yu H, Chen D, Su X, Zhang C. Effects of dietary fiber on human health. Food Sci Human Wellness 2021; 10: 545-554.

43. Demigné C, Sabboh H, Puel C, Rémésy C, Coxam V. Organic anions and potassium salts in nutrition and metabolism. Nutr Res Rev 2004; 17: 249-258.

44. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND, Kahleova H. The effects of vegetarian and vegan diets on gut microbiota. Front Nutr 2019; 6: 1-10.

45. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, Serrazanetti DI, Di Cagno R, Ferrocino I, Lazzi C, Turroni S, Cocolin L, Brigidi P, Neviani E, Gobbetti M, O'Toole PW, Ercolini D. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2016; 65: 1812-1821.

46. Wu G, Shi Y, Han L, Feng C, Ge Y, Yu Y, Tang X, Cheng X, Sun J, Le GW. Dietary methionine restriction ameliorated fat accumulation, systemic inflammation, and increased energy metabolism by altering gut microbiota in middle-aged mice administered different fat diets. J Agric Food Chem 2020; 68: 7745-7756.

47. Yang Y, Zhang Y, Xu Y, Luo T, Ge Y, Jiang Y, Shi Y, Sun J, Le GW. Dietary methionine restriction improves the gut microbiota and reduces intestinal permeability and inflammation in high-fat-fed mice. Food Funct 2019; 10: 5952.

48. Losno EA, Sieferle K, Perez-Cueto FJA, Ritz C. Vegan diet and the gut microbiota composition in healthy adults. Nutrients 2021; 13: 2402.

49. Wallis KF, Melnyk SB, Miousse IR. Sex-specific effects of dietary methionine restriction on the intestinal microbiome. Nutrients 2020; 12: 781.

50. Kitada M, Ogura Y, Monno I, Xu J, Koya D. Effect of methionine restriction on aging: its relationship to oxidative stress. Biomedicines 2021; 9: 130.

51. Nimni ME, Han B, Cordoba F. Are we getting enough sulfur in our diet? Nutr Metab (Lond) 2007; 4: 24.

52. Zhang Y, Jelleschitz J, Grune T, Chen W, Zhao Y, Jia M, Wang Y, Liu Z, Höhn A. Methionine restriction - Association with redox homeostasis and implications on aging and diseases. Redox Biol 2022; 57: 102464.

53. Miousse IR, Pathak R, Garg S, Skinner CM, Melnyk S, Pavliv O, Hendrickson H, Landes RD, Lumen A, Tackett AJ, Deutz NEP, Hauer-Jensen M, Koturbash I. Short-term dietary methionine supplementation affects one-carbon metabolism and DNA methylation in the mouse gut and leads to altered microbiome profiles, barrier function, gene expression and histomorphology. Genes Nutr 2017; 12: 22.

54 Ramos-Molina B, Sánchez-Alcoholado L, Cabrera-Mulero A, Lopez-Dominguez R, Carmona-Saez P, Garcia-Fuentes E, Moreno-Indias I, Tinahones FJ. Gut microbiota composition is associated with the global DNA methylation pattern in obesity. Front Genet 2019; 10: 613.

55. Kovalska M, Baranovicova E, Kalenska D, Tomascova A, Adamkov M, Kovalska L, Lehotsky J. Methionine diet evoked hyperhomocysteinemia causes hippocampal alterations, metabolomics plasma changes and behavioral pattern in wild type rats. Int J Mol Sci 2021; 22: 4961.

56. Zou K, Rouskin S, Dervishi K, McCormick MA, Sasikumar A, Deng C, Chen Z, Kaeberlein M, Brem RB, Polymenis M, Kennedy BK, Weissman JS, Zheng J, Ouyang Q, Li H. Life span extension by glucose restriction is abrogated by methionine supplementation: cross-talk between glucose and methionine and implication of methionine as a key regulator of life span. Sci Adv 2020; 6: eaba1306.

57. Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. Nat Rev Cancer 2019; 19: 625-637.

58. Sedillo JC, Cryns VL. Targeting the methionine addiction of cancer. Am J Cancer Res 2022; 12: 2249-2276.

59. Storz MA, Ronco AL, Hannibal L. Observational and clinical evidence that plant-based nutrition reduces dietary acid load. J Nutr Sci 2022; 11: 1-15.

60. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. J Biol Chem 2009; 284: 22507-22511.

61. Felig P, Marliss E, Cahill GF Jr. Plasma amino acid levels and insulin secretion in obesity. N Engl J Med 1969; 281: 811-816.

62. Simmons RM, McKnight SM, Edwards AK, Wu G, Satterfield MC. Obesity increases hepatic glycine dehydrogenase and aminomethyltransferase expression while dietary glycine supplementation reduces white adipose tissue in Zucker diabetic fatty rats. Amino Acids 2020; 52: 1413-1423.

63. Moliterno P, Donangelo CM, Borgarello L, Pécora M, Olascoaga A, Noboa O, Boggia J. Association of dietary patterns with cardiovascular and kidney phenotypes in an Uruguayan population cohort. Nutrients 2021; 13: 2213.