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ORIGINAL CONTRIBUTION

Association between salt intake and gastric atrophy by *Helicobacter pylori* infection: first results from the Epidemiological Investigation of Gastric Malignancy (ENIGMA)

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Abstract

Purpose Gastric atrophy (GA), usually linked to chronic infection with *Helicobacter pylori* (*H. pylori*), may over time evolve into gastric malignancy. Besides *H. pylori*, high salt intake may play a role in GA development. This study evaluates cross sectionally the association between salt intake and GA in Chilean adults.

Methods Population-based samples were recruited from two sites, Antofagasta and Valdivia, partaking in the Epidemiological Investigation of Gastric Malignancies. At recruitment, participants answered questionnaires and provided biospecimens. Salt intake (g/day) was estimated from casual spot urine samples using the Tanaka equation. GA was determined by serum pepsinogen levels. Only participants ≥ 40 to 70 years of age were considered in this analysis, $n = 565$. For the association between salt intake (as sex-specific quartiles) and GA, odds ratios (ORs) and the corresponding 95% confidence intervals (CI) were estimated through multivariable logistic regression.

Results In women, the multivariable-adjusted OR for GA comparing quartile 4 of the estimated salt intake (12.8 g/day) to quartile 1 (6.6 g/day) was 1.18 (95% CI 0.52–2.68, P-trend = 0.87). The corresponding OR in men was 0.49 (95% CI 0.19–1.27, P-trend = 0.17) with salt intakes of 12.8 g/day and 7.1 g/day for quartiles 4 and 1, respectively.

Conclusion There was little evidence for an association between salt intake estimated from spot urine and GA risk in our cross-sectional analysis of middle aged and older adults in Chile. Reverse causation bias cannot be ruled out and the sample size was limited to provide more precise estimates.

Keywords Atrophic gastritis · Stomach cancer · Sodium excretion · *H. pylori* · Tanaka equation

Introduction

Gastric cancer is the fourth leading cause of cancer deaths worldwide largely due to difficulty screening for its early signs, often resulting in late diagnosis and delayed treatment [1]. In 2020, over 1 million new cancers and 769,000 cancer-related deaths were attributed to gastric cancer worldwide [1]. Among the Latin American countries, Chile has the highest age-standardised incidence rate (ASR) of gastric cancer (ASR of 26.9/100,000 men; 10.3/100,000 women) and it is the leading cause of cancer deaths in Chilean men (ASR of 17.9/100,000 ASR) [2]. Gastric cancer is a heterogeneous and multifactorial disease. Chronic bacterial infection with *Helicobacter pylori* (*H. pylori*) is a well-established cause, particularly of the major intestinal subtype non-cardia adenocarcinomas [3], and it has shown to be a major determining factor in the premalignant stages involving gastric mucosal atrophy (GA) [4, 5]. *H. pylori* persisting in the gastric mucosa can lead to progressively abnormal tissue changes resulting in non-atrophic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia, and eventually neoplasia [5]. In addition to *H. pylori*, other modifiable risk factors, particularly those related to diet and lifestyle, are likely to be involved in gastric cancer

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aetiology [6]. Dietary salt may increase the risk of gastric cancer by damaging the gastric mucosal lining, leading to inflammation, atrophy and *H. pylori* colonisation [5, 6]. In animal models, high salt levels were shown to induce GA in rodents and be responsible for the primary cellular damage conducive to gastric cancer development [7–10]. Deleterious effects of a high salt diet on gastric mucosa may be exacerbated when infected with *H. pylori*, particularly the high-virulence cytotoxin-associated gene A (*cagA*) strains producing oncoproteins known as CagA [8, 10].

While numerous human studies of varying designs have investigated the association between salt intake and the risk of gastric cancer [11], the evidence is still inconclusive. Studies looking specifically at the associations between salt intake and precancerous lesions, such as GA, stratified by *H. pylori* infection are particularly scarce. When salt intake was assessed by self-reported dietary questionnaires a lack of association with GA (assessed by histology) was observed in *H. pylori*-infected individuals and overall [12, 13]. Salt intake assessed by self-reported dietary instruments is known to inadequately capture the hidden salt, particularly in processed foods [14] and the discretionary salt added at the table, likely contributing to those inconsistent findings [15]. For comparison, when 24-h urinary sodium (Na) excretion was used as a proxy for salt intake in a Korean study, a significantly higher odds of having atrophic gastritis with intestinal metaplasia was observed in the highest exposure group (OR 2.87; 95% CI 1.34–6.14 for salt intake of ~ 11 g/day) after adjusting for *H. pylori* infection [16]. Na excretion measured in 24-h urine is an objective measure and considered the gold standard for salt intake assessment; however, it is generally unfeasible to obtain from study participants. Salt intake estimated from spot urine is considered as the next best proxy [17]. Additionally, inadequate adjustment for *H. pylori* infection with the CagA-producing strains may be limiting our knowledge regarding the role of dietary salt in the development of GA and gastric cancer.

Two main studies estimated the mean salt intake in Chilean adults (≥ 15 years old) to be 10–11 g/day [18, 19], more than double the daily intake recommended by the WHO [20]. Both studies used the Tanaka prediction equation shown to adequately estimate mean salt intake from spot urine when the actual salt consumption is between 9 and 12 g/day [21, 22]. Despite the high salt consumption and a high burden of gastric cancer in Chile, studies assessing the relationship between salt intake and the risk of GA, a precursor of gastric cancer, are lacking in this population.

The aim of this study was to evaluate the association between daily salt intake estimated from Na excretion and GA in adults living in two areas of Chile, namely Antofagasta and Valdivia, with contrasting local GC risk. Both study sites partake in the Epidemiological Investigation of Gastric Malignancies (ENIGMA), a series of global prevalence surveys coordinated by IARC/WHO [23]. We hypothesized that a high daily intake of salt is positively associated with GA and that *H. pylori* infection has a synergistic effect with high salt intake for GA. We decided a priori to analyse men and women separately given the risk differences.

Materials/subjects and methods

Study population

The ENIGMA cohort in Chile has been described elsewhere [23]. Briefly, the ENIGMA study in Chile recruited a population-based sample of 1395 participants who were between 1 and 69 years of age by stratifying on site, sex, and 5-year age groups between May 2014 and August 2015 [23]. The study participants were selected to represent the general population in two areas of Chile, Antofagasta with gastric cancer ASR of 21/100,000 and Valdivia with ASR of 33/100,000 [24]. The sampling frame in Antofagasta was the whole city while it was the Barrios Bajos sector in Valdivia representing the city's socio-economic diversity; "socio-economically homogeneous blocks were defined and then randomly selected in each conglomerate" [23]. First, households within the blocks and then individuals within the selected households were randomly selected based on predefined eligibility criteria, which included (1) mental and physical competence to participate in the study and (2) no history of GC [23]. Individuals refusing to participate were replaced based on the sampling criteria until a sample size of ~ 700 per site was reached. The overall response rates for all ages (i.e.1–69 years) were 84.5% and 71.4% in Antofagasta ($n = 690$) and Valdivia ($n = 705$), respectively [23]. Fieldwork was conducted predominantly during standard working hours. At recruitment, all participants gave their informed consent prior to their inclusion in the study. The participants were then asked to provide biological samples (blood, urine, and stool) and answer study-specific questionnaires on risk factors of gastric cancer such as household crowding, lifestyle habits, and family history of gastric cancer. Because our outcome of interest was GA, which is a result of a series of long-term changes in the gastric mucosa caused by chronic infection with *H. pylori*, only participants who were ≥ 40 years of age in the ENIGMA study ($n = 618$) were tested for serum pepsinogen levels to detect GA and considered in this analysis.

Salt intake estimation

Salt intake was estimated in grams of salt per day (g/day) from predicted 24-h urinary Na excretion which, in turn, was estimated from Na and creatinine measured in spot urine

provided by the participants at their convenience but within 48 h of blood collection and completing the study questionnaires. Urinary Na was measured by ion-sensitive electrodes with the Cobas ISE indirect Na–K–Cl Gen. 2 assay on Cobas 8000 ISE analysers. Urinary creatinine was measured with the Cobas Creatinine Jaffe Gen.2 assay on Cobas c 702 clinical chemistry analysers (Roche Diagnostics, Mannheim, Germany) analysed at the Medical University of Vienna. To obtain 24-h urinary Na excretion, the crude urinary Na concentrations were adjusted for urinary creatinine levels using the Tanaka prediction equation [21]. The Tanaka equation first predicts 24-h creatinine excretion based on the subject's weight, height, age, and spot creatinine concentration. The predicted 24-h creatinine is then used to estimate 24-h urinary Na excretion (in mg) using spot urine concentrations of Na and creatinine. The 24-h urinary Na excretion in mg was converted to salt intake in g/day, considering that 1 g of table salt (NaCl) contains about 400 mg of Na and that, on average, 95% of Na from the diet is excreted in the urine, respectively [25, 26].

Determination of gastric atrophy

GA was the binary outcome variable determined by serum pepsinogens I (PgI) and II (PgII) levels and their ratio (PgI/PgII). With the progression of gastric atrophy, PgI levels decrease gradually, while PgII levels remain constant, resulting in a reduction of PgI/PgII [27]. PgI and PgII were blindly measured with a latex-agglutination test system (Eiken Chemical, Tokyo, Japan) at the University of Latvia. GA of any severity was defined as having both PgI $\leq$ 70 ng/ml and PgI/PgII ratio $\leq$ 3. Advanced GA was defined as having both PgI $\leq$ 30 ng/ml and PgI/PgII $\leq$ 2, according to the manufacturer's reference values and previous validation by collaborators [28].

Assessment of lifestyle and other variables

Data on socio-demographic and lifestyle factors including education level, smoking history, medical history, and diet were self-reported using a standardized questionnaire designed to identify risk factors related to gastric cancer [23]. For diet, a limited food frequency questionnaire was administered that aimed to investigate specific foods/food groups that were likely to be associated with gastric cancer in Chile. The food frequency questionnaire contained 22 different food items (at times groups of related foods) and standard serving portions were provided only for the alcoholic beverage items. Participants were asked to report on their habitual frequency of consumption of specific foods in the last 3 years. They were then asked to recall the habitual consumption of those same food items 20 years ago. Anthropometric measurements were taken by the local study team trained on the study-specific standard operating procedures. *H. pylori* status was assessed at IARC by measuring anti-*H. pylori* IgG in serum (Biohit Plc, Finland) where $\geq$ 30 IEU was defined to be as *H. pylori* positive. To detect IgG antibodies against specific proteins of *H. pylori*, including the CagA, Helicoblot 2.1 immunoblot kit (Genelabs Diagnostics, Singapore) was performed at Bordeaux University, France.

In this study, we excluded those subjects whose GA status was missing ($n = 13$) and who did not provide spot urine samples or if their crude urinary Na concentrations exceeded the upper limits of linearity defined as > 250 mmol/L ($n = 33$). We further excluded 7 participants because of missing weight, height, or age; information needed to calculate the 24-h Na excretion. The final dataset contained 565 participants.

Statistical analyses

Lifestyle, dietary and other characteristics of the study participants were summarised according to categories of salt intake estimated from spot urine. Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between salt intake (the lowest quartile as the reference) and GA status. All models were stratified by sex to account for differences in gastric cancer risk between men and women. In an exploratory analysis, we fitted models separately by study site, but no sizeable differences in point estimates were observed and all results presented are for both study sites combined. Models with two sets of adjustments were fit. Model 1 was adjusted for study site and age. Model 2 was further adjusted for education (years), smoking status (never, former, current), daily alcohol intake (g/day), BMI (kg/m^2), *H. pylori* infection (positive/negative), and weekly frequencies of fruit, vegetables, and chilli consumption (number of times/week). We also fitted Model 2 stratifying by *H. pylori* status to explore potential effect modification. We performed the following sensitivity analysis: (1) using pack-years (a continuous variable) instead of the categorical one to adjust for smoking; (2) using waist-to-height ratio computed as waist circumference (cm) divided by height (cm) to adjust for body fatness instead of BMI; (3) using CagA positive *H. pylori* infection; and (4) using, separately, quartiles of the crude Na-to-creatinine ratio as well as quartiles of systolic blood pressure as analogous exposures and proxies of salt intake to compare the OR to those obtained for the quartiles of estimated salt intake. We also replicated the above analysis by fitting the models using the dietary variables (daily alcohol intake and weekly frequencies of consumption of fruits, vegetables, and chilli) reported by the participants as consumed 20 years ago. Finally, to explore synergistic effects of salt intake and *H. pylori* we created a variable combining *H.*

pylori infection status with high or low salt intake, using the median salt intake as the cut-off. Results were considered statistically significant at a two-sided $p < 0.05$. All analyses were performed using R: A Language and Environment for Statistical Computing (version 4.0.1).

Results

The study population consisted of slightly more participants from Valdivia (53.8%, $n = 304$) and more women (57.2%, $n = 323$). Mean salt intake for all participants combined was estimated at 9.7 g/day (2.4 SD) and ranged from 3.8 to 20.7 g/day. Men and women had similar mean salt intake, 9.8 g/day (2.3 SD) versus 9.6 g/day (2.5 SD), respectively.

Tables 1 and 2 show the main characteristics of female and male participants, respectively, by quartiles of estimated salt intake. In both sexes, the proportion of participants from Antofagasta, the non-Hispanic Chileans, and current smokers, tended to increase with increasing salt intake. Participants with higher salt intake also smoked more (in pack-years) and had higher BMI and waist-to-height ratio (the latter in women only). No evident differences in the frequency of fruit or vegetable intake were observed across the quartiles of estimated salt intake. Infection with CagA positive *H. pylori* strains reflected closely the overall *H. pylori*-positive status across the quartiles of salt intake.

In women, the proportions of those with GA, with *H. pylori* infection and with habitual use of table salt, were inconsistent across the quartiles of salt intake (Table 1). However, women in the highest quartile of salt intake were generally younger, had higher alcohol intake (in g/day), and consumed chilli less frequently.

In men, the reported use of table salt, alcohol intake, and frequency of chilli consumption were inconsistent across the quartiles of estimated salt intake (Table 2). However, the proportion of men with any GA tended to decrease while the proportion with *H. pylori* infection increased with higher salt intake.

Table 3 shows the relationship between the estimated salt intake in quartiles and the odds of having GA by sex. In women, no association between salt intake and GA was observed in the minimally and fully adjusted models (i.e. models 1 and 2, respectively); however, both models suggested a positive association, albeit with wide confidence intervals.

In men, a suggestive inverse association between estimated salt intake and any atrophy was observed, which however did not reach formal statistical significance (Table 3). In the fully adjusted models (Model 2), the p-trend for linearity was not statistically significant in either women ($p = 0.87$) or men ($p = 0.17$).

Substituting CagA status for *H. pylori* seroprevalence (Model 3), or waist-to-height for BMI (Model 4) or pack-years for the categorical smoke variable (Model 5) did not change the OR estimates in women or men compared to those in Model 2 (Supplementary table 1). Moreover, fitting the fully adjusted model with quartiles of urinary spot Na-to-creatinine ratio as another plausible proxy of salt intake (Model 6) did not meaningfully alter the associations. On the other hand, fitting quartiles of systolic blood pressure as another proxy of salt intake (Model 7) reversed the direction of the associations in men. The test for linearity was not significant ($p = 0.49$) for the fully adjusted Model 7 in men. In summary, the sensitivity analyses did not materially modify these observed associations.

There was little evidence for synergistic association when analysing salt intake and *H. pylori* status jointly (Table 4).

Discussion

Understanding the association between salt intake and GA (a precursor of gastric cancer) may shed light on the role of salt in the early phases of gastric cancer development and provide clues for preventive strategies, particularly when coupling *H. pylori* eradication and salt intake reduction in high-risk populations such as that of Chile. Nevertheless, our findings did not show a clear association between estimated salt intake and GA determined by serum pepsinogen levels in Chilean women and men. Contrary to our hypothesis, we observed a suggestive inverse association between what would be considered high salt intake (9.0–13 g/day estimated from spot urine) and GA in men. A case–control study in Venezuela previously reported a strong inverse association (OR 0.1, 95% CI 0.1–0.2) between gastric cancer and high urinary Na excretion (adjusted for creatinine) after controlling for age, sex and socio-economic status [29]. Muñoz et al. attributed this inverse association to the cases, most with advanced lesions, who were likely to reduce their salt intake to control gastric symptoms. However, GA is mostly asymptomatic and unlikely to induce major dietary changes. We also worked under the premise that an individual's salt intake is quite habit-forming and therefore stable enough to rank the subjects prior to the onset of morbidities. When we further investigated this by fitting the models with dietary variables reported by the participants as consumed 20 years ago, the suggested inverse associations in men did not change. Recency bias may play a role in this finding as most participants will poorly recall their diet from 20 years ago tending to report a more recent diet. Moreover, due to the cross-sectional design of our study, we cannot exclude reverse causation bias (outcome causing a change in exposure). This bias could have been more pronounced among men than among women and may be one reason for

Table 1 Main characteristics of women in ENIGMA Chile according to the quartiles of salt intake

Characteristic	Q1	Q2	Q3	Q4	All
Number of participants	81	81	81	80	323
Salt intake, g/day	6.6 (1.1)	8.7 (0.4)	10.2 (0.6)	12.8 (1.5)	9.6 (2.5)
Site	(missing, $n=0$)				
Antofagasta, *n* (%)	32 (39.5)	44 (54.3)	46 (56.8)	47 (58.8)	169 (52.3)
Valdivia, *n* (%)	49 (60.5)	37 (45.7)	35 (43.2)	33 (41.3)	154 (47.7)
Ethnicity	(missing, $n=3$)				
Chilean Hispanic, *n* (%)	75 (92.6)	73 (90.1)	72 (88.9)	69 (86.3)	289 (89.5)
Other, *n* (%)	6 (7.4)	8 (9.9)	9 (11.1)	11 (13.8)	34 (10.5)
Age, years	55.1 (9.3)	54.5 (9.0)	54.9 (7.7)	54.9 (7.9)	54.9 (8.5)
BMI, kg/m^2	29.5 (5.1)	29.3 (4.8)	29.7 (4.7)	32.1 (6.6)	30.1 (5.4)
Waist-to-height ratio	0.60 (0.09)	0.60 (0.08)	0.61 (0.07)	0.64 (0.09)	0.61 (0.08)
Education, years	11.7 (4.1)	11.7 (4.2)	10.8 (3.8)	10.6 (3.8)	11.2 (4.0)
Smoking status (categorical)	(missing, $n=3$)				
Never, *n* (%)	44 (54.3)	37 (45.7)	41 (50.6)	30 (37.5)	152 (47.1)
Former, *n* (%)	15 (18.5)	20 (24.7)	20 (24.7)	24 (30.0)	79 (24.5)
Current, *n* (%)	21 (25.9)	24 (29.6)	20 (24.7)	25 (31.3)	90 (27.9)
Smoking, pack-years	5.0 (10.4)	5.8 (11.7)	5.4 (10.6)	5.5 (12.1)	5.4 (11.2)
Gastric atrophy status	(missing, $n=0$)				
No atrophy, *n* (%)	60 (74.1)	63 (77.8)	61 (75.3)	56 (70.0)	240 (74.3)
With any atrophy, *n* (%)	21 (25.9)	18 (22.2)	20 (24.7)	24 (30.0)	83 (25.7)
With advanced atrophy, *n* (%)	7 (8.6)	5 (6.2)	8 (9.9)	8 (10.0)	28 (8.7)
H. pylori infection	(missing, $n=5$)				
Negative, *n* (%)	16 (19.8)	26 (32.1)	17 (21.0)	17 (21.3)	76 (23.5)
Positive, *n* (%)	64 (79.0)	53 (65.4)	63 (77.8)	62 (77.5)	242 (74.9)
CagA infection	(missing, $n=3$)				
Negative, *n* (%)	24 (29.6)	26 (32.1)	17 (21.0)	18 (22.5)	85 (26.3)
Positive, *n* (%)	57 (70.4)	53 (65.4)	63 (77.8)	62 (77.5)	235 (72.8)
Use of table salt	(missing, $n=2$)				
Never, *n* (%)	59 (72.8)	62 (76.5)	56 (69.1)	63 (78.8)	240 (74.3)
Sometimes, *n* (%)	17 (21.0)	14 (17.3)	18 (22.2)	7 (8.8)	56 (17.3)
Always, *n* (%)	5 (6.2)	4 (4.9)	7 (8.6)	9 (11.3)	25 (7.7)
Chilli intake (categorical)	(missing, $n=3$)				
Never consumer, *n* (%)	29 (35.8)	27 (33.3)	21 (25.9)	24 (30.0)	101 (31.3)
Less than daily, *n* (%)	40 (49.4)	45 (55.6)	53 (65.4)	45 (56.3)	183 (56.7)
Daily and more, *n* (%)	12 (14.8)	8 (9.9)	7 (8.6)	9 (11.3)	36 (11.2)
Chilli intake frequency, times/week	4.8 (5.9)	4.4 (6.8)	4.3 (5.1)	3.6 (3.5)	4.3 (5.4)
Alcohol intake, g/day	2.4 (4.5)	2.2 (3.5)	2.6 (5.5)	2.6 (4.3)	2.5 (4.5)
Fruit intake (categorical)	(missing, $n=3$)				
Never consumer, *n* (%)	1 (1.2)	8 (9.9)	1 (1.2)	7 (8.8)	17 (5.3)
Less than daily, *n* (%)	38 (46.9)	40 (49.4)	52 (64.2)	46 (57.5)	176 (54.5)
Daily and more, *n* (%)	42 (51.9)	33 (40.7)	27 (33.3)	26 (32.5)	128 (39.6)
Fruit intake frequency, times/week	5.4 (5.0)	4.9 (4.3)	5.3 (6.0)	5.2 (5.9)	5.2 (5.3)
Vegetable intake (categorical)	(missing, $n=3$)				
Never consumer, *n* (%)	0 (0)	1 (1.2)	1 (1.2)	1 (1.3)	3 (0.9)
Less than daily, *n* (%)	41 (50.6)	42 (51.9)	48 (59.3)	43 (53.8)	174 (53.9)
Daily and more, *n* (%)	40 (49.4)	38 (46.9)	32 (39.5)	36 (45.0)	146 (45.2)
Vegetable intake frequency, times/week	7.6 (4.2)	8.1 (5.1)	7.8 (4.4)	7.3 (4.2)	7.7 (4.5)

Salt intake was estimated from casual spot urine using the Tanaka equation

Unless otherwise indicated, values for continuous variables represent the mean (SD)

CagA, *Helicobacter pylori* cytotoxin-associated gene A protein; *H. pylori, Helicobacter pylori*; Q1 to Q4, quartiles 1 to 4

Table 2 Main characteristics of men in ENIGMA Chile according to the quartiles of salt intake

Characteristic	Q1	Q2	Q3	Q4	All
Number of participants	61	61	60	60	242
Salt intake, g/day	7.1 (1.0)	9 (0.4)	10.5 (0.5)	12.8 (1.2)	9.8 (2.3)
Site					
Antofagasta, *n* (%)	21 (34.4)	22 (36.1)	23 (38.3)	26 (43.3)	92 (38)
Valdivia, *n* (%)	40 (65.6)	39 (63.9)	37 (61.7)	34 (56.7)	150 (62)
Ethnicity	(missing, $n=0$)				
Chilean Hispanic, *n* (%)	54 (88.5)	57 (93.4)	55 (91.7)	49 (81.7)	215 (88.8)
Other, *n* (%)	7 (11.5)	4 (6.6)	5 (8.3)	11 (18.3)	27 (11.2)
Age, years	55.7 (8.9)	55.6 (8.8)	53.7 (8.1)	52.2 (7.5)	54.3 (8.4)
BMI, kg/m^2	28.8 (4.2)	29.4 (4.5)	29.3 (4.1)	29.6 (3.9)	29.3 (4.2)
Waist-to-height ratio	0.59 (0.07)	0.60 (0.08)	0.59 (0.07)	0.59 (0.06)	0.59 (0.07)
Education, years	11.9 (3.1)	12.2 (4.5)	11.7 (4)	11.8 (3.9)	11.9 (3.9)
Smoking status (categorical)	(missing, $n=0$)				
Never, *n* (%)	23 (37.7)	24 (39.3)	17 (28.3)	16 (26.7)	80 (33.1)
Former, *n* (%)	27 (44.3)	20 (32.8)	18 (30.0)	23 (38.3)	88 (36.4)
Current, *n* (%)	11 (18.0)	17 (27.9)	25 (41.7)	21 (35.0)	74 (30.6)
Smoking, pack-years	5.8 (8.9)	9.3 (13.7)	8.5 (11.6)	14.9 (24.2)	9.6 (15.9)
Gastric atrophy status	(missing, $n=0$)				
No atrophy, *n* (%)	39 (63.9)	49 (80.3)	44 (73.3)	46 (76.7)	178 (73.6)
With any atrophy, *n* (%)	22 (36.1)	12 (19.7)	16 (26.7)	14 (23.3)	64 (26.5)
With advanced atrophy, *n* (%)	8 (13.1)	5 (8.2)	5 (8.3)	6 (10.0)	24 (9.9)
H. pylori infection	(missing, $n=0$)				
Negative, *n* (%)	13 (21.3)	13 (21.3)	9 (15.0)	7 (11.7)	42 (17.4)
Positive, *n* (%)	48 (78.7)	48 (78.7)	51 (85.0)	53 (88.3)	200 (82.6)
CagA infection	(missing, $n=1$)				
Negative, *n* (%)	13 (21.3)	12 (19.7)	17 (28.3)	12 (20.0)	54 (22.3)
Positive, *n* (%)	48 (78.7)	49 (80.3)	43 (71.7)	47 (78.3)	187 (77.3)
Use of table salt	(missing, $n=0$)				
Never, *n* (%)	47 (77.1)	47 (77.1)	45 (75.0)	43 (71.7)	182 (75.2)
Sometimes, *n* (%)	10 (16.4)	9 (14.8)	10 (16.7)	9 (15.0)	38 (15.7)
Always, *n* (%)	4 (6.6)	5 (8.2)	5 (8.3)	8 (13.3)	22 (9.1)
Chilli intake (categorical)	(missing, $n=1$)				
Never consumer, *n* (%)	18 (29.5)	15 (24.6)	14 (23.3)	14 (23.3)	61 (25.2)
Less than daily, *n* (%)	32 (52.5)	31 (50.8)	31 (51.7)	35 (58.3)	129 (53.3)
Daily and more, *n* (%)	11 (18.0)	14 (23.0)	15 (25.0)	11 (18.3)	51 (21.1)
Chilli intake frequency, times/week	4.9 (6.0)	6.8 (8.2)	7.5 (9.5)	5.0 (7.3)	6.0 (7.9)
Alcohol intake, g/day	8.8 (20.5)	6.3 (8.0)	6.9 (8.2)	7.8 (10.7)	7.4 (12.9)
Fruit intake (categorical)	(missing, $n=1$)				
Never consumer, *n* (%)	1 (1.6)	2 (3.3)	4 (6.7)	1 (1.7)	8 (3.3)
Less than daily, *n* (%)	27 (44.3)	40 (65.6)	39 (65.0)	41 (68.3)	147 (60.7)
Daily and more, *n* (%)	33 (54.1)	19 (31.2)	17 (28.3)	18 (30.0)	87 (36.0)
Fruit intake frequency, times/week	5.6 (6.6)	4.1 (4.3)	5.2 (7.9)	4.1 (4.5)	4.8 (6.0)
Vegetable intake (categorical)	(missing, $n=0$)				
Never consumer, *n* (%)	1 (1.6)	0 (0)	1 (1.7)	1 (1.7)	3 (1.2)
Less than daily, *n* (%)	35 (57.4)	39 (63.9)	42 (70.0)	35 (58.3)	151 (62.4)
Daily and more, *n* (%)	25 (41.0)	22 (36.1)	17 (28.3)	24 (40.0)	88 (36.4)
Vegetable intake frequency, times/week	8.3 (7.2)	6.8 (4.3)	6.5 (3.7)	7.8 (4.7)	7.4 (5.2)

Salt intake was estimated from casual spot urine using the Tanaka equation

Unless otherwise indicated, values for continuous variables represent the mean (SD)

CagA, *Helicobacter pylori* cytotoxin-associated gene A protein; *H. pylori, Helicobacter pylori*; Q1–Q4, quartiles 1 to 4

Table 3 Odds ratios and 95% confidence intervals for gastric atrophy according to quartiles of salt intake

	n	*n* with GA/without GA	Q1	Q2 (CI)	Q3 (CI)	Q4 (CI)
Women						
Model 1	323	83/240	1 (reference)	0.89 (0.43–1.85)	1.03 (0.50–2.11)	1.35 (0.67–2.73)
Model 2	275	75/200	1 (reference)	1.10 (0.49–2.46)	0.85 (0.38–1.90)	1.18 (0.52–2.68)
Model 2 *HP*−	69	15/54	1 (reference)	0.30 (0.03–2.62)	0.19 (0.02–2.30)	0.83 (0.11–6.60)
Model 2 *HP*+	206	60/146	1 (reference)	1.37 (0.54–3.44)	1.01 (0.41–2.48)	1.13 (0.44–2.88)
Men						
Model 1	242	64/178	1 (reference)	0.43* (0.19–0.99)	0.71 (0.32–1.56)	0.63 (0.28–1.43)
Model 2	219	55/164	1 (reference)	0.28* (0.11–0.75)	0.45 (0.18–1.14)	0.49 (0.19–1.27)
Model 2 *HP*-	38	8/30	1 (reference)	1.45 (0.07–30.36)	0.09 (0.00–10.55)	0.07 (0.00–10.34)
Model 2 *HP*+	181	47/134	1 (reference)	0.18* (0.06–0.56)	0.41 (0.15–1.15)	0.43 (0.15–1.25)

Model 1 was adjusted for the site and age

Model 2 was adjusted for the site, age, education (years), smoking status (never, former, current), BMI (kg/m^2), daily alcohol intake (g/d), and frequency of consumption of fruit, vegetable, and chili (number of times/week). P-trend for linearity was p = 0.87 for women and p = 0.17 for men

CI, 95% confidence intervals; GA, gastric mucosal atrophy of any severity; *HP-*, *Helicobacter pylori-negative*; *HP+*, *Helicobacter pylori-positive*; Q1 to Q4, odds ratios for quartiles 1 to 4

* $p < 0.05$

Salt intake was estimated from casual spot urine using the Tanaka equation

Table 4 Analysis of high and low salt intake, *Helicobacter pylori* infection status, and risk for gastric atrophy

High salt intake[a]	*HP* infection	Women		Men		All combined	
		n with GA/without GA	Odds Ratio (CI)	*n* with GA/without GA	Odds Ratio (CI)	*n* with GA/without GA	Odds Ratio (CI)
Yes	Yes	31/72	1.46 (0.55–3.91)	22/68	1.07 (0.32–3.58)	53/143	1.23 (0.59–2.56)
No	Yes	29/74	1.66 (0.63–4.39)	25/66	1.34 (0.43–4.18)	54/137	1.43 (0.70–2.94)
Yes	No	7/25	1.20 (0.35–4.09)	2/15	0.50 (0.08–3.34)	8/41	0.69 (0.25–1.90)
No	No	8/29	1 (reference)	6/15	1 (reference)	15/43	1 (reference)

Salt intake was estimated from casual spot urine using the Tanaka equation

CI 95% confidence intervals, *GA* gastric atrophy of any severity, *HP Helicobacter pylori*

[a]Salt intake was considered as high when above the median estimated daily salt intake for the respective group (9.3 g/day for women, 9.6 g/day for men and 9.5 g/day for both sexes combined)

the differential association observed. This is supported by the slightly higher prevalence of GA, particularly advanced atrophy (9.9% vs. 8.7%).

Alternatively, Song et al. reported a significant positive association (OR 2.87; 95% CI 1.34–6.14) between high urinary Na and GA with intestinal metaplasia in a Korean case–control study [16]. In both, our study and the study by Muñoz et al., Na excretions from single spot urine samples were used as proxies for salt intake which could be one reason for the divergent results from those reported by Song et al. where Na was measured directly in urine collected over 24 h (the gold standard). The Tanaka equation used seemed appropriate for our population; although validation studies also showed that this equation increasingly underestimated the predicted 24-h Na excretion when Na excretion was below 156 mmol of Na/day (8.5 g of salt/day) and when actual salt intake was above 12 g/day [21, 22]. In our study, this would mean that some proportion of the participants, particularly men with a generally higher 24-h Na excretion [30], could have their salt intake misclassified and could be one reason for the null result when testing for linearity. However, the sensitivity analysis using a crude Na-to-creatinine ratio from spot urine as a proxy of salt intake revealed very similar results to those based on the Tanaka equation. Only

when we used systolic blood pressure as an analogous exposure [31] and another proxy for salt intake, particularly in salt-sensitive individuals, we observed a suggestive positive association with GA. Therefore, not the Tanaka equation alone, but also using single casual urine samples could be suboptimal for ranking the subjects by their salt exposure. Repeat spot urine measurements may indeed be needed for estimating usual salt intake at individual level. Unfortunately, 24-h urine collection was not feasible in our study.

Given the mechanistic evidence, it is unlikely that moderately high salt intake in men has a protective effect on GA; rather the observed associations in men were spurious. Animal studies indicate a deleterious effect of salt on the gastric mucosa, particularly when infected with *H. pylori* [7–10, 32]. This effect may be exacerbated when infected with the highly-virulent strain via upregulation of *cagA* gene [8]. However, our analysis of CagA-specific infection showed similar risk associations because over 95% were infected with CagA positive strains in our population. Additionally, analysing salt intake and *H. pylori* infection jointly did not support our hypothesis that a synergistic association exists.

A few other limitations in our study need mentioning. First, we could not adjust for the participants' total energy intake in our models since the ENIGMA Chile food frequency questionnaire aimed to investigate a group of specific foods previously associated with gastric cancer. Total energy intake is generally higher in men. Also, salt intake has been positively associated with obesity, often linked to higher energy intake, therefore adjusting for total energy intake could have permitted more refined modelling of the relationship. Second, the Tanaka equation uses weight and height of subjects when predicting 24-h Na excretion, which may partially account for the differences in energy intake due to body size, but it may be still inadequate. Third, the outcome variable was not assessed directly through endoscopic or histological examination but rather indirectly using serum PgI and PgII levels. However, pepsinogen serology is currently the most suitable non-invasive test for assessing GA [33]. Due to a small number of participants identified with severe GA, perhaps a more robust indicator of GA when using serum pepsinogens, we were unable to provide more insight as to the association between salt intake and GA. Lastly, selection bias is a threat to any epidemiological study, specifically when the response rate is low. In ENIGMA studies, efforts were made to draw random samples of the target population and achieve a high response rate. In our study, the response rate was 70% or higher depending on the region, which together with the random sampling should have minimised selection bias in our study. However, our study may underrepresent adults, particularly men, who work all day and are more likely to have unhealthy habits such as eating processed/"street" foods with higher salt content. Our study presents several strengths. We use an objective measure for salt intake. We report the estimates for the association between salt intake and GA for men and women separately and by *H. pylori* status. In addition, we also estimated the risk for CagA-specific *H. pylori* infection proposed to induce changes in gastric mucosa in response to a high-salt diet when compared to the wild type. Finally, the participants represent a population-based sample free of gastric cancer disease rather than a patient-based symptomatic sample.

In conclusion, this study found little evidence of an association between salt intake and GA. As evidenced by the wide confidence intervals in the OR estimates, our study was underpowered to provide more precise estimates. We, therefore, encourage additional studies, which can then be meta-analysed together with our study and more precise pooled effect estimates provided. Additionally, histological confirmation of GA and multiple spot urine samples taken to better ascertain the level of salt exposure from the diet should be considered in future studies to shed more light on the association between salt intake and GA.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00394-023-03132-w.

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Data availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at IARC/WHO.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval The ENIGMA study was approved by the IARC Ethical Committee (IEC Project No. 14-17) and the local ethical review committees in Chile (CEI Med UC Project No. 13-122) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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