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Supplemental Material

Ingested Nitrate and Breast Cancer in the Spanish Multicase-Control Study on Cancer (MCC-Spain)

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Table of Contents

Figure S1. Exposure-response relationship between waterborne nitrate intake (mg/day) from age 18 to 2 years before the interview and breast cancer association. Generalized additive models (GAMs) by study area

Table S1. General characteristics of analyzed (N=2765) compared to excluded (N=642) population

Characteristic	Excluded	Analyzed	*p value*
	n (%)	n (%)	
Controls	302 (47.0)	1,520 (55.0)	
Cases	340 (53.0)	1,245 (45.0)	<0.001
Age			
Mean (SD)	55.8 (14.2)	58.4(12.7)	<0.001*
Range	24-85	23-85	
Education			
<Primary school	110 (17.1)	450 (16.3)	
Primary school	176 (27.4)	899 (32.5)	
Secondary school	206 (32.1)	871 (31.5)	
University	150 (23.4)	545 (19.7)	0.046
Body mass index			
<18.5	12 (1.9)	54 (2.0)	
18.5-24.9	294 (45.8)	1,272 (46.0)	
25-29.9	212 (33.0)	940 (34.0)	
≥30	109 (17.0)	499 (18.0)	
Missing	15 (2.3)	0 (0.00)	0.974
Physical activity			
Low	476 (74.1)	1,958 (70.8)	
Intermediate	65 (10.1)	356 (12.9)	
High	101 (15.7)	451 (16.3)	0.128
Family history of BC [a]			
No	458 (71.3)	2,005 (72.5)	
Yes	154 (24.0)	664 (24.0)	
Missing	30 (4.7)	96 (3.5)	0.883
Age at menarche			
Mean (SD)	13(1.6)	12.8(1.6)	0.040*
Range	8-18	7-20	
Age at first birth [b]			
≤30 years	386 (78.6)	1768 (78.6)	
>30 years	97 (19.8)	460 (20.5)	
Missing	8 (1.6)	20 (0.9)	0.781
Age at menopause [c]			
≤50 years	231 (56.6)	1,094 (52.6)	
>50 years	121 (29.7)	685 (32.9)	
Missing	56 (13.7)	303 (14.6)	0.144
Menopausal status			
Premenopausal	232 (36.1)	679 (24.6)	

Postmenopausal	410 (63.9)	2086 (75.4)	<0.001
Oral contraceptives use			
Never	330 (51.4)	1,450 (52.4)	
Ever	306 (47.7)	1,311 (47.4)	
Missing	6 (0.9)	4 (0.1)	0.774
Parity			
Nulliparity	151 (23.52)	517 (18.70)	
1 delivery	118 (18.38)	468 (16.93)	
2 delivery	210 (32.71)	1130 (40.87)	
>2 deliveries	157 (24.45)	642 (23.22)	
Missing	6 (0.9)	8 (0.3)	0.001
Energy intake			
≤1479 kcal/day	145 (22.6)	735 (26.6)	
>1479-1894 kcal/day	177 (27.6)	809 (29.3)	
>1894 kcal/day	200 (31.2)	880 (31.8)	
Missing	120 (18.7)	341 (12.3)	0.487
Red meat intake			
<16 g/day	183 (28.5)	758 (27.4)	
>16-29 g/day	153 (23.8)	793 (28.7)	
>29 g/day	186 (29.0)	873 (31.6)	
Missing	120 (18.7)	341 (12.3)	0.174
Processed meat			
<5.2 g/day	177 (27.6)	740 (26.8)	
>5.2-13.4 g/day	173 (27.0)	807 (29.2)	
>13.4 g/day	172 (26.8)	877 (31.7)	
Missing	120 (18.7)	341 (12.3)	0.242
Vitamin C intake			
<129 mg/day	180 (28.0)	843 (30.5)	
129-203 mg/day	141 (22.0)	764 (27.6)	
>203 mg/day	201 (31.3)	817 (29.6)	
Missing	120 (18.7)	341 (12.3)	0.060
Vitamin E intake			
<8.6 mg/day	158 (24.6)	803 (29.0)	
>8.6-12.2 mg/day	150 (23.4)	801 (29.0)	
>12.2 mg/day	214 (33.3)	820 (29.7)	
Missing	120 (18.7)	341 (12.3)	0.007
Waterborne ingested nitrate			
<2.6 mg/day	146 (22.7)	930 (33.6)	
≥2.6-6.0 mg/day	167 (26.0)	908 (32.8)	
>6.0 mg/day	186 (29.0)	927 (33.5)	
Missing	143 (22.3)	0 (0.00)	0.120

[a] BC (Breast cancer). [b] Distribution among non-nulliparous women. [c] Distribution only for postmenopausal women. *p*-values for Chi square test in categorical variables and for T test* in continuous variables.

Table S2. Average waterborne ingested nitrate in different exposure periods and breast cancer association (OR and 95% CIs) by menopausal status.

Exposure periods	Ingested nitrate	Cases	Controls	Postmenopausal OR [b] (95%CI)	Ingested nitrate	Cases	Controls	Premenopausal OR [c] (95%CI)
Long-term (age 18 years to 2 years before interview)[a]								
	<2.2 mg/day	227	294	Ref.	<1.8 mg/day	71	87	Ref.
	≥2.2-3.8 mg/day	229	293	1.07 (0.83, 1.40)	≥1.8-3.1 mg/day	87	87	1.33 (0.84, 2.11)
	>3.8-9.1 mg/day	226	293	1.07 (0.81, 1.42)	>3.1-6.0 mg/day	80	87	1.02 (0.63, 1.65)
	>9.1 mg/day	231	293	1.31 (0.91, 1.91)	>6.0 mg/day	94	86	1.27 (0.73, 2.23)
	p for trend			*0.23*				*0.61*
Recent (from 15 to 2 years before interview)								
	<2.2 mg/day	218	290	Ref.	<2.0 mg/day	85	87	Ref.
	≥2.2-3.7 mg/day	226	292	1.08 (0.83, 1.40)	≥2.0-3.2 mg/day	75	86	0.97 (0.61, 1.52)
	>3.7-8.5 mg/day	227	288	1.18 (0.89, 1.57)	>3.2-6.2 mg/day	79	86	0.80 (0.50, 1.29)
	>8.5 mg/day	228	289	1.30 (0.93, 1.82)	>6.2 mg/day	90	86	1.01 (0.60, 1.71)
	p for trend			*0.11*				*0.81*
Early adulthood (age 18 to 30 years)								
	<2.2 mg/day	220	292	Ref.	<1.7 mg/day	78	87	Ref.
	≥2.2-4.2 mg/day	227	271	1.16 (0.89, 1.52)	≥1.7-3.0 mg/day	80	86	1.05 (0.66, 1.67)
	>4.2-9.2 mg/day	230	281	1.14 (0.86, 1.52)	>3.0-5.6 mg/day	71	86	0.83 (0.51, 1.34)
	>9.2 mg/day	209	281	1.12 (0.78, 1.61)	>5.6 mg/day	102	86	1.33 (0.78, 2.27)
	p for trend			*0.46*				*0.58*

[a] Analysis with alternative waterborne ingested levels, calculated assuming that women with bottled water consumption in recent residences, actually used tap water before the year 2000 and bottled water thereafter. [b] Adjusted for: study area, age, education, body mass index, family history of breast cancer, age at menopause, age at first birth, oral contraceptives use, and energy intake. [c] Age at menopause was excluded from the adjustment for premenopausal women. Trend *p*-values derived from a likelihood ratio test that comparing a model with the categorical nitrate intake variable as an ordinal variable (0, 1, 2) with a model that excluded this variable.

Table S3. Interaction of waterborne ingested nitrate with relevant covariables and breast cancer association (OR and 95%CI) among premenopausal women [a]

Waterborne Ingested nitrate	Cases	Controls	OR [b] (95% CI)	Cases	Controls	OR [b] (95% CI)	Interaction *p value**
	Vitamin C		**<142 mg/day**			**≥142 mg/day**	
<2.3 mg/day	54	55	Ref.	45	50	0.69 (0.38, 1.26)	
≥2.3-4.7 mg/day	43	52	0.88 (0.49, 1.61)	43	56	0.56 (0.31, 1.02)	
>4.7 mg/day	51	48	0.94 (0.49, 1.83)	51	48	0.84 (0.44, 1.61)	0.71
	Vitamin E		**<10 mg/day**			**≥10 mg/day**	
<2.3 mg/day	50	55	Ref.	49	50	0.77 (0.41, 1.46)	
≥2.3-4.7 mg/day	39	56	0.73 (0.40, 1.32)	47	52	0.71 (0.37, 1.38)	
>4.7 mg/day	44	44	1.10 (0.56, 2.15)	58	52	0.78 (0.39, 1.55)	0.75
	Vitamin C+E		**<152 mg/day**			**≥152 mg/day**	
<2.3 mg/day	54	56	Ref.	45	49	0.72 (0.39, 1.32)	
≥2.3-4.7 mg/day	43	51	0.96 (0.53, 1.74)	43	57	0.60 (0.33, 1.08)	
>4.7 mg/day	49	47	1.00 (0.52, 1.93)	53	49	0.94 (0.50, 1.78)	0.64
	Folate		**<276 µg/day**			**≥276 µg/day**	
<2.3 mg/day	47	59	Ref.	52	46	1.08 (0.58, 1.99)	
≥2.3-4.7 mg/day	38	53	0.94 (0.51, 1.72)	48	55	0.78 (0.43, 1.44)	
>4.7 mg/day	43	43	1.17 (0.59, 2.32)	59	53	1.02 (0.54, 1.95)	0.81
	Red meat		**<25 g/day**			**≥25 g/day**	
<2.3 mg/day	44	46	Ref.	54	59	0.97 (0.53, 1.75)	
≥2.3-4.7 mg/day	39	54	0.75 (0.40, 1.42)	46	54	0.85 (0.46, 1.59)	
>4.7 mg/day	46	55	0.85 (0.44, 1.66)	56	41	1.23 (0.61, 2.45)	0.64
	Processed meat		**<14 g/day**			**≥14 g/day**	
<2.3 mg/day	49	52	Ref.	50	53	0.82 (0.45, 1.49)	
≥2.3-4.7 mg/day	49	51	0.98 (0.54, 1.77)	37	57	0.57 (0.30, 1.06)	
>4.7 mg/day	39	52	1.28 (0.39, 1.41)	63	44	1.22 (0.62, 2.41)	0.05
	Smoking		**No**			**Yes**	
<2.3 mg/day	37	44	Ref.	74	72	1.37 (0.77, 2.45)	
≥2.3-4.7 mg/day	35	47	0.96 (0.50, 1.86)	63	68	1.19 (0.65, 2.17)	
>4.7 mg/day	52	45	1.29 (0.65, 2,56)	70	70	1.31 (0.69, 2,49)	0.74

[a] Only women with complete information on dietary covariables (N=596) and smoking (N=677) were analyzed. [b] Adjusted for: study area, age, education, body mass index, family history of breast cancer, age at first birth, and energy intake. **p*-value for overall interaction calculated by comparing the multivariate models with and without the interaction term using a likelihood ratio test

Table S4. Waterborne ingested nitrate and breast cancer association (OR and 95%CI) stratified by other covariables among pre and postmenopausal women

Waterborne ingested nitrate	**Cases**	**Controls**	**OR [a] (95% CI)**	**Cases**	**Controls**	**OR [a] (95% CI)**
Histological type			**Ductal [b]**			**Others/undefined [c]**
<2.6 mg/day	332	507	Ref.	91	507	Ref.
≥2.6-6.0 mg/day	289	507	0.93 (0.75, 1.16)	112	507	1.16 (0.84, 1.60)
>6.0 mg/day	330	506	1.16 (0.88, 1.52)	91	506	1.06 (0.70, 1.61)
Estrogen receptor [d]			**Negative**			**Positive**
<2.6 mg/day	73	507	Ref.	333	507	Ref.
≥2.6-6.0 mg/day	68	507	0.93 (0.64, 1.34)	321	507	1.02 (0.83, 1.26)
>6.0 mg/day	77	506	1.01 (0.63, 1.61)	336	506	1.16 (0.89, 1.52)

[a] Adjusted for: study area, age, education, body mass index, family history of breast cancer, age at first birth, use of oral contraceptives and energy intake. [b] ICD-10 code: C50. [c] ICD-10 codes: D05.1 and D05.7. [d] Numbers do not add 2765 due to missing data (N=37).

Table S5. Breast cancer association (OR and 95%CI) with dietary ingested nitrate (mg/day) from different sources by menopausal status

			Postmenopausal [a]				Premenopausal [a]
Nitrate intake sources	**Cases**	**Controls**	**OR (95%CI)**	**Nitrate intake**	**Cases**	**Controls**	**OR (95%CI)**
Animal							
<3.8 mg/day	228	344	Ref. [b]	<4.4 mg/day	73	103	Ref. [c]
3.8-5.8mg/day	266	344	0.98 (0.76, 1.27)	4.4-6.4mg/day	108	104	1.38 (0.89, 2.13)
> 5.8mg/day	303	343	1.03 (0.78, 1.35)	> 6.4mg/day	106	102	1.10 (0.68, 1.76)
Vegetables							
<80mg/day	284	344	Ref. [b]	<63mg/day	91	103	Ref. [c]
80-127 mg/day	269	344	0.91 (0.72, 1.16)	63-106 mg/day	93	104	0.88 (0.58, 1.35)
>127mg/day	244	343	0.81 (0.63, 1.05)	>106mg/day	103	102	0.89 (0.58, 1.37)
Total diet							
<94mg/day	281	344	Ref. [b]	<76 mg/day	92	103	Ref. [c]
94-143 mg/day	263	344	0.90 (0.71, 1.15)	76-118 mg/day	88	104	0.83 (0.54, 1.27)
>143mg/day	253	343	0.84 (0.64, 1.08)	>118 mg/day	107	102	0.91 (0.59, 1.40)
Total diet+waterborne							
<100mg/day	293	344	Ref. [b]	<81 mg/day	125	136	Ref. [c]
100-150 mg/day	255	344	0.81 (0.63, 1.03)	81-122 mg/day	86	100	0.94 (0.62, 1.44)
>150mg/day	249	343	0.79 (0.61, 1.03)	>122 mg/day	76	73	1.00 (0.65, 1.55)

[a] Only women with available data from the food frequency questionnaire were analyzed. [b] Adjusted for: study area, age, education, body mass index, family history of breast cancer, age at first birth, oral contraceptives use, age at menopause and energy intake. [c] Adjusted for the same covariates than OR for postmenopausal women, excluding age at menopause.

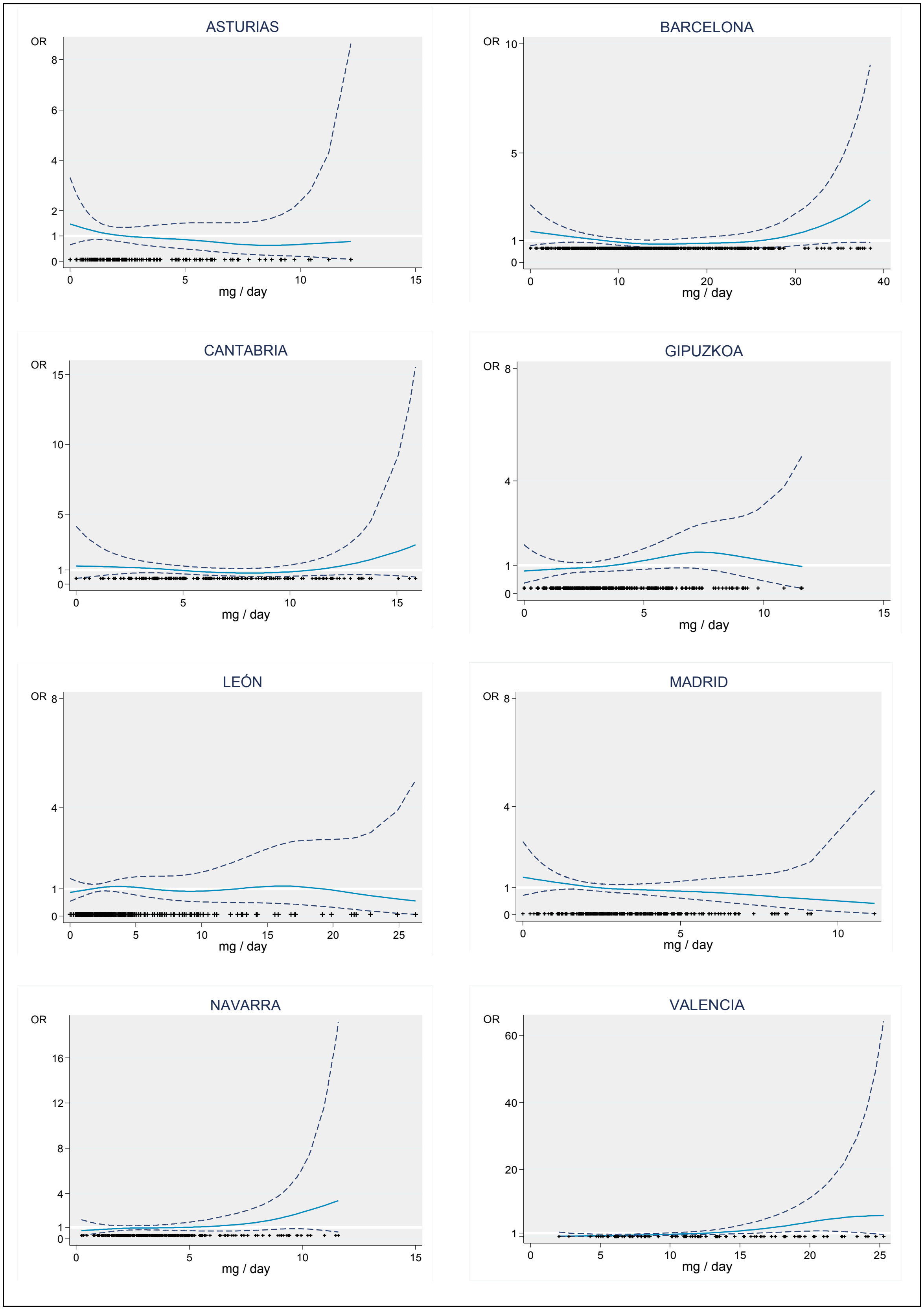

Figure S1. Exposure-response relationship between waterborne nitrate intake (mg/day) from age 18 to 2 years before interview and breast cancer. Generalized additive models (GAMs) by study area. Adjusted for: study area, age, education, body mass index, family history of breast cancer, age at first birth, use of oral contraceptives and energy intake.