Dietary determinants of urinary molybdenum levels in Mexican women: a pilot study

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Abstract

Objective. This study determined the main dietary sources of urinary molybdenum (Mo) concentrations in a sample of 124 pregnant women in Mexico. **Materials and methods.** Dietary data was collected during pregnancy, through a semi-qualitative food frequency questionnaire, with information of 84 foods. Urine Mo levels were determined by atomic absorption spectrometry, for at least two trimesters of pregnancy. The associations with Mo levels were estimated by generalized mixed effect regression models. **Results.** Between 5.8 to 12.7% of the samples were above the 95th percentile of urinary Mo distribution reported by National Health and Nutrition Examination Survey (NHANES) 2009-2010 for women (151 µg/L and 148 µg/g creatinine). After bootstrap resampling was conducted, women with high-consumption of hot peppers (β=1.34µg/g; 95% CI: 1.00-1.80; *p*= 0.05) had marginally higher urinary Mo concentration levels, creatinine adjusted, compared to women with low-consumption. **Conclusion.** Hot chili pepper consumption may contribute to body burden Mo levels in this population.

Keywords: diet; micronutrient; molybdenum

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Resumen

Objetivo. Determinar las fuentes dietéticas de molibdeno (Mo) urinario en 124 mujeres embarazadas residentes en el estado de Morelos, México. **Material y métodos.** Mediante un cuestionario de frecuencia de consumo de 84 alimentos, se obtuvo información dietética durante el embarazo. Las concentraciones urinarias de Mo se determinaron por espectrometría de absorción atómica, en al menos dos trimestres del embarazo. La asociación se estimó mediante modelos de efectos mixtos generalizados. **Resultados.** Entre 5.8 y 12.7% de las muestras superaron el P_{95} (151 µg/L y 148 µg/g creatinina) de la distribución de Mo urinario reportado para mujeres por la Encuesta Nacional de Nutrición y Salud de Estados Unidos (NHANES) 2009-2010. El mayor consumo de chile (β=1.34µg/g; IC95%: 1.00-1.80; *p*=0.05) se asoció con concentraciones marginalmente mayores de Mo. **Conclusión.** Probablemente debido a los fertilizantes o el sistema de riego utilizado en su cultivo, el consumo de chile es una posible fuente de exposición a Mo, en esta población.

Palabras clave: dieta; micronutrientes; molibdeno

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Molybdenum (Mo) is a trace element found in soil and is essential for the growth of most biological organisms including plants and animals. In humans, Mo has an essential role in metabolism, since it is required as a component of enzymes involved in the catabolism of sulfur amino acids and heterocyclic compounds, as well as in the metabolism of aromatic aldehydes.[1,2] In plants, Mo is used in the fertilization of various crops because it stimulates cellular development of the plant parenchyma, which prevents the deformation and development of necrotic regions on leaf blades.[3]

The main source of Mo exposure in humans is through oral consumption. Mo is present in nearly all foods in trace amounts as soluble molybdates, with high concentrations in legumes, cereal grains (and hence bread and baked products), leafy greens, milk, organ meat (liver, kidney) and nuts; however this varies from place to place depending on soil composition.[1,4-6] The absorption of the water-soluble molybdates from the digestive tract is very effective at a wide range of intakes indicating that molybdenum absorption is passive and not saturable, it is also not regulated at the level of intestinal absorption.[7] The body is able to adapt to this wide range of intake by regulating excretion via the urine, suggesting that molybdenum turnover is slow when dietary Mo intake is low and increases as dietary intake increases.[7] This makes urinary excretion a suitable matrix of biomarkers for short term molybdenum intake, since it is more directly related to recent intake.[8]

Mo toxicity has been mainly evaluated in animals with differences between species and seems to be mediated by copper (Cu) concentration. [9-12] In humans, Mo had been considered a compound of low toxicity, because its toxic effects had been reported in geographical areas with high intake of Mo via food,[13] and among workers from a Mo production plant highly exposed to air Mo levels.[14] A more recent study using data from the U.S. National Health and Nutrition Examination Survey (NHANES) 2007-2008 suggests a positive association between creatinine-adjusted Mo and self-reported liver conditions among adults older than 20 years of age;[15] finally in our cohort study, prenatal maternal urine Mo levels were negatively associated with psychomotor development over the first 30 months of life.[16] The objective of this study was to determine the dietary determinants of urinary Mo concentrations in this sample of pregnant women.

Materials and methods

Study population

In order to evaluate the association between some prenatal environmental exposure (e.g. organochlorine and molybdenum) and infant neurodevelopment, we assembled a perinatal cohort study [17]with 996 women identified and recruited in four different municipalities in the State of Morelos. All women were contacted every eight weeks until they became pregnant and during pregnancy they were monitored in relation to their pregnancy evolution and dietary information was collected at first and third trimester of pregnancy; while urine samples were obtained in each trimester. From 517 confirmed pregnancies, there were 442 births and 353 of them began a neurodevelopment follow-up until five years of age. By financial limitation, the association between prenatal Mo exposure and infant neurodevelopment[16] was evaluated in a random sample of 147 children who had at least five neurodevelopment evaluations between 1-30 months of age and at least one maternal urine sample during pregnancy. Finally, for this report we only included 124 women who completed the food frequency questionnaire (FFQ) and also had provided at least two urine samples during their pregnancy. The Ethics Committee of the National Institute of Public Health (CI: 515) approved the study protocol and all subjects gave informed consent.

Dietary assessment

A semi-qualitative previously validated FFQ[18] was used to assess the usual daily intake of 84 foods during the first and third trimester. The length of recall was over previous three months in each trimester. For each specified serving sizes, the questionnaire had 10 possible responses, ranging from 'never' to 'six per day'. Consumption of fruits and vegetables were adjusted for the seasonal availability in the market. For instance, since grapes were only available six months per year, the report of dietary consumption was divided by two. Portions per day were calculated by the weight corresponding to the frequency of use. The weights used were: six for reported frequencies of consumption of six per day; 4.5 for 4-5 per day; 2.5 for 2-3 per day; one for one per day; 0.78 for 5-6 per week; 0.428 for 2-4 per week, 0.065 for 2-3 per month, 0.016 for one per month or less and 0 for never.

The 84 foods were grouped into 11 food groups based on their nutrient profiles (table I) and they were then divided into subgroups. Analyzes were done with each food item in the questionnaire and with all food groups and sub food groups. Alcohol intake was not analyzed because only 10% reported consumption with a frequency of once a month. The food frequencies of each food items and groups were divided by the median (low and high) according to frequency of intake, except for manchego cheese, margarine, blackberry, strawberry, prickly pear, pear, mamey, *zapote*, bacon, beef liver, sardine, cauliflower, spinach, pumpkin flower, beet root, lentils, coffee and lard which was divided into 'intake' or 'no intake'.

Energy intake was estimated with the Food Intake Analysis System (FIAS) software developed by the University of Texas. Nutrient content reported for each food in the FIAS database was compared to the values of the composition of Mexican food tables developed by the National Institute of Nutrition in 1996.[19]

Covariates

Information on sociodemographic characteristics; age, schooling, occupation, spouse occupation, smoking status, parity and municipality were obtained from the baseline questionnaire. Smoking during pregnancy was categorized as 'never', 'smoking before pregnancy' and 'during pregnancy', occupation 'Paid working' or 'not paid working', parity 'first pregnancy' or 'more than one pregnancy', use of supplements with Mo during pregnancy 'yes' or 'no'. Because Mo is found in soil and it is often used in construction[20,21] spouse occupation was categorized as 'working in agriculture/construction' and 'other'. Place of resident (municipality) was defined from urban to less urban where I was more urbanized and IV less urbanized.

Table I

Food or food group by food item. Morelos, 2001-2005

Food groups	*Items*
Dairy	Cream cheese, sour cream, cheese (*oaxaca*, manchego, fresh), ice cream, yogurt, butter, margarine, whole milk
Eggs	Eggs
Meat	Non processed: Beef, pork, lamb (barbacoa), chicken, beef liver
	Processed: Ham, sausage, bacon, wieners, *cecina*
Fish	Canned fish: Tuna, sardines
	Fresh fish
Fruits	Orange, orange juice, tangerine, banana, plum, peach, apple, grapes, blackberry, strawberry, cantaloupe, watermelon, mango, pear, papaya, pineapple, prickly pear, mamey, *sapodilla (zapote)*
Vegetables	Green leafy vegetables: Spinach, lettuce, pigweed
	Other vegetables: Cauliflower, broccoli, carrot, beetroot, onion, potato, corn, zucchini, tomato sauce, fresh tomato, avocado, green beans, peas, hot pepper, pumpkin flower, *nopal*
Grains	Flour *tortilla*, bread, rolls, pasta, *tortilla* chips or potato chips, crackers, corn *tortilla*, corn flakes, rice, cake, cookie or sweet bread
Legumes	Beans, lentil, fava beans
Sugar and sweets	Chocolate bar, honey or jams
Drinks	Soda, coffee, herbal tea, *atole*
Fats	Vegetable oil, lard

Determination of molybdenum in urine

Each women participant provided a urine sample (10 mL) during each trimester of pregnancy; those samples were refrigerated immediately and transported to the National Institute of Public Health, where they were kept at -70°C in plastic vials, until their analysis in the Laboratory of Toxicology, Federal University of Bahia, Brazil. Mo determinations were made in a graphite furnace atomic absorption spectrometer AA240Z (Varian Inc., Australia). For quality control purposes, urine samples from Seronom Mo- U Trace elements urine (Norway, Lab Sero As, Billingstad), were used to check the reliability of the entire proposed analytical method. All the samples were above the detection limit of <0.2 μg/L and were analyzed by duplicate; the intra-assay and inter-assay precision relative standard deviation (RSD) were 2.4 and 7.8%, respectively. The accuracy for the reference material at concentration of 49.3 μg/L was 98.8%.

By a modification of the kinetic Jaffé method, creatinine was determinate using the LabTestDiagnostica (São Paulo, Brazil) reagent kit. The assay was carried out manually using a UV/Vis spectrophotometer (Biospectro, SP-220). For this method, the within-run precision was 2.9% at concentration of 97.9 mg/dL (Seronorm, Norway); the total assay precision was found to be 8.9% RSD, and the calculated accuracy was 101.9%. Urinary Mo concentrations were reported in μg/L or μg/g of creatinine, using the adjustment technique described by Barr.[22]

2.5 Statistical analysis

Urinary Mo levels were found to be positively skewed, thus natural log-transformed Mo levels were use and geometrics mean by trimester of pregnancy were estimated. Differences between urinary Mo concentrations, unadjusted and adjusted by creatinine, during pregnancy were evaluated by one-way analysis-of-variance (Anova). Possible associations between selected characteristics and log transformed creatinine-adjusted Mo urinary concentrations were estimated using linear regression at each trimester. The crude association between each food and food groups and creatinine-adjusted Mo urinary concentration was estimated using separate generalized mixed effect models. Only those foods with a p value higher than 0.20 in the unadjusted separate model were included in the final multivariate model:

$$Yij= Foodij\beta + Xi\beta + \varepsilon ij$$

dependent variable (Yij) were creatinine-adjusted Mo levels and independent variable (Foodij) each of selected foods (including caloric intake) in participant i and time j (where j= 1st, 2nd, 3rd trimester). As potential confounder factors with fixed effect (Xi) we evaluated: age and education (years), working (paid or not), parity (first pregnancy or more than one pregnancy), and municipality. Furthermore, the model assumes a random error (εij) with normal distribution, and an average equaling 0 and with constant variation. A linear random slope of trimester at evaluation and an unstructured covariance were considered for all models. Collinearity between these foods was evaluated using Spearman's rank correlation coefficients; green leafy vegetables was not included in the final model because it had a significant correlation with prickly pear (r=0.30, *p*=0.005). Hot chili pepper and coffee were not included in the same regression analysis, because they were positively and significantly correlate (Spearman correlation r= 0.14, *p*= 0.03). In order to ensure that the associations found were not random, bootstrapping of 100 replications was performed.

The diagnosis of the model consisted of the estimation of residuals by subtraction of the recorded values of urinary Mo levels from the model linear prediction of those values. Shapiro-Wilks and Shapiro-Francia tests and an evaluation using histograms and normal quantile graphs were used to evaluate residual normality. Statistical analysis was performed using STATA 12.0 and 5% significance level was considered.

Results

Selected subject characteristics are showed in table II. Women were around 22 years of age, 20% of them had more than one pregnancy; 7.3% reported taking supplements that including Mo and 46.8% resided in the most urbanized municipality.

Between the first and third trimester of pregnancy, average urinary concentrations of Mo unadjusted for creatinine varied between 40.3 and 37.0 µg/L, respectively. After adjustment for creatinine, the variation was between 45.7 and 54.2 µg/g of creatinine at the first and third trimester; however, these differences were not statistically significant (table III). All of the analyzed samples were above the detection limit (0.2 µg/L), and between 5.8 to 12.7% of the samples (depending on the trimester) were above the 95th percentile of Mo distribution reported for the US female population by NHANES (2009-2010).[23]

Table II

Characteristics of the study population. Morelos, 2001-2005

Characteristics	*(n=124)*
Age (years)	
Mean ± SD	21.9 ± 4.2
Education (years)	
Mean ± SD	10.7 ± 3.3
Parity >1 (%)	20.2
Body mass index (kg/m2) *	
Mean ± SD	22.9 ± 3.6
Smoking status (%)	
Never	59.4
Before pregnancy	35.0
During pregnancy	5.6
Energy (Kcal) Mean ± SD	
At 1st trimester	2845.0 ± 954.9
At 3rd trimester	2812.0 ± 747.5
Supplement with Mo (%)	
Yes vs. No	7.3%
Currently working (%)	43.6
Spouse occupation (%)	
Agriculture/Construction	25.4
Municipality (%)	
I	46.8
II	13.7
III	21.8
IV	17.7

* At 1st trimester of pregnancy

The associations between selected characteristics and urinary Mo levels varied according to trimester of pregnancy. In the first trimester, higher urinary Mo levels were observed among women with more than one pregnancy (β= 1.59 µg/g; 95% CI 1.05-2.42); while in the second trimester a positive association was observed between paid work and Mo urinary levels (β= 1.48µg/g; 95% CI 1.01-2.18). In contrast, inverse and significant associations were observed at third trimester among those women with higher education (β= -1.07 μg/g; 95% CI -1.14, -1.01) and higher caloric intake per day (β = -1.00 μg/g; 95% CI-1.01-1.00). No significant associations were found with maternal age, BMI, smoking status, prenatal supplements with Mo, spouse occupation and municipality (table IV).

Of the 84 food items, only the high consumption of hot chili peppers (β= 1.34 μg/g; 95% CI 1.04-1.72; p= 0.03) was associated with a statistically significant difference of creatinine-adjusted Mo urinary concentrations, after covariate adjustments. High intake of prickly pear (β= 1.31μg/g; 95% CI 1.01-1.68; p=0.05), fresh fish (β= 1.28 μg/g; 95% CI -1.01-1.65; p= 0.06) and coffee (β= 1.32μg/g; 95% CI 1.01-1.79; p= 0.05) were marginally associated with higher creatinine-adjusted Mo urinary concentrations.

In order to reject a possible type I error, bootstrap resampling analyzes (100 replications) was performed and only the hot chili pepper association (β= 1.34 μg/g; 95% CI 1.00-1.81; p=0.05) remained marginally significant (table V).

Table III
MEAN MOLYBDENUM LEVELS DURING PREGNANCY. MORELOS, 2001-2005

Urinary Mo levels	*Mean ± SD**	P_5	P_{50}	P_{95}	*%>P_{95} from* NHANES %
Unadjusted (µg/L)					
1st trimester (n=121)	40.3 ± 2.8	6.9	37.6	185.9	6.6
2nd trimester (n=110)	38.2 ± 3.0	5.2	40.0	229.2	9.0
3rd trimester (n= 120)	37.0 ± 3.1	4.5	34.8	183.7	5.8
Adjusted (µg/g of creatinine)					
1st trimester (n=121)	45.7 ± 2.6	9.8	46.7	155.4	5.8
2nd trimester (n=110)	55.0 ± 2.6	8.8	59.4	277.8	12.7
3rd trimester (n=120)	54.2 ± 3.0	7.1	59.2	291.8	10.8

* Geometric mean and SD

NHANES: U.S. National Health and Nutrition Examination Survey (2009-2010)

Table IV
CHARACTERISTICS OF STUDY POPULATION IN RELATIONS TO CREATININE ADJUSTED URINARY MO LEVELS μG/G. MORELOS 2001-2005

	1st Trimester (n=121)		*2nd Trimester (n=110)*		*3rd Trimester (n=120)*	
Characteristics	β	*CI95%*	β	*CI95%*	β	*CI95%*
Age (years)	1.01	(-1.03,1.05)	1.01	(-1.03,1.06)	-1.03	(-1.09,1.02)
Education (years)	-1.03	(-1.08,1.02)	1.01	(-1.06,1.07)	-1.07	(-1.14,-1.01)*
Parity (>1 vs. 1)	1.59	(1.05, 2.42)*	-1.10	(-1.77,1.47)	1.15	(-1.44,1.92)
Body mass index (kg/m2)	1.04	(-1.01,1.09)	-1.02	(-1.08,1.03)	1.02	(-1.03,1.09)
Smoking status						
(Yes vs. No)	1.19	(-1.19,1.69)	1.20	(-1.23,1.78)	1.17	(-1.29,1.77)
Energy (Kcal/day)	1.00	(-1.00,1.00)	1.00	(-0.99,1.00)	-1.00	(-1.01,-1.00)*
Supplement with Mo						
(Yes vs. No)	1.17	(-0.50,2.75)	2.22	(-0.92,5.32)	2.11	(-0.97,4.58)
Work status (Paid vs. not)	-1.16	(-1.65,1.22)	1.48	(1.01,2.18)*	-1.01	(-1.53,1.48)
Spouse occupation (Agriculture/Construction vs. other)	1.01	(-1.47;1.50)	1.17	(-1.35;1.87)	1.12	(-1.43;1.82)
Municipality						
I	Ref		Ref		Ref	
II	1.08	(-1.58,1.85)	1.15	(-1.58,2.08)	-1.02	(-1.97,1.89)
III	1.19	(-1.33,1.90)	-1.18	(-1.96,1.40)	1.01	(-1.67,1.73)
IV	1.18	(-1.37,1.90)	1.12	(-1.53,1.93)	1.18	(-1.49,2.09)

* p-value < 0.05

Table V

ASSOCIATION BETWEEN FOOD GROUPS AND URINE MOLYBDENUM LEVELS. MORELOS, 2001-2005

Food groups	*Prevalence of intake (%)*[*]	*Creatinine adjusted urine Mo levels μg/g*						*Corrected – Bootstrap*		
		β[‡]	*CI95%*	*p*	β[§]	*CI95%*	*p*	β	*CI95%*	*p*
Dairy	45.4	1.09	(-1.23,1. 46)	0.58						
Milk	59.7	1.23	(-1.06,1.62)	0.13	1.21	(-1.07,1.72)	0.15	1.18	(-1.12,1.58)	0.24
Fruits	50.0	-1.13	(-1.49,1.16)	0.37						
Prickly pear	37.0	1.32	(1.02,1.68)	0.04	1.31	(1.01,1.68)	0.05	1.25	(-1.03,1.63)	0.08
Eggs	61.2	-1.06	(-1.40,1.25)	0.69						
Meats	49.8	-1.16	(-1.39,1.24)	0.68						
Non processed meats	50.2	-1.12	(-1.47,1.17)	0.40						
Processed meats	49.0	-1.14	(-1.48,1.15)	0.34						
Fish	48.8	1.10	(-1.19,1.44)	0.49						
Canned fish	53.0	1.12	(-1.16,1.46)	0.38						
Fresh fish	47.8	1.30	(-1.00,1.68)	0.05	1.28	(-1.01,1.65)	0.06	1.19	(-1.04,1.51)	0.11
Vegetables	50.2	-1.02	(-1.37,1.31)	0.89						
Green leafy vegetables	46.6	-1.24	(-1.63,1.06)	0.13						
Other vegetables	61.5	1.00	(-1.35,1.35)	0.99						
Hot chili pepper[#]	42.6	1.34	(1.03,1.75)	0.03	1.34	(1.04,1.72)	0.03	1.34	(1.00,1.80)	0.05
Legumes	52.6	1.07	(-1.24,1.42)	0.64						
Grains	49.6	-1.07	(-1.50,1.32)	0.71						
Pasta	43.0	-1.35	(-1.79,-1.03)	0.03	-1.22	(-1.59,1.07)	0.14	-1.24	(-1.65,1.19)	0.14
Sugar and sweets	48.0	1.06	(-1.23,1.40)	0.66						
Drinks	48.4	-1.06	(-1.37,1.23)	0.66						
Coffee[#]	46.0	1.25	(-1.05,1.62)	0.11	1.32	(1.01,1.79)	0.05	1.34	(-1.01,1.79)	0.06
Fats	46.7	1.07	(-1.45,1.67)	0.75						

[*] Median value of intake; except for prickly pear (yes vs. no)

[‡] Intake high vs. low, adjusted for caloric intake

[§] Intake high vs. low, adjusted for age, education, caloric intake, parity, municipality and trimester food covariates (chili peppers, coffee, milk, prickly pear, fresh fish, pasta)

[#] Hot pepper and coffee were not run as covariates due to their significant correlation, Spearman rank correlation coefficients: r = 0.14 $p = 0.03$

Discussion

Molybdenum exposure in humans is mostly influenced by food consumption and our results suggest that among these women, the high-consumption of hot-chili pepper may be one of the main determinants of high urinary Mo concentration. In Mexico there is a wide variety of colored pepper fruits (sweet, semi hot, and hot varieties) and these are consumed either raw or cooked at different stages of maturity in fresh and canned forms, or air-dried and sun-dried.[24] Capsicum (chili/hot-chili peppers) is an excellent source of vitamins C (ascorbic acid), A, B-complex and E along with metals and minerals like molybdenum, manganese, folate, potassium and thiamine.[25,26] Data on the heavy metal content in hot-chili pepper fruits is very limited. The highest mean concentration of Mo in hot-chili peppers grown in the Kentucky, USA was found to be 0.65 μg/g dry weight.[27] Considering that hot-chili peppers contain about 88% water,[28] there are 8.33 g of fresh chili peppers in one g of dry chili peppers. Accordingly, the Mo concentration of 0.65 μg/g dry fruit equates to 0.08 μg/g in fresh fruits. The intake of hot chili peppers in the Mexican diet was estimated to be 15 kg/person/year in recent years.[29] Using this concentration of 0.08 μg/g in fresh chili peppers the daily consumption of Mo in fresh hot-chili pepper in the Mexican diet would average to 3.29 μg/d.

Molybdenum along with other micronutrients form part of bio-fertilizers that include cattle manure, milk and mineral salts (50 grams of molybdenum trioxide) and are used (through an irrigation system) to improve the plant growth[3] in hot chili pepper plantations.[30] It is unknown to us if our population consumed hot chili peppers that were locally cultivated locally or from different states within Mexico. However, in Mexico wastewater has been used as a source of crop nutrients over many decades. Even when treated, wastewater recycles organic matter and a larger diversity of nutrients than any commercial fertilizer can provide, biosolids, sludge and excreta in particular, provide numerous micronutrients such as cobalt, copper, iron, manganese, molybdenum and zinc, which are essential for optimal plant growth,[31] and can be absorbed by the hot-chili pepper and other vegetables from polluted soil, air and water.

To the best of our knowledge, there are no cut-off points established for the correct interpretation of the Mo urinary concentrations observed in our study population and this is mostly due to the current available exposure guidance values are established by daily intake levels.[32] Recently, for interpreting urine, plasma and whole blood Mo biomonitoring levels in the context of exposure guidance values, biomonitoring equivalents (BEs) have been designed to against inadequacy and toxicity.[33] BEs of Mo in urine associated with toxicity range between 200-7500 mg/L;[33] and taking into account these values, around only 5% of our women were into Mo toxicity exposure range.

Mo toxicity varies according to its compounds, the trioxide and ammonium molybdate possibly are more toxic than molybdenum disulfide, molybdenum metal or molybdenum dioxide[34] and we do not know which form of Mo or the quantity that is present in soil or is used for some crops particularly in hot-chili pepper crops. However, in children from these women we observed a negative association between prenatal Mo exposure and infant psychomotor neurodevelopment.[16] Similarly, two studies have observed a possible association between Mo exposure with spermatic damage,[35] reproductive hormone alterations[36] and liver disorders[15] at BEs levels associated with toxicity in whole blood (0.45-22mg/L) or lower than those urinary BEs proposal levels for Mo.

Some considerations must be taken into account to make an adequate interpretation of the results; for example, the estimation of Mo association with hot-chili peppers consumption is very conservative, because in this study the consumption of dry hot-chili peppers was not assessed; it was only asked about the consumption of fresh or canned hot-chili pepper with their meals. Additionally, the amount of Mo in vegetables depends on the kind of soil and we did not have information about the content of Mo in Mexican hot-chili pepper crops.

Dietary information and urine samples were collected at different times of pregnancy, allowing us to account for variability in the diet and Mo excretion throughout pregnancy. Although it is considered that the urinary determination of Mo is an acute marker of exposure, the fact that dietary exposure is continuous, urinary concentration of Mo may be a reflection of the magnitude and variation of exposure in the population.[33] Even though there was a good internal quality control in the Mo urinary analysis, the absence of an external quality control does not allow the ruling out the presence or the magnitude of this kind of error. However, if error exists, this could not be differential in regard to dietary information, because the person in charge of the analyses of urinary Mo did not know the study hypothesis and dietary conditions of each participant.

Finally it is unlikely that the association between urinary Mo levels and high hot-chili pepper intake would be a chance finding by multiple comparisons. The association involving hot-chili pepper intake appeared to be the most consistent after the Bootstrap correction was used. Likewise, to evaluate whether hot-chili pepper intake in this sample was representative of the rest of the cohort we compared the distribution of hot-chili pepper intake reported for women no included in the analysis. High consumption of hot-chili pepper during the 1st trimester (44.3 vs. 39.4, *p*= 0.34) and during 3rd trimester (43.9 vs. 40.6, *p*= 0.53) were similar between pregnant women included and no included.

Conclusion

Urinary Mo levels were increased by the consumption of hot-chili peppers. These results suggest that hot chili pepper consumption is positively associated with Mo exposure in pregnant women in this cohort. Mo is considered an essential trace element. Due to the small sample size of this study, the scarce evidence about its potential toxicity in humans,[13,15,16,35,36] further research with a larger sample size and a detail evaluation of other potential dietary and environmental sources of Mo is warranted.

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