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Interaction between mercury (Hg), arsenic (As) and selenium (Se) affects the activity of glutathione S-transferase in breast milk; possible relationship with fish and shellfish intake

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Abstract

Breast milk is regarded as an ideal source of nutrients for the growth and development of neonates, but it can also be a potential source of pollutants. Mothers can be exposed to different contaminants as a result of their lifestyle and environmental pollution. Mercury (Hg) and arsenic (As) could adversely affect the development of fetal and neonatal nervous system. Some fish and shellfish are rich in selenium (Se), an essential trace element that forms part of several enzymes related to the detoxification process, including glutathione S-transferase (GST). The goal of this study was to determine the interaction between Hg, As and Se and analyze its effect on the activity of GST in breast milk. Milk samples were collected from women between day 7 and 10 postpartum. The GST activity was determined spectrophotometrically; total Hg, As and Se concentrations were measured by atomic absorption spectrometry. To explain the possible association of Hg, As and Se concentrations with GST activity in breast milk, generalized linear models were constructed. The model explained 44% of the GST activity measured in breast milk. The GLM suggests that GST activity was positively correlated with Hg, As and Se concentrations. The activity of the enzyme was also explained by the frequency of consumption of marine fish and shellfish in the diet of the breastfeeding women.

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EFECTO DE LA INTERACCIÓN ENTRE MERCURIO (Hg), ARSÉNICO (As) Y SELENIO (Se) EN LA ACTIVIDAD DE GLUTATIÓN S-TRANSFERASA EN LECHE MATERNA; POTENCIAL RELACIÓN CON EL CONSUMO DE PESCADOS Y MARISCOS

Resumen

La leche materna es considerada como una fuente ideal de nutrientes para el crecimiento y el desarrollo de los recién nacidos, pero también puede ser una fuente potencial de contaminantes. Las madres pueden estar expuestas a diversos contaminantes como resultado de su estilo de vida y de la contaminación ambiental. Mercurio (Hg) y arsénico (As) pueden afectar negativamente el desarrollo del sistema nervioso fetal y neonatal. Algunos peces y mariscos son ricos en selenio (Se), un oligoelemento esencial que forma parte de diversas enzimas relacionadas con el proceso de desintoxicación, incluyendo glutatión S-transferasa (GST). El objetivo de este estudio fue determinar la interacción entre Hg, As y Se, así como analizar su efecto sobre la actividad de GST en la leche materna. Muestras de leche materna fueron obtenidas entre los días 7 y 10 después del parto. La actividad de la GST fue determinada espectrofotométricamente. Las concentraciones totales de Hg, As y Se fueron medidas por espectrometría de absorción atómica. Para explicar la posible asociación de las concentraciones de Hg, As y Se con la actividad de la GST en la leche materna, se construyeron modelos lineales generalizados. El modelo explicó el 44% de la actividad de GST medida en leche materna. El MLG sugiere que la actividad de GST se correlacionó positivamente con las concentraciones de Hg, As y Se. La actividad de la enzima se explica también por la frecuencia de consumo de peces marinos y mariscos en la dieta de las mujeres que se encuentran en periodo de lactancia.

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Palabras clave: *Leche materna. Modelos lineales generalizados. Glutation S-transferasa. Estrés oxidativo. Elementos traza.*

Introduction

Humans are exposed to different contaminants as a result of their lifestyle and environmental pollution.[1] Trace elements, including mercury (Hg) and arsenic (As), are some of the most harmful xenobiotics because they are widespread and persistent in the environment.[1,2] Selenium (Se), another trace element previously considered as toxic, is now known for its remarkable health benefits as an antioxidant, hormonal regulator, anticarcinogenic properties, enhancer of immune surveillance, cell-cycle effector, enhancer of apoptosis and inhibitor of angiogenesis.[3] Humans are exposed to Hg, As and Se from many sources.[4] The most important sources include diet and drinking water.[1,5,6] For some children the exposure starts *in utero* and continues during lactation[7]. Hg, a naturally occurring heavy metal known to be toxic for humans, is of particular concern for the fetus and neonate given its negative effects on neurodevelopment.[1,7] The toxicity of Hg strongly depends on its chemical form; elemental mercury (Hg°), inorganic (typically divalent, Hg^{+2}) when combined with other elements, or in organic compounds when combined with carbon (*e.g.* methylmercury, $MeHg^{+}$)[1]. Both $MeHg^{+}$ and Hg^{2+} are the most toxic due their high diffusion capacity through lipid membranes.[1] This process explains how Hg concentration can increase along the food web (marine ecosystems), a phenomenon referred to as biomagnification.[6,8] The provisional tolerable weekly intake (PTWI) for total Hg is 5 $\mu g/kg^{-1}$ bw (body weight) $week^{-1}$ with no more than 1.6 $\mu g/kg^{-1}$ bw $week^{-1}$ of $MeHg^{+}$, and some safe limits of Hg range from 0.001 mg kg^{-1} to 1 mg kg^{-1} depending on food or drink type.[5, 8-11]

The US Environmental Protection Agency[10] has classified As as a known carcinogen (category A)[3] associated with increased risk of cancer in the lung, skin, liver and bladder. Children that were exposed to As during early life or in-utero had marked increases in several chronic respiratory symptoms.[12] On the other hand, some studies suggest that As can be beneficial for animal growth and, in pharmacological amounts, As has been successfully used against some forms of leukemia.[13] Humans are mainly exposed to As through diet and drinking water. Arsenic exists in four oxidation states, As(+V) (arsenate), As(+III) (arsenite), As^{0} (arsenic), and AS(−III) (arsine). In addition to these forms, and their methylated derivatives, there are over 50 additional arsenic species identified in marine organisms, which show a wide range of toxicities, such as arsenocholine, arsenobetaine and arsenosugars and are considered innocuous to monomethyl (MMA) and dimethyl species (DMA) that are considered toxic.[14] Arsenic toxicity assessment is more complex when the degree of toxicity is compared between inorganic and organic species: MMA(+III) > DMA (+III) > As(+III) > As(+V) > MMA(+V) > DMA(+V)[15]. Just like Hg, there are many thresholds cited in the literature as safe limits of As consumption. The provisional tolerable weekly intake (PTWI) of As for children is 15 $\mu g/kg^{-1}$ body weight per week, and safe limits of As are considered between 1 $\mu g\ L^{-1}$ and 25 $\mu g\ L^{-1}$ in breast milk and drinking water.[10,16]

Prior to 1957, Se was considered a toxic element, but was subsequently recognized as an essential dietary trace element. Further, with the discovery of glutathione (GSH) and several other molecules that contain Se (selenoproteins), a biochemical function was assigned to this element.[17] Se has many biological effects; in the human body, it plays a role as an antioxidant, participates in hormone metabolism, in redox reactions, in reproduction and immune function[18]. The levels of this element depend on its intake; it is present in meats, fish, shellfish and vegetables.[17-19] A joint Food and Agriculture Organization/World Health Organization[20] expert committee on Human Vitamin and Mineral Requirements proposed a recommended minimal nutrient intake of 6 μg Se day^{-1} in infants aged 0 to 6 months weighing approximately 6 kg.[17]

Metal detoxification is an essential process for all organisms.[21] A number of mechanisms have been proposed to be involved in trace element detoxification. One of these is related to the selenoproteins, including glutathione S-transferase (GST) which has important antioxidant and detoxification functions.[21] The superfamily of GST is associated to metal detoxification. Some xenobiotics, such as As and Hg, are metabolized by conjugation with GSH, a reaction catalyzed by the GST enzyme. Usually, conjugation with GSH is the first step in the detoxification process. Selenium is found as a central part of this process. Therefore, the GST enzyme and Se play an important role *in vivo* in the metal detoxification process.[22]

The exposure to As and Hg presents important public health problems, especially for neonates when the possibility of contaminant transfer through breast milk is considered. Mothers are exposed to As and Hg by oral, inhalation and dermal routes. The oral route is considered to be the main exposure; therefore, a mother's nutrition during pregnancy and lactation period is very important. Fish and shellfish are rich sources of fatty acid and micronutrients such as Se, zinc and iron, especially in marine species. However, a diet rich in marine species may be regarded as a major pathway of exposure to contaminants including Hg and As.[1] Because a link between GST activity and Se concentration may participate in the detoxification process after exposure to Hg and As, the goal of this study was to determine the concentrations of [Se], [Hg] and [As], and evaluate its effect on the activity of GST measured in breast milk of women from Baja California Sur, Mexico.

Methods

Sampling

Breast milk samples were collected from women (n = 108) in Baja California Sur, Mexico. In the first

interview, informed consent was collected on the day of discharge from the hospital. In a second interview, 7 to 10 days after delivery, a survey was administered and milk samples were collected following the established sample collection procedure.[23] The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by Capítulo Baja California Sur de la Academia Nacional Mexicana de Bioética, A.C. All samples were stored and frozen at -80 °C in Centro de Investigaciones Biológicas del Noroeste, S.C. until Hg, As, Se determinations and GST activity analyses were performed.

Exposure assessment

The time-frame explored for food consumption analysis was 30 days prior to breast milk sample collection. No information was obtained regarding portion size, recipes, or preparation methods. Fish and shellfish consumption frequency data were grouped into four categories; never consumed, consumed once a month, consumed once every two weeks and consumed more than twice a week.[23]

Total concentration of mercury, arsenic and selenium analysis

Breast milk samples were transferred into Teflon vessels and digested with 70% nitric acid (HNO_3) and 30 % hydrogen peroxide (H_2O_2) in a microwave oven (Mars 5x, CEM, Matthew, NC, USA). Total concentration of Hg ([THg]), As ([TAs]) and Se ([TSe]) were quantified using a hydride system (HG 3000, GBC, Australia) coupled to an atomic absorption spectrophotometer (XplorAA, GBC, Braeside Australia). The cold vapor technique was used for [THg], and hydride generation for [TAs] and [TSe].[24] The detection limits (DL) were 0.05 µg L^{-1} for Hg and 0.02 µg L^{-1} for As. Analyses were performed in duplicate, including blanks; calibration standards and certified material (SRM1954 for Hg and GBW10017 for As and Se) of milk was included, with ≥ 90% recovery.

Activity of glutathione S-transferase (EC 2.5.1.18) analysis

GST activity was determined by measuring the change in absorbance caused by the formation of thioether glutathione dinitrobenzene complex as a product of the reaction between GSH and 1-chloro-2,4-dinitrobenzene (CDNB).[25] Working solution (0.1 M phosphate buffer, 10 mM GSH, and 60 mM EDTA), CDNB (10 mM) and the sample were mixed in a cuvette. Change in absorbance was recorded every 30 s during 6 min at 340 nm. Enzyme activity was expressed in units mg^{-1} of protein (U mg^{-1} protein). One unit of GST activity is defined as the amount of enzyme that catalyzes the production of 1 mol of CDNB per min.

Statistic analyses

Descriptive statistics were calculated, including means, medians, minimum, maximum, 10th and 90th percentiles, as well as the proportion of the values below the DL. In those cases in which the values were below the DL, a value corresponding to half the DL was used for statistical analysis[26]. GST activity, [TSe], [THg] and [TAs] values were not normally distributed (Kolmogorov-Smirnov $p < 0.01$). Therefore, non-parametric statistics (Kruskal-Wallis for four groups) were performed to evaluate differences in trace element concentration and GST activity between frequency categories of fish and shellfish intake.

A generalized linear model (GLM) was performed considering a *Gamma* distribution *error* to explain the activity of GST measured (response variable) in breast milk, using a *log* canonical link function.[27,28] The *Gamma* distribution can be used as an alternative of the *Gaussian* or *Poisson* distribution *error* for continuous positive data;[28,29] it extends over the range of where is the value of the variable of interest (GST activity).[30] The applicability of this distribution lies in its flexibility, from an inverse curve or right-hand skewed curve (when the dispersion parameter, v, is small relative to the μ^2) to a bell shaped and symmetric curve (for larger values of v).[28,30] The explanatory variables considered for modeling were [TSe], [THg], [TAs] and frequency categories of fish and shellfish intake; the former considered as factor variables included in the GLM. The simplification and selection of the minimal adequate model was performed starting with the maximal model containing all the factors, interactions and covariates of interest (k = 31 this study);[31] the simplification was done using the backward procedure evaluating all the alternative models by testing the contribution of each variable in turn ($p < 0.05$), and the change in the residual deviance at each step.[28,32] The deviance criterion is used as a measure of the goodness-of-fit of the model to the data.[28] Finally, the distribution of deviance residuals of the minimal-fitted model was evaluated as a diagnostic method and model validation.[28] Equations for the minimal-fitted models were generated in terms of the explanatory variables with significant contribution to GST activity.

Results

Total mercury, selenium and arsenic concentrations; GST activity

[THg], [TSe] and [TAs] concentrations and the GST activity were measured in breast milk of 108 women from Baja California Sur Mexico; results are summa-

Table I
Glutathione S-transferase (GST) activity (U mg^{-1} protein) and trace element levels (μg L^{-1}) in breast milk of women inhabiting Baja California Sur, México

Variable	*N*	*Minimum*	*Maximum*	*Mean*	*Median*	*P10*	*P90*	*% < LD**
GST U mg^{-1} prot	108	0.00001	0.070	0.007	0.002	0.0003	0.025	–
[THg] μg L^{-1}	108	0.03	24.87	2.52	1.54	0.03	5.51	14%
[TSe] μg L^{-1}	108	6.32	56.13	21.95	19.78	12.5	32.23	–
[TAs] μg L^{-1}	108	0.01	13.80	0.99	0.01	0.01	4.99	76%

% < LD: Percentage of values under the detection limit; P10: Percentile 10th; P90: Percentile 90th; GST: Glutathione S-transferase; [THg]: Total mercury concentration; [TSe]: Total selenium concentration; [TAs]: Total arsenic concentration.

rized in table I. The median of [THg] was 1.54 μg L^{-1} (range 0.03 to 24.87 μg L^{-1}); in 14% (15/108) of the samples the concentration was below the DL. In breast milk samples, the median [TSe] was 19.78 μg L^{-1} (range 6.32 to 56.13 μg L^{-1}). For [TAs] a median of 0.99 μg L^{-1} (range 0.01 to 13.80 μg L^{-1}) was found, with 76% (82/108) of the samples having concentrations below the DL. The median GST activity was 0.002 U mg^{-1} protein (range 0.00001 to 0.07 U mg^{-1} protein).

Trace element concentrations and GST activity by categories of intake

The median and percentiles (10 and 90%) of the continuous variables categorized by the frequency of intake for fish and shellfish are presented in table II. The group that never ate fish tends to present lower levels of [THg], [TSe] and GST activity compared with those who consumed fish more than twice a week; however, there was not a statistically significant difference associated to the frequency of fish and shellfish intake in the [THg], [TSe] ($p \geq 0.05$) nor GST activity in breast milk ($p \geq 0.05$). A significant difference in [TAs] was observed when the frequency categories of shellfish intake were evaluated ($p = 0.04$), with the higher levels of [TAs] found in women who never ate shellfish or ate it once a month (table II).

Relationship between trace elements and GST activity in breast milk

The variability in the GST activity measured in breast milk was explained in the GLM by the simultaneous effect of the frequency of fish and shellfish consumption, the concentration of [TSe], [THg] and [TAs], as well by the interaction between trace elements (table III). The minimal fitted model chosen with $k = 8$ covariates (GST activity ~ Intercept, [TSe], [THg], [TAs], shellfish and fish intake, [TSe] * [THg], [TSe] * [TAs], [THg] * [TAs]) presents a difference in residual deviance of 44% ($\beta = -7.528$, *Std Error* = 0.770, *residual deviance*= 193.27, $p < 0.01$, $k = 8$) in comparison with the residual deviance of the maximal model with $k = 31$ covariates ($\beta = -6.618$, *Std Error* = 1.7599, *residual deviance* = 96.426, $p < 0.01$, $k = 31$). The former means that by choosing only 8 covariates, statistically significant ($p < 0.05$), the variability in the activity of GST in breast milk was explained with greatest accuracy (fig. 1), in comparison with the variability explained (only 44% higher) by a maximal model with 31 covariates with no explicative power ($p \geq 0.05$). The equations for the fitted values of activity of GST are presented by categories of fish and shellfish frequency intake (table IV). The median values of the fitted data obtained by the model agreed in general terms with the median values of activity of GST observed (table IV). When the simultaneous effect of the eight covariates (frequency of intake of fish and shellfish, trace elements and the interaction between trace elements) are considered to explain the activity of the enzyme in breast milk, a tendency to increase of the GST activity is observed in the median fitted values together with the increase in the frequency of consumption of fish, with the lower values present in those women who never ate fish independently of their frequency of consumption of shellfish (table IV). The higher activity of GST fitted by the model are found in those women who consumed fish "once every two weeks" and "more than two times a week" together with the consumption of shellfish "once every two weeks" (table IV).

GST activity, trace elements and their regulatory thresholds

When the values of GST activity fitted by the model were plotted against the concentration of each trace element the majority of the values of the activity of the enzyme in breast milk were found under 0.02 U mg^{-1} proteins (fig. 2). In all samples [TSe] was above 6 μg L^{-1}, which is the minimum recommended intake for infants fed with human milk established by the Joint FAO/WHO Committee (1998; fig. 2a).[20] The highest [TSe] was 56.1 μg L^{-1}, and only 1.8% (2/108) of the samples had levels up to 45 μg L^{-1}, which was set as tolerable upper intake for infants aged 0 to 6 month[33] (fig. 2a). The median concentration of [THg] in breast milk for those women who frequently consumed shellfish (3.22 μg L^{-1}) and fish (3.35 μg L^{-1}) (table II) is lower than the threshold marked by Agency for Toxic

Table II

Median of glutathione S-transferase activity (GST, U mg^{-1} protein), total mercury ([THg]), total arsenic ([TAs]) and total selenium ([TSe]) concentration (µg L^{-1}) by fish and shellfish intake in breast milk of women inhabiting Baja California Sur, México

Frequency of intake	*GST U mg^{-1} prot*	*P10*	*P90*	**p*	*THg µg L^{-1}*	*P10*	*P90*	*p*	*TSe µg L^{-1}*	*P10*	*P90*	*p*	*TAs µg L^{-1}*	*P10*	*P90*	*p*
Fish																
Never	0.0019	0.00040	0.00758	> 0.05	1.87	0.03	3.35	> 0.05	18.84	10.14	31.59	> 0.05	0.01	0.01	–	> 0.05
Once a month	0.0010	0.00036	0.01617		1.10	0.03	5.40		19.86	11.87	36.10		0.01	0.01	5.04	
Once every two weeks	0.0033	0.00010	0.02872		1.43	0.03	5.67		19.72	9.39	31.85		0.01	0.01	5.63	
More than twice a week	0.0023	0.00030	0.01570		3.35	0.03	12.74		20.72	13.79	31.86		0.01	0.01	2.43	
Shellfish																
Never	0.0025	0.0002	0.0380	> 0.05	1.10	0.03	3.71	> 0.05	19.63	10.25	33.79	> 0.05	0.01	0.01	5.3	0.04
Once a month	0.0019	0.0004	0.0089		1.53	0.03	6.81		18.25	11.60	31.08		0.01	0.01	6.25	
Once every two weeks	0.0022	0.0002	0.053		1.91	0.03	5.49		25.41	13.56	35.28		0.01	0.01	0.01	
More than twice a week	0.0019	0.0002	–		3.22	0.03	–		23.39	15.34	–		–	–		

*Statistical significance by Kruskal-Wallis. P10: Percentile 10th; GST: Glutathione S-transferase; [THg]: Total mercury concentration; [TSe]: Total selenium concentration; [TAs]: Total arsenic concentration.

Substances and Disease Registry[10] (4 µg L^{-1}) and by Deutsche Forschungsgemeinschaft (5 µg L^{-1}) (fig. 2b).[34] By plotting the fitted values of the GST activity with [THg] is possible to show that [THg] in breast milk did not present values above 5 µg L^{-1} (fig. 2b). The [TAs] in 81.5% (88/108) of the samples was under 1 µg L^{-1}, in accordance with the recommendation by ATSDR (2007) for breast milk, and in 1.8% (2/108) of the samples was 10 µg L^{-1}, which is considered as a safe limit for drinking water by WHO[35] (fig. 2c).

Discussion

In this study, the GLM, a multivariate statistical analysis, helps to explain the activity of the GST measured in breast milk of women from Baja California Sur by the evaluation of the simultaneous contribution of many covariates, including the interaction between trace elements. The *Gamma* distribution *error* chosen during the GLM analysis resulted very useful to evaluate the activity of the GST due to its large coefficient of variation and the condition of the variable skewed to the right. Using *Gamma* distribution *error* avoided the issue of negative values being generated, which results unrealistic when the variable of interest is continuous, positive and has a large variability.[29]

Marine diet intake and trace element exposure

The minimal model chosen in this study explained the activity of GST by the simultaneous effect of the frequency of fish and shellfish consumption together with the concentration of [TSe], [THg] and [TAs], and also by the interactions between trace elements. Shellfish consumption did not have a significant contribution when the four categories were grouped ($p = 0.11$); however, shellfish consumed "once every two weeks" did contribute significantly ($p = 0.03$), generating the highest fitted values of GST activity together with the consumption of fish when consumed more than once every two weeks. With respect to the frequency of fish intake, the "never consumed" category presented statistically significant contribution in explaining the activity of GST, generating the lowest fitted values. However, there was not a direct effect of the frequency of intake of marine products over the GST activity of the women, when univariate analyses were performed (table II).

Fish is considered one of the main sources of Hg. Studies that included breast milk from women with high fish intake reported [THg] of 1.22 µg L^{-1} [26] to 4.1 µg L^{-1} [36]. WHO (1990), considers concentration ranges of 1.4 to 1.7 ng Hg g^{-1} as 'normal' in breast milk. The results from the bivariate analyses of this study did not show a clear pattern between [THg] in breast milk and the frequency of intake of fish and shellfish among women in Baja California Sur (table II), even when

Table III

Coefficients fifted by the generalized linear model (GLM) with Gamma error distribution for glutathione S-transferase (GST) activity (U mg^{-1} protein) in breast milk of women inhabiting Baja California Sur, México

Model	*Variable*	*Unstandardized coefficients*		*z*	*p*	*Deviance (df) minimal model*	*Scaled deviance (df) minimal model*	*95% Confidence interval of b*	
		b	*Std. Error*					*Lower*	*Upper*
GST	(Intercept)	-7.528	0.770	-9.777	< 0.01	193.27(95)	95 (95)	-9.068	-5.988
	[TSe]	0.061	0.021	2.919	< 0.01			0.019	0.103
	[THg]	0.772	0.169	4.557	< 0.01			0.433	1.111
	[TAs]	0.369	0.116	3.174	< 0.01			0.136	0.602
	[TSe] * [THg]	-0.029	0.006	-4.515	< 0.01			-0.042	-0.016
	[TSe] * [TAs]	-0.015	0.005	-3.170	< 0.01			-0.025	-0.006
	[THg] * [TAs]	-0.003	0.023	-0.112	0.91			-0.076	0.018
	Shellfish never consumed	0.857	0.446	1.923	0.05			-0.034	1.748
	Shellfish consumed once a month	0.332	0.427	0.777	0.44			-0.522	1.185
	Shellfish consumed once every two weeks	1.195	0.565	2.115	0.03			0.065	2.326
	Shellfish consumed more than twice a week	0[a]	-		-				
	Fish never consumed	-1.036	0.494	-2.100	0.04			-2.024	-0.049
	Fish consumed once a month	-0.163	0.504	-0.322	0.75			-1.171	0.846
	Fish consumed once every two weeks	0.221	0.509	0.434	0.66			-0.798	1.240
	Fish consumed more than twice a week	0[a]	–	–	–				

[a]Set to zero because this parameter is redundant. GST: Glutathione S-transferase; Std. Error: Standard error; df: degrees of freedom; [THg]: Total mercury concentration; [TSe]: Total selenium concentration; [TAs]: Total arsenic concentration.

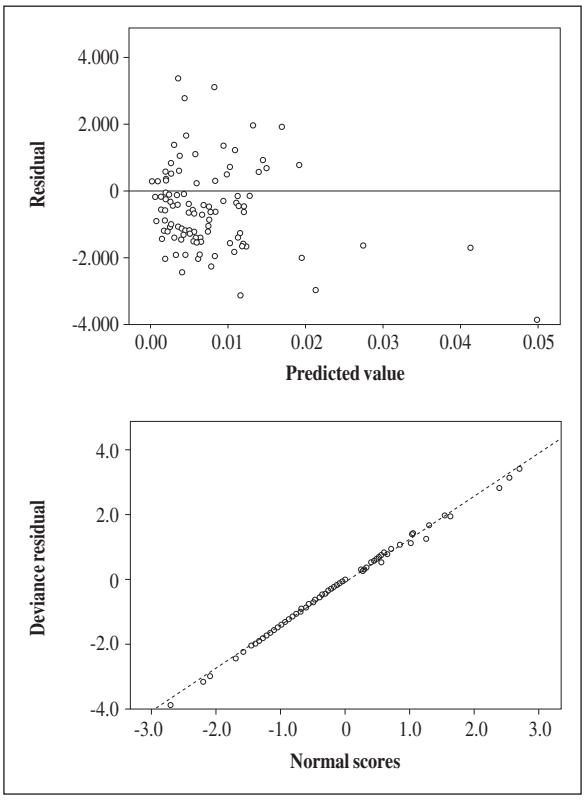


Fig. 1.—Residual plots of the minimal adequate model for glutathione S-transferase (GST) activity in breast milk of women inhabiting Baja California Sur, Mexico.

[THg] of up to 1.69 ± 0.18 μg g^{-1} and 0.01 to 0.51 μg g^{-1} have been reported, respectively, in muscle of blue shark and yellowfin tuna, both locally caught and consumed.[19,37] In this study, [TAs] was below the DL in 76% of the samples, under the concentration (1 μg L^{-1}) established by ATSDR (2007) as the safe limit for breast milk in 82% of the samples, and close to or above the threshold (10 μg L^{-1}) established as the safe limit for consumption of drinking water by WHO[35] in 2.7% of the samples. However, all samples were under the safe limit of 25 μg L^{-1} established in Mexico for drinking water[16]. In some areas of the world, drinking water may contain elevated [As]. Little As is excreted in breast milk, even in women with high exposure from drinking water. For example, in Argentina, around the Andes area, women are exposed to [As] of about 200 μg L^{-1} in drinking water, but their breast milk has approximately 2.3 μg As L^{-1} [38]. If babies were given formula with local water, arsenic exposure could be 87 times higher than for infants fed with breast milk. Therefore, exclusive breastfeeding protects infants from potential As exposure in the water used to reconstitute formula.[39] The frequency of fish intake did not show any effect on the [TAs] in this study. Statistically, there is an association between [TAs] and shellfish intake; however, a biological explanation is confounded by high variability in the data (table I), lack of information for the category of frequency of shellfish intake "more than twice a week", and values below the DL for the group with shellfish intake of "once every two weeks" (table II). Therefore, the apparent association found in this study between dietary habits and [TAs] could only be applicable to those women who rarely (once a month) or never eat shellfish.

The concentration of [TSe] in all breast milk samples at 7 to 10 days postpartum (transition milk) in this study was above the threshold for minimal adequate (6 μg L^{-1}) intake for infants who were exclusively and freely fed human milk.[17,18,20] Only 1.8% (2/108) of the samples showed [TSe] above the tolerable intake (45 μg L^{-1}).[33] The mean [TSe] in this study (21.9 μg L^{-1}) is 46% higher than the average recommended by the US Institute of Medicine for infants fed mainly with human milk (15 μg L^{-1}).[40] Both, geological factors and dietary habits, can be reflected in the elevated [TSe] found in breast milk in this study.

When the fish and shellfish intake frequency is considered, no association with [TSe] is observed ($p > 0.05$). However, [TSe] was 18% higher in those whose shellfish intake is more than twice a week as compared to those who never eat shellfish; similarly, [TSe] was 12% higher in those whose fish intake is more than twice a week as compared to those who never eat fish. The [Se] content in food can be extremely variable, depending on the combination of geological/environmental factors. The food items that are rich in [Se] are several species of fish and shellfish, approximately 1.5 to 6 times higher than in meats.[18] The geological factors and conditions make Baja California Sur a [Se]-rich area.[41] For example, [TSe] of 0.20 μg g^{-1} and 1.01 μg g^{-1} was recently reported in meat of yellowfin tuna, a fish species found in the coast of Baja California that is used for local consumption.[19]

Trace elements interactions and GST activity

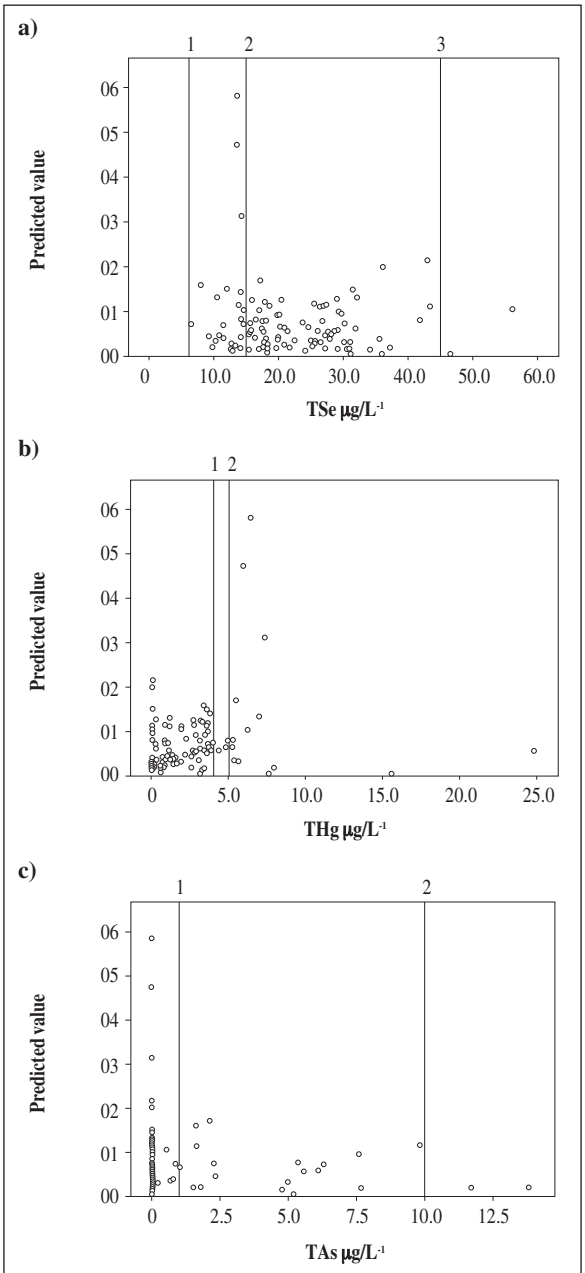
The present study was conducted with the objective of analyzing the potential link between GST activity and Se in the detoxification process following exposure to Hg and As. The GST activity was explained in the GLM chosen by the concentration of [TSe], [THg] and [TAs] together with the interactions between trace elements, specifically the interaction between [TSe] with [THg], [TSe] with [TAs] and [THg] with [TAs] additionally to the frequency of fish and shellfish intake. The interactions between [TSe] with [THg] and [TAs] appear to have an antagonistic effect, reducing GST activity (tables III and IV). The antagonistic effect of the interaction between [THg] and [TAs] did not show a statistically significant contribution in the model ($p = 0.91$) that helps to explain the activity of GST; even so, it contributed to explaining the variability of the adjusted model. The former results can be explained by the metal detoxification role of GST.

Some xenobiotics, such as As and Hg are metabolized by conjugation with glutathione (GSH), a reac-

Table IV

Fitted models for glutathione S-transferase activity and the total mercury (THg), selenium (TSe) and arsenic (TAs) concentration (μL^{-1}) in breast milk of women inhabiting Baja California Sur, Mexico, with fish and shellfish intake

Variable	*Shellfish intake*	*Fish intake*	*Model*	*Median GST measured*	*Median GST fitted model*
GST	Never	Never	$GST = e^{-7.707 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0020	0.0028
		Once in a month	$GST = e^{-6.833 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0010	0.0049
		Once e/2 weeks	$GST = e^{-6.450 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0070	0.0089
		> 2 times in a week	$GST = e^{-6.671 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	ND	ND
	Once in a month	Never	$GST = e^{-8.233 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0012	0.0014
		Once in a month	$GST = e^{-7.359 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0001	0.0028
		Once e/2 weeks	$GST = e^{-6.976 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0029	0.0048
		> 2 times in a week	$GST = e^{-7.196 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0043	0.0059
	Once e/2 weeks	Never	$GST = e^{-7.369 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0021	0.0022
		Once in a month	$GST = e^{-6.595 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	ND	ND
		Once e/2 weeks	$GST = e^{-6.112 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0036	0.0118
		> 2 times in a week	$GST = e^{-6.333 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0018	0.0120
	> 2 times in a week	Never	$GST = e^{-8.564 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	ND	ND
		Once in a month	$GST = e^{-7.690 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0028	0.0020
		Once e/2 weeks	$GST = e^{-7.307 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0015	0.0060
		> 2 times in a week	$GST = e^{-7.137 + 0.061\,[TSe] + 0.772\,[THg] + 0.369\,[TAs] - 0.029\,[TSe + THg] - 0.015\,[TSe + TAs]}$	0.0011	0.0023



Thresholds in breast milk:

a) **1.** Join FAO/WHO (1998). **2.** Institute of Medicine, Food and Nutrition Board (2000). **3.** Institute of Medicine, Dietary Reference Intakes: Applications in Dietary Assessment (2000).

b) **1.** Agency for Toxic Substances and Disease Registry (ATSDR, 1999). **2.** Deutsche Forschuengsgemeinschaft (DGF, 1984).

c) **1.** Agency for Toxic Substances and Disease Registry (ATSDR, 2007). **2.** World Health Organization (WHO, 2001).

Fig. 2.—Relationship between fitted values of glutathione S-transferase (GST) activity and total concentration of a) selenium [TSe]; b) mercury [THg]; and c) arsenic [TAs] in breast milk of women inhabiting Baja California Sur, Mexico.

tion catalyzed by GST, and is usually the first step in the detoxification process. Selenium is a central part of this process; e.g., SeO_3^{2-} reacts with GSH to form a mixed disulphide, 2 H^+ + 4 GSH + SeO_3^{2-} GSSG + GSSeSG + 3 H_2O.[22] The main effect of trace elements, such as Hg and As, with high affinity for the thiol groups (SH) of proteins and enzymes that are crucial in cell metabolism, is the production of reactive oxygen species (ROS), such as superoxide radical anion ($O_2^{\bullet-}$). The reaction with GSH can metabolize $O_2^{\bullet-}$ to protect cells from ROS-induced oxidative injury.[22]

The role of Se against organic or inorganic Hg may be different. It is possible that Se interferes with the metabolism of inorganic Hg by reacting with Hg^0 to form a less toxic compound.[42] The results from this study are in accordance with previous studies in which Hg accumulation is reduced in the presence of Se, while Hg accumulates to higher concentrations in the absence of Se.[43] This interaction between Se and Hg could be explained by the formation of a non-toxic Se-Hg complex with selenoprotein P, as was found in rat liver.[44] Although in this study only [THg] in breast milk was quantified and, thus, the fraction of it that corresponds to $HgMe^+$ is uncertain, the degradation of $MeHg^+$ to an inorganic form may be another protective mechanism that involves Se. When $MeHg^+$ is degraded to inorganic Hg, the methyl moiety can also be further degraded by homolysis to a methyl free radical.[42] These molecules may initiate a chain reaction of peroxidation of various lipid constituents; at this point, the reaction with GSH catalyzed by GST contributes to avoidance of oxidative damage induced by the methyl free radical to different organic structures.[22]

The formation of methylated metabolites is a critical step in the metabolism of inorganic and organic forms of Se and As, and it is generally assumed that the methylation pathway is directly related to the detoxification process (phase I and II). The metabolism and methylation of Se and As, are closely linked for the availability of GSH. As previously stated, inorganic forms of SeO_4^{2-} and SeO_3^{2-} are reduced by GSH to yield selenodiglutathione (GSSeSG) which is converted to hydrogen selenite (H_2Se). H_2Se is an intermediary metabolite for the synthesis of selenocysteine, which is further metabolized to the trimethylselenonium cation, the major urinary product of Se metabolism[3]. Similarly, As^{5+} is reduced to As^{3+} by arsenate reductase or purine nucleoside phosphorylase (PNP), which requires GSH;[45] subsequent methylation by As methyltranferase generates di- and trimethylated metabolites with the same excretion route as Se[3].

Other studies have described a direct interaction between Se and As[46] in aqueous solution (in the present study, milk) which may play a role in dissolution of these elements[3]. Many chemical forms of Se have been described in nature. In the diet, Se occurs in the +6 oxidation state as selanate (SeO_4^{2-}), +4 oxidation state as selenite (SeO_3^{2-}), 0 oxidation state as elemental (Se), -1 oxidation state as selenocystine, and -2 oxidation state as selenocysteine[3]. Similarly, As can occur in the As^{5+} oxidation state as arsenate, As^{3+} oxidation state as arsenite, 0 oxidation state as elemental As, and the -1 and -2 oxidation as arsenical pyrites[3]. Because Se and As have similar chemical and physical properties (e.g.

similar valance shell, electronic structure and atomic radio) they can be biologically antagonist to each other reducing their potential toxicity.

All previous alternatives can contribute to explain the finding in this study, that the GST activity in breast milk samples appears to be reduced according to the negative correlations found between the activity of the enzyme and the [TSe] * [TAs] and [TSe] * [THg] interactions suggested in the proposed model. Nevertheless, neither Se-Hg nor Se-As complexes were quantified in the present study. The GST activity was also explained in this study by positive correlations with concentrations of [THg], [TAs] and [TSe] and to fish and shellfish intake. These results suggest that a diet which includes fish and shellfish (rich in Hg and Se)[19] increases GST activity in breast milk, while the concentration of Se, interacting with Hg and As, has an antagonistic and protective effect reducing GST activity measured in breast milk.

Conclusion

The GST activity in human milk obtained from 108 breastfeeding women from Baja California Sur was positively correlated to [THg], [TAs] and [TSe], and the activity of the enzyme was also explained in the GLM by the frequency of consumption of marine fish and shellfish in the diet of the sampled women. Further, the generalized model constructed with the data from this study suggests that GST activity in breast milk samples is reduced by the interactions between [TSe] * [TAs], [TSe] * [THg] and [TAs] * [THg]. Potential interactions between these elements, speciation of each element, as well as the potential role of GSH and other antioxidants and their relative contribution to reduce the levels of xenobiotics in human milk warrant attention. Finally, the present study highlights the benefits of a marine fish and shellfish-based diet (rich in Se) during breastfeeding and enhances the notion that a marine diet should not represent a risk for neonates.

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