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Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon[☆]

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Abstract

This study aimed to examine the influence of the consumption of traditional foods on the relationship between fish consumption and mercury (Hg) exposure. A 12-month prospective dietary survey was carried out with 26 adult women from a fish-eating community in the Brazilian Amazon. Food ingestion was determined using a daily food diary, and total hair Hg levels were assessed for each month through sequential analyses using cold vapor atomic absorption spectrometry. Simple and multiple regression analyses showed that the strong relationship between fish consumption and Hg exposure was significantly modified by fruit consumption: for the same number of fish meals, those who ate more tropical fruits had lower hair mercury levels. The findings of this study indicate different ways of maintaining fish consumption while reducing Hg exposure in the Amazon. A number of phytochemicals and nutritional fibers present in fruits might be interacting with Hg in several ways: absorption and excretion, transport, binding to target proteins, metabolism, and sequestration. More studies are required on larger populations to further elucidate the extent and public health implications of the use of fruits to counteract the toxic action of methylmercury.

Keywords: Food consumption; Hg exposure; Hair Hg; Amazon; Brazil

1. Introduction

Mercury (Hg) is well recognized as a potential threat to human health due to its capacity to cause systemic toxicity. Methylmercury (MeHg) is the most toxic chemical form of the element and can have crucial effects on the central nervous system (WHO, 1990). The change in the chemical nature of Hg from inorganic to methylated forms is the first step in the aquatic bioaccumulation process, and therefore fish are a dominant source of human exposure to MeHg (WHO, 1991). It has been suggested that several factors may influence a population's vulnerability to the effects of MeHg. Among those that have received considerable attention are age, gender, health status, nutritional status, and the intake of other foods or nutrients that might influence the absorption, uptake, distribution, and metabolism of MeHg (NRC, 2000).

Despite the recognized importance of the influence of nutrition on MeHg intoxication, dietary information is not usually collected in most epidemiological studies examining the effects of MeHg exposure (Chapman and Chan, 2000). Studies carried out in different parts of the

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[☆] The present investigation was carried out in the context of a larger interdisciplinary study focusing on the sources and dispersion of mercury in the Amazonian environment, as well as its effects on the health of fish-eating populations living in that region. The research had financial support from the International Development Research Center (IDRC) of Canada. There are no financial or personal relationships between either the authors or their institutions and the funding institution that could have caused any conflicts of interest in the interpretation of the findings. In addition, the sponsor had no role in the study design, data collection, data analysis and interpretation, or the writing of the manuscript. Finally, we formally assure that this study involving humans was conducted in accordance with Brazilian Guidelines for the protection of human subjects.

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globe have shown different Hg toxicities in populations exposed to relatively similar doses of MeHg through the consumption of contaminated fish (Dolbec et al., 2000; Grandjean et al., 1997; Lebel et al., 1998; Myers et al., 1997; Wheatley and Paradis, 2000). These inconsistencies in MeHg toxicity are often attributed to the possible effects of dietary modulation, even though the mechanisms of interaction between nutrients and MeHg have not yet been fully elucidated (Chapman and Chan, 2000).

Hg contamination in the Brazilian Amazon was initially identified following the great gold rush in the late 1970s (Goulding et al., 1996). Since then, numerous studies have shown high levels of Hg in fish and human populations in many areas of the Amazon (Martinelli et al., 1988; Barbosa et al., 1995). Recent studies have reported dose-related deficits in neurological and neuropsychological functions, as well as cytogenetic properties among adults and/or children from this area (Amorim et al., 2000; Dolbec et al., 2000; Grandjean et al., 1999; Lebel et al., 1998). The problem of Hg is now known to be much more widespread than originally thought, since recent studies have demonstrated that deforestation resulting from massive colonization and "slash-and-burn" agricultural practices has caused intensive erosion of podzolic soils rich in naturally occurring Hg, contributing significantly to the contamination of waterways (Roulet et al., 1999). Thus, in the Amazonian basin, Hg from different sources is available for methylation processes contaminating the fish resources, which constitute the main source of animal proteins for the large population living along the riverbanks of this region (Boischio et al., 1995; Dolbec et al., 2001; Lebel et al., 1997).

Although several controlled experiments have estimated the effects of specific nutrients on Hg absorption and toxicity (Calabrese, 1978; Imura and Naganuma, 1985; Levander and Cheng, 1980; Peraza et al., 1998; Solomons and Viteri, 1982; Whanger, 1992), there is still a lack of information about the importance of the general diet in determining Hg concentrations in free-living populations. The purpose of this study was to examine whether specific foods in the local diet could influence Hg exposure through fish consumption among adult women from a riverside population in the Brazilian Amazon.

2. Materials and methods

2.1. Study design

A prospective dietary survey was undertaken with women from a village in the Brazilian Amazon, where fish is a dietary mainstay and previous studies had shown a strong relationship between fish consumption and hair mercury (HHg) levels (Lebel et al., 1997). Food consumption was recorded daily for a period of 12 months (March 1999–February 2000). At the end of this period, hair samples of at least 12 cm were taken for sequential analyses. In this way, food consumption frequency for a 12-month period could be examined over time with respect to HHg concentration levels.

2.2. Population

The study was carried out in the village of Brasília Legal (3°59′00″S, 55°30′00″W), which is situated on the Tapajós River, a major tributary of the Amazon. During a meeting with the villagers to discuss the general issues of an on-going larger study on the health effects of Hg, women of the village were invited to participate on a voluntary basis in a daily food intake survey that would last for 1 year. They were informed that over this period they should not cut their hair.

Thirty women were enrolled at the beginning of the study. Three subjects moved away during this period and 1 did not fill out the questionnaire correctly. Thus, at the end of the follow-up data were available for 26 women.

2.3. Assessment of food consumption frequency

A daily food diary was designed with input from the community in order to determine food consumption frequency. The questionnaire was pretested over a 1-month period prior to data collection. A food list was prepared with most of the regional food items covering several nutritional groups such as fish, red meats and chicken, dairy, eggs, breads, cereals, vegetables, tubercles, beans, spices, fruits, and tea and coffee. For each meal, the respondents checked the ones that they had eaten; a space was provided to write the specific type. For example, if a woman had checked "fish", she would then write the specific fish species. Spaces were likewise provided for food items that were not listed. These food diaries were verified daily by a trained community health agent, and special assistance was given to those who had difficulty writing. The food frequency was then calculated as the number of meals that included this food.

2.4. Hair sampling and analyses

Hair has been extensively used as a bioindicator of Hg exposure. Since hair grows at approximately 1 cm per month, it is possible to reconstitute recent past exposure history (Cernichiari et al., 1995) by cutting the hair into centimeter-long samples and analyzing the Hg content of each centimeter independently. In the present study, this retrospective assessment allowed us to examine in parallel the dietary habits and Hg contamination on a monthly basis for the period of 1 year.

Hair strands from the root were taken from the occipital region and then placed in plastic bags, with the root end stapled. The samples were analyzed at the Laboratory Services of the Research and Environmental Health Division, Health Canada. Analyses of total and inorganic Hg were performed according to the method described by Farant et al. (1981). Hair strands were cut in 1-cm segments, and each of the 12 segments was analyzed for total and inorganic Hg, using a stannous-chloride–cadmium chloride solution for the determination of total Hg and a stannous chloride solution for the determination of inorganic Hg. The concentrations of Hg were determined by cold vapor atomic absorption spectrometry (CVAAS), using a Hg UV monitor. Analytical quality control was ensured with standard hair samples, provided by the Hair Mercury Inter-laboratory Comparison Program of Health Canada.

2.5. *Statistical analysis*

For each food group, the consumption frequency was calculated by adding the reported frequency (number of meals) for each food item. The relationship between food intake and hair Hg was examined annually and seasonally. For the annual measures, the 12 monthly concentrations of hair Hg were averaged to provide a mean annual hair Hg and examined with respect to the total number of meals that included this food. For the seasonal measures, the year was divided into four seasons: high-water (March, April, May), descending-water (June, July, August), low-water (September, October, November) and rising-water (December, January, February). The mean hair Hg for each of the seasons was calculated and examined with respect to the number of meals during that season.

The associations between mean hair Hg and the total consumption of specific food groups was first assessed with univariate analyses. Correlation coefficients were used to estimate the association between individual food item consumption frequency and HHg concentrations. Simple and multiple linear regression models were used to estimate the association between food consumption frequency and HHg levels. The HHg level was the dependent variable in separate linear regression models which tested for the influence of the diverse food items present in the different nutritional groups studied; these were included as continuous independent variables. Analysis of covariance (ANCOVA) was used to test interactions.

Results were defined as statistically significant for a value of $P \leqslant 0.05$. Analyses were performed using the Statistical Analysis System (SAS), UNIX 6.12 version and Statview 5.0 (SAS Institute Inc.).

3. Results

The socio-demographic characteristics of the study population are shown in Table 1. Schooling varied between 0 and 11 years (mean: 6±3 years) and the age range was 23–62 years (mean: 41±11 years). Twenty-two of the women were born in a village on the Tapajós River and lived there most of their lives; the 4 others came from other areas in the Brazilian Amazon. Table 2 shows the reported food items throughout the study, grouped by their respective food groups.

Fig. 1 shows the mean HHg levels over 12 months and the standard deviation for each woman. Overall, the average HHg was 10.0 μg/g±4.7 (median: 9.1 μg/g, ranging from 4.0 to 20.0 μg/g). The average percentage of MeHg was 95.7%, ranging from 89.5% to 100%.

The women ate, on an average, approximately eight fish meals/week, ranging from about 4 to 12/week. Table 3 shows the annual fish consumption frequency for fish species that were eaten at least 1/month; those that were less frequently eaten were grouped as "others." Carnivorous fish made up an average of 43.5% of the fish diet, ranging from 22% to 65%.

Fig. 2 shows the relationship between total annual fish consumption (meals/year) and annual mean HHg levels ($r^2 = 0.33$; $P < 0.01$). Partial correlation analyses of fish consumption categorized by feeding habits and Hg levels show that the frequency of consumption of carnivorous fish is highly correlated to HHg (partial $r = 0.63$; $P < 0.001$), while the frequency of consumption of noncarnivorous fish was not significantly correlated to HHg (partial $r = 0.14$; $P = 0.18$). This is reflected in the individual species; Table 4 shows the Spearman correlations between the frequency of consumption of specific fish species and HHg. The highest correlations were observed for large carnivorous fish such as *Tucunaré*, *Pirarucu,* and *Filhote.* The consumption of *Pescada*, a widely eaten carnivorous fish, was not significantly

Table 1
Socio-demographic characteristics of the study population (26 women)

Characteristic	*n*	Mean±SD	Median	%
Age (years)	26	41.2±11.3	41	100
Education (years)	26	5.9±3.3	4	100
Alcohol consumption				
Drinks	16			61.5
No longer drinks	5			19.2
Never drank	5			19.2
Smoking habits				
Smokes	1			3.8
No longer smokes	6			23.1
Never smoked	19			73.1
Born by the Tapajós river	22			84.6

Table 2
Types of food reported by the study population

Food groups	Foods included in each food group
Fish	Pescada, barbado, dourada, filhote, pirarucu, sarda, surubim, tucunare, piranha, aracu, caratinga, pacu, cujuba, cara, tambaqui, jaraqui, sardinha
Meats	Beef, pork, chicken, game
Dairy	Milk, cheese, butter, yogurt
Eggs	Chicken eggs
Breads and cereals	From refined grain products: rice, crackers, biscuits, breads, pasta, corn flour, corn meal
Vegetables	Lettuce, chicory, cabbage, pumpkin, summer squash, beetroot, carrot, chayote, corn, cucumber, okra, tomato, kale, cauliflower, radish, spinach
Tubercles	Manioc and its derivatives, potatoes
Beans	Red, black, white beans
Spices	Onions, garlic, pepper, green pepper
Fruits	Bananas, ingá, oranges, tangerine, tapereba, coconut, avocado, pupunha, pineapple, mango, goyava, papaya, açai, ata, cupuaçu, muruci, tucuma, cacao, passionfruit, jambo

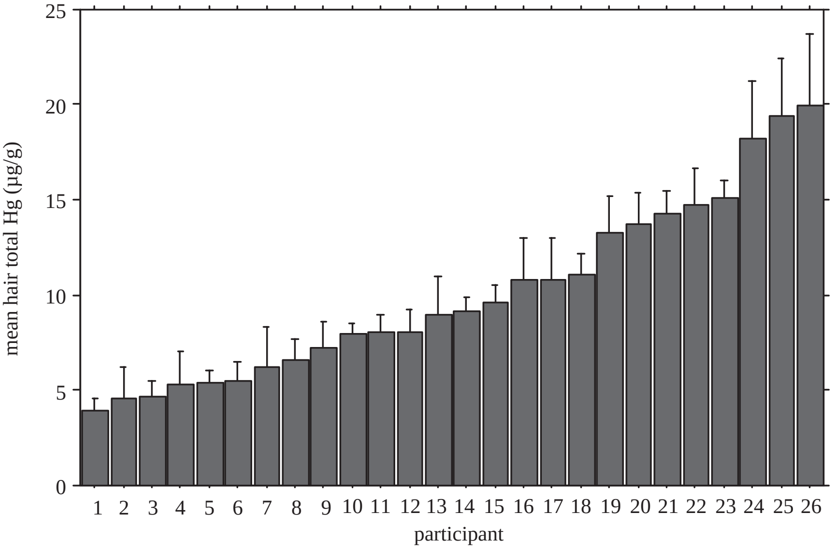


Fig. 1. Mean hair mercury levels over 12 months and the standard deviation for each woman.

correlated with HHg, while that of *Pacú* and *Tambaqui*, noncarnivorous fish, showed a tendency. These same relationships were observed when the fish were entered two-by-two into a multiple regression model.

The women in this study consumed an average of 7.5 meat meals/week, ranging from 4.8 to 12 meals/week (Table 5). Beef was included in an average of 4 meals/week, while chicken was eaten on an average of once a week. Annual meat consumption was negatively correlated to HHg ($r_p = -0.53$; $P < 0.05$). However, when multiple regression analyses were performed with both meat and fish in the model, only fish consumption remained significant. Moreover, there was an inverse relation between the consumption of fish and meat ($r_p = -0.58$; $P < 0.01$). Thus, the inverse relationship between meat and HHg was due to the fact that meat replaced fish meals, thus reducing Hg intake.

Fruits are widely consumed in this village and these women ate fruit at an average of one meal per day (Table 6). Many types of fruit are seasonally available, but the most commonly eaten are bananas (*Musa* spp., Musaceae), ingas (*Inga* spp., Leguminosae-Mimosoideae), and Oranges (*Citrus* spp., Rutaceae). A negative correlation was observed between the frequency of fruit consumption (all fruit) and HHg ($r = -0.39$; $P = 0.05$). However, contrary to meat, when both fruit and fish were included in the multiple regression model with HHg, both remained significant (fish: $\beta = 0.025$; $P < 0.001$; fruit: $\beta = -0.012$; $P < 0.01$; model $r^2 = 0.50$). The model remained significant when carnivorous and noncarnivorous fish were included separately. Furthermore, no relation was observed between total fruit and total fish consumption ($r_p = -0.13$; $P > 0.50$). Table 7 shows regression

Table 3
Descriptive statistics for the frequency of fish consumption (meals/year) for 26 women

Fish species	Fishes feeding habits[a]	Mean ± SD	Median	Range
Pacu (*Mylossoma* sp.)	n-c	87±34	85	41–167
Pescada (*Plagioscion* sp.)	c	80±31	74	49–164
Aracu (*Shizodon* sp.)	n-c	60±30	58	24–130
Caratinga (*Geophagus* sp.)	n-c	57±27	51	6–116
Tucunare (*Cichla* sp.)	c	50±24	47	10–121
Jaraqui (*Semaprochilodus* sp.)	n-c	19±12	18	4–55
Pirarucu (*Arapaima gigas*)	c	25±21	14	2–70
Piranha (*Serrasalmus* sp.)	c	8±8	6	0–34
Filhote (*Brachyplatystoma* sp.)	c	7±9	4	0–39
Dourada (*Brachyplatystoma flavicans*)	c	5±6	3	0–28
Tambaqui (*Colossoma macropomum*)	n-c	4±5	3	0–18
Surubim (*Pseudoplatystoma* sp.)	c	3±4	1	0–13
Cujuba (*Pseudodoras niger*)	c	3±5	0	0–22
Others	—	25±13	23	4–57
Total carnivorous fish	c	184±62	185	88–301
Total noncarnivorous fish	n-c	236±70	229	123–400
Total fish	—	434±108	424	222–675

[a] c, Carnivorous; n-c; noncarnivorous.

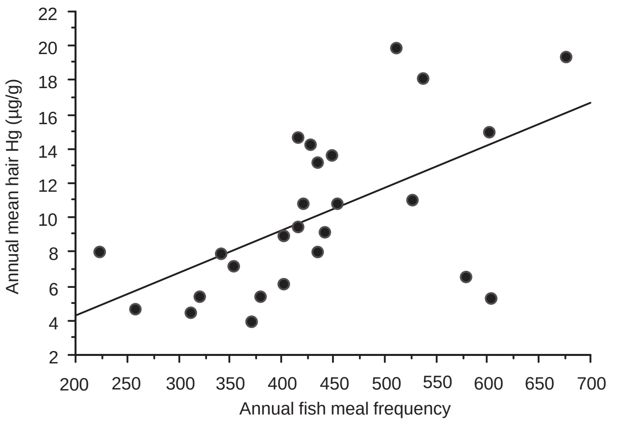


Fig. 2. The relationship between total annual fish consumption (meals/year) and mean hair Hg concentrations for 26 women.

coefficients for the fruit most frequently consumed and hair Hg levels from the multiple regression models, which include fish consumption.

Fig. 3 illustrates the influence of fruit consumption on the relationship between fish consumption and HHg levels. The regression lines are drawn for those who ate less than one fruit/day ($n = 13$) ($\beta = 0.034$; $P < 0.001$) and for those who ate fruit at least 1 meal/day ($n = 13$) ($\beta = 0.007$; $P = 0.44$). ANCOVA shows that the intercept of the two regression lines is not significant, but their slopes are, as the interaction term is significant ($F = 5.63$; $P < 0.05$). Thus, for the same quantity of fish intake, persons who ate at least one fruit/day had lower HHg levels. The relationship between fruit and fish consumption and HHg levels was tested for each season, which showed the same pattern for the entire year.

Dairy products, breads, cereals, vegetables, tubercles, and spices were consumed daily and did not show any relationship with HHg. Eggs were eaten more sporadically and no relationship was observed between eggs and HHg.

4. Discussion

In the Amazon, Hg exposure of riverine populations who depend on fish as a dietary mainstay presents a public health dilemma; on the one hand fish are the vehicle for this highly toxic substance and on the other hand fish constitute an essential and healthy food and there are few substitutes. The challenge is thus to maintain fish consumption while reducing Hg exposure. The findings of this study indicate different ways that this can be achieved.

As expected, the consumption of carnivorous fish, which are higher in the trophic chain and have higher levels of Hg, correlates positively with HHg (Dolbec et al., 2001; Lebel et al., 1997). On the other hand, the consumption of meat and chicken mediates the effect of Hg by substituting fish meals with meals that include meat and chicken. Indeed, the inverse relationship between meat and fish meals supports the fact that meat and chicken are replacing fish. It should be noted that there are not large quantities of meat or chicken available in these villages and that a mixed diet in which some meals are made up of small amounts of meat (usually cut up and served with rice) or chicken helps to reduce Hg exposure by replacing fish as a source of animal protein.

Table 4
Spearman correlation coefficients for frequency of fish consumption (meals/year) for 26 women in relation to mean hair Hg levels

Fish species	Fishes feeding habits[a]	Spearman coefficients	*P* values
Pescada (*Plagioscion* sp.)	c	0.06	0.78
Tucunare (*Cichla* sp.)	c	0.52	0.01
Pirarucu (*Arapaima gigas*)	c	0.42	0.04
Piranha (*Serrasalmus* sp.)	c	0.43	0.03
Filhote (*Brachyplatystoma* sp.)	c	0.50	0.01
Dourada (*B. flavicans*)	c	0.26	0.19
Surubim (*Pseudoplatystoma* sp.)	c	0.32	0.11
Cujuba (*P. niger*)	c	0.37	0.06
Pacu (*Mylossoma* sp.)	n-c	0.38	0.06
Caratinga (*Geophagus* sp.)	n-c	0.17	0.40
Aracu (*Shizodon* sp.)	n-c	0.14	0.47
Jaraqui (*Semaprochilodus* sp.)	n-c	−0.01	0.98
Tambaqui (*C. macropomum*)	n-c	0.35	0.08
Others	—	0.13	0.52
Total carnivorous fish	c	0.57	0.005
Total noncarnivorous fish	n-c	0.30	0.14
Total fish	—	0.60	0.003

[a] c, Carnivorous; n-c, noncarnivorous.

Table 5
Descriptive statistics for the frequency of meat consumption (meals/year) for 26 women

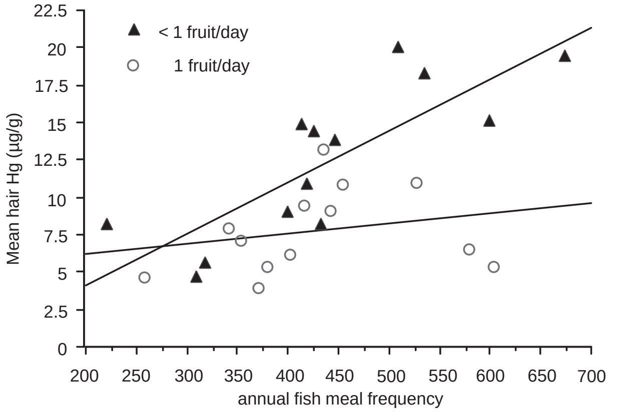
Meats	Mean ± SD	Median	Range
Beef	205 ± 71	181	99–412
Chicken	79 ± 30	79	13–137
Pork	20 ± 11	18	3–41
Game	87 ± 26	85	36–146
Total meat	393 ± 88	373	247–626

Table 6
Descriptive statistics for the frequency of fruit consumption (meals/year) for 26 women

Fruit species	Mean ± SD	Median	Range
Bananas	189 ± 93	177	66–425
Ingás	72 ± 52	61	16–279
Oranges	44 ± 30	38	13–146
Others	210 ± 76	210	84–348
Total fruit	574 ± 187	552	334–935

Table 7
Regression coefficients (β_1) for the frequency of fruit consumption (meals/year) in multiple linear models with fish consumption (meals/year) as an independent variable and hair Hg levels (μg/g)

Fruit species	β coefficient	*P* values
Bananas	−0.018	0.03
Ingás	−0.045	0.01
Oranges	−0.063	0.02
Others	−0.013	0.20
Total fruit	−0.012	0.003



Total hair Hg (µg/g) = 2.689 + 0.034 * fish meals; R^2 = 0.652 (< 1 fruit/day)
Total hair Hg (µg/g) = 4.874 + 0.007 * fish meals; R^2 = 0.054 (1 fruit/day)

Fig. 3. The influence of fruit consumption on the relationship between fish consumption (meals/year) and hair Hg levels (μg/g).

The most important finding of the present study is that fruit consumption appears to modulate the relationship between fish consumption and Hg exposure. The results suggest that for the same level of fish consumption, eating tropical fruit is associated with lower levels of HHg, and this relationship is observed for all periods of the year. There are several mechanisms by which fruits may affect Hg levels. Phytochemicals may interact with toxic metals in several ways in the body: absorption and excretion, transport, binding to target proteins, metabolism and sequestration (Peraza et al., 1998). A possible explanation is that the soluble dietary fiber of fruits could be interfering with absorption at the intestinal level. Although there appears to be an overall effect of fruit consumption, individual fruit may contain elements that can directly or indirectly reduce Hg levels; thus, it could be of interest to further examine the links between fruits with high levels of vitamin C (such as oranges and guavas) and Hg levels. In the present study only one woman smoked and six were ex-smokers; thus, we could not examine the relationship between smoking habits and Hg levels, but such a relationship should be examined in further studies with a larger population. Additionally, a number of studies suggest that an important group of health-promoting phytochemicals is the polyphenols (Bravo, 1998). These compounds are particularly abundant in fruits, and one of their important properties is that of antioxidants; they can form complexes with reactive metals, thus reducing their absorption.

Chapman and Chan (2000), in a review of the influence of nutrition on MeHg intoxication, report on studies that have been conducted on the effects of nutrients on Hg metabolism. Of these, one study has suggested that nutritional factors influence the reabsorption rate of MeHg rather than its primary absorption. They note that most epidemiological studies have not collected sufficient and detailed dietary information, and consequently it is still unclear how dietary factors can affect the absorption, metabolism, and/or excretion of MeHg.

From the viewpoint of public health, fruit consumption has been shown to be beneficial for well-being; studies have demonstrated that fruits reduce the risk of many chronic diseases (Botterweck et al., 1998; Joshipura et al., 1999). Here, bananas, ingás, and oranges showed the largest influence on HHg. Ingás are nitrogen-fixing trees which are much used in American humid tropics. Although this genus comprises about 180 species in the Brazilian Amazon, only 4 or 5 are actually consumed for the edible pulp around their seeds (Cavalcanti, 1996). Among these, *Inga edulis* Mart. (ingá-cipó), an important ingá cultivated in the home gardens of the study area, is valued in agroforestry for its rapid growth, tolerance of acid soils, and high production of leafy biomass that helps to control weeds and erosion (NFTA, 1993).

The daily prospective survey carried out in this study was based on the importance of knowing the fluctuations of food choices over time to assess the beneficial or deleterious effects of diet on health (Willett, 1994). Indeed, this study design allowed us to examine the relationships on a seasonal basis, which was very important in the identification of different fruit and fish species during different periods of the year.

This is the first study to observe, in a free-living population, that the consumption of tropical fruits can modify Hg levels in humans. Although the investigation focused on a small number of individuals, the results, which are consistent seasonally, offer support to the viewpoint that dietary modulation can be an important determinant of a population's Hg profile. This study did not allow us to determine whether the action of fruit nutrients is on the absorption, metabolism, or excretion of Hg, and experimental studies would be useful for identifying the specific agent. More studies are required on larger populations to further elucidate the extent and public health implications of the use of fruits to counteract the toxic action of MeHg.

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