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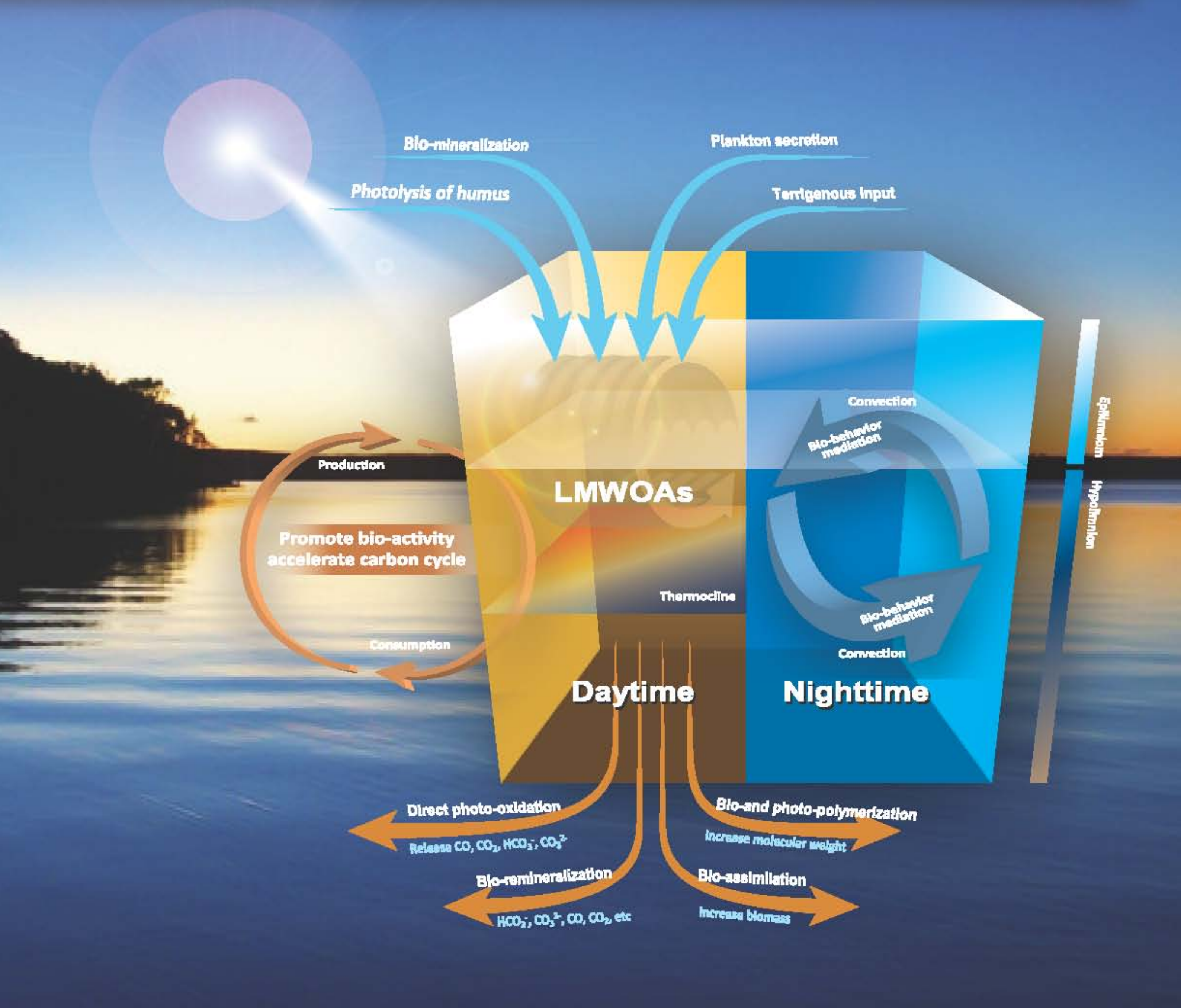

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Umbilical cord blood mercury levels in China

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Abstract

Mercury (Hg) is a well-known neurotoxicant. Hg exposure at high levels can harm individuals of all ages. Even low level exposure to Hg can damage the brain of fetuses and young children, and affect their central nervous system and cognitive development. The aims of our study were to measure total Hg levels in infant umbilical cord blood and to investigate the risk factors associated with total Hg cord blood levels in various cities in China. Our goal was to provide clues for the prevention of Hg exposure in utero. The results indicated that the average cord blood mercury levels (CBMLs) were (1.81 ± 1.93) μg/L, which were lower than those found in most previous studies. The concentrations also differed according to geographic region. The CBMLs were not only associated with family economic and living conditions, but also with diet in pregnant women, especially the intake of marine fish, shellfish, poultry, formula milk and fruits.

Key words: infant; mercury; risk factor; umbilical cord blood

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Introduction

Mercury (Hg) is one of the most common heavy metals, and has a high degree of accumulation and neuro-developmental toxicity. The general population may be exposed to three forms of mercury: elemental, inorganic, or organic (predominantly methyl) (Schober et al., 2003). Even though the placenta may accumulate an extremely high Hg content, it is not an absolute barrier and does not prevent the transmission of Hg from maternal blood to fetal blood (Pugach et al., 2009). Minamata disease, which occurred in Japan in the fifties and sixties of the previous century, was caused by methyl mercury (MeHg) pollution (Yorifuji et al., 2009). Both elemental Hg and MeHg can easily pass through the placenta to the fetus, and when elemental Hg is changed to polar compounds, it can not recross to the maternal circulation, resulting in accumulation of Hg in fetal tissues including the developing brain (Ramirez et al., 2003). Fetuses are known to be a high-risk group for MeHg toxicity due to the high susceptibility of the developing brain (Sakamoto et al., 2007).

In the past few decades, environmental Hg levels have significantly increased following increased industrial development. A wide range of anthropogenic activities emit large quantities of Hg into the environment, such as coal combustion in thermal power plants, industrial, commercial, and residential boilers, oil product combustion, cement production in wet and dry rotary kilns, and primary and secondary lead, zinc and steel production (Pacyna et al., 2006, 2010). Asian countries contributed about 64% of the global Hg emission from anthropogenic sources in 2007, followed by Europe (14%) and South America (10%) (Pirrone et al., 2010).

As China is the largest coal producer and consumer in the world, Hg emissions in China have been increasing rapidly in recent years and are receiving increased attention (Wang et al., 2010). With different levels of economic development and industrialization, and with varying population characteristics across different regions, the state of Hg exposure in each region and the associated risk factors remain undefined. The aims of this study were: firstly, to investigate the total Hg levels in infant umbilical cord blood samples across different cities in China; secondly, to assess the correlates of Hg exposure; thirdly, to provide strategies in order to prevent Hg exposure in utero.

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1 Materials and methods

1.1 Subjects

Geographically, China is composed of seven regions (Northeast, North, Northwest, Southwest, Central, East and South), with varying socio-economic development and population characteristics. We chose a major metropolitan city (capital city or central city) in each region, and one key maternity hospital in each city. These hospitals included Baoding Maternal and Child Care Hospital (North China), Women and Children's Hospital of Guangzhou (South), Kunming Maternal and Child Health Hospital (Southwest), Shengjing Hospital of China Medical University (Northeast), Wuhan Medical and Health Center for Women and Children (Central), Maternal and Child Health Hospital of Shanxi Province (Northwest) and Xiamen Maternal and Child Health Hospital (East). Approximately 200 pairs of mothers and full-term infants were recruited in each hospital between October and December 2008. Mothers who suffered from diabetes, chronic hypertension, chronic renal or cardiac disease during pregnancy were excluded. We initially recruited 1384 pregnant women with written consent forms. After delivery, 61 missed cord blood collection. Thus, 1323 mother-neonate pairs were included in the study with written consent and completed questionnaires. The study protocol was approved by the Medical Ethics Committee of Xinhua Hospital.

1.2 Questionnaire

A questionnaire was administered to the study subjects to obtain the information regarding: age, education level, and occupation of the pregnant women, the family income per capita, place of residence, years of residence in that location and residential story, reproductive history, smoking history of the family members and the pregnant women, and dental treatment during pregnancy. The dietary habits of the women during the gestation period were obtained using a food frequency questionnaire in which typical diet items were listed, including milk, eggs, beef, pork and mutton, poultry, kidney, liver, marine fish, freshwater fish, shrimp, shellfish, crab, bean products, barbecued foods, fried/flamed foods, leafy vegetables, root vegetables, and fruits. The dietary intake frequency was divided into four categories: 1 time/week, 1–3 times/week, 4–7 times/week, and ≥8 times/week.

1.3 Blood and data collection

Two well-trained nurses in each identified hospital obtained all blood samples according to the standard protocol. About 2 mL of umbilical cord blood was collected in an Eppendorf tube (10 μL of 7% EDTA was added in advance as an anticoagulant) using a sterilized syringe and a stainless steel needle, which was then homogenized and stored at –20°C immediately (Gao et al., 2007). All specimens were transported by air to the MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine in dry ice, and then stored at –20°C in the laboratory biobank until analysing. After delivery, all mothers were invited to complete a questionnaire consisting of about 100 questions concerning possible risk factors for Hg exposure during pregnancy.

1.4 Biochemical analysis

The concentration of total Hg in each umbilical cord blood sample was analyzed by cold vapor atomic absorption spectrometry (AAS) using an automatic Hg analyzer (Model DMA-80, Milestone, Italy). Samples were collected, stored, and analyzed using a standardized method (Gao et al., 2007). External quality assessment entailed the assay of control samples (Contox) purchased from Kaulson Laboratory, Inc., NJ, USA. After every 50 samples, a new boat was used after heating it twice to remove traces of Hg.

1.5 Statistical analysis

All statistical analyses were undertaken using SPSS 11.0 for Windows. Because the distribution of total Hg levels was skewed, analyses were performed on the natural logarithm of cord blood mercury levels (CBMLs), and all means reported were geometric. The descriptive statistics were analyzed for maternal and neonatal essential features. Multiple regression analyses were conducted in an attempt to construct an appropriate model to explore the related factors that affected the CBMLs. A stepwise approach was used, and the criteria for inclusion and exclusion were $P < 0.05$ and $P > 0.1$, respectively. A P-value less than 0.05 was considered statistically significant. The "exclude cases pair wise" method was used to handle the missing data.

2 Results

In total 1323 mother-neonate pairs were recruited into the study. The mean age of the mothers was 28.55 (SD: 3.98) years at delivery. The mean gestational duration was 38.99 (SD: 1.21) weeks. During pregnancy, 46 (3.48%) women had dental treatment. The prevalence of maternal smoking during pregnancy was low (6.73%), but 60.92% of the pregnant women's family members smoked, resulting in secondhand smoke exposure. Birth weight was < 2500 g in 11 infants (**Table 1**).

The average CBMLs in the seven cities was 1.81 μg/L (SD: 1.93), ranging from 0.91 μg/L in Baoding to 3.34 μg/L in Xiamen. We found that the CBMLs were statistically significantly different among the cities ($F = 633.961$, $P = 0.000$) (**Table 2**). Risk factors for Hg exposure included higher family income per person (odds ratio (OR) = 1.50), intake of poultry (OR = 1.91), marine fish (OR = 1.69), shellfish (OR = 2.21) and formula milk (OR = 2.32) during pregnancy. Protective factors included higher apartment floor (OR = 0.70), housewife/catering/unemployed in

Table 1 Maternal and neonatal essential characteristics

Variables		Mean	SD	Median	Min–Max	Number (percentage)
Mother's age (year)		28.55	3.98	28.42	17.42–42.42	1302
Gestational duration (week)		38.99	1.21	39.00	31.00–42.00	1228
Birth length (cm)	Male	50.35	1.63	50.00	42.00–60.00	624
	Female	49.86	1.56	50.00	40.00–56.00	558
Birth weight (g)	Male	3414.83	444.42	3400.00	1800.00–5780.00	650
	Female	3309.63	429.71	3300.00	1790.00–6600.00	579
Head circumference (cm)	Male	33.49	1.71	33.50	26.70–36.50	300
	Female	33.49	1.87	33.50	29.00–46.00	270
Dental treatment during pregnancy	No					1237 (93.50%)
	Yes					46 (3.48%)
	Refuse					40 (3.02%)
Smoking during pregnancy	No					1168 (88.28%)
	Yes					89 (6.73%)
	Refuse					66 (4.99%)
Family members smoking	No					509 (38.47%)
	Yes					806 (60.92%)
	Refuse					8 (0.61%)

Table 2 Cord blood mercury levels (CBMLs) in seven cities

City	Number	Mean ± SD (μg/L)*	Geometric mean (μg/L)	%> EPA RfD
Baoding	198	1.01 ± 0.49	0.91	0.00
Guangzhou	196	3.10 ± 1.43	2.80	6.10
Kunming	157	1.76 ± 1.23	1.55	0.60
Shenyang	195	2.37 ± 0.42	2.18	0.00
Wuhan	183	2.23 ± 1.03	2.05	1.60
Xi'an	196	1.22 ± 0.92	1.05	0.50
Xiamen	198	3.99 ± 2.54	3.34	18.70
Total	1323	2.26 ± 1.69	1.81	4.10

* F = 633.961, P = 0.000. %> EPA RfD: percentage above the U.S. Environmental Protection Agency's reference dose.

former job (OR = 0.37), and more intake of fruits (OR = 0.70) during pregnancy (**Table 3**).

3 Discussion

3.1 Cord blood mercury levels

This study showed that the geometric mean of the CBMLs in the seven cities was 1.81 μg/L (**Table 4**). Previously publications reported CBMLs the mean total Hg concentration in cord blood varied between 0.5 and 35 μg/L (Murata et al., 2007). China is therefore at the lower end of the distribution. Earlier local studies in China have also been reported. The Di'er Songhua River was polluted with Hg in the 1960s to 1980s. Songyuan is in the downstream region of the Di'er Songhua River. The geometric mean of CBMLs in Songyuan City was 2.66 μg/L (Zhang et al., 2008). Data from Dalian and Shanghai were both higher (Shi et al., 2010). Thus, the levels found in the present study were lower than that in those reports.

The U.S. Environmental Protection Agency's reference dose for Hg is based on a cord blood concentration of 5.8 μg/L (Rice, 2004; Hightower et al., 2006). According to this standard, about 4.1% of CBMLs in our study were at or above the reference dose, with the highest percentage elevated 18.70%, occurring in Xiamen. In contrast, Gao et al. (2007) found that 69.9% of CBMLs exceeded this threshold in the survey of Zhoushan, China. In addition, the CBMLs were related to the frequency of fish consumption. Similarly, we found that CBMLs in coastal areas (Xiamen, Guangzhou and Shenyang) were higher than those from inland areas (Wuhan, Kunming, Xi'an and Baoding). The reason may be that the consumption of seafood in these coastal cities is more than that in inland cities. Our results showed that 23.2% of women in Xiamen, 9.7% in Shenyang, and 7.6% in Guangzhou, ate marine fish more than 4 times a week, which was much higher than the inland cities: 5.4% in Wuhan, 2.5% in Baoding, 1.5% in Xi'an and 1.2% in Kunming. On the other hand, the proportion of women with fish consumption less than once a week was higher in the inland cities (**Fig. 1**).

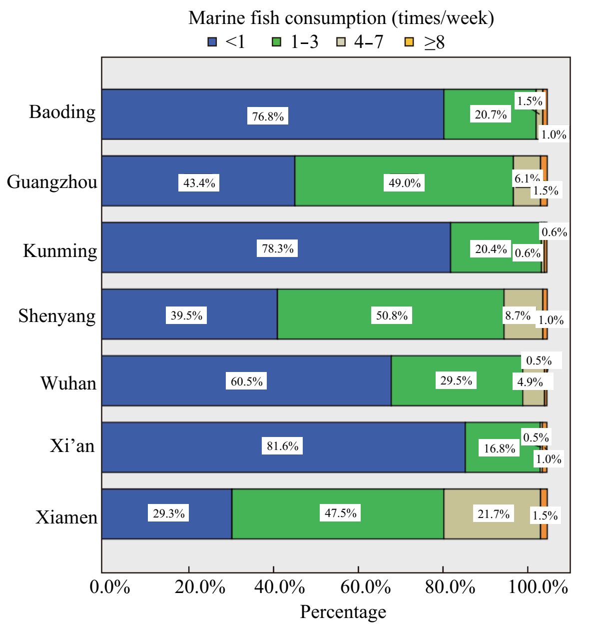

Fig. 1 Frequency of marine fish consumption during pregnancy in the seven cities.

Table 3 Cord blood mercury levels related factors of seven cities

Variables	Coefficients	Standard error	Wald	*P* value	OR	95% CI for coefficients	
						Lower bound	Upper bound
Family income per capita	0.40	0.14	8.23	0.004	1.50	0.13	0.68
Poultry	0.65	0.16	16.72	0.000	1.91	0.34	0.96
Marine fish	0.52	0.18	8.48	0.004	1.69	0.17	0.87
Shellfish	0.79	0.30	7.17	0.007	2.21	0.21	1.37
Formula milk	0.84	0.16	27.2	0.000	2.32	0.52	1.16
Residential storey	–0.35	0.12	8.52	0.004	0.70	–0.59	–0.12
Occupation before pregnancy							
Civilian staff*	–	–	8.68	0.277	1.00	–	–
Housewife/catering industry/unemployed	–0.99	0.49	4.12	0.042	0.37	–1.95	–0.03
Agriculture/forestry	–1.46	1.08	1.81	0.179	0.23	–3.576	0.67
Electronics industry/telecommunications/ electric power industry	–0.15	0.57	0.07	0.792	0.86	–1.26	0.96
Coal industry/ metallurgical industry/mining	–7.92	13.51	0.34	0.558	0.00	–34.39	18.55
Textile and chemical fiber industry/ chemical industry/ upholstery/transportation	–1.18	0.94	1.60	0.206	0.31	–3.02	0.65
Medicine/bio-industry	0.23	0.45	0.26	0.609	1.26	–0.64	1.10
Others	0.09	0.24	0.13	0.723	1.09	–0.39	0.56
Fruits	–0.37	0.17	5.09	0.024	0.69	–0.70	–0.05
Intercept	–0.83	0.53	2.53	0.112	0.43	–1.86	0.20

95% CI: 95% confidence interval; OR: odds ratio; * reference group dummy variable.

Table 4 Reported CBMLs in different cities

Countries or regions	CBMLs (μg/L)	Number	Reference
Seven region in China	1.81*	1323	This study
Disko Bay area in Greenland	35.6 ± 32.1	178	Bjerregaard and Hansen, 2000
Itaituba, Pará State, Brazil	16.68 ± 17.16	1510	Santos et al., 2007
Japan (Tsushima Islands, Fukuoka City and Katsushika)	9.81* (6.96–13.6)	115	Sakamoto et al., 2007
Kumamoto, Japan	7.6 ± 2.6	54	Sakamoto et al., 2012
Zhoushan City, China	5.58* (3.96–7.82)	406	Gao et al., 2007
Dalian City, China	3.57** (2.42–3.90)	107	Shi et al., 2010
Songyuan City, China	2.87 ± 1.13, 2.66*	58	Zhang et al., 2008
Shanghai, China	2.29** (1.62–2.62)	177	Shi et al., 2010
South Africa	1.2** (0.1–9.7)	62	Rudge et al., 2009
Krakow Inner City, Poland	0.88* (0.81–0.95)	220	Jedrychowski et al., 2006
Slovakia	0.8** (0.15–2.54)	99	Palkovicova et al., 2008
Montreal, Canada	0.69	92	Morrissette et al., 2004
Istanbul, Turkey	0.50 ± 0.64 (0–2.36)	143	Unuvar et al., 2007

* Geometric mean; ** median.

3.2 Factors related to cord blood mercury levels

3.2.1 Risk factors for Hg exposure

Consumption of fish and seafood is the primary Hg exposure route in humans. A positive correlation between maternal seafood consumption and neurotoxicity in exposed fetuses was first noted following the discovery of Minamata disease in the 1950s in Japan (Sato et al., 2006). The highest levels were found in fish that were apical predators of older age such as king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, scabbard, marlin and fish-consuming mammals such as seals and toothed whales. Biomarkers of low-level Hg exposure following fish consumption were observed in pregnant and lactating Slovenian women (Miklavčič et al., 2011). In this study, marine fish and shellfish consumption were also risk factors, but the consumption of freshwater fish was not a risk factor. The concentrations of Hg in fish thriving in rivers in north China (Songhua River and Di'er Songhua River) were generally higher than those in south China (Wujiang River). In contrast, Hg concentrations in fish collected from marine waters were different. Hg in fish from the Bohai Sea (0.09 mg/kg, North China) was lower than those from the Yellow Sea, Eastern Sea and the sea in the northern part of south China (0.21–0.36 mg/kg, South China) (Zhang et al., 2007). However, Zhang et al. (2010) reported that fish consumption composed only 1%–2% of an individual's MeHg exposure. Some studies confirmed the high levels of MeHg in rice, and in China the main human exposure to MeHg is related to frequent rice consumption in Hg-polluted areas (Li et al., 2010).

Hg is a constant component of municipal sewage, which may be used as a soil conditioner and agricultural fertilizer, allowing Hg bioaccumulation in the food chain (Tuzen et al., 2009). Poultry generally take aquatic plants and fish as food. Therefore, Hg is accumulated through the food chain, which results in poultry containing high levels of Hg. Ji et al. (2006) found that total mercury levels in the tissues of ducks from the Wanshan area in which the largest mercury mine is located were significantly higher than those of controls. Chicken consumption was also found to be related to cord blood MeHg levels in Swedish pregnant women (Bjornberg et al., 2003).

In this study, the intake of formula milk was a risk factor for CBMLs. This may be a rare phenomenon. Pregnant women in many developed countries drink raw milk and not powdered milk. The reason why formula milk contained Hg could be interpreted as follows: firstly, formula is made with tap water which is at the highest risk from metals contaminating the water supply; secondly, the can containing formula milk may be laminated using Hg; thirdly, formula milk nutrients are extracted from deep sea fish species, which often contain Hg.

Our findings are consistent with studies which also reported that low income was shown to be a positive predictor of Hg (Lederman et al., 2008; Mahaffey et al., 2009). Pregnant women with higher family income per capita may possibly pay more for food with high nutritional value during pregnancy. Therefore, their intake of poultry, fish, and pregnancy health care products (such as formula milk) will be greater than those with a lower family income per capita. These kinds of food not only consist of expensive nutritional ingredients, but are also polluted by Hg to different extents, which can increase CBMLs.

3.2.2 Protective factors of Hg exposure

Most human exposure to Hg is caused not only by ingestion of contaminated fish, but also by outgassing of mercury from dental amalgam and occupational exposure (Bernhoft, 2012). However, in this study, neither of these factors was considered to be risk factors. The outcome suggested that compared with civilian staff, only housewife/catering industry/unemployed women's babies' had relatively low CBMLs. Coal and mineral mining, which are considered to be major occupational Hg exposure routes, did not appear to be significant risk factors. A possible reason for this may be that the proportion of people employed in this profession was too small, only 0.2% of the total surveyed in this study.

The intake of fruits is a very good protective factor against increased CBMLs. Given the same quantity of fish intake, persons frequently consuming fruits had significantly lower blood ($\beta = -0.5$, $P = 0.001$) and hair ($\beta = -0.2$, $P = 0.000$) Hg concentrations (Passos et al., 2003, 2007). A number of physiochemical and nutritional fibers are present in fruits which might interact with Hg in several ways: absorption and excretion, transport, binding to target proteins, metabolism, and sequestration.

There is a lack of comprehensive information on Hg concentrations relating to vertical distribution in Chinese cities. The limited amount of data indicate that all the densely populated cities (Lanzhou, Beijing and Chongqing) tend to have higher concentrations of atmospheric particulate Hg and total gaseous Hg during the cold season (heating period) than during the warm season (non-heating period) (Zhang et al., 2007). The concentration in gradients decreased with increasing atmospheric turbulence (Lindberg et al., 1995). We found those who lived on higher apartment floors had lower CBMLs. Because of the high density of Hg, atmospheric Hg settles closer to the ground.

4 Conclusions

The average cord blood Hg level in this study was lower than that reported in most previous studies, including those conducted in other Chinese locations. The average Hg levels differed among the seven geographical regions. The CBMLs were not only associated with family economic and living conditions, but also associated with maternal diet during pregnancy.

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