Relationship between platelet monoamine oxidase-B (MAO-B) activity and mercury exposure in fish consumers from the Lake St. Pierre region of Que., Canada

Christopher John Stamler a,1, Nadia Abdelouahab b, Claire Vanier b, Donna Mergler b, Hing Man Chan a,*,1

a Centre for Indigenous Peoples’ Nutrition and Environment (CINE) and the School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Rd., Ste-Anne-de-Bellevue, Montreal, Que., Canada H9X 3V0
b Centre pour l’Etude des Interactions Biologiques entre la Santé et l’Environnement (CINBIOSE), Université du Québec à Montréal, Montréal, Canada

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Abstract

Mercury (Hg) is a widespread neurotoxic compound that bio-accumulates in fish and marine mammals. Monoamine oxidase (MAO; EC 1.4.3.4) regulates biogenic amine concentration in the brain and peripheral tissue and has been shown to be a molecular target of Hg compounds in animal models. Blood platelet monoamine oxidase-B (MAO-B) activity may reflect MAO function in the central nervous tissue. Therefore, the objective of this study was to evaluate the relationship between platelet MAO-B and Hg exposure in fish-eating adults (n = 127) living along the St. Lawrence River (Lake St. Pierre, Que., Canada). Hg concentrations were determined in blood and hair samples. A significant negative association was observed between platelet MAO-B activity and blood-Hg (r = −0.193, p = 0.029) but not with hair-Hg levels (r = −0.125, p = 0.169). Multiple linear regression analysis demonstrated that blood-Hg (β = 4.6, p = 0.011) and heavy smoking (β = −8.5, p = 0.001) were associated with reduced platelet MAO activity in the total population. In addition, this reduction in MAO-B activity appeared to be associated with blood-Hg concentrations above 3.4 µg/L (75th percentile). Possible gender related differences were also observed and are discussed. Our results suggest that MAO-B activity in blood platelets may be a useful tool to assess biochemical effects of Hg exposure in human populations. These changes in platelet MAO-B may reflect enzymatic changes in nervous tissue and should be further investigated as a surrogate marker of neurotoxicity.

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1. Introduction

Mercury (Hg) is a widespread neurotoxic pollutant (ATSDR, 1999). The dietary intake of contaminated fish and marine mammals is the main route of exposure to Hg in human populations (Van Oostdam et al., 1999). The methylated form, methylmercury (MeHg), is absorbed efficiently by the gastrointestinal tract and is able to cross the blood–brain barrier (NRC, 2000). Once in the brain, Hg compounds can disrupt protein function by interacting with sulfhydryl groups, potentially leading to cellular death and neuronal loss (Castoldi et al., 2001). Accidental poisoning episodes in Japan and Iraq have shown that exposure to high-levels of Hg causes neurological abnormalities including ataxia, paresthesia, tremors and visual and auditory impairments (Bakir et al., 1973; Harada, 1995). More recent studies conducted among adults in the Brazilian Amazon have suggested an association between Hg exposure and diminished motor and visual function at blood-Hg concentrations below 200 µg/L (Mergler, 2002), a guideline level associated with a low-risk of neurological damage (World Health and Organization, 1990). There is increasing interest in detecting early signs of neurotoxicity at low-levels of Hg exposure (NRC, 2000). The detection of Hg-related biochemical disruption has been proposed as a strategy to better understand these potential neurotoxic risks (Castoldi et al., 2001).
Biochemical changes in the nervous system generally occur prior to permanent damage, and therefore could be used to predict future stages of neurotoxicity (Costa and Manzo, 1995; Manzo et al., 1996). Numerous animal studies have shown that Hg exposure disrupts the function and transmission of the monoaminergic nervous system (Faro et al., 1997; Lindstrom et al., 1991; Oudar et al., 1989). Specifically, the enzyme monoamine oxidase (MAO, EC 1.4.3.4) has been shown to be disrupted by Hg compounds (Chakrabarti et al., 1998; Tsuzuki, 1981). MAO is involved in the oxidative deamination of amine neurotransmitters, including serotonin, dopamine and noradrenaline and exists as two isoenzymes, MAO-A and MAO-B (Shih, 2004). These isoenzymes differ in substrate specificity and tissue expression. MAO-B is the predominant isoenzyme in the brain, although both isoforms are important for neuronal function (Shih, 2004). Changes in brain MAO may reflect early stages of Hg neurotoxicity; however, due to the complexity and inaccessibility of the nervous tissue, direct brain measurements are not possible in large human studies.

Blood platelets are unique non-neuronal cells as they utilize similar cellular machinery as monoaminergic neurons (Manzo et al., 1996; Reed et al., 2000; Tayebati et al., 2002). MAO-B is present in platelets (Chen et al., 1993; Costa et al., 1988) and is similarly targeted by Hg compounds (Chakrabarti et al., 1998). Chakrabarti et al. (1998) reported that repeated oral exposure to MeHg in rats resulted in a parallel reduction of MAO activity in cortex, striatum and platelet tissue. Therefore, monitoring MAO-B activity in peripheral platelets may offer a strategy to screen for relevant neurochemical perturbation following Hg exposure in humans. Human studies have demonstrated that platelet MAO-B is reduced by alcohol use, heavy tobacco smoking (Whitfield et al., 2000) and by industrial exposure to styrene and manganese (Manzo et al., 1996). Positron emission tomography (PET) imaging studies have confirmed that brain MAO-A (Fowler et al., 1996b) and MAO-B (Fowler et al., 1996a) are reduced in heavy smokers when compared to non-smokers, suggesting that platelet MAO-B may serve as a surrogate marker for brain MAO in humans. Relationships between platelet MAO-B and Hg exposure have not been reported in human populations.

Lake St. Pierre is a widening of the St. Lawrence River system located between Montréal and Quebec City (Que., Canada), and is one of the largest sites for commercial and sport fishing in Canada. Reports of Hg concentrations in fish from the St. Lawrence River system suggest that several species may be above recommended guideline levels for Hg (Laliberté, 2003). Dietary consumption of these lake fish are associated with higher Hg exposure (Mahaffey and Mergler, 1998) and signs of neurotoxicity in humans (Mergler et al., 1998). Therefore, the objective of this study was to investigate the association between Hg exposure and platelet MAO-B activity in fish-eating adults living in the Lake St. Pierre region.

2. Materials and methods

2.1. Population and sampling

The study population inhabits the Lake St. Pierre region in the municipality of Sorel–Tracy (Que., Canada). The participants were recruited with the collaboration of the Lake St. Pierre Fisher’s Association. This cross-sectional study was carried out from February to April 2003. Participants, 18 years and older, were included in the study if they reported eating fish from Lake St. Pierre. Informed consent was obtained from the participants and coded questionnaires were completed to determine socio-demographic and lifestyle information. Fish intake and dietary information has also been collected from the participants and the relationship with Hg exposure is described elsewhere (Abdelouahab et al., in preparation). For the purpose of this study, age, gender, alcohol intake, smoking habits, potential exposure to industrial chemicals, body mass index (kg/m²) and neurological diseases of the participants were collected as potential confounders. Ethical approval to conduct this study was granted by McGill University and the Université du Québec à Montréal ethical review committees.

Blood samples (10 mL) were drawn into vacutainer tubes containing EDTA-K2. Blood was centrifuged at 200 x g for 10 min to obtain platelet-rich-plasma (PRP). PRP was centrifuged at 3000 x g for 25 min at 4 °C. Pellet containing the platelets were washed twice and suspended in phosphate buffer saline (50 mM NaH2PO4, 120 mM NaCl, pH 7.8). The concentration of protein in platelet preparations was determined by the Bradford method (1976). Mean protein concentration was 2.2 mg/mL and ranged from 0.5 to 7.0 mg/mL. Platelet samples were stored at −80 °C until biochemical assays were performed. The storage condition and stability of the samples had been previously tested (Stamler et al., 2005).

2.2. Platelet MAO-B measurement

Measurement of MAO-B activity was performed as described previously by Krajl (1965). Platelets (20 μg protein) were mixed in a Triton X-100 (0.5%) solution, and then diluted in 3 mL of phosphate buffer saline. Reaction was initiated by the addition of a concentration range of the MAO substrate, kynauramine dihydrobromide (1.5–50 μM; Sigma–Aldrich, St. Louis, MO, USA), followed by a 30 min incubation at 37 °C. Following the incubation, the reaction was stopped by the addition of 5 M perchloric acid, and centrifuged at 2000 x g for 10 min. The supernatant was diluted with 1 M NaOH and the fluorescence was read at 318 nm excitation and 380 nm emission on a spectrofluorometer (RF-551 Shimadzu, Japan). The concentration of the reaction was determined from the standard curve of 4-hydroxyquinoline (0–30 nM; Sigma–Aldrich) and results were expressed in nmol of product formed per minute per 20 μg of platelet protein.

2.3. Analysis for mercury, selenium, cadmium, manganese, lead and ferritin

Whole blood samples (7 mL) were drawn into heparinized tubes. Blood total-Hg concentrations were determined using cold-vapor atomic-absorption spectrometry. The detection limits were 0.2 μg/L for blood-Hg analysis. Certified reference material was analyzed for quality control purposes. Selenium
(Se), cadmium (Cd), manganese (Mn) and lead (Pb) blood concentrations were determined by using inductively coupled plasma-mass spectrometry (ICP-MS) technique. All analyses were carried out at the Centre de Toxicologie du Québec (Que., Canada).

Hair strands from the root were cut from the occipital region of the head. Hg analysis was conducted at the GEOTOP Laboratory of the University of Québec in Montréal, using cold-vapor atomic-fluorescence spectrometry. Hair strands were cut in 1 cm segments and analyzed for Hg as previously described (Bloom and Fitzgerald, 1988). The detection limit for hair-Hg analysis was 5 ng/g. Precision and accuracy of Hg determination were ensured with internal hair standards, which were provided for the Hair Mercury Inter-laboratory Comparison Program, Health Canada, Ottawa, Canada (Gill et al., 2002). Due to limited hair length in some participants, the Hg data from the 1 cm segment was used in order to maximize the number of usable samples.

Iron status was determined from serum ferritin levels, which were measured in duplicate using the ACTIVE® Ferritin Enzyme-Linked Immunosorbent Kit (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Ferritin concentration was expressed as ng per mL of serum. Participants classified as iron deficient if serum ferritin level was below 12 ng/mL.

2.4. Statistical analysis

Enzyme kinetic data were estimated using GraphPad Prism Version 3.02 Software (GraphPad Software Inc., San Diego, CA, USA). The apparent \( K_m \) and maximum velocity \( (V_{\text{max}}) \) values for MAO-B were estimated by non-linear regression analysis of the enzyme activity (V)-substrate concentration \([S]\) data according to the Michaelis–Menten equation: \( V_{\text{max}} \times [S]/(K_m + [S]) \). Reference made to MAO-B activity refers to the estimated \( V_{\text{max}} \) value.

All statistical analyses were conducted using SPSS statistical software (SPSS Version 11.5.0, San Rafael, CA, USA). A \( p \)-value less than or equal to 0.05 was considered statistically significant and all values were reported as means ± standard deviation (S.D.), unless otherwise noted. Data was analyzed for normality using the Kolmogorov–Smirnov test. Serum ferritin, hair-Hg and blood-Hg, -Cd, -Mn and -Pb data were log transformed for statistical analysis. MAO-B \( K_m \) was not normally distributed and therefore analyzed using non-parametric methods. Relationships between platelet MAO-B characteristics and blood-Hg or hair-Hg concentrations were evaluated using Pearson or Spearman correlations. Pearson’s correlations, Student’s \( t \)-test and one-way ANOVA for multiple comparisons were used to determine relationships between population variables and platelet MAO-B activity. Spearman correlations, Mann–Whitney \( U \)-tests or ANOVA performed on ranks, were used to evaluate the relationship between MAO-B \( K_m \) and population variables. Analyses were also performed separately in men and women.

A forward stepwise multiple linear regression analysis was performed to determine the predictors of platelet MAO-B activity in the population. Age, gender, heavy smoking (y/n), alcohol intake, potential occupational chemical exposure (y/n), serum ferritin (ng/L), Hg levels and all other blood element concentrations were considered as independent variables. A forward stepwise multiple linear regression analysis was also performed separately for men and women.

Additionally, the total population was divided into quartile groups based on blood-Hg levels, and mean MAO-B values were compared using a General Linear Model Univariate procedure adjusting for heavy smokers. Pair-wise comparison was performed using Bonferroni post hoc analysis. Student’s \( t \)-test was used to assess differences in MAO-B activity between participants above and below 3.4 \( \mu \)g/L of blood-Hg in the total, male and female population.

### Results

#### 3.1. Study population description and exposure measurements

The age of the participants ranged from 18 to 73 year with a mean of 49 ± 13 and 52 (40%) were female. Twenty two percent described themselves as current smokers, and a total of 20 individuals reported smoking more than 14 cigarettes per day. Mean body mass index (BMI) in the population was 26.5 ± 5.7 kg/m². Thirty percent of the population abstained...
from the use of alcohol, and 12% consumed greater than 420 g of ethanol/week (high alcohol consumption). Thirty participants reported having worked with industrial chemicals (i.e. metals, solvents). None of the participants reported having neurological diseases such as Schizophrenia, Parkinson’s or Alzheimer’s disease, and none were taking MAO inhibiting medication.

One male individual out of the total 130 participants was unable to donate blood for this study and two blood samples had insufficient platelets for the determination of MAO-B characteristics. Four men and one woman did not have sufficient hair sample to perform hair-Hg analysis. Mean (S.D.) median and range of platelet MAO-B activity, blood-Hg and hair-Hg concentrations in the study population are summarized in Table 1. Mean blood-Hg concentration was 2.4 ± 0.85 µg/g and ranged from 0.03 to 5.04 µg/g. The median (range) serum ferritin level was 169.2 (7.4–1000) ng/mL, and iron deficiency was observed in four participants.

3.2. Platelet MAO-B in the study population

Simple correlation analysis showed that platelet MAO-B activity was negatively correlated with blood-Hg \( (r = -0.193, p = 0.029) \), but not with hair-Hg \( (r = -0.125, p = 0.169) \) in the total population. Both hair-Hg and blood-Hg were negatively correlated with MAO-B activity in the males, however, no associations were observed in the females. No relationship was observed with either biomarker of Hg exposure and MAO-B \( K_m \) \( (p > 0.05) \) (data not shown).

Relationships between platelet MAO-B activities and other variables in the study population are summarized in Table 2. Negative correlations were observed between non-smokers and light-smokers in the total, male or female population \( (p < 0.05) \) but not in the female group. No difference in MAO-B was observed between non-smokers and light-smokers in the total group. MAO-B was not significantly affected by smoking status (y/n); however, heavy smokers \( (\geq 15 \text{ cigarettes per day}) \) displayed lower MAO-B activity \( (r = 0.724, p = 0.008) \) than moderate smokers \( (1–14 \text{ cigarettes per day}) \) and non-smokers combined. Significant effects of smoking on MAO-B activity were also seen in males, and similar trends were observed in females. No difference in MAO-B was observed between non-smokers and light-smokers in the total, male or female population \( (p > 0.05) \). Cigarettes smoked per day and blood-Cd concentrations were both negatively correlated with MAO-B activity in the total and male group, but not in the female group. Blood-Cd levels were strongly correlated with the number of cigarettes per day \( (r = 0.724, p < 0.001) \) and heavy smokers had over four times greater mean blood-Cd concentrations than non-smokers in the total population.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total population</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( r )</td>
<td>( r )</td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.008</td>
<td>0.110</td>
<td>-0.158</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.036</td>
<td>-0.017</td>
<td>-0.081</td>
</tr>
<tr>
<td>Cigarettes (no./day)</td>
<td>-0.199*</td>
<td>-0.272*</td>
<td>-0.058</td>
</tr>
<tr>
<td>Alcohol (g/week)</td>
<td>-0.050</td>
<td>0.147</td>
<td>-0.078</td>
</tr>
<tr>
<td>Ferritin (ng/L)</td>
<td>-0.177*</td>
<td>-0.285*</td>
<td>0.164</td>
</tr>
<tr>
<td>Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair-Hg (µg/g)</td>
<td>-0.125</td>
<td>-0.261*</td>
<td>0.120</td>
</tr>
<tr>
<td>Blood-Hg (µg/L)</td>
<td>-0.193*</td>
<td>-0.314**</td>
<td>0.057</td>
</tr>
<tr>
<td>Other elements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood-Cd (µg/L)</td>
<td>-0.235**</td>
<td>-0.314**</td>
<td>-0.070</td>
</tr>
<tr>
<td>Blood-Se (µg/L)</td>
<td>-0.179*</td>
<td>-0.185</td>
<td>-0.150</td>
</tr>
<tr>
<td>Blood-Mn (µg/L)</td>
<td>0.048</td>
<td>0.124</td>
<td>-0.144</td>
</tr>
<tr>
<td>Blood-Pb (µg/L)</td>
<td>-0.132</td>
<td>-0.109</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* Analyzed using Spearman correlation.
* Log transformed data.

Table 3

The distribution of mean platelet MAO-B characteristics in the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total population</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>MAO-B activity (S.D.)</td>
<td>( n )</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>98</td>
<td>28.7 (10.4)*</td>
<td>58</td>
</tr>
<tr>
<td>1–14 cigarettes/day</td>
<td>9</td>
<td>32.1 (12.6)*</td>
<td>5</td>
</tr>
<tr>
<td>≥15 cigarettes/day</td>
<td>20</td>
<td>21.3 (7.8)*</td>
<td>14</td>
</tr>
<tr>
<td>Heavy smoker (≥15 cigarettes/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>107</td>
<td>29.0 (10.6)*</td>
<td>63</td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>21.3 (7.8)*</td>
<td>14</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstainers</td>
<td>29</td>
<td>27.8 (7.5)*</td>
<td>17</td>
</tr>
<tr>
<td>1–420 g/week</td>
<td>84</td>
<td>28.0 (11.8)*</td>
<td>23</td>
</tr>
<tr>
<td>&gt;420 g/week</td>
<td>9</td>
<td>27.2 (9.7)*</td>
<td>37</td>
</tr>
<tr>
<td>Possible industrial chemical exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>97</td>
<td>27.1 (9.6)*</td>
<td>57</td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>30.2 (13.3)*</td>
<td>20</td>
</tr>
</tbody>
</table>

Superscripts (a and b) represent significant \( (p < 0.05) \) differences among the values in each category and column.

remaining population (3.8 ± 1.7 μg/L versus 0.9 ± 1.0 μg/L, p < 0.001). Serum ferritin levels were also negatively correlated with MAO-B activity (p < 0.01) in male and total population. There was no relationship between MAO-B characteristics and participant age, BMI or alcohol intake in females, males or in the total population.

To further investigate the relationship between platelet MAO-B and blood-Hg, a forward stepwise multiple regression analysis that considered relevant independent variables was performed. In the regression model (r² = 0.105, p < 0.001), both heavy smoking and blood-Hg explained 7.4 and 5.4% of the MAO-B variation in the total population, respectively (Table 4). To account for the potential effects of Fe deficiency the four iron deficient individuals were removed from the multiple regression analysis; the model was unaffected by their removal. Notably, gender, Se and serum ferritin were not significant predictors of platelet MAO-B activity. Similarly, blood-Hg (β = −7.05 ± 2.09) and heavy smoking (β = −9.95 ± 2.90) explained 22% (p < 0.001) of the variations in MAO-B activity in the men. However, when multiple regression was performed with the data from the women, none of the independent variables tested were significant.

MAO-B activity values, adjusted for the effects of heavy smoking, were stratified in blood-Hg quartile groups (Fig. 1). Blood-Hg levels in the lowest quartile group ranged from 0.2 to 0.5 μg/L while those in the highest group ranged from 3.4 to 17 μg/L. Mean MAO-B activity from participants in the upper Hg quartile group was significantly (p = 0.035) less than the mean values from the lowest quartile group.

Further analysis showed that participants with blood-Hg concentrations above the 75th percentile (3.4 μg/L) exhibited lower mean MAO-B activity when compared to the remaining population (Table 5). Males above 3.4 μg/L for blood-Hg had significantly (p < 0.05) lower mean MAO-B activity when compared to those below this level. A similar trend was also observed in females (Table 5).

### 4. Discussion

This study explores the relationship between Hg exposure and platelet MAO-B in a fish-eating community. The key finding is that low-level exposure to Hg was associated with reduced platelet MAO-B activity in humans. A reduction in platelet MAO-B was observed in participants with blood-Hg concentrations above 3.4 μg/L. It has been proposed that platelet MAO-B activity may reflect nervous system MAO (Costa and Manzo, 1995; Manzo et al., 2001), and therefore these enzyme variations may indicate early signs of Hg-related neurotoxicity.

MeHg exposure has been shown to alter parameters of the monoaminergic nervous system in rodent models. Administration of Hg compounds are known to increase noradrenaline and serotonin levels in various brain regions of rats (Lakshmana et al., 1993; Lindstrom et al., 1991). Similarly, repeated intrastrial administration of MeHg results in an increase in striatal dopamine levels with a concomitant decrease in dopamine metabolites (Faro et al., 2003). The function of the dopaminergic system in rats is also altered by low-level gestational exposure to MeHg (Dare et al., 2003; Gimenez-Llort et al., 2001). Reductions in both brain and platelet MAO activities have been previously observed in animal models following MeHg exposure (Berntssen et al., 2003; Chakrabarti et al., 1998; Tszuki, 1981). In the present study, while platelet MAO-B activity was negatively correlated with blood-Hg concentrations, it is unclear if this observed relationship is a result of lower MAO-B protein levels or reduced enzyme activity.

### Table 4

Predictors of platelet MAO-B activity (nmol/min/20 μg) in the total study population from multiple linear regression analysis

<table>
<thead>
<tr>
<th>Predictors</th>
<th>β-coefficient (±S.E.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy smoking</td>
<td>−8.55 ± 2.48</td>
<td>0.001</td>
</tr>
<tr>
<td>Blood-Hg</td>
<td>−4.52 ± 1.74</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Total model: adjusted r² = 0.105, p < 0.001

a β-coefficient were determined by stepwise multiple linear regression. Inclusion criteria: p < 0.05.

b Values for Hg were log transformed before analysis.

### Table 5

Mean platelet MAO-B activity (nmol/min/20 μg) in participants with blood-Hg levels above and below 3.4 μg/L

<table>
<thead>
<tr>
<th>Blood-Hg</th>
<th>Total population</th>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>MAO-B activity (S.D.)</td>
<td>n</td>
<td>MAO-B activity (S.D.)</td>
<td>n</td>
<td>MAO-B activity (S.D.)</td>
</tr>
<tr>
<td>&lt;3.4 μg/L</td>
<td>98</td>
<td>29.1 (10.9)a</td>
<td>57</td>
<td>27.8 (11.3)a</td>
<td>41</td>
<td>30.8 (10.2)a</td>
</tr>
<tr>
<td>≥3.4 μg/L</td>
<td>29</td>
<td>23.6 (8.6)b</td>
<td>20</td>
<td>22.5 (9.0)b</td>
<td>9</td>
<td>25.8 (6.9)a</td>
</tr>
</tbody>
</table>

Superscripts (a and b) represent (p < 0.05) significant differences between the values in each category and column.

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Costa and Manzo, 1995; Manzo et al., 2001; Berntssen et al., 2003; Chakrabarti et al., 1998; Tszuki, 1981; Dare et al., 2003; Gimenez-Llort et al., 2001; Lakshmana et al., 1993; Lindstrom et al., 1991; Faro et al., 2003.
specific activity. However, in vitro studies using rat brain synaptosomes and isolated platelets show that MAO is dose-dependently inhibited following incubation with MeHg (Chakrabarti et al., 1998). Earlier studies examining the structure of MAO, proposed that Hg compounds bind to essential cysteine residues, reducing activity and permanently damaging enzyme function (Gomes et al., 1976, 1969). While effects of Hg exposure on MAO protein levels cannot be ruled out, these in vitro studies provide a possible explanation for the observed reductions in platelet MAO-B activity. Given evidence that platelet MAO-B may act as a surrogate marker for brain MAO (Chakrabarti et al., 1998; Fowler et al., 1996b; Whitfield et al., 2000), it can be suggested that Hg-related effects on platelet MAO-B may also be observed in nervous tissue of the Lake St. Pierre population.

When data was analyzed by gender, significant associations between Hg exposure and platelet MAO-B were observed in men, but not women. There are several explanations for these gender differences. Controlled animal studies suggest that MeHg alters the function of the dopaminergic nervous system in male, but not in female rats (Gimenez-Llort et al., 2001). It was proposed that these differences may be related to the interaction of sex steroids on the dopaminergic system (Gimenez-Llort et al., 2001). Additionally, baseline levels for platelet MAO-B activity have been shown to be higher in women, when compared to men (Whitfield et al., 2000) and could explain gender specific associations with Hg compounds. However, it is likely that the size of the study population is the most important factor involved in the observed gender differences. These data suggest that the blood-Hg level above 3.4 µg/L (75th percentile for blood-Hg) may represent a threshold of effect for Hg on platelet MAO-B activity (Fig. 1). However, only 9 women were above this blood-Hg threshold level, compared to 20 men. Therefore, linear regression analysis may not be able to detect the effects of Hg on MAO-B activity in women, as an insufficient number were above this critical Hg exposure level. This hypothesis is strengthened by the fact that women with blood-Hg levels above 3.4 µg/L had lower mean platelet MAO-B activity compared to those below this Hg exposure level. Therefore, we cannot conclude from this study that the effects of Hg on MAO-B activity are gender specific.

Platelets are anuclear secretory cells that play an important role in hemostasis, thrombosis, vascular remodelling and repair (Reed et al., 2000). Platelets are considered a peripheral model of a serotonergic nerve ending, as they utilize similar transmitters, enzymes, receptors and transporters for cellular communication (Polgar et al., 2002; Reed et al., 2000). The lifespan of circulating blood platelets in humans has been estimated to be approximately 5–10 days (Najean et al., 1969). The short lifespan of platelets may explain why platelet MAO-B activity is associated with blood-Hg, and not with hair-Hg levels. The half-life of Hg in blood is approximately 50 days and is therefore thought to reflect relatively short-term exposure (Stern, 1997). Conversely, hair-Hg is considered a stable bio-indicator that reflects long-term MeHg exposure during a given period of time (Clarkson et al., 1988). While MAO-B activity in men was significantly associated with both hair-Hg and blood-Hg concentrations, the relationship was stronger with blood-Hg. These data suggest that platelet MAO-B activity may be influenced by recent Hg intake rather than overall long-term body burden.

The physiological consequence of reduced MAO activity in adults is not clearly established. Animal studies suggest that MAO inhibition in the brain can lead to changes in neurotransmitter concentrations and other cellular functions, which may result in altered behavioural effects (Murphy and Kalin, 1980). Genetic deficiencies in MAO activity have been associated with severe clinical disorders in humans including mental retardation (Lenders et al., 1996) and in some cases blindness and deafness (Shih and Thompson, 1999). Recent studies have also indicated that MAO is critical for proper brain development during early rodent embryogenesis (Cases et al., 1996; Whitaker-Azmitia et al., 1994). Some evidence suggest that reduced platelet MAO-B may have an effect on cardiovascular function, as platelet serotonin concentrations have been linked to the development of coronary artery disease (Vikenes et al., 1999). Serotonin released from activated platelets can trigger vasoconstriction, thrombosis and proliferation of arterial smooth muscle cells (Golino et al., 1989; Nemecek et al., 1986). Given that MAO-B regulates platelet serotonin concentrations, it is possible that a decrease in enzyme activity may result in higher blood serotonin concentrations and increased risk of cardiac events. This hypothesis could explain previously observed relationships between Hg exposure and increased risk of heart disease (Chan and Egeland, 2004; Stern, 2005) and should be further investigated.

Platelet MAO-B activity is influenced by a range of environmental and genetic factors. While genetic analysis was not performed in this study, a polymorphism has been shown to influence MAO-B activity in humans (Damberg et al., 2001). Environmental factors, including tobacco smoking (Berlin and Anthenelli, 2001; Whitfield et al., 2000), gender (Whitfield et al., 2000), iron deficiency (Youdim et al., 1975), alcohol intake (Rommelspacher et al., 1994) exposure to manganese (Smargiassi et al., 1995) and other industrial chemicals (Manzo et al., 1996) have been previously reported to affect platelet MAO-B activity in humans. Additionally, Pb exposure may also alter monoaminergic systems (Shellenberger, 1984). However, of the variables examined in the present study, only heavy smoking significantly contributed to reduced platelet MAO-B. Whitfield et al. (2000) has shown that heavy smokers (>10 cigarettes per day) have a reduction in platelet MAO-B when compared to non-smokers, former smokers, and light smokers (i.e. <10 cigarettes per day). Cigarettes are the major contributor to Cd levels in the blood (dell’Omo et al., 1999), and therefore MAO-B was also negatively associated with blood-Cd. Cd levels were strongly related to the reported number of cigarettes smoked per day, and were approximately four-fold higher in heavy smokers compared to moderate and non-smokers. While it has been shown that various constituents of tobacco smoke inhibit MAO-B activity in vitro (Castagnoli et al., 2002), the direct inhibition of MAO-B by Cd cannot be ruled out (Leung et al., 1992). In addition, the effects of smoking and blood-Cd on MAO-B activity were only observed...
in the male and not the female participants. This is most likely due to the relative low number of heavy smoking women \((n = 6)\) in this study group and not a gender dependent effect. Because, tobacco is a strong modulator of MAO-B activity, it is important to control for its effects when measuring platelet MAO-B in future population studies.

Platelet MAO-B activity was negatively correlated with blood-Se concentrations. However, this relationship was not significant after correcting for the effects of blood-Hg using the multiple linear regression models. Co-existence of Hg and Se in various tissues have been reported (Ganther et al., 1972; Watanabe, 2002), however, it is not clear whether the Se–Hg interaction is antagonistic or synergistic interaction (Magos, 1991). Similar to blood-Hg, Se levels in blood and plasma have been shown to be positively associated with dietary fish intake (Hansen et al., 2004; Karita and Suzuki, 2002; Svensson et al., 1992). Therefore, the observed association between Se and platelet MAO-B activity may be a result of covariance or a Hg–Se interaction.

Certain species of fish from the St. Lawrence River system have been shown to have levels of Hg above recommended guidelines, and consumption of these fish are the major determinant of Hg exposure in this area (Chan et al., 2000; Mahaffey and Mergler, 1998). Although Hg exposure was relatively low in this study group, associated biochemical variations in platelet MAO-B can be observed at blood-Hg levels that are below the lowest observable effect level (LOEL) (ATSDR, 1999). The physiological and clinical consequences of reduced platelet MAO-B activity are not clear, and must be further evaluated. These results suggest that MAO-B in blood platelets may be a useful tool to detect early biochemical effects of Hg exposure in human populations. Variations in platelets may reflect enzymatic changes in nervous tissue and therefore platelet MAO-B should be investigated as a potential surrogate marker of Hg neurotoxicity.

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