Prof. Pierre Ayotte

○アブストラクトデータ

Mercury and selenium speciation in marine mammals and mercury exposure among Inuit in Canada
Pierre Ayotte, Mélanie Lemire, Laurie Chan, Éric Dewailly, Adel Achouba, Pierre Dumas, Brian Laird, Michael Kwan

The traditional marine diet of the Inuit comprises sea mammals that may contain high concentrations of methylmercury (MeHg), a well known neurotoxic agent which in addition was recently shown to induce adverse cardiovascular effects. Conversely, several sea mammal tissues and organs frequently consumed by the Inuit are exceptionally rich in selenium (Se) and accordingly, Se intake in Inuit is among the highest in the world. Se is an essential element that is of key importance to the antioxidant defense system; evidence gathered in fish and marine mammal eating populations suggests that a high Se intake may play a role in offsetting some deleterious effects of MeHg exposure. In contrast, in populations other than marine food consumers, elevated plasma Se concentrations have been recently associated to type 2 diabetes, hypercholesterolemia and/or hypertension. Clearly, more information is needed on Se and Hg species found in food items and blood samples of the Inuit to better assess the risks and benefits of their traditional diet. Previous speciation analyses of marine mammal tissues consumed by the Inuit revealed that while meat contains mainly MeHg, Hg-Se insoluble crystals represents a large part of Hg present in liver, would reduce Hg bioavailability and related-toxicity for humans. In addition to total plasma Se levels, the most common biomarker of Se status, several other biomarkers (e.g. selenoproteins and small Se molecules such as selenoneine) have been identified and these may help to better characterize Se status and possible Hg-Se interactions among Inuit populations.

Our research program focuses on the Hg-Se balance and its relation to emerging health issues such as diabetes and cardiovascular diseases (CVD) risk factors in Inuit adults, taking into account the different species of Hg and Se. Speciation analyses are conducted in bio-banked blood samples from 900 Inuit adults (18-74y) who participated to the 2004 Inuit Health in Transition Study in Nunavik (Canada). Hyphenated HPLC-ICPMS techniques are used to measure plasma levels of selenoproteins (selenoprotein P, glutathione peroxidases and selenoalbumin), inorganic Se (Se+4 and Se+6) and small selenomolecules (e.g. methyl-selenocysteine, selenomethionine, selenoneine). Blood concentrations of MeHg and inorganic Hg are also determined. Participants also agreed to clinical measurements (e.g. fasting glucose and insulin, lipids profile, blood pressure, PON1 activity) and answered medical and food frequency questionnaires. The relations between biomarkers of exposure to Hg and Se and diabetes and CVD risk factors will be examined.　We will also identify the forms of Se and Hg present in various traditional foods as well as their bioavailability. These much needed data will improve our capacity to assess the risks and benefits of the traditional marine diet in this population.

○発表データ

Mercury And Selenium Speciation in Marine Mammals and Mercury Exposure among Inuit in Canada
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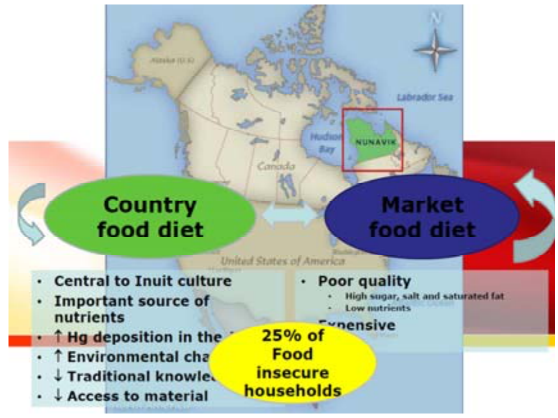
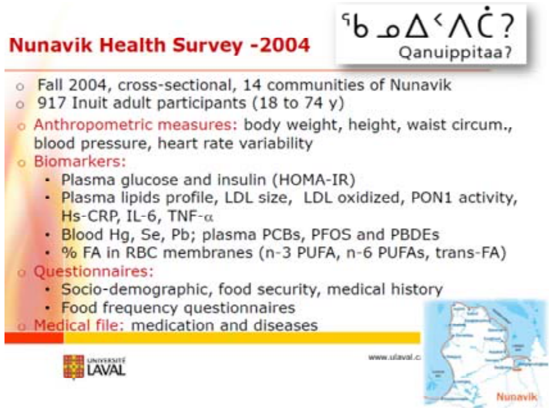


Labrador Sea
Hudson Bay
NUNAVIK
Canada
United States of America
Country food diet
Market food diet
Central to Inuit culture
Important source of nutrients
↑ Hg deposition in the
↑ Environmental cha
↓ Traditional knowle
↓ Access to material
Poor quality
High sugar, salt and saturated fat
Low nutrients
Expensive
25% of Food insecure households



Nunavik Health Survey -2004
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Qanuippitaa?
Fall 2004, cross-sectional, 14 communities of Nunavik
917 Inuit adult participants (18 to 74 y)
Anthropometric measures: body weight, height, waist circum., blood pressure, heart rate variability
Biomarkers:
Plasma glucose and insulin (HOMA-IR)
Plasma lipids profile, LDL size, LDL oxidized, PON1 activity, Hs-CRP, IL-6, TNF-α
Blood Hg, Se, Pb; plasma PCBs, PFOS and PBDEs
% FA in RBC membranes (n-3 PUFA, n-6 PUFAs, trans-FA)
Questionnaires:
Socio-demographic, food security, medical history
Food frequency questionnaires
Medical file: medication and diseases
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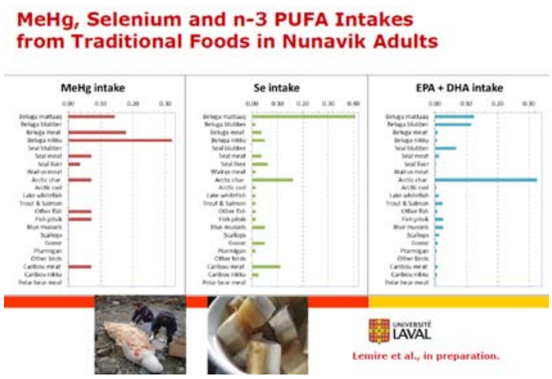
Nunavik

Table 1: Study population characteristics (weighted % or GM, range)

	All (n = 668)	≤ 34y (n = 332)	> 34y (n = 336)
Age (y)	34.3 (18 – 74)	25.0 (18 – 34)	47.2 (35 – 74)
Sex (F)	48.7	46.8	50.9
BMI (kg/m²)	26.8 (17.2 – 48.0)	26.2 (17.2 – 48.0)	27.5 (17.9 – 46.2)
Waist (cm)	90.4 (63.0 – 136.0)	88.2 (63.0 – 136.0)	92.8 (66.0 – 131.0)
Alcohol consumption (yes)	78.0	83.8	71.5
Smoking (yes)	67.8	71.8	63.3
Total country foods (CF) (g/day)	89.3 (1.1 – 2413.0)	78.0 (1.1 – 2413.0)	103.6 (1.1 – 1085.2)
Marine mammal CF (g/day)	10.2 (0 – 398.7)	8.4 (0 – 321.2)	12.7 (0 – 398.7)
Market foods (g/day)	2674 (0 – 12775)	2936 (0 – 10956)	2409 (264 – 12775)
% of CF intake of the total	3.1	2.5	3.9
Food insecurity (%)	25.0	25.4	24.7
Blood Hg (µg/L)	10.8 (0.1 – 240.7)	6.9 (0.1 – 164.5)	17.6 (0.5 – 240.7)
Blood Se (µg/L)	274 (134 – 3553)	251 (134 – 1579)	307 (166 – 3553)
% RBC DHA+DPA+EPA	9.1 (1.7 – 18.9)	7.3 (2.1 – 18.2)	11.1 (1.7 – 18.9)



Hg and Se Speciation in Traditional Foods
Beluga whales of the Western Arctic
Methylmercuric cysteinate
Methylmercuric glutathionate
Hg-Se complex
Several fish species
Selenoneine
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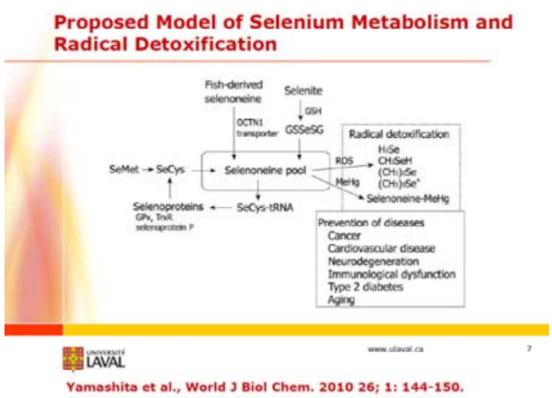
Lemes et al., ETC 2011; 12:2732;
Yamashita & Yamashita, J. Biol Chem. 2010 : 285: 18134.



Proposed Model of Selenium Metabolism and Radical Detoxification
Fish-derived selenoneine
Selenite
GSH
OCTN1 transporter
GSSeSG
Radical detoxification
SeMet → SeCys
Selenoneine pool
ROS
MeHg
Selenoneine-MeHg
Selenoproteins GPx, TrxR selenoprotein P
SeCys-tRNA
Prevention of diseases
Cancer
Cardiovascular disease
Neurodegeneration
Immunological dysfunction
Type 2 diabetes
Aging
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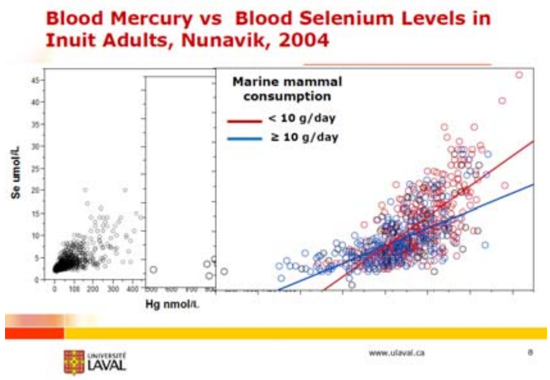
Yamashita et al., World J Biol Chem. 2010 26; 1: 144-150.



Hg and Se Associations with CV Risk factors/Biomarkers in Inuit

- **Blood pressure (Valera et al, 2009):** Hg ↑ Se ↓ n-3 ↓
- **Heart rate and HRV (Valera et al, 2008):** Hg ↑ Se ns n-3 ↓
- **PON1 activity (Ayotte et al, 2011):** Hg ↓ Se ↑ n-3 ↑
- **IsoP and IsoF (Alkazemi et al, 2013):** Hg ↑ Se ↓

- **TD2? Other cardiovascular risk factors in adults?**

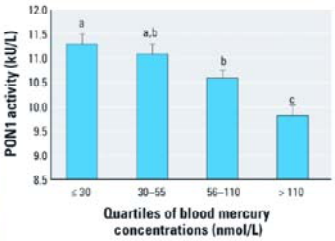
Multiple Linear Regression Model* of Plasma PON1 Activity in Inuit Adults, Nunavik, 2004

Variables	Beta	95% CI	P-value
Age (years)	-0.047	-0.066, -0.028	<0.001
Blood mercury (nmol/L)[b]	-0.063	-0.091, -0.035	<0.001
Blood selenium (µmol/L)[c]	0.067	0.045, 0.088	<0.001
HDL-C (mmol/L)[b]	0.077	0.061, 0.094	<0.001
EPA+DHA (%)	0.048	0.023, 0.074	<0.001
PON1 variants			
rs662 (Q192R)			
GG (CC)	-0.189	-0.241, -0.136	<0.001
AG (CT)	-0.101	-0.147, -0.055	<0.001
rs854560 (L55M)[d]			
TT (MM)	0.096	0.038, 0.153	0.001
rs705379 (-108C/T)			
GG (QQ)	0.366	0.279, 0.452	<0.001
AG (QR)	0.230	0.142, 0.317	<0.001

*31% of PON1 activity variance explained, N = 651

Ayotte et al., EHP, 2011; 119:1077–1083

Plasma PON1 Activity* and Blood Hg Levels



*Mean values were adjusted for age, blood selenium levels, plasma HDL-C concentrations, and PON1 genotypes (rs662, rs854560, rs705379).

Ayotte et al., EHP, 2011; 119:1077–1083

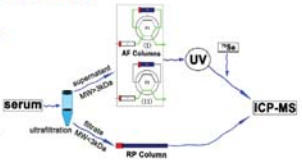
Objectives of the Study

***Part A* : Selenium speciation in plasma of Inuit adults**

- To measure **biomarkers of Se status** in relation to emerging health problems in Nunavik (diabetes, cardiovascular risk factors), taking into consideration possible interactions with **different fractions of Hg, omega-3 PUFAs and other contaminants**

Selenium containing proteins (GPx, SelP, Se-Albumin): affinity-chromatography ICP-MS

Small seleno-compounds (Se^{+4}, Se^{+6}, SeMet, MeSeCys, selenoneine): ion-pair reverse-phase chromatography ICP-MS

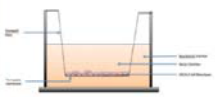


Objectives of the study (cont'd)

***Partie B* : Traditional foods of Nunavik Inuit**

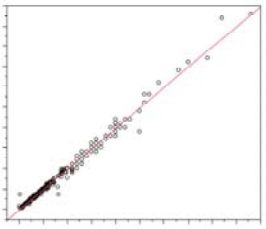
- To measure **concentrations** of **different Hg and Se species** in foods
- To determine the **bioaccessibility of Hg and Se species**
  - **Step 1:** *in vitro* gastro-intestinal model (Simulator of the Human Intestinal Microbial Ecosystem)
  - **Step 2:** Intestinal epithelium model (Caco-2 cells)





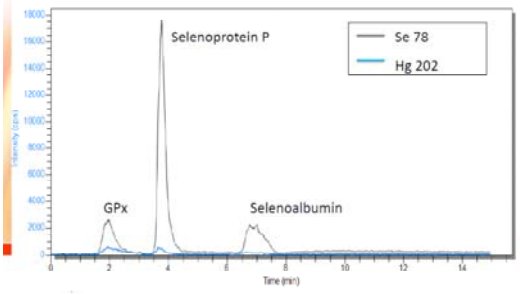
Preliminary results – Part A: MeHg vs THg in Blood Samples of 228 Inuit Adults from Nunavik, 2004

- MeHg (nmol /L)
  - Median = 52
  - 25th perc = 28
  - 75th perc = 110
- THg (nmol/L)
  - Median = 57
  - 25th perc = 31
  - 75th perc = 120

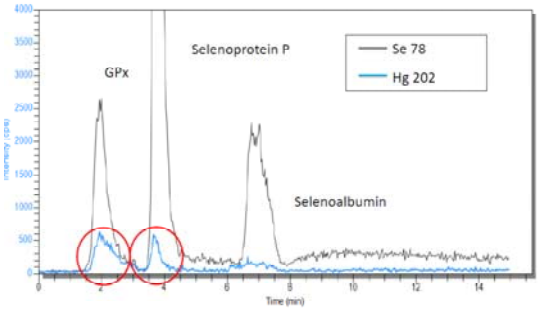


More than 90% is MeHg...

Preliminary results (Part A) : Se proteins AF-ICP-MS

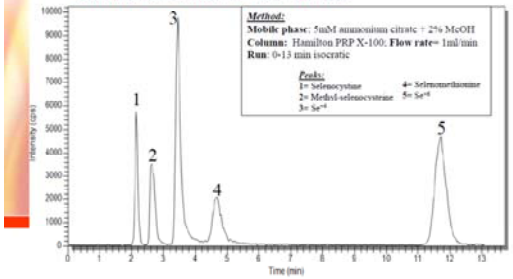


Preliminary results (Part A) : Se proteins AF-ICP-MS

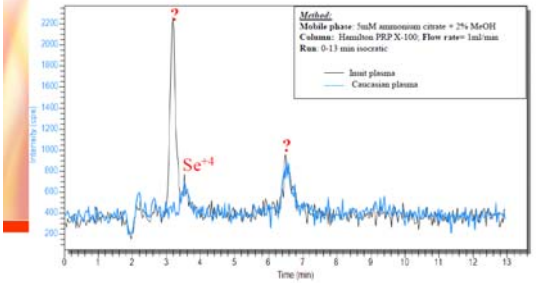


Separation and Detection of a Mixture of Seleno-compounds by Anion Exchange LC-ICPMS

Compounds spiked in mobile phase



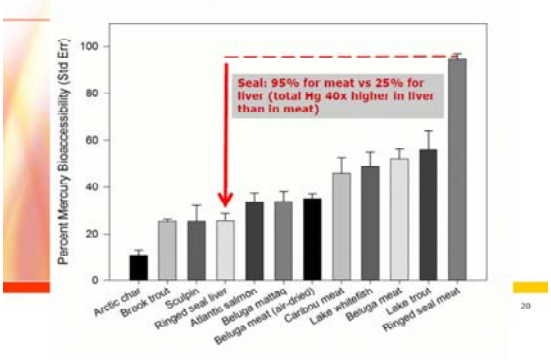
Comparative Analysis – Inuit vs Caucasian plasma samples



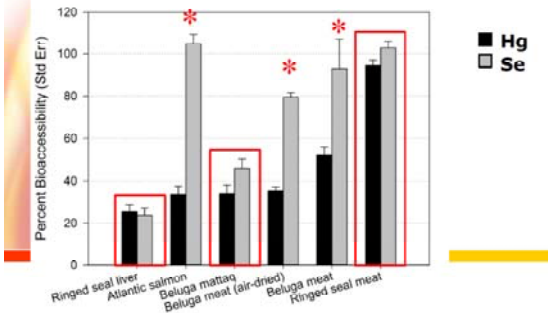
Preliminary results (part B) - Total Hg and Se levels in 291 traditional food samples from Nunavik (2008-2012)

Food Item	n	Total Hg (µg g⁻¹ w.w.)		Total Se (µg g⁻¹ w.w.)	
		Geometric Mean	Range	Geometric Mean	Range
Beluga meat (raw)	20	1.1	0.39 – 2.7	0.73	0.60 – 1.1
Beluga meat (air-dried or nikku)	10	4.0	1.9 – 4.9	1.3	1.1 – 1.4
Beluga mattaaq (skin)	16	0.44	0.25 – 1.6	4.4	2.4 – 7.5
Ringed seal meat	20	0.30	0.13 – 1.1	0.44	0.28 – 0.68
Ringed seal liver	20	12.0	2.5 – 90.0	8.7	3.1 – 38.0
Caribou meat	30	0.027	0.014 – 0.054	0.20	0.10 – 0.29
Blue mussels	32	0.018	0.0078 – 0.040	0.42	0.31 – 0.74
Arctic char (sea-run)	18	0.044	0.020 – 0.11	0.32	0.27 – 0.48
Lake whitefish	20	0.17	0.10 – 0.30	0.23	0.17 – 0.40
Brook trout	24	0.12	0.065 – 0.68	0.23	0.18 – 0.29
Atlantic salmon	17	0.041	0.028 – 0.062	0.27	0.20 – 0.35
Lake trout	20	1.10	0.66 – 1.70	0.17	0.021 – 0.41
Shorthorn sculpin meat	25	0.19	0.071 – 0.38	0.35	0.24 – 0.70
Shorthorn sculpin eggs	19	<LOD	<LOD	1.4	1.1 – 1.7

Preliminary results (part B) – Bioaccessibility of Hg from traditional food samples in the SHIME model



Preliminary results (Part B) : Bioaccessibility of Hg and Se from traditional foods in the SHIME model



Preliminary conclusions – Part A

- More than 90% of Hg in blood of Inuit adults is in the form of MeHg
- Hg signal coincides with Se (SelP and GPx), suggesting that MeHg is bound to these proteins
- An unknown seleno-compound is present in Inuit plasma (selenoneine?)

Preliminary conclusions – Part B

- Hg concentration in beluga meat and ringed seal liver are elevated
- Hg in ringed seal liver is of low bioaccessibility (25%)
- Beluga mattaaq is very rich in selenium
- Arctic Char is low in Hg; it is a good source Se and n-3 PUFAs

Future Work

- ***Part A:***
  - Biomarkers of Se status: selenoproteins and small seleno-compounds in plasma
- ***Part B:***
- Total Hg and Se in additional food samples:
  - Fish and bird eggs, walrus meat, etc.
  - Transformed foods: nikku (caribou), pitsik (fish), igunak, etc.
- Hg and Se speciation in food samples
- Bioaccessibility experiments:
  - Hg and Se speciation in "gastric" fluid
  - Step 2 – Caco-2 cells
  - Include wild berries, algae, Labrador tea, tomato products

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