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Seasonal variations in exposure to methylmercury and its dietary sources among pregnant Inuit women in Nunavik, Canada

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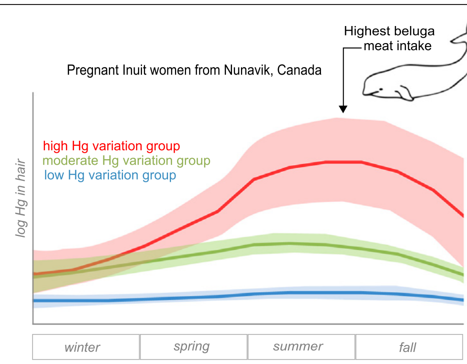
HIGHLIGHTS

- Elevated MeHg exposure remains a concern for pregnant women in Nunavik.
- Levels of Hg in hair were lower in winter and higher in summer/fall.
- Three different trajectories of monthly hair Hg variations were identified.
- Beluga meat was the primary source of MeHg exposure, particularly in summer.
- Only beluga meat consumption was associated with changing annual hair Hg trajectories.

GRAPHICAL ABSTRACT



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ABSTRACT

Among populations living in close connection with the sea, rivers and lakes for subsistence, diet varies according to local monthly wildlife species availability and food preferences. This may lead to variations in methylmercury (MeHg) exposure over a year, although no biomonitoring studies have documented this issue in Circumpolar populations, the most exposed to international Hg emissions. Our aim was to characterize seasonal variations

Abbreviations: AIC, Akaike information criterion; AMAP, Arctic Monitoring Assessment Programme; BIC, Bayesian information criterion; CHU de Québec, Centre hospitalier universitaire de Québec–Université Laval; CTQ, Centre de Toxicologie du Québec; FFQ, food frequency questionnaires; GM, geometric mean; Hg, mercury; ICP-MS, inductively 85 coupled plasma mass spectrometry; ITK, Inuit Tapiriit Kanatami; INSPQ, Institut National de Santé Publique du Québec; MeHg, methylmercury; NCDS, Nunavik Child Development Study; NCP, Northern Contaminants Program; NNHC, Nunavik Nutrition and Health Committee; NRBHSS, Nunavik Regional Board of Health and Social Services; NRC, Nunavik Research Centre; pTDI, provisional tolerable daily intake; TAT, total allowable take; WHO, World Health Organization.

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in MeHg exposure among pregnant Inuit women from Nunavik and to identify country foods responsible for these variations.

Between October 2016 and March 2017, 97 participants were recruited. Blood mercury (Hg) was tested and hair Hg was measured by centimeter as a surrogate for monthly MeHg exposure over the past year. Latent class growth analysis was conducted to identify groups of pregnant women with similar hair Hg monthly trajectories. Country foods consumption was documented by season. Seasonal daily intakes of MeHg were estimated based on concentrations in country foods.

Retrospective monthly hair Hg analyses revealed that MeHg exposure was lowest in winter, and highest in summer and early fall months. Three latent classes (groups) of pregnant women with similar trajectories of monthly hair Hg variations were identified: high (n = 20, 21%), moderate (n = 38, 41%) and low variation (n = 35, 38%). Beluga meat was the country food contributing to most of daily MeHg intake, primarily during summer and fall, and was the only one associated with the odds of being classified into moderate and high variation groups (OR 95% CI: 1.19 [1.01–1.39] and 1.25 [1.04–1.50]).

These findings underscore the importance of monthly variations in exposure to MeHg due to the seasonality of local foods consumed and responsible for elevated MeHg exposure. Further studies critically need to understand local diet fluctuations over a year to adequately assess MeHg exposure, adopt timely preventive interventions and evaluate the effectiveness of the Minamata Convention.

1. Introduction

Indigenous communities in the Arctic live in close connection with the sea, rivers and lakes for subsistence (ITK, 2017). Wildlife harvested from these environments, known as country foods, is central to cultural continuity, food security, and appreciated for it exceptional nutritional quality and taste (Council of Canadian Academies, 2014; Pirkle et al., 2016). However, some species are high in methylmercury (MeHg), a widespread environmental pollutant that is known to travel to global poles and accumulate in the aquatic food chain, primarily in top-predator fish species and marine mammals (Donaldson et al., 2010). Arctic Circumpolar populations are among the most exposed to MeHg in the world and monitoring their exposure is important to evaluate the effectiveness of the Minamata Convention, which aims to halve global mercury emissions (Arctic Monitoring Assessment Program (AMAP), 2015; UNEP, 2017).

Country food diets among the Inuit Nunangat, the homeland of the Inuit in Canada, vary depending on local cultural and individual food preferences, but also local ecological factors and quota system for some species, impacting the availability of, and access to, local wildlife species (ITK, 2017). Moreover, as previously reported in Qikiqtarjuaq (Nunavut, Canada), the availability and consumption of local food from hunting and fishing activities greatly varies according to season, which could lead to higher MeHg intakes in September, but lower in January, based on estimates from food questionnaire data in that region (Chan et al., 1997). In Nunavik (northern Quebec, Canada), MeHg intake estimations showed that beluga meat, eaten frozen, raw, cooked or air-dried (known as *nikku*), was the primary country food contributing to MeHg dietary exposure in Inuit adults, based on the *Qanuippitaa?* (How are we?) Inuit Health Survey conducted between August and October 2004 (Lemire et al., 2015). Conversely, some Inuit colleagues in Nunavik more recently observed that lake trout, a predatory fish species, is often consumed by women in some villages in Nunavik, especially in winter when other country foods are less available, although no dietary data exists to support this information. Moreover, to date, no studies in the Circumpolar Arctic have directly examined, using human exposure biomarkers, seasonal variations in MeHg exposure over the course of a year.

In populations with elevated consumption of fish and marine mammals, total Hg in whole blood reflects approximate MeHg exposure over the past three months (Berglund et al., 2005; National research Council, 2000). Total Hg in hair has also been used as a biomarker of maternal MeHg exposure (Cernichiari et al., 1995; Miklavčič et al., 2011; Schoeman et al., 2010), that is well correlated to the mother's diet and total Hg levels in blood (Clarkson, 2002; National research Council, 2000; World Health Organization, 2008). Furthermore, with hair growth averaging approximately 1 centimeter (cm) per month, retrospective sequential hair Hg analyses per cm of hair make it possible to reconstruct past monthly MeHg exposure over a specific period of time depending of the length of the hair bundle analyzed (Cernichiari et al., 1995; Clarkson, 2002; Dolbec et al., 2001; Gosselin et al., 2006; Miklavčič et al., 2011; National research Council, 2000).

In light of these observations, the aim of our study was: (1) to measure seasonal variations in MeHg exposure among Inuit women during pregnancy using retrospective sequential hair Hg analyses by cm; and (2) to identify the country foods responsible for these variations. We hypothesized that: (1) there is significant differences in MeHg exposure over the course of a year; (2) these variation patterns over time differ between pregnant women, who can be classified into groups with similar trajectories of monthly hair Hg variations over the year; and (3) the consumption of marine mammal meat and organs, and/or lake trout, explains seasonal variations in MeHg exposure, as well as sub-groups of monthly hair Hg variations with different risk exposure profiles.

2. Methods

2.1. Study population

This research is part of the project entitled "*Nutaratsaliit qanuingisiarningit niqituinnanut* - Pregnancy Wellness with Country Foods" (NQN), a cross-sectional study that was conducted from October 2016 to March 2017. The study targeted pregnant women in the 14 coastal communities of Nunavik (Fig. 1). Eligible participants included any pregnant Inuk (at any stage of pregnancy) over the age of 16 and living in Nunavik at the time of recruitment. Recruitment was conducted by convenience sampling and primarily based on lists of pregnant women provided by the Tulattavik and Innulitsivik Hospitals located in Kuujjuaq and Puvirnituq, respectively. Eligible participants were contacted by the research nurse who explained the study and sought informed consent.

The NQN project was approved by Nunavik Nutrition and Health Committee (NNHC) and carried out in collaboration with the Nunavik Regional Board of Health and Social Services (NRBHSS), midwives, nurses and physicians from Tulattavik and Inuulitsivik hospitals, maternity centres and local community services centres. Ethical approval was obtained from the Research Ethics Board of the *Centre hospitalier de l'Université Laval* (CHU de Québec, no. 2017-3176). Additionally, the research team was granted access to the list of pregnant women and their medical records at Tulattavik and Innuulitsivik hospitals via an authorization form. Confidentiality agreements were signed by the research team and interpreters were hired for fieldwork activities. All pregnant women who agreed to participate signed an informed consent form.

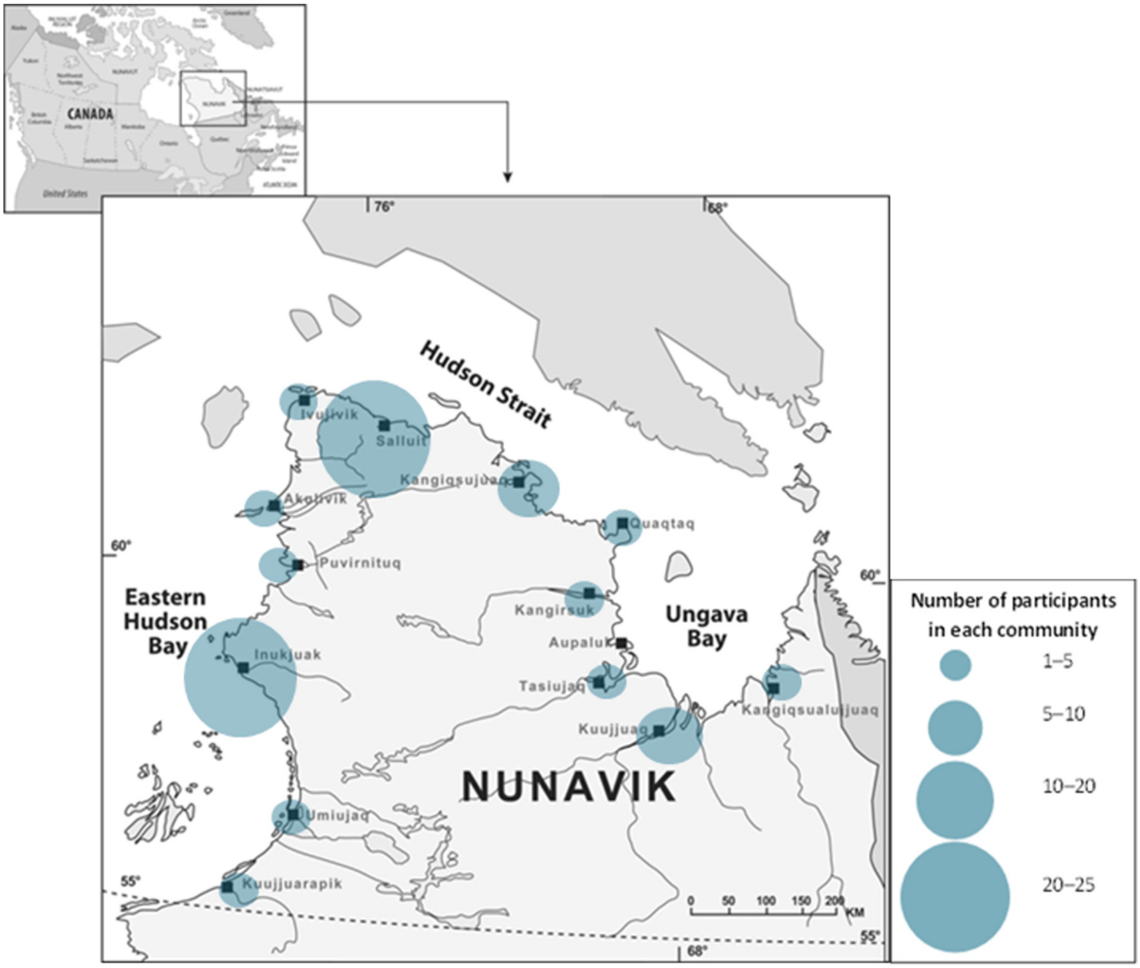

Fig. 1. Map showing the 14 communities of Nunavik and the number of participants recruited (N = 97) in each community for this study. Derived from Lemire et al. (2015).

2.2. Data collection

At the local community health centre of each community, a research nurse collected biological samples (blood and hair bundle) and administered a questionnaire to obtain pregnancy details and document frequency of country foods consumption by season.

Study design and questionnaires were developed based on previous biomonitoring projects including pregnant women in Nunavik, including the Arctic Char project (Gautier et al., 2016), and the 2017 *Qanuilirpitaa?* (How are we now?) Inuit Health Survey. An electronic version of the questionnaire was developed using Qualtrics® survey software. The food frequency questionnaire included pictures and asked participants to report the frequency of consumption of each country food by season (winter, spring, summer and fall) for the past 12 months. Medical records were reviewed to collect additional information with respect to pregnancy details.

Blood samples were collected from an antecubital vein in a 6 mL plastic vacutainer containing potassium ethylenediaminetetraacetic acid (EDTA, BD Medical, Mississauga, ON, CA), stored at −20 °C and sent frozen to the *Centre de Toxicologie du Québec* (CTQ) of the *Institut National de Santé Publique du Québec* (INSPQ) in Quebec City for total Hg analyses. Hemoglobin values were measured immediately after blood collection using a HemoCue Hb 201+ (Hemocue®, Kuvettgatan, Ängelholm, SE). A hair bundle (hair strand of 0.5 cm or more in diameter) was collected at the scalp from the occipital region using stainless steel scissors and stored in a plastic bag until shipment to the Nunavik Research Centre (NRC) of the Makivik Corporation in Kuujjuaq for total Hg analysis by cm. The hair strand had to be long enough to reconstruct the retrospective monthly MeHg exposure profile of each participant over the last year (about 12 cm starting from the scalp).

2.3. Laboratory analyses

Blood samples were analyzed for total Hg by inductively coupled plasma mass spectrometry (ICP-MS) at the CTQ using the method M-572 as previously reported in Fontaine et al. (Fontaine et al., 2008). Blood samples were diluted 20-fold in a diluent containing 0.5% (v/v) ammonium hydroxide and 0.1% (v/v) octylphenol ethoxylate. Mercury is brought to its elementary form by passing through argon plasma before being identified by mass spectrometry on the Elan DRC II ICP-MS instrument (Perkin Elmer, Woodbridge, ON, Canada). The limit of detection for blood Hg was 0.1 μg/L. Three reagent blanks were analyzed in each analytical sequence: one at the beginning, a second after the calibration curve, and a third at the end. The internal quality control (QC) of the analyses was ensured by analyzing non-certified reference materials inserted after the calibration curve, after every 10th sample and the end of each analytical sequence. The following reference materials from CTQ's Quebec Multielement External Quality Assessment Scheme (QMEQAS) were used: QM-B-Q1108, QM-B-Q1201, QM-B-Q1302, QM-B-Q1505 and QM-B-Q1512. In all analytical batches, concentrations obtained for reference materials were within 15% of the consensual values. The CTQ has ISO 17025 accreditation and participates in the following quality assurance and quality control programs: Comparison Program for Metals in Biological Matrices (PCI, Canada), QMEQAS (Canada), Priority Metals Quality Assessment Scheme (PMQAS, Canada), Lead and Multielement Proficiency Program (LAMP, USA), State of New-York Department of Health (USA) and the External Quality Assessment Scheme (G-EQUAS, Germany).

Hair samples were analyzed for total Hg at the NRC in Kuujjuaq (Nunavik), which is involved in the NCP's quality assurance and quality control inter-laboratory assessments. Hair strands were cut into sequential cm (maximum of 12 cm). Before analysis, each hair section was digested with a standard operation procedure of mixed-acid digestion developed at the NRC (Kwan, 2017). Nitric acid (16 M) was first to the sample for pre-digestion at room temperature overnight. The samples were then heated on a dry-bath at 73 °C for 3 h. A 2:1 mixture of sulfuric acid (18.3 M) and hydrochloric acid (11.6 M) was then added and the sample heated for a further 3 h on the dry-bath. A 2 mM solution of potassium dichromate in 30% v/v hydrochloric acid was then added to stabilize and dilute the digests, which were stored in a refrigerator and analyzed within 24 h. Total hair Hg concentration was determined by cold vapor atomic absorption using a mercury UV monitor (PinAAcle 900z atomic absorption spectrometer fitted with a heated quartz cell, Perkin Elmer Inc., Boston, MA, USA). The limit of detection for Hg in hair was 0.04 μg/g. Analytical quality control was ensured using three certified reference materials for metal analyses in human hair (QM-H-Q1203, QMEQAS09H-01, QMEQAS07H-07), provided by the CTQ. For each batch of samples, two reference materials were prepared alongside the participants' hair samples and analyzed at the beginning and the end of each analytical sequence. In all analytical batches, concentrations obtained for reference materials were within 10% of the certified values. To ensure adequate analytical precision, each sample and reference material was analyzed in duplicate. Results were accepted as valid only when the relative standard deviation of the duplicate measurements was less than 10%. Digestion blanks were prepared and analyzed alongside each batch of samples to check for contamination. All analyzed digestion blanks were below the limit of detection.

2.4. Participant follow-up

All participants received individual letters containing their blood and hair Hg results, along with factsheets about country foods detailing their nutrient composition and MeHg concentrations. Pregnant women with blood Hg levels exceeding the Health Canada guideline value (≥8 μg/L or 40 nmol/L) were advised to make an appointment with the health professional involved in their pregnancy follow-up (local midwife, nurse or other).

2.5. Calculation of daily MeHg intake

Food frequency questionnaires (FFQ) documented seasonal country food consumption, including which parts of the animals were eaten (meat, fat, skin, organs) and food preparation techniques for fish, marine mammals, seafood, land mammals, and wildfowl species. This included, air-dried beluga and caribou meat (*nikku*), dried fish flesh (*pitsik*) and raw, aged or boiled beluga skin and blubber (beluga *mattaaq*).

The daily country food intake was calculated by multiplying the daily consumption frequency by typical portion size for each food item (g/day). Typical portion sizes were based on average country foods serving size data (in grams) for women of childbearing age who participated in the 2004 *Qanuippitaa*? (How are we?) Nunavik Inuit Health Survey (C. Blanchet and Rochette, 2008) (Supplementary material – Table S1). Individual daily MeHg intake from each country food was estimated using the following equation: MeHg intake (μg/day) = $\sum$ amount of food consumed (g/day) X MeHg concentration in food (μg/g) (Supplementary material – Table S2). Total daily MeHg intake was calculated by summing MeHg intakes from all country foods.

MeHg concentrations for these estimations were derived from country food samples collected from 2008 to 2013 (as MeHg and/or total Hg concentrations) in different regions of Nunavik (Supplementary material – Table S2), and represent the most recent data available from the NRC. The highest concentrations of MeHg in country foods in Nunavik are found in beluga meat, marine mammal organs and piscivorous fish such as lake trout, northern pike and walleye. These foods may be eaten frozen, raw, or sometimes, cooked. Inuit also often eat dried beluga meat known as beluga *nikku*. As MeHg binds to proteins, the drying process greatly concentrates MeHg in this type of country food (Lemire et al., 2015). MeHg generally represents around 80 to 100% of total Hg in the meat or flesh of animals and total Hg concentrations were used as surrogates for MeHg concentrations in these country foods (Lemire et al., 2015). However, since a very large proportion of Hg in marine mammal organs is found as inorganic Hg, we used the proportion of 11% of total Hg as MeHg for the ringed seal liver (the marine mammal organ primarily consumed), based on a previous study in Nunavik (Lemire et al., 2015).

The provisional blood guidance value of Health Canada of 8 μg/L (Legrand et al., 2010) is based on the Provisional Tolerable Daily Intake (pTDI) of 0.2 μg/kg bw/day for daily MeHg intake in pregnant women, children, and women of childbearing age, and this value is known to correspond to 2 μg/g in hair (IPCS, 1990). As the average body weight for pregnant women in this project was 60 kg, a pTDI of 12 μg/day was used to assess whether the seasonal total daily MeHg intake estimated for each group of pregnant women exceeded provisional Canadian guidance values.

2.6. Statistical analysis

Descriptive statistics were used to describe levels of MeHg exposure (at recruitment, by month, and season), country food consumption and daily MeHg intakes. Log transformations were applied to normalize blood and hair Hg distributions, while $\log(x + 1)$ transformations were used for country food and MeHg intake variables.

Associations between blood and hair biomarkers were examined using the non-parametric Spearman correlation test. Models used to assess the seasonal variations in hair Hg were adjusted by age, and the trimester of pregnancy, based on previous literature on the topic (Arbuckle et al., 2016; Golding et al., 2013; Grandjean and Budtz-Jorgensen, 2007; Miranda et al., 2011; Morrissette et al., 2004; Stern and Smith, 2003).

Since it takes about 20 days for hair to appear on the surface of the skin, the first cm of hair from the scalp corresponds to a recall period of approximately 3 weeks prior to sample collection (National research Council, 2000). Accordingly, the date for the 1st cm of hair Hg (and the 11 subsequent monthly hair Hg cm) was retroceded 20 days before the recruitment date to estimate the corresponding retrospective monthly exposures over the past year. Hair Hg levels by season were established by grouping monthly hair Hg levels as follows: winter (January, February and March), spring (April, May and June), summer (July, August and September) and fall (October, November, and December).

Latent Class Growth Analyses were used to assess inter-individual differences in intra-individual variations in monthly hair Hg levels over time (Jung and Wickrama, 2008). We identified distinct latent classes (groups) of pregnant women having similar patterns or trajectories of hair Hg monthly variations over the year. Additionally, ordinal logistic regressions were used to test associations between the three latent classes of hair Hg monthly trajectories and the different intake of country food with high MeHg concentration. The exponentiated odds ratio coefficient was associated with a doubling of the predictor (country food variable). All these analyses were also adjusted by the relevant covariates previously identified in the literature – namely: age (Arbuckle et al., 2016; Golding et al., 2013; Miranda et al., 2011), trimester of pregnancy (Morrissette et al., 2004; Stern and Smith, 2003), and hemoglobin

Table 1
Selected characteristics of pregnant women and biomarkers of Hg exposure at recruitment (n = 93), Nunavik, 2016–2017.

	Geometric mean (GM) [95% CI] (range)
Age (years)[a]	24 [23–25] (16–37)
Weeks of pregnancy	21 [19–23] (8–39)
Blood Hg (μg/L)	4.35 [3.69–5.12] (0.80–40.12)
Hair Hg (μg/g) – Average of first 2 cm	2.04 [1.71–2.44] (0.34–22.41)
Hb (g/L)[a]	114 [112–117] (97–141)

[a] Arithmetic mean [95% CI] (range).

concentration (Arbuckle et al., 2016; Grandjean and Budtz-Jorgensen, 2007; Kim et al., 2014) at recruitment time.

Correlational statistics and ordinal logistic regressions were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA) whereas Latent Growth Analyses were carried out using the software Mplus (version 7.2.; Muthén & Muthén, Los Angeles, CA, USA). An α-level of 0.05 was used for hypothesis testing.

3. Results

A total of 97 women were recruited on a voluntary basis, representing approximately 42% of the estimated 231 pregnant women in Nunavik at the time of the study. A similar proportion of participants were recruited across the three coastal regions in Nunavik (Hudson Bay n = 38/99 (38%), Hudson Strait n = 37/77 (48%) and Ungava Bay n = 22/55 (40%)). Participants with short hair (less than 12 cm), or without hair samples, were excluded (n = 4).

The general characteristics of the pregnant Inuit women who participated in this study are summarized in Table 1. Mean age was 24 years old, and most participants were half-way through their pregnancy (average of 21 weeks of gestation). Geometric mean concentrations of blood and hair Hg (average of the first 2 cm) at recruitment were 4.35 μg/L and 2.04 μg/g, respectively, and both biomarkers were strongly correlated ($\rho = 0.94$, $p < 0.0001$). Whereas 40% of participants (n = 2/5) recruited in the fall between October and December 2016 had blood Hg levels above the Health Canada guideline (≥8 μg/L), only 22% of participants (n = 19/88) recruited in winter months between January to March 2017 had blood Hg above this guideline. Participants' hair Hg levels by cm were grouped by season (Table 2). Interestingly, hair Hg levels were the lowest in winter months and the highest in summer, secondly in fall, although the difference between summer and fall was only marginally significant ($p = 0.060$).

Using Latent Class Growth Analyses, three latent classes (groups) of pregnant women with similar trajectories of hair Hg monthly variations over a year were identified (Fig. 2): 21.5% of pregnant women grouped into the high Hg variation (hereafter referred to as "high variation", n = 20), 40.8% into the moderate Hg variation ("moderate variation", n = 38), and 37.6% into the low Hg variation ("low variation", n = 35). The three-class solution (high, moderate and low variation groups) was selected as the best model after exploring models with different numbers of classes. In other words, the three-class solution produced more distinct hair Hg level trajectories than other solutions, and the three-class solution had the smallest Bayesian information criteria (BIC) and Akaike information criterion (AIC) values (BIC = 3553.85; AIC = 3372.53) than the two-class solution (BIC = 3802.34; AIC = 3631.23).

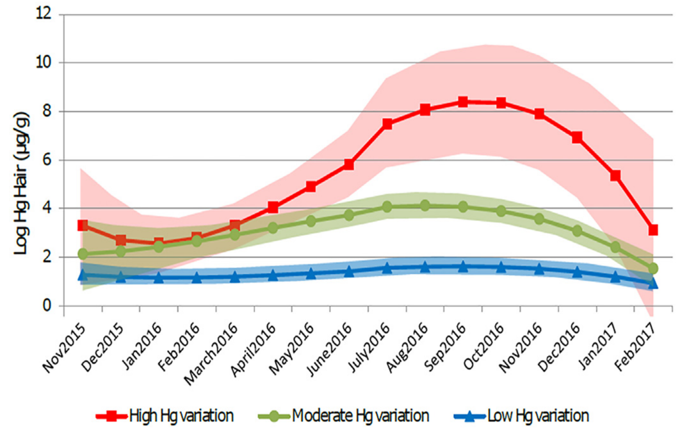

Fig. 2. Latent classes of hair Hg monthly variation trajectories with 95% CIs (shaded areas) for high (n = 20), moderate (n = 38) and low (n = 35) Hg variation groups found in Nunavik pregnant women (recruited between October 2016 and March 2017), and adjusted for age, hemoglobin levels and trimester of pregnancy.

Only pregnant women in the high and moderate variation groups presented significant monthly variations of hair Hg concentrations over time (Fig. 2; high and moderate groups, $p < 0.0001$ and low group, $p = 0.58$). A clear monthly variation in hair Hg concentrations was found in the high group, with a major peak during summer/fall months in 2016 (August until November). Indeed, during the summer months (July, August and September), a significant difference in hair Hg levels was found between the three groups (high: GM [95% CI] = 6.72 μg/g [4.99–9.05]; moderate: 3.88 μg/g [3.45–4.35]; low: 1.26 μg/g [0.99–1.60]; $p < 0.0001$). By contrast, during the winter months (January, February and March), hair Hg levels were the lowest, and no significant difference was found between the high and moderate variation groups (high: 2.48 μg/g [1.80–2.68]; moderate: 1.88 μg/g [1.49–2.38]; $p = 0.137$), although these two remained significantly higher than the low variation group (0.86 μg/g [0.67–1.12]; $p < 0.0001$). No differences for age, nor weeks of pregnancy were observed between groups.

In all three groups, the total daily country food intake (in g/day), the diversity of country food types consumed, and the total daily MeHg intake were highest during summer (Figs. 3 and 4 and Supplementary material Figs. S1 and S2). Beluga meat (raw, frozen or cooked) and beluga *nikku* were overall not highly consumed, except in the summer, and their consumption was consistently lower than other country foods. Nevertheless, beluga *nikku* and beluga meat (raw, frozen or cooked) were more often consumed in the high and moderate variation groups than in the low variation group (high: 8.54 g/day, moderate: 7.27 g/day, low: 2.46 g/day; differences for high vs low, $p = 0.0067$ and moderate vs low, $p = 0.021$). Moreover, the high variation group presented a higher consumption of beluga *nikku* (high = 5.91 g/day vs moderate = 3.23 g/day), whereas the moderate variation group had a higher consumption of beluga meat (high = 2.63 g/day vs moderate = 4.04 g/day), although the differences were not statistically significant. Lake trout and seal liver were not highly consumed in any of the seasons, and their consumption was not different between the three groups (seal liver did not feature among the fifteen most consumed country foods in the low group). Northern pike and walleye were not part of the fifteen most consumed country foods within each group.

Although beluga meat (raw, frozen or cooked) and beluga *nikku* were not among the country foods most highly consumed, they were responsible for most of the estimated daily MeHg intake across seasons,

Table 2
Seasonal variations of hair Hg among pregnant Inuit women, Nunavik, 2016–2017 (n = 93).[1]

Season	Hair Hg (μg/g) GM[2] [95% CI] (range)
Winter	1.49[a] [1.25–1.77] (0.19–8.09)
Spring	2.23[b] [1.88–2.65] (0.25–10.28)
Summer	2.86[c] [2.38–3.42] (0.19–22.41)
Fall	2.50[bc] [2.10–2.98] (0.25–13.59)

[1] Participants' monthly values for Hair Hg in 2015 were excluded from this analysis.
[2] Different letters represent statistically differences between seasons. Models were adjusted for age and trimester of pregnancy.

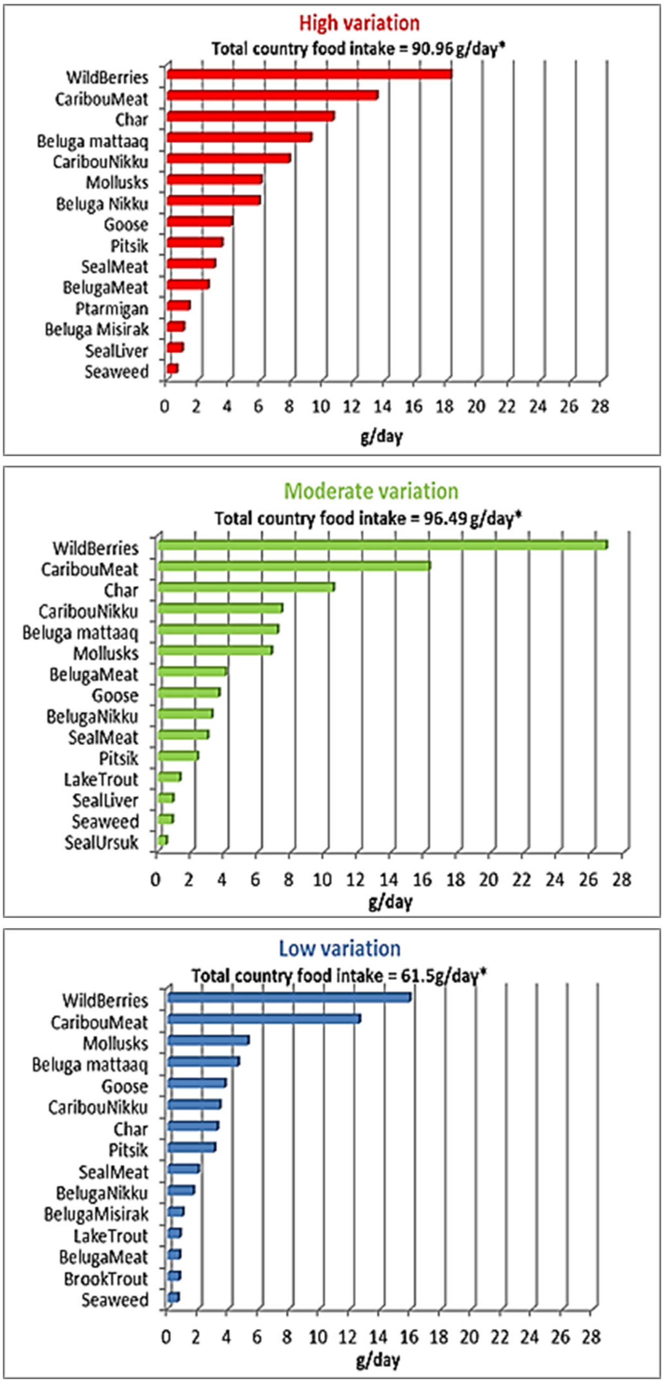

Fig. 3. Average daily country food intake among Nunavik pregnant women during summer 2016 within the three groups of hair Hg monthly variation trajectories (high n = 20, moderate n = 38, low n = 35) identified in the Latent Class Analysis. Fifteen most consumed country foods for each group are presented. *The total country food intake value presented is for all country foods in the FFQ, not only the fifteen most consumed.

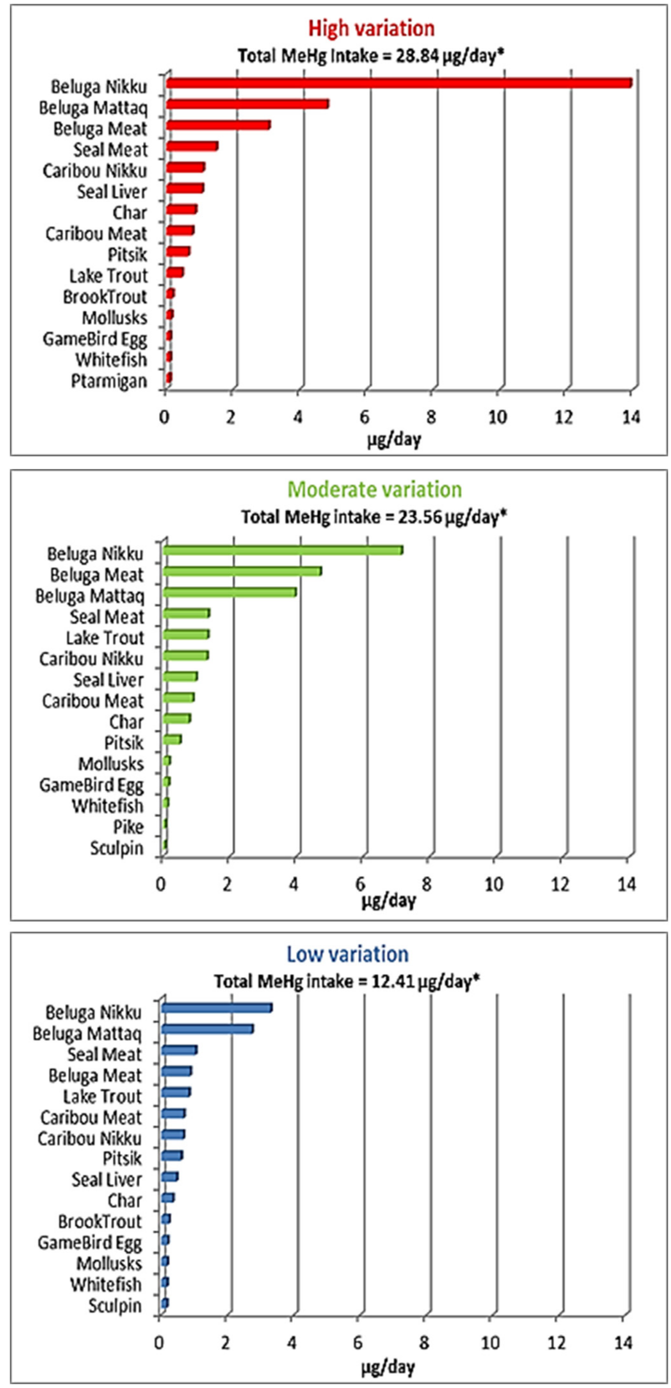

Fig. 4. Average MeHg intake by country food among Nunavik pregnant women during summer 2016 within the three groups of hair Hg monthly variation trajectories (high n = 20, moderate n = 38, low n = 35) identified in the Latent Class Analysis. Fifteen most consumed country foods for each group with the highest MeHg intake are presented. *The total MeHg intake value presented is for all country foods in the FFQ, not only the fifteen, with highest MeHg intake.

primarily in summer (Fig. 4, Supplementary Fig. S2). During that season, beluga meat and beluga *nikku* were responsible for more than half (59%) of total MeHg intake in the high variation group (moderate = 50%, low = 34%). By contrast, in the high and moderate groups, beluga *mattaaq* consumption was responsible for only 17% of MeHg intake (low group = 22%), although it was much more frequently consumed than beluga meat and *nikku*.

The estimated geometric mean of MeHg intakes based on total country food consumption in the high (29 µg/day) and moderate (24 µg/day) groups in the summer were two-fold greater than the provisional tolerable daily intake (pTDI) for pregnant women (12 µg/day). Furthermore, these two groups had average hair Hg values in the summer considerably higher than the World Health Organization (WHO) reference value of 2 µg/g (high = 6.72 µg/g; moderate = 3.88 µg/g). Conversely, the low group presented an average MeHg intake of 12 µg/day, and average hair Hg below the Canadian guidance value (1.26 µg/day).

The ordinal logistic regression models testing the contribution of each country food intake to the classification of pregnant women into the three Hg variation groups, showed that beluga meat (raw, frozen, cooked and *nikku*) was the only country food significantly associated with the monthly hair Hg variation pattern during summer (Table 3). There were no statistically significant associations in the other seasons of the year (Table S3). Beluga *nikku* consumption tended to be associated with the high variation group, whereas beluga meat (raw, frozen or cooked) was significantly associated with the moderate variation group.

4. Discussion

Sequential retrospective hair Hg analyses among pregnant Inuit women revealed significant monthly variations in MeHg exposure in Nunavik. MeHg exposure was the lowest in winter and highest in summer and early fall months, particularly among two sub-groups of participants which greatly exceeded recommended MeHg guidance values for pregnant women and which babies were more at risk of MeHg-related neurodevelopmental outcomes (Legrand et al., 2010). Our analyses confirm that these fluctuations in MeHg exposure in this Circumpolar region are due to the seasonality of local foods consumed and responsible for elevated MeHg exposure. Beluga meat (raw, frozen, cooked and/or *nikku*) was identified as the country food responsible for these monthly variations in hair Hg levels and as the country food that most contributed to daily MeHg intake, primarily in the summer and fall, and within the two more at-risk sub-groups. These findings underscore the importance of understanding local diet fluctuations over a year to adequately assess MeHg exposure, identify population sub-groups more at-risk of elevated MeHg exposure and adopt timely preventive interventions. Moreover, seasonality of MeHg exposure critically needs to be considered when assessing regional long-term time-trends in exposure, which are required to evaluate the effectiveness of the Minamata Convention.

4.1. Seasonal variations in MeHg exposure: regional specificities and Minamata Convention effectiveness evaluation

The retrospective component of our study provides a unique, and more integrated, assessment of year-round MeHg exposure and country food consumption in the Inuit Nunangat than has been reported previously in the literature. Our results are well aligned with previous findings with non-pregnant women from the 2004 *Qanuippitaa*? Nunavik Inuit Health Survey, showing that beluga meat was the country food most responsible for MeHg exposure among Inuit adults in Nunavik (Lemire et al., 2015). In our study, this was particularly the case for beluga *nikku*, which was much more consumed among the most at-risk sub-group of pregnant women (high variation group), with an average pTDI during summer months more than twice the recommended guidance value for maternal MeHg exposure in Canada (29 versus 12 μg/day). Furthermore, our study highlight that the seasonality of beluga whale availability, accessibility and consumption over time in conjunction with individual food preferences have a major impact on MeHg exposure in Nunavik.

Beluga whales are central to Nunavimmiut culture and diet. Consumption of beluga contributes to food security and nutrient intake due to their abundance in several nutrients such as protein, omega-3 polyunsaturated fatty acids, and selenium (including selenoneine) (Blanchet et al., 2000; Lemire et al., 2015; Little et al., 2019). Furthermore, beluga hunting is an important activity for cultural continuity and intergenerational transmission of traditional knowledge (Tyrrell, 2008). Seasonal hunting and consumption of beluga in Nunavik communities closely mirror the annual migratory patterns of the animals: beluga typically migrate from the Baffin region through the Hudson Strait in June to spend the summer in the Hudson Bay (either the Eastern or Western Hudson Bays depending of the stocks), and then migrate back in late October to early November. A beluga harvest management plan, overseen by the Nunavik Marine Region Wildlife Board, places a quota on whale harvesting by permitting a total allowable take (TAT) for each community (Nunavik Marine Region Wildlife Board, 2017). This system may impact consumption patterns, as it often results in hunters traveling to Hudson Strait communities to fulfill their community TAT, such that beluga are hunted, transported to hunters' home communities (if necessary), and shared within a small window of time between community members (typically one or two weeks in mid-June and late October). Quotas are renewed yearly (rather than seasonally) based on the calendar year, so the spring hunt tends to result in larger numbers of whales harvested, with the fall hunt being smaller as the TAT is often depleted following the spring hunt (Nunavik Marine Region Wildlife Board, 2017). Hunters have suggested that limited availability of beluga due to the quota also contributes to lost knowledge and skills of *igunaq* (fermented beluga meat) preparation, which may be driving the popularity of *nikku* as a means of preserving beluga meat (Tyrrell, 2008). Further, the quota system may impact MeHg exposure, since it encourages hunters to target larger whales to ensure more meat for their communities; and larger whales tend to be higher in MeHg (Loseto et al., 2008). Hunters have also reported shifting and less predictable migratory patterns due to climate change, which may pose issues for accessing beluga in the future. Thus, several environmental and socio-political factors influence beluga harvest and consumption, and subsequent MeHg exposure in Nunavik. All these need to be considered and adapted over the years in order to conduct adequate human biomonitoring studies (i.e. not during winter and early spring in Nunavik) and identify sub-groups for targeted and timely preventive interventions and clinical follow-ups when relevant.

It is of upmost importance of documenting MeHg exposure long-term time-trends for evaluating the effectiveness of the Minamata Convention ratified in 2017, particularly among Circumpolar populations who consume fish and marine mammals and are primarily exposed to MeHg from international Hg emissions, with often no local source (Basu et al., 2018). The present study shows that conducting biomonitoring studies when local foods responsible for most MeHg intake are off-season may lead to an under-estimation of MeHg exposure on an annual basis and lead to the possible erroneous conclusion MeHg is declining over the years in that region. To date, only two other previous studies have used retrospective hair Hg analyses to study seasonal variations in MeHg exposure, and these focused on documenting monthly fluctuations according to freshwater fish species consumed in the Amazon and South Central US (Dolbec et al., 2001; Dong et al., 2015). Thus, further studies on seasonal variation in MeHg exposure in Circumpolar countries and elsewhere are needed so that MeHg exposure assessment and time-trend analyses are consistently done during the most relevant

Table 3
Odds ratios for the association between country food intake and the three groups of hair Hg monthly variation trajectories during the summer among Nunavik pregnant women in 2016–2017 (n = 93).[a]

Country food intake in summer	Groups	Odds ratio [95% CI]	p
Beluga meat – all types	High vs. low	**1.25 [1.04–1.50]**	**0.017**
(raw, frozen, cooked and *nikku*)	Moderate vs. low	**1.19 [1.01–1.39]**	**0.033**
Beluga *nikku* (dried)	High vs. low	1.29 [0.96–1.72]	0.086
	Moderate vs. low	1.12 [0.87–1.44]	0.396
Beluga meat	High vs. low	1.28 [0.94–1.76]	0.118
(raw, frozen or cooked)	Moderate vs. low	**1.46 [1.10–1.93]**	**0.009**
Lake trout	High vs. low	0.67 [0.30–1.51]	0.336
	Moderate vs. low	1.24 [0.73–2.09]	0.423
Ringed seal meat	High vs. low	1.23 [0.82–1.59]	0.117
	Moderate vs. low	1.24 [0.99–1.54]	0.063
Ringed seal liver	High vs. low	1.23 [0.86–1.74]	0.251
	Moderate vs. low	1.11 [0.79–1.57]	0.542
Beluga *mattaaq*	High vs. low	1.06 [0.87–1.28]	0.574
	Moderate vs. low	1.09 [0.93–1.28]	0.278

Estimates in bold are significantly different between the Hg variation groups.
[a] For these analyses, the food items retained were those consumed by more than 25% of pregnant women and known to contain concentrations of MeHg above 0.2 μg/g.

months of the year and that are specific to each region. Furthermore, as Dong et al. (2015) stressed that local seasonal fluctuations in MeHg exposure limits comparisons of results between studies, time-sensitive biomonitoring studies are also needed for more accurate inter-region and countries comparisons, and ultimately, a more comprehensive effectiveness evaluation of the Minamata Convention.

4.2. Relevance for preventive and clinical interventions in Nunavik

The latent class growth analysis method used for the first time in the present study in an exposure assessment context allowed us to distinguish three groups of pregnant women with similar hair Hg monthly variation trajectories over time, deepening our understanding of local country foods responsible for the highest MeHg exposure among two particularly at-risk subgroups of pregnant women in Nunavik. MeHg has a short half-life of about 50 days (Clarkson, 2002; National research Council, 2000) and dietary changes to food with low MeHg have been shown to decrease body load of MeHg, and reduce potential fetal risks (Kirk et al., 2017; Knobeloch et al., 2011). Thus, until there is evidence for declining MeHg concentrations in the Arctic as a result of the Minamata Convention, a temporary solution for more at-risk pregnant women to reduce their exposure to MeHg is to decrease their consumption of country foods with high MeHg concentration (e.g., beluga meat, particularly beluga *nikku*) as early as possible during pregnancy. When possible, the consumption of these foods should be substituted by other culturally valued food low in Hg (Pirkle et al., 2016). Nevertheless, it is also important for local health professionals to understand that MeHg exposure can increase later pregnancy in forthcoming active fishing/hunting months. For this reason, it is important to integrate MeHg exposure assessments and counselling/education into clinical activities during all pregnancy follow-ups, but with an increased focus on preventive dietary counselling as summer approaches.

4.3. Strengths and limitations

Results from this study must be interpreted with caution since a convenience sampling approach was used and participants were not selected on a random basis. However, it must be emphasized that these data are for a specific, hard to reach population, that is as there are approximately only 340 pregnancies per year in Nunavik (*Ministère de la Santé et des Services Sociaux du Québec*, data file on births, update territorial division version M34–2019, based on the number of live births per year during the period from 2013 to 2017 in Nunavik), spread out among 14 villages across a vast territory. Still, 42% of pregnant women were recruited, making our sample size an important portion of the total population of pregnant women in Nunavik at that time.

Food frequency questionnaires and food portion size present the possibility of recall and social desirability bias. To minimize recall bias, we used a food frequency questionnaire for recording country food consumption by season, so the participant could more easily remember their consumption based on her knowledge of different seasonal harvests. We also used images to make it more user-friendly, to avoid misclassification, and to diminish language barriers. Local interpreters were available when needed. Average Hg concentrations in country foods used to estimate MeHg intake may have lacked precision, as there are important intra- and inter-species variations according to location or the age of the animal hunted for example. Moreover, there is only scattered data available for MeHg concentrations in country foods from Nunavik. Thus, we used total Hg concentrations in country foods as a surrogate for MeHg in most country foods (with the exception of seal liver). Further studies are needed to refine these estimates, while better understanding the impact of different country food preparation methods on MeHg concentrations and bioavailability as well as Inuit preferences for the age of marine mammals they prefer to consume. Conversely, a strength of our study was the use of specific total Hg and MeHg concentrations from locally-caught marine mammals, fish and others species. Thus, our assessment is highly relevant to local populations. Additionally, we found a fairly good agreement between hair Hg levels and estimated daily MeHg intake in each season, suggesting that total daily MeHg intake was adequately estimated.

Finally, monthly patterns of country food consumption and exposure to MeHg may change in the coming years. Indeed, the present project uses the premise that availability and consumption of country foods varies seasonally, and that these variations over time are similar from one year to the next. However, due to climate change, migration pattern and species availability may change from month to month in the coming years (Moore and Huntington, 2008). Moreover, MeHg bioaccumulation and distribution within Arctic aquatic ecosystems may change – in either direction – as MeHg methylation rates are predicted to increase and species abundance may change predator-prey relationships and overall biomagnification patterns in food webs (Moore and Huntington, 2008; Stern et al., 2012; Sundseth et al., 2015).

5. Conclusion

These findings underscore the importance of monthly variations in exposure to MeHg, notably among specific more at-risk population sub-groups, due to the seasonality of local foods consumed and responsible for elevated MeHg exposure. Further studies critically need to understand local diet fluctuations over a year, as well as the local environmental, cultural and socio-political factors influencing them, to adequately assess MeHg exposure, identify more at-risk sub-groups, adopt timely preventive and clinical interventions and evaluate the effectiveness of the Minamata Convention.

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CRediT authorship contribution statement

Mariana de Moraes Pontual: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Pierre Ayotte:** Conceptualization, Funding acquisition, Investigation, Writing - review & editing. **Matthew Little** Writing - review & editing. **Chris Furgal:** Conceptualization, Funding acquisition, Investigation, Writing - review & editing. **Amanda D. Boyd:** Conceptualization, Funding acquisition, Investigation, Writing - review & editing. **Gina Muckle:** Conceptualization, Funding acquisition, Writing - review & editing. **Ellen Avard:** Conceptualization, Data curation, Writing - review & editing. **Sylvie Ricard:** Conceptualization, Data curation, Writing - review & editing. **Marie-José Gauthier:** Conceptualization, Data curation, Writing - review & editing. **Elhadji Anassour Laouan Sidi:** Data curation, Formal analysis, Writing - review & editing. **Mélanie Lemire:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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