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Selenoneine is a major selenium species in beluga skin and red blood cells of Inuit from Nunavik

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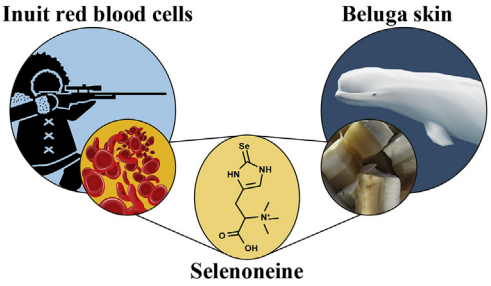
HIGHLIGHTS

- LC-ICP-MS/MS was used to quantify selenoneine and Se-methylselenoneine in RBCs.
- Selenoneine represented 54% of total Se content in Inuit RBCs.
- Selenoneine was also the major Se species in beluga mattaaq – an Inuit delicacy.
- Selenoneine may protect Inuit against methylmercury toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Nunavimmiut (Inuit of Nunavik, Northern Quebec, Canada) exhibit a high selenium (Se) status because of their frequent consumption of marine mammal foods. Indirect evidence from our previous studies had suggested that selenoneine – a novel selenocompound – may be accumulating in the blood of Nunavimmiut. We used a liquid-chromatography/inductively coupled tandem mass spectrometry (LC-ICP-MS/MS) method to measure concentrations of selenoneine and its methylated metabolite Se-methylselenoneine in archived red blood cells (RBC) obtained from 210 Nunavimmiut living in communities along the Hudson Strait, where marine mammal hunting and consumption are most frequent in Nunavik. This method was adapted to quantify selenoneine and its methylated metabolite in beluga mattaaq, an Inuit delicacy consisting of the skin with the underlying layer of fat and the major dietary source of Se for Nunavimmiut. Total selenium concentration was also measured in RBC and beluga mattaaq samples by isotope dilution ICP-MS/MS. The median selenoneine concentration in RBC was 413 μg Se/L (range = 3.20–3230 μg Se/L), representing 54% (median) of total Se content (range = 1.6–91%). Quantification of selenoneine in five beluga mattaaq samples (skin layer) from Nunavik revealed a median concentration of 1.8 μg Se/g wet wt (range = 1.2–7.4 μg Se/g), constituting 54% (median) of the total Se content (range = 44–74%). Se-methylselenoneine was also detected in Inuit RBC but not in beluga mattaaq, suggesting that selenoneine undergoes methylation in humans. Selenoneine may

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protect Nunavimmiut from methylmecury toxicity by increasing its demethylation in RBC and in turn decreasing its distribution to target organs.

1. Introduction

The Inuit of Nunavik (henceforth referred to as Nunavimmiut) reside in coastal communities along the Hudson Bay, the Hudson Strait and the Ungava Bay, north of the 55th parallel in Quebec, Canada (Fig. 1). Their dietary intake of selenium (Se) is among the highest in the world because of the exceptionally high Se content of country foods, especially those of marine origin including beluga, walrus, seal and fish eggs (Lemire et al., 2015). As a result, Nunavimmiut exhibit relatively high whole blood Se concentrations compared to the general Canadian population. The median blood Se concentration among Nunavimmiut who participated to the 2004 *Qanuippitaa*? Health Survey was 261 μg/L [interquartile range (IQR) = 166 μg/L], with values ranging from 126 to 3571 μg/L (Achouba et al., 2016). By comparison, the median concentration was 200 μg/L (IQR = 37 μg/L) among Canadians aged 6–79 years enrolled in the Canadian Health Measures Survey (Cycle 1, 2007–2009) (Health Canada, 2010). While all *Qanuippitaa*? participants had blood Se levels above that of 90 μg/L required for optimal Se-enzymes activity (Yang and Xia, 1995), approximately three percent displayed blood Se levels above the No Observed Adverse Effect Level (NOAEL) for selenosis of 1000 μg/L set by the U.S. Environmental Protection Agency (Poirier et al., 1994). There is therefore a need to better define the Se status in this population.

We first set out to measure concentrations of plasma Se-containing proteins and observed that despite the high whole blood Se status of Nunavimmiut, plasma Se levels were much less variable (IQR = 23 μg/L) and did not exceed 250 μg/L (Achouba et al., 2016). This is in sharp contrast to the linear relationship noted between blood and plasma Se levels in fish-eating communities of the Brazilian Amazon, which exhibit a high Se status resulting from their consumption of Brazil nuts (rich in selenomethionine) (Lemire et al., 2012). This suggests that marine country foods consumed by Nunavimmiut contains a different Se species that accumulates in blood cells and is responsible for the high blood Se status in this population (Achouba et al., 2016).

In 2010, Yamashita and Yamashita reported the identification of selenoneine (2-selenyl-N_{α},N_{α},N_{α}-trimethyl-L-histidine), a novel organoselenium compound which was extracted from the blood of bluefin tuna, *Thunnus orientalis* (Yamashita and Yamashita, 2010). Its methylated metabolite, Se-methylselenoneine, was subsequently identified in human urine (Klein et al., 2011). More recently, Yamashita et al. found that selenoneine was the major Se species in blood cells of a fish-eating population on remote Japanese islands (Yamashita et al., 2013a). We therefore hypothesized that selenoneine could also be a major Se species in blood cells of Nunavimmiut.

The objective of the present study was to identify and quantify selenoneine and Se-methylselenoneine in archived red blood cells (RBC) of 210 Nunavimmiut from Hudson Strait communities who participated to the 2004 *Qanuippitaa*? Health Survey. Residents from these communities exhibit the highest whole blood Se status of all Nunavimmiut (Lemire et al., 2015). We also investigated if these selenocompounds are present in beluga mattaaq, a delicacy consisting of the skin with the attached fat layer (Fig. 2), which is frequently consumed by residents from the Hudson Strait region and a major source of dietary Se for Nunavimmiut (Lemire et al., 2015).

Fig. 1. Map of Nunavik, Quebec, Canada. Hudson Strait communities are identified by a red star. Source: Makivik Corporation (http://www.makivik.org/wp-content/uploads/2013/02/nunavik1.gif.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Study population

In the fall of 2004, the *Qanuippitaa*? Nunavik Inuit Health Survey was conducted in the 14 communities of Nunavik. The targeted individuals were Inuit residents aged between 18 and 74 years. The study design and the sampling procedure were published elsewhere (Rochette and Blanchet, 2007). Briefly, a two-stage stratified random sampling method was used. In the first stage, a proportional random sample selection of private Inuit households was carried out, taking into account the size of each village. In the second stage, eligible members of each household were asked to participate. Participants were invited onboard the Canadian Coast Guard Ship Amundsen to fill out several questionnaires and attend a clinical session during which blood samples and different clinical and anthropometric measurements were collected. The overall participation rate was 50% and a total of 889 participants completed the clinical session (Rochette and Blanchet, 2007). Our study sample consisted of 210 participants from the four villages along the Hudson Strait (Ivujivik, Kangiqsujuaq, Quaqtaq and Salluit) from whom archived RBC samples were available. After venous blood collection in vacutainers containing EDTA, blood samples were centrifuged, the plasma and buffy coat removed and the resulting RBC pellet stored at −80 °C in the original collection tube at the Centre de Toxicologie du Québec (CTQ) of the Institut National de Santé Publique du Québec, Canada (INSPQ). The present

Fig. 2. Beluga mattaaq – an Inuit delicacy consisting of the skin with the underlying layer of fat. The fat tissue located above the dotted line was removed prior to analysis.

study was reviewed and approved by the Nunavik Nutrition and Health Committee and the Ethics Review Board of the CHU de Québec.

2.2. Beluga mattaaq samples

Beluga mattaaq samples from Nunavik were kindly provided by Dr. Ellen Avard and Dr. Michael Kwan from the Nunavik Research Center (Kuujjuaq, QC) while those from Nunavut were provided by Dr. Gary Stern from the Centre for Earth Observation Science (CEOS; Winnipeg, MB).

2.3. Reagents and chemicals

Tetrabutylammonium phosphate (TBAP) (99+%) ammonium hydroxide (99.99%; metal basis) and L-cysteine (98+%) were purchased from Alfa Aesar (Ward Hill, MA). Anhydrous ethyl ether (ACS Reagent Grade), heptafluorobutyric acid (HFBA) zero sulfate (99.5%; sequencing grade) and nitric acid (69–70%; ACS grade) were supplied by Fisher Scientific (Waltham, MA). Acetonitrile (LC-MS Omnisolv), methanol (LC-MS Omnisolv) and Triton X-100 were obtained from EMD (Omaha, NB), whereas ethanol was from Commercial Alcohols (Brampton, ON). Ammonium carbonate (99.999%; trace metals basis), dithiothreitol (DTT; ≥99%), formic acid (≥95%; reagent grade), leucine-enkephalin acetate salt hydrate (≥95% HPLC), seleno-DL-methionine (≥99%) and sodium selenate were purchased from Sigma-Aldrich (St. Louis, MO). Trimethylselenonium iodide (98%) was acquired from Prime Organics (product #1–9904; Woburn, MA) and Se-methylseleno-L-cysteine (98%) from Acros Organics (Geel, Belgium). Enriched metallic ^{77}Se (98%) was purchased from Isoflex (San Francisco, CA) and the gold (Au) standard solution 1000 μg/L (dilute HNO_3) was supplied by BDH VWR Analytical (Radnor, PA). Edinburgh Minimal Media (EMM2) was obtained from Sunrise Science Products (San Diego, CA). The SRM-1577c (bovine liver) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) and the CRM-DOLT-4 (dogfish liver) was obtained from the National Research Council of Canada (NRC, Ottawa, ON).

2.4. Instruments and materials

Yeast lysis was performed using a BeadBeater and 0.5 mm glass beads (BioSpec Products, Bartlesville, OK). Compounds identification was performed by an Acquity ultra performance liquid chromatography system (UPLC) (Waters, Milford, MA) equipped with a SeQuant™ Zic-HILIC, 100 × 2.1 mm, 3.5 μm column (Merck, Darmstadt, Germany) and coupled to a Xevo G2 time of flight mass spectrometer (QTOF-MS) (Waters). Selenoneine and Se-methylselenoneine quantification was carried out using an Agilent 1260 high performance liquid chromatography (HPLC) system (Agilent Technologies, Mississauga, ON) equipped with an Atlantis T3 column 3 μm, 4.6 × 100 mm column (Waters) and coupled to a ICP-QQQ 8800 mass spectrometer (Agilent Technologies). Filters 10 kDa cut-off were obtained from EMD (Omaha, NB). Evaporation was performed using a rapidvap system (Labconcco, Kansas City, MO). Milli-Q water was purified using the advantage A10 ultrapure water system (Merck Millipore, Billerica, MA).

2.5. Selenoneine standard biosynthesis and purification

The genetically modified fission yeast, *Schizosaccharomyces pombe* (strain *P3nmt1-egt1*$^{+}$), was kindly provided by Dr Mitsuhiro Yanagida (Pluskal et al., 2014). Cells were grown overnight at 28 °C in EMM2 media supplemented with 10 μM of sodium selenate. Cells were then harvested by centrifugation (30 min at 4000 rpm) and washed 3 times using ultrapure water. A total of 40 g of cells were suspended into cold (−30 °C) MeOH/H_2O (50:50) and lysed in the BeadBeater. The lysate was centrifuged during 30 min at 4000 rpm and the supernatant was collected and filtered through a 10 kDa cut-off centrifugal filter to remove proteins. The filtrate was finally concentrated under vacuum using a rapidvap system at 80 °C to yield 50 mL of lysate. Acetonitrile (50 mL) was added to the concentrated lysate prior to storage at −30 °C.

Selenoneine was purified by solid phase extraction (SPE) using an Oasis WCX weak cationic exchange cartridge (Waters) preconditioned with methanol and then 2% NH_4OH in water. After evaporation of acetonitrile, the lysate was diluted 1:10 in ultrapure water and loaded onto the cartridge under gravity flow. The cartridge was subsequently washed with 2% NH_4OH, methanol and 2% formic acid in methanol. Finally, selenoneine was gravity eluted

with a 50 mM DTT aqueous solution.

2.6. Se-methylselenoneine standard synthesis

A 1-mL aliquot of an aqueous solution containing our selenoneine standard (1 mg Se/L) was evaporated to dryness at 100 °C under nitrogen flow. One mL of ethyl ether was added to the precipitate under stirring and homogenized for 1 min, followed by the addition of 1 ml of diazomethane prepared as described elsewhere (Ngan and Toofan, 1991). The reaction with diazomethane was completed in 5 min at room temperature and the solution evaporated to dryness at 60 °C under nitrogen flow. The precipitate was then dissolved in water.

2.7. Compound identification

The identity of selenoneine and Se-methylselenoneine was confirmed by liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer (UHPLC-QTOF-MS) analysis. A volume of 20 μL of a diluted sample (1:5) in acetonitrile was injected in the UHPLC system. Compounds were separated on a Zic-HILIC column using acetonitrile (A) and 10 mM ammonium carbonate (B) as mobile phases at a flow rate of 250 μL/min. The elution gradient was as follows: 85:15 (A:B) from 0 to 2 min, increased linearly to 40:60 (A:B) from 2 to 10 min then reversed to 85:15 (A:B) from 10 to 14 min. Electrospray ionization was operated in positive mode (ESI+) and full-scan data were collected from $m/z = 50$ to 800 with a scan time of 0.65 s. Mass accuracy was corrected every 10 s by infusing a solution of 3.6 μmol/L of leucine-enkephalin (m/z 556.2771) at a flow rate of 10 μL/min via a LockSpray™ interface. QTOF-MS/MS acquisition was performed using nitrogen as the collision gas with a collision ramp from 15 to 30 V to generate the fragments. More details regarding instrumental settings are provided in Table S1. The observed ion mass spectrum for each compound was compared to the theoretical m/z value and Se isotope pattern was verified to confirm the presence of selenium. QTOF-MS/MS fragmentation was also used to confirm the structure of the compounds.

2.8. RBC sample preparation

Selenoneine and Se-methylselenoneine were extracted from RBC as follows: 50 μL of RBC were placed in a 1.5-mL polypropylene tube (Sarstedt, Nümbrecht, Germany) together with 10 μL of an internal standard solution containing Se^{+6} and $TMSe^{+}$ (10 μM each) in 0.5% HNO_3 and 440 μL of a 50-mM dithiothreitol (DTT) aqueous solution (pH = 9). The resulting solution was vortex mixed during 1 h. A 100-μL aliquot of the homogenate was diluted in 400 μL of ultrapure water and then filtered through a 10 kDa cut-off centrifugal filter at 12 000×g for 50 min at room temperature. Finally, 250 μL of the filtrate were added to 100 μL of a 2.5-fold concentrated mobile phase and the resulting solution analyzed using a high performance liquid chromatography-inductively coupled plasma tandem mass spectrometry (HPLC-ICP-MS/MS).

2.9. Beluga skin sample preparation

The fat layer was removed from the beluga mattaaq sample with a scalpel (see Fig. 2), leaving only the skin layer, which was then frozen in liquid nitrogen and pulverized with a mortar and pestle. For selenoneine extraction, 50 mg of the skin powder was homogenized in 15 mL of a 50-mM DTT aqueous solution using an ultra-probe sonication (Misonix Fisher Scientific Sonic Dismembrator 550, Fisher Scientific, Ottawa, ON) at 20% (100W) for 2 min. The homogenate was then centrifuged at 4000 rpm for 30 min at 4 °C and the supernatant was filtered through a 10 kDa cut-off centrifugal filter as described above. Finally, the filtrate was concentrated under vacuum and taken up in 1 mL of ultrapure water. For the HPLC-ICP-MS/MS analysis, samples were diluted 1:10 in the mobile phase and 10 μL of the internal standards solution used for RBC analysis were added.

2.10. Selenoneine and Se-methylselenoneine quantification by HPLC-ICP-MS/MS

The chromatographic separation of the selenocompounds was performed by ion paring HPLC by injecting 100 μL of the prepared sample through an Atlantis T3 reversed-phase column. The isocratic mode was used at a flow rate of 1 mL/min for 10 min using a mobile phase consisting of 0.5 mM of TBAP, 0.5% HFBA and 2% methanol (pH = 2.3). As two ion pairing agent were used, the conditioning of a new column is achieved using 700 mL of mobile phase. ICP-MS/MS operating conditions are presented in Table S1. Detection was performed by employing the kinetic energy discrimination mode (KED) in the collision cell using H_2/O_2 gas to eliminate the polyatomic interferences upon detection of isotopes ^{78}Se, ^{77}Se and ^{76}Se.

Concentrations of selenoneine and Se-methylselenoneine in RBC were determined using two external calibration curves constituted with selenoneine and Se-methylselenoneine standards; the combined signals of Se^{+6} and $TMSe^{+}$ were used as the internal standard. Recovery and global matrix effects were determined following the standardized procedure of Bienvenu et al. (2017) (see text, Supplementary material). Peak integration and concentration calculations were performed using the MassHunter software version 4.2 (Agilent Technologies, Mississauga, ON). As no certified reference materials were available for both selenoneine and Se-methylselenoneine, quality control (QC) was assessed using two RBC samples: 1) a low-level QC made up of RBC with non-detected levels of selenoneine/Se-methylselenoneine (obtained from a volunteer in our laboratory) that were spiked with both compounds; and 2) a high-level QC which was a pooled sample composed of RBC from Nunavimmiut displaying a total blood Se concentration greater than 1000 μg/L.

2.11. Total Se analysis by ID-ICP-MS/MS

We measured total Se concentration using a fully validated analytical method developed for human biomonitoring purposes at INSPQ following 17025 ISO guidelines. Fifty μL of RBC were dissolved in 5 mL of a solution containing 0.25% ammonia, 2% ethanol, 0.005% Triton X-100, 0.05% L-cysteine and 50 μL of the Au solution, spiked with 7.8 ng of ^{77}Se. The quantification was performed by isotope dilution (ID) calibration technique (Reyes et al., 2003) on an ICP-MS/MS operated in MRM mode with KED using H_2 gas to eliminate the polyatomic interferences upon detection of Se (see Table S1). We used the $^{78}Se/^{77}Se$ isotope ratio for Se quantification and verified the accuracy of the result with both $^{76}Se/^{77}Se$ and $^{80}Se/^{77}Se$ isotope ratios. In the absence of certified reference materials for Se in human blood, two Se proficiency testing materials (PTM) (QM-B-1108 and QM-B-1302) issued from the quality assessment scheme for trace elements (QMEQAS) operated by INSPQ were used throughout the analytical process.

For total Se determination in beluga skin, 250 mg of the powder (see above) were digested in 2 mL of HNO_3 for 18 h at 120 °C. Ultrapure water was then added to a final volume of 20 mL. Finally, 1 mL was diluted in 5 mL of the same solution used for RBC and spiked with the same quantity of ^{77}Se as mentioned above. Quality control (QC) was ensured by analyzing two reference materials (CRM-DOLT4 and SRM-1577c) with a certified total Se

concentration (see Table S2).

2.12. Statistical analysis

Because total Se and selenocompound concentrations in RBC were not normally distributed, we used the median and IQR as statistical descriptors and non-parametric statistics to perform correlation analyses (Spearman correlation coefficients). All analyses were performed using PASW Statistics for Windows, Version 22.0 (SPSS Inc., Chicago, IL). We selected an α-level of 0.05 for hypothesis testing.

3. Results and discussion

3.1. Standards synthesis and characterisation

Because no commercial standards are available for selenoneine and Se-methylselenoneine and in order to quantify these selenocompounds in RBC, selenoneine was produced through biosynthesis using a genetically modified strain of the African beer yeast *Schizosaccharomyces pombe* (*P3nmt-egt1*$^{+}$). This strain overexpresses the egt1 gene involved in the synthesis of ergothioneine, the sulfur (S) analog of selenoneine; the S/Se substitution occurs randomly when yeast is grown in a Se rich medium (Pluskal et al., 2014). We isolated selenoneine from the yeast lysate using weak cationic exchange solid phase extraction and confirmed the identity of the selenoneine standard by UHPLC-QTOF-MS. Selenocompounds bear a typical Se isotope pattern resulting from the conserved distribution of stable Se isotopes (Fig. S1). The selected ion chromatogram at *m/z* 278.040 (Fig. 3a) shows the selenoneine peak eluting at 3.65 min, whose mass spectrum exhibits a typical Se isotope pattern (Fig. 3b) matching exactly the theoretical pattern for selenoneine (Fig. 3c). We then synthesized Se-methylselenoneine through derivatization of selenoneine with diazomethane. The selected ion chromatogram at *m/z* 292.056 (Fig. 3d) shows the Se-methylselenoneine peak eluting at 3.27 min, whose mass spectrum exhibits a typical Se isotope pattern (Fig. 3e) matching exactly the theoretical pattern for Se-methylselenoneine (Fig. 3f). Additional confirmation of the identity of both compounds was obtained through MS/MS fragmentation experiments (Figs. S2a and S2b). The purity of our standards in terms of Se content was then verified by HPLC-ICP-MS/MS analysis. We first determined the chromatographic conditions required to achieve complete separation of selenoneine and Se-methylselenoneine as well as four other commercially available Se compounds, namely selenate (Se^{+6}), trimethyl-selenonium ion ($TMSe^{+}$), Se-methylselenocysteine and selenomethionine (Fig. 3g). Analysis of the selenoneine standard yielded a major peak eluting at 1.62 min (Fig. 3h) representing 98.3% of the total peaks area, while that of the Se-methylselenoneine standard produced a major peak at 3.47 min representing 95.6% of the total peaks area (Fig. 3i). After verifying selenium purity by HPLC-ICP-MS/MS, concentrations of the standards were certified by measuring their total selenium content using ID-ICP-MS/MS. Because our goal was to achieve only selenium purity of the standards required for LC-ICP-MS/MS quantification, we did not attempt to completely eliminate ergothioneine which is present in high concentrations in the *S. pombe* lysate (Pluskal et al., 2014; Turrini et al., 2018).

3.2. Concentrations of total Se, selenoneine and Se-methylselenoneine in Nunavimmiut RBC

We developed and validated a HPLC-ICP-MS/MS method for the quantification of selenoneine and Se-methylselenoneine in RBC extracts. So far, two methods have been developed to quantify selenoneine in blood cells but none of them included Se-methylselenoneine (Yamashita et al., 2013a; Kroepfl et al., 2019).We also measured total Se concentration in RBC by conventional isotope dilution (ID) ICP-MS/MS using enriched ^{77}Se (Reyes et al., 2003). The performance characteristics of both methods are presented in Table S3. We found that selenoneine is the major Se species in RBC from Hudson Strait Nunavimmiut. The identity of selenoneine in a pooled RBC sample was confirmed by UHPLC-QTOF-MS analysis: the retention time of the peak in the selected ion chromatogram at *m/z* 278.040 (Fig. 4a) and the corresponding Se isotope pattern (Fig. 4b) matching those of the selenoneine standard (Fig. 3a and b). The ion pair chromatogram obtained by HPLC-ICP-MS/MS analysis of an RBC extract from a participant exhibiting a high total Se concentration shows a selenoneine peak eluting at 1.62 min (Fig. 4e). The median selenoneine concentration in RBC was 413 μg Se/L (IQR = 327 μg Se/L), with values ranging from 3.20 to 3230 μg Se/L (Fig. 4f and Fig. S3) and 18% of participants exhibiting concentrations above 1000 μg Se/L (Fig. 4g). The median total Se concentration in RBC was 788 μg Se/L (IQR = 741 μg Se/L, with values ranging 205–3890 μg Se/L (Fig. 4f). Selenoneine represented 54% (median) of the total Se content in RBC (IQR = 36%; range = 1.4–91%). As previously reported by Yamashita et al. in RBC of a fish-eating population in Japan (Yamashita et al., 2013a), we noted a very strong positive correlation between concentrations selenoneine and total Se ($r_s = 0.984$, $P < 0.0001$; Fig. 4h), further indicating that selenoneine uptake and accumulation in RBC is responsible for the high blood Se status in Hudson Strait Nunavimmiut.

The UHPLC-QTOF-MS analysis also revealed the presence of Se-methylselenoneine in the RBC extract from a participant exhibiting a high total Se concentration. Indeed the retention time of the peak in the selected ion chromatogram at *m/z* 292.056 (Fig. 4c) and the corresponding Se isotope pattern (Fig. 4d) matched those of the Se-methylselenoneine standard (Fig. 3d and e). The small Se-methylselenoneine peak can be seen at RT = 3.57 min in the ion pair chromatogram (Fig. 4e), corresponding to a +0.1 min RT shift compared to the standard (Fig. 3i), due to a blood matrix effect. Se-methylselenoneine concentrations determined by HPLC-ICP-MS/MS analysis were much lower than those of selenoneine (Fig. 4f) with a median concentration of 17.9 μg Se/L [IQR = 28.4 μg Se/L; range = ND (<3.9) to 124 μg Se/L]. We observed a strong positive correlation between concentrations of Se-methylselenoneine and selenoneine ($r_s = 0.901$, $P < 0.0001$; Fig. 4i). Methylation of selenoneine may be a prerequisite for its excretion in urine; Se-methylselenoneine is most likely formed *in* vivo through hepatic and renal methyltransferases (Klein et al., 2011).

Our results indicate that selenoneine is the main Se species in RBC of Nunavimmiut from Hudson Strait communities, with concentrations reaching parts per million (ppm) levels in 18% of Nunavimmiut from this region. Selenoneine is actively transported in RBC via the organic cation/carnitine (OCTN1) membrane transporter, similarly to ergothioneine (Gründemann et al., 2005). Also accumulating in RBC of Nunavimmiut is methylmercury (MeHg), a well-known neurotoxicant that is biomagnified to reach concentrations of several ppm in marine mammals and predatory fish species of the Arctic (AANDC, 2012). We and others have recently provided some evidence to suggest that Se may afford protection against the toxicity of MeHg among Inuit from Nunavik and Nunavut (Ayotte et al., 2011; Hu et al., 2017). It is tempting to speculate that selenoneine could enhance the *in situ* demethylation of MeHg, as reported in laboratory experiments with fish embryos (Yamashita et al., 2013b). Granules of HgSe have been identified in organ and tissues of some marine species and likely constitute the endproduct of Se (selenoneine)-mediated MeHg detoxification (Ikemoto et al., 2004; Lailson-Brito et al., 2012). However evidence

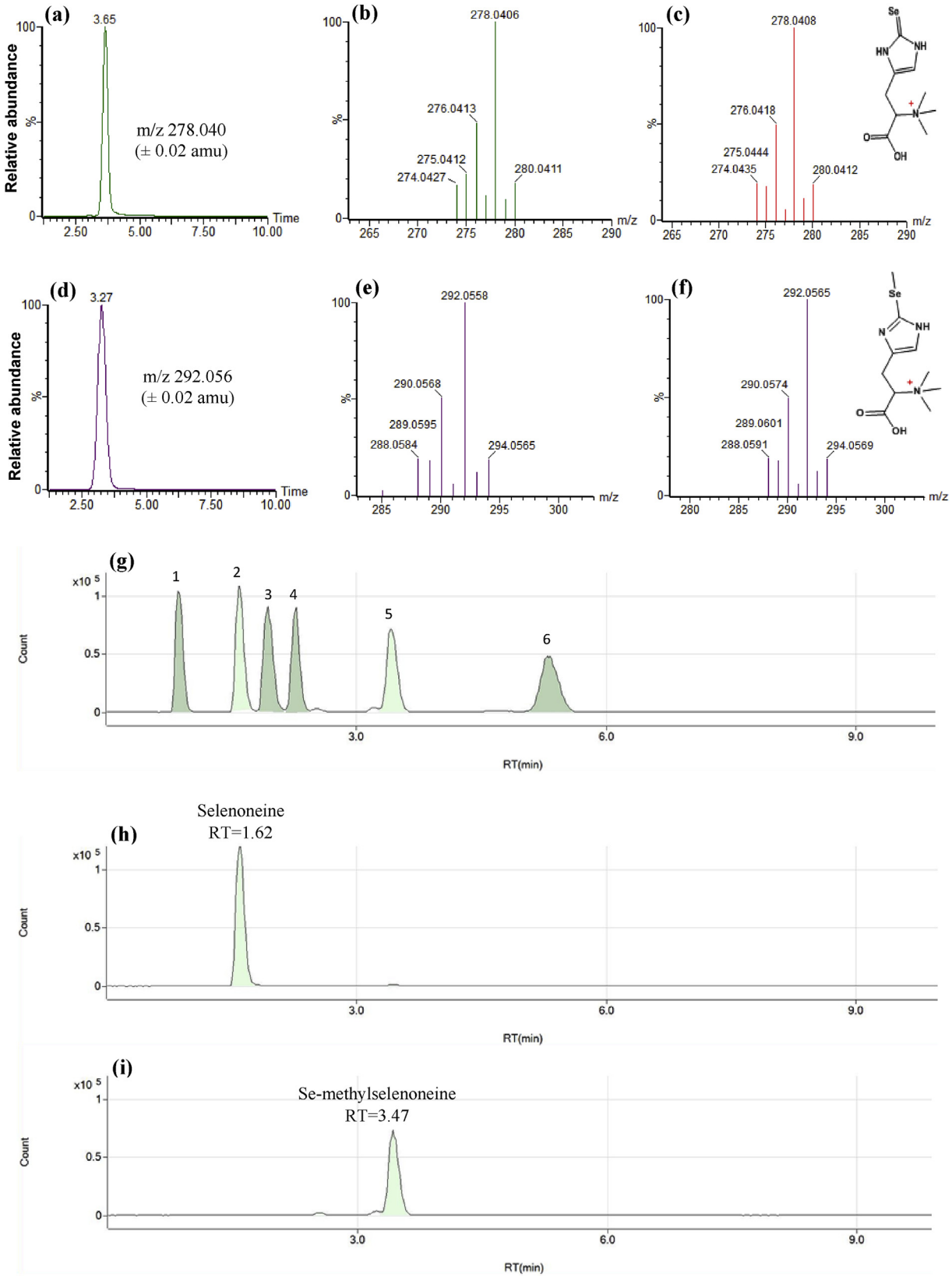

Fig. 3. Characterisation of selenoneine and Se-methylselenoneine standards. **(a)** Selected ion chromatogram of the purified selenoneine standard at *m/z* 278.040, **(b)** the corresponding selenoneine experimental mass spectrum and **(c)** the theoretical selenoneine mass spectrum. **(d)** Selected ion chromatogram of the Se-methylselenoneine standard at *m/z* 292.056, **(e)** the corresponding experimental Se-methylselenoneine experimental mass spectrum and **(f)** the theoretical Se-methylselenoneine mass spectrum. Ion pair chromatograms with ^{78}Se detection by ICP-MS/MS of **(g)** a standard mixture of selenoneine, Se-methylselenoneine and four other selenocompounds: (1) selenate (Se+6), (2) selenoneine, (3) trimethylselenonium ion (TMSe+), (4) Se-methylselenocysteine, (5) Se-methylselenoneine, (6) selenomethionine (all compounds at 1 μM); **(h)** the purified selenoneine standard; **(i)** the Se-methylselenoneine standard.

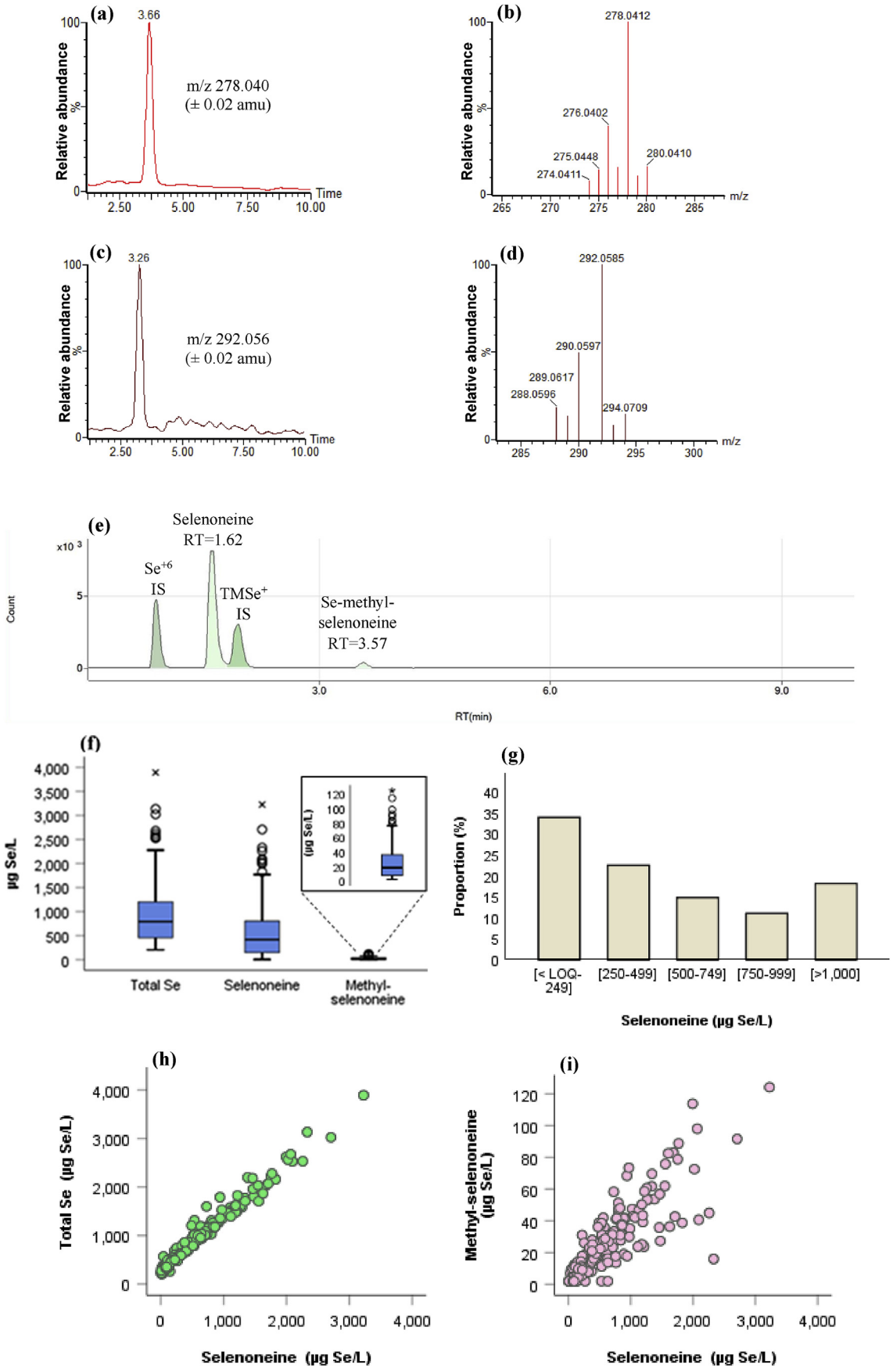

Fig. 4. Identification and quantification of selenoneine and Se-methylselenoneine in RBC of Nunavimmiut. **(a)** Selected ion chromatogram of selenoneine at *m/z* 278.040 in an extract of a pooled RBC sample from Hudson Strait Nunavimmiut, and **(b)** the corresponding selenoneine mass spectrum (UHPLC-QTOF-MS). **(c)** Selected ion chromatogram of Se-methylselenoneine at *m/z* 292.056 in an extract of a pooled RBC sample from Hudson Strait Nunavimmiut, and **(d)** the corresponding Se-methylselenoneine mass spectrum (UHPLC-QTOF-MS). **(e)** Ion pair chromatogram with ^{78}Se detection (HPLC-ICP-MS/MS) of a RBC extract from a participant exhibiting a high total Se concentration [Se^{+6} and $TMSe^{+}$: internal standards (IS)]. **(f)** Concentrations of selenoneine, Se-methylselenoneine and total Se measured by HPLC-ICP-MS/MS in RBC of Hudson Strait Nunavimmiut (N = 210). **(g)** Frequency distribution of selenoneine concentrations among Hudson Strait Nunavimmiut. **(h)** Correlation between concentrations of total Se and selenoneine in RBC of Hudson Strait Nunavimmiut ($r_s = 0.984$ $P < 0.0001$). **(i)** Correlation between concentrations of selenoneine and Se-methylselenoneine in RBC of Hudson Strait Nunavimmiut ($r_s = 0.901$, $P < 0.0001$).

supporting the formation of such granules in humans is lacking. OCTN1 is widely expressed in the human body (Gründemann et al., 2005; McBride et al., 2009; Nigro and Leonzio, 1996; Tamai et al., 1997, 2004) and accordingly, selenoneine is likely distributed to other cells in addition to RBC, where it may afford protection against oxidative stress. Selenoneine exists in a homodimeric oxidized form that can be reduced by glutathione (Yamashita et al., 2010), supporting the hypothesis that this organic form of selenium could play a role in the antioxidant defense system via its selenol (SeH) group, similarly to selenoproteins (Barbosa et al., 2017).

3.3. Concentrations of total Se and selenoneine in beluga mattaaq

Beluga mattaaq is a delicacy highly praised by Nunavimmiut that is frequently consumed by residents from the Hudson Strait region. In these communities, beluga mattaaq alone was shown to contribute to almost half (45%) of their daily total Se intake from country foods (Lemire et al., 2015). Therefore, we investigated whether selenoneine is present in mattaaq from beluga hunted in the Hudson Strait. We first removed the fat layer from a mattaaq sample (Fig. 2) and extracted Se species from the skin layer, as the fat layer contains little Se (Lemire et al., 2015; Binnington et al., 2017). We analyzed the resulting extract by UHPLC-QTOF-MS and observed on the selected ion chromatogram at *m/z* 278.040 a major peak eluting at 3.86 min (Fig. 5a) whose mass spectrum (Fig. 5b) matched that of our selenoneine standard (Fig. 3b). No peak was observed on the selected ion chromatogram at *m/z* 292.056 (not shown), indicating that Se-methylselenoneine is not present in beluga skin. We subsequently adapted the HPLC-ICP-MS/MS method developed for the analysis of RBC extracts to quantify selenoneine in beluga mattaaq extracts (Fig. 5c) and determined the total Se content of extracts by ID-ICP-MS/MS after an acid digestion. Results revealed that selenoneine is also the major Se compound in beluga mattaaq (Fig. 5d) with a median concentration of 1.8 μg Se/g wet wt (range = 1.2–7.4 μg Se/g; n = 5). Selenoneine represented 54% (median) of total Se, with values ranging from 44 to 74%. We obtained similar results for mattaaq samples from beluga whales hunted near Arviat (Nunavut), on the west coast of Hudson Bay (Fig. 5d). Similar to results from UHPLC-QTOF-MS analyses, HPLC-ICP-MS/MS analyses of beluga mattaaq extracts did not reveal the presence Se-methylselenoneine. Hence, beluga mattaaq consumption does not appear to be the source of Se-

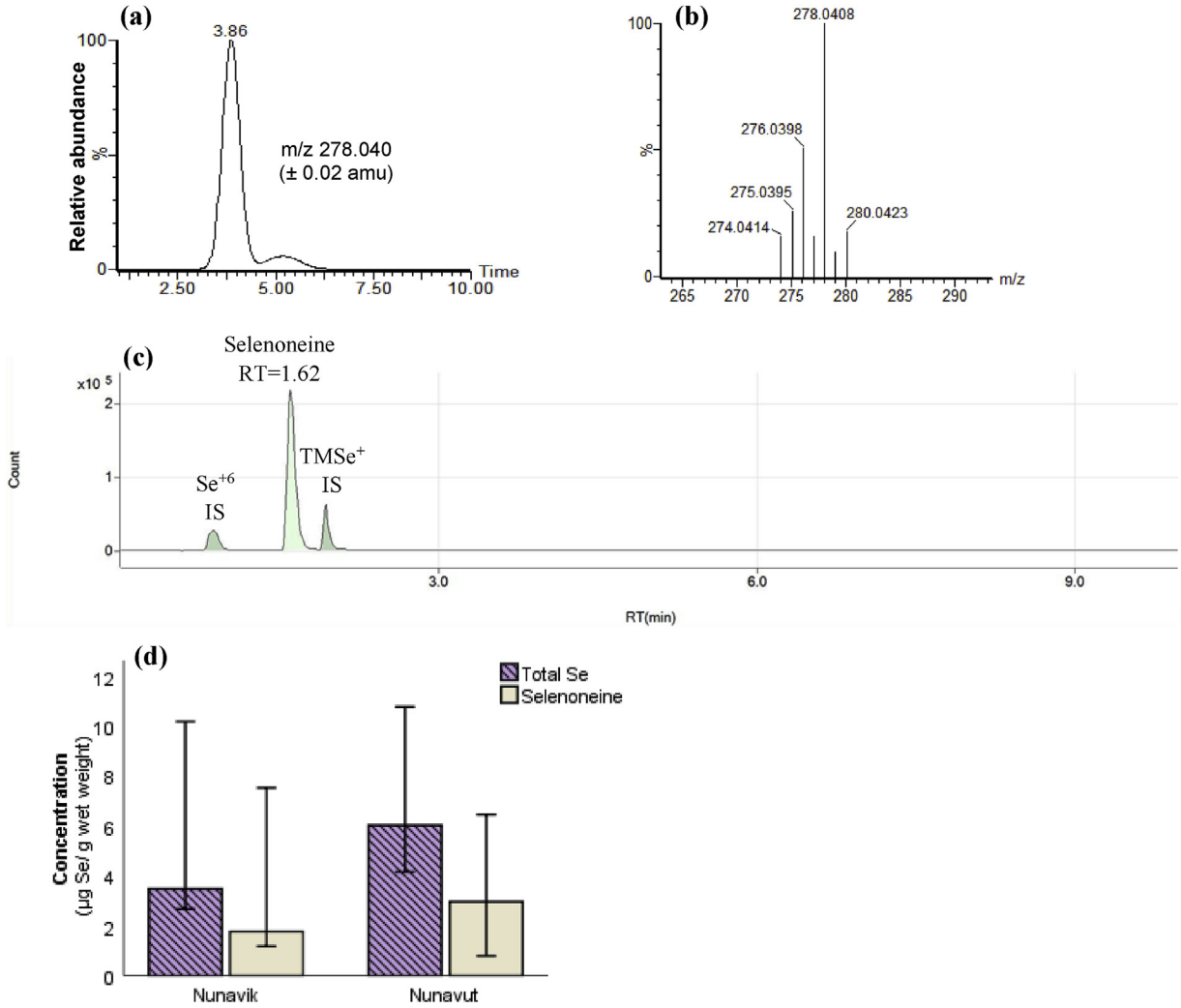

Fig. 5. Identification and quantification of selenoneine in beluga mattaaq. **(a)** Selected ion chromatogram of selenoneine at *m/z* 278.040 in an extract of beluga mattaaq (skin layer), and (**b**) the corresponding mass spectrum (UHPLC-QTOF-MS). **(c)** Ion pair chromatogram of a beluga mattaaq (skin) extract with ^{78}Se detection [HPLC-ICP-MS/MS; Se^{+6} and $TMSe^{+}$ as internal standards (IS)]. **(d)** Selenoneine and total Se concentrations (μg Se/g wet weight) in beluga mattaaq samples from Nunavik (N = 5) and Nunavut (N = 5) measured by HPLC-ICP-MS/MS. Each bar represents the median (±95% confidence interval).

methylselenoneine found in RBC of our participants.

Our results indicate that selenoneine is the major Se species in beluga mattaaq from two locations in the Canadian Arctic. The concentrations of selenoneine reported here, reaching several ppm, should be considered as lower-bound estimates of actual concentrations, because beluga skin is difficult to homogenize and selenoneine extraction likely incomplete. We recently reported that beluga mattaaq consumption was positively associated with RBC selenoneine concentration in a representative sample of the entire Nunavik population, suggesting that consumption of this highly praised country food is a major driver of selenoneine accumulation in Nunavimmiut RBC (Little et al., 2019).

The origin of selenoneine in beluga skin is not clear at the present time. Ergothioneine, the sulfur analogue of selenoneine, is primarily synthesized by fungal species and a number of bacterial species, not by plants or animals (Jones et al., 2014). One possibility is that some species consumed by belugas are rich in selenoneine that would be readily absorbed and distributed to the skin of the animal. Interviews conducted with experienced Inuit hunters from Nunavik communities indicated commonly reported prey species from the sculpin (*Cottidae*), cod (*Gadidae*), salmon (*Salmonidae*), and crustacean families (Breton-Honeyman et al., 2016). Analyses conducted in our laboratory on muscle samples from Arctic cod (*Boreogadus saida*) and Arctic sculpin (*Myoxocephalus scorpioides*), two main forage species of belugas, indicate non-detectable or very low selenoneine concentrations ($\leq$0.02 μg Se/g wet weight; unpublished data). Alternatively, the skin microbiome of belugas could be the source of selenoneine as a diverse range of bacteria and fungi can potentially synthesize ergothioneine (and selenoneine), including some marine bacteria (Jones et al., 2014).

4. Conclusions

Our study clearly indicates that selenoneine is the main Se species responsible for the elevated blood Se status of Nunavimmiut. Because this selenocompound is different from those previously associated with adverse health outcomes (inorganic selenium, selenomethionine), one should not conclude the high Se status of Nunavimmiut constitutes a health risk. To the contrary, current evidence albeit limited suggests that selenoneine may protect against MeHg-induced toxicity and oxidative stress. We are currently investigating whether or not this is the case for Nunavimmiut and other coastal populations consuming selenoneine-rich marine species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.04.191.

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