Environment International 96 (2016) 8–15

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Plasma levels of selenium-containing proteins in Inuit adults from Nunavik

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 3 August 2016
Accepted 22 August 2016
Available online xxxx

Keywords:
Arctic
Inuit
marine food chain
mercury
selenium
selenoproteins

ABSTRACT

Selenium (Se) is highly abundant in marine foods traditionally consumed by Inuit of Nunavik (Northern Quebec, Canada) and accordingly, their Se intake is among the highest in the world. However, little is known regarding the biological implications of this high Se status in this Arctic indigenous population. We used a method combining affinity chromatography and inductively coupled plasma-mass spectrometry with quantification by post-column isotope dilution to determine total Se levels and concentrations of Se-containing proteins in archived plasma samples of Inuit adults who participated to the 2004 Nunavik Inuit Health Survey (N = 852). Amounts of mercury (Hg) associated with Se-containing proteins were also quantified. Results show that glutathione peroxidase 3 (GPx3), selenoprotein P (SelP) and selenoalbumin (SeAlb) represented respectively 25%, 52% and 23% of total plasma Se concentrations. In addition, small amounts of Hg co-eluted with each Se-containing protein and up to 50% of plasma Hg was associated to SelP. Total plasma Se concentrations (median = 139 μg L^{-1}; interquartile range (IQR) = 22.7 μg L^{-1}) were markedly lower and less variable than whole blood Se concentration (median = 261 μg L^{-1}, IQR = 166 μg L^{-1}). A non linear relation was observed between whole blood Se and plasma Se levels, with plasma Se concentrations leveling off at approximately 200 μg L^{-1}, whereas 16% and 3% of individuals exhibited whole blood concentrations higher than 500 μg L^{-1} and 1000 μg L^{-1}, respectively. In contrast, a linear relationship was previously reported in communities consuming Brazil nuts which are rich Se, mainly present as selenomethionine. This suggests that a different selenocompound, possibly selenoneine, is present in the Arctic marine food chain and accumulates in the blood cellular fraction of Inuit.

1. Introduction

For thousands of years, the Inuit have relied for subsistence on country foods including fish, caribou, birds, whales, seal, seafood and berries. Although market-imported foods represent an increasing part of the modern Inuit diet, country foods are still considered essential for a healthy spirit, mind, intellect and body by the Inuit (ITK and ICC, 2012). Country foods are replete in proteins, vitamins, minerals and phytochemicals, and those of marine origin are exceptionally rich in long-chain omega-3 polyunsaturated fatty acids and selenium (Se) (Blanchet et al., 2000; Dudonné et al., 2015). Se is exceptionally abundant in certain marine foods such as beluga mattaaq (skin with underlying layer of fat), beluga and walrus meat, seal liver and fish eggs; accordingly, Se intake in this population is among the highest in the world (Lemire et al., 2015).

Se is an essential trace element for humans. It is mainly incorporated in selenoproteins in the form of selenocysteine (SeCys) which is encoded by the UGA codon in the human genome (Kryukov et al., 2003). A total of 25 selenoproteins have been identified, which contain a SeCys residue in their active site and play several biological functions, notably protection from oxidative stress (Kryukov et al., 2003; Labunskyy et al., 2014). In human plasma, three Se containing proteins, namely selenoprotein P (SelP), glutathione peroxidase 3 (GPx3) and selenoalbumin (SeAlb), have been identified so far (Plecko et al., 1999). Only SelP and GPx3 possess SeCys residues and belong to the family of selenoproteins while Se in SeAlb occurs unspecifically as selenomethionine (SeMet) by substitution of a methionine (Burk et al., 2001).

Abbreviations: AF, affinity column; CRM, certified human serum reference material; CTQ, Centre de Toxicologie du Québec; GPx3, glutathione peroxidase 3; ICP-MS, inductively coupled plasma mass spectrometry; ID, isotope dilution; INSPQ, Institut National de Santé Publique du Québec; KED, kinetic energy discrimination; MeHg, methylmercury; PTM, proficiency testing material; SeAlb, selenoalbumin; SeCys, selenocysteine; SelP, selenoprotein P; SeMet, selenomethionine; UPLC, ultra performance liquid chromatography.

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http://dx.doi.org/10.1016/j.envint.2016.08.015
0160-4120/

SelP is the most abundant plasma selenoprotein and contains more than 50% of the total Se in plasma (Labunskyy et al., 2014). Although its expression is observed in many tissues, SelP is mainly secreted by the liver and can contain up to 10 SeCys residues (Burk and Hill, 2005). It is mainly responsible for the transport of Se from the liver to peripheral tissues (Saito and Takahashi, 2002; Schomburg et al., 2003), especially to the brain and testis where specific SelP receptors were identified (Krol et al., 2012; Olson et al., 2008). In addition, SelP may also exhibit antioxidant functions, thereby protecting neuronal and astrocyte cells (Steinbrenner and Sies, 2013) and inhibiting the oxidation of low density lipoproteins (Traulsen et al., 2004). GPx3 is secreted by kidneys (Whitin et al., 2002) and plays a major role in the detoxification of extracellular hydrogen peroxide and fatty acid hydroperoxides (Papp et al., 2007). It is also expressed in other tissues including the heart, where it is the third selenoprotein in terms of mRNA abundance (Hoffmann et al., 2007). Conversely, SeAlb has no known Se dependent biological function; it likely represents a form of Se storage which can be used by the liver to synthesize SelP (Suzuki and Ogra, 2002).

In addition to contributing to the antioxidant defense system, Se may offset or counterbalance some of the deleterious effects induced by methylmercury (MeHg) (Chapman and Chan, 2000), which is bioaccumulated and biomagnified in marine mammals and predatory fish species of the Arctic (AANDC, 2012; Lemire et al., 2015). MeHg, with its capacity to cross the blood–brain and placental barriers and its gastrointestinal absorption approaching 100%, is a neurotoxic threat for Arctic residents (Hong et al., 2012). Data from several epidemiological studies conducted in Arctic populations and elsewhere in other fish-eating populations suggest that a high Se intake could protect against MeHg toxicity (Ayotte et al., 2011; Boucher et al., 2010; Fillion et al., 2013; Lemire et al., 2010, 2011; Valera et al., 2009). Khan and Wang (2009) hypothesized that Se is involved in the demethylation of MeHg and that granules of HgSe, which have been found in marine mammal and seabird liver (Ikemoto et al., 2004; Lailson-Brito et al., 2012; Nigro and Leonzio, 1996), is the final product of MeHg biotransformation (Khan and Wang, 2009). Conversely, MeHg may bound and inactivate selenoproteins (Khan and Wang, 2009).

Evidence in other parts of the world indicates that a high selenium status conveys health risks. In seleniferous areas of China (i.e. County of Enshi, Hubei Province), selenosis symptoms were observed in the population and SeMet was found as the main Se species in corn and rice grown in this high Se area (Beilstein et al., 1991; Rayman et al., 2008; Yang et al., 1983). Additionally, in other populations, an elevated Se status was associated to type 2 diabetes, hypercholesterolemia and/or hypertension, although findings still remains inconsistent (Rayman, 2012; Rayman and Stranges, 2013; Stranges et al., 2010).

While total Se concentrations in plasma or whole blood are the biomarkers most often used to evaluate associations between Se status and health effects, other biomarkers (e.g. selenoproteins and small Se molecules) may help to better characterize Se status (Xia et al., 2010). In Inuit adults from Nunavik, Northern Quebec, Valera and colleagues previously reported a mean whole blood selenium concentration of 3.70 μmol L^{-1} (292 μg L^{-1}) with values ranging from 1.5 to 45.0 μmol L^{-1} (119 to 3550 μg L^{-1}) in 732 participants to the 2004 Nunavik Inuit Health Survey (Valera et al., 2009). In order to better understand the effects of the high Se status on Inuit health, additional biomarkers other than total whole blood Se must be studied. Measuring the circulating levels of Se-containing proteins may help to better characterize the Se status and improve our capacity to identify the risks and benefits linked to the exceptionally high Se status found in Nunavik. We adapted in our laboratory the method of Li et al. (2011) combining affinity column (AF) chromatography and inductively coupled plasma mass spectrometry (ICP-MS) with post-column isotope dilution (ID-AF-ICP-MS) in order to determine plasma levels of GPx3, SelP and SeAlb in a representative sample of the Inuit population of Nunavik. We also quantified Hg associated to Se-containing proteins within the same analytical run.

2. Experimental

2.1. Study population

In the fall of 2004, the Nunavik Inuit Health Survey was conducted in the 14 communities of Nunavik, a territory located north of the 55th parallel in the province of Quebec (Canada) which is home to 10 750 Inuit according to the 2011 census (Statistique Canada, 2013). The aim of this study was to gather social and health information for this population and verify the evolution of its health status since the last survey conducted in 1992 (Jetté, 1998). 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 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 852 participants from whom plasma and whole blood samples were available. After venous blood collection in vacutainers containing EDTA and centrifugation, plasma was isolated and aliquoted in Sarstedt (Nümbrecht, Germany) 1.5-mL tubes and stored at −80 °C at the *Centre de Toxicologie du Québec* (CTQ) of the *Institut National de Santé Publique du Québec*, Canada (INSPQ). The present study was reviewed and approved by the Nunavik Nutrition and Health Committee and the Ethics Review Board of the CHU de Québec.

2.2. Reagents and chemicals

Ammonium acetate (ACS grade) was purchased from Anachemia (Montreal, QC, Canada), while methanol and ethanol (Omnisolv grade) were obtained from EMD (Omaha, NB) and Commercial Alcohols (Brampton, ON, Canada) respectively. The enriched metallic ^{77}Se (98%) was obtained from Isoflex (San Francisco, CA) and ^{204}Hg oxide (98%) from Cambridge Isotope Laboratories (Andover, MA). Milli-Q water was purified by the advantage A10 ultrapure water system (Merck Millipore, Billerica, MA).

Two Se certified human serum certified reference materials (CRM) (BCR-637 and SRM-1950) were used throughout. The SRM-1950 was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and the BCR-637 from the Institute for Reference Materials and Measurements (Geel, Belgium). Proficiency testing material (PTM) (QMEQAS 11-S-04) with a consensus value for Hg and Se was also used; they were obtained from the quality assessment scheme for trace elements operated by INSPQ.

2.3. Instrumentation

The complete chromatographic separation of the three Se-containing proteins was carried out using an Acquity ultra performance liquid chromatography system (UPLC) (Waters, Milford, MA) equipped with two 1-mL affinity columns, one containing heparin-sepharose and the other blue-sepharose, both purchased from GE Healthcare (Uppsala, Sweden). The columns were connected to a six-way Rheodyne automated switching valve (Model MXP9900-000; Oak Harbor, WA).

A NexION 300s ICP-MS (Perkin-Elmer, Waltham, MA) was coupled to the UPLC system for Se and Hg detection and quantification by post-column isotope dilution. A daily performance solution containing 1 μg L^{-1} of Be, Ce, Co, Fe, In, Li, Mg, Mn, Pb and U in 1% (v/v) nitric acid (HNO_3) was used for instrument optimization. The kinetic energy discrimination mode (KED) using H_2 gas was employed to eliminate the polyatomic interferences upon detection of both Se and Hg. For