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Biomarkers of seafood intake during pregnancy – Pollutants versus fatty acids and micronutrients

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ABSTRACT

Intake of fish and seafood during pregnancy may have certain beneficial effects on fetal development, but measurement of intake using questionnaires is unreliable. Here, we assessed several candidate biomarkers of seafood intake, including long-chain omega 3 fatty acids (n-3 LCPUFA), selenium, iodine, methylmercury, and different arsenic compounds, in 549 pregnant women (gestational week 29) in the prospective birth cohort NICE (Nutritional impact on Immunological maturation during Childhood in relation to the Environment). Proportions of the fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) in erythrocytes were measured using gas chromatography with flame ionization detector. Selenium was measured in blood plasma and erythrocytes, mercury and arsenic in erythrocytes, and iodine and several arsenic compounds in urine, using inductively coupled plasma mass spectrometry, arsenic compounds after first being separated by ion exchange high-performance liquid chromatography (HPLC). Each biomarker was related to intake of total seafood and to intake of fatty and lean fish, and shellfish in third trimester, estimated from a semi-quantitative food frequency questionnaire filled out in gestational week 34. The pregnant women reported a median total seafood intake of 184 g/week (5th-95th percentiles: 34–465 g/week). This intake correlated most strongly with erythrocyte mercury concentrations (rho = 0.49, $p < 0.001$), consisting essentially of methylmercury, followed by total arsenic in erythrocytes (rho = 0.34, $p < 0.001$), and arsenobetaine in urine (rho = 0.33, $p < 0.001$), the main form of urinary arsenic. These biomarkers correlated well with intake of both fatty fish, lean fish, and shellfish. Erythrocyte DHA and plasma selenium correlated, although weakly, mainly with fatty fish (rho = 0.25 and 0.22, respectively, both $p < 0.001$). In conclusion, elevated concentrations of erythrocyte mercury and urinary arsenobetaine can be useful indicators of seafood intake, more so than the n-3 LCPUFAs. However, the relative importance of the biomarkers may differ depending on the type and amount of seafood consumed.

1. Introduction

Seafood is rich in proteins, fatty acids, essential vitamins, and trace elements (Dale et al., 2019; EFSA, 2015). The intake of seafood has been linked to prevention of a broad spectrum of diseases among adults. In particular, fatty fish has been inversely associated with coronary heart disease as well as total mortality (EFSA, 2015; Mozaffarian and Rimm, 2006). More specifically, a regular intake of fish during pregnancy has been positively associated with birth weight and cognitive function (EFSA, 2015; Emmett et al., 2015; Hibbeln et al., 2019; Brantsæter et al., 2012), and inversely associated with development of food allergy and eczema in the child (Malmir et al., 2022; Barman et al., 2021).

The intake of seafood in epidemiological research is often measured using questionnaires, which have inherent problems to accurately reflect dietary intake (Bingham, 2002; Willett, 2013). Reliable biochemical markers of seafood intake would greatly improve the precision of intake measures and thereby the quality of such research (Hedrick et al., 2012; Jenab et al., 2009; Marckmann et al., 1995; Praticò

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et al., 2018). Obvious candidates for seafood biomarkers include long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFAs) (Solvik et al., 2021; Unger et al., 2021; Lund-Blix et al., 2016), which can be measured in blood plasma phospholipids or in erythrocyte membranes, the latter considered to represent the intake over a longer period of time (Escolano-Margarit et al., 2013). However, seafoods differ markedly in their fat content and intake of lean fish, such as cod, may not be adequately reflected by measurements of these fatty acids. Other nutrients that are found in seafood include iodine and selenium, and we have previously shown that the concentrations of these elements in urine and blood correlate with questionnaire-based data on seafood consumption during pregnancy (Stråvik et al., 2021). Also, plasma selenium concentrations were found to be elevated in Swedish fishermen, eating twice as much fish as the controls (Svensson et al., 1995).

Seafood may also contain environmental pollutants, such as methylmercury (EFSA, 2015; Scentific, 2012) and arsenic (Molin et al., 2015). Even though exposure to methylmercury occurs almost exclusively through seafood (Bjornberg et al., 2005; Castano et al., 2015), methylmercury has not been systematically evaluated as a biomarker of seafood intake (Brantsaeter et al., 2010; Ponce et al., 1998). Arsenic is quite abundant in marine fish, mainly in the form of arsenobetaine (Molin et al., 2015; Luvonga et al., 2020; Taylor et al., 2017; Francesconi and Edmonds, 1987), and elevated urinary concentrations of arsenobetaine following seafood intake have been reported (Molin et al., 2015; Navas-Acien et al., 2011; Stanstrup et al., 2014). Total arsenic concentrations in blood have previously been found to correlate with reported intake of fish among pregnant women (Brantsaeter et al., 2010), while concentrations in urine were unrelated to reported fish intake in French high-consumers of seafood (adult men and women) (Sirot et al., 2009). Inorganic arsenic, on the other hand, which is a highly toxic form of arsenic, is mainly found in drinking water, rice, and certain algae (EFSA, 2021). Less is known for other arsenic species present in seafood, such as arsenocholine and trimethylarsine oxide, particularly during pregnancy. Obviously, investigating different forms of arsenic separately may be necessary in order to evaluate their feasibility as biomarkers of seafood intake.

The aim of the present study was to assess a panel of potential biomarkers for self-reported intake of fatty and lean fish, shellfish, and total seafood in pregnant women in the Swedish NICE (Nutritional impact on Immunological maturation during Childhood in relation to the Environment) cohort. More specifically, the measured concentrations in blood or urine of DHA, DPA, EPA, selenium, iodine, methylmercury, total arsenic, arsenobetaine, arsenocholine, trimethylarsine oxide, and inorganic arsenic metabolites were related to the estimated intake of various seafood, using a semi-quantitative food frequency questionnaire.

2. Methods

2.1. Study population

The studied women participated in the birth cohort study *Nutritional impact on Immunological maturation during Childhood in relation to the Environment* (NICE). The study involved collection of a large number of biological samples, as well as questionnaires covering life-style parameters and diet of the pregnant woman as well as the child. Information regarding anthropometry and background characteristics was extracted from the birth record (Barman et al., 2018). In short, recruitment of pregnant women took place 2015–2018 in the catchment area of Sunderby Hospital in Norrbotten county, located in the most northern part of Sweden (65.3–66.4°N). The catchment area includes ten municipalities and covers the Southern part of the county (from the border to Norway in the west to the Gulf of Bothnia, i.e., the northern part of the Baltic Sea, in the east) and the Eastern part (along the coastline of the Gulf of Bothnia and the border to northern Finland).

Participants were recruited in connection with a routine sonography in gestational week 18–19 and had the first study visit for sampling of blood and urine around gestational week 29, after sending the written consent by mail. Participation criteria included signed consent, ability to communicate in spoken and written Swedish, a residential address in Norrbotten County and scheduled delivery at the Sunderby Hospital. In total, 655 pregnancies were included in the NICE cohort during the recruitment period (2015–2018). For the present study, second pregnancies during the study period (n = 18), twin-births (n = 3), and withdrawal from the study (n = 1) were excluded. Out of the remaining 633 women, 599 donated blood and/or urine in gestational week 29 (range 24–36), 554 of whom also had valid data on seafood consumption. The number of women included in each analysis varied depending on the availability of plasma, erythrocyte, and urine samples, as shown in Fig. 1.

The study was approved by the Regional Ethical Review Board, Umeå, Sweden. Women were informed that participation was voluntary and that they could renounce at any timepoint without giving a reason.

2.2. Intake of seafood and related nutrients

The dietary assessment has previously been described in detail (Stråvik et al., 2019, 2020). Concerning the seafood intake, information regarding intake of fatty fish (i.e., salmon, sushi, herring, mackerel, and tuna), lean fish (i.e., white fish such as cod, saithe, fish fingers and fish balls), and shellfish (e.g., shrimps and mussels) was collected in gestational week 34 using a web-based semi-quantitative food frequency questionnaire, Meal-Q, developed at the Karolinska Institutet and validated against a web-based seven-day weighted food diary (Christensen et al., 2013, 2014). The women were asked to report their intake frequency during the past month (ranging from 1 to 3 times/month to ≥5 times/day), and to specify their typical portion size based on fifteen example pictures, five for each of the food groups; meat, fish, or similar vegetarian option; potato, rice, pasta, or similar; and raw or cooked vegetables. In addition, information regarding consumption before pregnancy (not specified in time) was collected for freshwater Salmonids, such as brown trout, and predatory freshwater fish, such as northern pike and perch, as well as fish caught in the Baltic Sea, such as herring, salmon, or sea trout.

Based on the reported intake frequency of the whole diet (i.e., not only seafood products) and pictures of portion sizes provided in the questionnaire, or normal portions defined by the Swedish Food Agency (The Swedish Food Agency), the dietary intake of seafood was quantified, and the intake of fatty acids and micronutrients were calculated in a Java-based software (MealCalc) developed by the same research group which design the Meal-Q (Christensen et al., 2013, 2014). Dietary supplements were not included in the calculations. However, the Meal-Q questionnaire collected information regarding the use of dietary supplements ("Yes"/"Yes, sometimes"/"No"). Women who reported consumption of any supplement were asked follow-up questions regarding type of supplement (from a defined list, including e.g., multivitamins with minerals, fish oil, and selenium) and the intake frequency. Brand names were not included, and information regarding the specific content of the supplement was therefore not available. Depending on the number of follow-up questions, the Meal-Q comprised 102–174 questions and the response rate reached 94%.

2.3. Sample collection

Venous blood and urine samples were collected around gestational week 29 (5th-95th percentiles, 27–32) at the local maternity clinics that the women attended (Barman et al., 2018; Gustin et al., 2020). Blood was collected from the cubital vein in 10 mL EDTA tubes (Becton Dickinson, New Jersey, USA), used for fatty acid analysis, and in 6 mL trace element-free Na-heparin tubes (Greiner bio-one, Kremsmünster, Austria), used for trace element analysis. The blood samples were stored at 4 °C until transported cold (i.e., with ice packs in an insulated box), on

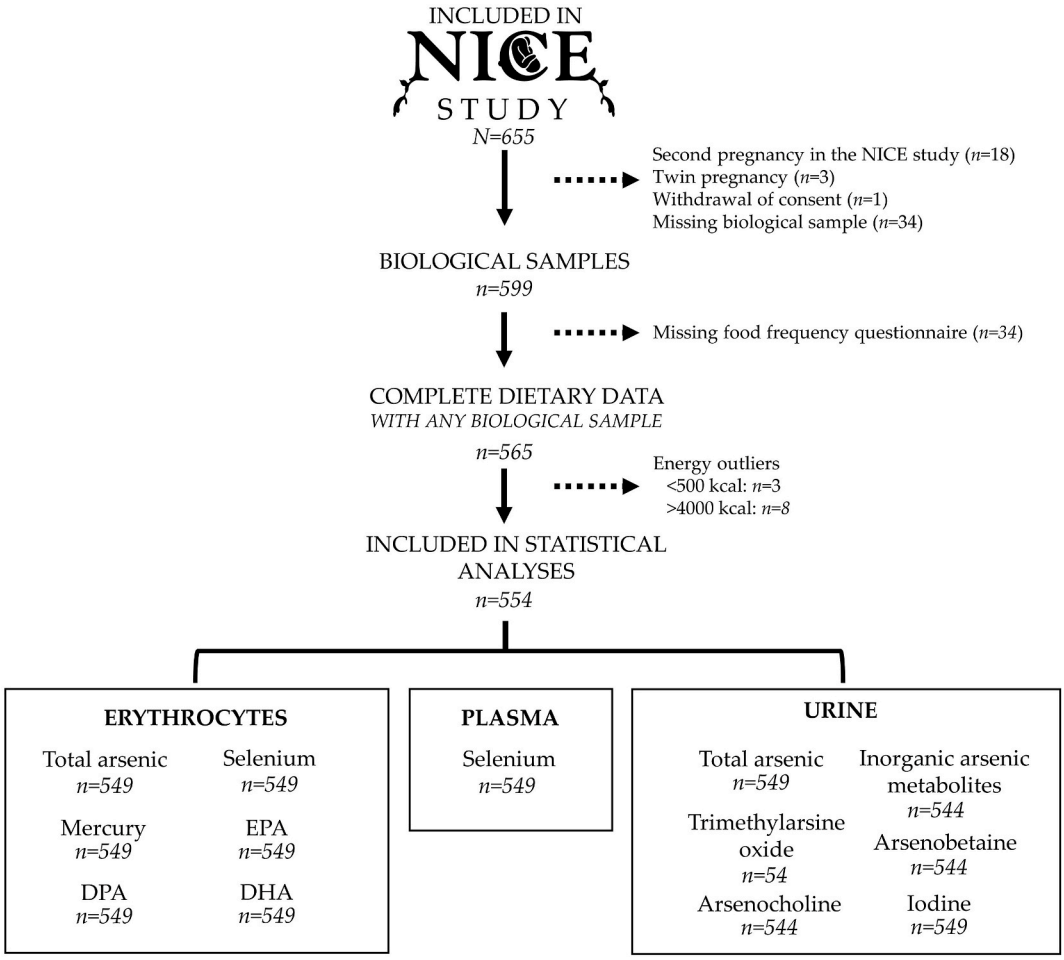

Fig. 1. Flow chart of inclusion of pregnancies and number of available biological samples. Abbreviations: EPA, eicosapentaenoic acid. DPA, docosapentaenoic acid. DHA, docosahexaenoic acid.

the same or following workday, to the hospital research laboratory, where they were centrifuged. Plasma was aliquoted to polypropylene tubes (Micronic, Nordic Biolabs AB, Sweden) and the erythrocyte fraction was left in the blood collection tubes. Spot urine samples (mid-stream) were collected in polypropylene urine collection cups (Sarstedt Inc, Newton, NC, USA) and transferred into trace element-free 24 mL polyethylene bottles. The urine samples were stored at 4 °C until transported, on the same or following workday, to the hospital research laboratory. All samples were stored temporarily at −20 °C until moved to −80 °C for long-term storage.

2.4. Analysis of fatty acids

The fatty acid composition of the erythrocytes was analyzed in our laboratory at Chalmers University of Technology, Gothenburg, and the method has previously been described in detail (Stråvik et al., 2020, 2021; Masood et al., 2005). In short, erythrocytes were mixed with internal standard (methyl tricosanoate, C23:0) and acetyl chloride-methanol solution 10% (v/v) fortified with butylated hydroxytoluene, and incubated for 1 h at 70 °C, for methylation of the fatty acids. Thereafter, the methyl esters were extracted with hexane and injected into the gas chromatography with flame ionization detector (GC-FID) system (Thermo Scientific, Waltham, MA, USA) for analysis. As quality control (QC) sample a mix of erythrocytes from five donors was used. The QC sample was analyzed after every tenth sample to check the performance of the GC-FID system. Fatty acids were analyzed in 33 different batches with the intra- and inter batch coefficient of variation (CV) of 6.2% and 16% for EPA, 6.0% and 12% for DPA and 7.5% and 11% for DHA. The external standard GLC-462 mixed fatty acid methyl esters (Nu-Chek Prep, Elysian, MN, USA) was used to create an external standard calibration curve (50, 35, 20, 10, 5 and 2.5 μg/mL). The internal standard C23:0 methyl ester was added to all erythrocyte samples (both study samples and QC samples) and to the external standard for derivation of the calibration curve. Concentrations of fatty acids in erythrocyte samples were quantified against the calibration curve. To calculate the proportion of a specific fatty acid, the peak area of that specific fatty acid was divided with the total peak areas of all fatty acids.

2.5. Analysis of trace elements

Plasma samples were analyzed for selenium, reflecting recent selenium intake. Erythrocytes samples were analyzed for selenium, total arsenic, and mercury, all of which are biomarkers of more long-term status (a few months). To note, erythrocyte mercury mainly consists of methylmercury (Berglund et al., 2005). The concentrations were measured in our laboratory at Karolinska Institutet, Stockholm, using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x and 7900, Agilent Technologies, Tokyo, Japan), with octopole reaction system in hydrogen (selenium), helium (arsenic in erythrocytes), or no gas (mercury in erythrocytes) mode. Prior to the analysis, the plasma and erythrocyte samples were diluted 1:20–1:25 in alkali solution [2% (w/v) 1-butanol, 0.05% (w/v) EDTA, 0.05% (w/v) Triton X-100, 1% (w/v) NH_4OH and 20 μg/L internal standard] and vortex mixed, sonicated for 5 min, and centrifuged 2 min at 1000 rpm (MSE centrifuge,

Super Minor, MSE (UK) Ltd, London, England) (Lu et al., 2015). Limit of detection (LOD; calculated as 3 times the standard deviation of the blank concentrations) was 0.36 μg/L for selenium in plasma, and 0.57 μg/kg, 0.01 μg/kg and 0.01 μg/kg for selenium, total arsenic, and mercury in erythrocytes. In total, two erythrocyte samples had a mercury concentration below the LOD for mercury and were replaced with LOD/$\sqrt{2}$.

Urine samples were analyzed for iodine, total arsenic, and different arsenic compounds. The procedure of analyzing iodine concentrations in urine (UIC), a short-term biomarker, with ICP-MS has previously been described in detail (Stråvik et al., 2021). Total urinary arsenic was analyzed using ICP-MS (Agilent Technologies, Tokyo, Japan) in helium mode and prior to the analysis the urine samples were diluted 1:10 in 1% nitric acid (67–69% w/w, NORMATOM®, VWR, Butterworth, UK). The LOD for iodine was 1.8 μg/L and for total arsenic 0.35 μg/L, and no urine sample had a concentration below these limits. In total, 12 different reference materials were analyzed together with the samples to assure accuracy of the measurements (Supplementary Table 1). Standard curves were prepared by dilution of standards of disodium methyl arsonate (MMA, Sigma-Aldrich), dimethylarsinic acid (DMA, NIST), sodium hydrogen arsenate heptahydrate [As(V), Sigma-Aldrich, arsenobetaine (LGC Standards, UK), TMAO (Toronto Research Chemicals, Canada) and arsenocholine (SRM 3034, NIST). Inorganic arsenic metabolites in urine, i.e., MMA, DMA, and remaining inorganic arsenic, were separated on an anion exchange chromatography column (Hamilton PRP-X110, Thermo Fisher Scientific), and analyzed by HPLC coupled to ICP-MS in helium mode for arsenic detection (Francesconi et al., 2002; Reid et al., 2020). The urine samples were filtered through 0.2 μm PES filters (Sarstedt, Germany) and then incubated with hydrogen peroxide for oxidation of arsenic (III) to arsenic (V) as described elsewhere (Scheer et al., 2012). Thus, measured arsenic (V) represented the sum of arsenic (III) and arsenic (V) in urine. The LOD values were 0.026 μg/L (MMA), 0.015 μg/L (DMA), and 0.028 μg/L [As (V)]. The sum of MMA, DMA, and arsenic (V) was used as a marker of exposure to inorganic arsenic.

Urinary arsenobetaine, trimethylarsine oxide, and arsenocholine were separated on a cation exchange chromatography column (Nucleosil SCX, Skandinaviska Genentch AB, Sweden) by HPLC and ICP-MS (7700x Agilent), as described previously (Reid et al., 2020; Xie et al., 2006; Torbøl Pedersen et al., 2020). The average LOD of 10 analyses were 0.27 μg/L for arsenobetaine and 0.28 μg/L for arsenocholine. Because trimethylarsine oxide was detected in very few samples, it was measured in the first run only with an LOD of 0.20 μg/L. Urine SRM 2669 (NIST) was used as a reference material to ensure accuracy of the measurements of arsenic metabolites and organic arsenic compounds. Reference material recoveries for all arsenic species are presented in Supplementary Table 1b. Detailed information of the used HPLC-ICP-MS methods for separation of the urinary arsenic compounds is presented in Supplementary Table 2a and Supplementary Table 2b.

2.6. Statistical analyses

Data were analyzed with IBM SPSS version 28 (IBM, New York, NY, USA) and R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) software packages. P-values below 0.05 were considered significant for all tests. Intake and biomarker distributions were investigated with the Shapiro-Wilk test of normality and cross-checked with histograms. Associations between the different biomarkers and between biomarkers and the estimated seafood intake were investigated with Spearman correlations and visualized in a correlation matrix and a heatmap. For the heatmap, unsupervised hierarchical cluster analysis was also conducted, which structures the variables and places correlated biomarkers next to each other. Further, $\log_2$-transformed biomarker concentrations were plotted against reported seafood intake using scatter plots with Lowess smoothing lines (Supplementary Figures 1-4).

3. Results

3.1. Seafood intake and its relation to background variables

The median age was 30 years and half of the women were expecting their first child (Table 1). Only 6% of the women smoked before pregnancy and almost all refrained from smoking during pregnancy, in accordance with recommendations presented to the women at their first visit to the antenatal clinics. Table 1 also compares background data for women with a total seafood intake below and above the group median value. Women with a higher seafood intake had a higher education level (75% had university degree, compared to 67% in those with lower fish intake, $p = 0.045$ in Linear-by-Linear Association) and fewer reported pre-pregnancy smoking (4% versus 8%, $p = 0.04$). No other differences could be found regarding background characteristics.

The median intake of total seafood in late pregnancy was 26 g/day (5th-95th percentiles: 4.8–66 g/day) and the median intake of fatty fish, lean fish, and shellfish was 9.1 (5th-95th percentiles: 0–36 g/day), 6.6 (5th-95th percentiles: 0–27 g/day) and 4.1 (5th-95th percentiles: 0–20 g/day) g/day, respectively. A majority of the women (55%) reported an intake of any type of seafood 1–2 times per week; 44% reported eating fatty fish 1–3 times per month, while 48% had lean fish and 47% shellfish 1–3 times per month. Only 22 (4%) women did not consume any seafood during late pregnancy, and the most common type to avoid was shellfish (46% without any consumption), followed by lean fish (17%) and then fatty fish (15%).

Freshwater Salmonids (e.g., brown trout) was consumed at least once per month by 121 (23%) women before pregnancy, which decreased to 60 (11%) during pregnancy. Freshwater predatory fish (e.g., northern pike and perch) was consumed by 54 (10%) before pregnancy and 14 (3%) during pregnancy. Fish from the brackish water of the Baltic Sea (e.g., Baltic herring) was consumed by 111 (21%) before pregnancy and 63 (12%) during pregnancy.

3.2. Seafood intake and biomarker concentrations

The concentrations of all potential dietary biomarkers are presented in Table 2 for all women, and women with relatively low and high total seafood intake (defined by median split, i.e., 26.29 g/day). As can be seen, DHA was the most abundant n-3 LCPUFA in erythrocytes during pregnancy, followed by DPA and lastly EPA. The range of the n-3 LCPUFA proportions in erythrocytes were narrow (5th-95th percentiles for EPA: 0.13–1.1%, DPA: 0.43–2.8%, and DHA: 1.4–6.8%), and did not vary remarkably with an increased seafood intake (low seafood intake: EPA: 0.12–1.1%, DPA: 0.42–2.8%, and DHA: 1.4–6.8%; high seafood intake: EPA: 0.13–1.2%, DPA: 0.43–2.8%, and DHA: 1.5–7.0%).

Women with a relatively high (above the median) intake of total seafood had significantly higher levels of all biomarkers, except for proportions of DPA in erythrocytes and urinary concentrations of iodine (Table 2). The most pronounced differences were seen for urinary concentrations of trimethylarsine oxide (although detected in few women) and arsenobetaine, which were 3.5 and 2.5 times higher, respectively, in urine of women with a higher intake of seafood. These women also had 2.2 times higher total urinary arsenic, the most common form of which was arsenobetaine, and 1.7 times higher erythrocyte mercury concentrations than the women with lower seafood intake. The least pronounced differences, but still significantly higher with a higher seafood intake, was seen for selenium in plasma and erythrocytes (8% and 3% higher, respectively). The women with no seafood consumption (i.e., 0 g/day, N = 22) had very low concentrations of erythrocyte mercury [median (5th-95th percentiles): 0.21 (0.03–2.23) μg/kg], erythrocyte arsenic [0.23 (0.09–2.45) μg/kg], and urinary arsenobetaine [0.36 (0.00–131) μg/L], corresponding to around 14%, 11% and 4%, respectively, of the levels presented for the whole cohort. In contrast, the proportions of the n-3 LCPUFAs in the erythrocytes of those not consuming any seafood reached 65% (EPA), 90% (DPA) and 74% (DHA)

Table 1
Background characteristics of the studied women divided into two groups by the median total seafood intake (26 g/day).

Maternal characteristics	All		Low seafood intake		High seafood intake		
	n	Median (5th-95th percentiles) *or* n (%)	n	Median (5th-95th percentiles)	n	Median (5th-95th percentiles)	p
Age (y)	554	30 (23–39)	300	30 (23–40)	254	30 (24–38)	0.593
Weight (kg; early pregnancy)	539	68 (53–100)	292	68 (53–102)	247	68 (54–97)	0.527
Height (cm)	554	167 (158–178)	300	167 (157–178)	254	167 (158–178)	0.720
BMI (kg/m^2; early pregnancy)	527	24.4 (19.5–34.9)	286	24.5 (19.3–36.7)	241	24.4 (19.7–34.3)	0.720
Parity	549	1 (0–2)	296	0 (0–2)	253	1 (0–1)	0.697
Nulliparous (yes)	549	273 (50)	296	150 (51)	253	123 (49)	0.669[f]
Education	547		296		251		0.045[t]
Elementary school, 9 year		12 (2)		8 (3)		4 (2)	
High school, 12 year		148 (27)		89 (30)		59 (24)	
University or other, >12 year		387 (71)		199 (67)		188 (75)	
Pre-pregnancy smoking (yes)	547	32 (6)	295	23 (8)	252	9 (4)	0.044[f]
Gestational week at sample collection	520	29 (27–32)	285	29 (27–32)	235	29 (27–33)	0.180
Seafood intake (g/day)							
Total seafood	554	26 (4.8–66)	300	17 (0.0–26)	254	40 (27–83)	<0.001
Fatty fish	554	9.1 (0.0–36)	300	6.6 (0.0–20)	254	20 (6.6–53)	<0.001
Lean fish	554	6.6 (0.0–27)	300	6.6 (0.0–20)	254	20 (3.1–30)	<0.001
Shellfish	554	4.1 (0.0–20)	300	0.0 (0.0–6.6)	254	6.6 (0.0–27)	<0.001
Supplement use (weekly)	554		300		254		
Fish oil (yes)		67 (12)		34 (11)		33 (13)	0.602[f]
Multivitamins with minerals (yes)		225 (41)		125 (42)		100 (39)	0.603[f]

Abbreviations: BMI, body mass index.
Differences in maternal characteristics between women with low seafood intake (≤26.29 g/day) and high seafood intake (>26.29 g/day) were tested with Mann-Whitney *U* test for continuous variables and with Fisher's Exact Test[(f)] or Linear-by-Linear association[(t)] for categorical variables.

Table 2
Biomarker concentrations in gestational week 29 for all and grouped by total seafood intake.

Biomarker	All		Low seafood intake		High seafood intake			
	n	**Median (5th-95th percentiles)**	**n**	**Median (5th-95th percentiles)**	**n**	**Median (5th-95th percentiles)**	**Ratio**	**p**
EPA								
Erythrocytes (% of total fatty acids) (μg/L)	549	0.51 (0.13–1.09)	296	0.49 (0.12–1.06)	253	0.54 (0.13–1.16)	1.10	0.004
DPA								
Erythrocytes (% of total fatty acids) (μg/L)	549	2.03 (0.43–2.77)	296	2.02 (0.42–2.78)	253	2.04 (0.43–2.77)	1.01	0.734
DHA								
Erythrocytes (% of total fatty acids) (μg/L)	549	5.12 (1.40–6.84)	296	4.95 (1.36–6.67)	253	5.49 (1.46–7.01)	1.11	<0.001
Selenium								
Plasma[a] (μg/L)	549	64.9 (44.9–91.3)	297	63.1 (42.6–90.0)	252	68.2 (48.0–92.9)	1.08	<0.001
Erythrocytes (μg/kg)	549	106 (78.4–145)	297	105 (78.1–140)	252	108 (79.8–146)	1.03	0.009
Iodine								
Urine[b] (μg/L)	549	114 (53.6–313)	296	112 (53.7–285)	253	116 (53.3–338)	1.04	0.378
Total arsenic								
Erythrocytes (μg/kg)	549	2.15 (0.31–11.5)	297	1.68 (0.21–9.76)	252	2.66 (0.61–13.5)	1.58	<0.001
Urine[b] (μg/L)	549	15.6 (2.59–201)	296	11.2 (2.25–158)	253	24.4 (3.63–272)	2.18	<0.001
Inorganic arsenic metabolites[c]								
Urine[b] (μg/L)	544	3.26 (1.19–13.3)	294	2.88 (1.14–10.9)	250	3.68 (1.26–14.9)	1.28	<0.001
Arsenobetaine								
Urine[b] (μg/L)	544	9.53 (0.35–168)	294	6.10 (0.20–123)	250	15.3 (0.72–265)	2.51	<0.001
Arsenocholine								
Urine[b] (μg/L)	544	0.11 (0.00–0.56)	294	0.09 (0.00–0.56)	250	0.13 (0.00–0.58)	1.44	0.020
Trimethylarsine oxide								
Urine[b] (μg/L)	54	0.08 (0.00–0.45)	30	0.04 (0.00–9.89)	24	0.14 (0.00–0.46)	3.50	0.004
Mercury								
Erythrocytes (μg/kg)	549	1.49 (0.28–4.27)	297	1.19 (0.15–3.36)	252	1.97 (0.80–4.77)	1.66	<0.001

Abbreviations: EPA, eicosapentaenoic acid. DPA, docosapentaenoic acid. DHA, docosahexaenoic acid.
Differences in biomarker concentrations between women with low seafood intake (≤26.29 g/day) and high seafood intake (>26.29 g/day) were tested with Mann-Whitney *U* test.
[a] Concentrations converted from measured μg/kg to μg/L by assuming a plasma density of 1.026 kg/L.
[b] Concentrations adjusted to the mean specific gravity of 1.017.
[c] Sum metabolites of inorganic arsenic (iAs + MMA + DMA).

of the levels reported for the whole cohort.

Correlations between reported consumption of different seafood (fatty fish, lean fish, shellfish, and total seafood) with the measured concentrations of the biomarkers are presented in a heatmap in Fig. 2 and in Supplementary Table 3. As can be seen, the reported intake of total seafood was most strongly correlated with erythrocyte

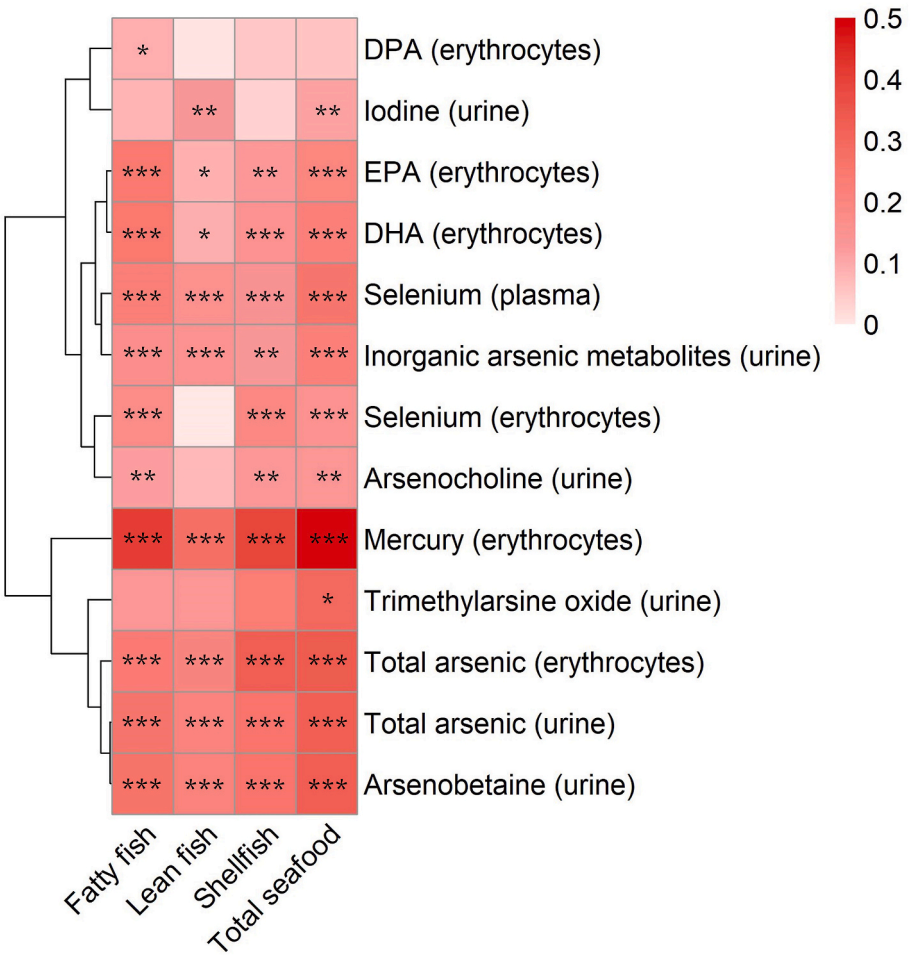

Fig. 2. Heatmap displaying Spearman correlations between reported seafood intake and potential biomarkers. Biomarkers were measured in gestational week 29 and food intake at gestational week 34 and intended to reflect maternal intake during the previous month. Significant correlations are denoted with asterisks as follows: $p < 0.001$ = ***, $p < 0.01$ = ** and $p < 0.05$ = *. Abbreviations: EPA, eicosapentaenoic acid. DPA, docosapentaenoic acid. DHA, docosahexaenoic acid.

concentrations of mercury (rho = 0.49, $p < 0.001$), followed by erythrocyte concentrations of arsenic (rho = 0.34), urinary arsenobetaine (rho = 0.33) and total urinary arsenic (rho = 0.32, all $p < 0.001$). For all these biomarkers, the correlations were similar regardless of type of seafood. Among the micronutrients and n-3 LCPUFAs, plasma selenium (rho = 0.26) and erythrocyte DHA (rho = 0.22) showed the strongest correlations with total seafood intake, although weaker correlations than those of mercury and arsenic. As expected, EPA and DHA correlated more strongly with fatty fish than with lean fish and shellfish. Also, plasma selenium showed stronger correlation with fatty fish than with lean fish and shellfish. Further, the concentrations of arsenic compounds and mercury were clustered together in the heatmap, indicating more similarities amongst them than with the nutrients (selenium, iodine, and the n-3 LCPUFAs), which, in turn, clustered together. Urinary iodine concentrations correlated weakly with lean fish intake (rho = 0.13), but not to the intake of fatty fish or shellfish.

3.3. Relationship between the different biomarkers

Correlations between the studied biomarkers are shown in Fig. 3 and Supplementary Table 4. As can be seen, erythrocyte mercury correlated strongly with erythrocyte arsenic (rho = 0.55), as well as with urinary arsenobetaine (rho = 0.48) and total urinary arsenic (rho = 0.47). The most abundant arsenic compound in urine, arsenobetaine, was highly correlated with the total arsenic concentrations in urine (rho = 0.96) and in erythrocytes (rho = 0.73). The sum of inorganic arsenic metabolites correlated fairly strongly with urinary arsenobetaine (rho = 0.47) and total arsenic (rho = 56), but not with the other arsenic measures.

Also, the n-3 LCPUFAs were highly correlated with each other (rho = 0.63–0.75). The correlations of EPA, DHA and DPA with selenium in plasma were weaker (rho = 0.29, rho = 0.24, and rho = 0.14, respectively), and with selenium in erythrocytes even weaker (rho = 0.13, rho = 0.11, and rho = 0.01, respectively, the latter not statistically significant). Similarly, the correlations of the n-3 LCPUFAs with the arsenic compounds were fairly weak (rho = 0.18–0.28), as were those with methylmercury (rho = 0.11–0.34).

3.4. Biomarkers and dietary supplements

To evaluate the potential impact of the use of supplements on the biomarker concentrations, all dietary biomarkers were listed separately for women regularly using supplements of fish oil or multivitamins with minerals (weekly use) and for those using such supplements more seldom or never (Table 3). Regarding the use of fish oil supplements, the only significant differences were seen for the fatty acids EPA, DPA and DHA which, as expected, were all higher in the supplement group (62%, 12% and 12% higher, respectively). The seafood intake did not differ significantly between these supplement groups.

When comparing levels of the biomarkers between the women taking multivitamins with minerals or not, the most pronounced differences were seen for urinary concentrations of arsenobetaine and total arsenic,

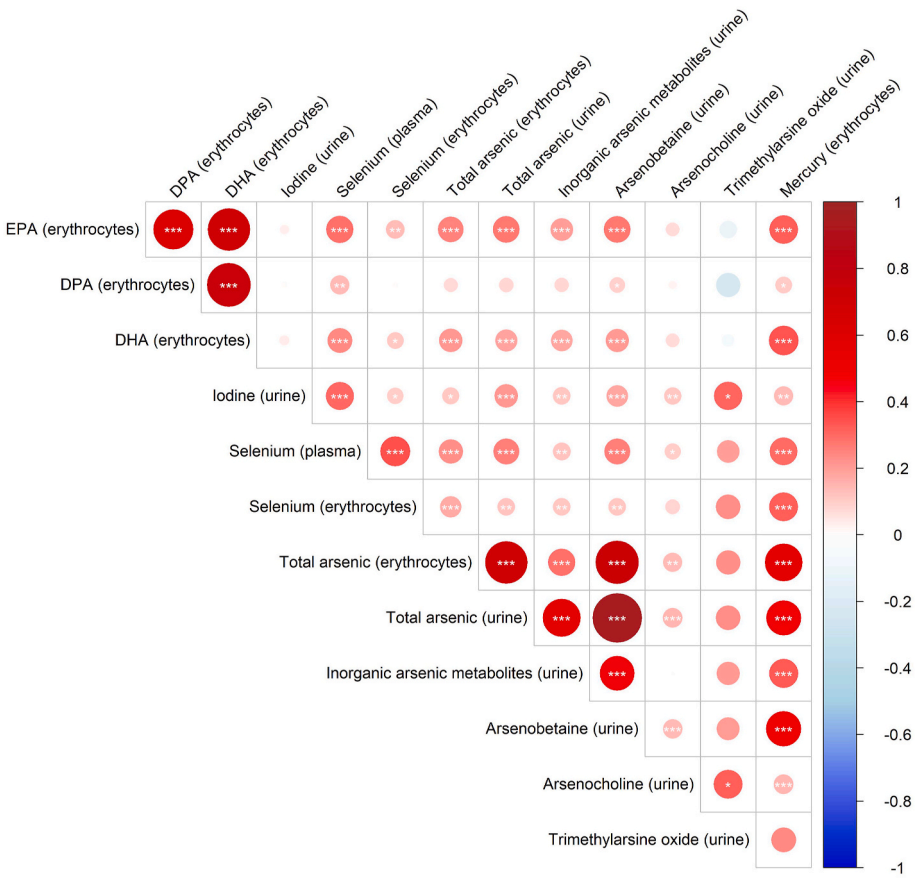

Fig. 3. Correlation matrix of potential biomarkers of seafood intake. Significant correlations are denoted with asterisks as follows: $p < 0.001$ = ***, $p < 0.01$ = ** and $p < 0.05$ = *. Abbreviations: EPA, eicosapentaenoic acid. DPA, docosapentaenoic acid. DHA, docosahexaenoic acid.

where women with a regular use of multivitamin supplements with minerals had 108% and 65%, respectively, higher concentrations. More expectedly, urinary concentrations of iodine were 54% higher in women with a regular use of multivitamins with minerals, and selenium concentrations in plasma and erythrocytes were also slightly higher among supplement users (13% and 5%, respectively). Further, no significant differences in seafood intake were found between the two groups, neither in total seafood intake nor intake of the different types of seafood.

4. Discussion

The present study aimed to evaluate a wide range of potential biomarkers for seafood intake in pregnant women in the north of Sweden. We found that erythrocyte mercury, mainly reflecting the exposure to methylmercury during the previous months, showed the strongest correlation with intake of all types of seafood, estimated using a semi-quantitative food frequency questionnaire in late pregnancy. Another biomarker that correlated strongly with seafood intake was urinary arsenobetaine, the main form of arsenic in urine of the studied women. Unexpectedly, both these exposures correlated much more strongly with fish intake than did the studied n-3 LCPUFAs, which, like methylmercury and arsenobetaine, are known to be found almost exclusively in fish and other seafood.

The median seafood intake found here, 182 g per week, represents almost 1.5 servings (one fillet commonly commercially sold in 125 g pieces), which is slightly lower than the estimated intake of around 210 g per week in the general population of Sweden (Ziegler and Bergman, 2017; Jordbruksverket, 2021). The Swedish Food Agency recommends pregnant women to eat fish 2–3 times a week, but to limit the intake of certain species (e.g., tuna, swordfish, and Baltic herring) to a maximum of 2–3 times a year, due to the frequent presence of environmental pollutants in such fish (The Swedish Food Agency, 2008). It is plausible that women decrease their fish intake during pregnancy due to the warnings regarding pollutants in the fish. Indeed, we did find evidence that the women decreased their consumption of fish caught in lakes or the Baltic Sea when they became pregnant. Farmed Atlantic salmon is increasingly consumed in Sweden and other countries (FAO, 2020) and has replaced herring as the most common fatty fish eaten in Sweden. It is now the most consumed fish regardless of classification (Borthwick et al., 2019). Among the lean fish, cod, saithe, and haddock represent the largest commercial value and can be assumed to be the most frequently consumed (Swedish Agency for Marine and, 2022).

The observed correlation of erythrocyte mercury with seafood intake is logical as human exposure to dietary methylmercury derives almost solely from fish, with some minor contribution from consumption of poultry and swine fed with fishmeal (Lindberg et al., 2004). In support of the latter, low concentrations of mercury were detected in erythrocytes (median 0.21 μg/kg, corresponding to about 0.08 μg/L in whole blood (Gustin et al., 2020)) of the women who reported that they did not consume any seafood (4% reporting intake of seafood more rarely than once a month). There is a biomagnification of methylmercury through the food chain of aquatic organisms, which is why predatory fish e.g., pike, perch, and pikeperch, often have the most elevated concentrations, which increase with age and size. Fresh tuna, shark, and swordfish, known to contain particularly high concentrations of methylmercury (EFSA, 2015), are rarely consumed in Sweden. Methylmercury concentrations in Swedish freshwater fish have declined over several decades due to markedly reduced mercury emissions (Åkerblom et al., 2014), and there are generally lower concentrations in the commonly

Table 3
Biomarker concentrations in gestational week 29 grouped by supplement use.

	Supplements		No supplements		Ratio	p
	n	Median (5th-95th percentiles)	n	Median (5th-95th percentiles)		
Supplement: Fish oil						
EPA						
Erythrocytes (% of total fatty acids) (μg/L)	66	0.81 (0.10–1.66)	483	0.50 (0.13–0.91)	1.62	<0.001
DPA						
Erythrocytes (% of total fatty acids) (μg/L)	66	2.26 (0.25–3.21)	483	2.02 (0.46–2.69)	1.12	0.008
DHA						
Erythrocytes (% of total fatty acids) (μg/L)	66	5.65 (1.27–7.52)	483	5.03 (1.41–6.69)	1.12	0.001
Selenium						
Plasma[a] (μg/L)	66	66.3 (42.5–96.3)	483	64.8 (45.4–90.8)	1.02	0.391
Erythrocytes (μg/kg)	65	106 (80.0–169)	484	106 (78.2–143)	1.00	0.354
Iodine						
Urine[b] (μg/L)	64	125 (55.2–332)	485	113 (52.8–308)	1.11	0.426
Total arsenic						
Erythrocytes (μg/kg)	65	2.14 (0.19–11.7)	484	2.15 (0.32–11.5)	1.00	0.712
Urine[b] (μg/L)	64	21.8 (2.08–267)	485	15.6 (2.65–198)	1.40	0.422
Inorganic arsenic metabolites[c]						
Urine[b] (μg/L)	63	3.79 (0.90–13.8)	481	3.19 (1.19–13.3)	1.19	0.290
Arsenobetaine						
Urine[b] (μg/L)	63	13.0 (0.31–210)	481	9.14 (0.37–169)	1.42	0.379
Arsenocholine						
Urine[b] (μg/L)	63	0.07 (0.00–0.41)	481	0.11 (0.00–0.58)	0.64	0.926
Trimethylarsine oxide						
Urine[b] (μg/L)	6	0.18 (0.09–0.24)[d]	48	0.06 (0.01–0.17)[e]	3.00	0.122
Mercury						
Erythrocytes (μg/kg)	65	1.73 (0.21–4.62)	484	1.47 (0.29–4.20)	1.18	0.511
Supplement: Multivitamins with minerals						
EPA						
Erythrocytes (% of total fatty acids) (μg/L)	222	0.52 (0.13–1.26)	327	0.50 (0.13–1.00)	1.04	0.284
DPA						
Erythrocytes (% of total fatty acids) (μg/L)	222	2.09 (0.42–2.77)	327	2.00 (0.43–2.78)	1.05	0.325
DHA						
Erythrocytes (% of total fatty acids) (μg/L)	222	5.27 (1.43–7.18)	327	5.01 (1.40–6.69)	1.05	0.098
Selenium						
Plasma[a] (μg/L)	223	69.6 (50.1–96.4)	326	61.8 (42.5–89.6)	1.13	<0.001
Erythrocytes (μg/kg)	223	109 (80.5–147)	326	104 (76.3–145)	1.05	0.002
Iodine						
Urine[b] (μg/L)	220	152 (60.9–346)	329	98.8 (49.3–201)	1.54	<0.001
Total arsenic						
Erythrocytes (μg/kg)	223	2.38 (0.30–12.2)	326	1.96 (0.31–11.0)	1.21	0.182
Urine[b] (μg/L)	220	22.6 (2.58–297)	329	13.7 (2.60–153)	1.65	0.003
Inorganic arsenic metabolites[c]						
Urine[b] (μg/L)	218	3.35 (1.26–14.8)	326	3.13 (1.03–12.3)	1.07	0.355
Arsenobetaine						
Urine[b] (μg/L)	218	15.4 (0.23–291)	326	7.41 (0.40–127)	2.08	0.008
Arsenocholine						
Urine[b] (μg/L)	218	0.11 (0.00–0.63)	326	0.11 (0.00–0.54)	1.00	0.484
Trimethylarsine oxide						
Urine[b] (μg/L)	27	0.10 (0.00–0.38)	27	0.06 (0.00–0.46)	1.67	0.835
Mercury						
Erythrocytes (μg/kg)	223	1.58 (0.22–4.32)	326	1.47 (0.30–4.24)	1.07	0.553

Abbreviations: EPA, eicosapentaenoic acid. DPA, docosapentaenoic acid. DHA, docosahexaenoic acid.
Differences between supplement group and non-supplement group were tested with Mann-Whitney *U* test. Supplement use was defined as regular consumption (every day or several days a week) of fish oil or multivitamins with minerals, women with a less regular intake of these supplements were categorized as not taking any supplements.

[a] Concentrations converted from measured μg/kg to μg/L by assuming a plasma density of 1.026 kg/L.
[b] Adjusted to the mothers mean urinary specific gravity ($SG_{mean} = 1.017$).
[c] Sum metabolites of inorganic arsenic (iAs + MMA + DMA).
[d] Presented with 25th-75th percentiles due to few cases. The 5th-95th percentiles are: 0.03-NA.
[e] Presented with 25th-75th percentiles for better comparison. The 5th-95th percentiles are 0.00–0.45.

consumed farmed Atlantic salmon than in the wild Atlantic salmon, because of the increasing use of plant-based feed (Lundebye et al., 2017; Jensen et al., 2020). A recent study found that only 3% of Swedish first-time mothers exceeded a hair mercury concentration of 1 mg/kg (also reflecting specifically methylmercury, samples collected three weeks postpartum), corresponding to the reference dose set by the US Environmental Protection Agency (0.1 μg MeHg/kg body weight per day) (Kippler et al., 2021). The median hair concentration in that study was 0.25 mg/kg, corresponding roughly to about 2.5 μg/kg in erythrocytes (assuming a blood to hair ratio of 250 (Scentific, 2012), and applying the whole blood to erythrocyte conversion formula previously reported (Gustin et al., 2020)). The presently studied NICE women had lower erythrocyte mercury concentrations (1.5 μg/kg), possibly partly related to pregnancy with an increase in red blood cell mass, driven by increased erythropoietin production (Chandra et al., 2012). Despite the fairly low exposure levels, erythrocyte mercury was the biomarker with the strongest correlation to the reported intake of all types of seafood.

The concentrations of total arsenic in both urine and erythrocytes

showed as strong correlations with reported total seafood intake (rho = 0.32 and rho = 0.34, respectively) as did urinary arsenobetaine (rho = 0.33), since arsenobetaine was the major form of ingested arsenic (rho = 0.96 for urinary arsenobetaine and total arsenic). Total arsenic in urine or erythrocytes as biomarkers of seafood intake does not have the same generalizability as urinary arsenobetaine, since they are influenced by exposure to multiple arsenic compounds through a variety of food sources, including drinking water. Only if such exposure is known, would total arsenic be a seafood biomarker of choice. An advantage is that the measurement is more straightforward than that of arsenobetaine. Erythrocyte arsenic is also a more long-term exposure marker than urinary total arsenic and arsenobetaine. Seafood is the main source of arsenobetaine, the N-analogue of glycine betaine (N,N,N-trimethyl glycine) (Navas-Acien et al., 2011; Edmonds et al., 1977; Hackethal et al., 2022), which serves as an osmolyte in marine organisms (Hoffmann et al., 2018; Yancey, 2001). The concentrations of arsenobetaine in freshwater fish are generally low, as such fish do not require osmolytes to the same extent. There was quite a range of arsenobetaine concentrations in urine (<0.27–1004 μg/L), which is in line with the large differences between and within seafood species (Molin et al., 2015; EFSA, 2021; Uneyama et al., 2007). The present study showed, however, similar correlations of urinary arsenobetaine with all types of seafood intake. Previous studies have shown that consumption of cod, plaice, and sole (i.e., comparable to lean fish in our study) result in particularly high urinary concentrations of arsenobetaine, higher than consumption of e.g., salmon and mussels (i.e., comparable to fatty fish and shellfish in our study) (Hackethal et al., 2022; Molin et al., 2014). Like for methylmercury, the arsenic concentrations seem to be lower in farmed Atlantic salmon compared to wild Atlantic salmon, largely due to the increasing use of plant-based feed (Jensen et al., 2020). Arsenobetaine has a short half-life in the body, usually less than 24 h (Molin et al., 2015). Whether it can mimic glycine betaine in the human body is not known. Glycine betaine is also a human osmolyte, accumulating in cells for volume and turgor regulation, and for DNA and protein stabilization (Ilyas et al., 2022; Wallace et al., 2008). Further, it is an important methyl donor in the methionine cycle and particularly important during pregnancy.

The urinary concentrations of trimethylarsine oxide were low and detected in a mere 10% of the women. The N-analogue, trimethylamine N-oxide (TMAO), is formed in the body from trimethylamine obtained by ingestion of fish or indirectly from bacterial metabolism of dietary compounds containing trimethylated nitrogen, such as choline, betaine, and L-carnitine, in the intestine (Zeisel and Warrier, 2017). It is possible that the measured trimethylarsine oxide in urine of the NICE women has a similar origin. If so, one explanation for the low frequency of detectable concentrations may be because TMAO is preferentially found in deep sea fish (Yancey et al., 2014). The fact that the urinary concentrations of trimethylarsine oxide correlated the strongest with arsenocholine, which also appeared in low concentrations in urine, may indicate a degradation of arsenocholine in the liver to trimethylarsine oxide, as reported from previous *in vitro* studies (Christakopoulos et al., 1988). Most of the ingested arsenocholine is, however, metabolized to arsenobetaine, which then is excreted in urine (Marafante et al., 1984). Thus, urinary concentrations of arsenocholine or trimethylarsine oxide are not reliable biomarkers of seafood intake.

The urinary concentrations of inorganic arsenic metabolites (median: 3.3 μg/L), commonly used as a biomarker of exposure to inorganic arsenic, the most toxic form of arsenic, corresponded to around one fifth of the total urinary arsenic concentration (median: 15.6 μg/L). Drinking water and rice are the most common sources of inorganic arsenic (EFS A Pane l on Contaminants in the Food Chain (CONTAM), 2009). The observed weak correlation with total seafood intake and the correlation with arsenobetaine and total arsenic in urine may be explained by the presence of inorganic arsenic mainly in algae (Taylor and Karagas, 2022) and bivalves (Molin et al., 2015), which are not broadly consumed in Sweden. Furthermore, arsenosugars, which are commonly found in marine algae and in filter feeders like mollusks and crustaceans, as well as arsenolipids, which appear to be common in fatty fish, are partly biotransformed in the human body to DMA (Molin et al., 2015; Taylor et al., 2017), the major metabolite of inorganic arsenic.

Although plasma selenium and urinary iodine correlated to some extent with the reported seafood intake, the suitability of using these measurements as biomarkers of seafood intake can be questioned. Iodine correlated weakly to lean fish intake but not to fatty fish or shellfish intake, whilst plasma selenium showed strongest association with fatty fish. Seafood is not a unique source of iodine or selenium and we have previously shown these concentrations to vary with the intake of several other food items and supplements (Stråvik et al., 2021).

We found fairly weak correlations of seafood intake with EPA and DHA, and essentially no correlation with DPA, all of which showed fairly narrow ranges. Importantly, even the women who did not consume any seafood had fairly high erythrocyte proportions of the n-3 LCPUFAs, particularly of DPA. Indeed, several studies have reported lower correlations between intake and measured levels of DPA in blood, compared to the associations between reported intake of DHA and EPA and their levels in blood (Serra-Majem et al., 2012). Importantly, the conversion that occurs between the different fatty acids, especially in women (Burdge and Wootton, 2002), contributes to the limited use of n-3 LCPUFAs as dietary biomarkers. During pregnancy, the endogenous production of DHA from non-marine dietary sources containing alpha-linolenic acid (e.g., nuts, seeds, and vegetable oils) is of high importance since DHA affects fetal growth and, more specifically, is incorporated into cell membranes of the fetal brain (Gibson et al., 2011). The n-3 LCPUFAs are transported via the placenta to the fetus, and the transport seems to be particularly enhanced during late pregnancy, which was shown in an intervention study where EPA and DHA correlated with fish intake in early, but not late pregnancy (Bosaeus et al., 2015). As expected, the fatty acids correlated more strongly with fatty fish than with lean fish and shellfish. Importantly, there are decreasing contents of EPA and DHA in farmed Atlantic salmon in the last few decades (>60%), as a result of the changes in the feed towards a more plant-based diet (Moxness Reksten et al., 2022).

Further in line with expectations, the women who consumed fish oil regularly (12%) had higher erythrocyte levels of the fatty acids EPA, DHA and DPA. Higher blood levels of these n-3 LCPUFAs have previously been reported for pregnant women in Norway, 77% of whom reported use of fish oil supplementation (Araujo et al., 2020). In a study of pregnant women in the US, a regular consumption of fish oil supplements (13.5% of the women) was associated with slightly higher urinary concentrations of total arsenic and arsenobetaine (Taylor and Karagas, 2022). We found no significant increase in these urinary concentrations among fish oil supplement users in our study. Instead, women taking supplements with multivitamins and minerals had more than twice as high concentrations of arsenobetaine in urine as compared to women not taking these supplements (median values 15.4 versus 7.4 μg/L). Interestingly, this difference could not be explained by a difference in seafood intake between these two groups. This warrants further studies of the content in supplements.

The strengths of the study include the large cohort of pregnant women and the use of several potential biomarkers of seafood intake (based on blood plasma, erythrocytes, and urine samples collected around gestational week 29), not solely focusing on nutritive components. However, considering the varying time periods between seafood intake and sampling (not assessed), there is a risk that the biomarker concentrations of compounds with short half-lives in the body (e.g., urinary iodine and arsenobetaine) were underestimated. Ideally, repeated sampling could compensate for such variations. Another limitation of the study is that we compared the biomarkers to the estimated dietary intake, which according to the premise of the study is unreliable. However, we performed a detailed investigation of dietary intake of fatty fish, lean fish, and shellfish, using a validated semi-quantitative food frequency questionnaire. Obviously, it may be difficult to

remember both portion size and frequency of a food that is not consumed regularly. Randomized controlled intervention studies in which the study meals are evaluated against the biomarkers are likely to give a more definitive answer concerning their ability to reflect the seafood intake, but such studies are difficult to perform, mainly due to the required extended study period. Using a food frequency questionnaire specifically targeting seafood intake, possibly repeated during pregnancy, or using a repeated weighted food record could perhaps give an indication closer to the truth.

In conclusion, erythrocyte mercury and urinary arsenobetaine were found to correlate strongly with the intake of seafood, regardless of the type of seafood consumed (fatty fish, lean fish, or shellfish) by the studied pregnant women. Thus, elevated concentrations of these seafood-specific compounds can be useful indicators of seafood intake, more so than the n-3 LCPUFAs. However, the relative importance of the biomarkers may differ depending on the type and amount of seafood consumed. In future studies assessing health outcomes of seafood intake, a combination of dietary assessments and objectively measured biomarkers would be preferred.

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Credit author statement

Mia Stråvik: Writing – original draft, Software, Formal analysis, Data curation, Investigation, Visualization. Klara Gustin: Investigation, Data curation, Writing – review & editing. Malin Barman: Data curation, Methodology, Writing – review & editing. Michael Levi: Investigation, Writing – review & editing. Anna Sandin: Cohort administration, Review & Editing. Agnes E. Wold: Review & editing, Cohort administration. Ann-Sofie Sandberg: Methodology, Writing – review & editing, Cohort administration, Funding acquisition. Maria Kippler: Review & editing, Methodology, Cohort administration, Funding acquisition. Marie Vahter: Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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.

Data availability

Explicit consent to deposit raw data was not obtained from the participants. The R code for the statistical analyses can however be obtained from: https://gitlab.com/miastravik/seafood-biomarkers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115576.

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