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Environmental mercury exposure and selenium-associated biomarkers of antioxidant status at molecular and biochemical level. A short-term intervention study

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A B S T R A C T

Mercury (Hg) is a potent toxicant. In the field of public health a chronic-low-level environmental Hg exposure resulting from fish consumption in general population is still being discussed. The objective of the study was to assess the influence of real Hg exposure on biomarkers of selenium (Se) status and selected biomarkers of pro-oxidant/anti-oxidant effects in healthy men (n = 67) who participated in the short-term intervention study consisting in daily fish consumption for two weeks. The analysis included Se level, Se-associated antioxidants at molecular (profile of 7 genes encoding selected proteins related to antioxidant defense) and biochemical levels (Se-dependent glutathione peroxidases activities and plasma selenoprotein P concentration). A pro-oxidant/anti-oxidant balance was explored using a biomarker of plasma lipid peroxidation and total antioxidant activity. The study revealed significant correlations ($p < 0.05$) between the biomarkers of exposure to Hg, Se level and Se-dependent antioxidants. Even though the risk of adverse effects of Hg for volunteers was substantially low, biomarkers of Hg altered levels of circulation selenoproteins and their genes expression. Changes in genes expression during study differed between the main enzymes involved in two systems: downregulation of thioredoxin reductase1 and upregulation of glutathione peroxidases. Hg exposure caused imbalance between the biomarkers of pro-oxidant/anti-oxidant effects.

1. Introduction

A seafood diet is commonly recommended by primary health care centers as a diet rich with proteins, omega-3 long chain polyunsaturated fatty acids (especially EPA and DHA), vitamins (particularly vitamin D) and also iodine (I) and selenium (Se). However, fish and shellfish consumption constitutes the main source of exposure to an organic form of mercury (Hg) - methylmercury (MeHg) (Filippini et al., 2018; You et al., 2018). Evaluation of exposure to MeHg can be executed on the basis of Hg levels measurement in food (e.g. fish, Kuras et al., 2017) or biological markers of environmental exposure to MeHg in biofluids: Hg in hair (Hg–H) (Brodzka et al., 2009) and Hg in blood (Hg–B). They are suitable tools in biological monitoring (WHO, 2018). MeHg is strongly lipophilic, thus, is easily accumulates in an organism and/or is biomagnified in the subsequent trophic chain. MeHg from fish and seafood consumption is next absorbed in the body by the gastrointestinal system with the efficiency up to 95% (EFSA, 2012; WHO, 2018). It is an approved neurotoxicant. Even at a low-level exposure, MeHg quickly penetrates biological barriers: blood\placenta and blood \brain. It may affect brain (selectively accumulated in the amygdala and hippocampus (Obiorah et al., 2015)) and the nervous system inducing direct or indirect neuropsychological disorders. MeHg may be transported into the tissues via MeHg-cysteine complex (Park and Mozaffarian, 2010), where it is next accumulated (mainly in the liver, hair and human brain).

Environmental exposure to MeHg is still constitutes an interesting part of scientific activity due to the redox imbalance and the resulting oxidative stress. Created reactive radicals affect, among others, epigenetic modifications at the level of DNA and reaction of oxidation of the major biomolecules, which contribute to the increase in cellular damage leading to a metabolic dysfunction. Toxicity mechanism of MeHg at a molecular level is probably connected with Hg binding to biological molecules via nucleophilic groups: thiol (-SH) and selenol (-SeH) that are necessary for the correct functioning of the enzyme systems responsible for maintaining the proper equilibrium of the organism. Natural adaptive abilities disorder may lead to the impairment of the detoxification process and next to the inefficient elimination of heavy metals from the intestines.

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Although, in certain cases, Se may lead to toxicity (Spallholz and Hoffman, 2002; MacFarquhar et al., 2010) it is a generally known as an antioxidant with immunological properties. A potential detoxification mechanism of this essential micronutrient may result from the higher affinity of Hg to Se (approx. 1 million-fold) than to sulfur (Ralston and Raymond, 2010; Melnick et al., 2010). Therefore, disruption of cellular processes associated with the regulation of the redox potential, depletion antioxidants the inhibition of protein synthesis or inactivation of enzymes may be detracted by Se in the structures of selenodependent enzymes (Franco et al., 2009; Ralston et al., 2012, 2018). The presence of Se in the selenocysteine (Sec) in the active center of selenoenzymes and selenoproteins is essentially related to its role in the body. Hg binds to Se in the active site of thioredoxin reductase (TrxR), glutathione peroxidase (GPx) and inhibits their activity in a consequence leading to a disruption of the intracellular redox and next oxidative stress (Branco et al., 2012, 2017a). Insights from experimental studies indicate that selenoproteins, mainly from the systems of thioredoxin reductase/thioredoxin (TrxR/Trx) and the glutathione (GSH), are particularly susceptible to Hg-induced malfunction (Branco et al., 2012). Studies signifying that the activity of GPx1-RBC as well as its expression is potentially regulated by availability of Se and depend on its status are plentiful (Sunde, 2018). Simultaneously mRNA expression levels for *TRXR1* may be mediated by reactive oxygen species (ROS) (Wu et al., 2003). Concentrations of Se in plasma/serum (Se–P/S) as well as in the urine (Se–U) are expressed as biomarkers of Se status and short-term dietary intake, although it is said that concentration of total Se does not reflect the real functional Se activity. Ralston et al. (2008) suggest that due to high affinity of Hg to -SeH group (Hg-dependent sequestration of Se), toxicity of MeHg may result from depletion of Se. Since Se is needed for inducing de novo selenoproteins synthesis, Hg-induced Se deficiency blocks regeneration of these selenoproteins (Ralston and Raymond, 2018). Benhar et al. (2016) suggest a molecular cross-talk between the two major antioxidant systems (TrxR/Trx and GSH). Their overlapping function consisting in maintaining cellular homeostasis indicates a compensation mechanism especially in the case when one system functioning is impeded (Du et al., 2012; Benhar et al., 2016). Notwithstanding, very often researchers focus only on one of these systems in their articles. Therefore, this combined aspect was also investigated in our study.

Biological monitoring is substantial for understanding human health effects of low-level MeHg exposure as a basis for future research efforts and risk assessment. Mechanism of gene-environment interaction may contribute to better understanding of genetics and realization of complex mechanisms between genetics and environment. The effects of Hg toxicity resulting from fish consumption associated with intensity of oxidative stress may affect alteration of the expression and/or activity of selenoproteins related with oxidative defense. Identification and validation of novel biomarkers of susceptibility might be an important part of the investigation of exposure-health relationships.

Because the study is a comprehensive assessment of the impact of environmental exposure of men to Hg at both mRNA and selenoprotein levels, with investigation of the selected biomarkers of the pro- and antioxidant effect as significant factors associated with the mechanism of Hg detoxification, it would allow better familiarization with the potential influence of Se and Se-dependent biomarkers of antioxidative defense on the mechanism of Hg inactivation.

2. Materials and methods

2.1. Study design

The intervention study including a Polish subpopulation was approved by the Local Ethics Committee of the Nofer Institute of Occupational Medicine (NIOM) in Lodz, Poland. The research, which was carried out from June 2015 to August 2015, involved 67 healthy, non-exposed to elemental mercury (Hg^0) vapor men with a mean age of 41 years (range 21–64 years) and BMI of 26.9 ± 4.3 kg/m^2 (range 17.8–40.2 kg/m^2). All the data were compared with reference to the baseline (values measured before the fish consumption). Details of the trial, subjects' and fish species exclusion criteria, concentration of Hg in the selected fish species, the estimated daily Hg intake, as well as dietary habits of the volunteers according to the validated Food Frequency Questionnaire (FFQ-6, http://www.uwm.edu.pl/edu/lidiawadolowska/) were described in a previous study (Kuras et al., 2017, 2018). In order to obtain the basic data needed for the research, a personal survey was conducted. The survey data were collected from the study volunteers at the first day of the intervention study and included questions about age, weight, BMI, current smoking status, diet, alcohol, dental amalgam fillings and medical history.

After obtaining written informed consent forms, biological samples were collected from the study participants at four time-points: I sampling - blood, urine and hair samples collected before fish meals consumption (also assigned as baseline), II sampling - blood and urine samples collected after one week of fish consumption, III sampling - blood and urine samples collected in the end of fish consumption (the second week) and finally IV sampling - blood and hair samples collected one month after the end of fish consumption (the washout period). Results from the hair samples collected in the washout period correspond to the results from biofluids in III sampling as hair growth is approximately 1 cm each month. A flowchart of the study design, sampling and data collection is presented in Fig. 1.

2.2. Specimen collection

The blood samples were collected into BD Vacutainer® Blood Collection Heparin Tubes, free from trace elements and heavy metals. They were then stored at −20 °C until the analysis.

Heparinized blood samples were centrifuged at 1500 × *g*, 4 °C for 10 min. Plasma samples were then stored at −20 °C until the analysis.

Erythrocytes were washed three times with a 0.9% solution of NaCl (with an excess of saline solution in the final), hemolyzed three times by thawing and freezing and then, in the end, frozen at −20 °C until the analysis (Zachara et al., 1984).

The urine samples were collected into cleaned disposable containers and acidified to pH < 1.

The hair samples (the occipital region of the head at the hair root) were cut and placed in polyethylene bags. They were washed with

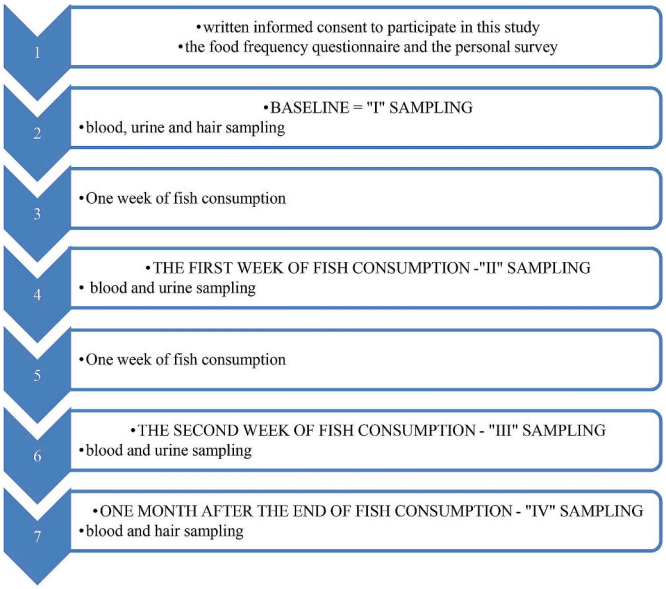

Fig. 1. Flowchart of intervention study design, sampling and data collection (n = 67).

acetone and deionized water and stored at a room temperature until the analysis.

2.3. Laboratory analysis

The detailed determination of total Hg and Se in the biological materials, creatinine in urine, total antioxidant activity in plasma (TAA-P), assays of activity of red blood cell and plasma glutathione peroxidases (GPx1-RBC and GPx3-P) and plasma selenoprotein P concentrations (SeP–P) as well as gene expression analysis in peripheral blood leukocytes were described in the above mentioned study (Kuras et al., 2017, 2018).

The biological samples were analyzed for total Hg concentrations by means of the thermal decomposition amalgamation atomic absorption spectrometry method (TDA-AAS) using DMA-80 (Milestone, Spectro-Lab, Poland). The correlation coefficient $r = 0.9998$ was achieved for the linear calibration curve (an absolute mass of Hg (nanogram) versus absorption peak area) in the range 0.5–10.0 ng. Limit of detection (LOD) and limit of quantification (LOQ) calculated based on a value of three times and six times of standard deviation (SD) amounted to 0.0025 ng and 0.0051 ng, respectively.

Diluted 150-fold (1.0% HNO_3) plasma samples were analyzed for total Se concentrations using inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e, PerkinElmer, SCIEX, USA). LOD and LOQ amounted to 0.19 µg/L and 0.39 µg/L, respectively.

The analysis quality was verified by the internal as well as external quality assessments (G-EQUAS) organized by the Institute of Occupational Social and Environmental Medicine of the University of Erlangen, Nuremberg.

2.4. Determination of thiobarbituric acid-reactive substances concentrations in plasma (TBARS-P)

Plasma concentration of TBARS was measured using a PerkinElmer Luminescence Spectrometer LS50-B (PerkinElmer, Shelton, CT, USA). TBA-reactive compounds were extracted to butanol. The value of fluorescence of butanol layer was read at an excitation wavelength of $\lambda = 525$ nm and emission wavelength of $\lambda = 547$ nm (Wasowicz et al., 1993).

2.5. Statistical analysis

All the data were expressed as arithmetic mean and standard deviation (SD) and the range (min.-max. values) to measure differences at the four time points. Normalization of genes-expression was carried out when acceptable normalizing genes (beta-actin (*ACTB*) and beta-2-microglobulin (*β2M*)) were optimized for the qPCR analysis. This procedure helps to disqualify variations in gene expression between the individuals that might have given incorrect data. The repeated measures analysis of variance (RM ANOVA) was performed to test the effects of time on the measured parameters. Post-hoc comparisons between the pair wise time points were performed using the NIR FISHER test. Normality was assessed by the Shapiro–Wilk test (non-normal data were log-transformed). Differences between concentrations of Hg in the biological materials and examined parameters, including smoking, dietary supplements, products containing fish oils and similar, alcohol consumption and amalgam fillings were assessed by the use of the *t*-test, while correlations between them were tested by the Pearson's correlation coefficient. Additionally, to compare the fatty as well as lean fish consumption frequency and concentrations of Hg–B as well as Hg–H in the four time points, the independent-samples *t*-test was used. The statistical analysis was conducted using STATISTICA 13.1 PL (StatSoft, Tulsa, OK, USA). Significance was established at a level of $p \leq 0.05$.

Table 1
Basic characteristics of the study group (67 subjects who ate fish).

Variable	All (n = 67)
Age (years)	41.0 ± 12.7[a] (21–64)[b]
BMI (kg/m^2)	26.9 ± 4.3 (17.8–40.2)
Smoking, n (%)	
Current	14 (20.9%)
Ever	28 (41.8%)
Never	53 (79.1%)
Dental amalgam, n (%)	23 (34%)
Dietary habits, n (%)	
Fat fish consumption:	
Never	6 (9%)
Once a month or less	33 (50%)
Several times a month	26 (40%)
Lean fish consumption:	
Never or almost never	3 (4%)
Once a month or less	35 (52%)
Several times a month	27 (41%)
Seafood:	
Never	62 (93%)
Once a month or less	3 (4%)
Several times a month	1 (1%)
Game meat consumption	
Never	51 (76%)
Once a month or less	14 (20%)
Several times a month	1 (1%)

[a] - Mean value ± standard deviation.
[b] - Range.

3. Results

3.1. Food frequency questionnaire

An extensive characterization of the study group including the semi-qualitative food frequency questionnaire was conducted in order to investigate of the food intake (times/person/day) during a year. Dietary habits of the subjects are presented in Table 1. In our validated FFQ-6 there were 62 of products, about which all the participants were asked open questions. There was a six-point scale, where number (1) meant never or nearly never, (2) once a month or less, (3) several times a month, (4) several times a week, (5) every day, (6) several times a day. According to the detailed information in FFQ-6, we found out that no individuals ate high-fat fish number of times per week, every day or number of times per day. The independent-samples *t*-test showed that there were statistically significant differences between the frequency of consumption of fatty fish and concentrations of Hg–B and Hg–H at the three time points with the exception of Hg–B, IV sampling (Table 2.). Such relationships were not found in the group that declared lean fish consumption frequency. Additionally, there were no significant differences in the urinary Hg level between the intake frequency. None of the study subjects reported any changes in their eating habits during the study.

3.2. Biomarkers of Hg exposure

Biomarkers of Hg exposure for the examined subjects (n = 67) measured at baseline, during the fish consumption and the washout period calculated using the ANOVA and next post-hoc tests are presented in Table 3. The increase of mean Hg–B concentration observed during this short exposure time comparing to the baseline, amounted to 45% and to 106% in the first and second weeks, respectively. However, the mean value of concentration of Hg–B in the washout period significantly decreased. It was still significantly higher than that at the baseline level ($p < 0.001$). Hair Hg concentration statistically elevated by 21% ($p < 0.001$ vs. the baseline). A strong positive correlation between Hg–B and Hg–H was found before the intervention (r = 0.81; $p < 0.0001$) and after two weeks of fish consumption (r = 0.76;

Table 2
Differences between the fatty fish consumption frequency and concentrations of Hg–B and Hg–H in the four time points.

Parameter	Time points	Mean ± SD (n = 6)	Mean ± SD (n = 33)	Mean ± SD (n = 26)	*p*	*p*	*p*
		never or nearly never (1)	once a month or less (2)	several times a month (3)	1–2	2–3	1–3
Hg–B μg/L	I	0.274 ± 0.166	0.630 ± 0.398	0.693 ± 0.440	**0.0389**	0.5582	**0.0304**
	II	0.519 ± 0.181	0.931 ± 0.494	0.951 ± 0.433	*0.0526*	0.8659	**0.0245**
	III	0.873 ± 0.134	1.364 ± 0.528	1.265 ± 0.441	**0.0308**	0.4416	**0.0416**
	IV	0.396 ± 0.297	0.840 ± 0.614	0.794 ± 0.615	0.0924	0.7715	0.1363
Hg–H μg/g hair	I	0.080 ± 0.058	0.246 ± 0.155	0.262 ± 0.176	**0.0141**	0.6923	**0.0185**
	IV	0.133 ± 0.067	0.308 ± 0.165	0.297 ± 0.121	**0.0157**	0.7929	**0.0035**

p-values obtained in the independent-samples *t*-test.

Table 3
Biomarkers of Hg exposure in the volunteers (n = 67) in four time points: I - baseline, II - after one week of fish consumption, III – after two weeks of fish consumption, IV - one month after the end of the study.
I: [a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$;
II: [A] - $p < 0.05$, [B] - $p < 0.01$, [C] - $p < 0.001$;
III: [*] - $p < 0.05$, [**] - $p < 0.01$, [***] - $p < 0.001$

Biomarkers of Hg exposure	Time points	Mean ± Standard Deviation (Min.-Max.)	*p* ANOVA
Hg–B (μg/L)[a]			$p < 0.0001$
	I	0.623 ± 0.412 (0.052–1.796)	
	II	**0.902 ± 0.463[c] (0.285–2.721)**	
	III	**1.282 ± 0.487 [c, C] (0.623–3.173)**	
	IV	**0.782 ± 0.599[c, B, ***] (0.053–2.852)**	
Hg–H (μg/g hair)[a]			$p = 0.00001$
	I	0.237 ± 0.164 (0.018–0.855)	
	IV	**0.288 ± 0.150[c] (0.043–0.924)**	
Hg–U (μg/g creat.)[a]			$p = 0.00025$
	I	0.231 ± 0.217 (0.054–1.105)	
	II	0.231 ± 0.189 (0.038–1.126)	
	III	**0.160 ± 0.166[c, C] (0.024–0.900)**	

Values in bold are significantly different as compared to the sampling.
[a] Non-normal data were normalized for the ANOVA test.

$p < 0.0001$). The mean hair to blood total Hg concentration ratios calculated as 1000*(Hair Hg/Blood Hg) were 390 ± 140 (SD) at baseline (range 80–820) and 230 ± 80 (SD) after two weeks of fish consumption in the range of 69–442 (after adjustment for the time lag).

Fig. 2 shows the relationship between Hg–H and Hg–B evaluated by the linear regression. The ratio was positively correlated with Se–P at baseline and in the washout period (r = 0.24; $p = 0.047$ and r = 0.27; $p = 0.028$, respectively).

3.3. Biomarkers of selenium status

The men who took dietary supplements (others than shark cartilage, fish oil, omega-3fatty acid or similar) had a significantly higher concentration of urinary Se (Se–U) adjusted for creatinine measured at baseline, it means before the fish consumption, than those who did not ($p = 0.018$). The mean plasma Se–P concentration was significantly higher before the intervention in the study subjects who drank alcohol than in those who did not ($p = 0.043$).

Significant changes in the mean value of Se–P concentration during the whole intervention study were observed (ANOVA $p < 0.0001$), (Table 4). The post-hoc comparisons showed that Se–P concentration was statistically increased after one week of fish consumption compared to the mean baseline and then remained almost at the same level ($p < 0.001$). Urinary Se concentration increased in the first week of fish consumption and next decreased after two weeks of fish consumption compared to the baseline and these changes were significant ($p < 0.001$).

Significant negative associations with BMI were found in the study individuals for Se–P (r = −0.30; $p = 0.015$), and for Se–U (r = −0.28; $p = 0.022$), measured before the intervention study. Additionally, we found negative associations between Se–U (μg/g creat.) and BMI during the fish consumption (r = −0.23; $p = 0.063$ (margin of statistical significance) after one week and r = −0.33; $p = 0.008$ after two weeks of fish consumption.

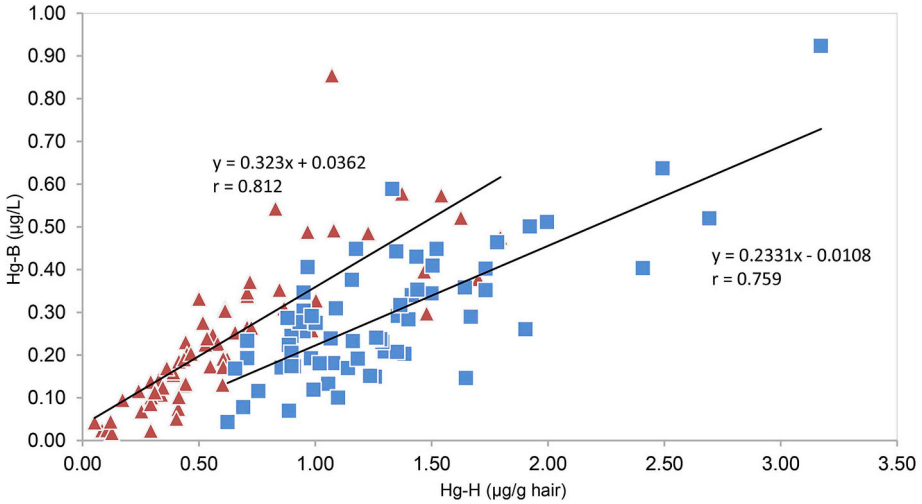

Fig. 2. The relationship between Hg–H and Hg–B evaluated by linear regression. Baseline (triangle) versus the second week of fish consumption (squares).

Table 4
Biomarkers of selenium status in the men (n = 67) in four time points: I - baseline, II - after one week of fish consumption, III – after two weeks of fish consumption, IV - one month after the end of the study. Relationship between the biomarkers of Hg exposure and selenium status.
I: [a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$;
II: [A] - $p < 0.05$, [B] - $p < 0.01$, [C] - $p < 0.001$;
III: [*] - $p < 0.05$, [**] - $p < 0.01$, [***] - $p < 0.001$

Parameters	Time points	Mean ± Standard Deviation (Min.-Max.)	*p* ANOVA	The Pearson's correlation coefficient (r, *p* value)
Se–P (μg/L)			$p = 0.00001$	
	I	73.346 ± 11.628 (43.545–99.439)		
	II	**81.636 ± 10.173 [c] (51.414–114.959)**		
	III	**80.809 ± 12.283 [c] (56.784–114.758)**		III: Se–P vs. Hg–B[*] (r = 0.29, $p = 0.019$)
	IV	**79.577 ± 11.710 [c] (46.233–101.227)**		
Se–U (μg/gcreat.)[&]			$\boldsymbol{p} < 0.0001$	
	I	13.061 ± 3.600 (7.588–23.164)		I: Se–U vs. Hg–U[***] (r = 0.61, $p < 0.0001$)
	II	**16.961 ± 4.088 [c] (8.556–24.680)**		II: Se–U vs. Hg–U (r = 0.36, $p < 0.0001$)
	III	**10.399 ± 2.805 [c, C] (5.632–19.549)**		

Values in bold are significantly different as compared to the sampling.
[*]μg/L.
[**]μg/g hair.
[***]μg/g creat.
[&]Non-normal data were normalized for the ANOVA test.

3.4. Relationship between the biomarkers of exposure to Hg and Se status

In the first week of fish meals consumption we observed a significantly increase in the concentration of Hg–B as well as Se–P and Se–U compared with the mean baseline ($p < 0.001$). At the same time the fish consumption did not influence on a urinary Hg level ($p > 0.05$), but along with a statistically increasing Hg–B concentration after the second week of fish consumption ($p < 0.001$), we observed in the same period a statistically significant decline in concentration of Hg–U and Se–U ($p < 0.001$). Se–P concentration remained higher through the whole period of the intervention study compared to the baseline ($p < 0.001$).

Association between the biomarkers of Hg exposure and Se status was assessed using the Pearson's correlation coefficient (Table 4). We found a strong correlation between urinary Hg and Se concentration ($p < 0.0001$) at baseline and moderate correlation between these elements after one week of fish consumption ($p < 0.0001$). In the end of fish consumption we found a weak correlation between Hg–B and Se–P ($p = 0.019$). The statistically significant relationship between concentrations of these elements in circulating bloodstream occurred only in the second week of fish consumption, thus, when the concentration of Hg–B was the highest, while the concentration of Se–P at that time remained almost at the same level compared to the first week of fish consumption. Nevertheless, the mean minimum value of Se–P in III sampling was higher than in II sampling (56.784 μg/L vs. 51.414 μg/L, respectively).

Se:Hg molar ratio calculated as a means ± standard deviation (min-max) of Se–P and Hg–B was 484.85 ± 489.66 μmol/L (75.34–2875.01) at baseline, 280.71 ± 128.86 μmol/L (73.15–742.58) after one week of fish consumption, 176.74 ± 55.12 μmol/L (77.89–308.67) after the second week of fish consumption and 205.95 ± 288,95 μmol/L (27.48–1657.36) in the washout period. Relationships between Hg and Se and their molar ratio in blood before the intervention was r = 0.594 for Hg and r = 0.024 for Se, $p < 0.05$ and after two weeks of fish consumption when the Hg body burden was the highest it was r = 0.828 for Hg and r = 0.165 for Se, $p < 0.05$.

3.5. Biomarkers of the pro- and anti-oxidant effect. Relationship between the biomarkers of exposure to Hg and oxidative stress

Assessment of the biomarker of lipoperoxidation showed significant changes during the fish consumption (Table 5). Concentration of TBARS-P increased in the first week of fish consumption when compared with the values observed at baseline ($p = 0.0032$). In the case of fish consumption one week longer, the concentration of the biomarker remained at a similar level but it was still higher compared to the baseline ($p = 0.0026$). A weak positive correlation was found between concentrations of Hg–H and TBARS-P after two weeks of fish consumption (r = 0.26, $p = 0.034$). The pro-oxidative effect of TBARS was visible with simultaneous reduction of an antioxidant status in plasma throughout the duration of the study. We found a successive slight decrease in the concentration of the pool of low molecular weight antioxidants in plasma, measured as total antioxidant activity throughout the whole period of the intervention study, but significant changes were observed only in the end of the fish consumption ($p = 0.005$) and the washout period ($p < 0.001$).

In the case of other biomarkers of antioxidant effect, the NIR Fisher test showed significant changes in the activity of Se-dependent enzymes at the three time points. Activities of Se-dependent enzymes (red blood cell as well as plasma GPx) increased after the volunteers started eating fish ($p < 0.001$). After the end of fish consumption, the activity of GPx1-RBC statistically decreased ($p < 0.001$). At the same time, the activity of GPx3-P remained almost unchanged, but it was still higher ($p < 0.001$ vs. the baseline) and then it slowly decreased until one month after the intervention study ($p < 0.001$). The activity of GPx1-RBC statistically increased again in the washout period ($p < 0.001$ vs. the baseline). No significant changes in the expression of SeP in both mRNA and protein levels were found during the whole trial ($p > 0.05$). Changes in SeP–P concentration at each time point were at the margin of statistical significance ($p = 0.0584$), when we included the influence of the baseline level of Se–P and Hg–B in the multivariate linear regression model (Table 5).

Comparison of the activities of GPx1-RBC and GPx3-P and concentrations of SeP–P in the four time points showed a weak relationship between the biomarkers of exposure to Hg and antioxidant effect measured at baseline, after one week of fish consumption and in the washout period. Significant positive correlations with GPx3-P were found for Hg–B (r = 0.25, $p = 0.043$) and Hg–H (r = 0.25, $p = 0.039$) at baseline. After one week of fish consumption we observed a positive significant correlation with Hg–U and SeP–P (r = 0.26, $p = 0.035$). In the washout period we noticed a positive significant correlation with Hg–B and GPx1-RBC (r = 0.27, $p = 0.026$). Despite these weak correlations, we did not observe any relationships between the biomarkers of environmental exposure to Hg and antioxidant selenoenzymes and SeP after two weeks of fish consumption.

Significant positive correlations were found between the activities of cellular GPx and the mRNA expression of *GPX1* during the whole trial (I sampling: r = 0.262; II sampling: r = 0.294; III sampling:

Table 5
Biomarkers of pro- and anti-oxidant effect in the men (n = 67) in four time points: I - baseline, II - after one week of fish consumption, III – after two weeks of fish consumption, IV - one month after the end of the study. Relationship between the biomarkers of Hg exposure and pro- and anti-oxidant effect.
I: [a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$;
II: [A] - $p < 0.05$, [B] - $p < 0.01$, [C] - $p < 0.001$;
III: [*] - $p < 0.05$, [**] - $p < 0.01$, [***] - $p < 0.001$

Parameters	Time points	Mean ± Standard Deviation (min.-max.)	*p* ANOVA (*p* adjustment[e])	The Pearson's correlation coefficient (r, *p* value)
TBARS-P (nmol/ml)	I	2.323 ± 0.609 (1.180–4.350)	***p* = 0.00730**	
	II	**2.513 ± 0.862 [b] (1.160–5.440)**		
	III	**2.517 ± 0.852 [b] (1.300–5.510)**		III:. TBARS-P vs. Hg–H[a] (r = 0.26, $p = 0.034$)
	IV	2.426 ± 0.797 (1.200–4.490)		
TAA-P (mmol/L	I	1.816 ± 0.308 (1.000–2.674	***p* < 0.0001**	
	II	1.789 ± 0.347 (0.912–2.817)		
	III	**1.680 ± 0.352 [b, A] (0.884–2.542)**		
	IV	**1.551 ± 0.319 [c, C, **] (1.001–2.812)**		
GPx1-RBC (U/gHb)	I	22.252 ± 4.504 (10.790–29.820)	***p* = 0.00011**	
	II	**23.983 ± 3.357[C] (17.420–29.910)**		II: GPx1-RBC vs. SeP–P (r = 0.42, $p < 0.001$)
	III	**22.201 ± 4.047[C] (11.340–29.650)**		III: GPx1-RBC vs. SeP–P (r = 0.35, $p < 0.001$)
	IV	**23.958 ± 3.557[c, ***] (16.250–29.630)**		IV: GPx1-RBC vs. Hg–B[b] (r = 0.27, $p = 0.026$)
GPx3-P (U/mL)[d]	I	0.186 ± 0.033 (0.129–0.321)	***p* < 0.0001**	I:. GPx3-P vs. Hg–B (r = 0.25, $p = 0.043$)
				I:. GPx3-P vs. Hg–H (r = 0.25, $p = 0.039$)
	II	**0.204 ± 0.043 [c] (0.151–0.474)**		
	III	**0.199 ± 0.039 [c] (0.149–0.430)**		
	IV	**0.173 ± 0.037[c, C, ***] (0.130–0.397)**		
SeP–P (μg/L)[d]	I	9.040 ± 6.309 (1.987–26.574)	$p = 0.57596$ (***p* = 0.05841**[e])	
	II	8.348 ± 6.185 (1.420–33.309)		II:. SeP–P vs. Hg–U[c] (r = 0.26, $p = 0.035$)
	III	8.433 ± 5.964 (0.985–28.206)		
	IV	8.301 ± 5.086 (1.620–27.231)		

Values in bold are significantly different as compared to the sampling.
SD standard deviation.
[a] μg/g hair.
[b] μg/L.
[c] μg/g creat.
[d] Non-normal data were normalized for the ANOVA test.
[e] The multivariate linear regression for Se–P μg/L (baseline), Hg–B μg/L (baseline).

r = 0.273, IV sampling: r = 0.326) ($p < 0.01$). We indicated a significant positive correlation between the activity of GPx1-RBC and concentration of SeP–P after one and two weeks of fish consumption (II sampling: r = 0.421; III sampling: r = 0.345, $p < 0.01$, respectively), whereas no such relationship was observed in the case of activity of GPx3-P and expression of *GPX3*, concentration of SeP–P and expression of *SEPP1* and the activity of GPx3-P and concentration of SeP–P.

3.6. Genes expression. Relationship between the biomarkers of exposure to Hg and expression of genes related to the antioxidant status

We tested differences in the mean values of the genes-normalized relative expression of selected selenoproteins: selenoprotein P (*SEPP1*), glutathione peroxidases (*GPX1, GPX3*), thioredoxin reductase 1 (*TRXR1*), thioredoxin 1 (*TRX1*), peroxiredoxins (*PRDX1, PRDX2*) during the four time points of the intervention study (Table 6). Four of the seven analyzed genes significantly changed during the fish consumption (ANOVA, $p < 0.05$).

We noticed a different pattern between the main enzymes involved in the antioxidant systems as revealed in downregulation of *TRXR1* and upregulation of *GPX1* expression. Levels of mRNA expression for *GPX1* increased after the fish consumption, but statistically increased in the third sampling, when Hg body burden was the highest. Moreover, we observed significant changes in the expression of *TRXR1* ($p < 0.001$) throughout the ongoing study, while *TRX1* remained unaffected. Gene expression of *PRDX1* statistically decreased after one and two weeks ($p < 0.001$) of fish consumption, whereas the mRNA level for *PRXD2* increased ($p < 0.001$). Not only *PRDX1* and *PRDX2* genes expression levels changed contradictory to each other during the intervention study. Also the analysis of their correlation with mercury revealed contradicting results. A positive significant correlation with expression of *PRDX1* was observed after one week of fish consumption only for Hg–U (r = 0.25, $p = 0.043$). A positive significant correlation with expression of *PRDX1* was observed after one week of fish consumption only for Hg–U (r = 0.25, $p = 0.043$). In the second week of fish consumption we found in the study individuals a significant negative correlation between Hg–H concentration and *PRDX1* mRNA (r = −0.44, $p < 0.001$). Moreover, expression of *PRDX1* in the washout period was inversely correlated with concentrations of Hg–B (r = − 0.25, $p = 0.039$).

No correlation was observed between Hg–B and selenoprotein-encoding genes after one and two weeks of fish consumption. In spite of the fact that differences between the level of gene expression of *TRXR1* were significant in the first as well as second week of fish consumption and in the washout period vs. the baseline, we did not observe correlations between the biomarkers of exposure to Hg and this selenoprotein-encoding gene in the whole intervention study. *GPX1*, *TRXR1, PRDX1* genes expression in the washout period was lower even before the fish consumption and differences were significant with the exception of *GPX1* expression.

Baseline Se–P was negatively correlated with *TRXR1* and *PRDX1* genes expression (r = −0.29; $p = 0.017$ and r = −0.27; $p = 0.032$, respectively) measured on the first day of the trial before the fish consumption. No correlation was seen between the biomarkers of Se status measured as Se–P and Se–U and antioxidant selenoenzymes and SeP as well as their genes expression after 1 as well as 2 weeks of fish consumption, with the exception of Se–P and the expression of thioredoxin-dependent *PRDX2* gene in the first week of the intervention study (r = 0.25; $p = 0.042$). Furthermore, plasma Se concentration in the washout period was associated with a significant reduction of genes expression of *TRXR1* and Se-dependent enzyme with redox property (r = −0.27; $p = 0.029$).

Table 6
Examination of mRNA expression profiles of selected genes for the subjects (n = 67) in four time points: I - baseline, II – after one week of fish consumption, III – after two weeks of fish consumption, IV - one month after the end of the study. Relationship between the biomarkers of Hg exposure and mRNA expression of selected genes.

Values in bold are significantly different as compared to the sampling:
I: [a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$;
II: [A] - $p < 0.05$, [B] - $p < 0.01$, [C] - $p < 0.001$;
III: [*] - $p < 0.05$, [**] - $p < 0.01$, [***] - $p < 0.001$

Parameters	Time points	Mean ± Standard Deviation (min.-max.)	*p* ANOVA	The Pearson's correlation coefficient (r, *p* value)
Ln *SEPP1*			$p = 0.68095$	
	I	3.930 ± 2.092 (0.000–6.293)		
	II	4.147 ± 2.256 (0.000–6.941)		
	III	3.739 ± 2.163 (0.000–6.498)		
	IV	3.958 ± 2.062 (0.000–7.011)		
Ln *GPX1*			$\mathbf{p = 0.00010}$	
	I	13.222 ± 0.415 (12.308–14.110)		
	II	13.325 ± 0.397 (12.468–14.220)		
	III	**13.450 ± 0.588 [c] (12.017–14.571)**		
	IV	**13.162 ± 0.420 [B, ***] (12.317–14.428)**		
Ln *GPX3*			$p = 0.70480$	
	I	4.662 ± 0.955 (0.000–7.183)		
	II	4.675 ± 0.757 (2.816–6.237)		
	III	4.651 ± 0.788 (2.778–6.484)		
	IV	4.560 ± 0.667 (3.187–6.587)		
Ln *TRXR1*			$\mathbf{p < 0.0001}$	
	I	5.833 ± 1.243 (0.942–7.868)		I: *TRXR1* vs. Se–P[a] (r = −0.29, $p = 0.017$)
	II	**4.917 ± 0.849 [c] (0.000–6.353)**		
	III	**5.076 ± 0.832 [c] (0.905–6.312)**		
	IV	**3.703 ± 1.889 [c, C, ***] (0.000–7.048)**		IV: TRXR1 vs. Se–P (r = −0.27, $p = 0.029$).
Ln *TRX1*			$p = 0.40673$	
	I	10.565 ± 0.497 (9.650–11.558)		
	II	10.551 ± 0.430 (9.778–11.558)		
	III	10.628 ± 0.464 (9.684–11.466)		
	IV	10.597 ± 0.330 (9.726–11.239)		
Ln *PRDX1*			$\mathbf{p < 0.0001}$	
	I	11.530 ± 0.278 (10.907–12.280)		I: *PRDX1* vs. Se–P (r = −0.27, $p = 0.032$)
	II	11.478 ± 0.247 (10.805–11.923)		II: *PRDX1* vs. Hg–U** (r = 0.25, $p = 0.043$)
	III	**11.349 ± 0.324 [c, B] (10.382–11.963)**		
	IV	**11.206 ± 0.378 [c, C, **] (9.216–11.964)**		IV: *PRDX1* vs. Hg–B[a] (r = − 0.25, $p = 0.039$)
Ln *PRDX2*			$\mathbf{p = 0.00942}$	
	I	10.978 ± 0.436 (9.883–11.947)		I: *PRDX2* vs. Hg–H*** (r = 0.44, $p < 0.0001$)
	II	**11.122 ± 0.357[a] (10.288–11.943)**		II:. *PRDX2* vs. Se–P (r = 0.25, $p = 0.042$)
	III	**11.201 ± 0.530[c] (10.188–12.283)**		
	IV	11.087 ± 0.431 (9.959–12.080)		

[a] μg/L, ** μg/g creat., ***μg/g hair.

4. Discussion

Assessment of exposure to Hg of the general population, which is exposed mainly by food consumption, is based on the analysis of basic biomarkers, i.e. Hg–B, Hg–H. Other biomarkers of effects and sensitivity assessing the impact of such exposure are also sought. In the present experimental study, we analyzed the effects of environmental exposure to Hg in the men through the study of oxidative stress markers. We included expression and activity of selected selenoproteins mostly depending on the Se status, with an additional extensive and detailed dietary intake information. Our results indicated significant differences in the concentrations of biomarkers of Hg exposure, Se status and pro- and antioxidant status between the considered averages, both during the first and the second week of fish meals consumption.

4.1. Biomarkers of Hg exposure

Similarly to other studies concerning such an environmental exposure we found that the concentration of Hg–H is proportional to the concentration of Hg–B (Budtz-Jørgensen et al., 2004; Basu et al., 2014; Ripley et al., 2018). This combination can be used to assess the concentration of Hg–B by measuring concentration of Hg–H. The hair-to-blood mercury concentration ratio (250:1) recommended by the Joint Food and Agriculture Organization of the UN (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives is in agreement with our outcomes found in the individuals in our study after two weeks of fish consumption. This ratio is frequently used for assessments of the risk and exposure to ease the conversion of results of MeHg–H samples to circulating levels of the MeHg–B (WHO, 1990; Joint FAO/WHO, 2004). The mean Hg–H to Hg–B ratios found in other studies amounted to 292 (after a single fish meal consumption from 18 to 22 μg Hg/kg b.w.) (Kershaw et al., 1980), to 194 (after 5 weeks of fish consumption approximately 1.0 μg/g MeHg, unadjusted ratio) and to 315 (after 5 weeks of fish consumption approximately 1.0 μg/g MeHg, adjusted ratio) (Yaginuma-Sakurai et al., 2012). Baseline mean Hg–H/Hg–B ratio amounted to 354 (Yaginuma-Sakurai et al., 2012) which was similar to our ratio. We suggest that the people with a higher fish meals consumption achieve a state of equilibrium. Our explanation is in resemblance to the results of Liberda et al. (2014), who claim that pharmacokinetic aspect including demethylation ratio of MeHg into inorganic form of mercury (IHg) as well as the rates of fecal excretion of MeHg and urinary excretion of IHg is essential (Clarkson and Magos, 2006; Berglund et al., 2005).

Because MeHg constitutes a very small part of urinary Hg (about 10%), Hg–U cannot reflect concentration of MeHg in the body. Hg–U reflects mainly IHg compounds. As we expected, the individuals with dental amalgam fillings had a significantly higher concentration of Hg–U before (0.313 ± 0.276 μg/g creat.) and after the fish consumption (0.327 ± 0.275 μg/g creat.) compared to the volunteers without amalgams before (0.179 ± 0.160 μg/g creat.) and after of the fish

consumption (0.175 ± 0.087 μg/g creat.), $p = 0.015$ and $p = 0.001$, respectively. It was confirmed that amalgam is a predominant source of metallic Hg in non-occupationally exposed individuals. Similar results have been observed in past literature (Kingman et al., 1998; Nur Ozdabak et al., 2008; Dutton et al., 2013). Moreover, concentration of Hg–U remained unchanged after the start of fish consumption compared to the baseline. Therefore, no significant increase in a Hg–B concentration was caused by fish consumption.

4.2. Relationship between the biomarkers of exposure to Hg and Se status

It is well-known that concentration of Hg–U is indicative of exposure to IHg. We used determination of Hg–U and Se–U only for the purpose of the elements interactions examination and not because Hg–U is the main biomarker of environmental exposure to MeHg. In the study of a relationship between the biomarkers of exposure to Hg and Se status we noticed that along with a statistically significant increase-in the Hg–B concentration after one as well as two weeks of fish consumption compared to the baseline, an increased plasma level of Se was observed. This is justified because marine fish constitute the source of not only MeHg but also Se and a higher concentration of this element is observed in people eating, among others, fish meals. Concentration of Se–P in the second week of fish consumption remained at a similarly higher level compared to the first week. Moreover, only in the second week of fish consumption (when the Hg body burden was the highest), we observed a statistically significant correlation between Hg–B and Se–P. Ser et al. (2017) showed a positive correlation between Hg–B and Se–P, but only when concentration of MeHg exceeded the median level (1.16 μmol/L). Researchers suggest the presumable existence of a threshold of exposure to MeHg, above which the metabolism of Se and/or its selenoproteins may alter (including the increase in the Se–P level). The authors also indicated that results from the FFQ revealed a correlation between fish/whales consumption and Hg–B, but not Se–P. Thus, the examined correlation between Hg–B and Se–P does not necessarily indicate the co-intake and following co-accumulation (Ser et al., 2017).

Nevertheless, it may suggest that exposure to Hg reduces the pool of bioavailable Se in the body. Se might be involved in the formation of the insoluble and metabolically inactive biological HgSe compounds simultaneously protecting against toxicity of Hg (Raymond and Ralston, 2004). The relationship between the biomarkers of exposure to Hg and Se status in relation to total Hg–B and Se–P, visible in our short-term study, manifests itself in differences in the molar ratio - the amounts in moles of Hg and Se involved in chemical reactions, between the first as well as the second week of fish consumption versus baseline. At the same time (III sampling), we noticed a significant difference in Se:Hg molar ratio as compared to the time before the intervention. The mean Se:Hg molar ratio was not constant through the whole intervention study, but decreased along with the increasing Hg concentration. Besides, we discovered that a higher concentration of Hg–B during the fish consumption was more strongly related to Se:Hg molar ratio than the concentration of Se–P related to this ratio. The results presented by Chen et al. (2006), Ralston et al. (2008), Peterson et al. (2009) indicate that beneficial effects of Se on MeHg toxicity raise especially when Se:Hg molar ratios approach or exceed one. The Se:Hg molar ratio above one may mean that Se is available to form the above mentioned stable and inert MeHg-selenol complexes (Berry and Ralston, 2008; Khan and Wang, 2009). In addition, the elements first form equimolar complexes and next bind specifically to proteins (Yoneda and Suzuki, 1997; Suzuki et al., 1998). Thus, this ratio suggests that complexes formation is a dominant mechanism of detoxification. According to Peterson et al. (2009), this mechanism also indicates a reduced MeHg bioaccumulation or increase in its elimination. Thus, the molar ratio between these elements is regarded as a critical factor in Hg toxicity (Berry and Ralston, 2008) and principal factor in the risk assessment (Raymond and Ralston, 2004; Peterson et al., 2009; Ralston and Raymond, 2010). Ralston et al. (2008) correlated toxicity of MeHg regarding MeHg exposure and dietary Hg:Se molar ratios in blood/tissues derived from exposed rats. This study on the interaction of Hg and Se based on the combined exposure to different levels of these elements in rats has revealed that concentrations of Hg–B and Hg levels in tissues did not reflect the direct Hg toxicity and the risk from its exposure. The observed toxicity was proportional to the molar ratios in blood. Thus, the information about the risk of harmfulness assessments from MeHg exposure results from the calculated Hg:Se relationship, rather than from the concentration of Hg–B (Ralston et al., 2008). Orct et al. (2009) noticed that higher Se:Hg molar ratio impact on redistribution of Hg from plasma to erythrocytes. Moreover, co-administration in equimolar doses of Hg and Se are decisive to the highest uptake of Hg by plasma and liver and the lowest retention of Hg in the kidney and erythrocytes. Watanabe (2002) claimed that due to human variability in toxicodynamics, practical importance of modifying effects of Se on MeHg toxicity may be unclear.

On the other hand, Se bioavailability, based on urinary excretion, was the greatest in the first week of fish consumption and only during that time (with the exception of baseline) we noticed an association between urinary Se and Hg ($p < 0.0001$). The urinary interaction may indicate a regulated metabolism of Se. Through a metabolic pathway dietary Se was converted into methylated metabolites that are excreted through, among others, urine. Se present in fish is next metabolized to selenide ion in the body and then incorporated as a Sec into selenoproteins (Papp et al., 2010; Rayman et al., 2012). The major selenocompounds excreted in the urine are methylated Se species, particularly Se sugars (up to 53% of urinary total Se), trimethylselenonium ion (TMSe, up to 5% of urinary total Se) (Skerfving, 1978; Kuehnelt et al., 2006). The association was found between Hg and Se in urine in the baseline and in the first week between Hg–U and Hg–B, but not between Se–U and Se–P. It may signify that the initial short-term exposure to Hg interfered with the metabolism and excretion of Se. A study on animal model has confirmed that dietary Se increased elimination of MeHg from the body and that this mechanism occurs in a dose dependent manner (Komsta-Szumska et al., 1983; Bjerregaard et al., 2011). Komsta-Szumska et al. (1983) found that excretion of organic Hg via urine after co-administration of MeHg and Se was the highest in the first day and significantly diminished of excretion of organic Hg–U was observed only on day 13 (compared to days 1, 3, 7). Significantly lower excretion of both elements in our study was observed in the second week of fish consumption compared to the first week and even to the baseline. The latest research indicates the presence of demethylation of MeHg process in the intestines of marine fish (Feng et al., 2015; Wang et al., 2017; Li et al., 2018; Bjerregaard et al., 2018). Other studies suggest that Se can play a role in this process in fish, waterbirds and marine mammals (Palmisano et al., 1995; Eagles-Smith et al., 2009; Wang et al., 2017). Eagles-Smith et al. (2009) found that concentrations of Se and IHg in livers of birds were positively correlated above the demethylation threshold (hepatic Hg concentration 8.51 ± 0.93 μg/g dry weigh). The correlation between Se and IHg was negative below the demethylation threshold. Those authors claimed that demethylation is initiated because Se may act as a binding site for demethylated Hg and hence, reduce the possible secondary toxicity (Eagles-Smith et al., 2009). It is, therefore, reasonable to explain the fact that limited Se excreted through the urine is due to the formation of HgSe complexes in blood. It can be confirmed in our study by a positive correlation of Hg–B and Se–P concentrations in the second week of fish consumption. The results obtained in the study confirm the existence of a biological interrelationship between Hg and Se.

4.3. Relationship between the biomarkers of exposure to Hg and the biomarker of the pro-oxidant effect

Fish consumption was associated with a significant increase in the of concentration of the biomarker of lipid peroxidation. Baseline

TBARS-P had the same level of mean concentration as the control group in our previous paper concerning occupational (Hg^0) exposure (Kuras et al., 2018). Individuals exposure to Hg^0 (n = 131) with the median of exposure time of 4.0 years (the interquartile range IQR: 1.5–15.0 years, in the same workplace) and 15.0 years (IQR: 5.0–30.0 years, in the whole plant) had a similar concentration to the levels found in the MeHg exposed subjects in the first and second week of fish consumption ($TBARS\text{-}P_{occupational\ exposure}$: 2.61 ± 0.73 (min.-max. 1.26–4.78) vs. $TBARS\text{-}P_{one\ week}$: 2.51 ± 0.86 (min.-max. 1.16–5.44), $TBARS\text{-}P_{two\ weeks}$: 2.52 ± 0.85 (min.-max. 1.30–5.51). It may suggest that regardless of the chemical form of Hg (depending on the route of exposure) we can observe the its pro-oxidant effect. Moreover, TBARS-P concentrations among the workers from the chemical plant compared with the control have differed statistically $p = 0.0104$.

4.4. Relationship between the biomarkers of exposure to Hg and the biomarkers of the anti-oxidant effect

Another issue discussed in this paper is the impact of exposure to Hg on Se-biomarkers of the antioxidant effect, including the activity of the Se-dependent enzymes. In our study we found no correlations between Hg–B, Hg–H and Hg–U and the biomarkers of the anti-oxidant effect (with the exception of Hg–U and SeP–P after the first week of fish consumption). That is similar to the results obtained by Huang et al. (1995) in men from Sweden with different levels of fish consumption. Concentrations of Hg–P, Hg–B, Hg-RBC and Hg–U did not correlate with the concentration of SeP–P and activity of GPx3-P. Furthermore, those authors showed that Se–P raised slightly with increased fish consumption among the Swedish men, but no such increases were noticed in SeP–P concentration and the activity of GPx3-P (Huang et al., 1995). Similarly to Huang et al. (1995) we found no correlation between Se status and the previously mentioned Se-dependent proteins, whereas a positive relationship was found between the activity of GPx3-P and concentration of SeP–P. According to those authors, Se level from fish consumption did not affect Se incorporated into selenoproteins, which may support the notion that these Se-dependent proteins may be more desirable as a functional biomarkers than Se–P (Huang et al., 1995). Additionally, the significantly positive correlation between Hg–B and Se–P observed in our study may, on the other hand, imply that Hg intake through fish consumption interferes with Se utilization for selenoprotein biosynthesis (Svensson et al., 1992).

The study on plasma Se speciation has shown a different pattern of responses of GPx and SeP–P (Ser et al., 2017). Exposure to a high level of MeHg has revealed a positive correlation between Hg–B and SeP–P, but not GPx. No correlation has been found between Hg–B and two plasma selenoproteins during exposure to a low level of MeHg. Those authors explain (beyond the hypothesis of a threshold MeHg exposure), that the elevated Se–P levels in the group with a higher concentration of Hg might be correlated with the concentration of SeP–P because its transporting function for Se supply is demanded in various organs (Ser et al., 2017). In our study no significant changes in expression of SeP at both mRNA and protein levels were found during the whole trial ($p > 0.05$). It may confirm that exposure to Hg was not high enough for any interaction with SeP–P to take place. Another study has shown that in Latvian community in the case of a moderate exposure to MeHg (Hg-RBC 0.1–43.7 μg/L) a significant positive correlation between Hg-RBC and both selenoproteins (SeP–P and GPx3-P) occurred (Hagmar et al., 1998). In an Amazonian population (Hg–B 47.8 ± 36.3 μg/L) the result of a correlation between the concentration of Hg–B and activity of GPx1-RBC has been opposited (Grotto et al., 2010). Because cytosolic GPx is an intracellular antioxidant enzyme acting in the first line of defense, its activities may imply initialized detoxification mechanisms in individuals exposed to MeHg, as a response to the disturbed balance resulting from excessive ROS production. First activated protection system in a form of the activity of antioxidant enzymes, in the case of ongoing exposure might be later impaired. Thus, the antioxidant enzyme GPx activity changes due to disturbances in the body as a result of oxidative stress. Moreover, the reduction in the activity of Se-dependent enzymes in the second week of fish consumption may be a result of the above mentioned decreased Se bioavailability that is required for enzymatic activity. As a consequence, a lower concentration of Se in a body alters selenoprotein synthesis and further, its expression (Yoneda and Suzuki, 1997; Suzuki et al., 1998).

Se consumed from fish, may form Hg–Se complexes having effect on the amount of Se incorporated into the functional selenoproteins of plasma. The lack of correlation in our study between the biomarkers of exposure to Hg and activities of antioxidant enzymes as well as concentration of SeP–P after the first and the second week of fish consumption may suggest that changes in the levels of these Se-biomarkers of the antioxidant effect were not high enough to correlate with changes in Hg. Chen et al. (2006) found that fractions of various measured Se-containing serum proteins collected from people exposed to Hg included the least (the detection limit) Hg concentrations. The greatest Hg concentrations were present in both fraction of SeP and GPx. In addition, those authors have shown that fractions with SeP–P bound more Hg, which confirmed the strong relationship between SeP–P and Hg, but only when exposure to Hg was high.

Borderline significant decrease in SeP–P upon time ($p = 0.0584$) was observed in our study when baseline plasma selenium and baseline blood mercury concentrations were included in the multivariate linear regression model. This observation may confirm that SeP–P is involved in the transport of Hg in the blood but this mechanism largely depends on baseline selenium status and baseline level of Hg exposure.

4.5. Relationship between the biomarkers of exposure to Hg and expression of gene related to the antioxidant status

Because numerous studies indicate a protective role of Se and selenoproteins in the transport and binding (detoxification) of Hg in animal models (Garcia-Sevillano et al., 2015; Liu et al., 2018), we investigated whether there exists a relationship between the biomarkers of Hg and expression of mRNA for selenoproteins in the case of environmental (MeHg) exposure. We evaluated differences in the levels of *TRXR1* gene expression at each time point and the changes were significant. Although, inhibition of the thioredoxin system (*TRXR1, PRDX1*) by exposure to Hg was proved in our study, the lack of correlation between the biomarkers of exposure to Hg and *TRXR1* does not necessarily mean that there is no causal relationship. A number of findings based on both *in vitro* and *in vivo* models have revealed that Hg inhibits antioxidant protein components of the TrxR/Trx system as well as reduces their mRNA level. This statement is confirmed in the animal model (Franco et al., 2009; Wagner et al., 2010; Branco et al., 2011, 2012, 2014), homogenized tissues (Wagner et al., 2010) as well as human cells, like cervical carcinoma (HeLa) cells and (HEK 293) embryonic kidney cells (Carvalho et al., 2008) or neuroblastoma (SH-SY5Y) cells (Franco et al., 2009; Branco et al., 2017a). Some authors claim that a molecular mechanism of MeHg toxicity and its inhibition of the TrxR/Trx system is the main cellular target of Hg (Carvalho et al. (2008); Branco et al. (2017b)). In the study by Carvalho et al. (2008), authors claimed that this inhibition was selective towards TrxR, because TrxR is more sensitive than other selenoproteins due to highly nucleophilic structure. Because Sec residue is present in the open C-terminal the catalytic active site of TrxR, MeHg may bind to Sec causing enzyme inhibition (Brandt and Wassjohann, 2005). Because Branco et al. (2017b) indicated that Hg toxicity is associated with inhibition of the TrxR enzyme, they deliberately used the activity of TrxR as a biomarker of an early adverse effect of Hg toxicity.

Nevertheless, inhibition of the TrxR function can be restored by GPx, which acts as a backup system (Franco et al., 2009; Lu and Holmgren, 2014). However, in practice the GPx may be also inhibited by Hg action (Farina et al., 2009; Franco et al., 2009). Franco et al. (2009) claimed that the GPx may be the second essential target for

MeHg toxicity, because it is the crucial enzyme in neutralizing pro-oxidative effects. Recent research involving assumption of the overlapping functions of both GSH and TrxR redox systems describes the mechanism supporting the maintenance of redox potential - when one of the systems is inhibited, the latter one increases its activity to restore the antioxidant capacity in a cell (Avval and Holmgren, 2009; Du et al., 2012; Benhar et al., 2016). Wagner et al. (2010) found a dose-dependent inhibition of TrxR activity in the case of the *in vitro* model. MeHg treatment caused significant inhibition of renal and hepatic TrxR activity, while TrxR activity in brain was only slightly inhibited. The *in vivo* model confirmed that exposure to MeHg in male mice caused significant inhibition in the liver and kidney, in contrast to the brain. It may cause less sensitive to MeHg inhibition in brain and as a consequence 10 times lower deposition of MeHg than in the kidney and liver (de Freitas et al., 2009; Wagner et al., 2010).

Branco et al. (2014) described various mechanisms of IHg and MeHg toxicity, which is in opposition to our previous study concerning occupational exposure to Hg^0 (Kuras et al., 2018). Regardless of the Hg chemical form, we have found that Hg coming from both occupational as well as environmental sources significantly decreased *TRXR1* expression. According to Branco et al. (2014) the mechanism of a protective action of Se depends on the chemical form of Hg. After exposure to IHg these authors observed an increase in *TRXR1* expression in human hepatoma (HepG2) cells, whereas *TRXR1* expression was diminished after exposure to MeHg. Additionally, these authors investigated if MeHg changed the regulation of *TRXR1* transcription, which was visible in the analysis of translocation of the transcription factor Nrf-2 to the nucleus. In contrast to IHg, exposure to MeHg caused slower *TRXR1* transcription. Moreover, MeHg affected expression and activity of the anti-oxidant Trx/TrxR system and decreased *TRXR1* expression in HepG2 cells. In the end, simultaneous exposure to Se and Hg indicated that Se supports the TrxR/Trx system under toxicity of IHg, whereas no such a protective effect was seen after exposure to MeHg (Branco et al., 2014). Opposite reactions between concentration of Se–P and expressions of *TRXR1* and *PRDX1* were also confirmed also in our study. Reduction in the pool of bioavailable Se in the individuals from our study manifested itself by the diminished genes expression of Se-dependent enzyme *TRXR1*. The following thioredoxin-dependent *PRDX1* may indicate that the complexity of the reciprocal action within human selenoproteome may result from the dependence on Se intake and Se-dependent redox regulation. The well-known hypothesis that the protective effect of Se against Hg toxicity may in fact be opposite means that the impact of Hg exposure may cause Se deficiency status and hence, the direct inhibition of selenium's role in controlling the intracellular redox environment in the body.

Because this Se-dependent enzyme has a redox property, inhibition of TrxR may elevate levels of ROS. Therefore, MeHg may impair the redox state of cells through elevating Trx1 oxidative bias (Wataha et al., 2008). This may explain of another mechanism by which MeHg may induce oxidative stress, i.e. via TrxR inhibition. Wataha et al. (2008) claimed that a higher concentration of Hg temporarily changed in the cellular redox in monocytes that next generated a change in the regulation of the Nrf2 pathway and levels of Trx1. These results have been shown that the human Trx/TrxR/Prdx system is of high vulnerability and extraordinarily complex. Further research involving the analysis of transcriptional regulation of components of the TrxR/Trx system is necessary. Gene expression of thioredoxin-dependent *PRDX2* revealed a positive association with the concentration of Hg (the difference concerns a different matrix) before the intervention study, which is in agreement with the control group from the previous paper $r_s = 0.41$; $p = 0.001$ vs. $r_s = 0.30$; $p = 0.0151$, respectively (Kuras et al., 2018). This functional endogenous antioxidant enzyme under physiological conditions is responsible for the cellular redox regulation and antioxidant protection. It may suggest that a change in the level of the expression of thioredoxin-dependent *PRDX1* gene is involved in the antioxidant mechanism and is induced when the organisms confront oxidative stress caused by Hg (Kim et al., 2005). Changes in the antioxidant gene expression throughout the intervention study differed between selenoenzymes involved in the system of GSH and TrxR. Assuming that TrxR and GPx are the primary cellular targets of Hg (except for other important selenoproteins, which were not included in this paper), it is surprising that in our study after the study subjects started the fish consumption not only GPx (a Se-dependent enzyme) activity was elevated but also its (*GPX1*) expression. Moreover, significantly elevated expression of *GPX1* gene in the second week of fish consumption substituted the inhibited GPx1 enzyme, de novo enzyme in order to minimize harmful effects of Hg, which confirmed this statement. Additionally, in our study mRNA encoding *GPX1* and *GPX3* genes revealed a different response to Hg exposure, suggesting that the influence of different levels of Hg on GPx expression may vary depending on the enzyme isoform.

Shankar and Mehendale (2005) described the mechanism of toxicant interference on a cell and its ability to maintain redox balance. If any toxic substance e.g. Hg, generates ROS, the growth of gene expression of antioxidant enzymes is expected, while diminished antioxidant gene expression may signify that Hg interferes with the cell's ability to metabolize current ROS present in the cell. Animal as well as *in vitro* models have shown, that overexpression of *GPX1* has a beneficial effect on the prevention of the pro-oxidative states. Thus, exposure to Hg in our study altered expression of oxidative-stress related genes, implying Hg caused oxidative stress in the study volunteers in two ways.

5. Conclusion

Despite the fact that the calculated in our previous paper (Kuras et al., 2017), permissible Hg concentrations in the fish meals consumed within two weeks were not exceeded, exposure to Hg caused a short-term imbalance between the biomarkers of pro- and anti-oxidant effects. It was observed by the increased lipid peroxidation with a simultaneous decrease in the total antioxidant activity in the plasma. A reversible disruption of oxidative balance in the study volunteers exposed to Hg may suggest partly functional deactivation manifested by a significant decrease in the levels of mRNA encoding *TRXR1* as well as *PRDX1*, which are notably sensitive to Hg. Nevertheless this mechanism is a short-term trend rather than a permanent phenomenon. At the same time, we noticed that selenoproteins not included in TrxR/Trx system, responded to Hg exposure differently. The significantly increased Se-dependent enzyme GPx1-RBC and GPx3-P activities at both mRNA and protein levels may indicate induction of an antioxidant response pathway in the exposed individuals, acting as a compensatory mechanism. Thus, interaction between Hg and Se through the activity of selenoproteins may include a variety of toxicological and biochemical processes. Future research should explain whether Se-dependent proteins, examined in our paper, may be used as functional biomarkers to assess exposure to MeHg in the general population as a significant part of the exposure dependence assessment - possible health effects.

Declaration of interests

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.

Conflict of interest

The authors declare that they have no conflict of interest.

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