

# THE EFFECT OF POLYUNSATURATED FATTY ACIDS ON CARDIOVASCULAR RISK FACTORS AND URINARY MERCURY IN WORKERS EMPLOYED AT ELECTROLYTIC PRODUCTION OF CHLORINE

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## Abstract

**Objectives:** While inorganic mercury is being gradually withdrawn from industry, environmental exposure to mercury is recognized as one of the greatest present toxicological problems. The aim of this study was to evaluate the effect of polyunsaturated fatty acids (PUFAs) supplementation on selected cardiovascular risk factors and the urinary mercury (Hg-U) concentration in workers occupationally exposed to mercury vapor. **Material and Methods:** Overall, 38 workers of an electrolyzer hall (Hg-U:  $46.6 \pm 35.7$   $\mu\text{g/g}$  creatinine) and a control group of 60 employees not exposed to Hg (Hg-U:  $4.3 \pm 15.5$   $\mu\text{g/g}$  creatinine) were included in a clinical cross-over study. Clinical and laboratory tests were carried out 4 times: before and after a 3-month period of PUFAs supplementation (1000 mg daily), then after a 3-month break, and then after another 3-month period of PUFAs supplementation. **Results:** The baseline heart rate (HR) and serum triglyceride levels were higher in the Hg-exposed workers than in the controls, whereas systolic blood pressure (SBP) and cholesterol (C) levels exceeded normal values in both groups. There was a positive correlation between high-density lipoprotein 3 cholesterol (HDL<sub>3</sub>-C) and Hg-U levels. The PUFAs use was associated with a decrease in both HR and SBP. After the first stage of supplementation, a decrease in the Hg-U concentration was observed. In a multivariate logistic regression model, decreases in Hg-U were associated only with exposure to mercury;  $\text{OR}_{\text{Hg}} = 0.562$  (95% CI: 0.323–0.979),  $p < 0.042$ . After the second 3-month period of PUFAs supplementation, a significant association between HDL<sub>3</sub>-C and a Hg-U decrease was shown:  $\text{OR}_{\text{HDL}_3} = 1.222$  (95% CI: 1.01–1.46),  $p < 0.033$ . **Conclusions:** In the workers exposed to mercury vapor, PUFAs supplementation led to some beneficial effects on HR and SBP. The first stage of supplementation was associated with a decrease in Hg-U in which HDL<sub>3</sub> metabolism probably plays an important role. *Int J Occup Med Environ Health.* 2021;34(4):551–64

## Key words:

blood pressure, heart rate, HDL-C, PUFA, mercury exposure, urinary Hg

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## INTRODUCTION

Anthropogenic release of mercury occurs from manifold industrial point sources and coal-fired power plants. It is estimated that over 2000 tons of mercury are emitted annually into the atmosphere [1]. According to EU Directives and the Minamata Convention, the use of mercury in industrial technologies should be withdrawn in all countries in the world [2].

In the EU, in the near past, occupational exposure to mercury vapor occurred mainly in electrolyzer halls during chlorine production. Until 2015, some employees of the chlor-alkali industry were still exposed to mercury vapor despite the implementation of mercury-free technologies. The main source of exposure to mercury were electrolyzers. On removal of the hardware and waste containing mercury, the contamination was spread to environment. Mercury in different chemical forms may be found in the air, such as elemental mercury in the fumes from coal-fired power plants, waste incinerators and crematoria, as well as in the water, e.g., methyl mercury compounds in fish and seafood, in the soil, e.g., in the landfills of industrial waste, and in the human body, e.g., in amalgamated dental fillings [3,4].

Metallic mercury vapors are highly toxic, affecting mainly the brain, the kidneys and the liver. Inhalation of mercury vapor causes severe pneumonia, which can often be fatal. Mercury has a high affinity for sulfhydryl groups (-SH) of aminoacids, proteins, enzymes, and sulfur-containing antioxidants such as glutathione, the most potent intracellular and mitochondrial antioxidant [5].

The least known is the effect of mercury on the cardiovascular system. However, it has been shown that mercury toxicity may be associated with hypertension, coronary heart disease, cerebrovascular accident, and carotid atherosclerosis [5,6]. It should be noted that in those factories in which mercury is used in the production process, factory workers are often people with many risk factors for cardiovascular diseases (CVDs), such as hypertension and lipid

disorders [6]. For this reason, CVDs prevention in workers exposed to mercury vapor is especially relevant.

Polyunsaturated fatty acids (PUFAs) seem to exert cardio-protective activity. Among them, omega-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may prevent CVDs through antiarrhythmic effects, blood viscosity reduction, platelet aggregation inhibition, inflammation suppression, blood vessel function improvement, as well as plasma fibrinogen and insulin resistance reduction [7]. However, people who have had a heart attack do not seem to benefit greatly from PUFAs supplementation [8]. On the other hand, in animal experiments, dietary fish oil supplements and DHA or EPA have been found to decrease myocardial infarct size [9], and to exert cardio-protective effects against ischemia and reperfusion in isolated hearts [10]. An omega-3 PUFAs-enriched diet diminishes infarct size also in humans but, to observe this effect, the omega-3/omega-6 PUFAs ratio should be >1:5 [11]. Multiple hypotheses are considered to explain the molecular mechanisms of cardio-protective omega-3 PUFAs action: the activation of the Akt pathway [8], the interaction with peroxisome proliferator-activated receptor gamma, the activation of G-protein-coupled receptors, the decrease in cyclooxygenase-2 gene expression, and the inhibition of nuclear factor  $\kappa$ B [12,13].

In addition to the effect of PUFAs on the circulatory system, there are indications that these acids reduce the toxicity of heavy metals in animals and humans [14,15]. Although the results of several systematic reviews and meta-analyses have provided insufficient evidence for the cardio-protective effects of PUFAs supplementation [16], in this study, the PUFAs wide action has become the rationale for adding PUFAs to the diet of the employees exposed to mercury in chlorine production. The aim of the study was to assess the effect of PUFAs supplementation on selected cardiovascular risk factors (heart rate [HR], blood pressure and serum lipids) and the urinary mercury (Hg-U)

concentration (as a measure of total mercury burden) in the workers exposed to mercury vapors, compared to the control group.

## MATERIAL AND METHODS

### Subjects

The study was conducted in a group of 98 male workers of a chemical factory in which mercury had been used for chlorine production. The study group consisted of 38 men aged 21–57 years, employed in the electrolyzer hall involving exposure to mercury vapors for a period of 1–40 years. They worked in a 4-shift work system as electrolyzer operators, mechanics and shift supervisors. The electrolyzer operators' tasks included controlling the electrolyzers, pumps, water flow and sumps filling, looking for leaks in the installation, as well as washing service platforms and floors in the production hall. The mechanics were engaged in the installation, de-installation and renovation of equipment, including equipment that had come into direct contact with mercury. The shift supervisors controlled the operations for handling the processing line in the chlorine production department. All the employees were provided with standard protection (clothing, shoes, gloves, goggles, helmets and masks with mercury absorbers). The control group consisted of 60 men aged 24–66 years, working in the same chemical plants over a period of 1–42 years but without exposure to mercury.

Each man from the study and control groups was asked not to consume fish for 3 days prior to urine sample collection. This information was included in the prepared questionnaire. The purpose of limiting fish consumption was to reduce the impact of mercury originating from its main dietary source.

Based on the questionnaire, lifestyle, daily diet, modes of food preparation and stimulants intake were evaluated. The majority (55%) of the employees who participated in the study led medium-active lifestyles. Their diet was usually rather monotonous (81%), rich in meals that included meat. Overall, the fat dietary intake was high and exceeded

the recommended daily share of fats in the majority of respondents (72%). The most common method of preparing meals was frying and stewing. Only a small number of workers (<1%) declared daily consumption of raw vegetables and fruit. There were no significant differences between the study and control groups in terms of diet and physical activity. The percent of people having  $\leq 5$  dental amalgam fillings was similar (44.7% and 48.3%, respectively) in the study and control groups. The body mass index (BMI) was calculated based on the respondents' anthropometric data (height and body weight) collected using calibrated equipment. Blood pressure was measured using a sphygmomanometer, according to the International Protocol of the European Society of Hypertension (2003), and HR was estimated from the electrocardiogram.

No worker reported any prior CVDs or symptoms typical of mercury poisoning. Physical examinations and laboratory tests were performed 4 times:

- at the beginning of the study (stage I),
- after a 3-month period of PUFAs supplementation (stage II),
- after a 3-month break from PUFAs (stage III),
- after another 3-month use of PUFAs (stage IV).

All the respondents provided their written informed consent to participate in the study. All the procedures used were in accordance with the ethical standards of the responsible local Bioethics Committee (No. KB-125/2015) and with the Helsinki Declaration as revised in 2013.

### Supplementation

In both the study and control groups, the supplemented PUFAs were Smart Omega (GALENA, Wrocław, Poland) used at a daily dose of 1000 mg for 3 months, twice, with a 3-month break. The product was taken every day, at the same time, after the main meal. Each capsule of the supplement contained 500 mg of fish oil including 36% of EPA and 24% of DHA. The concentration of heavy metals (lead, cadmium, mercury and arsenic) in the prepa-

ration did not exceed the permissible limits according to Commission Regulation (EC) No. 1881/2006 of December 19, 2006. Zinc in the supplement was found at trace concentrations (<30 ppm).

### Biochemical measurements

Venous blood samples were taken after 12 h of fasting, and centrifuged at 1000 g for 20 min at a temperature of 4°C. Serum samples were stored at a temperature of -80°C. The total serum cholesterol (TC), serum triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured using the Spinreact enzymatic assay (SantEsteve De Bas, Girona, Spain). The low-density cholesterol (LDL-C) level was estimated among patients with a TG concentration of <4.52 mmol/l (400 mg/dl) by means of the Friedewald formula. The QUANTOLIP® HDL precipitation test (Technoclone GmbH, Vienna, Austria) was used to measure the HDL<sub>2</sub>-C and HDL<sub>3</sub>-C levels. The serum non-HDL-C was calculated as a difference between the TC and HDL-C concentrations.

Urine samples were collected in the morning, before the work shift. The Hg-U concentration was determined using an atomic absorption spectrophotometer Solaar M6 by ThermoElemental Co., in addition to snap VP100 for the generation of cold vapor. The wave length used for this measurement was  $\lambda = 253.7$  nm with deuterium background correction using a quartz atomization chamber. The validation parameters in the Hg-U determination were as follows: the linear dependence of absorbance readings on mercury concentration in working solutions found for concentrations of 4–96 mg/l; the limit of detection of 0.63 mg/l; and the limit of quantification of 1.26 mg/l. Correctness, expressed as relative load (%), was calculated on the basis of measurements of the reference material ClinChec®-Controls Urine Control level I by Recipe (a concentration of 2.30 mg/l), and amounted to 1.30%. Correctness was also calculated on the basis of measurements of the reference material, i.e., Reference

Seronorm™ Trace Elements Urine L-2 (a concentration of 44.0 mg/l; whereby the sample was diluted 5 times in order to obtain a concentration of 8.8 mg/l), and amounted to 9.02%.

Repeatability, as a parameter of precision of the applied method, was 7.17%. In 2015–2017, the laboratory participated in the G-EQUALS external quality assurance program at the University of Erlangen-Nuremberg and obtained certificates. The serum concentrations of copper and zinc were determined by flame absorption spectrometry (FAAS) in air-acetylene flame at wave lengths of 324.8 nm and 213.9 nm, respectively, with deuterium background correction. Calibration curves were determined using CPI International Single-Element Cooper (Zinc) Standard at 1000 µg/ml. Seronorm™ Trace Elements Serum certified serum metal standards were used to control the content of copper and zinc.

### Statistical analysis

The results were presented as a mean  $\pm$  standard deviation ( $M \pm SD$ ) or a median and interquartile range (IQR). In the case of normal distribution, paired t-tests were applied and statistical significance between the means was calculated using ANOVA tests. Non-parametric tests were used in the case of non-normal distribution; the Mann-Whitney U test and the Kolmogorov-Smirnov test were used for the comparison of 2 independent samples, the Kruskal-Wallis test with a post hoc test was used for multiple independent samples, and the Wilcoxon test or the ANOVA rank Friedman test were used for the comparison of dependent samples. Contingency tables with Fisher's exact test and the  $\chi^2$  test were used for qualitative variables. The association between the functional and metabolic changes induced by PUFAs and Hg-U (as a continuous variable) was analyzed in the multivariable linear regression model. The association between qualitative changes in Hg-U (a decrease or no decrease, in the 0-1 system) and predictors was analyzed in the multivariable

linear regression model. The correlations between variables were checked using Spearman's rank coefficient. A p-value of  $<0.05$  was accepted as statistically significant. Statistical package SPSS v. 13.3 was used for statistical analysis.

## RESULTS

At the beginning of the study, the mean anthropometric data and the mean period of work experience were similar in both the study and control groups. However, the mercury-exposed workers were 3 years younger on average. In all workers, about 40% were tobacco smokers and the percentage of smokers was higher in the study group (60%) than in the control group (22%) (Table 1). About 90% of workers in the study and control groups reported small or moderate alcohol consumption: about 18%  $>5$  u/month, 67% 5–20 u/month, and 5%  $>2$  u/day. The most commonly consumed type of alcohol was beer.

In both groups, the median BMI value was in the overweight range. Simultaneously, the mean C levels exceeded normal values, with the mean TC level of  $>190$  mg/dl and the mean non-HDL-C level of  $>145$  mg/dl. The cholesterol subclasses levels were similar in the study and control groups. The mean TG concentration was significantly higher ( $p < 0.01$ ) in the group of workers exposed to mercury.

In both groups, the mean values of blood pressure, systolic (SBP) and diastolic (DBP), were similar and slightly above the norm. The resting HR was significantly faster in the study group ( $p < 0.01$ ).

The medium Hg-U concentration in the men exposed to mercury vapor was many times higher in comparison to the controls ( $p < 0.001$ ), whereas the serum copper and zinc concentrations were similar (Table 1).

In the group of all 98 workers, the 25th percentile of Hg-U was  $0.98 \mu\text{g/g}$  of creatinine, whereas the 75th percentile was  $32.96 \mu\text{g/g}$  of creatinine. The distribution of Hg-U values was log-normal.

The analysis of all parameters depending on cigarette smoking revealed a much higher HR ( $78 \pm 12$  vs.  $71 \pm 9$ ,  $p < 0.001$ ), serum TG ( $234 \pm 183$  vs.  $157 \pm 92$ ,  $p < 0.01$ ) and Hg-U ( $28.9 \pm 33.3$  vs.  $15.0 \pm 30.4$ ,  $p < 0.01$ ) in smokers compared to non-smokers. Five employees who stopped smoking in the last few months were not included in the analysis.

As the number of smokers in the study and control groups was different, and as there were some differences in HR, TG and Hg-U between smokers and non-smokers, as well as between Hg-exposed and non-exposed workers, the impact of smoking and occupational exposure to mercury on these parameters was analyzed. In the multifactorial linear regression models, HR was associated with smoking, TG with BMI, smoking and occupational exposure to Hg, whereas Hg-U was associated only with occupational exposure (Table 2).

After excluding smoking from confounders, occupational exposure to Hg was significantly associated with HR ( $p = 0.0047$ ,  $\beta = 0.2964$ , 95% CI: 0.09–0.49), as well as with TG ( $p = 0.0007$ ,  $\beta = 0.3304$ , 95% CI: 0.14–0.51) and Hg-U ( $p = 0.0000$ ,  $\beta = 0.6991$ , 95% CI: 0.47–0.80).

Two cycles of 3-month PUFAs supplementation were found to cause some functional and metabolic changes (Table 3).

Both in the study and control groups, the PUFAs-induced decrease in HR appeared together with a significant decrease in SBP. The t-tests for dependent samples showed that the changes in lipid metabolism were non-significant except for the HDL<sub>3</sub>-C level which increased after the first round of supplementation (in the study group, the difference between stages I and II was statistically significant;  $p < 0.05$ ) and decreased after the second PUFAs supplementation in both the study and control groups (Figure 1).

Simultaneously, in the group of all 98 workers, the first supplementation with PUFAs resulted in a significant reduction ( $p < 0.0001$  in the Wilcoxon pairs of order tests)

**Table 1.** Characteristics of the workers occupationally exposed to mercury vapor and a control group of non-exposed men who received polyunsaturated fatty acids preparation in April 2016–April 2017 at the Wrocław University Medical Center, Wrocław, Poland

Variable	Participants (N = 98)		p
	mercury-exposed (N = 38)	non-exposed (N = 60)	
Age [years] (M±SD)	37.6±10.2*	41.3±7.2	0.0392
Work experience [years] (Me (IQR))	12.0 (2.0–20.0)	17.0 (9.0–25.0)	0.0896
Height [cm] (M±SD)	1.77±0.06	1.79±0.06	0.1939
Body mass [kg] (M±SD)	82.7±12.8	86.8±11.8	0.1081
BMI [kg/m <sup>2</sup> ] (M±SD)	26.2±3.3	27.0±3.3	0.2527
Smokers [n (%)]	23 (60.5)***	13 (21.6)	0.0002
Low/moderate alcohol drinkers [n (%)]	34 (90)	54 (90)	0.5935
Amalgam fillings >5 [n (%)]	2 (5.2)	5 (8.3)	0.7026
Heart rate [min <sup>-1</sup> ] (M±SD)	78±12.2**	72±8.2	0.0048
Blood pressure [mm Hg] (M±SD)			
systolic	144±19.8	142±18.3	0.7148
diastolic	91±12.7	92±12.6	0.8563
Cholesterol [mmol/l] (M±SD)			
total	5.92±1.57	5.76±0.97	0.5804
LDL	3.07±1.16	3.43±0.91	0.1101
HDL			
total	1.6±0.36	1.55±0.34	0.4567
HDL <sub>2</sub>	0.49±0.2	0.42±0.2	0.1470
HDL <sub>3</sub>	1.1±0.25	1.11±0.21	0.8385
non-HDL	4.31±1.67	4.21±0.96	0.7416
TG [mmol/l] (M±SD)	2.68±2.1**	1.73±0.92	0.0030
Creatinine-U [mg/ml] (M±SD)	1.52±0.67	1.78±0.67	0.0600
Hg-U [µg/g cre] (Me (Q25; Q75))	40.7 (14.9; 73.9)***	1.1 (0.7; 1.8)	0.0000
Cu-S [µg/dl] (M±SD)	105.8±16.4	105.2±15.0	0.8524
Zn-S [µg/dl] (M±SD)	106.0±11.9	103.0±13.0	0.3208

Creatinine-U – urine creatinine concentration; Cu-S – copper in serum; HDL – high density lipoprotein; Hg-U – urine mercury concentration; IQR – interquartile range; LDL – low density lipoprotein; TG – triglycerides; Zn-S – zinc in serum.

Statistically significant differences in comparison to the control group: \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

in the Hg-U concentration: from 21.0±3.3 µg/g of creatinine in stage I to 16.3±3.2 µg/g of creatinine in stage II. After a 3-month break in PUFAs use, mercury concen-

tration increased to a level higher than the initial value (22.0±5.0 µg/g of creatinine in stage III) and remained nearly unchanged after the second cycle of PUFAs sup-

**Table 2.** An assessment of the relationship between the heart rate, triglycerides, urinary mercury, and smoking or occupational exposure to mercury vapor (with age and body mass index [BMI] as confounders), April 2016–April 2017, Wrocław University Medical Center, Wrocław, Poland

Variable	b	t	p	b±SD	95% CI
Heart rate					
age	0.0256	0.197	0.1976	0.0200±0.105	−0.18–(−0.22)
BMI	0.3220	1.004	0.3177	0.1024±0.101	−0.10–0.30
smoking	3.1724	2.644	<b>0.0096</b>	0.2907±0.109	0.07–0.50
exposure to Hg	1.9225	1.605	0.1119	0.1752±0.109	−0.04–0.39
Serum triglycerides					
age	0.1740	0.108	0.9140	0.0105±0.097	−0.18–0.20
BMI	11.9340	2.970	<b>0.0038</b>	0.2909±0.097	0.09–0.48
smoking	30.8010	2.074	<b>0.0408</b>	0.2169±0.104	0.00–0.42
exposure to Hg	35.8050	2.415	<b>0.0177</b>	0.2522±0.104	0.04–0.45
Urinary mercury					
age	0.0028	0.008	0.9928	0.0007±0.084	−0.16–0.16
BMI	0.5895	0.731	0.4664	0.0621±0.084	−0.10–0.23
smoking	−1.7178	−0.577	0.5652	0.0522±0.090	−0.23–0.12
exposure to Hg	21.8291	7.351	<b>0.0000</b>	0.6633±0.090	0.48–0.84

Parameterization with sigma-restrictions in the linear regression model. Bolded are  $p < 0.05$ .

plementation ( $21.9 \pm 6.4$   $\mu\text{g/g}$  of creatinine in stage IV). However, the Hg-U concentration distribution differed between the study and control groups. Although a significant decrease in Hg-U was observed after the first period of PUFAs supplementation in both mercury-exposed workers and the control group, measurements at stages III and IV revealed a persistent decrease in Hg-U in the study group and a return of Hg-U to initial values in the control group (Table 3, Figure 2).

Different Hg-U changes at individual time points were not accompanied by other intergroup differences in PUFAs effects (Table 3).

A multivariate logistic regression model was used to answer the question of whether there was an association between a decrease in Hg-U (with a decrease or no decrease in Hg-U expressed in the 0-1 system as the qualita-

tive variable) induced by the first or second PUFAs supplementation, and smoking or exposure to mercury (qualitative predictors). Age, BMI, the duration of exposure to mercury and lipid levels (TC, LDL-C, HDL-C, HDL<sub>2</sub>-C, HDL<sub>3</sub>-C and TG, measured before each PUFAs supplementations) were used as potential quantitative predictors. A multifactorial model was built by the backward stepwise approach in the logistic regression method using a validation mechanism based on a v-fold cross-scoring in 12 steps.

After the first 3-month period of PUFAs supplementation, only a significant association between the decrease in Hg-U concentration and exposure to mercury was observed:  $OR_{\Delta\text{Hg}} = 0.562$  (95% CI: 0.323–0.979),  $p < 0.042$ .

After the second 3-month period of PUFAs supplementation, no significant associations between changes in

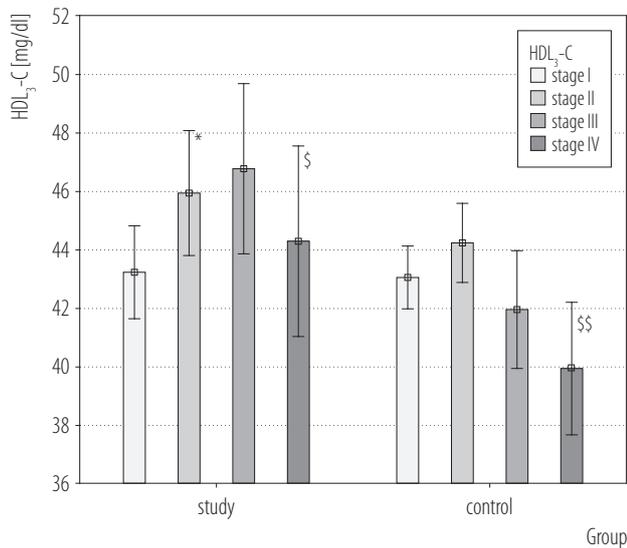
**Table 3.** The functional and metabolic parameters measured in the workers exposed to mercury vapor and the control group during polyunsaturated fatty acids supplementation in April 2016–April 2017 at the Wrocław University Medical Center, Wrocław, Poland

Variable	study group (N = 38)				Participants (N = 98)			
	stage I	stage II	stage III	stage IV	stage I	stage II	stage III	stage IV
Heart rate [min <sup>-1</sup> ] (M±SD)	78±12.2	68±6.1***	76±9.47	70±7.93**	72±8.2	61±6.3***	73±7.18	68±6.19**
Blood pressure [mm Hg] (M±SD)								
systolic	144±19.8	137±17.0***	145±25.1	138±19.0**	142±18.3	136±14.5***	145±19.2	140±17.3***
diastolic	91±12.7	87±8.73	89±16.5	88±7.17	92±12.6	89±6.65	93±14.5	90±7.11
Cholesterol [mmol/l] (M±SD)								
total	5.92±1.57	5.81±1.74	6.08±1.57	5.87±1.62	5.76±0.97	5.68±0.89	6.23±0.78	5.79±0.94
LDL	3.07±1.16	2.94±1.01	3.25±1.21	3.12±1.33	3.43±0.91	3.41±0.83	3.95±0.76	3.54±0.94
HDL								
total	1.60±0.36	1.60±0.45	1.62±0.41	1.52±0.42	1.55±0.34	1.52±0.34	1.45±0.35	1.39±0.37
HDL <sub>2</sub>	0.49±0.20	0.43±0.18	0.38±0.13	0.38±0.11	0.42±0.20	0.43±0.20	0.33±0.16	0.37±0.15
HDL <sub>3</sub>	1.10±0.25	1.17±0.29*	1.21±0.29	1.14±0.32*	1.11±0.21	1.14±0.25	1.12±0.21	1.03±0.23**
non-HDL	4.31±1.67	4.18±1.93	4.29±1.76	4.34±1.85	4.21±0.96	4.13±0.92	4.60±0.77	4.37±0.82
TG [mmol/l] (M±SD)	2.68±2.10	2.74±3.58	1.87±1.78	2.36±2.82	1.73±0.92	1.61±0.83	1.72±0.91	1.82±1.35
Hg-U [µg/g cre] (Me (Q25; Q75))	40.7 (14.9; 73.9)	28.6 (12.5; 62.1)*	27.9 (11.9; 64.3)	23.8 (11.8; 66.2)	1.10 (0.7; 1.8)	0.48 (0.13; 1.46)***	1.47 (0.69; 1.89)	1.27 (0.47; 1.7)

Abbreviations as in Table 1.

Stage I – at the beginning of the study, stage II – after a 3-month period of PUFAs supplementation; stage III – after a 3-month break in supplementation; stage IV – after a 3-month period of re-supplementation.

Statistically significant differences between the parameters measured at stages I and II or between the parameters measured at stages III and IV, \* p &lt; 0.05, \*\* p &lt; 0.01, \*\*\* p &lt; 0.001.



Data given as  $M \pm SD$ .

Stages as in Table 3.

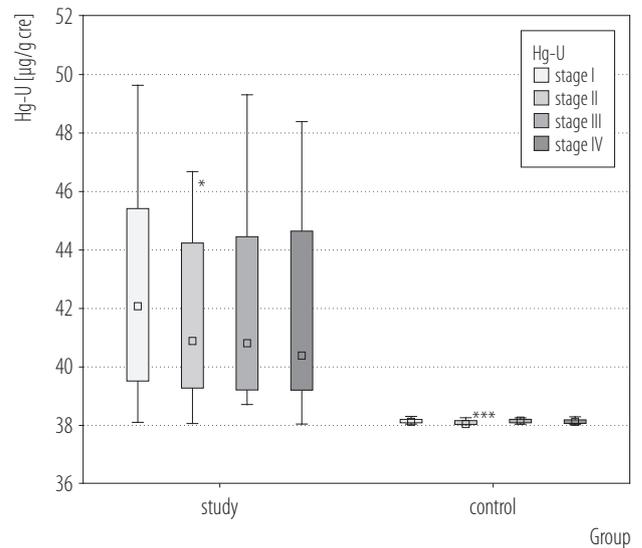
A statistically significant difference between HDL<sub>3</sub>-C values measured at stages I and II: \*  $p < 0.05$ .

A statistically significant difference between HDL<sub>3</sub>-C values measured at stages III and IV: §  $p < 0.05$ ; §§  $p < 0.01$ .

**Figure 1.** The high-density lipoprotein 3 cholesterol (HDL<sub>3</sub>-C) in the workers exposed to mercury vapor and the control group during polyunsaturated fatty acids supplementation in April 2016–April 2017 at the Wrocław University Medical Center, Wrocław, Poland

the Hg-U concentration and predictors were found. However, the parameter evaluation showed an association between HDL<sub>3</sub>-C and a Hg-U decrease:  $OR_{HDL_3} = 1.222$  (95% CI: 1.01–1.46,  $p < 0.033$ ). Attention should also be paid to the positive linear relationship between Hg-U and HDL<sub>3</sub>-C at the beginning of the study ( $p < 0.01$ ) (Figure 3).

There was no significant relationship between serum trace metals and Hg-U concentrations. Over the period of observations, serum copper and zinc levels remained similar to the initial values in both the study group (Cu:  $106.5 \pm 18.1$  mg% vs.  $105.7 \pm 13.9$  mg% and Zn:  $113.9 \pm 16.8$  mg% vs.  $106.0 \pm 11.9$  mg%) and the control group (Cu:  $105.7 \pm 13.9$  mg% vs.  $105.2 \pm 15.0$  mg% and Zn:  $109.1 \pm 13.6$  mg% vs.  $103.3 \pm 13.0$  mg%).



The box-plot represents median and Q25; Q75, whiskers in the box-plot – a range of min.–max values.

Stages as in Table 3.

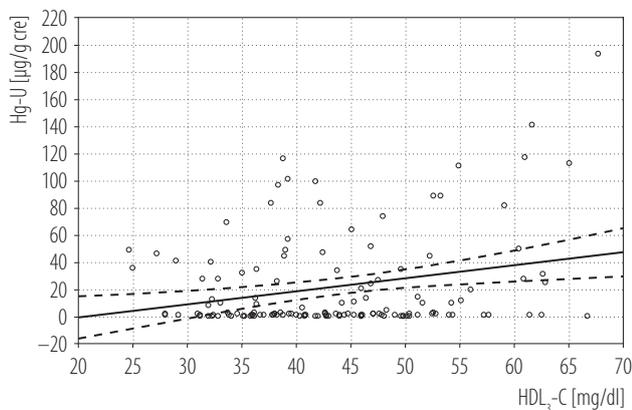
Statistically significant differences between the Hg-U measured at stages I and II: \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ .

**Figure 2.** The mercury urine concentration (Hg-U) in the workers exposed to mercury vapor and the control group during polyunsaturated fatty acids supplementation in April 2016–April 2017 at the Wrocław University Medical Center, Wrocław, Poland

## DISCUSSION

Occupational or environmental exposure to heavy metals such as lead, cadmium, and mercury is known to be associated with increased cardiovascular morbidity and mortality [5,6]. Some researchers suggest that mercury or lead toxicity should be evaluated in any patient with coronary atherosclerosis, cerebrovascular accident or hypertension [6].

Since PUFAs seem to have a cardio-protective role, in the authors' opinion, their preventive use in middle-aged men occupationally exposed to mercury vapor was justified. The PUFAs supplementation in the group of workers with high levels of Hg-U ( $46.6 \pm 35.7$  µg/g creatinine) was further justified as there were other cardiovascular risk factors present, such as smoking (60% of workers), elevated TG levels, increased HR and a tendency to elevated blood



**Figure 3.** The correlation between the serum high-density lipoprotein 3 cholesterol ( $\text{HDL}_3\text{-C}$ ) and mercury urine concentration (Hg-U) in the workers exposed to mercury vapor and the control group during polyunsaturated fatty acids supplementation in April 2016–April 2017 at the Wrocław University Medical Center, Wrocław, Poland

pressure. The main reason for the increased HR and TG may have been smoking, as the number of smokers among the mercury-exposed workers was significantly higher than in the non-exposed group. Moreover, in the multifactorial linear regression model, smoking was significantly associated with heart rate as well as with serum TG (Table 2). However, increased HR and TG may have also resulted from a higher mercury body burden in the workers exposed to mercury vapor. After excluding smoking from confounders, occupational exposure to Hg was still significantly associated with both HR and TG ( $p < 0.01$  and  $p < 0.001$ , respectively).

In other studies, populations occupationally exposed to mercury exhibited an increased resting HR and/or hypertriglyceridemia, even after considering traditional risk factors and exposure to other heavy metals [17,18]. Increased resting HR and affected HR variability in the mercury-exposed population have been explained as consequences of increased sympathetic or decreased parasympathetic activity [17]. The hypothesis about the impaired sympathovagal control of the heart was confirmed in experimental studies performed on rats poisoned with low-dose inor-

ganic mercury [18]. However, the results of other studies on adults with environmental exposure to mercury were heterogeneous and did not show any consistent associations between mercury concentrations and cardiac autonomic function [19].

In this study, workers exposed to mercury, similar to controls, displayed mean SBP values above the accepted norm. This is compatible with the results obtained in other studies [6,18]. In the meta-analysis, a significant positive association between mercury levels and blood pressure values was identified [7]. In this study, a correlation between mercury levels and blood pressure was not shown. Also, in some other studies, no significant association between blood mercury and SBP or DBP was observed [20,21].

Notably, PUFAs, when supplemented for 6 months as was done in this study, led to a significant decrease in HR and SBP, in both the study and control groups. These beneficial changes in the cardiovascular system were undoubtedly associated with PUFAs supplementation; they appeared after the first period of supplementation, were no longer observed after the break in the supplementation and then reoccurred after the second period of PUFAs supplementation. These effects of PUFAs on the circulatory system are known [22,23] and were expected. However, the surprising and more interesting result of this study was that PUFAs significantly reduced the Hg-U concentration after a 3-month supplementation period. This statistically significant Hg-U decrease was observed in both the study and control groups. In the logistic regression model, the PUFA-induced Hg-U decreases were significantly dependent only on occupational exposure to mercury. In the Hg-exposed workers, these Hg-U decreases were less frequent than in the control group. And, *vice versa*, in the control group (environmentally exposed to Hg), the chance for a reduction in Hg-U by PUFAs was greater than for people exposed professionally to mercury.

The Hg-U concentration is an indicator of chronic exposure to mercury, in contrast to the mercury concentration

in the blood, which is an indicator of acute exposure, or the concentration of mercury in the hair, which is a good indicator of fish consumption [24,25]. The association between PUFAs and Hg-U may be important in the context of the environmental exposure to mercury, e.g., a common use of fish as an important source of both PUFAs and mercury.

The mechanism behind the observed changes in the Hg-U concentration is unclear. It is worth noting that relatively small doses of PUFAs were used in the study, which did not affect lipid concentration, apart from the effect on HDL<sub>3</sub>-C. On the other hand, the authors demonstrated the positive linear correlation between HDL<sub>3</sub>-C and Hg-U at the beginning of the study.

Mortazavi et al. [26] showed a positive dependence between serum HDL-C and the blood mercury concentration in a group of 3713 Korean men with metabolic syndrome. The authors hypothesized that the elevated blood mercury concentration may have been a factor that led to an increase in the serum HDL-C concentration in these men. In fact, HDL<sub>3</sub> is the predominant HDL subclass with a density range of about 1.125–1.21 g/ml. It is a protein-enriched subclass (the mean protein-to-lipid ratio is 55:45 by weight), with apoA-I and apoA-II as the major structural peptides. There are currently some discrepancies between the preclinical and clinical data regarding the beneficial role of HDL<sub>2</sub> and HDL<sub>3</sub> in cardiovascular prevention, with more evidence pointing to HDL<sub>3</sub> [27]. On the other hand, an increase in these HDL subclasses does not always mean an improvement in the cardio-protective potential [27].

Mercury effects included the appearance of acute phase proteins [28], which might be associated with impaired HDL function [29]. Lately, it was shown that mercury might be responsible for the existence of dysfunctional HDL through inactivation of paraoxonase, an extracellular anti-oxidative enzyme related to HDL. Such a dysfunction in HDL can manifest itself as an impairment in the reverse cholesterol transport, anti-inflammatory, an-

tioxidant and antithrombotic functionalities [5]. In other studies, Hg-U was positively and significantly related to total- and oxy-LDL-C, indicating mercury as an indirect cardiovascular risk factor [30].

In this study, in the group of workers with high Hg-U, a small but significant increase in HDL<sub>3</sub> after the first PUFAs supplementation and, similar to the control group, a decrease in HDL<sub>3</sub> after the second PUFAs supplementation were observed. It is uncertain whether these changes in the serum HDL<sub>3</sub> are connected with the changes in Hg-U observed during PUFAs use. It is possible as there is a close connection between HDL<sub>3</sub> and PUFAs metabolism. On the level of the cellular membrane, HDL<sub>3</sub> stimulates the conversion of phosphatidylethanolamine into phosphatidylcholine through a sequence of 3 trans-methylation reactions, leading to PUFAs entry into the brain [31]. The HDL<sub>3</sub> particle, while participating in trans-methylation reactions, could also participate in PUFAs entry into the kidney. It is probable since the reno-protective effects of long-chain omega-3 PUFAs supplements have been observed [32].

Inhaled elemental mercury is absorbed through the mucous membrane of the lungs, rapidly oxidized to other forms, and stimulating the synthesis of metallothionein, it is cumulated mainly in the kidney [33]. Given that an approximately linear relationship between mercury in the kidney and mercury in urine was documented [34], the decrease in Hg-U after the first PUFAs supplementation may reflect a decreased mercury accumulation in the kidney. It would be consistent with the observation that omega-3 PUFAs attenuate mercury toxicity in the kidney [35]. In turn, an association between HDL<sub>3</sub>-C and the decrease in Hg-U after the second PUFAs supplementation, and the positive linear relationship between HDL<sub>3</sub>-C and the Hg-U levels at the beginning of the study, seem to confirm the interaction between mercury and HDL<sub>3</sub>-C.

The limitations of this study include a small size of the study group (38 workers exposed to mercury vapor).

Another limitation is the lack of a control group of subjects with no previous exposure to mercury, since the control individuals involved in the study were exposed to low mercury levels due to local pollution. Although the effect of PUFAs on Hg-U and the relationship between Hg-U and HDL<sub>3</sub> found in this study were statistically significant, the size effects were small suggesting that further studies would be necessary.

It needs to be highlighted that the presented study concerning the consequences of occupational exposure to mercury vapor may be one of the last such studies in Europe thanks to new technologies of mercury-free chlorine production. In the future, results of these studies can provide reference in the interpretation of the health effects of mercury present in the environment. The authors believe that the results of their study warrant research in a wider population.

In summary, the results of this study indicate that elevated baseline HR, TG and SBP in the workers with high Hg-U levels are associated with smoking as well as occupational exposure to mercury. A low-dose PUFAs supplementation (1000 mg/day), while having no significant effect on TG, reduced HR and SBP in the workers both with low and elevated Hg-U levels. In addition to the PUFAs beneficial effects on the cardiovascular system, after the first 3 months of supplementation a decrease in Hg-U levels, accompanied by an increase in serum HDL<sub>3</sub>-C, was observed both in the study and control groups. The relationship between HDL<sub>3</sub>-C and a decrease in Hg-U was confirmed in the multivariate logistic regression model.

## CONCLUSIONS

The PUFAs supplementation at a small dose of 1000 mg daily for 3 months in the workers exposed to mercury vapor leads to a decrease in HR and SBP, as well as to a decrease in the Hg-U concentration after the first cycle of PUFAs supplementation. The effect of PUFAs on Hg-U is possibly mediated by the HDL<sub>3</sub> metabolism. The PUFAs

supplementation might be beneficial in populations environmentally exposed to mercury which warrants further studies.

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