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REVIEWS: CURRENT TOPICS

Mercury as a risk factor for cardiovascular diseases☆,☆☆

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Abstract

Mercury is a heavy metal that exists naturally in the environment. Major sources include the burning of fossil fuels (especially coal) and municipal waste incineration. Mercury can exist in several forms, with the most hazardous being organic methylmercury. In waterways (lakes, rivers, reservoirs, etc.), mercury is converted to methylmercury, which then accumulates in fish, especially in large predatory fish. Fish and fish products are the major—if not the only—source of methylmercury in humans. Mercury has long been recognized as a neurotoxin for humans, but in the last 10 years, its potentially harmful effects on cardiovascular diseases (CVD) have raised a cause for concern, mostly due to the proposed role of mercury in oxidative stress propagation. Some epidemiological studies have indeed found an association between increased levels of mercury in the body and risk of CVD. There are several plausible mechanisms to explain the association; these are discussed in this review. We also review the epidemiological studies that have investigated the association between mercury and CVD.

Keywords: Cardiovascular diseases; Epidemiology; Heavy metals; Mercury; Risk factors

1. Introduction

Fish is an important source of nutrients for humans. It is an excellent source of proteins, vitamin D, selenium and, especially, long-chain $n-3$ fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The beneficial effects of fish consumption on the prevention of cardiovascular diseases (CVD), especially CVD-related mortality, have been increasingly recognized [1]. However, some fish species can also contain harmful substances such as polychlorinated biphenyls, dioxins and other environmental contaminants, such as methylmercury. These contaminants are present at low levels in water systems (lakes, rivers, reservoirs, oceans, etc.) but bioconcentrate in the aquatic food chain, reaching their highest levels in large and old predatory fish and marine mammals.

Mercury is a heavy metal that is naturally present in the environment. It is an environmental pollutant that exists in three forms: elemental or metallic mercury, inorganic mercury compounds and organic mercury. For most people, quicksilver, used, for example, in thermometers, may be the most familiar form of elemental mercury. Dental amalgam fillings, which consist of about 50% mercury, are an example of inorganic mercury compounds. Organic mercury is found mainly in fish as methylmercury and in some vaccines as ethylmercury (thimerosal).

The global cycle of mercury begins with the evaporation of mercury vapor into the atmosphere. The main sources of this mercury are burning of fossil fuels, particularly coal, and municipal waste incineration. Mercury is also released by natural evaporation from the sea and land surfaces and by volcanoes. Chloralkali plants and paper pulp factories discharge mercury compounds as waste into waterways, and there has also been an extensive use of mercury in gold mining [2]. Mercury vapor is stable and can reside in the

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Table 1
Mercury levels in selected fish

Species	Mercury concentration (mg/kg)		
	Mean	Range	Reference
Burbot	0.22–0.26	0.20–0.37	[4]
Catfish	0.05	ND–0.31	[5]
Cod	0.10–0.11	ND–0.42	[5]
Herring	0.04	ND–0.14	[5]
Mackerel, king	0.73	0.23–1.67	[5]
Perch, freshwater	0.14	ND–0.31	[4,5]
Perch, ocean	ND	ND–0.03	[5]
Pike	0.38–0.40	0.15–0.85	[4]
Pike–perch	0.11–0.30	0.06–0.37	[4]
Pollock	0.04	ND–0.78	[5]
Salmon	0.01–0.07	ND–0.19	[4,5]
Sardine	0.02	0.004–0.35	[5]
Shrimp	ND	ND–0.05	[5]
Shark	0.99	ND–4.54	[5]
Swordfish	0.98	ND–3.22	[5]
Tilefish	1.45	0.65–3.73	[5]
Trout, freshwater	0.07	ND–0.68	[4,5]
Tuna	0.38	ND–1.30	[5]
Whitefish	0.03–0.08	ND–0.31	[4,5]

ND = mercury concentration below the detection level (0.01 mg/kg).

atmosphere for about 1 year; thus, it is capable of being distributed to even the remotest regions [2]. Mercury eventually returns to the Earth's surface and finds it way into water sources, where it is converted by microorganisms to the highly toxic form, organic methylmercury. This form bioaccumulates in the aquatic food chain and is found in all species of fish and fish-eating animals [2]. In humans, too, major exposure to methylmercury occurs via food, with the major sources being fish and fish products [3]. Almost 100% of the mercury present in fish is methylmercury [3]. The highest concentrations are found in large and old predatory fish, such as sharks, swordfish, tuna and pike. These fish may have levels exceeding 1.0 ppm (1.0 mg/kg). Most fish accumulate <0.5 ppm. A list of methylmercury levels in selected commercial fish and shellfish is shown in Table 1. A more extensive list can be found on the US Food and Drug Administration (FDA) website [5]. Methylmercury in fish is primarily bound to proteins; thus, skinning and trimming of fish do not significantly reduce its concentrations [1].

Dietary methylmercury is well absorbed from the gastrointestinal tract, readily enters the bloodstream and is distributed to all tissues in about 30 h [2]. It also crosses the placental barrier with ease and enters the fetal circulation [6]. About 5% of the body load is found in the blood compartment and about 10% is found in the brain [2]. Methylmercury also accumulates in hair and toenails, which both can be used as indicators of long-term mercury exposure in population studies [7,8]. The hair-to-blood concentration ratio is about 250:1 [7]. In the body, methylmercury is mainly, if not exclusively, bound to the sulfur atom of thiol ligands [2]. Methylmercury is metabolized to inorganic mercury prior to elimination via feces, but the rate of conversion is slow (the half-life is about 70–80 days) [8]. This, together with the fact that the human body has no way of excreting mercury actively, means that mercury continues to accumulate in the body throughout life.

The concern about methylmercury in fish is linked to its high toxicity. It is known to exert major toxic effects on the central nervous system (CNS). The developing brain, in particular, is vulnerable to methylmercury toxicity, leading to several neurodevelopmental disorders [6]. The exposure of adults to high levels of methylmercury has also resulted in extensive neurological damage and mortality [6], with the most severe examples being the extensive outbreaks of mercury poisoning that occurred in Iraq in the early 1970s due to the consumption of seeds that had been treated with methylmercury to prevent fungal growth, and in Minamata Bay in Japan in the 1950s when the local population consumed highly contaminated fish after an industrial spill of methylmercury into that bay [2].

The concentration of mercury in fish, even for humans consuming only small amounts of fish (10–20 g/day), can markedly affect the intake of methylmercury [7]. In 1997, the US Environmental Protection Agency reduced the recommended safe daily intakes of methylmercury from 0.5 to 0.1 μg/kg body weight [9]. US Environmental Protection Agency and US FDA have also issued recommendations on fish consumption based on methylmercury content [10] (i.e., women of childbearing age and young children should avoid fish with methylmercury levels around 1.0 ppm). They should also limit their consumption of commonly eaten fish and shellfish that have lower levels of mercury to an average of two meals a week (total: 12 oz, ≈340 g). If no advisories for local waters are available, the consumption of game fish should be limited to one meal per week and no other fish should be consumed. For other individuals, the recommendations are not as strict, but regular consumption of fish with a high methylmercury content should nevertheless be avoided.

In recent years, more attention has been given also to other possible adverse effects of methylmercury exposure, in addition to CNS damage (i.e., its contribution to CVD). This stems predominantly from initial epidemiological findings from Finland that a high mercury content in hair was associated with an increased progression of atherosclerosis and risk of CVD [11,12]. Subsequent research has confirmed these findings [13,14]. It is noteworthy that these adverse effects on CVD have been observed at methylmercury levels much lower than those associated with neurotoxicity.

The mechanisms by which mercury exerts its negative effects are not fully understood. However, its high affinity for thiol groups and its ability to bind selenium into an insoluble complex could reduce antioxidative defenses and promote free radical stress and lipid peroxidation in the human body [12]. This review initially evaluates the studies that have

Table 2
Studies on the association of tissue levels of mercury with the risk of CVD and atherosclerosis

Study; nationality; author, year	Type of study; follow-up	Sex	*n*	Outcome	Mercury sample	Mean level	Main results
KIHD study; Finnish; Salonen et al., 1995 [12]	Prospective; 6.0 years	Male	1833	MI, CHD death, CVD death, any death	Hair	1.9 μg/g	Clearly higher risk of MI and all-cause death among men with high levels of mercury; nonsignificantly higher risk of CHD and CVD deaths among men with high levels of mercury
KIHD study; Finnish; Salonen et al., 2000 [11]	Prospective; 4.0 years	Male	1014	Progression of carotid atherosclerosis	Hair	1.8 μg/g	Mercury accumulation associated with accelerated progression of atherosclerosis, as assessed by CCA-IMT
KIHD study; Finnish; Rissanen et al., 2000 [16]	Prospective; 10.0 years	Male	1871	MI	Hair	1.9 μg/g	High content of mercury attenuated the protective effect of fish-oil-derived fatty acids on risk of MI
Swedish study; Swedish; Hallgren et al., 2001 [18]	Nested case–control; 1.7 years	Female, male	78+156	MI	Erythrocyte	4.44–5.42 ng/g	Inverse association between risk of MI and the biomarkers of fish intake, including erythrocyte levels of mercury
EURAMIC study; eight European countries and Israel; Guallar et al., 2002 [14]	Case–control	Male	684+724	MI	Toenail	0.14–0.57 μg/g	High mercury level directly associated with higher risk of MI
Health Professionals Follow-up Study; American; Yoshizawa et al., 2002 [17]	Nested case–control; 5.0 years	Male	470+464	CHD	Toenail	0.72–0.74 μg/g	Mercury level not significantly associated with risk of CHD
KIHD study; Finnish; Virtanen et al., 2005 [13]	Prospective; 13.9 years	Male	1871	MI, CHD death, CVD death, any death	Hair	1.9 μg/g	High mercury level was associated with increased risk of MI, CHD, CVD and any death, and attenuated the protective effect of fish-oil-derived fatty acids

described the association between mercury and CVD end points (Table 2). We have limited our review to only include studies that have also reported data about fish consumption (either dietary intake, markers of intake or long-chain $n-3$ fatty acids). Subsequently, we explore possible mechanisms behind the negative effects of mercury on CVD.

2. Mercury and the risk of CVD: epidemiological observations

The potential harmfulness of mercury in CVD was first observed in the Kuopio Ischemic Heart Disease Risk Factor (KIHD) study cohort, which was published in 1995 by Salonen et al. [12]. The KIHD study is an ongoing prospective population-based study that is designed to investigate risk factors for CVD, atherosclerosis and related outcomes in a population-based randomly selected sample of men from eastern Finland [15]. A total of 2682 men aged 42–60 years participated in baseline examinations in 1984–1989 when hair samples were collected for mercury analyses. Men were followed up, on average, for approximately 6 years. During this time, 73 acute coronary events and 78 deaths occurred, of which 24 were CVD deaths and 18 were coronary heart disease (CHD) deaths, in 1833 men free from prior CVD. The mean hair mercury content was 1.9 μg/g, ranging from 0 to 15.7 μg/g. The adjusted risk of acute coronary events and deaths from any cause was significantly increased in men in the highest third of hair mercury content ($\geq$2.0 μg/g) compared with other men [risk ratio (RR)=1.96, 95% confidence interval (CI)=1.23–3.13 for acute coronary events; RR=2.26, 95% CI=1.43–3.56 for any deaths]. The risk of CVD and CHD mortality was also increased but did not reach significance, probably due to the smaller number of events. Similar elevations in risks, along with 56% higher mean hair mercury content ($P<.001$), were also observed in men consuming $\geq$30 g/day fish compared with men consuming less than that amount. In addition, in a subsample of subjects ($n=187$) for whom measurements of serum immune complexes containing oxidized low-density lipoprotein (LDL) were available, both hair mercury content and high urinary mercury excretion were significantly associated with elevated titers of these complexes, supporting the theory of a role for mercury in the promotion of lipid peroxidation.

In the follow-up study by Rissanen et al. [16], the observation time of the KIHD study cohort was extended by

4 years. During the average follow-up time of 10 years, 194 acute coronary events occurred in 1871 CVD-free men. One important observation from this paper was that a high content of mercury in hair attenuated the beneficial effects of fatty acids from fish on the risk of acute coronary events. In the whole cohort, the adjusted risk of an acute coronary event was 1.44 (95% CI=1.11–1.65) in men in the highest fifth of the proportion of serum DHA+DPA compared with men in the lowest fifth. However, when the cohort was stratified according to hair mercury content, the risk of an event was reduced by 67% (95% CI=19–87%) in men in the highest fifth of serum DHA+DPA but with hair mercury <2.0 μg/g, while the risk was reduced only by 24% in men with hair mercury ≥2.0 μg/g.

The latest follow-up study of this cohort supports both of these findings. In the study by Virtanen et al. [13], the follow-up time was extended by four additional years, with the mean follow-up time now being 13.9 years. During this time, 282 acute coronary events, 132 CVD, 91 CHD and 525 all-cause deaths had occurred. As in previous studies, the men were divided into thirds based on their hair mercury content. In the whole cohort, men in the highest third of hair mercury content (≥2.0 μg/g) had an extensively adjusted 1.60-fold (95% CI=1.24–2.06) risk of acute coronary event, 1.68-fold (95% CI=1.15–2.44) risk of CVD, 1.56-fold (95% CI=0.99–2.46) risk of CHD and 1.38-fold (95% CI=1.15–1.66) risk of any death, compared with men in the lower two thirds. Furthermore, for each microgram of mercury in hair, the risk of acute coronary event increased, on average, by 11% (95% CI=6–17%), the risk of CVD death increased by 10% (95% CI=2–19%), the risk of CHD death increased by 13% (95% CI=3–23%) and the risk of any death increased by 5% (95% CI=1–9%).

Men were then divided into two groups based on hair mercury content, and the effect of DHA+DPA on the risk of the abovementioned outcomes was studied. In men with low hair mercury content (<2.0 μg/g), the risk of an acute coronary event was reduced by 31% (95% CI=9–48%), the risk of CVD death was reduced by 41% (95% CI=11–51%) and the risk of CHD death was reduced by 57% (95% CI=16–75%) for each percentage unit increase in DHA+DPA proportion of all fatty acids in serum. The interaction between hair mercury and serum DHA+DPA was also found to be statistically significant. No reductions in risks were seen in men with high hair mercury content (≥2.0 μg/g). In addition, the risk of any death was not statistically significantly reduced in men with low hair mercury content, most likely due to the large number of non-CVD-related deaths occurring in this category.

The fish intake of men in the highest third of hair mercury content, as assessed by a 4-day food recording, was over two times higher than that consumed by men in the lowest third (65 vs. 30 g/day, $P<.001$), and hair mercury correlated with the intake of fish ($r=.27$, $P<.001$) and serum DHA+DPA concentration ($r=.25$, $P<.001$). This suggests that fish is the main source of mercury in this study population.

So far, the KIHD study has been the only one to report about the negative effects of mercury on the progression of atherosclerosis. Salonen et al. [11] studied 1014 men from the KIHD study cohort for whom complete information about hair mercury content and carotid atherosclerosis was available. Carotid atherosclerosis was determined by ultrasonographic assessment of common carotid artery intima–media thickness (CCA-IMT). In the multivariate linear regression model, a high hair mercury content was the second strongest predictor of CCA-IMT progression during the 4-year follow-up from baseline examinations, being superceded only by systolic blood pressure. For each microgram per gram of hair mercury, there was an increment of 8 μm (7.3% of the mean increase) in the 4-year increase in the mean CCA-IMT ($P<.001$). When the men were divided into fifths of hair mercury content, the increase in the mean CCA-IMT was 32% higher in the highest fifth than in the lower fifths after adjustment for relevant confounders.

In 2002, the same issue of the *New England Journal of Medicine* contained two papers about mercury and risk of CVD. In the first paper, Guallar et al. presented results from the EURopean community multicenter study on Antioxidants, Myocardial Infarction and breast Cancer (EURAMIC) study from eight European countries and Israel [14]. Using a case–control design, they evaluated the joint association of mercury and DHA with the risk of myocardial infarction (MI) in 684 cases and 724 control men aged 70 years old or younger. Mercury was measured from toenail clippings, and DHA was measured from subcutaneous adipose tissues. After adjustment for multiple CVD risk factors, the case:control ratio of mercury concentration was 1.15 (95% CI=1.05–1.25). The odds ratio (OR) in the highest toenail mercury fifth compared to the lowest toenail mercury fifth was 2.16 (95% CI=1.09–4.29), after adjustment for traditional risk factors and antioxidants. One other interesting finding was that, although a high DHA concentration was not associated with a decreased risk of MI after adjustment for age and center, introduction of mercury into the model resulted in OR=0.66 (95% CI=0.42–1.03) in the highest DHA fifth compared to the lowest fifth. This suggests that a high mercury content may diminish the beneficial effects of fish consumption on cardiovascular health, as was originally observed in our KIHD cohort.

The other study, published by Yoshizawa et al. [17], used a nested case–control design with 470 cases and 464 controls consisting of 40- to 75-year-old men from the Health Professionals Follow-up Study to investigate the association between toenail mercury concentration and risk of CHD. Dentists, who have an occupational exposure to mercury vapor via amalgam, accounted for 63% of controls. Mean toenail mercury levels were also higher in dentists than in nondentists (0.91 vs. 0.45 μg/g, respectively; $P<.001$). After adjustment for CHD risk factors, the RR in the highest mercury fifth was 0.97 (95% CI=0.63–1.50)

compared to the lowest mercury fifth. Adjustment for the intake of $n-3$ fatty acids from fish (EPA and DHA) did not change the result. The authors then excluded the dentists from the analyses and found a multivariate-adjusted RR=1.27 (95% CI=0.62–2.59) for the highest fifth of mercury compared with the lowest fifth. After additional adjustment for EPA and DHA, RR=1.70 (95% CI=0.78–3.73). Statistical power was substantially reduced in the analyses, which excluded the dentists (220 cases), explaining the statistically nonsignificant results. However, the increase in risk after controlling for $n-3$ fatty acids from fish is consistent with the results from the KIHD and EURAMIC studies. A repeat study with a larger nondentist study population would be of great interest.

One study not supporting the negative effects of mercury in CVD was conducted by Hallgren et al. [18] in Sweden. This was a prospective nested case–control study of 78 cases of first MI and 156 controls. Unlike previously discussed studies, this study included women ($n=48$). Erythrocyte mercury was used as a marker for mercury exposure. Both erythrocyte mercury and plasma EPA and DHA levels were higher in subjects reporting high fish intake than in those reporting low intake. A high plasma EPA+DHA concentration was associated with a decreased risk of MI. However, in contrast with previously discussed studies, a high mercury concentration (>6 ng/g erythrocyte), compared with a low concentration (≥6 ng/g), was also associated with a decreased risk of MI, although the association was not statistically significant (multivariate OR=0.51, 95% CI=0.21–1.24). There are at least two explanations for the apparent discrepancy. First, the mercury levels were low. Only in two subjects did the concentration of erythrocyte mercury exceed the corresponding hair mercury value of 2.0 μg/g. Thus, the range of erythrocyte mercury may have been too narrow to exert sufficient statistical power to detect an association. Alternatively, if mercury exerts its negative effects only after a certain threshold value has been exceeded, then the levels in this study may have been too low and erythrocyte mercury may have been simply a marker of the beneficial effects of fish intake against CVD. Currently, there are insufficient data to set a threshold value after which mercury would exert its negative effects. This threshold might be fraught with difficulties since it might well be population-dependent due to differences in the population intake of dietary antioxidants and selenium, or differences in their genetic-based defenses. In the KIHD study, the risk of CVD was increased after the hair mercury content reached about 2.0 μg/g [10,11].

3. Possible mechanisms of mercury toxicity in CVD

3.1. Mercury–selenium interaction

The trace mineral selenium is an essential nutrient for humans. As selenocysteine, it is a component of selenoproteins; about 35 selenoproteins have been identified so far [19]. Some of these selenoproteins have important enzymatic functions; generally, they contain selenocysteine at their active site. Perhaps the best-known example of a selenoprotein with enzymatic activity is glutathione peroxidase, which has an important role in the protection against lipid peroxidation (discussed in more detail in section 3.2). Severe selenium deficiency is rare in humans, but it has been seen in some parts of China, where selenium levels in soil are extremely low. Keshan disease, an endemic cardiomyopathy, and Kashin–Beck disease, a deforming arthritis, were first identified in these regions [19]. Less severe selenium deficiency has also been linked to several conditions, such as cancer and CVD [19]. In addition to regions in China, in comparison with North America, selenium levels are also low in Europe. For example, Finland started supplementing agricultural fertilizers with selenium in the mid-1980s.

Mercury has a high affinity for selenium, and it readily binds selenium to form insoluble mercury selenide complexes [20]. This interaction was first observed by Ganther et al. [21] in 1972. They discovered that sodium selenite had an alleviating effect against methylmercury-induced mortality in rats. Another early study found that both mercury and selenium coaccumulated in the autopsied tissues of mercury miners [22]. This interaction between mercury and selenium may represent one mechanism through which mercury increases the risk of CVD. Mercury could reduce the bioavailability of selenium and, in that way, impair the activity of glutathione peroxidase, thus promoting lipid peroxidation and, subsequently, atherosclerosis. In addition, one way for the body to eliminate mercury is by binding to glutathione, further decreasing cellular defenses against oxidation. Glutathione–mercury complexes appear to be the primary form in which mercury is transported and eliminated from the body [23]. However, at present, there is very little evidence from human studies to support the hypothesis. In fact, in the study by Yoshizawa et al. [17], the significantly increased risk of CHD, which was associated with high mercury levels, was found among those men with the highest levels of toenail selenium. The combination of high mercury and low selenium was not associated with higher risk. However, as the authors stated, these observations could be due to chance. The numbers of cases and controls in these stratified analyses were rather low.

On the other hand, this interaction could also function in the opposite direction (i.e., high selenium levels could protect against excess mercury). The inorganic selenium compound, selenium dioxide, has been shown to be effective in the inactivation of mercury in the intestinal tract of rats when the two compounds were administered simultaneously (i.e., the absorption of mercury was reduced) [24]. The organic compound seleno-DL-methionine was ineffective in this respect. However, in humans, supplementation with 100 μg/day selenomethionine for 4 months has been shown to lower mercury levels in the

body, as indicated by a 34% decrease in mercury levels in pubic hair [25]. Since selenium is the only potential naturally occurring chemical that could regulate mercury levels in the body, these findings could have significant public health importance.

3.2. Promotion of lipid peroxidation

Oxidation of LDL is considered to be a key event in the development of atherosclerosis, and oxidized LDL particles are found in atherosclerotic lesions [26–29]. An increased plasma concentration of LDL leads to an increased deposition of LDL particles inside the artery wall, where they are prone to suffer from oxidative damage [30]. Oxidized LDL levels in the circulation show a positive relationship with IMT and with the progression of atherosclerosis in carotid arteries, as well as with plaque occurrence in the carotid and femoral arteries in men [31–33]. They also exhibit a positive relationship with the severity of acute coronary syndromes [28] and are biomarkers for CHD risk [34,35].

Lipid peroxidation is an autocatalytic process initiated by free radicals (e.g., superoxide anion, hydrogen peroxide and lipid peroxide), which are produced in the body primarily as a result of aerobic metabolism [36]. Transition metal ions, particularly divalent ions such as iron and copper, can further catalyze the reaction [37]. These molecules contain one or more unpaired electrons, which can react with other molecules and create new radical molecules. Lipid peroxidation occurs when lipids are damaged by free radicals [38]. In this process, polyunsaturated fatty acids in cell membranes (e.g., phospholipids in LDL) undergo degradation via a chain reaction. The presence of oxidized LDL particles attracts monocytes into the artery wall, where they eventually differentiate into macrophages. Macrophages scavenge oxidized LDL particles and accumulate intracellular lipids in artery walls [30]. This induces macrophages to release proinflammatory cytokines, which further promote monocyte recruitment and accumulation of lipid-rich macrophages or foam cells. These are the most predominant cell types present in early atherosclerotic lesions [30]. Antioxidants (e.g., vitamin E, vitamin C, selenium and glutathione) and antioxidative enzymes (e.g., glutathione peroxidase, catalase and superoxide dismutase) scavenge free radicals and thus protect LDL against peroxidation.

No published data demonstrating an association between mercury exposure and blood lipid or lipoprotein levels were found. However, mercury has been shown to promote atherosclerosis both in rats and in humans [11,39,40]. There are several mechanisms by which it can promote lipid peroxidation and subsequent atherosclerosis. First, mercury has a very high affinity for sulfhydryl groups (i.e., it can bind to and inactivate antioxidative thiolic compounds such as glutathione [23,41], which plays a critical role in regenerating vitamins C and E from their oxidized byproducts) [42]. Glutathione is particularly important in antioxidative defenses, since it is the most abundant low-molecular-weight intracellular thiol [42]. For example, acute administration of mercury chloride to rats has been shown to evoke toxic effects in the kidney, liver and lungs, with this damage being associated with increases in lipid peroxidases and a significant reduction in glutathione levels [43]. An increase was also observed in myeloperoxidase activity — an index of neutrophil infiltration in oxidant-induced tissue injury. Administration of two antioxidants, melatonin and *N*-acetylcysteine, could reverse these effects [43]. Mercury poisoning, which is associated with increased lipid peroxidation in the liver and kidneys, also results in inactivation of superoxide dismutase and catalase [44], which are important enzymes in scavenging hydrogen peroxide. Likewise, mercury can inactivate paraoxonase, an important high-density lipoprotein-bound antioxidative enzyme that has been associated with the risk of CHD [45,46].

Second, by binding to selenium, mercury can reduce the bioavailability of selenium for incorporation into glutathione peroxidase. There are at least four different glutathione peroxidases, and all contain selenium in their active site [47]. Glutathione peroxidase 1 uses glutathione to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols [48]; together, they constitute the principal antioxidant defense system in mammalian cells [49,50]. A dietary deficiency in selenium has been shown to markedly decrease tissue glutathione peroxidase activity and to result in peroxidative damage and mitochondrial dysfunction [51]. Furthermore, in patients with coronary artery disease, a low level of activity of red cell glutathione peroxidase 1 has been observed to be independently associated with an increased risk of fatal and nonfatal cardiovascular events [52].

Third, mercury, as a transition metal, can act as a catalyst in Fenton-type reactions, which result in the formation of highly reactive hydroxyl free radicals. This is supported by observations that antioxidants and oxygen radical scavengers can protect against methylmercury toxicity both in vitro and in vivo [53–56]. In vitro, mercury ions increased the production of superoxide anions in human neutrophils [57] and caused an increase in mitochondrial hydrogen peroxide production [58]. The electron transport chain in the mitochondria is the principal site of cellular production of reactive oxidants superoxide and hydrogen peroxide. It has been demonstrated that mercury alters the structural integrity of the mitochondrial inner membrane, resulting in loss of normal cation selectivity [59]. Addition of mercury to mitochondria isolated from the kidneys of rats has resulted in a concentration-related depolarization of the inner mitochondrial membrane, increased hydrogen peroxide formation, glutathione depletion and the formation of thiobarbituric acid reactive substances [60]. Mercury-induced oxidative damage to the mitochondria has also been observed in several other tissues in vitro, including myocardial tissues [60]. Exposure of monocytes to methylmercury chloride led to reactive oxygen species (ROS) generation, thiol depletion, altered mitochondrial function

and apoptosis [61]. However, there are some indications that mercury may not have any major effect on the direct nonenzymatic peroxidation of LDL at physiological concentrations, but may promote peroxidation via some indirect mechanism, such as the peroxidase system [62].

The oxidation hypothesis is also supported by our earlier report from the KIHD cohort, where high hair mercury content and high urinary mercury excretion were associated with elevated titers of immune complexes containing oxidized LDL [12]. Thus, mercury can induce lipid peroxidation through a variety of mechanisms and, in these ways, promote the progression of atherosclerosis and subsequent CVD.

3.3. Hypertension

There are limited data on the association between methylmercury and hypertension. In a study with 917 seven-year-old children from the Faroe Islands, prenatal methylmercury exposure was associated with increased blood pressure [63]. Diastolic blood pressure and systolic blood pressure increased by 13.9 and 14.6 mmHg, respectively, when cord blood mercury concentrations increased from 1 to 10 μg/L. No further increase was seen above this level. The effect was found to be greater in children with a birth weight below the group median and to be smaller in those with a birth weight above the median. Similar but weaker relationships were seen with maternal hair methylmercury. Especially in boys, the increase from 1 to 10 μg/L was also associated with a 47% decrease in heart rate variability (HRV), but no effect on heart rate was observed. These effects were suggested to be attributable to the action of methylmercury on the parasympathetic nervous system [63].

The study was repeated in the same cohort when the children were 14 years of age [64]. The follow-up consisted of 878 children. In that study, the effect of fetal methylmercury exposure on blood pressure was no longer apparent. However, prenatal methylmercury exposure was still associated with decreased HRV, although methylmercury exposure at 7 years of age (assessed from the children's hair mercury concentration) was not associated with HRV and only a marginal association of one component of HRV was seen with exposure at 14 years of age. The significant associations of prenatal methylmercury exposure with HRV at 14 years of age were attenuated after adjustment for 7-year outcomes, suggesting that postnatal exposure had had little effect on HRV [64].

Similar findings on cardiovascular function were observed in a small study with nine adult patients who suffered from prenatal methylmercury poisoning (fetal Minamata disease) [65]. Compared to 13 age-matched controls, the subjects had a significantly decreased pulse pressure, although blood pressure did not differ between groups. They also had a significantly elevated resting heart rate, but HRV did not differ significantly between the groups. Similar to the authors of the previous study [63], they also proposed that a parasympathetic nervous system dysfunction had occurred after prenatal methylmercury exposure.

Overall, the evidence on mercury exposure and blood pressure is not very strong. There are no epidemiological studies in adults to support these preliminary findings. However, based on these observations, it is possible that prenatal exposure to mercury could have a role in the development of cardiovascular complications during adult life.

3.4. Other possible effects

The high thiol-binding capacity of mercury may also inhibit the activation of nuclear factor κB (NF-κB). NF-κB is a transcription factor that activates the expression of a variety of genes, including those involved in inflammatory and immune responses [66,67]. It can be activated by several stimuli, such as oxidative stress, and it plays a significant role in the production of many inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) [66,67]. Mercury may bind to sulfhydryl groups present in the NF-κB and thus impair the activation of NF-κB and attenuate its effects on gene expression [68,69]. There may also be impairment of other steps in the NF-κB activation pathway [68]. In workers occupationally exposed to mercury, the levels of TNF-α and IL-1 have been shown to be lower than those in controls [70,71]. NF-κB is also a major transcription factor of the inducible nitric oxide synthase (iNOS) gene [72]. iNOS catalyzes the production of nitric oxide (NO), which has an important role in the maintenance of vascular regulation and immune system [73]. Mercury has been shown to suppress NO production in a macrophage cell line by inhibiting the NF-κB pathway [74]. On the other hand, NF-κB can be activated by several stimuli, such as oxidative stress, which in turn is driven by the presence of the pro-oxidant mercury [12,57,58,60]. Due to the role of mercury in the propagation of oxidative stress, it is possible that suppression of NO production by mercury could also be mediated by the modulation of asymmetric dimethylarginine (ADMA)-demethylating enzyme, dimethylarginine dimethylaminohydrolase (DDAH). The presence of a reactive cysteine residue (Cys-249) in the active site of DDAH renders this enzyme susceptible to diminished activity as a result of its oxidation by ROS. ADMA is recognized not only as an effective NOS inhibitor but also as a risk marker for CVD [75–78], and oxidative (as well as nitrosative) stress can evoke significant changes in ADMA levels by decreasing DDAH enzyme activity [79]. The role of mercury in CVD-related endothelial, inflammatory and immune functions warrants further investigation.

Atherosclerosis has been suggested to be caused by vascular endothelial cell injury [80,81]. Small injuries to the endothelial monolayer lining the inner surface of blood vessels are rapidly repaired by the migration and proliferation of endothelial cells in the vicinity of the damage [82,83]. Mercury may interfere with this process and, in that way, contribute to the pathogenesis of atherosclerosis. This

is supported by a study that found that methylmercury, in a dose-dependent manner, inhibited the migration of cultured human umbilical vein endothelial cells after an injury to the endothelial monolayer [84].

Since mercury has a high affinity for sulfhydryl groups, it may bind to and inhibit several biomolecules. For example, there is some evidence from in vitro studies that mercury can induce changes in platelet aggregation by binding to the thiol groups present in the platelet membrane Na^+–K^+ ATPase [85–87]. Furthermore, compared to controls, workers occupationally exposed to mercuric vapors exhibited a statistically significant increase in several parameters related to blood coagulation, which is indicative of a hypercoagulative effect for mercury [88].

Mercury may also alter cardiac sodium handling by promoting the oxidation of cysteinyl residues and by bridging adjacent sulfhydryl groups to form a sulfur–mercury–sulfur bridge, thus blocking sodium channels [89,90]. This may have an important role in modulating sodium channel permeability in myocytes, which could facilitate the development of arrhythmias [90].

4. Conclusions

There is evidence from epidemiological studies to support the hypothesis that high mercury levels in the body may increase the risk of CVD, and there are also plausible mechanisms that may explain these effects. However, some issues need to be taken into consideration before one can draw any definitive conclusions. One obvious consideration is to examine whether body mercury is associated with other CVD risk factors (rather than being an independent predictor of risk) and thus could reflect the status of these. In our articles, confounding has been performed via adjustments in multivariate models [11–13,16]. This does not, however, ensure the independence of the association, as it is not possible to measure every conceivable parameter. On the other hand, some or several of these risk factors may act as effect mediators. The latter issue warrants more detailed analyses and is a topic of further study.

Briefly, in our KIHD cohort of about 2000 middle-aged men with respect to classical CHD risk factors, hair mercury was directly and statistically significantly associated with age, smoking, serum LDL and total cholesterol, plasma fibrinogen and type 2 diabetes, but not with systolic or diastolic blood pressure, serum homocysteine concentration or a positive family history of ischemic heart disease. Furthermore, hair mercury exhibited a statistically significant inverse association with maximal oxygen uptake capacity. Clearly, mercury, an environmental pollutant, an oxidative stress promoter and a toxin, is associated with a multitude of disadvantageous impacts on human health.

There are no available comparable data on the dietary intake of mercury between populations. However, it is clear that if one attempts to find an association between exposure and disease, then a wide range of exposure has to be examined. The fact that Finnish study subjects also have high levels of dietary mercury intake increases the range of mercury levels found in hair and thus improves statistical power to detect associations between mercury level and the risk of coronary event and other outcomes. In populations with either low or high dietary intakes of mercury, it will be difficult to find and test such associations.

Although fish may contain harmful compounds, they are also a very important source of nutrients, especially long-chain $n-3$ fatty acids, which may prevent chronic diseases like CVD. The American Heart Association's current recommendations to eat fish (particularly fatty fish) twice each week may be enough, allowing an individual to obtain the beneficial health effects of fish consumption without excessive exposure to possible environmental contaminants in fish. The best practice is to vary the type of fish consumed, to prefer smaller-sized fish and to avoid consuming fish from natural sources with a high content of environmental pollutants.

In a recent paper, an expert panel evaluated the risks of hypothetical shifts in fish consumption based on recommendations for fish consumption for women of childbearing age [91]. It was earlier found that women had decreased their total fish consumption by 17% after the US FDA's 2001 fish advisory for pregnant women [92]. The expert panel calculated that if this decline were to occur in the entire population, then it would have an unfavorable impact on public health, especially with respect to detrimental effects on CVD among elderly men [91]. Instead, if the general population increased its fish consumption, this would have a beneficial impact on public health. Therefore, governmental organizations, the media and health professionals must be careful in the presentation of alarmist messages to the public, so that unintentional shifts in fish consumption can be avoided.

To conclude, there is accumulating research pointing to the harmful effects of mercury exposure on the risk of CVD. In addition, there are several possible biological mechanisms that support this theory. Further studies in different populations are still needed to confirm the results and to find other possible factors that may modify the harmful effects of mercury on the risk of CVD.

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