Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 630–636
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Seasonal mercury exposure and oxidant-antioxidant status of James Bay sport fishermen

Marie-Claire Bélanger[a,b], Marc-Edouard Mirault[b,c], Eric Dewailly[c,d], Michel Plante[e], Line Berthiaume[a], Micheline Noël[b], Pierre Julien[a,c,*]

[a]*Québec Lipid Research Centre, CHUL Research Centre, Centre Hospitalier Universitaire de Québec, Québec City, Québec, Canada G1V 4G2*
[b]*Health and Environment Research Unit, CHUL Research Centre, Centre Hospitalier Universitaire de Québec, Québec City, Québec, Canada G1V 4G2*
[c]*Faculty of Medicine, Université Laval, Québec City, Québec, Canada G1K 7P4*
[d]*Public Health Research Unit, CHUL Research Centre, Centre Hospitalier Universitaire de Québec, Québec City, Québec, Canada G1V 2M2*
[e]*Hydro-Québec, Montréal, Québec, Canada H2Z 1A4*

Received 27 August 2007; accepted 4 December 2007

Abstract

The effects of a moderate *seasonal* exposure to methylmercury on plasma low-density lipoprotein (LDL) oxidation and cardiovascular risk indices are not known. The objective of the study was to assess the effects of a seasonal exposure to mercury at similar dose reported to increase cardiovascular risk through fish consumption. Effects on lipoprotein cholesterol and fatty acid profiles, LDL oxidation, and blood oxidant-antioxidant balance were to be assessed in sport fishermen presenting normal blood selenium and ω-3 fatty acid contents. Thirty-one healthy James Bay sport fishermen were assessed for within-subject longitudinal seasonal variations in hair and blood mercury, plasma oxidized LDL, lipophilic antioxidants, homocysteine, blood selenium, and glutathione peroxidase and reductase activities determined before and after the fishing season and compared by matched-pair tests. Hair mercury doubled during the fishing season (2.8 ± 0.4 μg/g, $P < .0001$). Baseline blood selenium, homocysteine, and erythrocyte fatty acid profiles did not change. Plasma high-density lipoprotein cholesterol increased (+5%, $P = .05$), whereas very low-density lipoprotein cholesterol and oxidized LDL decreased (−8%, $P = .05$; −18%, $P = .008$). Blood glutathione peroxidase (+9.7%, $P = .001$), glutathione reductase (+7.2%, $P < .0001$), and total glutathione (+45% $P < .0001$) increased during the fishing season. Plasma total coenzyme Q10 (+13%, $P = .02$), ubiquinone-10 (+67%, $P = .03$), and β-carotene (+46%, $P = .01$) also increased, whereas vitamin E status was unaffected. Pairwise correlations revealed no association between mercury exposure and any of the biomarkers investigated. In contrast, strong predictors of cardiovascular risk such as high-density lipoprotein cholesterol, oxidized LDL, and glutathione peroxidase improved during the fishing season despite elevated methylmercury exposure. The beneficial effects of seasonal fishing activity and fish consumption on cardiovascular health may suppress detrimental effects of concomitant moderate methylmercury exposure.

1. Introduction

Among all contaminants present in aquatic ecosystems of various fish consuming populations, methylmercury (MeHg) remains a major concern to World Health Organization and national public health authorities. Although fish consumption is considered protective for coronary heart disease (CHD), MeHg intake from fish may potentially reduce if not annihilate beneficial effects of ω-3 fatty acids. In contrast to the neurotoxic effects of MeHg, which are well established, the cardiovascular effects are still matters of intense debate (reviewed by Clarkson and Magos [1] and by Clarkson et al [2]). Several studies have reported statistical associations between cardiovascular disease (CVD) and MeHg exposure. One study found a direct relation between mercury concentrations and the risk of myocardial infarction [3], whereas a case-control study of more than 300 000 male health professionals found no such association [4]. Another study from Finland reported that consumption of nonfatty freshwater fish and consequent accumulation of mercury

* Corresponding author. Centre de recherche sur les maladies lipidiques, CHUL-CHUQ Research Center, Québec City, Québec, Canada G1V 4G2. Tel.: +1 418 656 4141x47802; fax: +1 418 654 2145.
E-mail address: pierre.julien@crchul.ulaval.ca (P. Julien).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.12.006

in Eastern Finnish men were associated with an increased risk of CHD. Of interest, MeHg exposure was also correlated with serum titers of immune complexes containing oxidized low-density lipoprotein (OxLDL) [5]. A further prospective study by Salonen et al [6] showed that mercury levels in Finnish men were associated with accelerated progression of carotid atherosclerosis, as revealed by ultrasonographic assessment of carotid intima-media thickness. Considering that oxidative modification of LDL in the arterial wall is a key process in atherosclerosis (reviewed by Chisolm and Steinberg [7] and by Stocker and Keaney [8]), it was proposed that accelerated progression of atherosclerosis and excess risk of CHD may have resulted from MeHg-stimulated lipid peroxidation [5,6]. Mercury and MeHg were proposed to enhance lipid peroxidation, but nevertheless do not seem to promote direct nonenzymatic peroxidation of LDL as copper and iron [9]. On the other hand, low selenium status was reported to contribute to the excess risk of CVD detected in the Finnish cohort [10,11]. Whether such an effect can be related to lower selenium-dependent antioxidant defenses, a condition supposed to favor enhancement of lipid peroxidation by mercury [9], is not known. In contrast to the Finnish studies, the Inuit from Nunavik present a lower incidence of CVD than the Southern Québec population, despite heavy exposure to MeHg (and other persistent organic pollutants [12]) and high prevalence of obesity and smoking [13]. This population, however, is characterized by unusually high blood selenium and n-3 polyunsaturated fatty acid (n-3 PUFA) contents in lipids. Furthermore, these Inuit show a robust antioxidant defense status and low plasma OxLDL; and no association was found between LDL oxidation and MeHg exposure [14]. These observations suggest that the phenotypic responses to MeHg exposure may ultimately depend on complex interactions between mercury and dietary factors such as n-3 PUFAs, selenium, and antioxidant vitamins (eg, vitamin E, coenzyme Q10 [CoQ10]), besides intrinsic genetic factors. They also raise the possibility that the selenium/vitamins-dependent antioxidant defense status may be an important determinant of the effects of MeHg, a documented source of oxidative stress [15-19].

The objective of the present study was to assess the effects of a seasonal exposure to MeHg on lipid and fatty acid profiles, LDL oxidation, and blood antioxidant status in sport fishermen working in a hydroelectric power plant (Hydro-Québec) at James Bay. This target population group was selected on the basis of previously documented *normal* selenium status and elevated seasonal exposure to mercury during summer (M Plante, Hydro-Québec, unpublished data). Exposure and biochemical end points were determined at 2 time points, that is, before and after the fishing season, in the same group of fishermen. This study design provided the unique opportunity to assess seasonal differences for any end point in the same subject, that is, in the absence of genetic variability.

2. Methods

2.1. Subjects

A total of 36 white men initially agreed to participate in the study, which involved 2 interventions: hair and blood sample collection before (June) and after (November) the fishing season. The donors were all healthy sport fishermen between the ages of 22 and 61 years (46.7 ± 1.3, mean ± SEM) working at James Bay (Northern Québec) as white collar employees of Hydro-Québec. Twelve of them were current smokers. Any individual taking vitamin supplements or medication affecting lipid profile and prooxidant/antioxidant status such as statins, fibrates, angiotensin-converting enzyme inhibitors, and anti-inflammatory drugs was excluded from the study, reducing the final number of participants to 31. All of them signed a consent form approved by the Université du Québec à Montréal Ethic Committee and were interviewed to determine their current health status, lifestyle, and medical history.

2.2. Blood collection

Fasting venous blood was taken in June (before fishing season) and in November (after fishing season). Each blood sample was split into 3 EDTA tubes. Sulfosalicylic acid and phenylmethylsulfonyl fluoride were added to one of them for glutathione (GSH) determinations [14]. Another tube was immediately centrifuged to separate the plasma from blood cells (500*g*, 5 minutes). All collection tubes, containing plasma, blood cells, or total blood, were then immediately filled with argon (to prevent ex vivo oxidation) and stored at −80°C until analyzed. Samples were frozen within a 15-minute period after withdrawal.

2.3. Contaminants

Contaminant analyses were performed by the Laboratoire de toxicologie de Québec. Blood and the first centimeter of hair, corresponding to mercury exposure during the last month, were analyzed for mercury by cold vapor atomic absorption technique [20]. Blood selenium was analyzed by inductively coupled plasma–mass spectrometry [21].

2.4. Lipoproteins and fatty acids

Plasma lipoproteins were separated by sequential ultracentrifugation: very low-density lipoprotein (VLDL) (density <1.006), LDL (density = 1.035-1.063), and high-density lipoprotein (HDL) (density >1.063), according to previously published methods [22]. Cholesterol and triacylglycerol concentrations were measured enzymatically using an RA-500 analyzer (Technicon, Tarry Town, NY). Levels of apolipoprotein B (apo B) were determined by automated immunonephelometry (Behring Nephelometer 100 Analyzer; Dade Behring, Marburg, Germany) [23]. Lipids from erythrocyte membranes were extracted with chloroform-methanol (2:1 by vol) [24], and fatty acids were methylated using methanol-benzene (4:1 vol/vol) and acetyl chloride as

previously published [25]. Fatty acid profiles were obtained by gas-liquid chromatography (HP 5890; Hewlett Packard, Toronto, Ontario, Canada) using an Innowax capillary column (30m × 0.25 mm × 0.25 μm; Agilent, Mississauga, Ontario, Canada). Chromatography was calibrated using a mixture of 37 different fatty acids (FAME 37; Supelco, Bellefonte, PA). Erythrocyte fatty acid profiles were expressed as percentage of total fatty acids.

2.5. Determination of plasma OxLDL

Plasma OxLDL was measured by enzyme-linked immunosorbent assay using the monoclonal antibody mAb-4E6 (Mercodia AB, Uppsala, Sweden). The mAb-4E6 is directed against a conformational epitope of the apo B-100 moiety of LDL [26].

2.6. Determination of blood glutathione peroxidase, glutathione reductase, GSH, and plasma homocysteine

Glutathione peroxidase (GPx) and glutathione reductase (GR) activities were determined by commercial enzymatic assays (Randox Laboratories, Mississauga, Ontario, Canada). Total blood GSH was measured using the enzymatic recycling assay [27]. Total L-homocysteine in plasma was quantitatively measured by fluorescence polarization immunoassay using an IMx analyzer (Abbott Laboratories, Abbott Park, IL) [28].

2.7. Determinations of lipophilic antioxidants

Simultaneous monitoring of ubiquinol-10, ubiquinone-10, tocopherols, and carotenoids was carried out by high-performance liquid chromatography (HPLC) with coulometric electrochemical detection as described by Finckh et al [29]. The following calibration standards were purchased from Sigma-Aldrich (St Louis, MO) or VWR Scientific: α-tocopherol (α-TOH), α-tocopheryl quinone (α-TQ), γ-tocopherol (γ-TOH), ubiquinone-10, ubiquinone-9, tocotrienol, and β-carotene. Ubiquinol-9 and ubiquinol-10 were prepared from their respective ubiquinones according to Yamashita and Yamamoto [30]. Plasma samples were extracted by a method adapted from Menke et al [31]. Briefly, after addition of 4 ng β-tocotrienol and 5 ng ubiquinol-9 as internal standards for post-HPLC quantification purpose, 300 μL plasma was thawed at 4°C in the dark and processed immediately by addition of 2 mL of a methanol-ethanol (1:1) mixture and vigorous shaking, followed by addition of 10 mL hexane. The solvent was evaporated under a nitrogen stream, and the dry sample was redissolved in 700 μL ethanol and injected in a Gold HPLC system (Beckman, Coulter Canada Inc, Mississauga, Ontario, Canada) with an autosampler connected to a Prontosil column (150 × 4.0 mm; Bischoff Chromatography, Atlanta, GA). The mobile phase contained methanol-ethanol-isopropanol (88:24:10) and 15 mmol/L lithium perchlorate. Oxidized and reduced forms of vitamins and CoQ10 were detected using a Coulochem III (ESA, Bedford, MA) coulometric electrochemical detector, as described [29,31]. Each compound's concentration was determined by use of calibration standard curves. No oxidation of the ubiquinol-9 internal standard was detected after plasma extraction and HPLC analysis.

2.8. Statistical analyses

Statistical analyses were carried out using JMP 4.0 software (SAS institute, Cary, NC). Although untransformed values are shown in Tables 1 and 2, data were log transformed to normalize their distribution. Matched-pairs analyses were used to compare data before and after the fishing season. Afterward, transformed continuous variables were compared using multivariate analysis (pairwise correlations). Correlations were considered statistically significant when $P < .05$.

3. Results

The 31 adult fishermen were nonobese men (baseline body mass index, 27.7 ± 0.7 kg/m^2; mean ± SEM). No significant change in body mass index was observed after the fishing season. As shown in Table 1, blood and hair mercury concentrations increased during the fishing season by 63% and 100% ($P < .0001$), respectively. In contrast, whole blood selenium status was not altered and was similar to that found in the Southern Québec population (E Dewailly, unpublished data). No differences in plasma lipids, apolipoproteins, and erythrocyte fatty acids were observed, except for a slight decrease in VLDL cholesterol (−8%, $P = .05$) and VLDL

Table 1
Mercury exposure, lipid and fatty acid profiles

End point	Fishing season		P[a]	Δ change
	Before	After		
Blood Hg (nmol/L)	21.9 ± 3.7	35.6 ± 5.2	.0002	+63%
Hair Hg (μg/g)	1.4 ± 0.3	2.8 ± 0.4	<.0001	+100%
Blood selenium (μg/L)	242.9 ± 6.2	247.7 ± 5.6	NS	–
Cholesterol (mmol/L plasma)				
$Total_{Plasma}$	4.89 ± 0.15	5.09 ± 0.17	NS	–
VLDL	0.60 ± 0.04	0.55 ± 0.04	.05	−8%
LDL	2.43 ± 0.15	2.48 ± 0.14	NS	–
HDL	0.77 ± 0.04	0.81 ± 0.05	.01	+5%
Triacylglycerols (mmol/L plasma)				
$Total_{Plasma}$	2.01 ± 0.18	2.06 ± 0.17	NS	–
VLDL	1.69 ± 0.18	1.54 ± 0.15	.01	−9%
Apolipoproteins (g/L plasma)				
Apo B-VLDL	0.29 ± 0.05	0.26 ± 0.06	NS	–
Apo B-LDL	0.80 ± 0.04	0.80 ± 0.04	NS	–
Apo A1-HDL	1.23 ± 0.04	1.32 ± 0.07	.1	+7.3%
LDL-C/apo B–LDL	3.09 ± 0.14	3.35 ± 0.26	NS	–
Erythrocyte fatty acids (%)				
SFA	43.11 ± 0.16	43.85 ± 1.27	NS	–
MUFA	19.36 ± 0.29	19.47 ± 0.73	NS	–
n-3 PUFAs (EPA + DHA)	4.92 ± 0.20	5.30 ± 0.60	NS	–

Mean ± SEM, n = 31. NS indicates nonsignificant difference ($P > .05$); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[a] All comparisons were made with matched-pair test. Statistically significant difference for $P < .05$; trend for $.05 < P < .1$.

Table 2
Oxidative and antioxidant/redox biomarkers

Biomarkers	Fishing season		P[a]	Δ change
	Before	After		
Oxidation				
OxLDL (U/L)	60.5 ± 3.6	49.6 ± 3.3	.008	−18%
OxLDL/apo B–LDL	73.6 ± 4.5	68.1 ± 5.0	.1	−7.5%
Homocysteine (μmol/L)	9.7 ± 1.0	9.9 ± 0.9	NS	–
Blood antioxidant defense				
GR (U/g Hb)	13.9 ± 0.4	14.9 ± 0.4	<.0001	+7.2%
GPx (U/g Hb)	75.1 ± 2.3	82.4 ± 2.8	.001	+9.7%
GSH (μmol/g Hb)	4.4 ± 0.2	6.4 ± 0.3	<.0001	+45%
Plasma antioxidant/redox				
α-TOH (μmol/L)	26.5 ± 1.9	28.6 ± 1.4	NS	–
α-TQ (μmol/L)	4.7 ± 0.4	4.2 ± 0.3	NS	–
γ-TOH (μmol/L)	3.7 ± 0.3	3.9 ± 0.3	NS	–
$Tocopherols_{Total}$ (μmol/L)	34.9 ± 1.5	36.0 ± 1.8	NS	–
α-TQ/α-TOH	0.18 ± 0.08	0.15 ± 0.06	NS	–
Ubiquinol-10 (μmol/L)	1.3 ± 0.06	1.4 ± 0.08	NS	–
Ubiquinone-10 (μmol/L)	0.15 ± 0.03	0.25 ± 0.03	.03	+67%
$CoQ10_{Total}$ (μmol/L)	1.5 ± 0.1	1.7 ± 0.1	.02	+13%
Ubiquinone-10/$CoQ10_{Total}$	0.10 ± 0.02	0.14 ± 0.02	NS	+39%
β-Carotene (μmol/L)	0.37 ± 0.04	0.54 ± 0.07	.01	+46%

Mean ± SEM, n = 31. Hb indicates hemoglobin.
[a] All comparisons were made with matched-pair test. *$Tocopherols_{Total}$* is defined as the sum of α-TOH, α-TQ, and γ-TOH. Statistically significant difference for $P < .05$.

triacyglycerols (−9%, $P = .01$), with a concomitant increase in HDL cholesterol (+5%, $P = .01$). As shown in Table 2, the mean concentration of plasma OxLDL was lower (−18%, $P = .008$) after the fishing season, with concomitant trend toward lower extent of LDL oxidation per LDL particle (OxLDL/apo B–LDL, −7.5%, $P = .1$). Homocysteine plasma levels, another biomarker of oxidation associated with cardiovascular risk, were unaffected and in the reference range reported for white persons [32]. All blood GSH-related antioxidant biomarkers, GR and GPx enzymatic activities, and total GSH (GSH + GSSG glutathione disulfide), increased during the fishing season (+7.2%, 9.7%, and 45%, respectively). No significant changes in concentrations of plasma tocopherols including α-TOH, α-TQ, and γ-TOH; total tocopherols; and α-TOH redox state (α-TQ/α-TOH) were detected. Of note, although baseline α-TOH plasma levels were in the reference range [33], baseline α-TQ levels and baseline α-TQ/α-TOH ratios were both unusually high in this group of men, about 3-fold higher than in other studies (Discussion). All plasma samples contained exogenous β-tocotrienol and ubiquinol-9 added as internal standards (*a*) for quantification purpose and (*b*) for assessment of potential artifactual oxidation of ubiquinols during plasma sample preparation and analysis, which would yield ubiquinone-9. The chromatogram shown in Fig. 1 provides an example of lipophilic antioxidant HPLC profile showing that no ubiquinone-9 was detected, even in a case with unusually large amount of plasma ubiquinone-10.

In contrast to the unaffected tocopherol status, total CoQ10 concentration in plasma was higher after the fishing season (+13%, $P = .02$), the rise being associated with a major increase in ubiquinone-10 (+67%, $P = .03$) but not ubiquinol-10 (Table 2). The seasonal oxidation of ubiquinol-10 produced a 39% rise in ubiquinone-10–$CoQ10_{Total}$ redox ratio, which, however, did not reach statistical significance because of the limited number of participants available in the study. Depending on which published study is used for comparison, the baseline ubiquinone-10–$CoQ10_{Total}$ redox ratio was in the reference range [31] or about 2-fold higher than in other populations [29,30,34]. Finally, plasma β-carotene also showed a major seasonal rise (+46%, $P = .01$) above a normal baseline level [33].

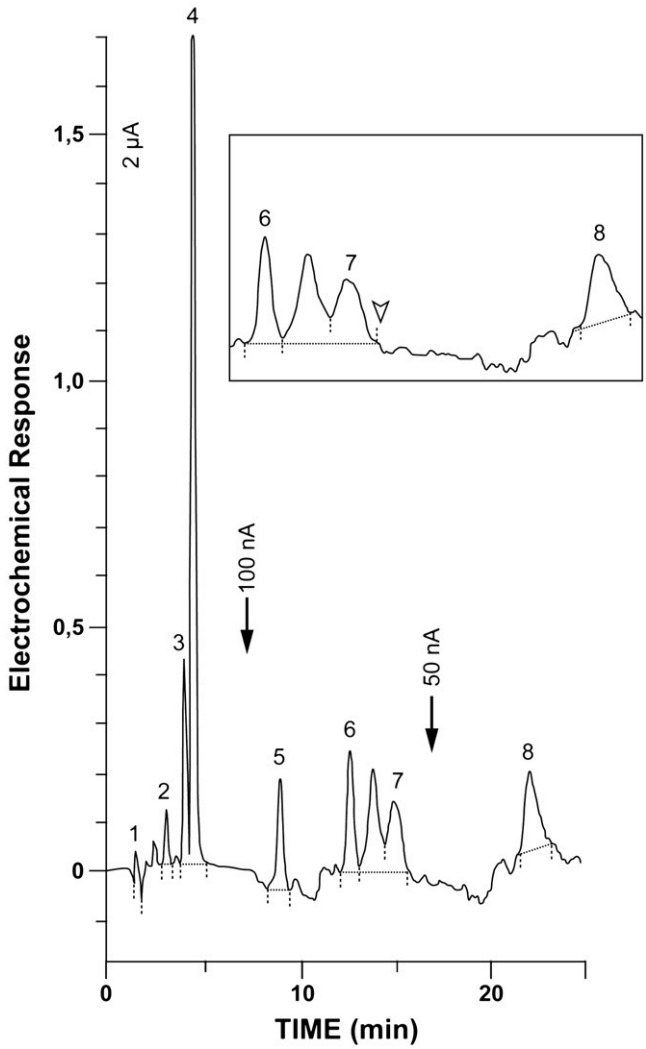

Fig. 1. High-performance liquid chromatogram of plasmatic lipophilic antioxidants from a James Bay fisherman presenting high level of ubiquinone-10 (8). The plasma sample (300 μL) contained the following: 4 ng β-tocotrienol (internal standard) (1), 4.5 μmol/L α-TQ (2), 7.71 μmol/L γ-TOH (3), 41.8 μmol/L α-TOH (4), 5 ng ubiquinol-9 (internal standard) (5), 2.22 μmol/L ubiquinol-10 (6), 0.45 μmol/L β-carotene (7), and 1.37 μmol/L ubiquinone-10 (8). All compounds were separated according to their retention time. Detection sensitivity scale was adjusted to 2 μA for tocopherols, 100 nA for ubiquinols and β-carotene, and 50 nA for ubiquinone-10. The insert shows extension of the chromatogram region upstream of ubiquinone-10 (8), where ubiquinone-9 elution was expected (white arrow), but not detected.

Pairwise correlations revealed no significant associations (Pearson coefficients) between blood mercury and any of the biomarkers assessed before and after the fishing season. Oxidized LDL, on the other hand, correlated with α-TQ (0.39, $P < .05$ before; 0.62, $P < .005$ after) and α-TQ/α-TOH redox state (0.40, $P < .05$ after), but not with ubiquinone-10 or ubiquinol-10–$CoQ10_{Total}$ redox ratio. In addition, ubiquinol-10 correlated with α-TOH (0.73, $P < .0001$ before; 0.50, $P < .005$ after), whereas β-carotene correlated with both ubiquinol-10 (0.41, $P = .02$) and ubiquinone-10 (0.36, $P < .05$) after the fishing season.

4. Discussion

Our objective was to assess the effects of a moderate seasonal exposure to MeHg through fish consumption on blood oxidant-antioxidant balance, lipid and fatty acid profiles, and LDL oxidation status of James Bay sport fishermen featuring "normal" selenium and fatty acid status. Although several seasonal changes in antioxidant and LDL oxidation status were observed, none of these were found associated with mercury exposure. How significant was this exposure? Before the fishing season, baseline hair and blood mercury contents were 2- to 3-fold higher than those reported for North American white populations [35-37]; and their hair mercury increased by a factor of 2, to reach 2.8 ± 0.4 μg/g (mean ± SD) by the end of the fishing season. This mercury level was thus 4- to 6-fold higher than those reported for North American and European populations [3] and about 3-fold higher than that reported for sport fishermen from Montreal who were eating fish more than once a week [37]. However, it was 3-fold lower than that in Inuit from Nunavik [14], but comparable with that of relatively exposed Finnish fishermen (third tertile) [38] for which excess risk of CVD has been associated with mercury exposure.

Baseline tocopherol plasma content of the James Bay fishermen was in the reference range reported for other white populations [39,40], whereas mean baseline $CoQ10_{Total}$ was 30% to 50% higher than those reported for healthy subjects of different countries [30,34,39-42]. It seems likely that the elevated baseline and seasonal CoQ10 levels in these fishermen were largely due to fish consumption all year round, which is an established source of exogenous CoQ10 [43]. Baseline α-TQ plasma content and α-TQ/α-TOH redox ratio, which were not affected by the fishing season, were surprisingly high, that is, 4- to 10-fold those reported in other studies [44,45]. The reason for this unusual α-TOH redox state is not known. It was unlikely to be artifactual oxidation of α-TOH during plasma sample preparation and analysis because all precautions were taken to minimize ubiquinol oxidation during sample preparation and HPLC analysis, as recommended [29,31,34], and no oxidation of ubiquinol-9 (added as internal standard, more susceptible than α-TOH to oxidation) was detected (Fig. 1). Moreover, normal α-TQ/α-TOH ratios were obtained for an Inuit population from Nunavik, as determined in our laboratory using exactly the same experimental protocol (Bélanger et al, unpublished data).

In contrast to the α-TOH redox status, which was unaffected by the fishing season, a major rise in ubiquinone-10 and ubiquinone-10–$CoQ10_{Total}$ redox ratio was observed, suggesting that the James Bay fishermen experienced some kind of seasonal oxidative challenge. The increase in ubiquinone-10–$CoQ10_{Total}$ ratio has been used as a sensitive biomarker of oxidative stress in various pathological conditions [34,46,47]. The selective rise of CoQ10 redox ratio (vs constant α-TQ/α-TOH redox state) observed in the James Bay fishermen is thus in agreement with the previous demonstration that ubiquinol-10 is an endogenous antioxidant of LDL featuring higher reactivity toward oxidants than either tocopherols or carotenoids in inhibiting LDL oxidation [48]. Whether this rise in ubiquinol-10 oxidation was related to antioxidant protection of LDL or other redox-related function(s) is not known. On the other hand, the concomitant increase in 3 major components of the GSH redox cycle, that is, the GSH, GPx, and GR activities, may be consistent with seasonal oxidative stress. It is conceivable that the rise in GSH and related enzymes reflected an adaptive response to a seasonal oxidative challenge. Whichever was the origin of the putative oxidative challenge, our results suggest that it was not related to mercury exposure. Increased physical activity is one of the potential lifestyle changes associated with the fishing season that may have induced adaptive responses of antioxidant defense systems [49,50]. On the other hand, the seasonal rise in β-carotene plasma content (~50%) is likely to reflect a change in diet associated with increased consumption of fruits, vegetables, and fishes such as rainbow trout that are rich in both carotenoids and CoQ10. A seasonal increase in serum β-carotene of similar magnitude was reported for Spanish men [51] and for United Kingdom smokers after a fish oil–supplemented diet rich in fruits and vegetables [52]. The correlation observed between β-carotene and CoQ10 plasma contents of the James Bay fishermen *after* but not *before* the fishing season suggests that the major source of seasonal β-carotene was probably fish.

Despite increasing mercury exposure of the James Bay fishermen, the fishing season appeared to have several beneficial effects related to cardiovascular health. Positive effects included lowering OxLDL plasma content and elevating blood GPx activity and HDL cholesterol, 3 major predictors of cardiovascular risk [26,53-55]. Mean OxLDL plasma concentrations after the fishing season (49.6 ± 3.3 U/L) was lower than that reported for healthy controls in a German study (70.4 U/L) [56] but close to that determined in Inuit from Nunavik (44.4 ± 1.7 U/L) [14]. Increased consumption of dietary antioxidants during summer may have contributed to reduce LDL oxidation by elevating LDL ubiquinol-10 and carotenoid contents and by preserving or increasing paraoxonase-1 activity, an HDL-associated

esterase that can hydrolyze and reduce lipid peroxides in lipoproteins and in arterial cells [57]. Modest but statistically significant seasonal increase in HDL cholesterol and concomitant decrease in VLDL cholesterol were additional effects expected to reduce the risk of coronary artery disease [55]. Moreover, the 10% increase in blood GPx activity that was in the range of GPx activity inversely associated with cardiovascular events [53] is another factor that may contribute to lower plasmatic OxLDL and decrease cardiovascular risk. Finally, the seasonal increase in plasma β-carotene (about 50%) may also be beneficial for cardiovascular health because enrichment of LDL with this antioxidant was shown to protect it from endothelial cell-mediated oxidation [58]. These observations are applicable to this particular group of James Bay fishermen, but other studies are required to assess the effects of seasonal mercury exposure in the general population.

All these cardiovascular positive effects despite a mean hair mercury content of 2.8 μg/g at the end of the fishing season stand in sharp contrast with the suggestion that the risk of myocardial infarction may double when hair mercury level reaches approximately 2 μg/g [3]. Our results, however, are in excellent agreement with data from monitoring programs in Canada suggesting that Cree Indians with mean hair mercury of 10 μg/g have a lower risk of death from CVD than the rest of the population in Quebec with 20-fold lower baseline hair mercury [59].

Acknowledgment

This study was supported by grants from the Toxic Substances Research Initiative, the Northern Contaminant Program, Indian and Northern Affairs; by Hydro-Québec for logistic support; and by a doctoral studentship from the FRSQ Cardiovascular Health Network. We are grateful to Francine Noël for assistance for on-site collection of blood samples and clinical data, and to Suzanne Gingras for help in the statistical analysis.

References

[1] Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 2006;36:609-62.
[2] Clarkson TW, Magos L, Myers GJ. The toxicology of mercury—current exposures and clinical manifestations. N Engl J Med 2003;349:1731-7.
[3] Guallar E, Sanz-Gallardo MI, van't Veer P, Bode P, Aro A, Gomez-Aracena J, et al. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 2002;347:1747-54.
[4] Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D, et al. Mercury and the risk of coronary heart disease in men. N Engl J Med 2002;347:1755-60.
[5] Salonen JT, Seppanen K, Nyyssonen K, Korpela H, Kauhanen J, Kantola M, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. Circulation 1995;91:645-55.
[6] Salonen JT, Seppanen K, Lakka TA, Salonen R, Kaplan GA. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population-based prospective 4-year follow-up study in men in eastern Finland. Atherosclerosis 2000;148:265-73.
[7] Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. Free Radic Biol Med 2000;28:1815-26.
[8] Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004;84:1381-478.
[9] Seppanen K, Soininen P, Salonen JT, Lotjonen S, Laatikainen R. Does mercury promote lipid peroxidation? An in vitro study concerning mercury, copper, and iron in peroxidation of low-density lipoprotein. Biol Trace Elem Res 2004;101:117-32.
[10] Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982;2:175-9.
[11] Salonen JT, Salonen R, Seppanen K, Kantola M, Parviainen M, Alfthan G, et al. Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischaemic heart disease in Eastern Finnish men. Atherosclerosis 1988;70:155-60.
[12] Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL. Determinants of polychlorinated biphenyls and methylmercury exposure in Inuit women of childbearing age. Environ Health Perspect 2001;109:957-63.
[13] Dewailly E, Blanchet C, Lemieux S, Sauve L, Gingras S, Ayotte P, et al. n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. Am J Clin Nutr 2001;74:464-73.
[14] Belanger MC, Dewailly E, Berthiaume L, Noel M, Bergeron J, Mirault ME, et al. Dietary contaminants and oxidative stress in Inuit of Nunavik. Metabolism 2006;55:989-95.
[15] LeBel CP, Ali SF, McKee M, Bondy SC. Organometal-induced increases in oxygen reactive species: the potential of 2¢,7¢-dichlorofluorescin diacetate as an index of neurotoxic damage. Toxicol Appl Pharmacol 1990;104:17-24.
[16] LeBel CP, Ali SF, Bondy SC. Deferoxamine inhibits methyl mercury–induced increases in reactive oxygen species formation in rat brain. Toxicol Appl Pharmacol 1992;112:161-5.
[17] Lund BO, Miller DM, Woods JS. Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 1993;45:2017-24.
[18] Yee S, Choi BH. Methylmercury poisoning induces oxidative stress in the mouse brain. Exp Mol Pathol 1994;60:188-96.
[19] Sarafian T, Verity MA. Oxidative mechanisms underlying methyl mercury neurotoxicity. Int J Dev Neurosci 1991;9:147-53.
[20] Farant JP, Brissette D, Moncion L, Bigras L, Chartrand A. Improved cold-vapor atomic absorption technique for the microdetermination of total and inorganic mercury in biological samples. J Anal Toxicol 1981;5:47-51.
[21] Labat L, Dehon B, Lhermitte M. Rapid and simple determination of selenium in blood serum by inductively coupled plasma–mass spectrometry (ICP-MS). Anal Bioanal Chem 2003;3:3.
[22] Ordovas JM. Fast ultracentrifugation methods for the separation of plasma lipoproteins. Methods Mol Biol 1998;110:93-103.
[23] Connelly PW, Poapst M, Davignon J, Lussier-Cacan S, Reeder B, Lessard R, et al. Reference values of plasma apolipoproteins A-I and B, and association with nonlipid risk factors in the populations of two Canadian provinces: Quebec and Saskatchewan. Canadian Heart Health Surveys Research Group. Can J Cardiol 1999;15:409-18.
[24] Shaikh NA, Downar E. Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. Circ Res 1981;49:316-25.
[25] Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986;27:114-20.
[26] Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2001;21:844-8.
[27] Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548-55.

[28] Fortin LJ, Genest Jr J. Measurement of homocyst(e)ine in the prediction of arteriosclerosis. Clin Biochem 1995;28:155-62.
[29] Finckh B, Kontush A, Commentz J, Hubner C, Burdelski M, Kohlschutter A. High-performance liquid chromatography–coulometric electrochemical detection of ubiquinol 10, ubiquinone 10, carotenoids, and tocopherols in neonatal plasma. Methods Enzymol 1999;299:341-8.
[30] Yamashita S, Yamamoto Y. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. Anal Biochem 1997;250:66-73.
[31] Menke T, Niklowitz P, Adam S, Weber M, Schluter B, Andler W. Simultaneous detection of ubiquinol-10, ubiquinone-10, and tocopherols in human plasma microsamples and macrosamples as a marker of oxidative damage in neonates and infants. Anal Biochem 2000;282: 209-17.
[32] Schnabel R, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Torzewski M, Lubos E, et al. Glutathione peroxidase–1 and homocysteine for cardiovascular risk prediction: results from the AtheroGene study. J Am Coll Cardiol 2005;45:1631-7.
[33] Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, alpha-tocopherol, lutein/zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection. Clin Chem 1994;40:411-6.
[34] Lagendijk J, Ubbink JB, Vermaak WJ. Measurement of the ratio between the reduced and oxidized forms of coenzyme Q10 in human plasma as a possible marker of oxidative stress. J Lipid Res 1996;37: 67-75.
[35] Kingman A, Albertini T, Brown LJ. Mercury concentrations in urine and whole blood associated with amalgam exposure in a US military population. J Dent Res 1998;77:461-71.
[36] Rhainds M, Levallois P, Dewailly E, Ayotte P. Lead, mercury, and organochlorine compound levels in cord blood in Quebec, Canada. Arch Environ Health 1999;54:40-7.
[37] Kosatsky T, Przybysz R, Armstrong B. Mercury exposure in Montrealers who eat St. Lawrence River sportfish. Environ Res 2000;84:36-43.
[38] Virtanen JK, Voutilainen S, Rissanen TH, Mursu J, Tuomainen TP, Korhonen MJ, et al. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. Arterioscler Thromb Vasc Biol 2005;25:228-33.
[39] Perugini C, Bagnati M, Cau C, Bordone R, Paffoni P, Re R, et al. Distribution of lipid-soluble antioxidants in lipoproteins from healthy subjects. II. Effects of in vivo supplementation with alpha-tocopherol. Pharmacol Res 2000;41:67-74.
[40] Tomasetti M, Alleva R, Solenghi MD, Littarru GP. Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ10 ratio as a possible marker of increased risk for atherosclerosis. Biofactors 1999;9:231-40.
[41] Kaikkonen J, Nyyssonen K, Salonen JT. Measurement and stability of plasma reduced, oxidized and total coenzyme Q10 in humans. Scand J Clin Lab Invest 1999;59:457-66.
[42] Yalcin A, Kilinc E, Sagcan A, Kultursay H. Coenzyme Q10 concentrations in coronary artery disease. Clin Biochem 2004;37:706-9.
[43] Bliznakov EG. From sharks to coenzyme Q10. Adv Exp Med Biol 1976;73:441-50.
[44] Murphy ME, Kolvenbach R, Aleksis M, Hansen R, Sies H. Antioxidant depletion in aortic crossclamping ischemia: increase of the plasma alpha-tocopheryl quinone/alpha-tocopherol ratio. Free Radic Biol Med 1992;13:95-100.
[45] Palan PR, Woodall AL, Anderson PS, Mikhail MS. Alpha-tocopherol and alpha-tocopheryl quinone levels in cervical intraepithelial neoplasia and cervical cancer. Am J Obstet Gynecol 2004;190:1407-10.
[46] Hasegawa G, Yamamoto Y, Zhi JG, Tanino Y, Yamasaki M, Yano M, et al. Daily profile of plasma %CoQ10 level, a biomarker of oxidative stress, in patients with diabetes manifesting postprandial hyperglycaemia. Acta Diabetol 2005;42:179-81.
[47] Yamamoto Y, Yamashita S. Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress. Mol Aspects Med 1997;18:S79-84.
[48] Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. Proc Natl Acad Sci U S A 1991;88:1646-50.
[49] Sen CK. Glutathione homeostasis in response to exercise training and nutritional supplements. Mol Cell Biochem 1999;196:31-42.
[50] Elosua R, Molina L, Fito M, Arquer A, Sanchez-Quesada JL, Covas MI, et al. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. Atherosclerosis 2003;167:327-34.
[51] Olmedilla B, Granado F, Blanco I, Rojas-Hidalgo E. Seasonal and sex-related variations in six serum carotenoids, retinol, and alpha-tocopherol. Am J Clin Nutr 1994;60:106-10.
[52] Roberts WG, Gordon MH, Walker AF. Effects of enhanced consumption of fruit and vegetables on plasma antioxidant status and oxidative resistance of LDL in smokers supplemented with fish oil. Eur J Clin Nutr 2003;57:1303-10.
[53] Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003;349:1605-13.
[54] Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). Arterioscler Thromb Vasc Biol 2002;22:1162-7.
[55] Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. Faseb J 2001;15:2073-84.
[56] Kopprasch S, Pietzsch J, Kuhlisch E, Fuecker K, Temelkova-Kurktschiev T, Hanefeld M, et al. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes 2002;51:3102-6.
[57] Aviram M, Kaplan M, Rosenblat M, Fuhrman B. Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development. Handb Exp Pharmacol 2005:263-300.
[58] Dugas TR, Morel DW, Harrison EH. Impact of LDL carotenoid and alpha-tocopherol content on LDL oxidation by endothelial cells in culture. J Lipid Res 1998;39:999-1007.
[59] Plante M, Babo S. Mercury and the risk of myocardial infarction. N Engl J Med 2003;348:2151-4 [author reply 2151-4].