Fish consumption and myocardial infarction: a second prospective biomarker study from northern Sweden¹–⁴

Maria Wennberg, Ingvar A Bergdahl, Göran Hallmans, Margareta Norberg, Thomas Lundh, Staffan Skerfving, Ulf Strömberg, Bengt Vessby, and Jan-Håkan Jansson

ABSTRACT
Background: A beneficial role of fish consumption on the risk of myocardial infarction (MI) has been reported and is mostly ascribed to n-3 (omega-3) fatty acids. However, fish also contains methylmercury, which may increase the risk of MI.

Objective: The objective was to determine how fish consumption and erythrocyte concentrations of mercury (Ery-Hg) and selenium (Ery-Se) are related to the risk of MI and whether n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) in plasma phospholipids (P-EPA+DHA) are protective.

Design: This was a case-control study nested within the northern Sweden cohort, in which data and samples were collected prospectively. The study included 431 cases with an MI after data and sample collection, including 81 sudden cardiac deaths (SCDs) and 499 matched controls. Another 69 female cases with controls from a breast cancer screening registry were included in sex-specific analyses.

Results: Odds ratios for the third compared with the first tertile were 0.65 (95% CI: 0.46, 0.91) for Ery-Hg, 0.75 (95% CI: 0.53, 1.06) for Ery-Se, and 0.78 (95% CI: 0.54, 1.11) for P-EPA+DHA. Ery-Hg and P-EPA+DHA were intercorrelated (Spearman’s R = 0.34). No association was seen for reported fish consumption. Multivariate modeling did not change these associations significantly. High concentrations of Ery-Se were associated with an increased risk of SCD.

Conclusions: The biomarker results indicate a protective effect of fish consumption. No harmful effect of mercury was indicated in this low-exposed population in whom Ery-Hg and P-EPA+DHA were intercorrelated.

INTRODUCTION
Fish is considered a healthy food, especially because of its content of the long-chain omega-3 (n-3) fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3); however, other nutrients might also be beneficial, such as selenium. The beneficial role of fish consumption on the risk of cardiovascular disease (CVD) has been extensively studied (1–3). On the other hand, fish also contains environmental contaminants, such as methylmercury (MeHg) and persistent organic pollutants. Several epidemiologic studies [eg, a multicenter study (4) and studies from eastern Finland (5, 6)] have found an association between the risk of CVD or risk factors for CVD (7) and concentrations of mercury in hair or nails.

In contrast with the Finnish studies, a prospective nested case-control study of 78 cases of myocardial infarction (MI) and of 156 controls in northern Sweden reported an inverse association between risk of MI and mercury concentrations in erythrocytes (Ery-Hg) and EPA and DHA in plasma phospholipids (P-EPA+DHA) (8). It was suggested that this unexpected “beneficial” association could be explained by Ery-Hg being a marker of fish consumption. The exposure level of MeHg is relatively low in northern Sweden (median Ery-Hg: 3.6 μg/L) (9), particularly when compared with eastern Finland [mean hair-Hg: 1.9 μg/g (6), corresponding to an Ery-Hg of ≈13 μg/L] (7, 10–12). The multicenter study showed a somewhat higher exposure [mean nail-Hg: 0.25 μg/g (4), corresponding to an Ery-Hg of ≈6 μg/L] (7, 10–12).

In this larger, prospective, nested case-control study with 431 cases of first MI and 499 matched controls in northern Sweden, we aimed to examine the influence of Ery-Hg on MI as well as the effects of fish consumption, Ery-Se, and P-EPA+DHA. To study sex differences, another 69 female cases with MI and controls were identified from a breast cancer screening registry and were included in the sex-specific analyses. We also examined the relation between the study variables and the risk of sudden cardiac death (SCD).

SUBJECTS AND METHODS
Study population and study design
The study population was derived from the Northern Sweden Health and Disease Study (NSHDS), which consists of 3 sub-

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cohorts: the Västerbotten Intervention Program (VIP) (13), the WHO’s Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Study in northern Sweden (14), and the Mammography Screening Project (MSP) (15). Both VIP and MONICA are health examination programs for CVD and diabetes. In VIP, the mean participation rate was 59%; until 1995, some health centers discontinued VIP in periods, and thus the participation rate varied between health centers. In the northern Sweden MONICA Study, the mean participation rate was 77%. Data collection in VIP and MONICA regarding CVD risk markers were very similar. The designs of VIP and MONICA were described in detail previously (13, 14). To increase the number of female cases, participants in the MSP were included in the sex-specific analyses. The participation rate in the MSP was 85% in the screening phase, and 57% of the participants donated blood samples. By 31 December 1999, ~73,000 unique subjects had been screened in these 3 subcohorts in the NSHDS.

Participants in VIP or MONICA were asked to complete a questionnaire concerning social background, medical history, and various lifestyle factors, including diet and smoking habits. A medical examination of the participants was conducted. Participants consented to donate blood samples, which were stored at −80°C in a biobank for future research. Health screening information was stored in a database. The participants in the MSP did not fill out a questionnaire, but blood samples for future research were collected and information on smoking habits was obtained.

This study consists of subjects participating in any of the health examinations mentioned above from years 1987 to 1999. Because very limited multivariate adjustment was possible for the participants in the MSP, they were not included in the main analyses. They were included in additional sex-specific analyses, in which fewer variables were considered. In the main analyses, 91% of the cases were participants from VIP and 9% were from the MONICA Study. A prospective, nested, case-control study design was used (Figure 1).

Consecutive cases of MI occurring from 1 October 1994 to 31 December 1999 were identified through the northern Sweden MONICA incidence registry (16). Of these cases, SCD was defined as survival time from onset of symptoms to death of <24 h. Controls (one control for men and 2 controls for women) were matched to the cases for sex, age (±2 y), date of health survey (±4 months), type of health survey, and geographic region (municipality). Because of a smaller number of female participants, 2 controls were matched to female cases to increase statistical power. Cases and controls were excluded if previous MI, stroke, or malignant disease could not be excluded according to the questionnaire or case records. In addition, subjects without biobanked erythrocyte samples were excluded, leaving 431 cases and 499 controls (at least one control per case) from VIP and MONICA. Of these cases, 81 were defined as SCD. Another 69 cases and 126 controls from the MSP were included in the sex-specific analyses. The study was approved by the Research Ethics Committee of Umeå University. All participants gave informed consent.

Baseline variables

Smoking habits were classified into “daily smoking” or “nonsmoking” (including previous smokers and occasional smokers). Body mass index (BMI) was calculated as weight (kg)/height squared (m²). Hypertension was defined as a systolic blood pressure ≥140 mm Hg, a diastolic blood pressure ≥90 mm Hg, or the reported use of antihypertensive medication during the past 14 d. Diabetes was defined as self-reported

FIGURE 1. Selection and exclusion algorithm for study subjects from the Västerbotten Intervention Program (VIP) and the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Study from northern Sweden. Data were sampled between 1987 and 1999. MI, myocardial infarction; Ery-Hg, erythrocyte mercury; Ery-Se, erythrocyte selenium; P-EPA+DHA, sum of proportions of eicosapentaenoic acid and docosahexaenoic acid in plasma phospholipids. *One referent for men; 2 referents for women.
disease from the questionnaire or a fasting glucose ≥7 mmol/L and/or a 2-h postload plasma glucose ≥11.0 mmol/L (≥12.2 mmol/L in the VIP, as capillary plasma was drawn). Total cholesterol was measured in serum, sampled after >4 h of fasting. Educational level was categorized into fulfilled academic education (university or college) or not.

Dietary data

Information on average dietary intake over the past year was acquired from a food-frequency questionnaire (FFQ). There have been some changes over time in the FFQ. The version currently used in the MONICA study (84-items) has been validated with ten 24-h diet records (17) and with determination of fatty acid content in erythrocyte membranes as biomarkers (18). In the VIP, a somewhat shorter version (64–66 items) of the FFQ has been used since 1996. Each of the FFQ’s used 9 predefined responses and were answered by most of the participants (n = 705). Another 217 participants completed older versions of the FFQ with 6 predefined responses. The participants from the MSP (n = 195) did not fill out any FFQ, whereas another 8 subjects also lacked dietary data.

Fish consumption

The questions regarding consumption of fatty and lean fish were identical in the different FFQ versions. The questions asked were as follows: “How often do you eat lean fish (eg, perch and cod)?” and “How often do you eat fatty fish (eg, herring, lavaret, and salmon)?”. In addition, the question “How often do you eat salty fish (salt herring)?” was asked in most of the FFQ versions. Because herring is a fatty fish, we assumed that this was reflected in the respondents’ replies regarding fatty fish, but for those who reported a more frequent consumption of salty fish than of fatty fish (n = 54), the estimation of salty fish consumption was interpreted as fatty. Because it is uncertain how people report fatty and salty fish consumption, alternative analyses were carried out in instances in which higher consumption of salty fish was reported. The frequencies in the questionnaires with 9 predefined responses were quantified to meals per week: never = 0, a few times per year = 0.05, 1–3 times/mo = 0.50, once per week = 1.00, 2–3 times/wk = 2.50, 4–6 times/wk = 5.00, once per day = 7.00, 2–3 times/d = 17.5, and >4 times/d = 28.0. In the versions of the FFQ with 6 alternatives, the frequencies were quantified to meals per week: never = 0, <1 time/wk = 0.5, 1–2 times/wk = 1.5, 3–5 times/wk = 4.0, 6–7 times/wk = 6.5, and >1 time/d = 10.5. Consumption of lean and fatty fish was computed to total fish meals per week. Consumption of total fish was also categorized in 4 groups of fish consumption: <1 time/mo (n = 32 cases and 28 controls), 1 time/mo to <1 time/wk (n = 74 cases and 87 controls), 1–2 times/wk (n = 260 cases and 319 controls), and >2 times/wk (n = 26 cases and 40 controls).

Alcohol consumption

Questions on consumption of wine, spirits, and strong beer were included in all versions of the FFQ and were encoded to intakes per week of each beverage.

Consumption of fruit and vegetables

Because there were different versions of the FFQ, consumption of fruit and vegetables was only estimated as a dichotomized variable. First, all questions on fruit and vegetables were converted to intakes per day and then computed. With the same response alternatives as described for fish consumption, the possible categorization was intake of fruit and vegetables ≤1 time/d or >1 time/d. If data were missing on one or several questions, data were coded as insufficient if the available information did not allow categorization of the subject to the >1 time/d per group.

Physical activity level

The different versions of the questionnaire used over time complicated the estimation of physical activity level. It was possible to identify subjects reporting low levels of physical activity. Subjects who on questions regarding leisure-time physical activity chose the answer “never,” “hardly ever,” or “mostly inactive” were included in this category.

Blood sampling

Venous blood samples were drawn without stasis into evacuated tubes after a minimum of 4 h of fasting in the VIP and the MONICA Study. In the MSP, blood samples were collected throughout the day, not necessarily fasting. Erythrocytes, buffy coat, and plasma were separated by centrifugation at 1500 × g for 15 min, and aliquots were stored at −80°C until used (19). Storage time (time between sampling and analyses) ranged from 8 to 20 y.

Measurement of apolipoprotein B and apolipoprotein A-I

Apolipoprotein (apo) A-I and apo B were analyzed in 2007 by using blood samples stored in the biobank since screening; apo A-I and B were measured by immunoturbidimetry with reagents from Dako (Glostrup, Denmark) and a calibrator (0947) on a Hitachi 911 multianalyzer (Roche Diagnostics GmbH, Mannheim, Germany). The ratio of apo B to apo A-I (apo B/apo A-I) was calculated and used instead of serum cholesterol in the statistical analyses.

Measurement of fatty acids

Fatty acids were measured in 2007 by gas-liquid chromatography after separation of lipids by thin-layer chromatography and transmethylation (20) and were expressed as a percentage of all fatty acids in phospholipids (Unit for Clinical Nutrition Research, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden). The method imprecision (calculated as the CV for duplicate preparations and measurements) has been reported to be <1–5.5% (21).

Measurement of mercury

Mercury was measured in duplicate in acid-digested erythrocytes by cold-vapor atomic fluorescence spectrometry (22). The limit of detection (3 times the SD of the blank) was 0.14 μg/L, and the CV was 5.3%. The analytic accuracy was checked by using Seronorm Trace Elements Whole Blood [SERO AS,
Billingstad, Norway; lots MR4206 and 404109y; obtained: 
0.16 (mean ± SD) and 13.8 ± 0.60 μg/L, respectively; 
recommended: 2.0–2.4 and 13.5–16.4 μg/L, respectively] and 
human blood quality control from Centre de Toxicologie du 
Quebec, Canada (lot M-04–01; obtained: 1.9 ± 0.15 μg/L;
certified: 2.0 μg/L).

Measurement of selenium

The concentration of selenium in the erythrocyte samples was measured in duplicate by inductively coupled plasma mass spectrometry (ICP-MS; Thermo ×7; Thermo Elemental, Wins-
ford, United Kingdom) (23). The detection limit was 0.55 μg/L. 
The method imprecision was 3.2%. The analytic accuracy 
checked against reference material. For Seronom Trace Elements Whole Blood (lot 0503109; SERO AS, Billingstad, 
Norway), the results (n = 126) obtained were 125 ± 6.8 
(mean ± SD) compared with the Recommended concentration 
of 113–133 μg/L. For human blood quality control with samples 
obtained from Centre de Toxicologie du Quebec, Canada (lot 
ICP-01B-03), the obtained values were 176 ± 12 (n = 78) 
compared with the certified value of 191 ± 60 μg/L.

Statistics

The crude effect of each potential risk factor was examined by 
univariate conditional logistic regression (ie, including a single 
potential risk variable) based on the matched case-control sets 
(24, 25) in the VIP and the MONICA Study. To proceed with 
multivariate analyses, we analyzed Spearman’s correlations (26) 
between the candidate risk factors in controls from the VIP and 
the MONICA Study; these correlations provide a descriptive support for understanding potential confounding effects in the 
multivariate settings.

Fairly extensive multivariate models were possible for the 
participants in the VIP and the MONICA Study. Because the 
crude effect estimates of traditional risk factors (except BMI) and 
other study variables were in the same direction for both sexes 
and the effect modification by sex was not evident (except for 
BMI, but BMI showed only weak correlations with the exposure 
variables and, consequently, no strong confounding effects in the 
multivariate analyses), we decided to analyze combined data 
from men and women. Multivariate models were first built for 
each exposure variable (ie, fish consumption, Ery-Hg, P-EPA 
+DHA, and Ery-Se). The data-driven adjustment covariates were 
first sorted out. These covariates changed the point estimate, 
reflecting a crude exposure effect of ≥10% (27), and were as 
follows: 1) fish intake, apo B/apo A-I, smoking, diabetes, edu-
cation, physical activity level, consumption of strong beer, and 
consumption of fruit and vegetables; 2) Ery-Hg, systolic blood 
pressure, smoking, education, consumption of strong beer, and 
consumption of fruit and vegetables; 3) P-EPA+DHA, apoB/ 
apoA1, smoking and consumption of fruit and vegetables; and 4) 
Ery-Se, apo B/apo A-I, smoking, education, consumption of 
strong beer, and consumption of wine. To facilitate directly 
comparable models for the exposure variables, each data-driven 
model included all adjustment covariates sorted out (ie, diabetes, 
smoking, systolic blood pressure, apo B/apo A-I, education, 
physical activity level, and consumption of strong beer, wine, 
and fruit and vegetables (referred to as model 1). Extended 
multivariate models were planned considering the Framingham 
Study, in which cholesterol concentration, systolic blood pres-
sure, smoking, and diabetes were established as important (28). 
However, instead of using total cholesterol and HDL cholesterol 
as in the Framingham Heart Study, we used apo B/apo A-I, 
which has proved to be a better risk marker for MI (29). How-
ever, because all of the Framingham covariates were included in 
model 1, no extended multivariate model was built.

In the main analyses, the exposure biomarkers were categori-
as low, medium, or high by the tertile limits based on 
all controls from the VIP and the MONICA Study. We report 
the P value for trend (Wald test) of each trichotomized bio-
marker variable. In addition, we considered finer categorizations 
(quitiles) of the biomarker variables and continuous (not catego-
ized) Ery-Hg, P-EPA+DHA, and Ery-Se. Fish consumption 
was analyzed in 4 groups of consumption and as a continuous variable.

Also, we report the results from a multivariate model including 
Ery-Hg and P-EPA+DHA together, along with all adjustment 
covariates (referred to as model 2). We also performed statistical 
tests of no (multiplicative) interaction between Ery-Hg and P-
EPA+DHA. We also analyzed the effects on SCD with the same 
multivariate models as for all MI and sex-specific effects using 
the covariates smoking and apo B/apo A-I—the covariates 
available for the females in the MSP. The statistical computations 
were carried out by using SPSS for Windows (version 15 and 17; 
SPSS Inc, Chicago, IL).

RESULTS

Baseline characteristics concerning background variables and 
study variables for 431 cases of MI with 499 matched controls in 
the VIP and the MONICA Study and 69 cases with 126 controls 
in the MSP are presented in Table 1. For the whole study group, 
the mean consumption (and range) of fish was 1.26 meals/wk 
(0–8 meals/wk). Median (and range) concentrations of mercury 
and selenium in erythrocytes were 3.54 μg/L (0.01–87 μg/L) and 
126 μg/L (72.5–713 μg/L), respectively, and the median relative 
level of P-EPA+DHA was 5.84% (2.78–14.5%). Average time 
table between baseline (Table 1) and the event was 3 y and 11 mo 
(range: 4–4036 d), and the mean age at MI was 58.7 y (34.1– 
77.1 y). Established risk factors for MI were more common in 
cases (Table 1).

Storage time for blood samples ranged from 8 to 20 y. No 
significant difference in median P-EPA+DHA values was ob-
erved in groups with different blood sample storage times (8–
11 y: 5.86%; 17–20 y: 5.75%).

Correlations between fish consumption variables were ex-
amined. Significantly positive, although not strong (Spearman’s 
R < 0.20), correlations were observed between reported fish 
consumption and Ery-Hg, P-EPA+DHA, and Ery-Se (Table 2). 
The strongest correlation among fish consumption-related vari-
bles was seen between Ery-Hg and P-EPA+DHA (Table 2).

In univariate analyses for cases and controls from VIP and 
MONICA, academic education, physical activity, and con-
sumption of fruit and vegetables were associated with a decreased 
risk of MI. Smoking, consumption of strong beer, diabetes, BMI, 
systolic blood pressure and apoB/apoA1 were associated with an 
increased risk of MI (Table 3). No notable protective effect of 
self-reported fish consumption was found. Ery-Hg was clearly
### TABLE 1
Baseline characteristics of 500 cases from the Northern Sweden Health and Disease Study with myocardial infarction during follow-up until 31 December 1999 and for 625 matched controls

<table>
<thead>
<tr>
<th>Study variables</th>
<th>The Västerbotten Intervention Program and the MONICA Study from northern Sweden</th>
<th>The Mammography Screening Project</th>
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<td>Controls</td>
<td>Cases</td>
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<td>Values</td>
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</table>
| **Year of sampling**
| **Fish consumption (meals/wk)**
Men | 331 | 1.00 (0–5.50) | 1.00, 1.50 | 317 | 1.00 (0–8.00) | 1.00, 1.05 | 126 | 3.16 (0.20–26.8) | 2.48, 4.60 |
Women | 143 | 1.05 (0.10–4.50) | 1.00, 1.50 | 75 | 1.05 (0–4.50) | 1.00, 1.50 | 69 | 2.57 (0.18–86.9) | 1.90, 3.43 |
| **Erythrocyte Hg ($\mu$g/L)**
Men | 350 | 3.95 (0.15–81.4) | 2.89, 5.21 | 350 | 3.47 (0.01–45.9) | 2.64, 4.75 | 126 | 3.16 (0.20–26.8) | 2.48, 4.60 |
Women | 149 | 3.68 (0.19–16.7) | 2.95, 4.72 | 81 | 3.04 (0.43–23.4) | 2.38, 4.18 | 69 | 2.57 (0.18–86.9) | 1.90, 3.43 |
| **Erythrocyte Se ($\mu$g/L)**
Men | 350 | 123 (75.5–250) | 116, 132 | 350 | 120 (72.5–302) | 112, 130 | 126 | 135 (99.0–713) | 128, 146 |
Women | 147 | 132 (81.0–400) | 122, 140 | 81 | 126 (86.0–207) | 119, 136 | 69 | 135 (98.5–198) | 129, 146 |
| **P-EPA (%)**
Men | 308 | 1.37 (0.45–5.44) | 1.19, 1.60 | 306 | 1.38 (0.50–3.75) | 1.24, 1.60 | 122 | 1.40 (0.68–5.54) | 1.23, 1.84 |
Women | 126 | 1.37 (0.68–4.52) | 1.19, 1.53 | 68 | 1.25 (0.41–3.62) | 1.04, 1.48 | 69 | 1.33 (0.49–4.81) | 1.14, 1.64 |
| **P-DHA (%)**
Men | 308 | 4.56 (2.22–8.41) | 4.11, 5.08 | 306 | 4.41 (2.31–8.26) | 4.06, 4.83 | 122 | 4.37 (1.77–8.29) | 4.00, 4.91 |
Women | 126 | 4.70 (2.12–8.55) | 4.28, 5.24 | 68 | 4.68 (3.23–7.54) | 4.27, 5.07 | 69 | 4.14 (2.19–9.72) | 3.60, 4.90 |
| **P-EPA+DHA (%)**
Men | 308 | 5.91 (2.94–13.5) | 5.28, 6.66 | 306 | 5.72 (3.47–11.5) | 5.28, 6.34 | 122 | 5.77 (2.78–13.3) | 5.32, 6.69 |
Women | 126 | 6.06 (3.11–12.3) | 5.62, 6.70 | 68 | 5.93 (4.15–11.2) | 5.31, 6.60 | 69 | 5.43 (3.19–14.5) | 4.85, 6.21 |
| **Smokers (%)**
Men | 338 | 21.0 | 329 | 38.9 | 126 | 15.9 | 69 | 59.4 |
Women | 145 | 21.4 | 76 | 36.8 | | | |
| **Academic education (%)**
Men | 333 | 14.1 | 326 | 7.98 | 126 | 15.9 | 69 | 59.4 |
Women | 146 | 22.6 | 77 | 11.7 | | | |
| **Physically active (%)**
Men | 324 | 64.8 | 310 | 57.7 | | | |
Women | 138 | 57.2 | 69 | 46.4 | | | |
| **Fruit and vegetables >1 time/d (%)**
Men | 333 | 82.3 | 319 | 75.2 | | | |
Women | 147 | 89.1 | 75 | 88.0 | | | |
| **Strong beer (times/wk)**
Men | 324 | 0.05 (0–1.00) | 0.00, 0.05 | 313 | 0.05 (0–2.50) | 0.05, 0.05 | 122 | 5.77 (2.78–13.3) | 5.32, 6.69 |
Women | 135 | 0.00 (0–1.00) | 0.00, 0.02 | 70 | 0.00 (0–1.00) | 0.00, 0.05 | 69 | 5.43 (3.19–14.5) | 4.85, 6.21 |

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The Va¨ sterbotten Intervention Program and the MONICA Study from northern Sweden

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<th>Serum cholesterol (mmol/L)</th>
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<td>Value</td>
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<thead>
<tr>
<th>apo B/apo A-I</th>
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<td>Value</td>
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1 MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; P-EPA+DHA, sum of proportions of EPA and DHA in plasma phospholipids; BP, blood pressure; apo, apolipoprotein.

2 Values correspond to the 33rd and 67th percentiles.

3 Values are medians; ranges in parentheses.

4 Values are means; ranges in parentheses.

5 Hypertension defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or reported use of antihypertensive medication during the past 14 d.
TABLE 2
Covariation between variables: Spearman’s rank correlations (partial analyses adjusted for age and sex) between risk factors for myocardial infarction and study variables in 499 controls from the Northern Sweden Health and Disease Study.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Erythrocyte Hg</th>
<th>P-EPA+DHA</th>
<th>Erythrocyte Se</th>
<th>Fish</th>
<th>Lean fish</th>
<th>Fatty fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte Hg</td>
<td>499</td>
<td>0.34(^2)</td>
<td>0.20(^2)</td>
<td>0.095(^3)</td>
<td></td>
<td></td>
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<tr>
<td>P-EPA+DHA</td>
<td>434</td>
<td>0.15(^2)</td>
<td>0.055</td>
<td>0.048</td>
<td>0.79(^2)</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte Se</td>
<td>497</td>
<td>0.18(^2)</td>
<td>0.19(^2)</td>
<td>0.086</td>
<td>0.66(^2)</td>
<td>0.063</td>
</tr>
<tr>
<td>Fish</td>
<td>474</td>
<td>0.10(^2)</td>
<td>0.023</td>
<td>0.002</td>
<td>0.018</td>
<td>0.019</td>
</tr>
<tr>
<td>Lean fish</td>
<td>479</td>
<td>0.16(^2)</td>
<td>0.066</td>
<td>0.057</td>
<td>0.023</td>
<td>0.086</td>
</tr>
<tr>
<td>Fat fish</td>
<td>480</td>
<td>0.005</td>
<td>0.037</td>
<td>0.002</td>
<td>0.018</td>
<td>0.019</td>
</tr>
<tr>
<td>Smoking</td>
<td>483</td>
<td>0.004</td>
<td>0.042</td>
<td>0.052</td>
<td>0.036</td>
<td>0.090</td>
</tr>
<tr>
<td>apo B/apo A-I</td>
<td>497</td>
<td>0.10(^2)</td>
<td>0.096(^2)</td>
<td>-0.005</td>
<td>-0.033</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI</td>
<td>482</td>
<td>0.005</td>
<td>0.057</td>
<td>0.057</td>
<td>-0.023</td>
<td>-0.086</td>
</tr>
<tr>
<td>Diabetes</td>
<td>493</td>
<td>-0.021</td>
<td>0.037</td>
<td>0.002</td>
<td>-0.018</td>
<td>-0.019</td>
</tr>
<tr>
<td>SBP</td>
<td>477</td>
<td>-0.099(^4)</td>
<td>0.004</td>
<td>-0.039</td>
<td>-0.12(^4)</td>
<td>-0.096(^2)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>462</td>
<td>-0.031</td>
<td>-0.016</td>
<td>0.058</td>
<td>0.16(^2)</td>
<td>0.14(^2)</td>
</tr>
<tr>
<td>Fruit and vegetables</td>
<td>480</td>
<td>-0.053</td>
<td>0.029</td>
<td>0.044</td>
<td>0.12(^2)</td>
<td>0.15(^2)</td>
</tr>
<tr>
<td>Wine</td>
<td>466</td>
<td>0.011</td>
<td>0.13(^2)</td>
<td>0.13(^4)</td>
<td>-0.011</td>
<td>-0.026</td>
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<tr>
<td>Beer</td>
<td>459</td>
<td>0.027</td>
<td>0.080</td>
<td>0.076</td>
<td>0.028</td>
<td>-0.025</td>
</tr>
<tr>
<td>Academic education</td>
<td>479</td>
<td>-0.035</td>
<td>0.17(^4)</td>
<td>0.13(^4)</td>
<td>0.043</td>
<td>0.021</td>
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</table>

\(^1\) P-EPA+DHA, sum of proportions of eicosapentaenoic acid and docosahexaenoic acid in plasma phospholipids; apo, apolipoprotein; SBP, systolic blood pressure.

\(^2\) Significant correlation: \(^2P \leq 0.001, ^3P \leq 0.05, ^4P \leq 0.01.\)

associated with a decreased risk of MI. Point estimates for P-EPA+DHA and Ery-Se were also < 1, but they were not statistically significant (Table 4).

Ery-Hg remained related to a decreased risk of MI in the multivariate models (P for trend = 0.025 in model 1) (Table 4). When data were analyzed by quintile (Q), the crude odds ratios (ORs) with 95% CIs for Ery-Hg were as follows, with Q1 as comparison group; Q2 = 0.86 (0.56, 1.32), Q3 = 0.61 (0.39, 0.94), Q4 = 0.70 (0.45, 1.08), and Q5 = 0.58 (0.37, 0.91). After adjustment according to model 1 in Table 4, there was still a significant association for Ery-Hg in Q5 (OR: 0.48; 95% CI: 0.23, 0.99).

An investigation of how inclusion of the competing fish-consumption biomarkers affects the relation to MI showed that the associations with MI for Ery-Hg were only moderately attenuated (Table 4, model 2). We found no firm evidence of interaction effects between these 2 biomarkers (P = 0.059). An attempt to further describe the potential interaction through stratified analyses did not clarify this issue. An attempt to further describe the potential interaction through stratified analyses did not clarify this issue (see Table S1 under “Supplemental data” in the online issue).

Women from the MSP were included in sex-specific analyses. A total of 500 cases were studied. Crude analyses were made separately for women in the MSP and combined for women in the VIP and MONICA, and the results were found to be similar. Therefore, groups were combined in multivariate analyses, which compared the effects among men and women. Associations were found to be similar for men and women (see Table S2 under “Supplemental data” in the online issue).

With SCD as an endpoint (81 cases), protective relations appeared for Ery-Hg and P-EPA+DHA, which were similar to those of MI (see Table S3 under “Supplemental data” in the online issue), but only in the crude models. In contrast, a significant association was found with Ery-Se in the adjusted model, which indicated an increased risk at high levels.

DISCUSSION
Ery-Hg was associated with a decreased risk of MI, but self-reported fish consumption was not. Both P-EPA+DHA and Ery-Se showed the same tendencies as Ery-Hg, but their associations with risk of MI were not statistically significant. A probable explanation is that all biomarkers in some way reflect fish consumption.

We do not know of any mechanism that would make mercury protective against MI. On the contrary, some mechanisms of mercury might increase the risk of MI (30). It may seem contradictory that an association appears for a marker of fish consumption, but not for reported fish consumption. However, dietary surveys are affected by subjective factors causing errors. In the present study, the variation in reported fish consumption was low. The predefined frequency alternatives may have contributed to this lack of contrast, which also have another consequence: we could not test for benefits at very low concentrations of EPA+DHA (3). With few subjects reporting no consumption of fish (n = 7), any possible association would be difficult to demonstrate. Another potential limitation was the differences between FFQs between the cohorts and over time. This, however, is of minor concern because the questions on fish consumption were the same throughout (except for the MSP, for which no questionnaire was used). The main limitation with the FFQ was the lack of contrast mentioned above. In the present study, we therefore used biomarkers to get a better contrast for the exposure variables.

The higher correlation between Ery-Hg and P-EPA+DHA than between fish consumption and these biomarkers supports the presumption that biomarkers reflect real fish consumption better than does self-reporting. It is probable that Ery-Hg reflects the general fish consumption better than does P-EPA+DHA. The latter has been suggested to reflect intake from only the last days, whereas it has been suggested that erythrocytes reflect the past...
It may thus be that EPA+DHA in erythrocyte membranes reflects a longer time span than does EPA+DHA in plasma phospholipids (31); however, this has been questioned, suggesting a similar time course (1–2 wk, ie, longer than a few days) for EPA+DHA in both matrices (32). Furthermore, levels of EPA and DHA are expressed as a percentage of fatty acids analyzed; thus, it is affected by the amount of other fatty acids in plasma. Ery-Hg, on the other hand, is a concentration. Even though total mercury in erythrocytes was measured in our study, we believe that it reflects exposure to MeHg reliably. Twenty-three samples from the northern Sweden MONICA Study were previously analyzed for total and inorganic mercury (median inorganic mercury: 0.5 μg/L; ranging from a detection limit up to 7.5 μg/L). It was found that inorganic mercury made up only 8% (range: 2–57%) of the total mercury (33). Thus, as our data suggest, Ery-Hg may be a better marker of fish consumption than P-EPA+DHA, even though levels of MeHg vary within and between fish species. The weakest correlation was seen between fish consumption and selenium concentrations. This is not surprising because many other foods are sources of selenium. Swedes have a higher intake of selenium from meats and milk products than from fish (34), even though a portion of fish contains more selenium than does an equal portion of meat.

It has been suggested that Ery-Hg may be a marker of a healthy lifestyle (35). In this study, no correlation was seen between Ery-Hg and the consumption of fruit and vegetables, physical activity, or education. This suggests that the association between Ery-Hg and decreased MI risk is due to Ery-Hg being a marker of fish consumption rather than to a healthy lifestyle.

Selection bias was assessed by comparison studies of participants and nonparticipants in VIP (36) and in the MONICA Study (37). Only small differences were found regarding social and health factors, which indicated that selection bias was small. A limitation of this study was that only single measurements were performed, which increased the risk of misclassification. Also, the information for some confounders from the questionnaire was coarse, especially for consumption of fruit and vegetables and level of physical activity. We observed only a tendency (P = 0.059) for an interaction, and the consumption of fruit and vegetables, physical activity, or education. This suggests that the association between Ery-Hg and decreased MI risk is due to Ery-Hg being a marker of fish consumption rather than to a healthy lifestyle.

A limitation of this study was that only single measurements were performed, which increased the risk of misclassification. Also, the information for some confounders from the questionnaire was coarse, especially for consumption of fruit and vegetables and level of physical activity. However, we saw no relevant change in point estimates after adjustment for these variables, which excluded this as a source of statistical variation. Individuals with missing data on one or several adjustment variables, and therefore excluded from the multivariate analyses, may have differed from those with complete data. Again, reasonable consistency of point estimates after adjustment does not support this as a cause of statistical variation.

Another limitation was the limited range of Ery-Hg concentrations, which means that an effect of mercury on MI risk may have been obscured by the protective effect of fish. It would be of interest to study the interaction between Ery-Hg and P-EPA +DHA. We observed only a tendency (P = 0.059) for an interaction.
The storage time of blood samples must be considered, especially for fatty acids because they are sensitive to oxidation. We observed no significant time-dependent differences when levels of fatty acids in groups with different durations of storage were compared. Therefore, we do not believe that storage time influenced the results.

Only limited adjustment (for apo B/apo A-I and smoking) was possible in sex-specific analyses (see Table S3 under “Supplemental data” in the online issue), because the participants in the MSP did not fill out the same questionnaires. Associations between markers of fish consumption and risk of MI were similar in men and women in these analyses.

In a population-based cohort in eastern Finland, levels of mercury in hair were associated with increased risk of cardiovascular outcomes (6). Also, Guallar et al (4) found an increased risk of MI in a multicenter study using nail-Hg. In contrast, the study by Yoshizawa et al (38) from the United States (38) showed no association between toenail mercury and risk of coronary heart disease. However, when dentists were excluded, a nonsignificant association between toenail mercury and elevated risk of coronary heart disease was observed. In a study from the Faroe Islands, mercury exposure was associated with increased blood pressure and carotid intima-media thickness (7). The general population in northern Sweden has low mercury concentrations as compared with populations in whom an increased risk of cardiovascular morbidity or risk factors for CVD has been observed with higher mercury concentrations (4, 6, 7). The differences in exposure level might explain these seemingly conflicting results.

The increase in risk of SCD at higher concentrations of Ery-Se is surprising, but may have been a chance finding because of the small number of cases (see Table S3 under “Supplemental data” in the online issue). No significant association was found in the crude model, but was found in the adjusted one. Previous studies of selenium and risk of CVD have been inconclusive, but suggest a weak protective effect (39). However, Bleys et al (40) found indications for a decrease in cardiovascular mortality with increasing serum selenium concentrations up to 120 μg/L and increased mortality at higher concentrations. We found no reports on selenium and SCD specifically. However, our data suggest an association that ought to be followed up in larger studies.

In conclusion, overall, the biomarker results indicate a protective effect of fish consumption, even though data on self-reported fish consumption show no clear protective association. No harmful effect of mercury was indicated in this low-exposed population, in whom mercury and EPA+DHA were intercorrelated. Any harmful effect of mercury must therefore be overridden by protective nutrients from fish.
participated in the analysis and interpretation of the data and the drafting and finalizing of the manuscript; GH and MN: participated in the discussion initiating the study, collection and administration of data, and finalizing of the manuscript; TL: carried out the chemical analysis of mercury and selenium and participated in drafting of the manuscript; SS: participated in interpreting the data, and the drafting and finalizing of the manuscript; US: participated in designing and carrying out the data analysis, interpretation of the data, and the drafting and finalizing of the manuscript; BV: carried out the chemical analysis of fatty acids and participated in drafting of the manuscript; and J-HJ: participated in the discussion initiating the study, data analysis, interpretation of the data, and the drafting and finalizing of the manuscript. The authors had no conflicts of interest to report.

REFERENCES