

## MERCURY, FISH OILS, AND THE RISK OF MYOCARDIAL INFARCTION

EISEO GUALLAR, M.D., DR.P.H., M. INMACULADA SANZ-GALLARDO, M.D., M.P.H., PIETER VAN'T VEER, PH.D., PETER BODE, PH.D., ANTTI ARO, M.D., PH.D., JORGE GÓMEZ-ARACENA, M.D., PH.D., JEREMY D. KARK, M.D., PH.D., RUDOLPH A. RIEMERSMA, PH.D., JOSÉ M. MARTÍN-MORENO, M.D., DR.P.H., AND FRANS J. KOK, PH.D.,  
FOR THE HEAVY METALS AND MYOCARDIAL INFARCTION STUDY GROUP\*

**ABSTRACT**

**Background** It has been suggested that mercury, a highly reactive heavy metal with no known physiologic activity, increases the risk of cardiovascular disease. Because fish intake is a major source of exposure to mercury, the mercury content of fish may counteract the beneficial effects of its n-3 fatty acids.

**Methods** In a case-control study conducted in eight European countries and Israel, we evaluated the joint association of mercury levels in toenail clippings and docosahexaenoic acid (C22:6n-3, or DHA) levels in adipose tissue with the risk of a first myocardial infarction among men. The patients were 684 men with a first diagnosis of myocardial infarction. The controls were 724 men selected to be representative of the same populations.

**Results** The average toenail mercury level in controls was 0.25 µg per gram. After adjustment for the DHA level and coronary risk factors, the mercury levels in the patients were 15 percent higher than those in controls (95 percent confidence interval, 5 to 25 percent). The risk-factor-adjusted odds ratio for myocardial infarction associated with the highest as compared with the lowest quintile of mercury was 2.16 (95 percent confidence interval, 1.09 to 4.29; P for trend=0.006). After adjustment for the mercury level, the DHA level was inversely associated with the risk of myocardial infarction (odds ratio for the highest vs. the lowest quintile, 0.59; 95 percent confidence interval, 0.30 to 1.19; P for trend=0.02).

**Conclusions** The toenail mercury level was directly associated with the risk of myocardial infarction, and the adipose-tissue DHA level was inversely associated with the risk. High mercury content may diminish the cardioprotective effect of fish intake. (N Engl J Med 2002;347:1747-54.)

Copyright © 2002 Massachusetts Medical Society.

**M**ERCURY is a highly reactive heavy metal with no known physiologic activity.<sup>1,2</sup> Exposure to toxic levels of mercury results in neurologic and renal damage, but the consequences of long-term exposure to low levels of mercury are poorly understood.<sup>1,2</sup> Mercury may predispose people to atherosclerotic disease by promoting the production of free radicals or by inactivating several antioxidant mechanisms through binding to thiol-containing molecules or to selenium.<sup>3-5</sup> In 1995, Salonen et al. reported an increased risk of

coronary heart disease among residents of the Kuopio area in Finland whose hair samples had increased levels of mercury.<sup>6,7</sup> The participants in that study, however, had relatively high levels of mercury, which were derived largely from locally contaminated freshwater fish.

Fish intake is a major source of exposure to mercury, mainly in the form of methylmercury.<sup>2</sup> Intake of fish or fish oils (long-chain n-3 polyunsaturated fatty acids) has long been hypothesized to prevent cardiovascular events.<sup>8</sup> Two large, randomized clinical trials have shown reduced mortality after myocardial infarction among patients assigned to a diet rich in fatty fish<sup>9</sup> or to fish-oil supplements,<sup>10</sup> but the generalizability of these findings to subjects without coronary heart disease is uncertain. The results of epidemiologic studies relating fish intake or fish-oil levels to coronary events have been contradictory,<sup>11</sup> and it has been suggested that mercury may counteract the beneficial cardiovascular effects of n-3 fatty acids in fish.<sup>2,6,7</sup>

To evaluate the association of mercury with the risk of myocardial infarction, and to test the hypothesis that high mercury levels may offset the inverse association between fish oil consumption and myocardial infarction, we assessed the joint association of mercury levels in toenail clippings and docosahexaenoic acid (C22:6n-3, or DHA) levels in adipose tissue with the risk of a first myocardial infarction among men who were participants in the European Multicenter Case-

From the Department of Epidemiology and Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore (E.G.); the Department of Epidemiology and Biostatistics, National School of Public Health, Institute of Health Carlos III, Madrid (E.G., M.I.S.-G., J.M.M.-M.); the Service of Preventive Medicine, Hospital 12 de Octubre, Madrid (M.I.S.-G.); the Division of Human Nutrition and Epidemiology, University of Wageningen, Wageningen, the Netherlands (P.V., F.J.K.); the Interfaculty Reactor Institute, Delft University of Technology, Delft, the Netherlands (P.B.); the Department of Health and Functional Capacity, National Public Health Institute, Helsinki, Finland (A.A.); the Department of Preventive Medicine, University of Málaga, Málaga, Spain (J.G.-A.); the Epidemiology Unit, Department of Social Medicine, Hadassah Medical Organization and Hebrew University-Hadassah School of Public Health and Community Medicine, Jerusalem, Israel (J.D.K.); the Cardiovascular Research Unit, University of Edinburgh, Edinburgh, United Kingdom, and the Department of Medical Physiology, University of Tromsø, Tromsø, Norway (R.A.R.); and the Department of Preventive Medicine, Universidad Autónoma de Madrid, Madrid (J.M.M.-M.) Address reprint requests to Dr. Guallar at the Welch Center for Prevention, Epidemiology, and Clinical Research, 2024 E. Monument St., Suite 2-639, Baltimore, MD 21205-2223, or at eguallar@jhsp.edu.

\*Other investigators are listed in the Appendix.

Control Study on Antioxidants, Myocardial Infarction and Cancer of the Breast (EURAMIC).<sup>12,13</sup>

## METHODS

## Design and Subjects

The target population consisted of men 70 years of age or younger who were native residents of any of eight European countries or Israel.<sup>12,13</sup> Subjects were excluded if they had a previous diagnosis of myocardial infarction, drug or alcohol abuse, or a major psychiatric disorder; if they were institutionalized; or if they had modified their dietary pattern in the previous year.

The patients were men with a first acute myocardial infarction (code 410 of the *International Classification of Diseases, 9th Revision*), confirmed by characteristic electrocardiographic changes and elevated enzyme levels,<sup>14</sup> who had been hospitalized within 24 hours after the onset of symptoms. They were recruited from the coronary care units of participating hospitals.

The controls were men without a history of myocardial infarction, recruited from the population of the catchment areas from which the patients originated, and frequency-matched for age in five-year intervals. In Finland, Israel, Germany, Scotland, and Switzerland, random sampling from local population registers was used to select controls. In Russia and in the two Spanish centers, population registries could not be used, because of the lack of complete census data or because of legal restrictions. Therefore, controls were selected from among hospitalized patients with disorders not known to be associated with dietary factors (renal colic, hernia, acute appendicitis or mesenteric adenitis, volvulus or subocclusion due to fibrosis, noninfectious prostatism, and rectal or anal disorders other than cancer, hemorrhoids, or chronic infections).<sup>12</sup> When low participation rates from population samples were anticipated, controls were selected by random sampling from the catchment area of the patient's general practitioner (in the Netherlands) or by inviting apparently healthy friends and relatives of the patient to participate (in Norway).<sup>12,13</sup>

Patients and controls were recruited concurrently during 1991 and 1992. The participation rates among potential subjects were 81 percent for patients and 64 percent for controls. Local institutional review boards approved the study, and written informed consent was obtained from study participants.

## Data Collection

Information on smoking, hypertension, and diabetes was collected by standard questionnaires.<sup>12,13</sup> A history of hypertension or diabetes was based on the patient's report of a physician's diagnosis. A family history of coronary heart disease was defined by a self-reported fatal or nonfatal myocardial infarction in a parent. Clippings from all 10 toenails were collected within eight weeks of enrollment.<sup>13</sup> The mean ( $\pm$ SD) weight of the samples was  $53.8 \pm 39.0$  mg. A subcutaneous specimen of adipose tissue was taken from the buttock by needle aspiration.<sup>12</sup> The adipose-tissue sample was taken from patients within seven days after admission to the hospital. A nonfasting sample of venous blood was also obtained. Blood samples were drawn from patients within 24 hours after hospital admission.

## Analysis of Biologic Samples

Toenail mercury was measured by instrumental neutron-activation analysis at the Interfaculty Reactor Institute of Delft University of Technology, Delft, the Netherlands.<sup>15</sup> Toenail clippings were irradiated for four hours in a thermal flux of  $5 \times 10^{12}$  neutrons per second per square centimeter. After a decay time of 21 days, the gamma radiation of mercury was measured in a well-type Ge(Li) detector for one hour. Irradiation of study samples was conducted from April 1998 through June 1999. Samples from patients and

controls were analyzed together, randomly distributed across batches, and masked with respect to case-control status.

For each sample, the limit of detection was defined as the level at which mercury could be detected with 97.5 percent certainty. For a sample of average weight (53 mg), the limit of detection was  $0.11 \mu\text{g}$  per gram. In the 76 samples with mercury levels below the detection limit, we imputed a mercury level of one half the detection limit. For quality control, a sample of freeze-dried plankton reference material (BCR CRM-414, Community Bureau of Reference, Commission of European Communities) was included in each analytic batch. The average of 48 measurements of this material was  $0.26 \mu\text{g}$  per gram (95 percent confidence interval, 0.24 to 0.28), against a certified mercury level of  $0.276 \pm 0.018 \mu\text{g}$  per gram. The interassay coefficient of variation for this reference material was 13.6 percent.

Fatty acids in adipose tissue were assayed at the National Public Health Institute, Helsinki, Finland, by gas chromatography (model HRCG412, HNU Nordion Oy).<sup>16,17</sup> The portion of the fatty-acid peak area containing DHA, as determined by gas chromatography, was calculated and expressed as a fraction of the total fatty-acid peak area. Because the levels of eicosapentaenoic acid (C20:5n-3) in adipose tissue were below the detection limit of the chromatograph for most samples, fish-oil fatty acids were represented exclusively by DHA.<sup>18</sup> The interassay coefficient of variation for DHA in adipose tissue was 25 percent. The serum total cholesterol levels were determined by standard methods.<sup>12</sup>

## Statistical Analysis

Because the distribution of mercury was right-skewed, logarithmic transformation was used to improve normality. The distribution of mercury in controls was used to compute cutoff points and medians for quintiles of exposure. For multivariate analysis, the association of mercury with the risk of myocardial infarction was estimated by multiple logistic regression. The odds ratios in quintiles 2, 3, 4, and 5 were estimated by using the lowest quintile as the reference category, and tests for trend across quintiles of mercury were performed. The reported P values are two-tailed. Statistical analyses were performed with S-Plus software.<sup>19</sup>

## RESULTS

In comparison with the controls, the patients had significantly higher body-mass index and lower high-density lipoprotein cholesterol levels and were more likely to have hypertension, to have diabetes, to smoke, and to have a family history of myocardial infarction (Table 1).<sup>12</sup> The total cholesterol level was lower among patients than among controls, almost certainly reflecting the effect of acute myocardial infarction. Therefore, total cholesterol was not further considered in case-control comparisons.

Controls from Zeist, the Netherlands, and Berlin, Germany, had the lowest average levels of mercury among controls ( $0.14$  and  $0.17 \mu\text{g}$  per gram, respectively), whereas those from the two Spanish centers had the highest ( $0.57 \mu\text{g}$  per gram in Granada and  $0.51 \mu\text{g}$  per gram in Málaga) — a 4.1-fold range of variation (Table 2). The level of DHA in adipose tissue was strongly correlated with the toenail mercury level (Table 3). The age- and center-adjusted correlation coefficient between the levels of DHA and mercury was  $0.34$  ( $P < 0.001$ ).

After adjustment for age, center, and DHA level,

MERCURY, FISH OILS, AND MYOCARDIAL INFARCTION

**TABLE 1. CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH MYOCARDIAL INFARCTION AND IN CONTROLS.\***

| RISK FACTOR                                   | PATIENTS (N=684) | CONTROLS (N=724) | P VALUE |
|---|------------------|------------------|---------|
| Age (yr)                                      | 54.7±8.9         | 53.2±9.3         | 0.002   |
| Body-mass index†                              | 26.5±3.9         | 25.9±3.4         | 0.004   |
| Total cholesterol (mmol/liter)‡               | 5.46±1.11        | 5.56±1.10        | 0.11    |
| HDL cholesterol (mmol/liter)‡                 | 0.98±0.25        | 1.09±0.29        | <0.001  |
| Hypertension (%)§                             | 26.0             | 17.4             | <0.001  |
| Current smoker (%)                            | 61.3             | 37.5             | <0.001  |
| Diabetes mellitus (%)§                        | 8.4              | 3.9              | <0.001  |
| Alcohol intake (g/day)                        | 18.2±27.2        | 17.8±24.0        | 0.75¶   |
| Parental history of myocardial infarction (%) | 57.6             | 45.3             | <0.001  |

\*Plus-minus values are means ±SD.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡To convert values for total cholesterol and high-density lipoprotein (HDL) cholesterol to milligrams per deciliter, divide by 0.02586.

§The history was based on the subject's report of diagnoses by a physician.

¶The association of alcohol with the risk of myocardial infarction was J-shaped, with the lowest risk at 13.1 g of intake per day (P<0.001).

the patients had higher mercury levels than the controls (case-control ratio, 1.10; 95 percent confidence interval, 1.03 to 1.18) (Table 2). This association persisted after the exclusion of the two Spanish centers, which were the centers with the highest mercury levels (DHA-adjusted case-control ratio, 1.09; 95 percent confidence interval, 1.02 to 1.17), and after adjustment for multiple cardiovascular risk factors (case-control ratio, 1.15; 95 percent confidence interval, 1.05 to 1.25).

Analysis with adjustment for age and center showed an increased risk of myocardial infarction at high mercury levels (P for trend=0.01) (Table 4). Adjustment for DHA markedly increased the association and elicited a graded, positive dose-response pattern. This trend was further strengthened after adjustment for traditional risk factors and levels of antioxidants, resulting in an odds ratio of 2.16 for patients in the highest quintile of mercury level, as compared with the lowest (95 percent confidence interval, 1.09 to 4.29; P for trend=0.006). When mercury was introduced as a continuous variable in the regression models, the multivariate odds ratio associated with a change

**TABLE 2. MEANS AND PATIENT: CONTROL RATIOS FOR MERCURY LEVELS IN TOENAILS.**

| CENTER                    | NO. OF PATIENTS/<br>NO. OF CONTROLS | PATIENT PARTICIPATION RATE/<br>CONTROL PARTICIPATION RATE | TOENAIL MERCURY LEVEL*     |                  | MEAN TOENAIL MERCURY LEVEL IN PATIENTS/<br>MEAN TOENAIL MERCURY LEVEL IN CONTROLS<br>(95% CI)† |
|---------------------------|-------------------------------------|---|----------------------------|------------------|--|
|                           |                                     |   | %                          |                  |  |
|                           |                                     |   | PATIENTS                   | CONTROLS         |  |
|                           |                                     |   | μg/g (interquartile range) |                  |  |
| Helsinki, Finland         | 56/62                               | 97/51   | 0.27 (0.20–0.36)           | 0.23 (0.15–0.38) | 1.16 (0.93–1.44)   |
| Berlin, Germany           | 75/97                               | 82/73   | 0.19 (0.12–0.25)           | 0.17 (0.13–0.24) | 1.08 (0.90–1.30)   |
| Jerusalem, Israel         | 57/59                               | 60/53   | 0.30 (0.21–0.41)           | 0.25 (0.18–0.36) | 1.20 (0.99–1.46)   |
| Zeist, the Netherlands    | 64/57                               | 75/50   | 0.14 (0.09–0.26)           | 0.14 (0.08–0.19) | 0.98 (0.75–1.28)   |
| Sarpsborg, Norway         | 96/101                              | 96/98   | 0.31 (0.21–0.49)           | 0.30 (0.23–0.41) | 1.03 (0.89–1.19)   |
| Moscow, Russia            | 92/97                               | 97/79   | 0.20 (0.13–0.27)           | 0.20 (0.13–0.27) | 1.02 (0.85–1.24)   |
| Edinburgh, United Kingdom | 39/25                               | 98/61   | 0.15 (0.11–0.22)           | 0.18 (0.13–0.23) | 0.85 (0.65–1.10)   |
| Granada, Spain            | 55/52                               | 45/67   | 0.53 (0.38–0.77)           | 0.57 (0.34–0.85) | 0.92 (0.70–1.21)   |
| Málaga, Spain             | 94/100                              | 89/77   | 0.68 (0.39–1.09)           | 0.51 (0.29–0.80) | 1.33 (1.08–1.63)   |
| Zurich, Switzerland       | 56/74                               | 93/26   | 0.20 (0.12–0.28)           | 0.21 (0.15–0.29) | 0.97 (0.76–1.23)   |
| Overall                   | 684/724                             | 81/64   | 0.27 (0.15–0.45)           | 0.25 (0.15–0.40) | 1.07 (1.00–1.14)   |
| Overall‡                  |                                     |   |                            |                  | 1.10 (1.03–1.18)   |
| Overall§                  |                                     |   |                            |                  | 1.15 (1.05–1.25)   |

\*Mercury concentration was analyzed on a log scale, and the results were transformed back to the natural scale.

†The ratios have been adjusted for age (continuous variable) and center (indicator variable). The P value for heterogeneity by center was 0.22. CI denotes confidence interval.

‡The ratios have been adjusted for age, center, and docosahexaenoic acid level (continuous).

§The ratios have been adjusted for age, center, docosahexaenoic acid level, body-mass index (continuous), smoking (indicator variables for current smokers and former smokers), high-density lipoprotein cholesterol (continuous), history of hypertension (indicator variable), diabetes (indicator variable), alcohol intake (indicator variables for current and former drinkers), adipose-tissue α-tocopherol level (continuous), adipose-tissue β-carotene level (continuous), toenail selenium (continuous), and toenail weight (continuous).

**TABLE 3. RISK FACTORS ACCORDING TO QUINTILE OF TOENAIL MERCURY LEVEL AMONG CONTROLS, ADJUSTED FOR AGE AND CENTER.\***

| RISK FACTOR                                   | QUINTILE |       |       |       |       | P VALUE |
|---|----------|-------|-------|-------|-------|---------|
|   | 1        | 2     | 3     | 4     | 5     |         |
| Age (yr)                                      | 54.3     | 52.3  | 53.3  | 54.1  | 53.5  | 0.97    |
| Body-mass index†                              | 25.5     | 26.0  | 25.9  | 26.0  | 26.0  | 0.44    |
| Total cholesterol (mmol/liter)‡               | 5.5      | 5.5   | 5.5   | 5.6   | 5.6   | 0.25    |
| HDL cholesterol (mmol/liter)‡                 | 1.07     | 1.07  | 1.13  | 1.11  | 1.08  | 0.89    |
| α-Tocopherol (μg/g)§                          | 188.4    | 164.7 | 195.3 | 205.7 | 201.3 | 0.12    |
| β-Carotene (μg/g)§                            | 0.42     | 0.41  | 0.49  | 0.45  | 0.41  | 0.64    |
| Selenium (mg/kg)§                             | 0.59     | 0.59  | 0.59  | 0.62  | 0.64  | <0.001  |
| Docosahexaenoic acid (%¶)                     | 0.16     | 0.21  | 0.23  | 0.27  | 0.35  | <0.001  |
| Current smoker (%)                            | 42.3     | 34.9  | 41.2  | 34.9  | 36.5  | 0.52    |
| Alcohol intake (g/day)                        | 12.2     | 17.5  | 21.2  | 19.4  | 21.9  | 0.02    |
| Hypertension (%)                              | 10.6     | 12.9  | 11.9  | 14.4  | 17.3  | 0.12    |
| Diabetes mellitus (%)                         | 2.1      | 5.3   | 3.9   | 1.8   | 1.7   | 0.26    |
| Parental history of myocardial infarction (%) | 45.0     | 48.7  | 46.7  | 37.3  | 40.6  | 0.25    |

\*The cutoff values for quintiles of mercury in the controls were 0.14, 0.21, 0.29, and 0.45 μg per gram.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡To convert values for total cholesterol and high-density lipoprotein (HDL) cholesterol to milligrams per deciliter, divide by 0.02586.

§Values are geometric means.

¶Docosahexaenoic acid (C22:6n-3) values are expressed as the percentage of total fatty-acid peak area.

||The history was based on the subject's report of diagnoses by a physician.

**TABLE 4. ODDS RATIOS FOR A FIRST MYOCARDIAL INFARCTION, ACCORDING TO QUINTILE OF TOENAIL MERCURY LEVEL OR ADIPOSE-TISSUE DOCOSAHEXAENOIC ACID (DHA) LEVEL.\***

| MEASURE       | QUINTILE            |                  |                  |                  |                  | P FOR TREND |
|---------------|---------------------|------------------|------------------|------------------|------------------|-------------|
|               | 1                   | 2                | 3                | 4                | 5                |             |
| Mercury       |                     |                  |                  |                  |                  |             |
| Median (μg/g) | 0.11                | 0.17             | 0.24             | 0.36             | 0.66             |             |
|               | odds ratio (95% CI) |                  |                  |                  |                  |             |
| Model 1†      | 1.00                | 0.86 (0.61-1.22) | 1.01 (0.71-1.44) | 1.08 (0.76-1.55) | 1.47 (0.99-2.14) | 0.01        |
| Model 2‡      | 1.00                | 0.93 (0.64-1.36) | 1.11 (0.76-1.63) | 1.24 (0.83-1.84) | 1.86 (1.20-2.91) | 0.001       |
| Model 3§      | 1.00                | 0.86 (0.49-1.50) | 1.18 (0.67-2.07) | 2.08 (1.12-3.84) | 2.16 (1.09-4.29) | 0.006       |
| DHA           |                     |                  |                  |                  |                  |             |
| Median (%FA)  | 0.10                | 0.16             | 0.22             | 0.28             | 0.44             |             |
|               | odds ratio (95% CI) |                  |                  |                  |                  |             |
| Model 1†      | 1.00                | 1.13 (0.80-1.61) | 1.26 (0.87-1.83) | 1.27 (0.87-1.88) | 0.80 (0.53-1.23) | 0.23        |
| Model 2‡      | 1.00                | 1.07 (0.75-1.54) | 1.15 (0.79-1.66) | 1.07 (0.72-1.58) | 0.66 (0.42-1.03) | 0.01        |
| Model 3§      | 1.00                | 1.07 (0.63-1.84) | 1.12 (0.64-1.94) | 0.83 (0.47-1.49) | 0.59 (0.30-1.19) | 0.02        |

\*For all odds ratios, the lowest quintile for toenail mercury level or adipose-tissue DHA level served as the reference category. CI denotes confidence interval, and %FA the percentage of total fatty-acid peak area.

†Model 1 is adjusted for age (continuous variable) and center (indicator variable).

‡Model 2 is further adjusted for DHA (docosahexaenoic acid) (continuous variable) when the relative risk in mercury quintiles is modeled and is adjusted for mercury (continuous variable) when the relative risk in DHA quintiles is modeled.

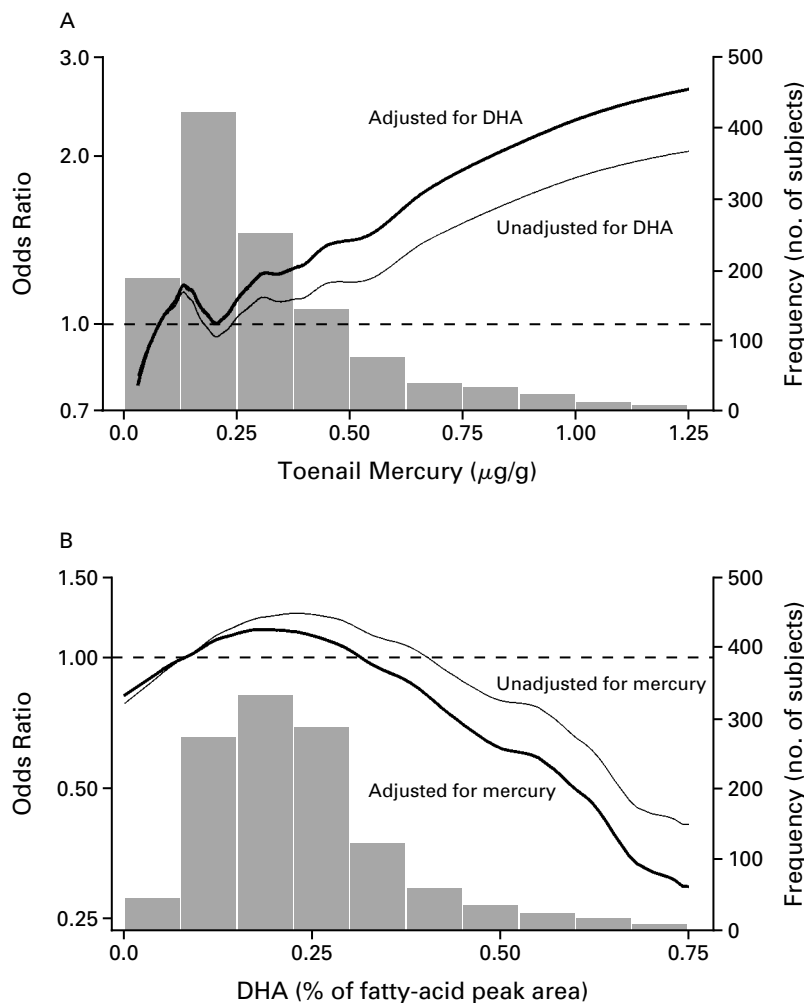
§Model 3 is further adjusted for body-mass index (continuous), waist:hip ratio (continuous), smoking status (indicator variables for current smokers and former smokers), alcohol intake (indicator variables for current and former drinkers), high-density lipoprotein cholesterol (continuous), diabetes (indicator variable), history of hypertension (indicator variable), parental myocardial infarction (indicator variable), α-tocopherol level (continuous), β-carotene level (continuous), toenail selenium level (continuous), and toenail weight (continuous).

from the 25th to the 75th percentile of the mercury distribution was 1.63 (95 percent confidence interval, 1.22 to 2.18;  $P=0.001$ ).

The dose-response curve for the relation between the mercury level and the risk of myocardial infarction was further examined by nonparametric logistic regression (Fig. 1).<sup>19</sup> There was a positive, monotonic increase in risk associated with mercury levels above 0.25  $\mu\text{g}$  per gram, which was steeper after adjustment for DHA levels.

The average levels of DHA, expressed as a percentage of the total fatty-acid peak area, were  $0.24 \pm 0.13$

percent in patients and  $0.25 \pm 0.13$  percent in controls. In analyses adjusted for age and center, there was no consistent relation between increasing DHA levels and the risk of myocardial infarction (Table 4).<sup>17</sup> After adjustment for the mercury level as well, there was a significant trend toward a lower risk of myocardial infarction with higher DHA levels ( $P$  for trend = 0.01). This trend was confirmed in the nonparametric analyses (Fig. 1). There was no interaction between mercury and DHA with respect to their associations with the risk of myocardial infarction ( $P$  for the interaction = 0.61).



**Figure 1.** Nonparametric Estimates of the Risk of Myocardial Infarction According to the Levels of Mercury in the Toenails (Panel A) and of Docosahexaenoic Acid (DHA) in Adipose Tissue (Panel B).

All curves have been adjusted for age and center. The nonparametric regression models used a lowess smoother with 40 percent span.<sup>19</sup> The reference value (odds ratio = 1.0) was set at 0.08  $\mu\text{g}$  per gram for mercury and 0.08 percent of the total fatty-acid peak area for DHA, both values corresponding to the 5th percentile of their respective distributions among controls. The bars represent the frequency distribution of mercury and DHA in the study sample.

We performed several sensitivity analyses to assess the consistency of our findings. First, we reanalyzed the data while excluding the results from Málaga, the center with the strongest effect of mercury. When we did so, the association of mercury with the risk of myocardial infarction persisted: the DHA-adjusted case-control ratio of mercury levels was 1.08 (95 percent confidence interval, 1.01 to 1.15). In addition, there were no significant differences in the association of mercury and myocardial infarction among study centers ( $P$  for the interaction between center and mercury level=0.20). Second, we found similar results in centers that used controls from the general population and in those that selected other types of controls (data not shown). Third, we confirmed that the participation rates in each center, both for patients and for controls, were not correlated with the association between mercury level and myocardial infarction ( $P=0.66$  for the correlation in controls and  $P=0.97$  for the correlation in patients). Finally, we assessed the association between mercury level and myocardial infarction, restricting our analyses to the five centers with the highest response rates among controls; the results were similar to our overall results (the ratio of the mercury level in patients relative to that in controls, after adjustment for DHA levels, was 1.12;  $P=0.005$ ).

#### DISCUSSION

In this international case-control study, we found an independent and graded association between toenail mercury levels and the risk of myocardial infarction. Furthermore, mercury masked an inverse association between DHA levels and the risk of myocardial infarction that became evident only after adjustment for the mercury level.

Several factors add to the strength of our findings. First, toenail and adipose-tissue samples were collected from patients shortly after they had had a myocardial infarction. These measurements are therefore unlikely to have been affected by the development of disease, a common limitation of case-control studies. Second, only patients with a first myocardial infarction were examined, so it is unlikely that they had changed their diet before the event. Finally, toenail mercury is a reliable biologic marker of long-term exposure to mercury.<sup>2,20,21</sup> The validity of the mercury measurements in our study is further reinforced by the finding of a strong association between mercury and DHA, a biologic marker of fatty-fish intake.<sup>18</sup>

Mercury exists in three forms: elemental mercury, inorganic mercury compounds, and organic mercury, primarily methylmercury.<sup>1,2</sup> Exposure to inorganic mercury occurs occupationally; people can also be exposed to inorganic mercury from silver-mercury amalgam in dental fillings. Exposure to methylmercury results almost exclusively from the consumption of

fish, shellfish, and marine animals; these foods are a major source of exposure to mercury for the general population.<sup>2</sup> Large, predatory fish, such as swordfish and sharks, have the highest concentrations of mercury (around 1  $\mu\text{g}$  per gram); tuna, trout, pike, and bass have intermediate concentrations (0.1 to 0.5  $\mu\text{g}$  per gram); and most shellfish have low concentrations.<sup>1,2</sup> In populations eating large quantities of fish from locally contaminated lakes or rivers, however, other species may be the main contributors to the total intake of mercury.<sup>6</sup>

Mercury may promote atherosclerosis and hence increase the risk of myocardial infarction in several ways. Mercury promotes the production of free radicals in experimental models,<sup>3-5</sup> and it may bind selenium to form mercury selenide, an insoluble complex that cannot serve as a cofactor for glutathione peroxidase.<sup>22</sup> In addition, methylmercury has a very high affinity for thiol groups, and it may inactivate the antioxidant properties of glutathione, catalase, and superoxide dismutase.<sup>23</sup> Mercury may induce lipid peroxidation,<sup>24</sup> and mercury levels were a strong predictor of oxidized low-density lipoprotein levels in the Kuopio Ischemic Heart Disease Study.<sup>6</sup> Mercury compounds may also promote platelet aggregability<sup>25</sup> and blood coagulability,<sup>26</sup> inhibit endothelial-cell formation and migration,<sup>27</sup> and affect apoptosis and the inflammatory response.<sup>28</sup> Increased rates of cardiovascular disease were found among mercury-exposed workers,<sup>29,30</sup> and mercury levels in hair predicted the progression of carotid atherosclerosis in a longitudinal study.<sup>31</sup> Toenail mercury, however, did not predict the incidence of coronary heart disease in a nested case-control study in U.S. health professionals reported elsewhere in this issue of the *Journal*.<sup>32</sup>

Some limitations also need to be considered in the interpretation of our findings. Our analyses were based on single measurements of mercury and DHA, and they are subject to random measurement error. In addition, the levels of mercury or DHA were low in many study participants, thus increasing the likelihood of analytical error. It is likely that the results of our analyses underestimate the associations of both mercury and DHA levels with myocardial infarction.

Another potential limitation of our study is that the participation rate was higher for patients than for controls. Although this raises the possibility of selection bias, the association of mercury levels with myocardial infarction was higher in centers with higher participation rates, making selection bias an unlikely explanation of our results. Furthermore, because both mercury and DHA are derived primarily from fish in the diet, selection bias would be expected to influence associations of the levels of both of these substances with myocardial infarction in the same direction, not in opposite directions.

We did not have information on the sources of mercury or DHA or on the amount and type of fish consumed by the study participants. However, the high mercury levels in the two Spanish centers are consistent with the high consumption of fish in that country<sup>33</sup> and the high levels of mercury in fish caught in the Mediterranean<sup>34,35</sup> and consumed in those cities. The correlation between mercury and DHA suggests that fish is probably the main source of mercury in toenails in our populations, although other sources of exposure are possible. Finally, our patient population was restricted to patients with myocardial infarction who survived until hospitalization. The observed associations thus cannot be generalized to patients with acute cardiac events who die before hospitalization.

Fish intake is currently recommended to reduce the risk of cardiovascular diseases<sup>36</sup> and as part of a Mediterranean-type diet.<sup>37</sup> However, the findings of epidemiologic studies of fish intake or fish-oil levels and coronary heart disease are contradictory, ranging from clearly inverse associations<sup>38-40</sup> to virtually null associations<sup>17,41-45</sup> and to positive associations.<sup>6</sup> Protective effects of fatty fish<sup>9</sup> and fish-oil supplements<sup>10</sup> have been found in two secondary-prevention trials. In both trials, the protection was largely limited to fatal coronary events, whereas we found an inverse association between DHA levels and nonfatal myocardial infarction. It is possible that, although the antiarrhythmic effects of fish oils may prevail in the prevention of recurrent events in patients who have had a myocardial infarction or in the prevention of sudden death from cardiac causes,<sup>46,47</sup> the antiaggregant and other antiatherogenic properties of fish oils may also have a substantial preventive effect.

The risk of cardiovascular disease in a population may depend on the balance between n-3 fatty acids and methylmercury in the fish consumed. Exposure to methylmercury is already a concern in specific high-risk groups; the Food and Drug Administration has advised pregnant women and women who may become pregnant not to eat swordfish, king mackerel, tilefish, shark, or fish from locally contaminated areas.<sup>48</sup> Our results raise the possibility that this advice should be extended to the general adult population. However, our findings do not imply that people should stop eating fish. Our mercury-adjusted analysis is consistent with a protective effect of dietary fish, provided it is not heavily contaminated.

The Heavy Metals and Myocardial Infarction Project was supported by a BIOMED-2 Concerted Action from the European Commission (research contract BMH4-CT98-3565) and was an ancillary project to the EURAMIC Study. The national studies were financed by grants from the British Heart Foundation, the Dutch Ministry of Health, the Spanish Fondo de Investigaciones Sanitarias, the German Federal Health Office, the Norwegian Research Council, the Russian Ministry of Science, the Swiss National Science Foundation, the Yrjö Jahnsson Foundation, and the Israel Science Foundation.

Presented in part at the 42nd Annual Conference on Cardiovascular Dis-

ease Epidemiology and Prevention of the American Heart Association, Honolulu, April 23-26, 2002.

*We are indebted to the members of the EURAMIC Study Group for making available the original data from the myocardial infarction part of their study. In addition to several of the authors, other members of the EURAMIC Study Group were Lenore Arab (Project Management Group), Ramón Gálvez-Vargas (Project Leader), Jussi K. Huttunen (Project Management Group), Alwine F.M. Kardinaal (Project Management Group, Project Leader), Blaise C. Martin (Project Leader), Vladimir P. Mazaev (Project Leader), J.J. Ringstad (Project Leader), and Michael Thamm (Project Leader).*

## APPENDIX

Other investigators of the Heavy Metals and Myocardial Infarction Project were Lydia Gorgojo, Institute of Health Carlos III, Madrid; Alwine F.M. Kardinaal, TNO Nutrition and Food Research, Zeist, the Netherlands; Jussi K. Huttunen, National Public Health Institute, Helsinki, Finland; Joaquín Fernández-Crehuet, Universidad de Málaga, Málaga, Spain; José F. Guillén, Universidad de Granada, Granada, Spain; Michael Thamm, Robert Koch Institute, Berlin, Germany; Blaise C. Martin, Zurich University, Zurich, Switzerland; Jetmund Ringstad, Østfold Central Hospital, Fredrikstad, Norway; and Vladimir Mazaev, Russian Ministry of Health, Moscow, Russia.

## REFERENCES

1. Keating MH, Mahaffey KR, Schoeny R, et al. Mercury study report to Congress. Vol. I. Executive summary. EPA-452/R-97-003. Washington, D.C.: Environmental Protection Agency, December 1997.
2. Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, Commission on Life Sciences. Toxicological effects of methylmercury. Washington, D.C.: National Research Council, 2000.
3. Magos L. Physiology and toxicology of mercury. *Met Ions Biol Syst* 1997;34:321-70.
4. Jansson G, Harms-Ringdahl M. Stimulating effects of mercuric- and silver ions on the superoxide anion production in human polymorphonuclear leukocytes. *Free Radic Res Commun* 1993;18:87-98.
5. Clarkson TW. The toxicology of mercury. *Crit Rev Clin Lab Sci* 1997;34:369-403.
6. Salonen JT, Seppanen K, Nyyssonen K, 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.
7. Rissanen T, Voutilainen S, Nyyssonen K, Lakka TA, Salonen JT. Fish oil-derived fatty acids, docosahexaenoic acid and docosapentaenoic acid, and the risk of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Circulation* 2000;102:2677-9.
8. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr* 2000;71:Suppl:171S-175S.
9. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* 1989;2:757-61.
10. GISSI-Prevenzione Investigators (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico). Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447-55. [Erratum, *Lancet* 2001;357:642.]
11. Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality: a systematic review of prospective cohort studies. *Eur J Clin Nutr* 1999;53:585-90.
12. Kardinaal AF, Kok FJ, Ringstad J, et al. Antioxidants in adipose tissue and risk of myocardial infarction: the EURAMIC Study. *Lancet* 1993;342:1379-84.
13. Kardinaal AF, Kok FJ, Kohlmeier L, et al. Association between toenail selenium and risk of acute myocardial infarction in European men: the EURAMIC Study: European Antioxidant Myocardial Infarction and Breast Cancer. *Am J Epidemiol* 1997;145:373-9.
14. Tuomilehto J, Kuulasmaa K. WHO MONICA Project: assessing CHD mortality and morbidity. *Int J Epidemiol* 1989;18:Suppl 1:S38-S45.
15. Bode P. Automation and quality assurance in the NAA facilities in Delft. *J Radioanal Nucl Chem* 2000;245:127-32.
16. Aro A, Kardinaal AF, Salminen I, et al. Adipose tissue isomeric trans

- fatty acids and risk of myocardial infarction in nine countries: the EURAMIC study. *Lancet* 1995;345:273-8.
17. Guallar E, Aro A, Jimenez FJ, et al. Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study. *Arterioscler Thromb Vasc Biol* 1999;19:1111-8.
  18. Marckmann P, Lassen A, Haraldsdottir J, Sandstrom B. Biomarkers of habitual fish intake in adipose tissue. *Am J Clin Nutr* 1995;62:956-9.
  19. S-PLUS 2000 user's guide. Seattle: Data Analysis and Products Division, Mathsoft, 1999.
  20. Garland M, Morris JS, Rosner BA, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. *Cancer Epidemiol Biomarkers Prev* 1993;2:493-7. [Erratum, *Cancer Epidemiol Biomarkers Prev* 1994;3:523.]
  21. MacIntosh DL, Williams PL, Hunter DJ, et al. Evaluation of a food frequency questionnaire-food composition approach for estimating dietary intake of inorganic arsenic and methylmercury. *Cancer Epidemiol Biomarkers Prev* 1997;6:1043-50.
  22. Cuvin-Aralar ML, Furness RW. Mercury and selenium interaction: a review. *Ecotoxicol Environ Saf* 1991;21:348-64.
  23. Naganuma A, Koyama Y, Imura N. Behavior of methylmercury in mammalian erythrocytes. *Toxicol Appl Pharmacol* 1980;54:405-10.
  24. Rungby J, Ernst E. Experimentally induced lipid peroxidation after exposure to chromium, mercury or silver: interactions with carbon tetrachloride. *Pharmacol Toxicol* 1992;70:205-7.
  25. Kostka B. Kinetic evaluation of ADP-induced platelet aggregation potentiation by methylmercuric chloride. *J Trace Elem Exp Med* 1991;4:1-9.
  26. Wierzbicki R, Prazanowski M, Michalska M, Krajewska U, Mielicki WP. Disorders in blood coagulation in humans occupationally exposed to mercuric vapors. *J Trace Elem Exp Med* 2002;15:21-9.
  27. Kishimoto T, Oguri T, Abe M, Kajitani H, Tada M. Inhibitory effect of methylmercury on migration and tube formation by cultured human vascular endothelial cells. *Arch Toxicol* 1995;69:357-61.
  28. Insug O, Datar S, Koch CJ, Shapiro IM, Shenker BJ. Mercuric compounds inhibit human monocyte function by inducing apoptosis: evidence for formation of reactive oxygen species, development of mitochondrial membrane permeability transition and loss of reductive reserve. *Toxicology* 1997;124:211-24.
  29. Barregard L, Sallsten G, Jarvholm B. Mortality and cancer incidence in chloralkali workers exposed to inorganic mercury. *Br J Ind Med* 1990;47:99-104.
  30. Boffetta P, Sallsten G, Garcia-Gomez M, et al. Mortality from cardiovascular diseases and exposure to inorganic mercury. *Occup Environ Med* 2001;58:461-6.
  31. 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.
  32. Yoshizawa K, Rimm EB, Morris JS, et al. Mercury and the risk of coronary heart disease in men. *N Engl J Med* 2002;347:1755-60.
  33. FAOSTAT 2001 CD-ROM: FAO statistical databases. Rome: Food and Agriculture Organization of the United Nations, 2001.
  34. Dorozynski A. Mediterranean poison fish forecast. *Nature* 1975;254:549-51.
  35. Von Burg R, Greenwood MR. Mercury. In: Merian E, ed. *Metals and their compounds in the environment: occurrence, analysis, and biological relevance*. Weinheim, Germany: VCH Verlag, 1991:1045-88.
  36. Krauss RM, Eckel RH, Howard B, et al. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Stroke* 2000;31:2751-66.
  37. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamele N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999;99:779-85.
  38. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985;312:1205-9.
  39. Daviglus ML, Stamler J, Orenica AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997;336:1046-53.
  40. Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002;287:1815-21.
  41. Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. *N Engl J Med* 1995;332:977-82.
  42. Morris MC, Manson JE, Rosner B, Buring JE, Willett WC, Hennekens CH. Fish consumption and cardiovascular disease in the Physicians' Health Study: a prospective study. *Am J Epidemiol* 1995;142:166-75.
  43. Gillum RF, Mussolino M, Madans JH. The relation between fish consumption, death from all causes, and incidence of coronary heart disease: the NHANES I Epidemiologic Follow-up Study. *J Clin Epidemiol* 2000;53:237-44.
  44. Wood DA, Riemersma RA, Butler S, et al. Linoleic and eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. *Lancet* 1987;1:177-83.
  45. Guallar E, Hennekens CH, Sacks FM, Willett WC, Stampfer MJ. A prospective study of plasma fish oil levels and incidence of myocardial infarction in U.S. male physicians. *J Am Coll Cardiol* 1995;25:387-94.
  46. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995;274:1363-7.
  47. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113-8.
  48. Center for Food Safety and Applied Nutrition. Consumer advisory: an important message for pregnant women and women of childbearing age who may become pregnant about the risks of mercury in fish. College Park, Md.: Food and Drug Administration, March 2001. (Accessed November 1, 2002, at <http://vm.cfsan.fda.gov/~dms/admehg.html>.)

Copyright © 2002 Massachusetts Medical Society.