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Markers of high fish intake are associated with decreased risk of a first myocardial infarction

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High intake of fish has been associated with reduced risk of CHD. The high content of *n*-3 polyunsaturated fatty acids (PUFA) in fish has been suggested to be a protective factor. In addition, fish is the entirely dominating source of methylmercury for the general population, and the concentration of Hg in erythrocytes (Ery-Hg) is often used as an index of fish consumption. Our aim was to study the relationships between a first-ever myocardial infarction, Ery-Hg, activity of gluthathione peroxidase in erythrocytes (Ery-GSH-Px) and plasma concentration of the *n*-3 PUFA eicosapentaenoic and docosahexaenoic acids (P-PUFA). In a population-based prospective nested case–control study within Northern Sweden seventy-eight cases of a first-ever myocardial infarction were compared with 156 controls with respect to Ery-Hg, P-PUFA and Ery-GSH-Px. Both Ery-Hg and P-PUFA, but not Ery-GSH-Px, were significantly ($P < 0{\cdot}0001$) higher in subjects reporting high fish intake (at least one meal per week) than in those with lower intake. This finding suggests that Ery-Hg and P-PUFA reflect previous long-term fish intake. Low risk of myocardial infarction was associated with high Ery-Hg or high P-PUFA. In a multivariate model the risk of myocardial infarction was further reduced in subjects with both high Ery-Hg and high P-PUFA (odds ratio 0·16, 95 % CI 0·04, 0·65). In conclusion, there is a strong inverse association between the risk of a first myocardial infarction and the biomarkers of fish intake, Ery-Hg and P-PUFA, and this association is independent of traditional risk factors.

Fish intake: Mercury: *n*-3 polyunsaturated fatty acids: Myocardial infarction

A decreased risk of CHD has been reported in subjects with high fish intake (Kromhout *et al.* 1985; Shekelle *et al.* 1985; Norell *et al.* 1986; Svensson *et al.* 1995*a*; Daviglus *et al.* 1997). The presently favoured theory is that the reduced risk is caused by *n*-3 polyunsaturated fatty acids (PUFA) that are present in high concentrations in fish. The effect has been especially attributed to the PUFA eicosapentaenoic acid (20:5 *n*-3; EPA) and docosahexaeonic acid (22:6 *n*-3; DHA) (de Deckere *et al.* 1998), which are closely associated with fish intake (Svensson *et al.* 1993). Dietary supplementation with these fatty acids has been shown to reduce the risk of cardiovascular complications in survivors of myocardial infarction (GISSI-Prevenzione Investigators, 1999) and recently it was found that high intake of fish and *n*-3 fatty acids was associated with decreased risk of ischaemic stroke in women (Iso *et al.* 2001). Significant correlations between the habitual fish intake and the proportions of EPA and DHA in the plasma phospholipids, respectively, of the same

Abbreviations: AMI, acute myocardial infarction; DHA, docosahexaeonic acid; EPA, eicosapentaenoic acid; Ery-GSH-Px, activity of glutathione peroxidase in erythrocytes; Ery-Hg, Erythrocyte Hg; GSH-Px, glutathione peroxidase; MeHg, methylmercury; MONICA, Monitoring Trends and Determinants in Cardiovascular Disease; P-PUFA, plasma concentrations of the *n*-3 PUFA EPA and DHA; PUFA, polyunsaturated fatty acid.

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magnitude for both fatty acids, were demonstrated (Andersen *et al.* 1996). A parallel protective factor of fish intake may be its importance as a source of Se (Åkesson *et al.* 1991; Svensson *et al.* 1992, 1995*b*; Bensryd *et al.* 1994; Hagmar *et al.* 1998), which is considered to protect against CHD (Salonen, 1987), possibly due to its presence in the antioxidant enzyme glutathione peroxidase (GSH-Px).

However, not all studies have shown a protective effect of fish consumption on CHD (Ascherio *et al.* 1995; Morris *et al.* 1995). Notably, Hg, for which fish is the dominating source (in the form of methylmercury (MeHg); World Health Organization, 1990), has been suggested to increase the risk of CHD (Salonen *et al.* 1995). Many studies have shown that a high consumption of MeHg-contaminated fish may cause neurotoxic effects (World Health Organization, 1990), but there are no additional reports of any impact of MeHg on the risk of CHD. Metabolically, Hg can inactivate antioxidant agents, such as glutathione, by binding to sulfhydryl groups. Furthermore, Hg can bind to Se, thereby reducing the activity of Se-containing factors including GSH-Px. Also, Hg may promote formation of free radicals (World Health Organization, 1990). The concentration of Hg in erythrocytes (Ery-Hg) is a strong indicator of MeHg uptake, and is a good and often-used index of fish consumption (Svensson *et al.* 1992), as fish in most populations is the major source of MeHg (World Health Organization, 1990). Ery-Hg has not been studied in relation to CHD. Surprisingly, hair Hg, which is also an index of MeHg intake (World Health Organization, 1990), has been claimed to be associated with increased risk of CHD (Salonen *et al.* 1995).

A problem in studies of the relationship between fish intake and CHD has been poor reliability of the data on fish consumption. Information about fish obtained by a questionnaire lacks precision and may even be biased (Rylander *et al.* 1998). Thus, there is a need to supplement such information by the use of relevant biomarkers, e.g. the PUFA EPA and DHA in plasma (P-PUFA) and Ery-Hg.

The aim of the present study was to evaluate if there is a relationship between the risk of acute myocardial infarction (AMI) and Ery-Hg, P-PUFA, or GSH-Px in erythrocytes (Ery-GSH-Px).

Materials and methods

Study population

A prospective case–control study design was used, nested within an ongoing community intervention programme on cardiovascular disease and diabetes prevention; The Västerbotten Intervention Programme launched in 1985. In this programme all men and women were invited to a health survey when they reached 30, 40, 50 and 60 years of age. At the same time, the two northernmost counties in Sweden, Västerbotten and Norrbotten, joined the WHO Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study. The Västerbotten Intervention Programme health survey was designed to fit the MONICA criteria so that it would be possible to refer to MONICA data when evaluating the community intervention programme. Participants in these two surveys were requested to donate blood samples to be stored for future research purposes. In September 1994, 36 405 subjects had been screened for cardiovascular risk factors, and more than 91 % of the participants had donated blood samples. Details about the methods have been given elsewhere (Weinehall *et al.* 1998). Of the 243 reported cases with myocardial infarction in the MONICA incidence registry (World Health Organization MONICA Project Principal Investigators, 1988), eighty-six fulfilled the criteria of a first-ever myocardial infarction. Of these, six were excluded because they had a cancer diagnosis according to the Swedish National Cancer registry and another two cases were excluded as their blood samples were inadequate for analysis. In the remaining seventy-eight cases that were included in the present study the myocardial infarction occurred on average 18 months after participation in the health survey (median 15 months). The control subjects were randomly selected from the same population-based health surveys. For the present study, they were matched for sex, age (±2 years), date of health survey (±1 year), and geographical region. Control subjects were excluded if they had reported a previous AMI or stroke, according to the survey questionnaire, or if AMI or stroke before the health survey could not be excluded from the case records, or if they had moved out of the MONICA region or died before 30 September 1994. They were asked to complete a questionnaire concerning, among other things, social background, medical history, intake of drugs, education (years) and various lifestyle factors, including diet, daily current smoking, drinking habits and stress.

Baseline examination and laboratory procedures

Smokers were defined as those reporting daily smoking of cigarettes, cigarillos, cigars or a pipe. Those participants who reported that they were ex-smokers or occasional smokers were classified as non-smokers. BMI was calculated as weight (kg)/ height $(m)^2$. Blood pressure was recorded after 5 min rest by means of an Hg random zero sphygmomanometer with the subject in a sitting posture. Korotkoff's 5th phase was used as the diastolic pressure. Hypertension was defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg, or reported use of anti-hypertensive medication during a period of 14 d before the health survey. Samples were obtained after a minimum of 4 h of fasting. Total cholesterol was measured using a benchtop analyser (Reflotron; Boehringer Mannheim GmbH, Mannheim, Germany) at each health survey centre at the time of the health survey. To evaluate these results, blood samples stored at −80°C were analysed using an enzymic method (CHO-PAP; Boehringer Mannheim GmbH, Mannheim, Germany) from 180 of the 234 cases and control subjects. The mean values for the two analyses differed by 0·04 mmol/l (r 0·90); adjustments were made based on these differences, using the enzymic method as the standard. Venous blood was obtained in Venoject tubes (Terumo Leuven, Belgium) containing sodium heparin for determination of GSH-Px and Hg, and EDTA for lipid determination. The blood cells and plasma were separated by centrifugation at 1500 ***g*** for 15 min, divided into aliquots, and stored frozen at −80°C until analysis. To avoid bias and interassay variability, the blood specimens

from cases and control subjects were analysed at random within triplets as described earlier.

Fish intake

The fish consumption was estimated from the questionnaires in the Västerbotten Intervention Programme, where the participants were asked about the number of fat and lean fish meals per week consumed at any time of the day. From these data, the subjects were divided into categories consuming ≥one or <one meal of fat fish, lean fish or total fish respectively per week.

Mercury determination

Ery-Hg was determined in acid-digested samples using the atomic fluorescence technique (Sandborg-Englund *et al.* 1998). The detection limit was 0·2 ng Hg/g erythrocyte. All samples were analysed in duplicate. The accuracy of the method is checked regularly by participation in an inter-laboratory programme (Centre de Toxicologie du Québec, Canada). During the year when the present samples were analysed, our results averaged 97 (range 90–102) % (*n* 18) of the target values within a concentration range of 2·5–35 ng Hg/ml erythrocytes. The precision of the method, calculated as the CV for the duplicate results, was 4 % in the concentration range <3 ng Hg/ml and 3 % in the ranges 3–6 and >6 ng Hg/ml. For internal method control, two commercial reference samples of lyophilised whole blood (Seronorm; Nycomed, Oslo, Norway) were included in each analytical series. For batch 205052, our result was 2·2 (SD 0·15) ng Hg/ml (*n* 26), and for batch 205053, 13·4 (SD 087) ng Hg/ml (*n* 27). The recommended values were 3 and 12·4 ng Hg/ml respectively. In addition, two samples, M-9710 and M-9711, from the inter-laboratory comparison programme were included repeatedly in the analytical series. Our results averaged 2·5 (SD 0·16) ng Hg/ml (*n* 13; target value 2·6 ng Hg/ml) and 5·9 (SD 0·60) ng Hg/ml (*n* 9; target value 6·0 ng Hg/ml).

Lipid analyses

Total cholesterol was measured by enzymic methods with Reflotron bench-top analysers (Boehringer Mannheim GmbH) at each health survey centre at the time of the health survey. The fatty acid composition of the plasma phospholipids EPA and DHA was analysed and results given as P-PUFA. Blood samples for fatty acid analysis were available from 211 subjects. The fatty acids were separated by GLC after separation of the lipids by TLC and transmethylation as described elsewhere (Boberg *et al.* 1985). The fatty acid methyl esters were separated on a 25 m wall-coated open-tubular glass capillary column coated with SLP OV-351 (Quadrex Corporation, New Haven, USA), with He as a carrier gas. A Hewlett-Packard (Avondale, PA, USA) system was used and the fatty acids were identified by comparing retention times with those of NuCheck Prep (Elysian, MN, USA) fatty acid methyl ester standards and PUFA mix no. 2 (Supelco, Bellefonte, PA, USA). The relative amounts of the fatty acids were expressed as a percentage of all fatty acids analysed.

Glutathione peroxidase determination

Ery-GSH-Px activity was determined using a coupled spectrophotometric technique (Hardell *et al.* 1993). To inhibit the activity of haemoglobin, potassium ferricyanide and cyanide were added to the haemolysates (Güntzler *et al.* 1974).

Table 1. Baseline characteristics of seventy-eight individuals with myocardial infarction and 156 matched controls subjects (Mean values and standard deviations)

	Cases		Controls subjects		Subjects with missing values	
	Mean	SD	Mean	SD	Cases	Control subject
Males:female	62	16	124	32	0	0
Age (years)	54·8	7·2	54·6	7·2	0	0
Education (≤12 years:≥12 years)	67:2		126:18		9	12
Fish intake (<one meal/week:≥one meal/week)	41:35		69:84		2	3
Current smoker: yes:no	30:39		41:107		9	8
% total	43		28			
Diabetes: yes:no	6:67		1:149		5	6
% total	8		0·7			
Hypertension: yes:no	31:41		40:113		7	3
% total	43		26			
Systolic blood pressure (mmHg)	143·4	20·3	137·2	17·7	3	0
Diastolic blood pressure (mmHg)	89·2	9·6	86·1	8·8	3	0
BMI (kg/m^2)	27·5	4·2	25·6	3·6	4	0
Total serum cholesterol (mmol/l)	6·75	1·41	6·39	1·22	1	0
n-3 PUFA (20:5 and 22:6) in plasma phospholipids (%)	5·8	1·5	6·3	1·6	8	15
Hg in erythrocytes (ng/g)	4·44	3·8	5·42	5·9	0	1
Gluatathione peroxidase activity in blood (μkatal/g haemoglobin)	1·22	0·30	1·22	0·28	0	1

PUFA, polyunsaturated fatty acids.

Table 2. Concentrations of mercury in erythrocytes (Ery-Hg), and sum of *n*-3 polyunsaturated fatty acids 20:5 and 22:6 in blood plasma phospholipids (P-PUFA), and activity of glutathione peroxidase in erythrocytes (Ery-GSH-Px) in groups with varying fish intakes (cases and control subjects combined)

Fish consumption (no. of meals per week)	Ery-Hg (ng Hg/g erythrocyte)			P-PUFA (%)			Ery-GSH-Px (μkatal/g haemoglobin)		
	n	Median	80 % Central range	*n*	Median	80 % Central range	*n*	Median	80 % Central range
Fatty fish									
<1	164	3·6***	1·9–7·6	148	5·6**	4·3–7·9	164	1·2	0·84–1·5
≥1	64	5·6	2·2–12	58	6·5	4·8–9·1	64	1·2	0·85–1·9
Lean fish									
<1	146	3·6**	1·8–7·6	136	5·6**	4·3–7·6	146	1·2	0·81–1·6
≥1	82	5·0	2·1–12	70	6·1	4·8–9·0	82	1·2	0·89–1·6
Total									
<1	109	3·3***	1·8–6·6	102	5·4***	4·3–7·6	109	1·2	0·83–1·6
≥1	119	5·2	2·1–10	104	6·2	4·7–8·5	119	1·2	0·88–1·6

Median values were significantly different from those for the group eating ≥one meal of fish per week (Mann–Whitney test): $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.
†For details of subjects and procedures, see p. 398.

Statistical analysis

Levels of the biomarkers were compared between groups with different fish intakes using the Mann–Whitney test. Also, Spearman's correlation coefficients (*r*S) for the biomarkers were calculated. Two-tailed $P < 0{\cdot}05$ was regarded as statistically significant. Univariate conditional logistic regression was employed to estimate the effect of each variable on the risk of a first-ever myocardial infarction (Hosmer & Lemeshow, 1989). Effect estimates are presented as odds ratios with 95 % CI. The continuous variables (Ery-Hg, P-PUFA, Ery-GSH-Px, serum cholesterol, BMI and diastolic blood pressure) were trichotomised, in order to evaluate approximately 'dose–response' relationships. Depending on the relationships obtained, these variables were sometimes further collapsed into dichotomous variables. Linear tests for trend, without categorising the continuous variables, were also performed. The variables which turned out to be influential ($P < 0{\cdot}2$) were considered in a multivariate conditional logistic regression analysis (Hosmer & Lemeshow, 1989). In the multivariate analysis only the combined P-PUFA variable was used, due to high correlation between the two fatty acids. Missing values were not replaced; the numbers of subjects with missing values per variable are shown in Table 1.

Results

Baseline characteristics of the seventy-eight subjects with a first-ever myocardial infarction and the 156 control subjects are shown in Table 1. Ten died within 28 d from the onset of the infarction, of these seven died before reaching the hospital. There were 119 subjects reporting 'high' (here defined as ≥one meal/week) intake of fish, and 110 reporting low intake (Table 1). The range levels of Ery-Hg

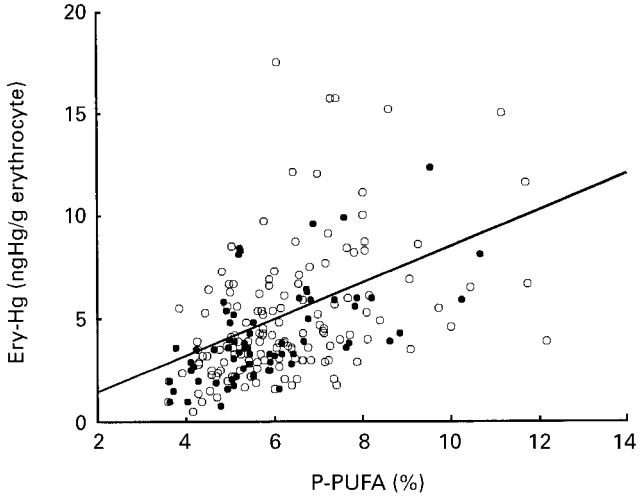

Fig. 1. Relationship between mercury levels in erythrocytes (Ery-Hg) and sum of concentrations of polyunsaturated fatty acids 20:5 and 22:6 in plasma (P-PUFA) in seventy-eight cases of acute myocardial infarction (●) and 156 matched control subjects (○). For details of subjects and procedures, see p. 398. Spearman's r 0·50, $P < 0{\cdot}001$.

was 0·5–67 ng Hg/g erythrocyte, P-PUFA 3·6–12·2 %, and Ery-GSH-Px 0·6–2·3 μkatal/g haemoglobin. Ery-Hg, as well as P-PUFA, were significantly higher in subjects who reported a 'high' fish intake, compared with those of subjects with 'low' intake, irrespective of whether total, fat or lean fish was considered (Table 2). On the other hand, Ery-GSH-Px activities did not differ significantly between those groups. In Fig. 1. the relationship between P-PUFA and Ery-Hg is shown (Spearman's r 0·50; $P < 0·001$). Ery-Hg correlated weakly with Ery-GSH-Px (Spearman's r 0·16; $P = 0·014$). There was no significant correlation between P-PUFA and Ery-GSH-Px. We found weak negative correlations between P-PUFA and systolic blood pressure (rS −0·15; $P = 0·029$) and BMI (rS −0·19; $P = 0·007$), but not with diastolic blood pressure and cholesterol.

The univariate analyses revealed that the risk of a first-ever myocardial infarction decreased with increasing Ery-Hg and P-PUFA, but increased with increasing serum cholesterol, BMI and diastolic blood pressure (Table 3). When evaluating EPA and DHA separately as continuous variables in univariate conditional regression analysis both were significantly related to the risk of AMI (odds ratio 0·56; 95 % CI 0·33, 0·99 and odds ratio 0·74; 95 % CI 0·55, 0·99 respectively). Also, smokers as well as subjects with less than 12 years of education showed a higher risk. The other variables (fatty and lean fish consumption and Ery-GSH-Px) showed no clear evidence to be influential.

The multivariate analysis revealed that subjects with both high Ery-Hg (>6 ng Hg/g erythrocyte) and high P-PUFA (>5·5 %) had a markedly lower risk (Table 4). When compared with subjects with both 'non-high' Ery-Hg and 'non-high' P-PUFA the odds ratio was 0·16 (95 % CI 0·04, 0·65), taking into account other influential variables.

Discussion

The present primary prospective study shows that the risk of a first-ever myocardial infarction was reduced in subjects with high levels of P-PUFA and Ery-Hg. The impact of the combination of P-PUFA and Ery-Hg levels on the AMI risk was strong. This finding indicates that the combination of the two estimates is a better index of fish intake than each of the estimates separately. More specifically, they would reflect different aspects of fish intake; P-PUFA is mainly an index of intake of fatty fish, while Ery-Hg reflects intake of all fish.

The chemical determinations of both P-PUFA and Ery-Hg are both accurate. However, they have limitations as biomarkers of fish intake. P-PUFA reflect the intake of fish

Table 3. Univariate conditional logistic regression analyses of relationships between acute myocardial infarction and potential risk or confounding factors

		n				
Factor	Category	Cases	Control subjects*	OR	95 % CI	Test for linear trend: $P=$
Fatty fish consumption (meals/week)	<1	56	109	1·0	–	
	≥1	20	44	0·85	0·45, 1·62	
Lean fish consumption (meals/week)	<1	52	95	1·0	–	
	≥1	24	58	0·76	0·42, 1·36	
Ery-Hg (ng Hg/g erythrocyte)†	≤3	27	43	1·0	–	
	3–6	39	68	0·91	0·49, 1·69	
	>6	12	44	0·43	0·19, 0·95	0·07
P-PUFA (%)‡	≤5·5	38	49	1·0	–	
	5·5–6·5	13	41	0·44	0·21, 0·94	
	>6·5	19	51	0·43	0·21, 0·88	0·02
Ery-GSH-Px (μkatal/g haemoglobin)	≤1	18	35	1·0	–	0·9
	1·0–1·5	50	97	1·00	0·52, 1·94	
	>1·5	10	23	0·80	0·31, 2·06	
Serum cholesterol (nmol/l)§	≤6	25	59	1·0		0·05
	6–7	20	40	0·99	0·49, 2·00	
	>7	33	49	1·61	0·84, 3·09	
Smoking status	Non-smoker	39	107	1·0	–	
	Smoker	30	41	2·01	1·00, 3·59	
BMI (kg/m^2)‖	<25	23	78	1·0	–	
	25–28	22	43	1·95	0·92, 4·16	
	>28	29	35	3·03	1·49, 6·16	0·007
Diastolic blood pressure (mmHg)¶	<80	14	45	1·0	–	
	80–90	26	57	1·11	0·67, 3·22	
	>90	35	54	2·34	1·05, 5·23	0·09
Education (years)	>12	2	18	1·0	–	
	≤12	67	126	4·70	1·06, 20·7	

OR, odds ratio; Ery-Hg, Hg level in erythrocytes; P-PUFA, Sum concentration of *n*-3 polyunsaturated fatty acids 20:5 and 22:6 in blood plasma phospholipids; Ery-GSH-Px, glutathione peroxidase activity in blood.

* In total seventy-eight cases and 156 control subjects were included; however, information was not complete.

† When Ery-Hg was collapsed into a dichotomous factor (≤6 *v.* >6) OR was 0·45 (95 % CI 0·22, 0·92).

‡ When P-PUFA was dichotomised (≤5·5 *v.* >5·5) OR was 0·43 (95 % CI 0·24, 0·79).

§ When serum cholesterol was dichotomised (≤7 *v.* >7), OR was 1·61 (95 % CI 0·91, 2·86).

‖ When BMI was dichotomised (≤25 *v.* >25) OR was 2·50 (95 % CI 1·32, 4·72).

¶ When diastolic blood pressure was dichotomised (≤90 *v.* >90) OR was 1·82 (95 % CI 0·98, 3·39).

Table 4. Multivariate conditional logistic regression analyses of relationships between acute myocardial infarction and potential risk or confounding factors

		Model 1*		Model 2†		Model 3‡	
Factor	Category	OR	95 % CI	OR	95 % CI	OR	95 % CI
Ery-Hg (ng Hg/g erythrocyte)	≤6	1·0	–				
	>6	0·51	0·21, 1·24				
P-PUFA (%)	≤5·5	1·0	–				
	>5·5	0·49	0·26, 0·91				
Ery–Hg × P–PUFA	≤6 and ≤5·5			1·0	–	1·0	–
	≤6 and >5·5			0·58	0·30, 1·11	0·87	0·37, 2·02
	>6 and ≤5·5			1·46	0·31, 6·94	1·09	0·19, 6·23
	>6 and >5·5			0·18	0·06, 0·56	0·16	0·04, 0·65
Smoking status	Non-smoker					1·0	–
	Smoker					2·20	0·95, 5·10
BMI (kg/m^2)	≤25					1·0	–
	>25					3·62	1·44, 9·15

OR, odds ratio; Ery-Hg, Hg level in erythrocytes; P-PUFA, sum concentration of *n*-3 polyunsaturated fatty acids 20:5 and 22:6 in blood plasma phospholipids.

* Ery-Hg and P-PUFA considered as two independent factors in the same model. The interaction between these two factors was tested by the likelihood ratio test ($P = 0{\cdot}10$). Thus, there was a tendency for a multiplicative interaction effect between these two factors (in contrast to model 2).

† Ery–Hg × P–PUFA considered as one factor (each individual was classified according to his or her Ery-Hg and P-PUFA). The numbers of individuals (cases:control subjects) in each category were: ≤6 and ≤5·5,: 34:42; ≤6 and >5·5, 26:60; >6 and ≤5·5, 4:6; >6 and >5·5, 6:32.

‡ Ery–Hg × P–PUFA, smoking status and BMI considered as three independent factors in the same model. The factors serum cholesterol, diastolic blood pressure and education did not contribute significantly to the model (test for linear trend; $P = 0{\cdot}5$, $P = 0{\cdot}16$ and $P = 0{\cdot}9$ respectively).

over the preceding weeks (Katan *et al.* 1991). Ery-Hg has a longer time perspective; several months (World Health Organization, 1990). Thus, the long-term fish intake, which is possibly the major risk indicator, was not reflected. However, the fish intake is probably fairly constant over time. Further, any variation would bias towards the null. This bias also holds for the fact that the MeHg content of fish from different sources varies (World Health Organization, 1990). There was no statistically significant association between myocardial infarction and questionnaire-reported intake of fish. This finding is probably due to the number of fish meals reported at baseline being a poor measure of the amount of fish consumed. Thus, the two presently-used biomarkers of fish intake, despite their limitations, are likely to give a much more valid measure of the long-term fish intake (Rylander *et al.* 1998).

We have not been able to identify significant confounding. There is a possibility that fish intake, as reflected by P-PUFA and Ery-Hg, is associated with sports fishing and thus with a physically active lifestyle, but this factor is probably of limited importance.

The present Ery-Hg values were generally low, in accordance with those seen earlier in Swedes (Svensson *et al.* 1987, 1992, 1995*b*; Åkesson *et al.* 1991; Bensryd *et al.* 1994; Oskarsson *et al.* 1996), although one subject had a level sufficiently high (67 ng Hg/g erythrocyte) to indicate a considerable intake of MeHg-contaminated fish. However, the levels are much lower than those associated with neurotoxic effects in adults (about 400 ng Hg/g erythrocyte) (World Health Organization, 1990).

Our results are in disagreement with the report from Finland by Salonen *et al.* (1995), who found strong associations between increased risk of AMI, CHD and total mortality, on the one hand, and fish intake, hair Hg (an index of MeHg intake) and urinary Hg (an index of exposure to inorganic Hg; Åkesson *et al.* 1991; Svensson *et al.* 1992; Oskarsson *et al.* 1996), on the other. In the highest hair Hg tertile (>2 μg Hg/g hair), there was a 2·9-fold (95 % CI 1·2, 6·6) increase in cardiovascular deaths. The results are interesting, but confusing in several ways. The average hair Hg level found in this Finnish population is very high. Thus, hair Hg of 2 μg Hg/g corresponds to an Ery-Hg of about 15 ng Hg/g erythrocyte (World Health Organization, 1990), a concentration which was only exceeded by two of our subjects. Hypothetically, there may be a negative effect of such a high exposure, possibly through formation of free radicals. However, this possibility needs confirmation.

While partially in contrast to the results of Salonen *et al.* (1995), the present negative association between AMI and Ery-Hg is in accordance with the results of a prospective study on a cohort of Swedish women in the whom the risk of CHD decreased with increasing MeHg and inorganic Hg in serum (Ahlqwist *et al.* 1993, 1999; Bergdahl *et al.* 1998).

There is a positive association between serum levels of Se and fish intake (Åkesson *et al.* 1991; Svensson *et al.* 1992, 1995*b*, Bensryd *et al.* 1994; Hagmar *et al.* 1998), and in populations with moderate Se intake, there is a correlation between Ery-GSH-Px and the blood Se content (Rea *et al.* 1979; van Callie-Bertrand *et al.* 1986). Se in selenocysteine forms the active site of GSH-Px. Despite this factor, we did not find any relationship between Ery-GSH-Px and fish intake. Such a relationship has previously been found for the plasma GSH-Px isoenzyme in a Latvian population (Hagmar *et al.* 1998). One explanation may be that the Se levels in Swedes are higher, and the importance of fish as a Se source is smaller in Sweden than in Latvia.

Important protective effects are associated with the P-PUFA. Several mechanisms are assumed to be involved; anti-thrombotic or anti-arythmic effects (de Deckere *et al.* 1998) and macrophage function and, thus, possibly plaque

stability (Lee *et al.* 1985; Grönholdt *et al.* 1998; Ross, 1999) and lowering of serum triacylglycerols (Harris, 1997). The possibility of the latter association is the reason why triacylglycerols were not included in the multivariate models. As for MeHg, it is unlikely that it would exert a protective effect against AMI. However, through intake of fish, it is associated with P-PUFA, and with Se concentrations in blood, (Svensson *et al.* 1992; Huang *et al.* 1995; Hagmar *et al.* 1998). Se has been claimed to reduce the risk of CHD (Salonen, 1987). On the other hand, we found no relationship between AMI and Ery-GSH-Px activity.

We conclude that there is a strong inverse association between the risk of a first-ever myocardial infarction and the biomarkers of fish intake, Ery-Hg and P-PUFA, and this association is independent of traditional risk factors.

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References

Ahlqwist M, Bengtsson C & Lapidus L (1993) Number of amalgam fillings in relation to cardiovascular disease, diabetes, cancer and early death in Swedish women. *Community Dentistry and Oral Epidemiology* **21**, 40–44.

Ahlqwist M, Bengtsson C, Lapidus L, Bergdahl I & Schutz A (1999) Serum mercury concentration in relation to survival, symptoms, and diseases: results from the prospective population study in women in Gothenburg. *Acta Odontologica Scandinavica* **57**, 168–174.

Åkesson I, Schütz A, Attewell R, Skerfving S & Glantz P-O (1991) Status of mercury and selenium in dental personnel: Impact of amalgam work and own fillings. *Archives of Environmental Health* **46**, 102–109.

Andersen LF, Solvoll K & Drevon CA (1996) Very-long-chain *n*-3 fatty acids as biomarkers for intake of fish and *n*-3 fatty acid concentrates. *American Journal of Clinical Nutrition* **64**, 305–311.

Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL & Willett WC (1995) Dietary intake of marine *n*-3 fatty acids, fish intake, and the risk of coronary disease among men. *New England Journal of Medicine* **332**, 977–982.

Bensryd I, Rylander L, Högstedt B, Aprea P, Bratt I, Fåhraéus C, Holmén A, Karlsson A, Nilsson A, Svensson B-L, Schütz A, Thomassen Y & Skerfving S (1994) Effect of acid precipitation on retention and excretion of elements in man. *The Science of the Total Environment* **145**, 81–102.

Bergdahl IA, Schütz A, Ahlqvist M, Bengtsson C, Lapidus L, Lissner L & Hultén B (1998) Methylmercury and inorganic mercury in serum – correlation to fish consumption and dental amalgam in a cohort of women born in 1922. *Environmental Research* **77**, 20–24.

Boberg M, Croon L-B, Gustafsson I-B & Vessby B (1985) Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. *Clinical Science* **68**, 581–587.

Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D & Shekelle RB (1997) Fish consumption and 30-year risk of fatal myocardial infarction. *New England Journal of Medicine* **336**, 1046–1053.

de Deckere EAM, Korver O, Verschuren PM & Katan MB (1998) Health aspects of fish and *n*-3 polyunsaturated fatty acids from plant and marine origin. *European Journal of Clinical Nutrition* **52**, 749–753.

GISSI-Prevenzione Investigators (1999) Dietary supplementation with *n*-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* **354**, 447–455.

Grönholdt M-L, Dalager-Pedersen S & Falk E (1998) Coronary atherosclerosis: determinants of plaque rupture. *European Heart Journal* **19**, Suppl. C, C24–C29.

Güntzler WA, Kremers H & Flohe L (1974) An improved coupled test procedure for glutathione peroxidase (EC 1-11-1-9) in blood. *Zeitschrift für Klinische Chemie und Klinische Biochemie* **12**, 444–448.

Hagmar L, Persson-Moschos M, Åkesson B & Schutz A (1998) Plasma levels of selenium, selenoprotein P and glutathione peroxidase and their correlations to fish intake and serum levels of thyroropin and thyroid hormones. A study on Latvian fish consumers. *European Journal of Clinical Nutrition* **52**, 796–800.

Hardell L, Danell M, Ängqvist C-A, Marklund SL, Fredriksson M, Zakari A-L & Kjellgren A (1993) Levels of selenium in plasma and glutathione peroxidase in erythrocytes and the risk of breast cancer. *Biological Trace Element Research* **36**, 99–108.

Harris W (1997) N-3 fatty acids and serum lipoproteins: human studies. *American Journal of Clinical Nutrition* **65**, S1645–S1654.

Hosmer DW & Lemeshow S (1989) *Applied Logistic Regression*, New York: Wiley & Sons.

Huang W, Åkesson B, Svensson BG, Schütz A, Burk RF & Skerfving S (1995) Selenoprotein P and glutathione peroxidase (EC 1.11.1.9) in plasma as indices of selenium status in relation to the intake of fish. *British Journal of Nutrition* **73**, 455–461.

Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Hennekens CH & Willett WC (2001) Intake of fish and omega-3 fatty acids and risk of stroke in women. *Journal of the American Medical Association* **285**, 304–312.

Katan MB, van Birgelen A, Deslypere JP, Penders M & van Staveren WA (1991) Biological markers of dietary intakes, with emphasis on fatty acids. *Annals of Nutrition and Metabolism* **35**, 249–252.

Kromhout D, Bosschieter EB & Coulander CL (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205–1209.

Lee TH, Hoover RI, Williams JD, Sperling RI, Ravalese J III, Spur BW, Robinson DR, Corey EJ, Lewis RA & Austen KF (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *New England Journal of Medicine* **312**, 1217–1224.

Morris MC, Manson JE, Rosner B, Buring JE, Willett WC & Hennekens CH (1995) Fish consumption and cardiovascular disease in the physicians' health study: a prospective study. *American Journal of Epidemiology* **142**, 166–175.

Norell SE, Ahlbom A, Feychting M & Pedersen NI (1986) Fish consumption and mortality from coronary heart disease. *British Medical Journal* **293**, 426.

Oskarsson A, Schütz A, Skerfving S, Palminger Hallén I, Ohlin B & Json Lagerkvist B (1996) Total and inorganic mercury in breast milk and blood in relation to fish consumption and amalgam fillings in lactating women. *Archives of Environmental Health* **51**, 234–241.

Rea HM, Thomson CD, Campbell DR & Robinson MF (1979) Relation between erythrocyte selenium concentrations and glutathione peroxidase (*EC* 1.11.1.9) activities of New Zealand residents and visitors to New Zealand. *British Journal of Nutrition* **42**, 201–208.

Ross R (1999) Atherosclerosis – an inflammatory disease. *New England Journal of Medicine* **340**, 115–126.

Rylander L, Strömberg U & Hagmar L (1998) Agreement between reported fish consumption obtained by two interviews and its impact on the results in a reproduction study. *European Journal of Epidemiology* **14**, 93–97.

Salonen JT (1987) Selenium in ischaemic heart disease. *International Journal of Epidemiology* **16**, 323–328.

Salonen JT, Seppänen K, Nyyssönen K, Korpela H, Kauhanen J, Kantola M, Tuomilehto J, Esterbauer H, Tatzber F & Salonen R (1995) Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. *Circulation* **91**, 645–655.

Sandborg-Englund G, Elinder C-G, Langworth S, Schutz A & Ekstrand J (1998) Mercury in biological fluids after a malgam removal. *Journal of Dental Research* **77**, 615–624.

Shekelle RB, Missell LV, Paul O, Shryock AM & Stamler J (1985) Fish consumption and mortality from coronary heart disease. *New England Journal of Medicine* **313**, 820.

Svensson B-G, Åkesson B, Nilsson A & Skerfving S (1993) Fatty acid composition of serum phosphatidylcholine in healthy subjects consuming varying amounts of fish. *European Journal of Clinical Nutrition* **47**, 132–140.

Svensson BG, Björnham Å, Schütz A, Lettewall U, Nilsson A & Skerfving S (1987) Acidic deposition and human exposure to toxic metals. *The Science of the Total Environment* **67**, 101–115.

Svensson B-G, Mikoczy Z, Strömberg U & Hagmar L (1995*a*) Mortality and cancer incidence among Swedish fishermen with a high dietary intake of persistent organochlorine compounds. *Scandinavian Journal of Work, Environment and Health* **21**, 106–115.

Svensson B-G, Nilsson A, Jonsson E, Schütz A, Åkesson B & Hagmar L (1995*b*) Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. *Scandinavian Journal of Work, Environment and Health* **21**, 96–105.

Svensson B-G, Schütz A, Nilsson A, Åkesson I, Åkesson B & Skerfving S (1992) Fish as a source of exposure to mercury and selenium. *The Science of the Total Environment* **126**, 61–74.

van Callie-Bertrand M, Degenhart HJ & Fernandes J (1986) Influence of age on the selenium status in Belgium and the Netherlands. *Pediatric Research* **20**, 574–576.

Weinehall L, Johnson O, Jansson JH, Boman K, Huhtasaari F, Hallmans G, Dahlen GH & Wall S (1998) Perceived health modifies the effect of biomedical risk factors in the prediction of acute myocardial infarction. An incident case-control study from northern Sweden. *Journal of Internal Medicine* **243**, 99–107.

World Health Organization (1990) *Methylmercury. Environmental Health Criteria* no. 101. *International Programme on Chemical Safety*, Geneva: WHO.

World Health Organization MONICA Project Principal Investigators (1988) The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. *Journal of Clinical Epidemiology* **41**, 105–114.