

Blood indices of selenium and mercury, and their correlations with fish intake, in young people living in Britain

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Relationships between Se and Hg in erythrocytes, and between these indices and intakes of fish and other foods, were studied as an adjunct to the British National Diet and Nutrition Survey (NDNS) of young people aged 4–18 years. Hg was measured in 965 packed erythrocyte samples by inductively coupled plasma mass spectrometry. Fe measurements permitted the calculation of whole-blood Hg. Erythrocyte and plasma Se, and 7 d weighed dietary intake estimates, were available. Erythrocyte Hg was positively skewed, normalised by log-transformation. It was 20% higher in girls than boys (3.17 v. 2.65 nmol/l, $P=0.004$), and increased with age in boys but not girls. It was directly and strongly correlated with erythrocyte or plasma Se. Hg and Se concentrations were directly correlated with fish intake. Certain other food groups were also directly correlated with Se and Hg concentrations, but less strongly than for fish. The strength and consistency of the relationship between erythrocyte Hg and Se suggests an important chemical link. Previous studies suggest that Se protects against the toxicity of Hg, and that fish is an important source of both. No toxic levels of Hg were found, which is reassuring because of the known health benefits of fish consumption, especially oily fish. Hg intakes need to be monitored, especially in women of child-bearing age, to ensure that Food Standards Agency guidelines are met.

Mercury: Selenium: Biochemical indices: Food intakes: Fish: Children: British National Diet and Nutrition survey

Hg and Se co-exist in certain foods that are used for human consumption, especially in fish (Clarkson *et al.* 1988; Ministry of Agriculture, Fisheries and Food, 1999; Combs, 2001; Cabanero *et al.* 2005). Previous studies have shown that dietary fish intake correlates strongly with plasma Hg concentration and with Se status indices in some human populations (Sherlock *et al.* 1984; Brune *et al.* 1991; Karita & Suzuki, 2002). Fish, especially those species which are near the top of the food chain, concentrate environmentally derived Hg via their prey (Ministry of Agriculture, Fisheries and Food, 1998; Food Standards Agency, 2002). Hg has no known essential or beneficial biological role and can be highly toxic, especially for the developing brain (Cuvin-Aralar & Furness, 1991). Se, on the other hand, is an essential nutrient, being specifically encoded and incorporated into a number of selenoproteins, many of which have known enzymic functions (Combs, 2001). Se intake has declined in the UK during recent decades, mainly because of a reduction in grain imports from Se-rich regions of North America, and may now be suboptimal, especially for protection against some cancers (Rayman, 2000, 2005).

An opportunity to study the relationships between these two elements in the blood and in the diet of British young people arose in the context of the National Diet and Nutrition Survey (NDNS) of young people aged 4–18 years, which was

performed on a representative cross-sectional population sample drawn from mainland Britain in 1997 (Gregory *et al.* 2000). Plasma Se concentrations were measured as part of the original survey (Gregory *et al.* 2000), and erythrocyte Se concentrations were measured in packed erythrocyte preparations from the same set of blood samples as part of a subsequent linked study (Bates *et al.* 2002). Hg concentrations in stored packed erythrocytes from the same blood sample-set have now been measured for the present study. These Hg measurements have permitted an exploration of the strength of the relationship between Se and Hg in blood, and of the relationships between each of these biochemical indices and the dietary choices that were recorded by 7 d weighed dietary intake records during the survey.

Materials and methods

Survey fieldwork

The survey and its procedures have been described in detail elsewhere (Gregory *et al.* 2000); therefore, only a brief summary is provided here. The population sample was obtained by random selection from eligible individuals living in 132 randomly chosen postcode sectors, which were randomly allocated to one of four sequential 3-month 'rounds' of fieldwork,

from January to December 1997. Participation in each part of the survey was invited but was not compulsory; therefore, not all of the participants complied with all of the parts of the survey. The survey included a demographic-socioeconomic-lifestyle questionnaire, a weighed dietary record that was kept for 7 consecutive days, and an early morning, usually fasting, blood sample that was taken by a trained phlebotomist.

The blood samples used for Se analysis were collected in EDTA containers (Monovettes; Sarstedt, Leicester, UK), and those used for Hg analysis were collected in contamination-controlled heparinised Monovettes (Sarstedt). The blood collection was performed by phlebotomists in the subjects' own homes; the blood subsamples were distributed and were used for a wide range of biochemical status measurements in three different laboratories.

Permission was given for the survey procedures, including the additional biochemical analyses, by individual National Health Service Local Research Ethics Committees representing each of the participating postcode sectors, and also by the South-East Multi-centre Research Ethics Committee and the Ethics Committee of the Medical Research Council Dunn Nutrition Unit, Cambridge, of which the Micronutrient Status Laboratory of Medical Research Council Human Nutrition Research was formerly a part.

Food intake records and food group estimates

Trained interviewers assisted the respondents to keep weighed records of their intakes of all foods eaten at home, plus diary records of foods eaten outside the home, for 7 consecutive days. These records included dietary supplements and information about brands of manufactured foods, methods of food preparation and leftovers. Computer and face-to-face checks for completeness and consistency were performed. The interviewers then coded the food items into about 6000 food codes, each code being allocated to one of 115 subsidiary food groups that were in turn aggregated into fifty-seven main food groups and then into eleven major food types. For the purpose of the present study, the 115 subsidiary food groups provided the principal basis for the analysis of food choices. The 7 d estimates of food group intakes in g/7 d were rounded to zero or to integral values of 1 or more, so there were no values between 0 and 1.

Se status analyses

The Survey Report (Gregory *et al.* 2000) includes summary results for plasma Se concentration, measured by inductively coupled plasma mass spectrometry in the laboratory of Dr Trevor Delves (SAS Trace Element Unit, Southampton General Hospital, Southampton, UK). The same laboratory also measured erythrocyte Se by a similar method (Delves & Sieniawska, 1997), and an analysis of the Se results of these erythrocytes has been previously described (Bates *et al.* 2002).

Erythrocyte Hg concentration analysis

Saline-washed erythrocyte pellets were used; these were prepared in hospital laboratories close to the sites of blood collection in the respondents' homes within 4 h of phlebotomy and

were stored frozen at -40°C and then at -80°C until analysed, apart from 2–3 brief freeze–thaw cycles for aliquot removal. The resuspended erythrocytes were thawed and vortex-mixed, and a 200 μl aliquot was added to 0.1% HNO_3 (200 μl), internal standard solution (Au 80 ng/l, Ga 3 $\mu\text{g/l}$; 200 μl) and diluent (0.01M- NH_3OH , 0.01% cysteine, 1% butanol; 3 ml).

Inductively coupled plasma mass spectrometry analysis was performed using an Elan 6100 Dynamic Reaction Cell instrument (Perkin Elmer, Beaconsfield, UK). Hg was quantified in standard mode using the ^{202}Hg isotope with five 2500 ms replicates. Fe was also quantified in standard mode using the ^{58}Fe isotope with five 10 ms replicates. Se was quantified in Dynamic Reaction Cell mode using CH_4 at 0.4 ml/min as the reagent gas, and the ^{78}Se isotope was measured with five 1000 ms replicates. Au (five 1000 ms replicates in both standard and Dynamic Reaction Cell mode) was used as the internal standard for Hg and Se, and Ga (five 100 ms replicates) was used as the internal standard for Fe. Calibration standards were prepared with human blood samples in place of the sample, and standard solutions were prepared in 0.1% HNO_3 .

Three Seronorm Trace Element Whole Blood samples at each of two concentrations were included with the analysis of each batch of samples. Throughout the analysis, the mean accuracies for Hg were 100.7% (CV 7.3%) at 2.2 ng/l and 103.1% (CV 5.0%) at 7.7 ng/l. Samples were also analysed blind for Hg as part of the Centre du Toxicologie du Quebec 'round-robin' interlaboratory comparison programme. The mean of eighteen analyses of six different samples agreed to within 0.5% of the Centre's participants' median concentrations, with a CV of 6.4%.

From the Fe content measurement, it was possible to calculate the Hb content of the diluted erythrocyte samples, and hence the whole-blood-equivalent concentration of Hg, in nmol/l, by the following formula:

$$\text{Hg (nmol/l)} = (\text{Hg} \times \text{Hb} \times 224 \times 10\,000\,000) / (\text{Fe} \times 64\,500 \times 200.6),$$

where Hg is measured (diluted-sample) Hg concentration in $\mu\text{g/l}$, Hb is original whole blood Hb concentration in g/dl, measured by the cyanmethaemoglobin assay, Fe is measured (diluted-sample) Fe concentration in mg/l, 64 500 is the molecular weight of Hb, of which four atoms of Fe contribute 224 Da, 200.6 is the atomic weight of Hg and 10 000 000 converts μg and mg to g, and dl to litres.

In populations whose exposure to Hg is mainly from their diet, rather than Hg amalgam tooth fillings or other inorganic sources (e.g. industrial Hg), the majority (approximately 95%) of total blood Hg has been shown to be in the erythrocytes (Kershaw *et al.* 1980; Sanzo *et al.* 2001). Therefore, the erythrocyte Hg concentration divided by the haematocrit is nearly the same as the whole-blood Hg content, thus enabling a comparison to be made between the Hg data set from the present study and a set of whole-blood Hg concentration estimates that were obtained for the recent NDNS of British adults (Ruston *et al.* 2004).

A useful check of intersample and interassay comparability was achieved by comparing the erythrocyte Se assay results from the 965 matched erythrocyte aliquots from the NDNS

young people aged 4–18 years that were analysed in both Southampton and Cambridge. The overall median Se for each of these two sets agreed to within 4%, and their individual values were closely correlated with a Pearson's r value of 0.8. No significant linear correlation between the individual differences between the two erythrocyte Se data sets and the mean individual values could be detected ($P=0.98$, NS); therefore, the small (4%) observed bias between the two data sets was constant throughout the range of values of the index. This was considered to indicate satisfactory interassay agreement, especially in view of the fact that different subsamples, and slightly different inductively coupled plasma mass spectrometry assay methodologies, were used in the two laboratories.

For the data analyses that are reported in the present paper, the erythrocyte Se (and plasma Se) estimates are those which were measured in Southampton, which are part of the same analytical data set that was used for our publication on Se status (Bates *et al.* 2002). The blood Hg measurements were performed in Cambridge.

Statistical methods

Statistical analyses were performed by DataDesk (Data Descriptions Inc., Ithaca, NY, USA) statistical programme and included both univariate and multivariate linear regression. For the Survey Report (Gregory *et al.* 2000), weighting factor adjustments were used to correct for the known sociodemographic differences between the composition of the survey sample and the entire (census) population of Great Britain. These adjustments were, however, unnecessary for the present study (which is mainly focused on cross-sectional comparisons between different indices), and they have therefore not been used here. The Hg, and to a lesser degree the Se, status data sets were positively skewed; therefore, \log_e -transformation was used in order to achieve a Gaussian distribution of values. For Hg, the skewness coefficient (for all data points) was +6.6 before log-transformation but fell to -0.5 after log-transformation, which is within the acceptable limits (for a Gaussian distribution) of -1.0 to +1.0.

The analytical approach to the study of the relationships between the blood indices and the food group intakes was tailored, for the present study, so as to be compatible with the large number of zero intakes in the 7 d diet records for many of the subsidiary food groups that were of interest. For the intake:status comparisons shown later in Tables 3 and 4, the intakes of each individual food group were scored as 1 for a zero recorded intake, and 2, 3 or 4 for ascending thirds of non-zero intake, these scores being entered into linear regression analysis *v.* the blood indices. For the analysis in Table 4 later, the square root of each 7 d food group intake was entered into linear regression *v.* the blood indices. Square-root transformation of the numerical values of the food group intakes was used to reduce any disproportionate contributions to the regressions by high outliers. Square-root transformation was used because many of the estimated intake values were zero, which is incompatible with log-transformation, and there were no values below 1.0; therefore square-root transformation reduced the positive skewness in these data sets. These two alternative approaches yielded similar conclusions

with respect to the relationships between food intake and blood index.

A value of $P<0.05$ was used as the cut-off for significant differences, except where large numbers of food groups were being compared (in Table 4 later), in which case $P<0.01$ was substituted.

Results

Of 2672 randomly selected subjects who were eligible for inclusion in the survey, 2127 (80%) co-operated with the initial interview, 1701 (64%) completed the diet record, and 1193 (45%) provided blood samples. Of these 1193 bloods, 965 (36% of the initial eligible sample) yielded valid results for all three biochemical indices: erythrocyte Se, plasma Se and blood Hg.

Table 1 shows the blood Hg concentrations in each age and gender subgroup and in the combined groups. A significant difference in mean \log_e Hg concentration was detected between the genders, with a higher value in girls, and there was a significant positive trend with increasing age in boys but not girls. The highest blood Hg value observed was 77.5 nmol/l (approximately 15.5 $\mu\text{g/l}$), and twenty-seven subjects (2.8% of the sample) had blood Hg levels greater than 15 nmol/l. There were no significant differences in Hg level between social classes 1, 2 and 3. There was a trend towards higher Hg concentrations in London and the south-east than in other major regions, but there were no significant regional differences (data not shown).

Erythrocyte and plasma Se values, subdivided by gender and age group, were included in our previous publication (Bates *et al.* 2002) and are therefore not tabulated here. For 1112 samples in which erythrocyte Se was measured, the previously reported mean value was 1.51 (SD 0.30) $\mu\text{mol/l}$; for 1127 samples in which plasma Se was measured, the mean was 0.87 (SD 0.15) $\mu\text{mol/l}$, and there was little indication of a skewed distribution for either of the Se indices. Similar mean Se index values were obtained for the slightly smaller sample set for which Hg measurements were also available. About 40% of blood Se was in the plasma compartment, with about 60% in the erythrocytes. The median molar ratio of Hg:Se in the erythrocyte fraction was 0.005, and the maximum observed value was 0.06; thus, no values approaching a molar ratio of unity were encountered.

Table 2 shows the results of linear regression analysis, comparing blood Hg concentrations with plasma or erythrocyte Se concentration. For each gender and age group considered separately, and for each of the combined groups, there were highly significant direct relationships between the blood Hg index and each of the Se indices. Figure 1 shows this relationship for blood Hg *v.* erythrocyte Se for all subjects. The plot suggests that the relationship is non-linear, with a stronger correlation between the two indices at higher values of both. Thus, for values of \log_e blood Hg concentration greater than 1.0, the relationship between the Hg and Se indices is highly significant ($t = 12.8$, $P<0.0001$), whereas for values of \log_e blood Hg concentration of less than 1.0, the relationship is not significant ($t = 0.4$, $P=0.6$).

Table 3 explores the relationship between blood Hg concentration and fish intake. Four categories of fish intake, each considered separately, and the sum of all four combined,

Table 1. Blood Hg concentrations by gender and age group

Gender and age group	<i>n</i>	Blood Hg concentrations (nmol/l)					
		Mean	SD	Median	Geometric mean	2.5th percentile	97.5th percentile
Boys							
4–6 years	66	3.17	2.22	2.76	2.50	0.57	9.13
7–10 years	150	3.86	6.73	2.92	2.49	0.27	10.26
11–14 years	154	3.51	3.42	2.66	2.45	0.42	13.86
15–18 years	125	4.44	3.70	3.48	3.25	0.52	15.20
All boys	495	3.80	4.64	2.91	2.65	0.38	12.42
Girls							
4–6 years	69	4.52	4.36	2.97	3.22	0.60	18.53
7–10 years	114	4.87	4.55	3.54	3.38	0.53	19.14
11–14 years	143	4.68	6.39	3.46	2.93	0.45	17.25
15–18 years	144	4.76	4.63	3.67	3.22	0.25	14.49
All girls	470	4.73	5.16	3.46	3.17	0.51	16.91
All boys and girls	965	4.25	4.92	3.12	2.89	0.44	15.27

Hg concentrations were measured in diluted samples of packed erythrocytes and have been expressed as nmol/l whole blood, assuming that all the Hg in blood is contained in the erythrocytes.

Significance of gender difference (girls > boys) for log_e blood Hg concentration overall: $t = 2.87$, $P = 0.004$.

Significance of linear regression of log_e blood Hg concentration v. age: for boys, $t = 2.09$, $P = 0.04$; for girls, $t = 0.22$, $P = 0.8$, NS.

were each subdivided into four ascending levels of intake. From this categorical analysis, it was clear that the blood Hg level was directly correlated with fish intake. A larger t value was seen with oily fish than with the other three fish categories, and oily fish also gave the biggest spread (greater than threefold) of blood Hg concentration between zero fish intake and the highest third of non-zero fish intake level.

Table 3 also explores the relationships that exist between erythrocyte Se concentration and fish intake. Three of the four individual categories of fish intake, plus the combination of all four categories, exhibited a strong direct correlation with erythrocyte Se concentration. The same was true for plasma Se concentration (data not shown). However, the fish intake for one fish category, that of fried white fish, was not significantly correlated with either of the Se indices. The relationships between fish intake and Se status were consistently less strong, in terms of t value, than the relationships between fish intake and blood Hg concentration in each of the fish categories.

Table 4 examines the significant ($P < 0.01$) relationships found between other food group intakes and the blood indices of Se and Hg. For the blood Hg index, by far the strongest correlation among the different food groups was that seen with total fish intake. A small number of other food groups were also positively related to the Hg index, and a single food group (non-whole grain, non-high-fibre breakfast cereal) was inversely related to it. For erythrocyte Se, there were nearly equal numbers of direct and inverse relationships with the intake estimates of these other food groups. The direct relationship with total fish intake was again stronger than for any other food group. The inclusion of total fish intake in the regression model for the other food groups shown in Table 6 had virtually no influence on the strength of the correlations between erythrocyte Se and these food group intakes; we therefore concluded that they did not solely reflect fish intake patterns but were independently linked to erythrocyte Se.

Table 2. Linear regression of log_e blood Hg concentration v. erythrocyte Se concentration and v. plasma Se concentration, by gender and age group

Gender and age group	Log _e blood Hg concentration v. erythrocyte Se concentration				Log _e blood Hg concentration v. plasma Se concentration			
	Degrees of freedom	Slope	SE (slope)	t	Degrees of freedom	slope	SE (slope)	t
Boys								
4–6 years	63	0.97	0.28	3.5**	65	1.98	0.49	4.0**
7–10 years	147	1.45	0.29	4.9**	145	2.50	0.53	4.7**
11–14 years	151	1.43	0.25	5.7**	152	1.71	0.55	3.1*
15–18 years	122	1.21	0.28	4.3**	122	2.00	0.51	3.9**
All boys	492	1.33	0.14	9.4**	495	2.03	0.27	7.6**
Girls								
4–6 years	66	1.32	0.32	4.1**	67	2.50	0.65	3.8**
7–10 years	111	1.26	0.21	6.0**	112	2.44	0.50	4.9**
11–14 years	140	1.47	0.26	5.6**	141	2.42	0.59	4.1**
15–18 years	141	1.36	0.23	6.0**	141	2.28	0.57	4.0**
All girls	467	1.39	0.12	11.4**	470	2.45	0.28	8.7**
All boys and girls	962	1.33	0.09	14.8**	968	2.23	0.19	11.5**

For each of these calculations, log_e blood Hg concentration was the dependent variable and the Se index was an independent variable, adjusted for age. In all cases the relationship was positive (i.e. direct).

* $P < 0.01$, ** $P < 0.001$.

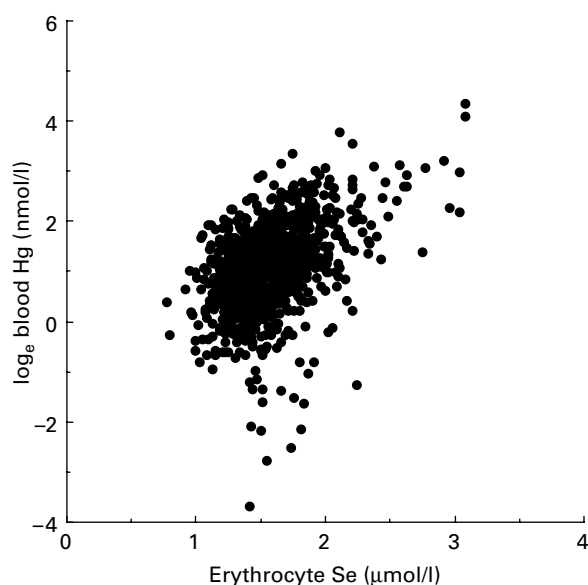


Fig. 1. Blood Hg plotted v. erythrocyte Se for all subjects.

The relationships observed between plasma Se concentration and food group intakes (data not shown) were generally in the same direction as those observed for erythrocyte Se concentration. The intake of the following food groups was directly and significantly ($P < 0.01$) correlated with plasma Se concentration: all fish combined, lamb dishes, chicken and turkey dishes, eggs, green beans, ‘other’ vegetables,

‘other’ fruit, fruit juice, rice and wholemeal bread. Intake of the following food groups was inversely and significantly correlated with plasma Se concentration: potato chips, savoury snacks, carbonated soft drinks.

The estimated intake of the following individual nutrient categories was significantly and directly correlated ($P < 0.01$) with the blood Hg index, after adjusting for age and gender: protein, Zn, total carotenoids, vitamin D, niacin, vitamin B₁₂, cholesterol and non-haem Fe. However, with the exception of total carotenoids and non-haem Fe, the significant relationship with each of these nutrient intakes became non-significant ($P \geq 0.01$) when the square root of total fish intake was also included in the regression model.

Discussion

Fish contribute, on average, about one third of the British population’s total dietary intake of Hg, which amounts to about 3 µg/d for an average British adult (Ministry of Agriculture, Fisheries and Food, 1999; Ysart *et al.* 2000). Most of the Hg in fish occurs as methyl-Hg cysteine (Harris *et al.* 2003) or as methyl-Hg (Cabanero *et al.* 2005). The methyl-Hg that fish absorb and contain is mainly derived from the action of bacteria upon inorganic Hg in the environment (Clarkson *et al.* 2003). Methyl-Hg cysteine may be converted to methyl-Hg chloride in the acid conditions of the stomach (Harris *et al.* 2003). This Hg derivative readily passes across cell membranes.

Table 3. Blood Hg and Se concentrations by defined ranges of fish intake

Categories and scores for fish intake	Range of intakes (g/7 d)	Mean intake (g/7 d)	n	Geometric mean blood Hg (nmol/l)	t for linear regression (Hg)	Mean erythrocyte Se (µmol/l)	t for linear regression (Se)
Fried white fish							
1	0	0	558	2.53		1.558	
2	0.1–117.0	79.8	123	2.96		1.557	
3	117.1–199.9	150.1	150	3.19		1.565	
4	> 199.9	327.2	134	4.48	6.3**	1.621	1.8
Other white fish							
1	0	0	860	2.72		1.553	
2	0.1–122.6	70.7	31	3.97		1.615	
3	122.7–244.8	188.8	39	5.31	5.9**	1.688	
4	> 244.8	507.0	35	5.19		1.751	4.7**
Shellfish							
1	0	0	899	2.83		1.560	
2	0.1–56.5	28.5	17	4.36		1.634	
3	56.6–109.6	72.3	28	3.82		1.665	
4	> 152.8	199.6	21	3.87	2.4*	1.702	2.9*
Oily fish							
1	0	0	707	2.37		1.526	
2	0.1–64.2	48.1	86	3.99		1.630	
3	64.3–152.8	106.6	80	5.16		1.671	
4	> 152.8	242.1	88	6.40	12.1**	1.750	7.8**
All fish combined							
1	0	0	345	1.84		1.499	
2	0.1–122.8	77.6	200	2.98		1.559	
3	122.9–251.3	182.5	212	3.50		1.572	
4	> 251.3	452.4	208	4.94	13.2**	1.685	7.0**

From the entire survey data set of food group intakes, each category of fish intake (fried white fish, other white fish, shellfish, oily fish) was subdivided into zero recorded intake and three ascending thirds of intake, so as to generate a score of 1–4 for each food group. The numbers in each third of non-zero intake are not exactly equal in the table because only a subset had Hg and Se measurements available. The linear regressions relate the log₁₀ blood Hg or erythrocyte Se values to the fish intake scores for each category of fish, and there are 963 degrees of freedom. The estimates are adjusted for age and gender. Although canned tuna may be considered relatively non-oily, it is included in the category ‘oily fish’ because whole tuna is classified as an oily fish.

* $P < 0.01$, ** $P < 0.001$.

Table 4. Linear regression of square-root food group intake v. log_e blood Hg concentration and v. erythrocyte Se concentration

Regression v. log _e blood Hg concentration		Regression v. erythrocyte Se	
Food group	<i>t</i>	Food group	<i>t</i>
All fish combined	+14.2**	All fish combined	+7.8**
Lamb and lamb dishes	+4.2**	Lamb and lamb dishes	+5.5**
Chicken and turkey dishes	+5.2**	Chicken and turkey dishes	+4.2**
Salad	+4.2**	Salad	+4.3**
Leafy green and 'other' vegetables	+3.7 to +4.3**	Leafy green and other vegetables; vegetable dishes	+4.0 to +5.6**
Cooked carrots	+3.2**		
Rice	+4.6**	Pasta, rice	+3.0 to +8.7*
		Soup	+3.3**
		Oranges, 'other' fruit	+3.3 to +3.8**
		Polyunsaturated oil	+3.4**
Breakfast cereal (excluding whole grain, high fibre)	-3.1*	Breakfast cereal (excluding whole grain, high fibre)	-3.0*
		Sausages, meat pies, bacon and ham	-3.2 to -4.1**
		Potato chips	-3.6**
		Biscuits, savoury snacks	-3.0*
		Sugar or chocolate confectionary	-2.6 to -3.2*
		Ice cream	-3.7**
		Canned fruit in syrup	-2.8*

Hg and Se indices were the dependent variables; square-root food group intakes were independent variables. The estimates shown are all adjusted for age and gender. Other subsidiary food groups, listed in full in the Survey Report (Gregory *et al.* 2000) were not correlated ($P > 0.01$) with either Hg or Se concentration. These included eleven categories of bread, cakes, sweet puddings and cereal products; ten categories of dairy products; nine categories of butter and different types of margarine; eggs and egg dishes; beef; pork and liver; burgers and kebabs; several types of fruit and vegetables that are not listed in the table; sugar and preserves; fruit juices and soft drinks; and beer, wine and beverages.

* $P < 0.01$; ** $P < 0.001$.

Within the body, methyl-Hg is carried as water-soluble complexes, mainly attached to the S atom of thiols, especially L-cysteine and glutathione. It is carried across cell membranes, including the blood-brain barrier and placenta, on the large neutral amino-acid carrier, which also carries L-methionine (Clarkson, 2002). Within the erythrocyte, methyl-Hg binds to cysteine residues of Hb. Hb also contains a major proportion of the Se in erythrocytes, in the form of selenomethionine, and a selenomethionine-rich meal contributes more Se to Hb than to seleno-enzymes in blood (Thomson *et al.* 1993). The half-life of Hg is about 70 d in human adults, and most of it is eliminated in the faeces after conversion to inorganic Hg (World Health Organization, 1976). Hg can cause damage and malfunction, especially in the developing brain and the kidney, and may also cause congenital malformations and immune function abnormalities. The most vulnerable population group is pregnant women because of serious potential damage to the developing fetal brain (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004). Children are considered to be more vulnerable than adults, partly because their food intake per kg body weight is higher (Clarkson *et al.* 2003).

A Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives and Contaminants (JECFA) has concluded (mainly from contamination accident evidence) that 200 µg Hg/l blood is the lowest concentration that is associated with detectable functional toxicity in some individuals. In 1972, the committee defined provisional tolerable weekly intakes (PTWI) of Hg as 0.3 mg total Hg for adults, of which not more than 0.2 mg should be methyl-Hg (World Health Organization, 1972). This is equivalent to 5 and 3.3 µg/kg body weight per week for a 60 kg subject. The 3.3 µg/kg value for methyl-Hg was reconfirmed in 1989 and 2000. However, in 2003 the PTWI upper limit was reduced to 1.6 µg/kg per week for pregnant women and those about

to become pregnant (World Health Organization, 2003). This can be calculated to correspond to a blood Hg concentration of 16 µg/l or 80 nmol/l (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004), whereas the highest concentration encountered in the present study was 77 nmol/l. The US Environmental Protection Agency established a reference dose for methyl-Hg that was about one fifth of the 1972 JECFA PTWI, namely 0.1 µg/kg body weight per d (0.7 µg/kg per week; Environmental Protection Agency, 1997) and this was upheld by the National Research Council (2000). The 2003 JECFA PTWI and the Environmental Protection Agency reference doses correspond to blood Hg levels of approximately 80 and 35 nmol/l, respectively.

In the present study, only 2.8% of the population sample had Hg values over 15 nmol/l; therefore, the population as a whole appears to be at low risk of Hg toxicity. The UK Food Standards Agency's Committee on Toxicity (2003) considers that the 1972 JECFA PTWI of 3.3 µg/kg per week is sufficiently protective for the general (British) population, including children, but that for women who are pregnant or breast-feeding or are about to become pregnant within a year, the new JECFA PTWI of 1.6 µg/kg per week is more appropriate. The NDNS data were considered to be reassuring with respect to average- and high-level consumption of fish, insofar that it appears that the new (2003) JECFA PTWI for methyl-Hg is generally not being exceeded (Scientific Advisory Committee on Nutrition/Committee on Toxicity (2004) Advice on fish consumption: benefits & risks, 2004).

Strong positive correlations have been reported between Hg concentrations and Se levels in human tissues, for example following exposure to inorganic Hg (Kosta *et al.* 1975), and similar positive correlations between the two elements have also been encountered in the livers of marine mammals (Koeman *et al.* 1975). It was therefore proposed, 25–30 years ago, that Se interacts directly with Hg (and other

heavy metals) in marine foods, and that it may protect against the toxic effects of Hg (Magos & Webb, 1980), although the exact mechanisms for the protective effect have not been elucidated. Free-radical limitation was one suggested mechanism (Ganther, 1978), based on observations of protection by vitamin E as well as by Se against methyl-Hg toxicity. An additional mechanism for the formation and binding of 1:1 molar Hg:Se complexes to selenoproteins is likely to be the basis of another important protective mechanism, as discussed in the following paragraphs.

Several more recent studies have also commented on the strong correlation that is encountered between Se and Hg in human blood and tissues (Cuvin-Aralar & Furness, 1991; Whanger, 1992). A study of Faroe islanders (Grandjean *et al.* 1992, 1997), whose intake of marine food, and hence of methyl-Hg, is relatively high, demonstrated a positive correlation between erythrocyte Hg and plasma Se. A Japanese study (Hongo *et al.* 1985) found that both erythrocyte and plasma Se were positively correlated with erythrocyte Hg. A Swedish study (Svensson *et al.* 1992) recorded a direct correlation between erythrocyte Hg and plasma Se, as did a study in northern Finland (Luoma *et al.* 1992). In autopsied human organs, including pituitary and kidney, studied in Germany (Drasch *et al.* 2000), the analytical evidence indicated a 1:1 molar association of Hg with Se, such that at high Hg burdens, a major fraction of total Se was bound to Hg, and the Hg:Se molar ratio approached 1.0. Selenoprotein P in plasma can bind Hg–Se as a 1:1 molar complex, accumulating up to over 1000 such units per mol protein (Yoneda & Suzuki, 1997), and a 1:1 molar ratio of accumulated Hg and Se was recorded in autopsied pituitaries of Swedish dental staff (Nylander & Weiner, 1991).

Some studies (e.g. Svensson *et al.* 1992) have attributed the observed Hg:Se correlations in human tissues and blood to a common dietary source of both elements in fish. People with amalgam dental fillings excreted less Se than those without amalgam fillings (Hol *et al.* 2002). About 90% of the Hg in erythrocytes occurs as methyl-Hg (Berglund *et al.* 2005). Methyl-Hg can be converted to inorganic Hg in several human tissues, notably the kidney (Clarkson *et al.* 1988). It is, however, not clear whether any of the methyl-Hg that enters the erythrocytes from dietary sources such as fish is converted to inorganic Hg before it can interact with Se within this tissue, nor whether it thereby establishes a Hg–Se complex and hence a correlation with Se, specifically within the erythrocytes. The nature of the interactions between methyl-Hg and Se (both dietary and endogenous), and the possible significance of Se for the detoxification of methyl-Hg, are not yet well understood (Watanabe, 2002).

The main purpose of the present study was to explore the relationships between blood Hg, Se status and dietary choices in British young people, in order to establish whether the blood Se and Hg indices are as strongly correlated in this population group as they are in other populations, and whether there are associations with fish intake, which could account for the correlations that are observed in the blood. Although an association of Hg in blood with Hg amalgam tooth fillings is possible, this was considered to be of uncertain importance as a determinant of blood Hg (International Programme for Chemical Safety, 1991). Fewer than 20% of the participants in the survey had any Hg amalgam dental fillings (Walker *et al.* 2000).

Blood Hg varied significantly with age in boys over the age range studied, and was slightly but significantly higher in girls than in boys. Our previous study (Bates *et al.* 2002) also established that the erythrocyte Se concentrations were slightly higher in girls than in boys, although plasma Se did not differ significantly between the genders. In the recent NDNS of British adults, conducted during 2000–2001 (Ruston *et al.* 2004), the median blood Hg concentration increased from 2.3 nmol/l in men aged 19–24 years to 6.6 nmol/l in men aged 35–49, and from 4.3 nmol/l in women aged 19–24 years to 7.0 nmol/l in those aged 35–49. Although the sampling and analytical methodologies were not identical between the young people's and the adults' NDNS surveys, these data suggest that blood Hg concentration probably continues to increase with age above 18 years and that it is consistently higher in women than men. In the same adult population sample (Ruston *et al.* 2004), the mean erythrocyte Se concentration increased from 1.42 $\mu\text{mol/l}$ in men aged 19–24 years to 1.64 $\mu\text{mol/l}$ in men aged 35–49, and from 1.73 $\mu\text{mol/l}$ in women aged 19–24 to 1.80 $\mu\text{mol/l}$ in women aged 35–49, with a median erythrocyte Se:Hg ratio in the region of 250 in adults. Thus, the erythrocyte concentrations of Hg and Se appear to increase in parallel with increasing age in British subjects and exhibit a modest but consistent gender difference (female > male).

A Danish (Grandjean *et al.* 1992) and a Japanese study (Hongo *et al.* 1985) have also reported a higher erythrocyte Se concentration in women than in men, but a Canadian study (Mahaffey & Mergler, 1998) found that men had a higher blood Hg concentration than women. A strong direct relationship was observed between the blood Hg concentration and both of the Se status indices in all age groups in the present study of young people. This correlation may have a number of explanations, among which could be a common dietary source of these two elements, and/or a chemical interaction involving both elements, which results in an accumulation of both of them at key tissue sites, including the erythrocyte compartment.

The present study has confirmed the prediction that the Hg concentration in blood, which is commonly used as a proxy estimate of the whole-body burden of Hg, was strongly correlated with the fish intake of young people in Britain. It was especially strongly correlated with the intake of oily fish, even though oily fish are not necessarily located at the top of the food chain. Some oily fish, such as fresh tuna, undoubtedly have higher Hg levels than most white fish, whereas others, such as sardines, mackerel and salmon, probably do not (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004; Cabanero *et al.* 2005). Other food groups that were significantly correlated with blood Hg included green vegetables. Several individual nutrient intakes were directly correlated with blood Hg concentration; however, 75% of these correlations became non-significant when adjusted for total fish intake, which suggests that they may have been acting as proxy indicators for a relatively fish-rich diet.

The association of the Se status indices with the intake of specific food groups shared some similarities with those seen for Hg. The intake of fried white fish, however, did not significantly predict Se status, whereas the intake of the other three fish groups was predictive. Although Hg that is ingested with fish may increase the erythrocyte content of

Se, by relocating or stimulating the production of Hg-sequestering Se and selenoproteins, in practice the molar ratio of Se to Hg in blood is so high in the UK that a moderate increase in erythrocyte Hg is unlikely to alter its Se concentration.

From a public health viewpoint, it is encouraging that no toxic levels of Hg were found. The known benefits of fish consumption, especially oily fish, are likely to outweigh any potential risks of Hg contamination at current levels of consumption (Bouzan *et al.* 2005; Smith & Sahyoun, 2005). However, monitoring of Hg intakes, especially of women of child-bearing age, should continue in order to meet Food Standards Agency guidelines.

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