Selenium content of wheat for bread making in Scotland and the relationship between glutathione peroxidase (*EC* 1.11.1.9) levels in whole blood and bread consumption

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The selenium content of the 1989 harvest of wheat used for bread making in Scotland ranged from 0·028 μg/g dry weight for home-grown wheat to 0·518 μg/g for Canadian wheat. The tonnage values indicate that 13·8% of the wheat used in bread making came from Canada. This reflects in a calculated dietary intake of 31 μg/d which is well below the recommended levels of 70 and 55 μg for adult males and females respectively (National Research Council, 1989). The average glutathione peroxidase (*EC* 1.11.1.9) level in 478 samples of human whole blood was 6·08 (SE 0·065) units/ml. This increased to 6·65 (SE 0·321) in sixty-two subjects consuming brown or wholemeal bread but was unaffected by oily fish consumption. Analysis of a small number of samples of whole milk, eggs and meat indicated slightly higher concentrations than previously published values but this trend was insufficient to compensate for the lower cereal provision of Se.

Selenium: Wheat flour: Bread: Human RDA: Glutathione peroxidase

For more than 25 years selenium has been recognized as an essential nutrient to mammals and birds, with white muscle disease in lambs and calves, exudative diathesis in poultry and hepatosis dietetica in pigs arising from Se deficiency (Underwood, 1977). In recent years it has been established that Se is also essential to man, and diseases resulting from Se deficiency have been described (Zhu, 1981; Diplock, 1987). Epidemiological studies, especially from Finland, have linked a lower Se status to an increased risk of cancer in man. The relative risk in these studies was from 2 to 5·8 in lower Se status alone, and up to 11·4 in subjects with combined lower Se and vitamin E status (Salonen *et al.* 1985). Associations were established between cardiovascular death and myocardial infarction and serum Se by Salonen *et al.* (1982). Kok *et al.* (1989) found decreased Se levels in acute myocardial infarction, while Korpela *et al.* (1989) noted a beneficial effect of Se supplementation after acute myocardial infarction. Oster *et al.* (1983) suggested that low serum Se was associated with congestive cardiomyopathy and Reeves *et al.* (1989) showed reversible cardiomyopathy due to Se deficiency in a single case study. There is evidence that Se has an antioxidant role in man (Stadtman, 1990), especially with respect to a low Se status in the diseases already mentioned (Pearson *et al.* 1990). Wayner *et al.* (1987) has shown that the total (peroxyl) radical-trapping antioxidant parameter (TRAP) of plasma would point to up to 50% being from plasma proteins with the plasma sulphydryl groups providing the first line of defence during peroxyl radical attack.

The average intake of Se in Britain was calculated as 60 μg/d by Thorn *et al.* (1978) and as 43 μg/d by the present authors in 1986. The recommended levels of Se intake quoted by the National Research Council (1989) are 70 and 55 μg/d for adult males and females respectively.

The availability of Se in Scottish soils is low, as in many parts of Northern Europe, due to the acid nature of the geological parent material (Berrow & Ure, 1989). Traditionally the wheat that has been grown in Scotland has been generally only low-protein winter varieties unsuitable for bread production. Bread-making wheat has been largely imported from Canada. This wheat is associated with higher levels of Se (Arthur, 1972). In recent years the protein content of the wheat grown in Scotland has improved, with the result that a high proportion of the wheat now used in bread making is no longer Canadian. This trend towards decreasing use of Canadian wheat was identified by Barclay & MacPherson (1987) and, as it is continuing, we have measured the Se concentrations in flours and breads and considered the nutritional effect by calculation of the average dietary intake of Se and by determining glutathione peroxidase (*EC* 1.11.1.9; GSH-PX) activity in human blood.

MATERIALS AND METHODS

Sources of samples

Flour. The samples, obtained from Allied Mills Ltd, Glasgow (4); Carrs Flour Mills, Silloth, Cumbria (7); Robert Hutcheson & Co., Kirkcaldy (5); Rank Hovis Ltd, Caledonian Mills, Edinburgh (6) and Rank Hovis, Solent Mills, Southampton (10), represented flour in routine production and used in the baking of various types of bread from wholewheat to sliced pan. Triplicate analyses were carried out on each flour sample.

Bread. The samples were of fourteen commonly consumed brands, purchased locally.

Milk. Single samples were obtained from the bulk silos of six Scottish creameries.

Eggs. Samples were obtained locally: three from supermarket chain stores, two from the College farm and one from a local free-range flock.

Beef. Four different cuts were obtained locally.

Analytical

Sample preparation for Se determination was carried out using the nitric acid–perchloric acid digestion procedure recommended in *The Analysis of Agricultural Materials* (Ministry of Agriculture Fisheries and Food, 1986). Se in the digest solution was then determined by atomic absorption spectrometry via hydride generation. The flour and bread samples were analysed as received, while the milk, eggs and beef were initially freeze-dried with the final result calculated in all cases on a 100° dry matter basis. The analytical method was verified by carrying out the analysis of a Community Bureau Reference wholemeal flour (BCR no. 189, supplied by the Laboratory of the Government Chemist, Teddington, Middlesex). The results (140 (SE 5·0) ng/g) agreed with the certified Se level of 132 (SE 10·0) ng/g.

Protein was determined by modified Kjeldahl digestion.

GSH-PX was measured in whole blood by the method of Paglia & Valentine (1967) as modified by Lawrence & Burk (1976). The reaction was carried out at 30°. The final glutathione (GSH) concentration in the assay mixture was 4 mM and this was buffered at pH 7·2. The reaction was started by the addition of 0·05 ml cumene hydroperoxide (10 mM). Internal quality control was maintained by use of a standardized GSH-PX preparation supplied in lyophilized form by Boehringer Mannheim. External quality control was maintained by participation in a Ministry of Agriculture, Fisheries and Food scheme involving twenty laboratories.

Ayrshire Heart Health Study 1987

Volunteers (478) were selected from two regions within Ayrshire, i.e. Kilmarnock and Loudoun, and Cumnock and Doon Valley.

The participants were matched for age and sex. On attending their local clinic they

Table 1. *Selenium content (μg/g dry weight) in flours used in bread**

(Mean values with their standard errors)

Flour		No. of samples	Se concentration Mean	SE	Range
Protein content:	< 130 g/kg	5	0·079	0·05	(0·026–0·148)
	130–150 g/kg	19	0·168	0·103	(0·028–0·439)
	> 150 g/kg	8	0·234	0·148	(0·046–0·518)

* For details of sources, see p. 262.

provided a blood sample and were invited to complete a questionnaire on their health and diet. The questionnaire used was the standard Scottish Heart Health Study questionnaire, the dietary section being that developed by the MRC Epidemiology Unit in Cardiff (Yarnell *et al.* 1983). The questions used related to sex, age, social class, district of residence, cardiac record, proportion and type of bread and fish in the diet. This was done in conjunction with the Ayrshire and Arran Coronary Prevention Programme – Baseline Risk Factor Survey (Cairns Smith *et al.* 1989).

Weights of food consumed

The weights of different foods consumed on average in the UK and Scotland were abstracted from the Annual Report of the National Food Survey Committee (Ministry of Agriculture, Fisheries and Food, 1989). These data are based on household consumption rather than individual estimates.

Statistical analysis

The differences between means of groups were tested by the two-tailed two sample Student's t test. Correlation coefficients were obtained and the significance between them tested. The statistical packages used were Minitab 7 and Genstat V.

RESULTS

Table 1 shows the mean Se concentration in flour used for bread making in Scotland relative to its protein content. Se concentrations ranged from 0·028 to 0·518 μg/g. Flour of 100% Scottish origin had an average Se content of 0·059 μg/g while flour of 100% Canadian origin had a calculated value of 0·511 μg/g.

Se contents of bread made in Scotland from EEC-blend wheat and that of partly Canadian origin are detailed in Table 2. Also shown are the Se contents of whole milk, egg and beef.

In Fig. 1 the weights of foods consumed according to the National Food Survey, (Ministry of Agriculture, Fisheries and Food, 1989) are detailed together with their provision of Se to the diet. Se content of foods, other than those in Table 2 and wheat-based foods, are taken from Thorn *et al.* (1978). Assuming bread from EEC-blend flour is consumed, this would bring the average daily intake for the UK to 30 μg and for Scotland to 31 μg. Using Canadian flour the intake would rise to 40 and 42 μg respectively. The percentage of the total dietary Se coming from cereals using EEC-blend wheat in bread is 22 and 42 for Canadian wheat.

Table 2. *Selenium content (μg/g) of foods available in Scotland**

(Mean values with their standard errors)

	No. of samples	Se content: Dry wt Mean	Dry wt SE	Fresh wt Mean	Fresh wt SE
White bread (average, tin)					
EEC blend	3	0·033	0·007	0·021	0·005
Canadian blend	4	0·188	0·03	0·118	0·02
Wholewheat bread (average)					
EEC blend	4	0·047	0·007	0·030	0·005
Canadian blend	3	0·210	0·025	0·130	0·018
Milk, whole	6	0·173	0·017	0·015	0·001
Egg, whole	6	0·710	0·032	0·175	0·008
Beef, raw, lean and fat, 86% lean	4	0·254	0·035	0·038	0·006

* For details, see p. 262.

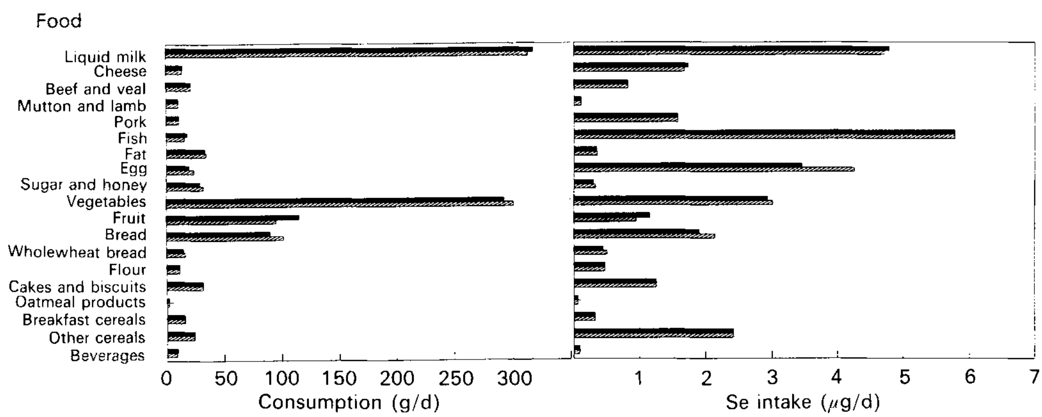

Fig. 1. UK (■) and Scottish (▨) average daily consumption of individual foods from Ministry of Agriculture, Fisheries and Food (1989) National Food Survey and their contribution to dietary selenium intakes.

As oily fish and bread were thought to be good sources of Se in the UK diet it was decided to determine whether their level of consumption was correlated with whole-blood GSH-PX activity.

Table 3 shows the relationship between (a) bread consumption and (b) oily fish consumption and GSH-PX levels in the blood of 478 volunteer members of the Ayrshire population. Men eating less than ten slices of white bread each week had significantly ($P = 0·006$) higher GSH-PX concentrations (6·23 (SE 0·30) U/ml) than those consuming ten to nineteen slices (5·16 (SE 0·22) U/ml). This was also true for the whole population, although not for women by themselves. Significance was reached for women when more than thirty slices were eaten each week. When an equal amount (ten to nineteen slices/week) of white or brown and wholemeal bread were consumed, GSH-PX

Table 3. *Relationship between whole blood glutathione peroxidase* (EC *1.11.1.9*; *GSH-PX*) *and diet of Scottish subjects participating in Ayrshire Health Study**

(Mean values with their standard errors)

Food	Group	GSH-PX (U/ml) Mean	SE	*n*	Statistical significance of difference: *P* =
White bread (no. of slices/week)					
< 10	Men	6·23	0·30	16	0·006
10–19	Men	5·16	0·22	29	
< 10	Women	6·37	0·323	23	0·007
> 30	Women	5·10	0·297	8	
< 10	Both	6·31	0·22	39	0·028
10–19	Both	5·69	0·165	61	
< 10	Both	6·31	0·22	39	0·149
> 30	Both	5·83	0·245	40	
10–19 slices					
White bread	Men	5·16	0·22	29	0·009
Brown and wholemeal	Men	6·29	0·356	24	
White bread	Women	6·17	0·214	32	0·163
Brown and wholemeal	Women	6·87	0·447	38	
White bread	Both	5·69	0·165	61	0·009
Brown and wholemeal	Both	6·65	0·321	62	
Oily fish consumed weekly					
Yes	Men	6·01	0·17	70	NS
No	Men	5·96	0·11	173	
Yes	Women	6·18	0·156	74	NS
No	Women	6·19	0·12	161	
Yes	Both	6·10	0·115	144	NS
No	Both	6·07	0·08	334	

NS, not significant.

* For details, see p. 262.

concentrations were significantly (*P* = 0·009) higher for the whole population (6·65 (SE 0·32) U/ml *v*. 5·69 (SE 0·17)) and for men (6·29 (SE 0·36) *v*. 5·16 (SE 0·22)) consuming brown or wholemeal bread but again not for women (6·87 (SE 0·45) *v*. 6·17 (SE 0·21)). No correlation was found between the frequency of oily fish consumption and GSH-PX activity.

Social class influenced GSH-PX activity in the whole population and in men but not in women (Table 4), with non-manual workers (classes 1–3) having significantly higher values (*P* = 0·017) for the whole population and for men *P* = 0·014. Place of residence affected the GSH-PX concentration with the Kilmarnock area men having significantly (*P* = 0·024) higher values (6·13 (SE 0·11)) than those from Cumnock and Doon Valley (5·72 (SE 0·14)). No differences were found for women.

Although women generally had higher GSH-PX activities, no significant sex differences were found. There was a tendency for GSH-PX to increase with age and the youngest men in the survey (40–44 years) had significantly (*P* = 0·033) lower concentrations (5·72 (SE 0·22)) than those aged 55–59 years (6·34 (SE 0·20)). Other relationships examined but which showed no significance included a history of angina (or not) or a previous heart attack (or not).

Table 4. *Relationship between glutathione peroxidase* (EC *1.11.1.9*; *GSH-PX*) *in blood and a range of variables measured in Scottish subjects participating in the Ayrshire Health Study*

Variable	Group	GSH-PX (U/ml whole blood) Mean	SE	*n*	Statistical significance of difference: *P* =
	Mean of whole population	6·08	0·065	478	
Sex	Male	5·98	0·089	243	0·106
	Female	6·19	0·095	235	
Age					
40–44	Men	5·66	0·187	57	0·033
55–59	Men	6·19	0·159	66	
Social class (based on husband's occupation)					
1–3	Men	6·28	0·15	78	0·014
4–6	Men	5·82	0·11	160	
1–3	Women	6·29	0·15	80	0·581
4–6	Women	6·13	0·13	140	
1–3	Both	6·28	0·11	158	0·017
4–6	Both	5·96	0·08	303	
Angina					
Yes	Men	5·82	0·24	26	0·485
No	Men	6·00	0·10	217	
Area					
Kilmarnock	Men	6·13	0·114	154	0·024
Cumnock	Men	5·72	0·140	89	
Kilmarnock	Women	6·10	0·125	136	0·304
Cumnock	Women	6·30	0·148	99	

Table 5. *Origin of wheat purchased by Scottish flour millers* ($\times 10^3$ *tonnes*)

(From The Scottish Agricultural College (Aberdeen) survey of wheat users 1989)

	1986/87	1987/88	1988/89
Scotland	78	160	73
England	210	117	187
EEC	120	127	128
North America	88	68	62

DISCUSSION

In a previous survey conducted in 1985 (Barclay & MacPherson, 1987) the range of Se in wheat was similar (0·023–0·576 μg/g) to that recorded in the present study, but there was then a good correlation with protein content. This correlation dropped from 0·79 to 0·47 and was significantly ($P < 0·05$) lower than it was in the earlier study, reflecting the higher-protein wheat now grown on soil of low Se availability. As the protein content of home-grown wheat has risen, the requirement to import bread-making wheat has fallen. This is emphasized by the values shown in Table 5. One of the home-grown wheats had a high protein content of 171 g/kg but a Se concentration of only 0·046 μg/g. The traditional source of high-protein wheats in Scottish bread has been Canada, but Canadian flours now represent only 13·8% of the bread flour used compared with 26% in 1985. While the

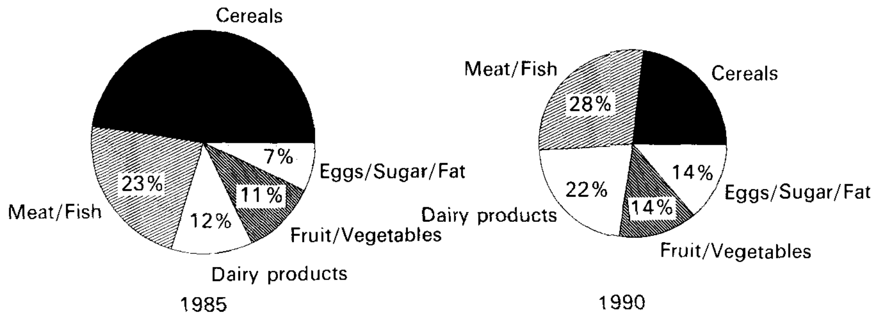

Fig. 2. The change in proportion of dietary selenium supplied by different food groups between 1985 and 1990. Total intake 43 μg (1985) and 30 μg (1990).

Scottish: English wheat used will vary with harvest conditions, the North American tonnage seems likely to continue falling.

Most bread-making flour is a blend from several sources. Thus, in Table 2 the breads are classified as 'EEC blend' where no North American wheat is used, in contrast to 'Canadian blend' where from 10 to 55% is of Canadian origin. Taking into consideration the total tonnages used, the heavy bias (86%) to lower Se blends is emphasized. Brueggermann & Ocker (1990) indicated that the 1988 harvest in Germany resulted in breads of a similar low Se content.

Fig. 1 reflects the intake of foods on a UK and Scottish basis showing the contribution that each makes to total dietary Se. Analysis of a few samples of foods other than bread and flour revealed results which showed small increases over those of Thorn *et al.* (1978), indicating that the major difference still lies in the source of the wheat. This can be readily appreciated by reference to Fig. 2 which shows the changing contribution of the different food groups to total dietary Se between 1985 and 1990. Cereal-based foods now provide just 22% compared with 47% 5 years earlier, leaving meat and fish as the major source. Dairy products and eggs/sugar/fat have almost doubled their provision but this has been insufficient to compensate for the dramatic decline in cereal Se.

Different foods may have different levels of bioavailability of Se, but van der Torre *et al.* (1991) could find no difference in this respect between meat and bread. For the present study Se has been assumed to be equally available in all foods.

The average Se intake of 30 μg/d is lower than the recommended level quoted by the National Research Council (1989) at 70 and 55 μg/d for adult males and females respectively. Stewart *et al.* (1978) argued that 24 μg/d was adequate to maintain Se balance in healthy young New Zealanders, but intakes of 25–30 μg/d were accepted as inadequate by Finland in 1984. Sodium selenate is now added to the main fertilizers sold in Finland to increase the grain content of Se to 100 μg/kg (Koivistoinen & Huttunen, 1986). Brueggerman & Ocker (1990) and Oster & Prellwitz (1989) have examined daily Se intake in West Germany. Oster & Prellwitz (1989) estimate this at 47 μg for males and 37 μg for females. Bunker *et al.* (1988) have studied the Se intake in two groups of elderly people in Southampton. The apparently healthy group (*n* 24) had an average intake of 64·7 μg/d compared with 37·5 μg/d in the housebound group (*n* 20), the foods having been analysed in 1987. There was considerable within-group variation (healthy 24·5–129 μg/d, housebound18·4–89·7 μg/d), but respective theoretical daily requirements were calculated as 35·3 μg/d and 33 μg/d. Possibly because of the variation in intake there was no difference noted in whole-blood GSH-PX activities between the two groups.

Lyon *et al.* (1985) compared the trace mineral content of 'traditional' and 'convenience' foods sampled in Glasgow. In the same study the traditional diet, which included wholewheat bread, provided a daily intake of 73·6 μg compared with 50·4 μg for convenience foods. (T. D. B. Lyon, personal communication).

Bread has traditionally been a major source of Se in the UK diet. In more recent years wholemeal flour has been more likely to have a North American origin and so wholemeal bread has been likely to provide more Se to the diet. Lower blood Se levels have been shown to correlate directly with GSH-PX activity (Levander *et al.* 1983; Thomson *et al.* 1985), but in the present study increasing white bread consumption did not show an increase in GSH-PX activity which was probably due to the very low Se content of white bread based on EEC flours, since on the basis of equivalent consumption of wholemeal bread there was an effect ($P = 0{\cdot}009$). Nevertheless, the *Dietary and Nutritional Survey of British Adults*, carried out by the Office of Population Censuses and Surveys, Social Survey Division (1990) states that only 49% of UK adults eat wholemeal bread thus leaving half the population with dietary Se intakes well below those currently recommended.

Fig. 1 indicates that fish is a good source of dietary Se. The *Household Food Consumption and Expenditure* (Ministry of Agriculture, Fisheries and Food, 1989) figures do not separate white fish from oily fish but *The Dietary and Nutritional Survey of British Adults* (Office of Population Censuses and Surveys, Social Survey Division, 1990) shows that while 48% of the population eat white fish, only 34% on a UK average and 29% on a Scottish average eat oily fish. This was possibly reflected in the lack of response in GSH-PX levels of oily fish eaters in this survey.

Pearson *et al.* (1990) calculated that 25% of their healthy volunteers and 50% of their medical patients from the North-West of England had serum Se values below those required for full expression of Se-dependent enzyme activity. In our previous survey, also in Ayrshire (Barclay *et al.* 1986), there was a correlation of 0·883 between serum Se ($\leqslant$ 900 μg/l) and GSH-PX in whole blood. The mean GSH-PX value of 354 patients was then 8·16 U/ml under the same standard conditions as applied here. These analyses were carried out in 1985. The present average GSH-PX concentration for the population sampled under the Ayrshire and Arran Heart Health Study was 6·08 U/ml. However, no attempt was made to match the two populations and, while they were broadly similar in age and sex distribution and in area of residence, they cannot be regarded as directly comparable.

In conclusion, the level of Se in wheat used for bread making in Scotland in 1989–90 ranged from 0·028 μg/g for home-grown grain to 0·518 μg/g for Canadian grain. This was calculated to result in a dietary intake of 31 μg/d for average Scottish consumers using EEC-blend flour in bread and 42 μg/d for those using Canadian-blended flour. The GSH-PX levels determined in oily fish eaters do not show any significant difference from others which could be due to the confounding effects of differences in their consumption of wholewheat bread.

The variety of foods consumed by the Scottish public ranges from the high Se levels of some breads to the extremely low levels of others, with no indication being given to Se content. On average wholewheat breads have a higher Se content than white breads and this was reflected in the higher GSH-PX levels obtained ($P = 0{\cdot}009$).

The different trends in dietary Se provision by cereal-based and animal-derived food groups identified here strongly suggest the need for a more comprehensive survey to establish the Se status of the Scottish people.

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