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Correlates of plasma homocysteine, cysteine and cysteinyl-glycine in respondents in the British National Diet and Nutrition Survey of Young People Aged 4–18 Years, and a comparison with the Survey of People Aged 65 Years and Over

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Plasma total homocysteine (tHcy), cysteine and cysteinyl-glycine were measured in a representative sample of 922 young people aged 4–18 years, participating in the National Diet and Nutrition Survey in mainland Britain in 1997. Both tHcy and cysteine increased markedly with age; cysteinyl-glycine less so. Neither tHcy nor cysteine differed between genders; cysteinyl-glycine was higher in males. tHcy concentrations were lowest in the winter; cysteine and cysteinyl-glycine varied only slightly with season. In respondents aged >15 years, tHcy was higher in smokers, but in respondents aged 7–11 years, tHcy was higher in those whose mothers smoked. tHcy was inversely correlated with serum folate, serum vitamin B_{12} and vitamin B_6 status, but neither cysteine nor cysteinyl-glycine shared these relationships. The relationships between tHcy and B-vitamin status indices ran parallel with those of the 65 years and over survey, but at much lower tHcy concentrations for any given B-vitamin concentration. Age-adjusted tHcy was not correlated with anthropometric indices, blood pressure, haematology, plasma creatinine, urea or cholesterol, but was directly correlated with fasting triacylglycerol. We conclude that disease-risk indices, like tHcy and perhaps cysteine, if established during early life, may be modulated by diet and lifestyle, thereby providing an opportunity for public health intervention.

Homocysteine: National Diet and Nutrition Survey: Vitamins: Children

Plasma total homocysteine (tHcy) is well established as a powerful risk indicator and probable risk determinant for a wide range of vascular diseases in all age groups of Western society (Ueland *et al.* 1992; Alfthan *et al.* 1994; Arnesen *et al.* 1995; Boushey, 1995; Danesh & Lewington, 1998; Refsum *et al.* 1998; Nygard *et al.* 1999; Selhub, 1999). A number of dietary and status index correlates or determinants of tHcy have been identified, the most powerful and robust of which include the B-vitamins: folate, vitamin B_{12} and vitamin B_6, whose metabolic functions are intimately connected with the metabolic pathways of homocysteine disposition (Brattstrom *et al.* 1988; Rosenberg, 1996; Verhoef *et al.* 1996; Homocysteine-Lowering-Triallists'-Collaboration, 1998; Malinow *et al.* 1998; Omenn *et al.* 1998; Pietrzik & Bronstrup 1998; Robinson *et al.* 1998; Selhub, 1999). Research has so far focussed mainly on the determinants of tHcy in later life, and there is a paucity of information about the determinants of tHcy in representative samples of healthy children and young people, especially in Britain.

Homocysteine, cysteine and cysteinyl-glycine are all metabolically inter-related (Mansoor *et al.* 1992), and these thiol species in plasma are probably important in determining the redox milieu and free radical generation rates (Mansoor *et al.* 1992, 2000; Ueland *et al.* 1996; Enoiu *et al.* 2000). A correlation between plasma cysteine and vascular disease risk, independent of its correlation with tHcy, has recently been described (Mansoor *et al.* 1995; El-Khairy *et al.* 1999, 2001). Therefore, the measurement of plasma cysteine and cysteinyl-glycine (which can be

Abbreviations: NDNS, National Diet and Nutrition Survey; tHcy, total homocysteine.

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performed in the same chromatography run as tHcy) is of potential interest for the understanding of biological interrelationships and for disease prediction. The possibility of risk-related geographical variations in tHcy (Bates *et al.* 1997), of smoking-related variations (Refsum *et al.* 1998), and of seasonal variations in cardiovascular risk factors (Woodhouse *et al.* 1994) provided the rationale for also examining these potential socio-demographic determinants.

The National Diet and Nutrition Survey (NDNS): Young People Aged 4–18 Years was commissioned jointly by the Department of Health and the Ministry of Agriculture, Fisheries and Food in mainland Britain, and carried out during 1997 (Gregory *et al.* 2000). Responsibility for this survey and the NDNS Programme transferred from the Ministry of Agriculture, Fisheries and Food to the Food Standards Agency on its establishment in April 2000. The present publication describes the population distribution of tHcy, cysteine and cysteinyl-glycine, and thus provides reference values for the British population for this age range. It also compares them with values observed in British people aged 65 years and over, from a previous NDNS (Bates *et al.* 1997; Finch *et al.* 1998), which used similar survey procedures and assay methodologies, and can thus provide a robust comparison between representative samples of contrasting age groups of the British population.

Respondents and methods

The design and execution of the NDNS of Young People Aged 4–18 Years has been described (Gregory *et al.* 2000); therefore only the main features are summarised here. The Social Survey Division of the Office for National Statistics recruited young people from 132 postcode sectors, randomly selected from mainland Britain, in four waves corresponding to the four seasons, during 1997. Within each sector, a sample of addresses was selected and was approached by post to establish the number of private households at each address and the age and gender of each of the residents in these households. From the responses, a further random selection then achieved the required numbers of each sex, for the four age groups: 4–6 years, 7–10 years, 11–14 years and 15–18 years (one respondent in the latter age group had just passed his nineteenth birthday when his blood sample was taken, but the data are nevertheless included in the 15–18-year age group).

Of 2672 young people who were eligible to participate, 2127 (80 %) cooperated by completing an interview with a trained interviewer in their own home. Current smoking habit (yes/no) was ascertained by questionnaire, from both the young person (respondent) and their parent(s) respectively, but biochemical confirmation was not used. Of the respondents, 1701 completed a consecutive 7 d weighed dietary record of all food and drink consumed, and 1193 provided a blood sample which, in most cases, was an early morning, fasting sample.

As noted in the survey report (Gregory *et al.* 2000) the survey refusals (non-respondents) were more commonly in the 15–18-year age group than were the partial or full respondents, and of those who did cooperate, a smaller proportion in the 4–6-year group than in the other groups provided a blood sample. There was also some variation in cooperation between waves and between regions, particularly in the proportion of respondents who provided a blood sample. However, a detailed comparison between those respondents who provided a blood sample that was subsequently used for the analysis of the thiol species and those who did not (either because they provided no blood sample, or because there was insufficient for this particular analysis, or because permission for it was not given) showed no evidence of differences in socio-economic indices, in nutrient intakes or in other blood status indices, including those of the key B vitamins. Therefore we consider that there is unlikely to have been any selection bias which could have caused significant confounding of the conclusions of the present study.

Blood was taken by a trained phlebotomist in the respondents' own homes. Two sub-samples were sent by first class post to Great Ormond Street Hospital Haematology Laboratory for routine haematology measurements and for analyses of serum and whole-blood folate and serum vitamin B_{12}. A sample of heparinised blood was immediately chilled in a cold-box, and was taken by the phlebotomist to a local hospital laboratory within 0.5 to 2 h, where it was immediately separated to yield plasma and saline-washed erythrocytes. These samples were stored frozen for up to 3 months at −40°C or lower, and then at −85°C until analysed. Except for the thiol species, most of the biochemical status analyses were performed at Medical Research Council Human Nutrition Research in Cambridge, within 2 years of collection.

The measurements of serum and whole-blood folate and serum vitamin B_{12} were performed by Abbott IMx analyses at Great Ormond Street Haematology Laboratory (Fiore *et al.* 1988; Kuemmerle *et al.* 1992; Wilson *et al.* 1995). The measurements of vitamin B_6 (pyridoxal phosphate and its breakdown product, pyridoxic acid) were performed by HPLC after oxidation with alkaline cyanide (Bates *et al.* 1999*a*). The tHcy, cysteine and cysteinyl-glycine analyses were carried out on heparinised plasma samples within 3 years of sample collection, by an HPLC-based method (Fiskerstrand *et al.* 1993), in the Department of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway. It was confirmed that the inter-assay CV for the tHcy, cysteine and cysteinyl-glycine assay in control samples was less than 10 % in all cases (see Fiskerstrand *et al.* 1993). The assay procedure has been shown to yield results that agree closely with those of the procedure (Mansoor *et al.* 1992) that was used previously in the same laboratory for the assay of samples from the survey of people aged 65 years and over (Bates *et al.* 1997) and also with the results from other laboratories participating in an international external quality assurance scheme (Moller *et al.* 1999). It has also been demonstrated that plasma tHcy remains stable even at −20°C, for at least 6 years (Israelsson *et al.* 1993). Therefore, no significant degradation was likely during 3 years storage a −80°C. The other relevant survey assays are described in the survey report (Gregory *et al.* 2000). The vitamin B_6 status values obtained for the NDNS 4–18-year and the NDNS 65 years and over surveys are described elsewhere (Bates *et al.* 1999*b*).

The data from the survey of people aged 65 years and over were confined to those living in the community ('free-living sample') (Finch *et al.* 1998).

Permission for the survey procedures was obtained from the Local Research Ethics Committees associated with each postcode sector. Separate permission from each of the Local Research Ethics Committees was obtained for the analysis of tHcy, cysteine and cysteinyl-glycine, and analysis was limited to those respondents who gave their written permission for the further analysis of stored plasma residues.

Statistical methods

Statistical analyses were performed by DataDesk (Data Description Inc.) statistical package. The plasma tHcy dataset was positively skewed (skewness coefficient 5·1), therefore all calculations involving tHcy used $\log_e$-transformed values, followed by back-transformation where appropriate. Although not strictly necessary in terms of skewness, for consistency the calculations involving plasma cysteine and cysteinyl-glycine in Tables 1 and 2 also used $\log_e$-transformed values followed by back-transformation. In Tables 2, 4 and 5, the potential determinants of tHcy, cysteine and cysteinyl-glycine were entered as either continuous (for age only) or discrete-value variables in a general linear (one-way ANOVA after grouping where necessary) multivariate model, which reported mean-adjusted values and the overall significance of the contribution of each variable to the ANOVA model. In Table 3, a range of biochemical covariates (continuous variables) was entered, together with age and season, in a multivariate regression model, followed by backwards elimination until only those which remained significant contributors, at $P<0{\cdot}05$, remained. For Fig. 1, the B-vitamin index determinants were categorised into ascending tenths of their distribution, the geometric mean values of tHcy and of the B-vitamin index were then calculated for each tenth, and these were plotted. For all significance calculations, $P<0{\cdot}05$ was accepted as significant.

Results

Table 1 presents the geometric mean and inner 95 % ranges by age group for the three thiol species: tHcy, cysteine, and cysteinyl-glycine, in plasma. Both tHcy and cysteine exhibited progressive increases with age throughout the age range, and these were highly significant for both genders ($P<0{\cdot}0001$). Cysteinyl-glycine, however, did not share this strong relationship with age; moreover it was the only one of the three species to exhibit a major gender difference, which was, in turn, largely confined to the oldest (15 years and over) age group. Of the other species, tHcy exhibited a marginally significant gender difference, with slightly higher values in males in the oldest age group.

Although the population sample had been randomly selected, it did not exactly match the entire (census) population. In order to test whether this minimal deviation had any measurable effect on the results, a 'weighting variable', designed to represent the ratio of numbers in each population category of the survey sample to those in the entire census population (Gregory *et al.* 2000), was applied. This was achieved by using the reciprocal of the weighting variable as the 'variance variable' in the DataDesk general linear model. However, this adjustment was found to make less than 1 % difference to 85 % of the calculations in Table 1, and in no case did the adjusted value differ by more than 3 % from the corresponding unadjusted value. Therefore, it was concluded that the weighting adjustment could be dispensed with, for the purposes of the present study.

Table 1. Distribution of geometric means (GM) for total homocysteine (tHcy), cysteine and cysteinyl-glycine, by gender and age group, in four subgroups of the National Diet and Nutrition Survey 4–18-years-old respondents*

Age group (years)†	*n*	tHcy (μmol/l)‡		Cysteine (μmol/l)‡		Cysteinyl-glycine (μmol/l)‡	
		GM	95 % range	GM	95 % range	GM	95 % range
Males							
4·00–6·99	49–51	5·16	2·7–9·5	188	114–238	32·3	18·8–49·7
7·00–10·99	125–131	5·59	3·1–9·5	203	145–264	36·1	22·8–53·7
11·00–14·99	156–157	6·18	2·9–11·5	214	161–273	38·3	25·0–58·7
15·00–18·99	140	8·54	4·1–20·1	227	165–286	38·6	24·7–55·3
Females							
4·00–6·99	62	4·79	2·2–8·1	194	123–253	34·9	17·2–50·4
7·00–10·99	107–109	5·69	2·7–10·6	207	152–271	35·8	24·1–53·1
11·00–14·99	137–140	6·40	3·5–11·8	216	160–267	36·1	23·4–55·9
15·00–18·99	140–144	7·80	3·9–14·3	232	164–289	33·4	21·3–53·6

* For details of respondents and procedures, see p. 72.

† The age groups are given to two decimal points in this table, but are rounded to integers elsewhere.

‡ All the tabulated data were obtained by back-transformation from the means and ranges of $\log_e$ values, so as to normalise skewed distributions, especially for tHcy. By linear regression with adjustment for age and season, only cysteinyl-glycine exhibited a significant overall gender difference, which was confined mainly to the oldest age group, males having higher values than females ($P<0{\cdot}0001$). There was also a marginally significant ($P=0{\cdot}044$) gender difference for tHcy in the oldest age group; however cysteine exhibited no significant gender difference for any age group. The significance of the overall linear regression of each log-transformed analyte with age was: for tHcy, $P<0{\cdot}0001$; for cysteine, $P<0{\cdot}0001$; for cysteinyl-glycine, $P=0{\cdot}045$.

Table 2. Seasonal variation of homocysteine, cysteine and cysteinyl-glycine values in young people aged 4–18 years and in free-living adults aged 65 years and over in Britain*

Season	*n*	Homocysteine (μmol/l)†	Cysteine (μmol/l)†	Cysteinyl-glycine (μmol/l)†
Young people aged 4–18 years				
Winter (January–March)	185–188	5·20	218	35·9
Spring (April–June)	159–165	6·37	215	35·2
Summer (July–September)	294	7·22	207	32·2
Autumn (October–December)	282	6·60	217	41·4
F ratio for seasonal difference		41·3	7·7	74·5
P		<0·0001	<0·0001	<0·0001
Adults aged 65 years and over (free-living)				
Winter (January–March)	153–161	12·86	272	34·0
Spring (April–June)	163–166	15·47	255	35·3
Summer (July–September)	186	15·06	254	36·2
Autumn (October–December)	238	15·06	261	35·7
F ratio for seasonal difference		9·39	11·55	2·19
P		<0·0001	<0·0001	0·09

* For details of respondents and procedures, see p. 72.
† All values are back-transformed from means of $\log_e$-transformed values and are adjusted for age (within the two age groups), and the values in adults aged 65 years and over are also adjusted for gender.

Table 2 reveals the existence of a significant seasonal variation in all three thiol species. As shown also in Table 2, this seasonal variation was seen not only in the young people's survey, but also in the survey of people aged 65 years and over. In both age groups, the lowest mean values of tHcy were observed in the winter months, with generally higher values during spring, summer and autumn. Although there was some evidence of seasonal variations in the key B-vitamin status indices (but not of their intakes) that could partly account for the observed variation in tHcy concentrations (not shown), this accounted for only a small proportion of the seasonal variance on plasma tHcy. In contrast to tHcy, cysteine exhibited the lowest values in the summer, and this was also true for cysteinyl-glycine in the young people, although in the 65 years and over survey there was no significant seasonal variation in cysteinyl-glycine. Amongst other blood status indices which are known to determine vascular disease risk in older people, total and LDL-cholesterol and diastolic blood pressure exhibited their maximum levels in the winter, and plasma vitamin C exhibited minimum levels in the winter, in the 4–18-year survey (not shown). In contrast, HDL-cholesterol, systolic blood pressure and white cell count did not exhibit any significant seasonal variations in the 4–18-year survey (not shown).

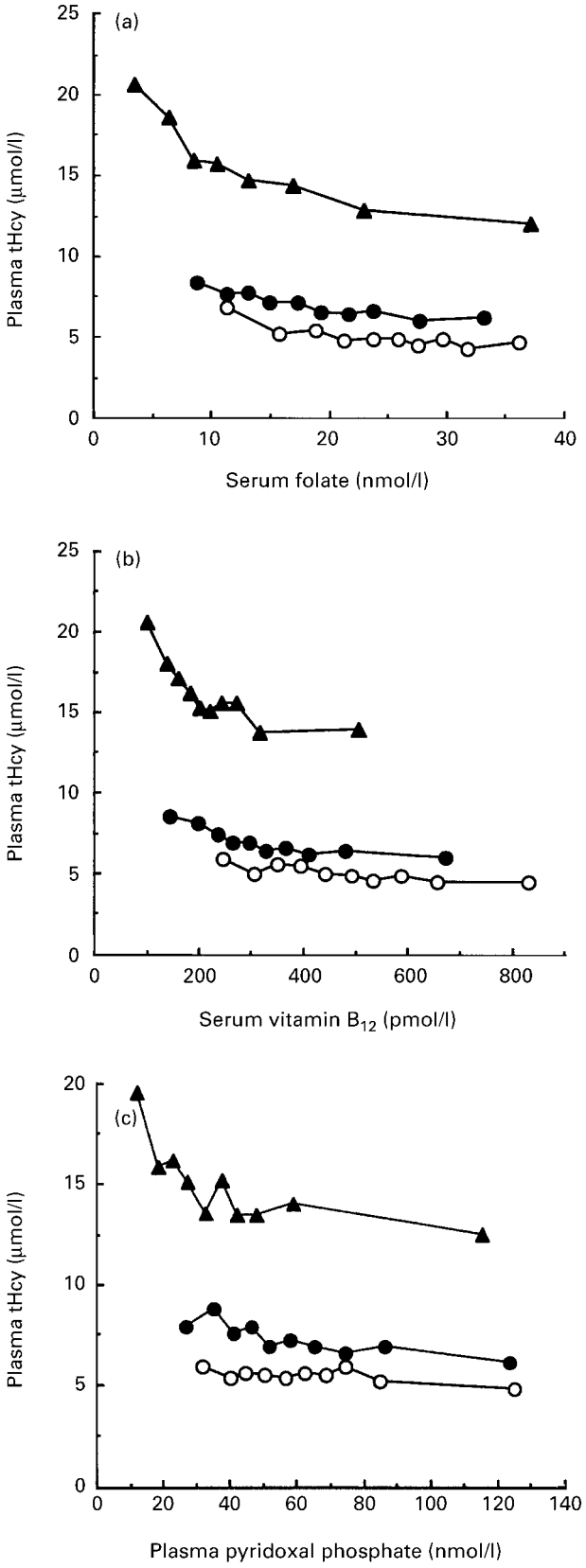
Table 3 lists the biochemical status indices which were significantly correlated with tHcy in a multivariate model in which age, season, and forty-six blood status indices (comprising haematology indices; plasma fat-soluble vitamin status indices for vitamins A, D, E and carotenoids; water-soluble vitamin indices in serum, plasma or erythrocytes; mineral indices including bone-related minerals, Fe, Zn, Cu and Se; plasma total- and HDL-cholesterol and plasma α_1-antichymotripsin (index of acute phase status)) were introduced, and were then removed by backwards elimination, until those remaining all contributed at a significance of $P<0{\cdot}05$. The significance of the five indices in the multivariate model, including triacylglycerol, was unaffected by the omission of fourteen respondents who reported not complying with the fasting conditions for the collection of the blood sample. The significance of the relationships between tHcy and these five indices (including pyridoxic acid) was very little affected by adjustment for both plasma creatinine and plasma urea (not shown), suggesting that kidney function was not a major confounding factor in this age group. The overall correlations between tHcy and folate, vitamin B_{12} and vitamin B_6 intakes, adjusted for age, season and energy intake, were inverse and significant at $P<0{\cdot}0001$ in all three cases (not shown).

tHcy was strongly and directly correlated with both cysteine and cysteinyl-glycine, after adjustment for age and

Table 3. Multivariate linear regression* between $\log_e$-transformed homocysteine and biochemical status indices in young people aged 4–18 years†

Status index	*F* ratio	Coefficient ($\times10^{-4}$)	SE ($\times10^{-4}$)	*P*
Serum folate	92·3	−134	16·5	<0·0001
Serum vitamin B_{12}	11·8	−2·52	0·73	0·0015
Plasma triacylglycerol	8·4	+640	220	0·006
Plasma pyridoxic acid	6·0	−51·2	20·8	0·012
Erythrocyte superoxiden dismutase	6·7	−1·07	0·41	0·013

* All regression calculations were adjusted for age and for season. The multivariate analysis (d.f. 759) was by backwards elimination, until all remaining relationships were significant at $P<0{\cdot}05$.
† For details of respondents and procedure, see p. 72.



gender (not shown). The biochemical correlates of cysteine and cysteinyl-glycine differed from those of tHcy insofar that they were both much less strongly correlated with B-vitamin status indices, especially with serum folate and vitamin B_{12}.

Fig. 1 depicts the relationships observed between tHcy and (a), serum folate; (b), serum vitamin B_{12}; (c), plasma pyridoxal phosphate, for two age ranges of the young people, and for the free-living survey participants aged 65 years and over. Although there is a large vertical difference between the three age groups, there is also a strong parallelism with respect to the relationships between tHcy and the B-vitamin status indices at all ages. Among the older respondents, there were many with low B-vitamin status indices, but there was also a small subgroup with high serum B-vitamin concentrations, resulting from the regular use of dietary supplements.

Table 4 explores the relationships between tHcy and smoking habit (respondent's own and maternal smoking). There was a marginally-significant difference in plasma tHcy between smokers and non-smokers in the oldest age group, and unexpectedly, a more highly significant difference in tHcy between those whose mothers smoked and those whose mothers did not, in one of the younger age groups.

Table 5 explores geographical variations in mean tHcy concentrations. Although less highly significant than in the survey of older people (Bates *et al.* 1997), there was evidence for higher values in the north of Britain. After adjustments for age and season, there were also significant north–south gradients for serum folate (P=0·005), for serum vitamin B_{12} (P=0·05) and for plasma pyridoxic acid (P=0·05). All of these gradients indicated better vitamin status in the south than in the north of the country.

Possible relationships between plasma tHcy and the following socioeconomic, anthropometric and health indices were also examined: social class of head of household; household income; receipt of income supplements or credits; maternal educational qualification level; maternal age; ethnic group; whether the subject was vegetarian; consumption of tea or coffee; body weight; height; BMI; mid-upper-arm circumference; systolic and diastolic blood pressure. After adjustment for age and

Fig. 1. Plasma total homocysteine(tHcy) *v.* serum folate (a), vitamin B_{12} (b), and plasma pyridoxal phosphate (c), subdivided by tenths of their distribution, for young people aged 4–10 years (○) and 10–18 years (●) and free-living elderly adults over 65 years of age (▲). In each part of the figure, the relationship between tHcy and the corresponding status index of one of the three key determinant B-vitamins: folate, vitamin B_{12} or vitamin B_6 (pyridoxal phosphate), is depicted. The B-vitamin status values from all respondents in each age group who had both a B-vitamin and a tHcy measurement were arranged in ascending order of the B-vitamin values, and assigned a number from 1 to 10 according to the tenths of this distribution. The geometric mean value of the corresponding tHcy values was calculated for each tenth of the B-vitamin distribution and the ten resultant pairs of values were then plotted for each age group. There were approximately thirty-four values for each data-point for the 4–10-year age group; fifty-seven values for each data-point for the 11–18-year age group and seventy-five values for each data-point for the 65 years and over age group. For details of respondents and procedures, see p. 72.

Table 4. Relationships between respondent's reported current smoking status or their mother's smoking status, and their plasma homocysteine concentrations*†

(Geometric mean values)

Age group (years)	Homocysteine (μmol/l) Smokers Mean	Smokers *n*	Non-smokers Mean	Non-smokers *n*	*F* ratio	*P*
Respondents' own reported smoking status						
7–10	5·85	2	5·52	202	0·08	0·8
11–14	6·48	16	6·25	247	0·20	0·7
15–18	8·53	78	7·90	171	2·38	0·12
All	7·11	96	6·54	620	4·76	0·03
Maternal smoking status						
4–6	4·58	32	4·66	70	0·11	0·74
7–10	5·95	69	5·59	144	7·9	0·006
11–14	6·51	81	6·16	181	1·6	0·21
15–18	8·34	80	7·92	158	1·0	0·31
All	6·56	262	6·17	555	6·7	0·01

* For details of respondents and procedures, see p. 72.

† Plasma homocysteine was $\log_e$-transformed for the analysis and then back-transformed for reporting. Smoking habit was self-reported as 'yes' or 'no', without details of number of cigarettes/d and without biochemical confirmation. All models were adjusted for age and season. The significant relationship with maternal smoking status in the 7–11 year age group remained significant even after excluding those (*n* 2) respondents who were themselves smokers. It also remained significant after further adjustment for variations in household income, receipt of benefits, gender and geographical region. Paternal smoking was not significantly correlated with total homocysteine, and neither maternal nor own reported smoking habit were correlated with cysteine and cysteinyl-glycine. The difference in total homocysteine between non-smokers whose mother was a smoker, and those whose mother was not, was also significant by the Scheffé test at $P=0·034$.

Table 5. Geographical distribution of homocysteine in young people aged 4–18 years*

(Mean values)

Geographical subdivision	Homocysteine concentration (μmol/l)†	*n*
North–south		
Scotland and Northern England	6·95	238
Central, South West, Wales, London, South East	6·58	486
F ratio	4·6	
P	0·03	
Four main regions		
Scotland	7·29	57
Northern England	6·90	181
Central, South West, Wales	6·67	249
London and South East	6·55	237
F ratio	2·1	
P	0·10	
Eleven regions		
Scotland	7·30	57
Northern England	6·57	23
North West	6·44	74
Yorkshire + Humberside	7·46	84
East Midlands	5·89	55
West Midlands	7·03	51
East Anglia	6·64	45
London	6·40	74
South East	6·63	163
South West	7·15	61
Wales	6·75	37
F ratio	2·9	
P	0·002	

* For details of respondents and procedures, see p. 72.

† Plasma homocysteine was $\log_e$-transformed for the analysis and then back-transformed. The models were all adjusted for age, season, household income, maternal highest qualification level, ethnic group, reported own and maternal smoking habits. Missing values in some of these categories were responsible for the reduced numbers of respondents represented, compared with previous tables. If adjusted for age and season only, the significance of the difference between subdivisions in the first two models was non-significant ($P=0·12$ and 0·4 respectively), but that in the eleven-region model remained significant ($P=0·01$).

season, none of these indices exhibited any significant relationships with tHcy, in young people aged 4–18 years.

Discussion

Previously published studies in the literature of tHcy in clinically-normal children are broadly compatible with those of the present study although they vary according to the populations studied, to whether or not the subjects were divided by sex, and to whether the results were reported as arithmetic means, geometric means or medians. Several studies (de Laet *et al.* 1999; Jacques *et al.* 1999; Osganian *et al.* 1999) reported sex differences, with higher values in boys, especially in the older, post-pubertal age groups. Studies of children aged between 5 and 11 years have found median or geometric mean values of 5·8 μmol/l (Vilaseca *et al.* 1997), 5·5 μmol/l (Greenlund *et al.* 1999) and 6·2 μmol/l (de Laet *et al.* 1999). For those aged 11–15 years the medians or geometric means were 5·7 μmol/l (Vilaseca *et al.* 1997), 6·5 μmol/l (Jacques *et al.* 1999), 7·1 μmol/l (de Laet *et al.* 1999) and 5·0 μmol/l (Osganian *et al.* 1999). Tonstad *et al.* (1997) found 5·1 μmol/l in 8–12-year-old children. For adolescents aged 15–19 years, medians or geometric means have been 8·1 μmol/l (Vilaseca *et al.* 1997), 7·6 μmol/l (Jacques *et al.* 1999), 6·3 μmol/l (Greenlund *et al.* 1999) and 9·0 μmol/l (de Laet *et al.* 1999). Other studies of children of unspecified ages, or with less than thirty per group, or with arithmetic means only, included Reddy (1997), Balasa *et al.* (1999), Koch *et al.* (1999) and Raslova *et al.* (2000). All of these studies, which have included the USA and several west and east European countries, are consistent with the values found in the present study of British children. Several of these, and other, studies (Tonstad *et al.* 1996, 1997; de Laet *et al.* 1999; Osganian *et al.* 1999; Selhub, 1999; Delvin *et al.* 2000) have recorded inverse relationships between homocysteine and B-vitamin status indices in children. The study by Osganian *et al.* (1999) recorded a weak direct relationship between tHcy and systolic blood pressure and BMI. There have not been any previously-published studies on cysteine or cysteinyl-glycine in children, to the authors' knowledge.

Variation of plasma tHcy with season (Table 2) has received little attention in previous studies. In the present study of young people, as in the survey of people aged 65 years and over, significantly lower tHcy levels were observed during the winter months than at other times of the year. The other two thiol species, which were measured in the same HPLC run in the same samples, exhibited dissimilar seasonal cycles. The seasonal variation in tHcy was not matched by a corresponding seasonal cycle in B-vitamin intakes, and was only partly explained by a

seasonal cycle in B-vitamin status indices (not shown). In older people, vascular risk factors such as high blood pressure, high fibrinogen and high cholesterol levels, and also the highest prevalence of symptomatic vascular disease, generally occur in the winter months (Woodhouse *et al.* 1993*a*,*b*, 1994). The participants in the 4–18-year survey, in common with older people in other studies (Woodhouse *et al.* 1993*a*,*b*, 1994 Khaw & Woodhouse, 1995), had significantly higher median cholesterol concentrations (4·2 *v.* 4·0 mmol/l), lower median vitamin C concentrations (57 *v.* 62 μmol/l), and higher median diastolic blood pressures (56·4 *v.* 54·2 mmHg) in the winter than in the summer.

The relationships between tHcy and B-vitamin status (Table 3; Fig. 1), which were demonstrated in the present study, concur with previous observations in adults (Brattstrom *et al.* 1988; Verhoef *et al.* 1996; Bates *et al.* 1997; Homocysteine-Lowering-Triallists'-Collaboration, 1998; Omenn *et al.* 1998; Pietrzik & Bronstrup, 1998; Mansoor *et al.* 1999). The observation in the present study that plasma pyridoxic acid was a stronger covariate with tHcy than plasma pyridoxal phosphate was unexpected, since the former is an excretory degradation product whereas the latter is one of the active coenzyme forms of vitamin B_6 and is usually deemed to be the better index of status. However, extra-cellular (e.g. plasma) pools of this vitamin may be less closely related to its functional status than intracellular turnover rates. The inverse relationship between tHcy and erythrocyte superoxide dismutase (Table 3) contrasts with the direct relationship observed in older respondents in two recent studies (Moat *et al.* 2000; Wilcken *et al.* 2000), suggesting that there may be differences between different populations, and possibly between age groups, in this respect. The direct relationship with fasting triacylglycerol (Table 3) may have arisen because both indices decline progressively during the postprandial period, so that even in fasting blood samples, both of them may be influenced by the interval since, and macronutrient content of, the previous meal. Neither total nor HDL cholesterol shared this relationship. Serum or plasma triacylglycerol concentrations have been shown, in recent studies, to predict coronary disease (Hokanson & Austin, 1996; Stampfer *et al.* 1996).

A relationship between plasma tHcy and smoking has been reported only infrequently (Refsum *et al.* 1998). In the oldest age group of young people in the present study there was a weak direct relationship between plasma tHcy and their own reported smoking habit (Table 4). The other smoking-related observation in the present study, namely a strong direct relationship between maternal smoking habit and plasma tHcy, principally seen in the 7–11-year-old children (Table 4), was unexpected. A direct effect of passive smoking seems inherently unlikely. Alternatively, maternal smoking habit may covary with other lifestyle risk factors, which may impinge particularly strongly on younger children, who spend a high proportion of their time at home. This relationship with maternal smoking was not, however, attenuated by adjustment for a number of indices of socio-economic status. Clearly the mechanism requires further investigation.

Geographical gradients in tHcy were less strong in the present study (Table 5) than in the survey of older people (Bates *et al.* 1997), but they were in the same direction, namely higher concentrations in the north than the south. Moreover, the B-vitamin status indices which were correlated inversely with tHcy were consistently higher in the south than the north. Further information about the geographical variation of nutritional status indices and the corresponding nutrient intakes can be found in the survey reports (Finch *et al.* 1998; Gregory *et al.* 2000).

Many recent studies have indicated that plasma tHcy is a potent risk indicator for vascular disease (Boushey, 1995; Refsum *et al.* 1998; Welch & Loscalzo 1998; Nygard *et al.* 1999; Selhub, 1999) and is largely independent of other major risk indicators such as blood cholesterol, blood pressure and platelet function. Explanatory hypotheses have been formulated, usually based on endothelial damage, and it has been argued that those interventions which can lower tHcy levels may also reduce the risk of vascular disease in later life. This remains an unproven, though attractive, hypothesis, and it is now being tested by several ongoing intervention studies. Recent studies (Mansoor *et al.* 1995; El-Khairy *et al.* 1999, 2001) have indicated an important, possibly separate, role for plasma cysteine. By exploring the range of influences that impinge on both plasma tHcy and cysteine early in life, it may be possible to identify those factors which (a) contribute to later risk, and (b) are modifiable by dietary or lifestyle modification.

Because the population sample used for the present study was a representative sample of young people drawn by random selection from all parts of mainland Britain, it provides reference values for future studies, provided that the assay methodologies are comparable. The existence of widely available external quality assurance schemes (for example, see Moller *et al.* 1999) to which the present study was linked, will help to ensure inter-laboratory and inter-study comparability. Our comparison between the three plasma thiol species in young and older people illustrates the considerable magnitude and statistical significance of the change in plasma tHcy that is attributable to ageing. It is likely that the progressive increase in plasma tHcy with age has a variety of determinants, not all of which have yet been identified.

In conclusion, the present study has demonstrated a progressive increase in plasma tHcy with age, and strong inverse relationships with blood indices of folate, vitamin B_{12} and vitamin B_6 status, but it has also revealed some unexpected relationships, including a seasonal variation, with lowest values in the winter, and a direct relationship with maternal smoking habit in 7–10-year-old children. It has provided normative values for plasma tHcy, cysteine and cysteinyl-glycine for young people living in mainland Britain. The relevance of these indices for later disease risk, and their response to intervention, remains an important subject for future research.

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