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Plasma homocysteine, folate and vitamin B_{12} compared between rural Gambian and UK adults

Sophie E. Moore[1,2], M. Azam Mansoor[3]†, Christopher J. Bates[4]* and Andrew M. Prentice[1,2]

[1]*Medical Research Council, Keneba, The Gambia*
[2]*Medical Research Council International Nutrition Group, London School of Hygiene and Tropical Medicine, London, UK*
[3]*Division of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway*
[4]*Medical Research Council Human Nutrition Research, Cambridge, UK*

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The disease risk indicator plasma total homocysteine (tHcy) is influenced by genetic and environmental factors, including folate and vitamin B_{12} status. Little is known about the determinants of tHcy in rural West Africa. We explored the hypothesis that tHcy in rural Gambian adults might vary between the sexes and physiological groups, and/or with folate and vitamin B_{12} status. Comparisons were made with a British national survey. Non-pregnant Gambian women (*n* 158) had tHcy concentrations (geometric mean 9·0 μmol/l) similar to those of non-pregnant UK women (*n* 449; 9·4 μmol/l), whereas pregnant Gambian women (*n* 12) had significantly lower values (6·2 μmol/l). Gambian men (*n* 22) had significantly higher values (14·7 μmol/l) than British men (*n* 354; 10·8 μmol/l). Gambian lactating women and British men and women exhibited significant inverse relationships between $\log_e$(tHcy) and folate status; however, only the British subjects exhibited significant inverse relationships between $\log_e$(tHcy) and vitamin B_{12} status. In the British sample, and in Gambian lactating women, folate and vitamin B_{12} status variations together accounted for 20–25 % of the variation in $\log_e$(tHcy). Within the UK, black-skinned adults had folate and tHcy levels similar to those of their white-skinned counterparts, but significantly higher vitamin B_{12} values. We conclude that, whereas folate and vitamin B_{12} status are similar between British and rural Gambian populations, tHcy is higher in Gambian men and lower in pregnant Gambian women, and that serum vitamin B_{12} values appear to be higher in black-skinned than white-skinned British subjects. Possible reasons are discussed.

Homocysteine: Folate: Vitamin B_{12}: Gambia: West Africa: United Kingdom

Homocysteine is a S-containing amino acid that is an intermediary product in methionine metabolism. Its plasma concentrations are directly correlated with vascular disease risk in Western populations (Ueland *et al.* 1992; Welch & Loscalzo, 1998); however, its relationship with risk in developing countries is less clear. In The Gambia, whereas obesity and associated cardiovascular risk factors are increasingly common in urban populations, such vascular risk factors occur relatively rarely in the rural environment (van der Sande *et al.* 2001).

The transfer of the methyl group from methionine is an important step in the metabolism of nucleic acids and in other key biochemical processes (Pancharuniti *et al.* 1994). In the course of these transmethylation reactions, homocysteine is normally remethylated to methionine by the enzyme methionine synthase. Folate (as N^5,N^{10}-methylenetetrahydrofolate) and vitamin B_{12} are both coenzymes for methionine synthase, and they are therefore necessary for the removal of homocysteine in the body, by transmethylation. Reduced concentrations of these two cofactors, resulting from nutritional deficiencies or genetic defects, results in homocysteine not being removed; an elevation in plasma total homocysteine (tHcy) level is then observed (hyperhomocysteinaemia). tHcy concentrations in plasma are therefore a sensitive indicator of vitamin B_{12} and folate deficiencies, and an inverse relationship between plasma tHcy and both plasma folate and vitamin B_{12} concentrations is observed, even in populations with generally adequate nutrition (Blom, 1998). The situation in developing countries, including those of Africa, where populations may have poor folate and vitamin B_{12} nutrition (Topley, 1968, Abdalla *et al.* 1986; Bates *et al.* 1986; Stabler & Allen, 2004; Siekmann *et al.* 2003) is less clearly established.

The present study was designed to compare and contrast plasma tHcy, folate and vitamin B_{12} concentrations in a sample of rural, subsistence farmers (i.e. women, including those at an early stage of pregnancy and during lactation, and men) in The Gambia, West Africa, and to compare them with a representative sample of adults living in Great Britain, who took part in the National Diet and Nutrition Survey (NDNS) of adults aged 19–64 years in 2000–1. It thus addressed two contrasting lifestyles and dietary patterns, to reveal characteristic metabolic and status differences between a developing, West African country and a Western country.

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

* **Corresponding author:** Dr Christopher J. Bates, fax +44 (0) 1223 437515, email Chris.Bates@mrc-hnr.cam.ac.uk
† **Current address:** Faculty of Mathematics and Natural Sciences, Agder University College, Kristiansand, Norway.

Methods

Gambian study population

Adults from the villages of Keneba, Kanton Kunda and Manduar in the West Kiang region of The Gambia (which is representative of many of the rural farming communities of the Sahelian region of West Africa) were recruited into the study. Detailed descriptions of nutritional and environmental conditions in this region have been provided elsewhere (Prentice *et al.* 1981, 1987). Briefly, the subsistence farming existence is heavily influenced by a single rainy season lasting from July to October, and, as a consequence, many markers of nutritional status exhibit major seasonal variations (Bates *et al.* 1982; Cole, 1993).

The adults recruited for the present study were part of a larger study examining the early-life programming of CVD, whose protocol has been described in detail elsewhere (Moore *et al.* 2001). All adults with a known month of birth, born between 1949 and 1977 and still residing in the three villages, were invited to participate. To meet the requirements of the main study (Moore *et al.* 2001) women with an already-diagnosed and well-established pregnancy were excluded; however, twelve subjects with undiagnosed pregnancies, eleven of whom were over 23 weeks before parturition and two of whom were still breast-feeding a previous child, were included. Their pregnant state was identified only at a later time-point, at which time they were allocated to a separate (pregnant) group.

The study took place during the months of April, May and June 1997, during the latter half of the dry season.

Experimental protocol: fieldwork

Gambian study. The subjects were brought to Keneba's Medical Research Council clinic following an overnight fast. A fasting heparinised venous blood sample was taken for the analysis of plasma homocysteine, folate and vitamin B_{12}. The blood samples were immediately centrifuged, and the plasma/serum samples were aliquotted and frozen at -70°C until the end of the fieldwork, when subsamples were transported to the UK and Norway on dry ice for analysis. Ethical permission for the study was granted by the joint Gambian Government and Medical Research Council Ethical Committee.

British survey. The main British NDNS survey procedures and outcomes are described in the published Survey Reports (Henderson *et al.* 2002; Ruston *et al.* 2004), so only a brief summary is included here.

The population sample was obtained by random selection of eligible individuals living in 152 randomly chosen postcode sectors, which were randomly allocated to four sequential 3-month 'rounds' of fieldwork, beginning in July 2000. Pregnant and breast-feeding women were excluded. Participation was invited but was not compulsory, and partial participation was possible. The survey included a demographic-socioeconomic-lifestyle questionnaire, a weighed dietary record kept for 7 consecutive days, and a non-fasting blood sample taken by a trained phlebotomist in the participant's home.

Three subsamples of blood were distributed, which were used for a wide array of biochemical status measurements, including serum folate and vitamin B_{12}, and plasma homocysteine. Immediately after collection of the sample for tHcy analysis, in Li-heparin, it was placed in a cold-box containing a freezer pack that cooled the sample to nearly 0°C. It was transported to a local hospital laboratory, usually within 2 h and in all cases in less than 4 h, and was immediately centrifuged at 4°C to separate the plasma, which was then stored at -40°C or lower.

For the present study, the subset with an age range confined to 22–50 years was selected, so as to match the age range of the Gambian population studied. Permission was given for the NDNS survey procedures by a Multi-centre Research Ethics Committee and by individual National Health Service Local Research Ethics Committees representing each of the participating postcode sectors.

Laboratory analyses

The Gambian plasma folate and B_{12} concentrations were measured at the Medical Research Council Human Nutrition Research, Cambridge using Abbott IMx kit assays (IMx Systems, Abbott Laboratories, IL, USA). The British subjects' serum folate and vitamin B_{12} concentrations were measured at Great Ormond Street Haematology Laboratory, London, also using the Abbott IMx kit assays. For the folate and vitamin B_{12} assays, agreement with UK National External Quality Assurance Scheme or commercial serum samples with assigned values was essentially identical at both centres; compared with the assigned or all-laboratory-mean results, the observed means were, on average, 5–7 % lower for both analytes.

The Gambian plasma homocysteine concentrations were measured at the Division of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway, by the method of Fiskerstrand *et al.* (1993), which is a reverse-phase HPLC assay. British tHcy concentrations were measured in Cambridge by the Abbott IMx fluorescence polarisation immunoassay kit assay (Shipchandler & Moore 1995). Homocysteine determinations by the Fiskerstrand *et al.* (1993) HPLC assay have been independently shown to give results that are essentially identical to those obtained by the Abbott IMx (fluorescence polarisation immunoassay) assay (Nexo *et al.* 2000). A comparison, in our laboratory, between IMx homocysteine round-robin assay results and those of all participants in the Moller *et al.* (1999) external quality assessment scheme indicated very close agreement, with a mean deviation of less than 1 % for twenty-eight distributed samples.

Statistical procedures

Plasma tHcy and serum or plasma folate and vitamin B_{12} concentrations all showed some evidence of a skewed distribution and were therefore logarithmically transformed to achieve a normal distribution. Statistical tests were performed on the untransformed or transformed variables, with the results being reported as antilogs, i.e. geometric means, wherever transformation was required. Comparisons between group means were made using the Scheffe test following a demonstration of group differences by ANOVA. Associations between continuous variables were examined by Pearson's correlation. Adjustments for age, anthropometric variables and B vitamin status indices were made by multivariate linear regression. P values of less than 0·05 were considered statistically significant. All statistical analyses were performed using DataDesk statistical

program for computers (Data Description Inc., Ithaca, NY, USA).

Results

Homocysteine, folate and vitamin B_{12} status in Gambian adults

Of 301 Gambian subjects who were invited to participate, twenty-six (9 %) were excluded because of an already diagnosed pregnancy, forty-one (14 %) refused and fifteen (5 %) were excluded on the grounds of ill-health. From the three villages, 219 adults (73 % of the total population available under the selection criteria) agreed to participate and gave their informed and signed consent. Men (11 % of the sample) were underrepresented, partly because of urban migration for paid employment in the period before the harvest season.

The age profiles of the study participants are shown in Table 1. As there were some significant intergroup age differences, all subsequent analyte comparisons between the groups were performed after age adjustment, by multivariate linear regression.

The distribution of tHcy in both the Gambian and British samples was positively skewed. The overall Gambian geometric mean plasma tHcy concentration was 9·23 μmol/l, with a range of 2·4–33·4 μmol/l and an interquartile range of 4·4–20·7 μmol/l. tHcy concentrations were positively correlated with age (20–29 years 7·79 μmol/l; 30–39 years 9·16 μmol/l; 40–49 years 10·45 μmol/l; 50–59 years 12·62 μmol/l; P for trend=0·0004). The overall geometric mean British plasma tHcy concentration was 10·0 μmol/l, with an interquartile range of 5·8–19·5 μmol/l. Table 1 shows the geometric mean values of tHcy in the four subgroups of Gambian participants (pregnant, lactating and non-pregnant, non-lactating women, and men), and in the two subgroups of UK participants (non-pregnant, non-lactating women, and men). There are significant intergroup differences, the highest tHcy concentrations being observed in the Gambian men and the lowest in the pregnant Gambian women.

Table 2 shows the folate concentrations by subgroup in the two populations. The lowest folate concentrations were found in the Gambian men, and the highest in the pregnant Gambian women; but there were fewer significant intergroup differences than for tHcy. The lower limit of normal plasma folate concentration in an otherwise well-nourished population has been defined as 6·8 nmol/l (3 ng/ml; Department of Health, 1991). Only one Gambian and five British subjects had plasma folate levels below this lower limit of normality.

Table 2 also shows vitamin B_{12} concentrations by subgroup in the two populations. The highest vitamin B_{12} concentrations were found in British men, and the lowest in the pregnant Gambian women, but there were no significant intergroup differences. A recommended criterion for biochemical vitamin B_{12} adequacy is a plasma vitamin B_{12} concentration above 96 pmol/l (130 pg/ml; Department of Health, 1991). Only one Gambian and nine British subjects had a plasma level below this cut-off point.

Significant linear inverse associations were found between the British log-transformed tHcy and folate concentrations and between the Gambian log-transformed tHcy and folate concentrations (Table 3 and Fig. 1). There was also

Table 1. Mean age and plasma total homocysteine (tHcy) concentrations in Gambian and British (National Diet and Nutrition Survey) population samples (Mean values and standard deviations)

	n	Mean age (years)	SD	Age range (years)	tHcy geometric mean (μmol/l)	tHcy interquartile range (μmol/l)	tHcy (folate- adjusted)* (μmol/l)	tHcy (multiple- adjusted)† (μmol/l)
Gambian pregnant women	12	31·2[a]	4·7	22–37	6·22[a]	4·6–8·3	6·35[a]	6·42[a]
Gambian lactating women	68	31·9[a]	6·8	23–46	9·07[b]	6·9–10·8	8·93[b]	9·19[bc]
Gambian non-pregnant, non-lactating women	90	39·3[b]	8·1	23–51	8·92[b]	6·9–11·5	8·57[ab]	8·63[ab]
Gambian men	22	36·6[abc]	8·6	24–51	14·73[d]	12·0–17·9	13·68[d]	13·76[d]
British women	449	37·2[bc]	7·3	22–50	9·43[b]	7·8–11·3	9·55[b]	9·65[b]
British men	354	37·2[bc]	7·6	22–50	10·87[c]	9·0–12·9	10·84[c]	10·69[cd]

Subset age range selection of the British men and women was confined to 22–50 years to match the age range of the Gambian study population. Values of *n* in the first numerical column refer to the unadjusted tHcy values in the fifth and sixth numerical columns, whereas for the adjusted values in the seventh and eighth numerical columns, values of *n* are somewhat smaller because of missing values of the adjusters. All tHcy values were age-adjusted by linear regression to calculate geometric means and ranges and for the significance calculations.

*Geometric mean values for tHcy were adjusted for both age and folate status (serum or plasma folate) by linear regression.

†Geometric mean values for tHcy were adjusted for age, folate and vitamin B_{12} status and for body weight and height, by linear regression.

[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different by Scheffe *post hoc* test ($P<0·05$). For all models, Gambian pregnant *v.* all non-pregnant Gambian women, $P=0·01$ or less; Gambian men *v.* all groups of Gambian women, $P<0·00001$ or less; Gambian men *v.* British men, $P=0·08$ or less.

For details of subjects and procedures, see p. 509.

Table 2. Plasma or serum folate and vitamin B_{12} concentrations in Gambian and British (National Diet and Nutrition Survey) population samples

	Folate geometric mean (nmol/l)	Folate *n*	Folate interquartile range	Vitamin B_{12} geometric mean (pmol/l)	Vitamin B_{12} *n*	Vitamin B_{12} interquartile range
Gambian pregnant women	22·0[ab]	12	16·1–26·2	206[a]	11	155–257
Gambian lactating women	17·7[ab]	66	9·1–33·4	269[a]	60	199–350
Gambian non-pregnant, non–lactating women	17·5[ab]	89	12·4–24·7	262[a]	81	181–365
Gambian men	14·6[a]	22	7·3–26·1	231[a]	13	183–308
British women	20·1[b]	441	10·0–39·8	255[a]	437	191–341
British men	19·2[ab]	347	10·8–34·4	281[a]	345	217–371

Gambian samples were plasma; UK samples were serum. For both the folate and vitamin B_{12} indices, some of the distributions were skewed; therefore geometric means are shown throughout. All geometric mean values were age-adjusted by linear regression.
[a,b] Mean values within a column with unlike superscript letters were significantly different by Scheffe *post hoc* test ($P<0·05$).
For details of subjects and procedures, see p. 509.

a significant inverse relationship between log-transformed values of tHcy and serum vitamin B_{12} in the British sample (Table 3). No significant linear association was, however, found between Gambian plasma tHcy and vitamin B_{12} concentrations (Table 3). Correlations between tHcy and anthropometric indices (body weight, height, BMI) were generally weak and inconsistent. However, the inclusion of weight and height as well as the folate and vitamin B_{12} indices as adjusters in the tHcy intergroup comparisons (Table 1) did have some influence on the significance values: for example, the combined effect of these adjusters reduced the significance of the tHcy differences between Gambian men, British men and British women.

Comparisons within the British sample

Within the entire British adults' NDNS survey sample, age range 19–64 years, there were sixteen black-skinned respondents (ten women, six men) whose status indices could be compared with those of the 629–649 white Caucasian females and 521–537 white Caucasian males with values for the three relevant status indices (i.e. slightly different numbers for each index). The age range of the female respondents was similar between the black and white subjects, but the mean age of the white Caucasian respondents was 7.6 years greater than that of the black male respondents. Age adjustment was therefore applied, by linear regression, to the intergroup comparisons (by Scheffe test) that are described later.

For tHcy and serum folate, there were no significant differences between the black-skinned and white-skinned respondents (*P* for intergroup comparisons ranging between 0·4 and 0·94). The geometric mean concentrations were as follows: tHcy (μmol/l): black women 8·83, white women 9·71, black men 11·58, white men 11·11; serum folate (nmol/l): black women 20·2, white women 20·4, black men 17·1, white men 19·7. For serum vitamin B_{12}, however, there was a marked and significant difference between the groups for both sexes. The geometric mean concentrations of vitamin B_{12} (pmol/l) were: black women 353, white women 262, *P* for difference 0·04; black men 390, white men 281, *P* for difference 0·04.

Discussion

Homocysteine, folate and vitamin B_{12} status in Gambian and British adults

The present study, conducted in a sample of rural Gambian adults, is the first to provide data on three interrelated status indices – plasma tHcy, plasma folate and plasma vitamin B_{12} concentrations – from this region of sub-Saharan Africa. The mean plasma tHcy concentration of 9·2 μmol/l observed in the Gambian adults is similar to that reported from a group of South African black adults (Ubbink *et al.* 1996). Twenty-three (12 %) of the Gambian adults had a tHcy concentration in excess of 15 μmol/l, and like the tHcy

Table 3. Pearson correlations between $\log_e$(plasma total homocysteine) and $\log_e$(folate or vitamin B_{12} concentrations) in Gambian and British (National Diet and Nutrition Survey) population samples

	Degrees of freedom (folate)	Correlation coefficient (folate)	P (folate)	Degrees of freedom (vitamin B_{12})	Correlation coefficient (vitamin B12)	P (vitamin B_{12})
Gambian pregnant women	10	−0·14	0·7	9	+0·25	0·4
Gambian lactating women	64	−0·44	0·0002	58	−0·19	0·15
Gambian non-pregnant, non–lactating women	87	−0·17	0·12	79	−0·17	0·14
Gambian men	20	−0·31	0·16	11	−0·36	0·2
British women	439	−0·39	<0·0001	435	−0·28	<0·0001
British men	345	−0·38	<0·0001	343	−0·39	<0·0001

The first three columns of data show the degrees of freedom, Pearson's correlation coefficients and significance *P* values for the linear regression of $\log_e$(total homocysteine) *v.* serum or plasma folate, for each population group, and the second three columns of data show the same information for the linear regression of $\log_e$(total homocysteine) *v.* $\log_e$(serum of plasma vitamin B_{12}). Inclusion of age adjustment made little or no difference to these relationships. For the Gambian lactating women, the variation in plasma folate and vitamin B_{12} indices combined explained 25·5 % of the variance in the homocysteine index, and for the British women and men, the variation in the vitamin indices explained 20·1 and 25·2 %, respectively, of the variance in the homocysteine index.
For details of subjects and procedures, see p. 509.

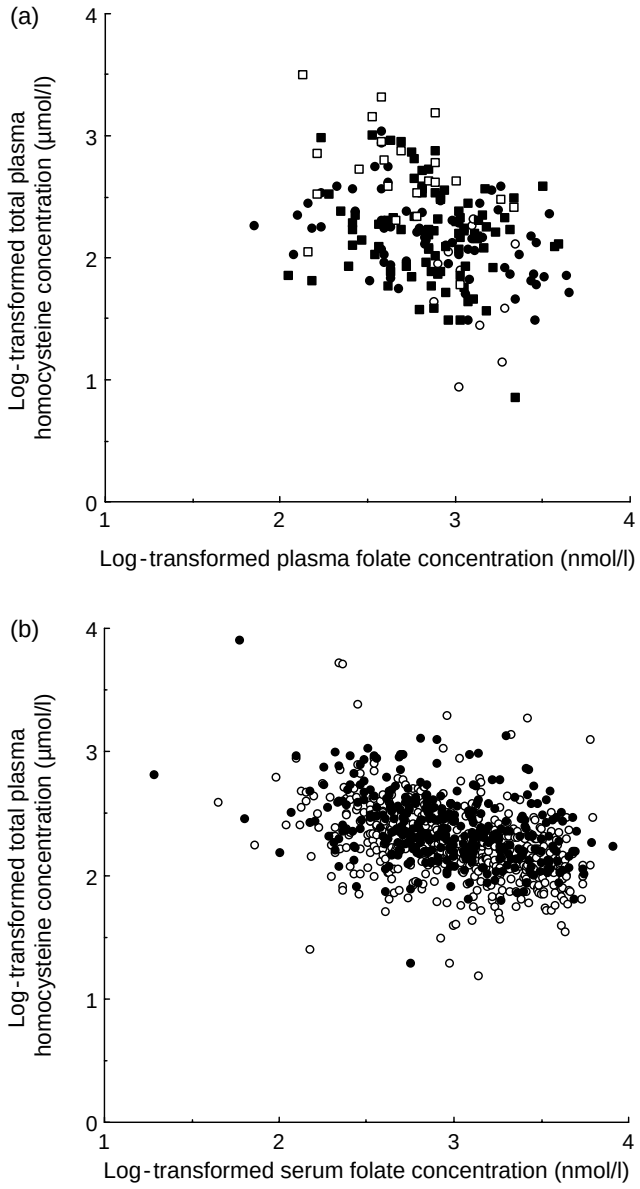

Fig. 1. $\log_e$(plasma homocysteine) plotted against $\log_e$(serum or plasma folate), for (a) Gambian and (b) British (National Diet and Nutrition Survey) subjects. (a) Gambian subjects: (○) pregnant women; (●) lactating women; (■) non-pregnant, non-lactating women; (□) men. (b) British subjects: (○) women; (●) men. For details of subjects and procedures, see p. 509. See Table 3 for the correlation coefficients for each of the population groups in this figure.

profile in Western populations (Ueland *et al.* 1993), their plasma tHcy had a frequency distribution that was positively skewed. Plasma tHcy levels were significantly higher in Gambian men than in Gambian women, and a significant increase was observed in both sexes with increasing age. The Gambian men had significantly higher plasma tHcy levels than the UK men, although the significance of the difference was considerably reduced after adjusting for folate and vitamin B_{12} status and body weight and height (Table 1). The non-pregnant Gambian women had tHcy levels similar to those of the UK women. Consistent with numerous previous reports from Western populations (Boushey *et al.* 1995), the plasma tHcy concentrations in the Gambian and British adults were inversely related to their plasma folate concentrations, although this relationship was significant only for lactating women among the Gambian groups (Table 3). However, the plasma tHcy concentrations in Gambian subjects were not significantly related to their plasma B_{12} concentrations, whereas the tHcy concentrations of the British subjects were inversely related to vitamin B_{12} status (Table 3). The comparatively low plasma tHcy concentrations observed in the pregnant Gambian women, which were only minimally affected by adjustment for B vitamin status and anthropometric indices (Table 1), are consistent with previous studies that have reported a substantial reduction in tHcy during pregnancy in women living in Western countries (Andersson *et al.* 1992; Walker *et al.* 1999; Ueland *et al.* 2000).

Plasma tHcy concentrations are influenced by a number of environmental and genetic factors, and although much is now known about the determinants of plasma tHcy in Western populations (Jacques *et al.* 2001; Vollset *et al.* 2001), far less is known about its determinants in populations in developing countries. Homocysteine metabolism is intimately connected with S metabolism and the dietary availability of S amino acids (Ingenbleek & Young, 2004), and with creatine synthesis (Gamble *et al.* 2005). In addition to the intakes and blood concentrations of the vitamins that are involved in homocysteine metabolism, smoking, exercise, alcohol intake and the consumption of caffeine-containing drinks have all been shown to influence blood homocysteine concentrations in Western populations (Nygard *et al.* 1998; Jacques *et al.* 2001). Despite their comparatively low folate intake and status, the physically active lifestyle of rural Gambians, their abstinence from alcohol and the low frequencies of smoking and of consumption of caffeine-containing drinks may all help to protect some members of this population from raised plasma tHcy levels. Gambian men, however, frequently consume cola nuts, which appear to be implicated in the markedly raised tHcy concentrations that have been reported in Bangladeshi men (Gamble *et al.* 2005). As a stimulant, cola nuts may have an effect on tHcy that is analogous to that of caffeine-containing drinks.

Previous studies in the West Kiang region of The Gambia, conducted mainly in groups of pregnant and lactating women, have suggested that folate status is generally precarious (Topley, 1968; Bates *et al.* 1986). A prenatal folate supplement is recommended for pregnant Gambian women to prevent the further decline in folate concentration that is usually observed during pregnancy, but in practice this is not received by all the pregnant women, and it is usually not received in early pregnancy, before the pregnancy has been confirmed. Furthermore, as this supplementation usually ceases at or soon after parturition, plasma folate concentrations have been found to decline considerably by the third month of lactation (Bates *et al.* 1986).

The present study was conducted at a time of year when the mean adult Gambian dietary folate intake was estimated as 110–140 μg/d (Bates *et al.* 1994), considerably less than the estimated folate intake of the British adults, which was 250–280 μg/d in women and 350–380 μg/d in men (Henderson *et al.* 2003). It needs to be remembered, however, that the dietary estimation techniques and food table nutrient values differed between these two studies. In the British

adults aged less than 50 years, on average 4 % of the folate intake for men and 8 % for women came from dietary supplements (Henderson *et al.* 2003). In the British adults, the average vitamin B_{12} intake was estimated as 5·1 μg/d in women and 6·8 μg/d in men (Henderson *et al.* 2003); there is, however, no information available about Gambian vitamin B_{12} intakes, although they seem likely to be lower than those of British subjects because of the paucity of meat in the Gambian diet (Bates *et al.* 1994). One reason for selecting the dry season for the Gambian study was that, at this time of year, the prevalence of malaria is low (Prentice *et al.* 1999). A decline in functional folate adequacy may occur during the malaria season as a consequence of both malaria infection (Shankar, 2000) and lower dietary folate intakes during the rainy season.

The current study has shown that, paradoxically, Gambian vitamin B_{12} concentrations appeared to be adequate and were not significantly different from those from the British population. By contrast, however, black subjects living in Britain had significantly higher serum vitamin B_{12} concentrations than white British Caucasian subjects (see later).

Whereas the Gambian blood samples were obtained after an overnight fast, the British samples had to be collected throughout daylight hours; i.e. the subjects were not necessarily fasting. A previous study (Fokkema *et al.* 2003) has found that early morning fasting blood samples typically contain about 4 % more tHcy than the mean concentration of subject-matched samples collected throughout daylight hours. Also, whereas the Gambian blood samples were separated very soon after collection, the British samples had to be kept for 1–4 h in a cold-box before separation. Available evidence (Ueland *et al.* 1987) indicates that tHcy remains constant in chilled blood samples for more than 4 h. A small upward bias in the British samples caused by the delay in separation may be offset by the small downward bias caused by non-fasting collection. Two different assay methods were used for the homocysteine assays, but the quality control checks indicated that their performances were essentially identical. Nevertheless, some of the smaller differences observed between the Gambian and UK data sets may need to be verified in order to eliminate the possibility of minor protocol, storage or assay-related differences.

Recent studies in other West African countries, such as Nigeria, Togo, Benin and Burkina Faso (Vanderjagt *et al.* 2000; Glew *et al.* 2002, 2004; Simpore *et al.* 2002; Adjalla *et al.* 2003; Amazou *et al.* 2004), have revealed a picture of relatively high tHcy concentrations, especially in men, associated with relatively poor folate and/or vitamin B_{12} intakes and status, but some apparent genetic advantage over white Caucasians with regard to 5,10-methylene tetrahydrofolate reductase polymorphism patterns and rates of homocysteine metabolism (Simpore *et al.* 2002). A recent comparison of West African, European and Mexican populations has reported considerably higher plasma concentrations of tHcy and vitamin B_{12}, and lower folate levels, in West Africa (Togo, Benin) than in the other countries studied (Gueant-Rodriguez *et al.* 2006). In addition, a lower prevalence of the methylene tetrahydrofolate reductase 677T and 1298C alleles was observed in the West African populations, leading to the suggestion that certain polymorphisms may confer a survival advantage in populations where dietary folate is inadequate (Gueant-Rodriguez *et al.* 2006).

Homocysteine, folate and vitamin B_{12} status in British (Caucasian and black) adults

The number of black-skinned adults participating in the British NDNS survey was small; therefore, the comparison between white Caucasian and black participants is preliminary. Nevertheless, the picture obtained from the NDNS-based comparison is similar to that obtained by Cappuccio *et al.* (2002) in England, and by Ganji & Kafai (2003) in the USA, with regard to homocysteine, namely that black-skinned participants living in Western countries have similar (or slightly lower) tHcy concentrations when compared with white Caucasians. One US study (Gerhard *et al.* 1999) reported higher homocysteine concentrations, together with lower folate concentrations, in black than in white premenopausal women, the tHcy and folate differences here being attributed to a greater use of multivitamin preparations by the Caucasian participants.

The most striking difference between black-skinned and white-skinned Caucasian survey participants in the present study was the significantly higher vitamin B_{12} concentration observed in the black-skinned participants, a difference that has been recorded in several previous studies in Africa, England and the USA (reviewed by Carmel, 1999). The explanation for this is not known, but we concur with Carmel's suggestion that it is more likely to have a genetic than a dietary origin, and we suggest that it is consistent with the absence of low plasma vitamin B_{12} concentrations in the rural Gambian subjects of the present study.

Conclusion

Within a sample of rural Gambian adults, tHcy concentrations were highest in men and lowest in pregnant women. Lactating and non-lactating, non-pregnant Gambian women had tHcy concentrations similar to those of British women participating in a national survey. Gambian men had higher tHcy concentrations than men in the British survey, but the significance of this difference was reduced by adjustment for folate and vitamin B_{12} status and body weight and height. In the British sample, tHcy was inversely correlated with both folate and vitamin B_{12} status in both sexes, but in the Gambian sample a significant inverse relationship was confined to lactating women and to the folate status index. Further studies are needed to explain the intergroup differences in tHcy in Gambian subjects and an apparent difference in vitamin B_{12} index values between black-skinned and white-skinned British subjects.

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