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Plasma folate as marker of folate status in epidemiological studies: the European Investigation into Cancer and Nutrition (EPIC)-Potsdam study

D. Drogan[1], K. Klipstein-Grobusch[1]*, S. Wans[2], C. Luley[2], H. Boeing[1] and J. Dierkes[2]

[1]*Department of Epidemiology, German Institute of Human Nutrition, Nuthetal, Germany*
[2]*Department of Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke University Magdeburg, Germany*

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Folate deficiency is often discussed as a potential risk factor for CVD and some cancers. Reliable assessment of folate status in large-scale epidemiological studies is therefore of major importance. The present study assessed the value of plasma folate (PF) compared with erythrocyte folate (EF) as a marker of folate status in 363 participants in the European Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort. EF and PF, total homocysteine (tHcy), pyridoxine, cobalamin, creatinine, total protein and packed cell volume were determined; glutamate carboxypeptidase (GCP) C1561T, reduced folate carrier (RFC) G80A and methylenetetrahydrofolate (MTHFR) C677T polymorphisms were analysed. Anthropometric measurements were taken and dietary intake was assessed with the EPIC-Potsdam food-frequency questionnaire. Comparison of EF and PF with factors that may modulate their concentrations was performed. Cross-classification of blood folates in quintile categories resulted in correct classification into the same or adjacent category of 75·5 % of all subjects. Age, BMI, pyridoxine and cobalamin, fruit and vegetable intake, and vitamin supplementation 24 h before blood draw were positively associated with EF and with PF. For tHcy an inverse association was found. Participants with the MTHFR 677TT genotype showed significantly elevated EF concentrations compared with those with 677CT genotype; EF and PF were more strongly correlated (r 0·78, $P < 0·0001$) for participants with MTHFR 677TT genotype than for those with the 677CC or 677CT genotype. In summary, our present results indicate that plasma folate seems to be a suitable marker for assessment of folate status for use in large-scale epidemiological studies.

Plasma folate: Erythrocyte folate: Biomarker: Polymorphism: EPIC study

Folate coenzymes play a key role in C_1 metabolism including nucleic acid synthesis, methionine regeneration and specific redox reactions. Among other disorders, low folate status or deficiency may be associated with CVD (Ward, 2001) and various cancers (Fairfield & Fletcher, 2002). Plasma folate (PF) and erythrocyte folate (EF) are both indicators of folate status, although differing in significance and properties. EF is thought to reflect tissue concentration and long-term supply of the vitamin. EF is taken up only by the developing erythrocytes in the bone marrow and thus circulates in blood during the 120 d lifespan of a mature erythrocyte. No recent or transient changes in dietary folate intake can be detected by EF analysis. In human subjects, EF becomes subnormal after 17 weeks of experimental folate deficiency (Herbert, 1982). In contrast, PF seems to be a sensitive indicator of short-term dietary folate intake and usually decreases within 1–3 weeks of experimental folate deficiency (Herbert, 1982). The use of PF or EF as an appropriate measurement to define folate status has been widely debated (Sauberlich, 1999).

Factors that influence PF and/or EF are folate intake and absorption, alcohol intake and drug use, and other largely unknown factors. Recently, genetic factors were discovered that might influence EF and PF. Among these, the C → T transition at position 677 in the gene encoding for methylenetetrahydrofolate reductase (MTHFR) C677T has received much attention, since it also influences total homocysteine (tHcy) and risk for CVD (Klerk *et al.* 2002). In addition, polymorphisms in genes encoding for proteins involved in folate absorption and transport have been discovered: a frequent base exchange in the gene of the reduced folate carrier (RFC) G80A and a less frequent base exchange in the gene encoding for the glutamate carboxypeptidase (GCP) C1561T, which is involved in cleavage of polyglutamates in the bowel before they are absorbed (Chango *et al.* 2000; Devlin *et al.* 2000). At present the influence of these polymorphisms on folate status and tHcy concentration is less clear than that of MTHFR C677T.

We therefore examined the significance of PF in comparison with EF as a possible biomarker reflecting folate status in large-scale epidemiological studies and their association with genetic, biochemical, anthropometric and lifestyle variables that may modulate one or both of them.

Abbreviations: EF, erythrocyte folate; EPIC, European Investigation into Cancer and Nutrition; FFQ, food-frequency questionnaire; GCP, glutamate carboxypeptidase; MTHFR, methylenetetrahydrofolate reductase; PF, plasma folate; PLP, pyridoxal-5′-phosphate; RFC, reduced folate carrier; tHcy, total homocysteine.

* **Corresponding author:** Dr Kerstin Klipstein-Grobusch, fax +49 33200 88 722, email Klipst@mail.dife.de

Subjects and methods

Subjects

Subjects participated in the Potsdam part of the European Investigation into Cancer and Nutrition (EPIC) study, a multi-centre prospective study conducted in ten European countries (Riboli *et al.* 2002). Details of the recruitment procedure, informed consent and approval for all study procedures given by the Ethical Committee of the State of Brandenburg for the EPIC-Potsdam study are described elsewhere (Boeing *et al.* 1999*a*). Briefly, men aged 40–64 years and women aged 35–64 years were randomly chosen from the general population. Subjects (*n* 27 616) recruited between August 1994 and September 1998 underwent a baseline examination, including a physical examination, self-administered questionnaires, PC-guided interviews and donation of 30 ml blood.

The present cross-sectional study comprised 203 men and 160 women in the EPIC-Potsdam cohort (Dierkes *et al.* 2001*b*). Eligible participants were identified by a computer program using the following inclusion criteria: aged 40–65 years, non-smoker or smoking less than ten cigarettes per d. People with a history of skin or of atherosclerotic diseases were excluded. The general recruitment procedure for the present study was part of the EPIC-Potsdam study recruitment and took place between March and September 1997.

Biochemical and genetic analyses

For the current study, all eligible subjects gave 30 ml blood in addition to the EPIC study protocol. Blood samples were taken by standardized procedures (Boeing *et al.* 1999*b*). The packed cell volume was determined using a packed cell volume centrifuge. The plasma was separated from the blood cells within 2 h. For EF analysis, whole blood was mixed with freshly prepared lysis reagent (Abbott Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions and frozen. The packed cells were frozen for DNA preparation. All samples were frozen at -80°C. The samples were transferred to the Institute of Clinical Chemistry on dry ice for analysis of tHcy, PF and EF, cobalamin, pyridoxal-5′-phosphate (PLP), protein, testosterone and oestradiol.

Total cobalamin, PF and EF were measured using commercial ion-capture-assay kits (IMx Abbott Diagnostics, Wiesbaden, Germany). Vitamin B_6 was measured as PLP by HPLC with fluorescence detection (Immunodiagnostik, Bensheim, Germany). Plasma tHcy was analysed using a HPLC system with fluorescence detection (Dierkes *et al.* 1998). Testosterone measurement was done by a RIA labelled with ^{125}I (DPC Biermann; Bad Nauheim, Germany). 17-β-Oestradiol was determined by a chemoluminescence–immunoassay system (Immulite 2000; DPC Biermann, Bad Nauheim, Germany). Total plasma protein was measured by the Biuret method and creatinine was measured by an enzymic assay (Roche Diagnostics, Mannheim, Germany). Within-run CV and between-run CV were respectively (%): tHcy 4 and 6; PF 3 and 6; EF 6 and 9; cobalamin 3 and 6; PLP 5 and 8.

The analyses of the single nucleotide polymorphisms were made by PCR. The PCR products were analysed by subsequent restriction fragment length polymorphism analysis in the case of the MTHFR C677T polymorphism, and by temperature-modulated heteroduplex analyses (Transgenomic Inc., Omaha, NE, USA) in the case of the RFC G80A and GCP C1561T. This method uses the formation of heteroduplexes in case of genetic alterations and separation of the DNA fragments by temperature modulated HPLC (Xiao & Oefner, 2001). Primers and PCR conditions were used as previously described (Dierkes *et al.* 2001*a*) or extracted from the original publications (Chango *et al.* 2000; Devlin *et al.* 2000), while the temperature-modulated heteroduplex analyses protocols were developed individually for each single nucleotide polymorphisms.

Anthropometry

Anthropometric measurements were performed by trained interviewers following standardized procedures (Klipstein-Grobusch *et al.* 1997). In light underwear and without shoes, height, weight, hip and waist circumference were measured, and subsequently BMI and waist:hip ratio were calculated. Using a standard calliper (Lange, Cambridge, MD, USA), skinfold thicknesses were measured on the right side of the body at four sites (biceps, triceps, subscapular and suprailiac). Measurements were taken three times and the mean value was taken to estimate skinfold thickness and subsequently % body fat (Durnin & Womersley, 1974).

Assessment of diet, lifestyle and medical history

Study participants completed a self-administered food-frequency questionnaire (FFQ) about their dietary habits during the past year. The FFQ comprised questions on frequency and portion size of 148 single food items and questions on regular supplement use. A detailed description of the FFQ and information on validity and reproducibility of the FFQ are given elsewhere (Boeing *et al.* 1997; Bohlscheid-Thomas *et al.* 1997*a*,*b*; Kroke *et al.* 1999). Nutrient intake was calculated by use of the German food code and nutrient database (BLS II.3; Klemm *et al.* 1999). Dietary folate was expressed as 'dietary folate equivalents' (1·0 μg dietary folate equivalent = 1·0 μg dietary folate = 0·5 μg synthetic folic acid; National Research Council, 1989; Deutsche Gesellschaft für Ernährung, 2000). Further information on physical activity, smoking history and use of medication during the previous 4 weeks was collected by a PC-guided interview.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (release 8.02; SAS Institute Inc, Cary, NC, USA). Major characteristics of the study participants were expressed as mean values and standard deviations, the *t* test for independent samples was applied to test for differences between men and women. For assessment of the relation of PF and EF the Pearson product-moment correlation coefficient (r) was calculated. The values of EF and PF were plotted against each other in a scatter-gram and the least-squares regression line was calculated.

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To investigate whether individuals were classified into similar categories based on their EF and PF measurements cross-classification of blood folates into quintile categories was carried out. Quintile categories for EF and PF were assigned separately and the overall percentage of individuals classified into the same, the adjacent or the extreme quintile category was determined. Furthermore, the relationships between either PF or EF to other variables was examined by calculating the corresponding Pearson product-moment correlation coefficients. For some variables, such as current smoking or genetic polymorphisms, only categorical data were available. Thus, blood folates of the corresponding categories were compared using the *t* test for independent samples or ANOVA adjusted for multiple comparisons by the Scheffé test. As neither PF nor EF was normally distributed, both variables had to be transformed logarithmically to the base of 10 before performing the tests. After the log-transformation, both skewness and kurtosis were between −1 and 1.

Results

Table 1 describes the characteristics of the male (*n* 203) and female (*n* 160) study participants for the biochemical, anthropometrical and lifestyle variables examined in the present study. No significant differences according to age, BMI and percentage of subjects who were smokers were observed between men and women.

Comparison of the nutritional variables demonstrated significantly higher levels of PLP and tHcy and non-significantly lower levels of cobalamin for men compared with women. None of the study participants had PF <7 nmol/l at the time the blood sample was drawn, i.e. none of the study participants was considered to be in negative folate balance. The same applied to EF, where a cut-off point of 305 nmol/l is considered to be a marker for adequate folate status. Blood folates were similar between the genders, whereas dietary folate intake expressed as 'dietary folate equivalents' was significantly higher in men. Furthermore, men had significantly higher dietary intakes of pyridoxine and cobalamin.

A scatter-gram (Fig. 1) illustrates the linear relationship between PF and EF described by the regression equation EF = 277·3 + 27·4 × PF. Both indices were significantly correlated (*r* 0·63, *P*<0·0001). Cross-classification of blood folates into quintile categories showed similar classification into the same or the adjacent quintile for 75·5 % of the study participants (Table 2). Extreme misclassification occurred for 1·7 % of the study participants.

Correlations between tHcy and other variables have been previously described (Dierkes *et al.* 2001*b*). Correlations between EF and PF to a number of variables are presented in Table 3, including age, anthropometrical and biochemical data. Significant positive correlations to PF as well as to EF were observed for the variables age, BMI, PLP and cobalamin. A significant inverse association was found between PF and tHcy. Similar findings were made for EF though to a smaller degree. Body fat correlated with PF but not with EF. In contrast, the waist:hip ratio showed a positive correlation to EF only. No significant effects on EF or PF were observed for BMR, fat-free mass and the physical activity level. Furthermore, EF and PF were unaffected by total plasma protein, creatinine, testosterone and 17-β-oestradiol (results not shown). Although heavy smokers were excluded from the present study, current smoking (up to ten cigarettes per d) was still significantly associated with lower concentrations of PF (15·0 *v.* 18·1 nmol/l) and EF (705 *v.* 772 nmol/l). Adjustment for age and gender did not change these associations or their strengths.

Frequencies for GCP C1561T, RFC G80A and MTHFR C677T polymorphisms for the study population are given

Table 1. Characteristics of the 363 male and female study participants
(Mean values and standard deviations)

	Men		Women		
	Mean	SD	Mean	SD	Statistical significance of effect (*t* test): *P*
Subjects (*n*)	203		160		
Smoker (%)	9·4		10		0·860
Age (years)	52·4	7·2	52·9	7·3	0·551
BMI (kg/m^2)	27·1	3·2	26·8	4·7	0·560
Fat-free mass (kg)	62·1	6·2	44·2	5·0	<0·0001
Body fat (%)	25·2	5·3	36·7	5·5	<0·0001
BMR (MJ/d)	7·5	0·6	5·9	0·4	<0·0001
Physical activity level	1·9	0·4	1·8	0·4	0·127
Plasma folate (nmol/l)	17·3	5·3	18·4	6·2	0·124
Erythrocyte folate (nmol/l)	772	249	758	246	0·488
Plasma pyridoxal-5′-phosphate (nmol/l)	46·8	29·5	40·7	35·0	0·0001
Total cobalamin (pmol/l)	237	85	256	113	0·184
Total homocysteine (μmol/l)	11·7	4·1	9·4	2·8	<0·0001
Protein (g/l)	66·2	5·8	68·2	4·3	<0·0001
Creatinine (μmol/l)	77	30	59	12	<0·0001
Plasma testosterone (nmol/l)	11·2	4·0	0·5	0·4	<0·0001
Plasma 17-β-oestradiol (pmol/l)	112	30	237	258	<0·0001
Dietary folate equivalent (μg/d)*	241	60	209	55	<0·0001
Dietary vitamin B_6 (mg/d)	1·8	0·5	1·4	0·4	<0·0001
Dietary vitamin B_{12} (μg/d)	7·5	3·3	5·5	2·2	<0·0001

* 1·0 μg dietary folate equivalent = 1·0 μg dietary folate = 0·5 μg synthetic folic acid.

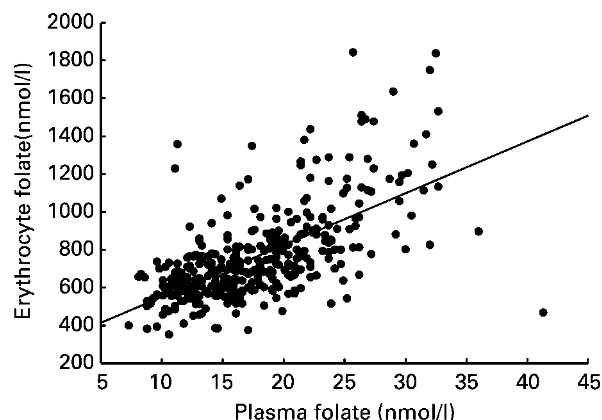

Fig. 1. Linear regression of plasma folate (PF) *v.* erythrocyte folate (EF) for 363 study participants. For details of subjects and procedures, see Table 1 and p. 490. EF = 277·3 + 27·4 × PF; *r* 0·63, *P*< 0·0001).

in Table 4. The frequency of each of the polymorphisms was in line with the Hardy–Weinberg equilibrium. By the Hardy–Weinberg law, a randomly mating population will eventually reach equilibrium as long as there are no selection pressures on the population.

PF, EF and tHcy concentrations according to genotypes are shown in Figs 2, 3 and 4. Subjects with the homozygous MTHFR genotype (677TT) had elevated EF and tHcy concentrations compared with those with the wild type (677CC) or heterozygous (677CT) form of the polymorphism (Figs 3 and 4). GCP C1561T genotypes revealed no effect on PF, EF or tHcy, whereas for the RFC G80A polymorphism tHcy concentrations were significantly elevated for subjects with the homozygous genotype (80AA) compared with subjects with the heterozygous form of the polymorphism (80GA) (Fig. 4).

MTHFR C677T polymorphism had a pronounced effect on the correlation between PF and EF: the Pearson correlation coefficient between EF and PF was 0·57 for the wild type (677CC), 0·66 for the heterozygous (677CT) and 0·78 for the homozygous (677TT) form (all correlations were highly significant, *P*< 0·0001). GCP C1561T and RFC G80A genotypes had no effects on the correlation coefficients between EF and PF.

In our present study population, the daily intake of folate expressed as dietary folate equivalents estimated from the FFQ was only very weakly associated with EF and PF (Table 2). Both PF and EF correlated positively with the consumption of total fruits, vegetables or Brussels sprouts within the study population. Increasing consumption of alcoholic beverages went along with higher levels of EF, but not with PF. Further major folate sources, such as bread, cereals, milk or milk products, or orange juice, were not associated with PF or EF (results not shown).

Dietary supplement intake during the previous 4 weeks was assessed by the FFQ. Significantly higher levels of EF and PF were noted for participants generally reporting use of vitamin supplements (*n* 52) and reporting in detail supplementation with B-group vitamins (n 25) within the scope of their medication practices (*P*< 0·05). Short-term vitamin supplement use (*n* 56; 24 h before blood drawing) was also associated with significant increases in EF and PF values (*P*< 0·05), whereas consumption of fortified juices (*n* 91; 24 h before blood drawing) had no effect on blood folate concentrations.

Discussion

Folate is an essential cofactor for methylation reactions and DNA synthesis within the cell. Inadequate folate status is therefore associated with a broad range of clinical symptoms ranging from haematological abnormalities to stomatitis or glossitis. Furthermore, there is increasing although so far inconsistent evidence for a role of folate in carcinogenesis and the development of CVD or neurological disorders. Although part of these effects may be mediated by hyperhomocysteinemia in folate-depleted subjects, low folate is now regarded as a risk factor in its own right (Morrison *et al.* 1996; Bazzano *et al.* 2002).

Folate can be measured as EF, PF or serum folate, leading to different conclusions about folate status (Sauberlich, 1999). A cross-sectional study, performed among 363 members of the EPIC-Potsdam cohort, was undertaken to compare EF and PF measurements in relation to genetic, biochemical, anthropometric and lifestyle variables. As expected, EF and PF were significantly correlated with each other (Fig. 1). According to the cross-classification performed, 76 % of the subjects were classified in the same or adjacent quintiles of EF or PF measurements (Table 2). Significant associations of EF and PF were found for tHcy, PLP and cobalamin, all of which are involved in folate metabolism. EF concentrations were significantly greater for participants with the MTHFR 677TT genotype compared with the 677CT genotype,

Table 2. Cross-classification by quintiles of erythrocyte folate (EF) and plasma folate (PF) for 363 study participants*

	Quintiles of EF				
	I (< 584 nmol/l)	II (584–656 nmol/l)	III (657–755 nmol/l)	IV (756–903 nmol/l)	V (≥ 904 nmol/l)
Quintiles of PF					
I (< 12·60 nmol/l)	30	22	15	3	3
II (12·60–15·59 nmol/l)	24	22	13	12	3
III (15·60–18·39 nmol/l)	13	17	20	14	7
IV (18·40–22·19 nmol/l)	6	8	19	25	15
V (≥ 22·0 nmol/l)	3	1	5	19	44

* For details of subjects and procedures, see Table 1, and p. 490.

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Table 3. Pearson product-moment correlation coefficients (*r*) and significance (*P*) between erythrocyte folate (EF) and plasma folate (PF), and anthropometrical, biochemical and dietary variables for study participants*

	PF		EF	
	r	*P*	*r*	*P*
Age (years)	0·14	0·008	0·11	0·043
BMI (kg/m^2)	0·16	0·002	0·17	0·001
Body fat (%)	0·14	0·008	0·04	0·398
Waist:hip ratio	0·00	0·997	0·14	0·009
Plasma pyridoxal-5′-phosphate (nmol/l)	0·26	<0·0001	0·21	<0·0001
Plasma cobalamin (pmol/l)	0·22	<0·0001	0·21	<0·0001
Total plasma homocysteine (μmol/l)	−0·30	<0·0001	−0·17	0·001
Dietary folate equivalent (μg/d)†	0·06	0·244	0·08	0·126
Fruit consumption (g/d)	0·15	0·004	0·12	0·026
Vegetable consumption (g/d)	0·15	0·004	0·11	0·039
Brussels sprouts (g/d)	0·15	0·004	0·13	0·017
Alcoholic beverages (ml/d)	0·07	0·192	0·12	0·017

* For details of subjects and procedures, see Table 1 and p. 490.
† 1·0 μg dietary folate equivalent = 1·0 μg dietary folate = 0·5 μg synthetic folic acid.

and MTHFR C677T polymorphism showed a modulating effect on the correlation between blood folates. Analysis of the participants' diets, and smoking and drinking histories, mainly revealed similar effects on PF and EF.

Table 4. Frequency of glutamate carboxypeptidase (GCP) C1561T, reduced folate carrier (RFC) G80A and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms for 363 study participants*

	GCP C1561T†		RFC G80A‡		MTHFR C677T§	
	n	%	*n*	%	*n*	%
Wild type	322	92·3	99	30·3	193	53·9
Heterozygous	25	7·2	170	52·0	140	39·1
Homozygous	2	0·6	58	17·7	25	7·0

* For details of subjects and procedures, see Table 1 and p. 490.
† GCP CT1561: wild type CC, heterozygous CT, homozygous TT.
‡ RFC G80A: wild type GG, heterozygous GA, homozygous AA.
§ MTHFR C677T: wild type CC, heterozygous CT, homozygous TT.

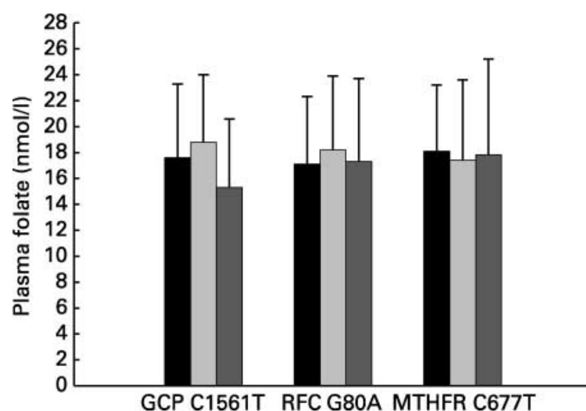

Fig. 2. Plasma folate concentration for glutamate carboxypeptidase (GCP) C1561T, reduced folate carrier (RFC) G80A and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms for 363 study participants. GCP C1561T: wild type CC, heterozygous CT, homozygous TT; RFC G80A: wild type GG, heterozygous GA, homozygous AA; MTHFR C677T: wild type CC, heterozygous CT, homozygous TT. ■, Wild type; ■, heterozygous; ■, homozygous. For details of subjects and procedures, see Table 1 and p. 490. Values are means with standard deviations shown by vertical lines.

In the present study EF and PF were significantly correlated (*r* 0·63, *P*<0·0001). Other studies reported mostly lower correlation coefficients, ranging from 0·41 to 0·52 (Jaffe & Schilling, 1991; Phekoo *et al.* 1997; Thamm *et al.* 1999; Pufulete *et al.* 2002), possibly as a result of different study populations investigated, i.e. different eligibility criteria for study inclusion applied. A number of anthropometrical, biochemical and dietary variables were investigated with regard to their possible association with blood folates. The strongest association with both EF and PF was seen for biochemical variables including PLP, cobalamin and tHcy; this denotes the tight metabolic interactions between folate, tHcy and the analysed B-group vitamins (Bailey & Gregory, 1999) and probably reflects consumption of foods rich in folate and B-group vitamins. Previously, smoking has been associated with a negative folate status (Benton *et al.* 1997). Although our present

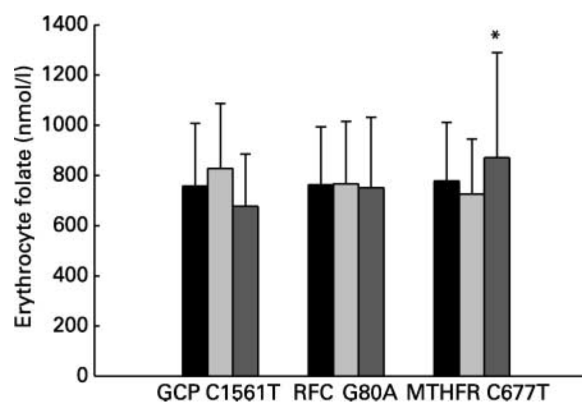

Fig. 3. Erythrocyte folate concentration for glutamate carboxypeptidase (GCP) C1561T, reduced folate carrier (RFC) G80A and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms for 363 study participants. GCP C1561T: wild type CC, heterozygous CT, homozygous TT; RFC G80A: wild type GG, heterozygous GA, homozygous AA; MTHFR C677T: wild type CC, heterozygous CT, homozygous TT. ■, Wild type; ■, heterozygous; ■, homozygous. For details of subjects and procedures, see Table 1 and p. 490. Values are means with standard deviations shown by vertical lines. Mean value was significantly different from that of the heterozygous group (ANOVA adjusted for multiple comparison (Scheffé test)): *P=0·025.

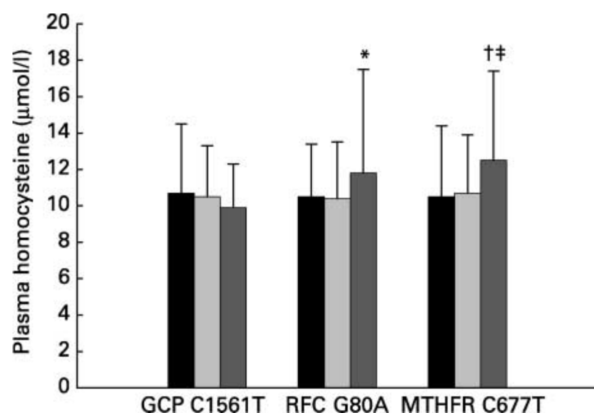

Fig. 4. Plasma homocysteine concentration for glutamate carboxypeptidase (GCP) C1561T, reduced folate carrier (RFC) G80A and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms for 363 study participants. GCP C1561T: wild type CC, heterozygous CT, homozygous TT; RFC G80A: wild type GG, heterozygous GA, homozygous AA; MTHFR C677T: wild type CC, heterozygous CT, homozygous TT. ■, Wild type; ■, heterozygous; ■, homozygous. For details of subjects and procedures, see Table 1 and p. 490. Values are means with standard deviations shown by vertical lines. Mean value was significantly different from that of the heterozygous group (ANOVA adjusted for multiple comparison (Scheffé test)): $^{*}P=0{\cdot}049$. Mean value was significantly different from that of the wild type group (ANOVA adjusted for multiple comparison (Scheffé test)): $\dagger P=0{\cdot}030$. Mean value was significantly different from that of the heterozygous group (ANOVA adjusted for multiple comparison (Scheffé test)): $\ddagger P=0{\cdot}062$.

study sample included only participants smoking less than ten cigarettes per d, smoking was associated with lower values of blood folates (Table 3).

MTHFR 677TT had effects on EF and on tHcy as well as a modulating effect on the correlation between PF and EF. It cannot be excluded that the increase in EF in these subjects is due to the assay used for determination. It has been reported previously that in individuals with MTHFR 677TT, a portion of the intracellular folate is found as formylated tetrahydrofolate, which shows a higher affinity to the antibodies used in the immunological assay, thus giving higher results (Bagley & Selhub, 1998). This is supported by comparisons with the microbiological assay, which did not give higher EF levels in 677TT individuals (Molloy *et al.* 1998).

Other authors also report that the RFC G80A polymorphism has no effect on blood folates (Chango *et al.* 2000; Fodinger *et al.* 2003; Winkelmayer *et al.* 2003). The genetic variation causes a change of arginine to histidine residue at position 27 of the protein. Recently, it was shown *in vitro* that both proteins exhibit similar transport properties to various forms of folate (Whetstine *et al.* 2001). Taken together, these results suggest that this single nucleotide polymorphism is not a major determinant of folate bioavailability and transport and thus of folate status.

Recent findings concerning the effect of the GCP C1561T polymorphism are less clear. Devlin *et al.* (2000) reported higher homocysteine, lower serum folate and no effect on EF folate concentrations in heterozygotes than in wild type individuals. This finding was not confirmed in other studies. Vargas-Martinez *et al.* (2002) reported higher serum folate levels in older men with the CT and TT genotype than in wild type subjects (CC) in the Framingham offspring cohort. This difference was only found in older men, and not in younger men and in women. A higher PF in CT and TT genotypes was also reported by Afman *et al.* (2003). Lievers *et al.* (2002) reported higher EF and PF concentrations in CT and TT carriers compared with wild type individuals and no effect for tHcy. At present, it seems that this polymorphism has no major effect on folate status and plasma homocysteine; however, present data do not allow us to draw a clear conclusion.

Unfortunately, the size of the present investigation and the frequency of the polymorphisms do not allow us to study interactions between these single nucleotide polymorphisms. This would have required a much greater number of subjects.

Blood folates were only weakly associated with dietary folate intake estimated by FFQ. Some previous studies have shown blood folates to be correlated significantly to dietary folate intake (Jacques *et al.* 1993; Green *et al.* 1998), whilst other studies have failed to do so (Thamm *et al.* 1999; McKinley *et al.* 2001; Melse-Boonstra *et al.* 2002). Likely explanations applicable to the current study are that folic acid is a quite unstable vitamin, its content in food being highly dependent on storage conditions and preparation methods, and its bioavailability depends on the length of the glutamate chain and the food matrix. Supplements and fortified foods contain folylmonoglutamate, which is thought to be absorbed twice as well as folylpolyglutamates, the major component of 'natural' folate in our diet (Suitor & Bailey, 2000). This may explain our observation that use of vitamin supplements (short- and long-term) resulted in higher values of PF and EF in the current study, whereas the association of blood folates with dietary intake assessed by FFQ was rather modest. It is possible that folate estimates by FFQ might only insufficiently reflect real dietary folate intake. Average daily dietary folate (dietary folate equivalents) for the present study population was 227 (SD 60) μg, ranging from 89 to 432 μg, and was thus lower than the RDA of 400 μg dietary folate equivalents/d for adults (Deutsche Gesellschaft für Ernährung, 2000). However, despite low reported dietary folate intake, none of the study participants showed subnormal levels of EF and PF.

In summary, EF and PF were significantly correlated with each other and most study participants were classified into similar categories by EF and PF measurements. For the majority of analysed variables that might modulate blood folates, similar associations to both EF and PF were observed. These findings suggest that plasma folate could be used as a marker of folate status in epidemiological studies estimating chronic disease risk.

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