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ORIGINAL ARTICLE

Validity of food frequency questionnaire estimated intakes of folate and other B vitamins in a region without folic acid fortification

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Background/Objectives: B vitamins have been implicated in major chronic diseases but results have been inconsistent. This study evaluated the accuracy of dietary intakes of folate, vitamin B12, riboflavin and vitamin B6 as measured by the Northern Sweden Food Frequency Questionnaire (FFQ) against repeated 24-h recalls (24HR) and plasma levels, taking into consideration the *MTHFR* 677C>T polymorphism.
Subjects/Methods: B vitamin intakes assessed by a semi-quantitative FFQ designed to measure the intake over the previous year were compared with those from 10 24HR, as well as to plasma levels of folate and vitamin B12, in randomly selected men ($n=96$) and women ($n=99$) aged 30–60 years. FFQ-based B-vitamin intakes were also compared with plasma levels of B-vitamins and with MTHFR 677C4T genotype in 878 men, aged 40–61 years.
Results: Intakes of vitamins B12 and riboflavin were similar, whereas folate and B6 intakes were 16–27% higher, as estimated by FFQ versus 24HR. Spearman correlation coefficients between the two methods ranged from 0.31 to 0.63 (all $P\leqslant 0.002$), and were lowest for vitamin B12. Intakes estimated by FFQ were correlated with plasma levels, but coefficients were lower (range: 0.13–0.33), particularly for vitamin B12 in men (0.15–0.18). Folate intake was not correlated with plasma levels in subjects with the *MTHFR* 677 T/T genotype.
Conclusions: The validity of the Northern Sweden FFQ for assessing B vitamin intake is similar to that of many other FFQs used in large-scale studies. The FFQ is suitable for ranking individuals by intake of folate, riboflavin, vitamin B6 and to a lesser extent vitamin B12.
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Keywords: folate; vitamin B; validity; food frequency questionnaire; 24-h recall; *MTHFR*

Introduction

Self-administered food frequency questionnaires (FFQs) are the most economical method to estimate dietary intake of foods or nutrients in large-scale epidemiological studies. However, FFQs are prone to both systematic and random errors, which may lead to misclassification and attenuation of diet–disease associations (Willett, 1998; Cade *et al*., 2004).

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A semi-quantitative FFQ has been used to estimate dietary intake in two population-based cohorts in northern Sweden, the Västerbotten Intervention Project (VIP, Weinehall *et al*., 1998), which is part of the prospective European Prospective Investigation into Cancer and Nutrition (EPIC; Riboli and Kaaks, 1997), and the northern Sweden World Health Organization (WHO) MONICA (Multinational monitoring of trends and determinants in cardiovascular disease) study (Stegmayr *et al*., 2003). The Northern Sweden FFQ has been validated against 24-h dietary records (24HR) and biological markers (Johansson *et al*., 2002; Wennberg *et al*., 2009). The FFQ had high reproducibility, and in comparison with 24HR, acceptable ranking capacity for energy and several nutrients, that is, correlation coefficients above 0.50 and intake ratios

close to 1. Similar to other dietary recording methods, underreporting in relation to estimated basal metabolism was frequent (Johansson *et al*., 2001).

The Northern Sweden FFQ has not been validated for B vitamins, including folate. The importance of folate for neural tube defects and megaloblastic anemia is well established. Folate has also been implicated in several chronic diseases, although results are inconsistent (Verhoeff *et al*., 1998; Toole, 2002; Liem *et al*., 2005; Bønaa *et al*., 2006; Lonn *et al*., 2006; Wang *et al*., 2007; Kim, 2008; Ulrich, 2008; Malouf and Grimley Evans, 2008; Maruti *et al*., 2009; Mathers, 2009). Folate acts as an essential methyl donor in one-carbon metabolism, providing methyl groups for the synthesis of methionine from homocysteine, and for the synthesis of nucleotides, particularly purines. Other B vitamins also contribute to methyl availability through co-enzyme activity.

The enzyme 5,10- methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate. In the *MTHFR* gene, a functional single nucleotide polymorphism (SNP; 677C>T, rs1801133) results in a thermolabile enzyme with reduced activity of up to 45% in individuals with the rare T/T genotype compared with the common C/C genotype. The *T* allele has been associated with reduced circulating levels of folate and increased levels of homocysteine (Frosst *et al*., 1995; Tanaka *et al*., 2009).

Measurements of B-vitamin intake are generally intended to be markers of a person's biological status. However, factors such as reduced conversion to circulating forms of folate due to the *MTHFR* 677C>T SNP or a narrow range of plasma concentrations, as occurs in countries with low intake (such as Sweden), may contribute to misclassification of subjects in higher intake quintiles.

The aim of this study was to extend the previous validation studies of the Northern Sweden FFQ to include folate, vitamin B12, riboflavin and vitamin B6. The FFQ was evaluated against repeated 24HR recalls, as well as against plasma levels of the B-vitamins, taking into consideration the *MTHFR* 677C>T genotype.

Subjects and methods

The Västerbotten intervention project

The study population is based on participants in the population-based VIP cohort in northern Sweden. In the VIP, established in 1985, all residents in the county of Västerbotten are invited for a health examination at their primary healthcare clinic on attaining 30, 40, 50 or 60 years of age. Participants completed a lifestyle questionnaire that included questions on diet, and donated a blood sample for research. Annual participation rates over the period relevant for this study ranged from 57 to 67%. No systematic differences in social or demographic variables were seen between participants and non-participants (Weinehall *et al*., 1998).

Study subjects

This study included two subgroups nested within the VIP cohort—the basic validation study subgroup and the prostate cancer subgroup.

The basic validation subgroup included a stratified random subsample of individuals attending the VIP in 1992 (Johansson *et al*., 2002). Of 246 individuals invited, 43 declined participation due to lack of time. Thus, 102 men and 101 women, equally distributed over the ages 30, 40, 50 and 60 years, consented to participate. The participants completed a self-administered FFQ shortly before and after a 1-year period of 24HRs. In this study, the first FFQ was used. Plasma samples from these subjects were analyzed for folate and vitamin B12.

The prostate cancer subgroup included men from a previous case–control study of B vitamins and prostate cancer (Johansson *et al*., 2009), with data and sample collection over the period 1992–2004. The original study included 568 prostate cancer cases and 1034 matched controls of which 899 subjects with dietary intake data were included in this validation study. Plasma samples from these subjects were analyzed for folate and vitamins B12, B6 and riboflavin, and DNA samples for the *MTHFR* 677C>T polymorphism.

The study was approved by The Regional Ethics Review Board in Umeå, Sweden, and all participants signed an informed consent form at the time of recruitment into the VIP study.

Food frequency questionnaire (test method)

An FFQ, designed to be semi-quantitative and optically readable for data input, was used. The FFQ was designed to capture habitual intake over the previous year. Two FFQs were distributed 1 year apart to capture reliability. Frequencies of consumption of 84 food items (64 items in part of the prostate cancer subgroup) were reported on a nine-level scale, as previously described (Johansson *et al*., 2002). In 1994, the management of the County Council of Västerbotten introduced a 64-item version of the FFQ for financial reasons, but frequency alternatives, meal size illustrations and several sections of the FFQ were left intact.

Energy and vitamin B intakes were calculated as described elsewhere (Johansson *et al*., 2002). The folate content of foods has recently been updated in the National Food Administration database (www.slv.se), and the updated values were used to estimate folate intake by the FFQ (reference method) and to recalculate intakes estimated by the reference 24HR recalls (Johansson *et al*., 2002).

Intake of B vitamins from supplements was derived from questions addressing the use of dietary supplements during the previous year and the previous 14 days. The response alternatives were: none, multivitamins, multiminerals, iron, selenium and/or other, for which subjects were asked to describe the product. For multivitamin users, 200 μg folate, 3 μg vitamin B_{12}, 1.5 mg vitamin riboflavin and 2 mg vitamin B_6 (conservative estimates based on supplements available in Sweden) were added to the dietary intakes from foods.

Repeated 24-h recalls (dietary reference method)
The 24HR interviews in the basic validation study subgroup were conducted by telephone by nine trained interviewers. Intakes of food and beverages during the preceding 24 h were recorded on 10 unannounced occasions per subject. The 24HRs were approximately equally dispersed over the year between FFQ1 and FFQ2 and covered all weekdays. Food portion estimation was facilitated by a booklet with life-size pictures (Bergström, 1979; Håglin *et al*., 1995). Daily energy and nutrient intakes were calculated using the food composition database of the National Food Administration. The use of dietary supplements and remedies was examined in detail in the 24HRs.

Blood sampling and analyses (biomarker reference method)
Venous blood samples were drawn without stasis into evacuated glass tubes after at least 4 h of fasting. Blood was drawn before the baseline FFQ was completed. Plasma, obtained by centrifugation at 1500 *g* for 15 min, was aliquoted and stored at −80 °C.

In the basic validation study, plasma folate and vitamin B12 were analyzed at the Department of Medical Biosciences, Clinical Chemistry, Umeå University, Sweden, using the Quantaphase II radioassay (Bio-Rad Diagnostic Group, Hercules, CA, USA). The total coefficients of variation (%) for vitamin B12 were 5.9 and 6.5% at levels 122 and 483 pmol/l, respectively, and for folate were 3.9 and 6.9% at levels 3.8 and 21.5 nmol/l, respectively.

In the prostate cancer study, plasma was analyzed for folate, vitamin B12, B6 (pyridoxal 5′-phosphate) and riboflavin levels at Bevital AS (Bergen, Norway). Concentrations of folate were determined by a microtiter assay using *Lactobacillus casei* (O'Broin and Kelleher, 1992). The within- and between-run coefficients of variations were 6.0 and 6.3%, respectively. Concentrations of vitamin B12 were determined by a *Lactobacillus leichmannii* microbiological assay (Kelleher and Broin, 1991; Molloy and Scott, 1997). The within- and between-run coefficients of variation were 5.4 and 6.7%, respectively. Vitamin B6 and riboflavin were determined by liquid chromatography-mass spectrometry/mass spectrometry (Midttun *et al*., 2005). The within- and between-run coefficients of variation were from 3 to 20 and 6 to 22%, respectively.

For 579 samples from the prostate cancer study, plasma folate and vitamin B12 concentrations were analyzed by both methods, which allowed for comparison. Values of folate and vitamin B12 were consistently higher with the Quantaphase II radioassay. The relations were: $\text{folate}_{\text{Microbiological Assay}} = 0.77 \times \text{folate}_{\text{Quantaphase}} - 0.457$ ($R^2 = 0.76$), and $\text{B12}_{\text{Microbiological Assay}} = 0.82 \times \text{B12}_{\text{Quantaphase}} + 73.4$ ($R^2 = 0.71$).

Analysis of MTHFR *677C>T genotypes*
The *MTHFR* 677C>T genotypes were identified using the TaqMan allelic discrimination method at the Center for Genome Research, Department of Medical Biosciences, Umeå University, Sweden. TaqMan assays and reagents were obtained from Applied Biosystems (Foster City, CA, USA). The PCR reactions were performed on GeneAmp PCR system 9700, and PCR programs were according to the manufacturer (Applied Biosystems). PCR products were analyzed on the ABI PRISM 7900HT Sequence Detection System.

Final participation rates and missing values
Subjects were excluded if any portion size question or >10% of the FFQ questions were unanswered (Johansson *et al*., 2002). Participants with unreasonably low or high estimated energy intake, defined as lowest 5% or highest 2.5% food intake levels in the Northern Sweden FFQ database (93 000 observations in 2008) were excluded. Food intake level values were calculated as estimated energy intake divided by estimated basal metabolic rate (based on age, sex and body mass; Schofield, 1985).

In the basic validation subgroup, six participants completed less than three 24HR and two more did not return the baseline frequency questionnaire; these participants were excluded. The remaining 96 men and 99 women completed all 10 24-HR and the baseline frequency questionnaire (79% participation rate). No respondent was excluded due to incomplete FFQ or unreasonable energy intake, but data on plasma analyses were missing for 11 men and 9 women.

In the prostate cancer study group, 899 men had completed the FFQ (540 the longer version, 359 the shorter version). None met the exclusion criteria for FFQ completeness, but 21 men were excluded due unreasonably high energy intakes, leaving 537 respondents with the longer FFQ and 341 with the shorter. Plasma sample could not be obtained for one of these 878 men, whereas DNA sample could not be obtained for 35 men.

Statistical analyses
All statistical analyses were performed separately for men and women (basic validation study) and for the two FFQ versions (prostate cancer study). No subjects from the basic validation subgroup, and 14.5% of the subjects from the prostate cancer subgroup reported multivitamin use. Adjustment for reported energy intake was by the residual method (Willett, 1998). All statistical analyses were based on total intakes, that is, including estimated intake from supplements.

All tests were non-parametric, except for linear regression analyses in which logarithmically transformed data were used. Central measures are presented as medians with 10th and 90th percentile limits. The attenuation coefficients for the various B vitamins were calculated as $\rho = 1/(1 + \text{var}_W/\text{var}_B)/n)^{0.5}$ where n is the number of repeated 24HRs, var_W is the variance within subjects, and var_B is the variance between subjects (Ocké *et al*., 1997). Associations between individual intakes were assessed by Spearman rank correlations and by ratios between intakes based on the test and reference method. All statistical tests were two sided and *P*-values below 0.05 were considered statistically significant.

The software SPSS version 16.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

Results

Median intakes, intake ratios, calibration coefficients and Spearman correlation coefficients between B vitamin intakes estimated by the FFQ (84 items) and 24HR in subjects from the basic validation study are reported in Tables 1 and 2. Daily intakes estimated by the FFQ, in men and women, respectively, for folate were 251 and 225 μg, for vitamin B12 were 7.0 and 4.6 μg, for riboflavin were 1.7 and 1.4 mg, and for vitamin B6 were 2.2 and 1.9 mg. Spearman correlations between intakes recorded by the FFQ at baseline (FFQ1) and one year later (FFQ2) ranged from 0.60 (vitamin B6 in men) to 0.76 (folate in women), all $P<0.001$.

Intakes of vitamins B12 and riboflavin were similar, whereas folate and B6 intakes were 16–27% higher, as estimated by FFQ versus 24HR (Table 1). Linear regression of FFQ intake on 24HR intake yielded β-values $\geqslant 0.5$ for all vitamins (both sexes) except vitamin B12, for which the β-values were 0.19 and 0.23 for men and women, respectively (Table 1). Correlation coefficients (non-energy adjusted) ranged from 0.31 (vitamin B12 in men and women) to 0.63 (riboflavin in women; Table 2). Energy adjustment did not improve the correlation coefficients (data not shown).

Correlation analyses between intakes and plasma levels of the B vitamins are presented in Tables 2–4. All correlation analyses between intakes and plasma levels used energy-adjusted intake data. Non-energy-adjusted data generally yielded lower coefficients (data not shown).

The correlation coefficients between FFQ-based intakes and plasma levels of folate and vitamin B12 followed the same pattern in the basic validation study subgroup and the prostate cancer study subgroup. The correlation coefficients for folate in stratified analyses based on sex and FFQ version varied between 0.20 and 0.31, whereas the correlation coefficients for vitamin B12 were lower in men ($r_{Spearman}$: 0.11–0.18), but not in women ($r_{Spearman}$: 0.30; Tables 2 and 3). The correlation coefficients between 24HR-based intake and plasma levels followed the same pattern as for the FFQ (Table 2), but in scatter plots of intakes versus plasma levels of folate, the FFQ displayed a wider distribution than the 24HR in both men and women (Figure 1). Median plasma levels for folate and vitamin B12 were significantly higher in the highest versus lowest quintile based on FFQ intake distributions (Tables 2 and 3), but continuous increases by ranking group were found only when the 2nd to 4th quintiles were combined (data not shown). Cross-classification of the subjects in the basic validation subgroup of FFQ versus 24HR estimated intakes in quartile categories resulted in correct classification into the same or adjacent category of 84% for folate, 83% for vitamin B6, 78% for riboflavin and 71% for vitamin B12. The corresponding values for FFQ estimated intakes versus plasma levels were 67 and 71% for folate and vitamin B12, respectively. In the prostate subgroup, correct classification

Table 1 Intake of folate, riboflavin and vitamins B12 and B6

Nutrient (daily intakes)	*Estimated intakes*[a]				*Ratio FFQ/24HR*[a]	*Linear regression*[b]	
	Median (10–90 percentile limits)			*Mann–Whitney test P-value*	*Median (10–90 percentile)*	*β-value (95% CI limits)*	*P-value*
	Sex	*FFQ*	*24HR*				
Energy (MJ and kcal)	Men	8.2 (5.8–12.8) 1974 (1385–3050)	9.2 (5.6–12.2) 2190 (1342–2919)	0.538	0.98 (0.67–1.50)	0.62 (0.41–0.83)	<0.001
	Women	6.9 (4.8–9.5) 1653 (1152–2263)	6.8 (4.7–8.9) 1621 (1128–2125)	0.488	1.02 (0.74–1.47)	0.50 (0.28–0.64)	<0.001
Folate (μg)	Men	251 (151–368)	208 (128–282)	<0.001	1.27 (0.79–1.96)	0.50 (0.27–0.70)	<0.001
	Women	225 (145–346)	181 (127–241)	<0.001	1.24 (0.91–1.83	0.73 (0.51–0.95)	<0.001
Vitamin B12 (μg)	Men	7.0 (3.4–10.7)	6.4 (3.6–14.0)	0.431	1.05 (0.53–1.96)	0.19 (0.02–0.35)	0.026
	Women	4.6 (2.8–7.2)	4.6 (2.7–9.9)	0.543	0.96 (0.49–1.78)	0.23 (0.09–0.36)	0.001
Riboflavin (mg)	Men	1.7 (1.1–2.8)	1.8 (1.2–2.6)	0.272	0.96 (0.60–1.40)	0.58 (0.36–0.80)	<0.001
	Women	1.4 (0.85–2.1)	1.5 (1.0–1.9)	0.173	0.93 (0.58–1.44)	0.54 (0.28–0.80)	<0.001
Vitamin B6 (mg)	Men	2.2 (1.4–3.6)	2.1 (1.4–2.9)	0.034	1.16 (0.71–1.85)	0.64 (0.39–0.89)	<0.001
	Women	1.9 (1.3–2.8)	1.6 (1.1–2.0)	<0.001	1.21 (0.90–1.74)	0.83 (0.61–1.04)	<0.001

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire.
Intake of folate, riboflavin and vitamins B12 and B6 as estimated by the longer version of the Northern Sweden FFQ (84 items) and 24HR (basic validation study group with 96 men and 99 women).
[a]Calculated on crude data (that is, not adjusted for energy). None took supplements.
[b]Calibration coefficients estimated by linear regression of the 24HR on the FFQ1 measurements; logarithmically transformed data were used in regression models.

Table 2 Intake and plasma levels of B vitamins in tertile groups

Vitamin[a]	*Sex*	*Median values in tertile groups ranked by FFQ estimated intakes*[a,b]									*Spearman correlation coefficients*					
		FFQ intake			*24HR intake*			*Plasma conc*			*FFQ vs 24HR*[b]		*FFQ vs plasma*[c]		*24HR vs plasma*[c]	
		1st	*2nd*	*3rd*	*1st*	*2nd*	*3rd*	*1st*	*2nd*	*3rd*	*Corr coeff*	P-*value*	*Corr coeff*	P-*value*	*Corr coeff*	P-*value*
Folate	Men	179	251	341	185	196	233	7.2	6.8	8.2	0.41	<0.001	0.24	0.03	0.18	0.088
	Women	166	225	311	152	181	221	9.0	9.7	10.1	0.57	<0.001	0.20	0.066	0.33	0.001
Vitamin B12	Men	5.44	7.22	8.35	4.10	6.42	10.48	132	170	155	0.31	0.002	0.11	0.325	0.19	0.077
	Women	4.27	4.97	5.01	3.04	4.60	7.44	144	146	173	0.31	0.002	0.30	0.004	0.19	0.076
Riboflavin	Men	1.26	1.75	2.42	1.56	1.86	2.24	—	—	—	0.47	<0.001	—	—	—	—
	Women	0.93	1.39	1.82	1.31	1.37	1.62	—	—	—	0.39	<0.001	—	—	—	—
Vitamin B6	Men	1.53	2.20	3.49	1.79	1.93	2.40	—	—	—	0.49	<0.001	—	—	—	—
	Women	1.36	1.88	2.55	1.32	1.55	1.79	—	—	—	0.63	<0.001	—	—	—	—

Abbreviation: FFQ, food frequency questionnaire.
Intake and plasma levels of B vitamins in tertile groups based on the distribution of intakes estimated by the Northern Sweden FFQ (basic validation study group with 96 men and 99 women), and correlations between measured intakes and plasma levels.
[a]Men and women were ranked separately for tertile classification. Intake of folate and vitamin B12 are expressed in μg/day and riboflavin and vitamin B6 in mg/day. Plasma levels of folate and B12 are expressed in nmol/l and pmol/l, respectively.
[b]Calculated on crude data (that is, not adjusted for energy).
[c]Calculated on energy-adjusted intake data. Non-adjustment for energy generally yielded lower correlation coefficients between intake and plasma levels.

Table 3 Median intake and plasma concentration of folate and B vitamins in quintile groups

Vitamin	*Median values in quintile groups ranked by FFQ-estimated intakes*[a]										*Spearman correlation*	
	FFQ intake[b]					*Plasma concentration*[c]					*FFQ*[d] *vs plasma*	
	Q1 (low)	*Q2*	*Q3*	*Q4*	*Q5 (high)*	*Q1 (low)*	*Q2*	*Q3*	*Q4*	*Q5 (high)*	*Corr coeff*	P-*value*
Folate												
Longer version FFQ	166	212	249	303	430	5.31	5.43	5.55	5.65	6.79	0.28	<0.001
Shorter version FFQ	153	191	234	287	417	5.18	5.58	6.07	5.73	7.80	0.31	<0.001
Vitamin B12												
Longer version FFQ	3.36	4.86	6.02	7.34	9.30	312	363	346	315	380	0.15	0.001
Shorter version FFQ	3.22	4.33	5.25	6.46	8.77	318	321	358	363	356	0.18	0.001
Riboflavin												
Longer version FFQ	1.06	1.39	1.67	2.01	2.86	9.17	10.12	10.51	11.81	12.02	0.13	0.02
Shorter version FFQ	0.99	1.32	1.56	1.94	2.96	7.37	8.92	9.62	11.43	13.61	0.28	<0.001
Vitamin B6												
Longer version FFQ	1.40	1.77	2.15	2.56	3.71	32.3	31.1	37.2	38.2	48.2	0.27	<0.001
Shorter version FFQ	1.33	1.71	2.00	2.45	3.77	31.3	33.9	39.3	35.0	43.1	0.24	<0.001

Abbreviation: FFQ, food frequency questionnaire.
Median intake and plasma concentration of folate and B vitamins in quintile groups (prostate cancer study group, 899 men) based on the distribution of intakes estimated by the Northern Sweden FFQ.
[a]Ranking was done separately for the two FFQ versions on absolute intakes, that is, reported intakes from supplements included. A total of 531 men, who had answered the longer FFQ version, and 339, who had answered the shorter version, had plasma analyses.
[b]Intakes of folate and vitamin B12 are expressed in μg/day and riboflavin and vitamin B6 in mg/day.
[c]Plasma levels are expressed in nmol/l for folate, and riboflavin and vitamin B6 and in pmol/l for B12.
[d]Calculated on energy-adjusted intake data.

based on quintiles of FFQ estimated intakes and plasma levels were found for folate 56% and B12 52%, and for both riboflavin and vitamin B6 57%.

The correlation coefficients between intakes and plasma levels, for the longer and shorter version of the FFQ, respectively, were: for riboflavin, 0.13 ($P=0.02$) and 0.28

Table 4 Correlations between B-vitamin intakes

		Spearman correlation coefficient by MTHFR 677C>T polymorphism[a]		
	Numbers[b]	*C/C*	*T/C*	*T/T*
Folate (μg/day)[c]				
Longer version FFQ[b]	280/185/49	0.31***	0.38***	0.07
Shorter version FFQ[b]	153/137/32	0.30***	0.34***	0.15
Vitamin B12 (μg/day)[c]				
Longer version FFQ[b]	279/186/49	0.08	0.20**	0.35*
Shorter version FFQ[b]	153/138/32	0.12	0.24**	0.18
Riboflavin (mg/day)[c]				
Longer version FFQ[b]	275/184/49	0.14*	0.11	0.07
Shorter version FFQ[b]	152/136/32	0.42**	0.12	0.26
Vitamin B6 (mg/day)[c]				
Longer version FFQ[b]	275/184/49	0.26***	0.36***	0.18
Shorter version FFQ[b]	152/136/32	0.29***	0.25***	0.09

Abbreviation: FFQ, food frequency questionnaire.
Correlations between B vitamin intakes estimated by the Northern Sweden FFQ and corresponding plasma concentrations by *MTHFR* 677C>T genotype (prostate cancer study group, 843 men with genotype data).
[a]Among the 843 men for whom a DNA sample was available, the *MTHFR* 677 C→T genotype distribution was: C/C = 434 men (51%), T/C = 338 men (38%) and T/T = 81 men (10%).
[b]Numbers refer to number of subjects in each genotype stratum (CC/TC/TT) with plasma analyses and an FFQ fulfilling quality criteria.
[c]Energy adjusted total daily intake, that is, intake from supplements is included. Non-energy-adjusted values yielded slightly lower correlations for nearly all evaluations.
*$P<0.05$, **$P<0.01$, and ***$P<0.001$.

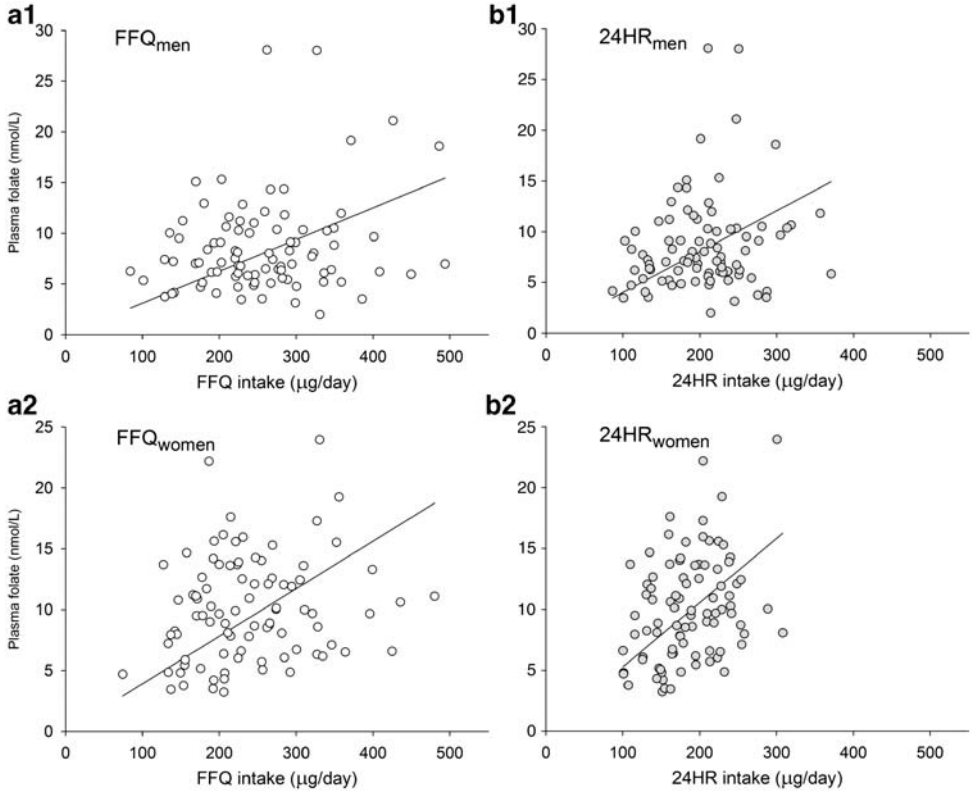

Figure 1 Scatter plot of (**a1**, **a2**) food frequency questionnaire (FFQ) and (**b1**, **b2**) 24 HR estimated intake by plasma concentration in (**a1**, **b1**) men and (**a2**, **b2**) women in the basic validation subgroup.

($P<0.001$); and for vitamin B6: 0.27 ($P<0.001$) and 0.24 ($P<0.001$; Table 3). Median plasma levels of both riboflavin and B6 were significantly higher in the highest versus lowest quintile based on FFQ intake distribution, and continuous increases by quintile groups were observed for riboflavin (both FFQ versions; Table 3).

To evaluate the effect of the *MTHFR* 677C>T polymorphism on the relationships between B vitamin intakes and circulating levels, we calculated correlations stratified by genotype. Among the 843 men genotyped for *MTHFR* 677C>T, the genotype distribution was 51, 39 and 10% for C/C, C/T and T/T, respectively. Mean plasma folate levels were lower in subjects homozygous for the minor T allele (5.8 nmol/l) than in C/T heterozygotes (7.3 nmol/l) or C/C homozygotes (7.4 nmol/l), although the differences were not statistically significant ($P=0.06$). Neither intake of folate nor of any other B vitamin varied by genotype (data not shown). In both the C/C and C/T groups, plasma folate levels correlated significantly with intake ($r_{Spearman}=0.30$–0.38, $P<0.001$), whereas intake and plasma levels were not correlated in the homozygous mutant T/T genotype group ($r_{Spearman}\leqslant 0.15$ and not statistically significant) (Table 4). The results were similar for both longer and shorter version of the FFQ (Table 4).

Discussion

The FFQs are widely used in large-scale studies on diet and disease, though there are arguments both for and against their use (Kristal and Potter, 2006; Willett and Hu, 2007). The advantages of FFQs include measuring 'usual' food intake over an extended period at comparably low costs, allowing for large study samples and repeated measurements. The FFQs are also less demanding for respondents than methods relying on interviews, which favor response rates. Disadvantages are limitations by restrictions in the food items and frequency alternatives provided, and that cognitive abilities may influence outcome more when self-administered FFQs are used (Marks *et al*., 2005). The FFQ data should therefore be validated and calibrated against true gold standard measures. However, none of the commonly used methods for validation/calibration, that is, other dietary recording methods or the use of biomarkers, provides a gold standard. Other dietary recording methods are deficient in that several sources of error correlate with those for FFQs. Biomarkers are prone to different sources of error than FFQs, such as sample handling, biochemical analysis, metabolism and variation over time.

In this study, we compared B vitamin intakes by FFQ with both 24HR-estimated intakes and plasma levels. Plasma levels reflect intake over the previous days to months. Erythrocyte folate levels would theoretically reflect a mean folate exposure over the previous 120 days (normal lifespan of erythrocytes). However, a greater number of manual laboratory procedures, and calculations to compensate for dilution, plasma folate levels and, if available, hematocrite (not available in our cohort) result in a higher analytical imprecision than for plasma folate. Plasma folate levels correlate well with total homocysteine levels (coefficient −0.47, $P<0.001$; in this study), and have also been reported to be sufficiently representative of long-term status in large epidemiological studies (Drogan *et al*., 2004). Still, repeated plasma sampling might have improved the correlations.

Overall, the results of this study are in accordance with other FFQ validation studies. Several FFQ validation studies report correlation coefficients between intakes estimated by FFQ and food dairies or records to be above 0.5 for folate, and around 0.3 for vitamin B12 (Erkkola *et al*., 2001; French *et al*., 2001; Flood *et al*., 2004), whereas correlation coefficients of approximately 0.25 for folate and similar or lower for vitamin B12 are noted for FFQ-based intakes compared with circulating levels (Brantsaeter *et al*., 2007, 2008). The lower correlations for vitamin B12 were therefore not unique to our study, and may represent inter-individual differences in absorption, due to differences in gastric acidity or intrinsic factor secretion. Fewer studies report on riboflavin and B6 in adults in Western countries. Willett *et al*. (1985) and Erkkola *et al*. (2001) noted correlation coefficients between FFQ and food record intakes of approximately 0.6 for vitamin B6 and slightly lower for riboflavin in women, which is similar to our data.

In this study, repeated 24HR were used as the reference method, with amounts eaten estimated with the aid of pictures. Due to similarities in possible sources of measurement errors for the selected reference method and the test method, such as cognitive abilities, type of illustrations used for portion estimation and willingness to report true intakes, the test and reference method were affected by similar measurement errors. However, the use of a less demanding method, that is, compared with a weighted food record, may have contributed to the high rate of compliance (97% of those who consented to participate completed all 10 interviews), and thus low selection bias, in our study. For example, all education levels and both rural and urban areas were represented. Seasonal fluctuation in B vitamin intake is not likely to have affected results, as FFQ was designed to reflect intake over the year before survey, and the 10 24HRs were approximately equally dispersed over the year.

The addition of intake from supplements generally increases the correlation between recorded intakes and biomarkers. Notably, none of the participants in the basic validation subgroup reported intake of multivitamins in the FFQ or any of the 10 24HRs, whereas 14.5% of the prostate study group reported use of multivitamins during the year or 14 days before survey. This may reflect a trend of increasing dietary supplement use in northern Sweden, as has been reported for the period 1981–1997 in a study from middle Sweden (Messerer *et al*., 2001), as the prostate study group represented a time period stretching several years after that of the basic validation study group. We did not account for the higher bioavailability of supplemental folic acid compared with natural folate. However, the difference in bioavailability may be considerably lower than previously

appreciated (Winkels *et al*., 2007). Furthermore, recalculating folate intakes according to Suitor and Bailey (2000), that is, including naturally occurring folate plus 1.7 times the amount of supplementary folic acid, only affected Spearman correlation coefficients at the third decimal (data not shown).

A matter of concern was the introduction of shortened version of the Northern Sweden FFQ in 1994. None of the participants in the basic validation subgroup, but 341 men (39%) in the prostate cancer subgroup, had completed the shorter version of the FFQ. As expected, the shorter version yielded a slightly lower intake of energy and B vitamins, but the correlations between intakes and plasma levels were very similar for the two versions, as were trends in quintiles, and relations to the genotype groups. It was therefore concluded that the validity to rank subjects regarding folate and B vitamin intake is similar for both the longer and shorter versions of the Northern Sweden FFQ.

All individuals in the prostate study group with the rare T/T genotype of the *MTHFR* 677C>T polymorphism had low folate plasma levels (<10 nM) regardless of intake. The T/T homozygosity tends to be associated with lower plasma folate levels, although increased folate consumption can stabilize the enzyme and increase circulating folate levels (Guenther *et al*., 1999; Casas *et al*., 2005). The fortification of foods is not mandatory in Sweden and voluntary fortification is rare. The consumption of vegetables and fruits in this region is low (Agudo *et al*., 2002), and, accordingly, both intake and plasma folate levels in this study were also low. Our findings may therefore indicate that the impact of the *MTHFR* 677C>T SNP on the relationship between intake and circulating levels of folate varies between populations, depending on total exposure. If folate intake is used to represent biological folate status, then T/T subjects in high quintiles of intake may risk misclassification in studies from populations with low folate intakes.

We conclude that the Northern Sweden FFQ is valid for ranking individuals by dietary intake of folate and vitamin B6, but to a lesser extent vitamin B12.

Conflict of interest

The authors declare no conflict of interest.

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