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Validation of a short food frequency questionnaire to assess folate intake

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A short quantitative food frequency questionnaire (FFQ) to assess folate intake was developed and validated against a 7-d weighed food intake record (7d-WR) and biochemical indices of folate status. Thirty-six men and women completed the self-administered FFQ on two occasions a month apart, kept a 7d-WR and gave two fasting blood samples at the beginning and end of the study for measuring serum and erythrocyte folate, respectively. Mean folate intakes were similar by repeat FFQ and correlated strongly (r 0·77 and r 0·72, $P<0·001$, for men and women, respectively). All other comparisons were done using the results of the FFQ administered on the first occasion. Men reported similar folate intakes on the FFQ and 7d-WR, but women reported greater intakes on the FFQ compared with the 7d-WR ($P<0·05$). There was a statistically significant correlation (partial, controlling for gender) between folate intakes reported by FFQ and 7d-WR (r 0·53, $P<0·01$). Folate intakes estimated by FFQ correlated significantly with serum (r 0·47, $P<0·01$), but not erythrocyte folate (r 0·25, $P>0·05$); the strength of the association was greater in men than in women. Validity coefficients estimated using the method of triads were higher for the FFQ than for the 7d-WR when serum folate was used as the biomarker. Overall, these results suggest that this short FFQ is a useful method for assessing folate intake, particularly in men.

Folate: Food frequency questionnaire: Validation: Biomarkers

Folate is required for DNA synthesis and for single carbon transfer reactions. Deficiency results in megaloblastic anaemia and intestinal villous atrophy. Low folate status in women is also associated with an increased risk of neural tube defects in the absence of megaloblastic anaemia and supplements of folic acid decrease the risk of neural tube defects (Department of Health, 2000). Low folate status may be related to an increased risk of CHD. Elevated plasma levels of homocysteine, which increase when dietary folate intake is low and are lowered by folic acid supplements, are associated with an increased risk of CHD (Boushey *et al.* 1995). Low dietary intakes of folate may also be implicated in the causation of cancer (Kim, 1999). Consequently, there is a need to develop methods to assess habitual folate intake reliably.

The problems associated with the assessment of habitual food intake are well documented (Bingham *et al.* 1994). Weighed food records require high subject motivation, training and lengthy data handling, while diet histories and 24-h recalls are prone to interviewer bias, need skilled interviewers and can be costly for large studies. Food frequency questionnaires (FFQ) are easy to administer and inexpensive to process, and they have become the preferred method of dietary assessment in many epidemiological studies. Folate intake assessed by either FFQ or food records have been shown to predict serum folate (Garry *et al.* 1984; Jacques *et al.* 1993; Selhub *et al.* 1993; Green *et al.* 1998) or erythrocyte folate (Giovannucci *et al.* 1993; Green *et al.* 1998). However, FFQ that attempt to cover all foodstuffs can be rather long, leading to loss of subject motivation and decreased reliability. On the other hand, shortening the FFQ by grouping foods together can lead to a loss of detailed information. Where only one nutrient is being measured it is possible to construct an effective FFQ that concentrates on the major sources of that nutrient. This approach is especially appropriate where the dietary sources of the nutrient are limited and has been successfully used to assess Ca intake in the elderly (Nelson *et al.* 1989).

Many FFQ are limited by the lack of quantitative information on portion sizes. This can be overcome by administering the FFQ in conjunction with colour photographs of food portion sizes such as *A Photographic Atlas of Food Portion Sizes* (Nelson *et al.* 1997). This atlas comprises a

Abbreviations: 7d-WR, 7-d weighed food intake record; FFQ, food frequency questionnaire.

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series of eight photographs for seventy-eight foods plus a selection of household measures, tin sizes and foods presented in different ways. Previous studies have shown that the use of a series of photographs is associated with smaller errors in portion size perception than when using single average photographs or no photographs at all (Nelson *et al.* 1994).

For the present study a short FFQ was developed to record the frequency of consumption of major sources of folate. It was designed to be administered in conjunction with the *Photographic Atlas of Food Portion Sizes*. Its reproducibility was tested by administering it on two occasions 4 weeks apart. Its relative validity was measured by comparing results with those obtained from a 7-d weighed food intake record (7d-WR) and biochemical indices of folate status (serum and erythrocyte folate concentrations). A power calculation showed that at least twenty-nine subjects would be needed to give 80 % power to detect a correlation coefficient between FFQ and 7d-WR of 0·5 as significant at the 5 % level.

Methods

Food frequency questionnaire design

Major dietary sources of folate intake in the UK population were identified through an analysis of *The Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990). Subjects in the survey were stratified into fifths of folate intake and individual foods that contributed more than 10 % of the folate intake among subjects in the top fifth were selected for inclusion in the FFQ. The main sources of folate in the UK diet were fortified breakfast cereals, bread, liver, potatoes, dark green vegetables, orange juice and yeast extract. The final FFQ comprised forty-one food groups including ninety individual food items. The FFQ was designed on the basis of these findings and other known rich sources of folate derived from food table information.

The instructions for completing the FFQ required subjects to describe their eating habits over the past year by ticking one of ten columns for frequency of consumption of each food item. The columns included 'never eaten' plus a range of frequencies from 'once per month or less' to '7-d per week'. For each item the subjects were then asked to describe the amounts consumed by reference to the *Photographic Atlas of Food Portion Sizes*. Where a food was not depicted in the atlas, subjects were referred to the photographs of foods that had a similar appearance and density to the food in question. The subjects were also prompted to write down weights or volumes of frequently consumed packaged items if they knew them.

Subjects

Thirty-seven men and women aged 22–65 years were recruited from the staff and student population of King's College London and through publicity in sports centres, libraries and other public venues. Subjects were recruited from May to September 1998. Exclusion criteria included pregnancy, the use of drugs known to interfere with folate metabolism and the presence of chronic disease. The study was approved by the Research Ethics Committee at King's College London. Subjects were not told of the precise purpose of the study, although they were given enough details to comply with Ethics Committee requirements.

On their first visit, subjects gave a fasting venous blood sample for the determination of serum folate, their heights and weights were measured and they completed the FFQ. They were then supplied with a food-recording diary and electronic digital scales (Soehlne; Chasmores Ltd, London, UK) and were given instructions on how to weigh and record all food and drink consumed for seven consecutive days. All subjects completed the 7d-WR within 10 d of their first visit and posted it to the department.

Four weeks later, the subjects returned for a second visit at which they completed the FFQ again (to assess reproducibility) and gave a further fasting blood sample for the determination of erythrocyte folate concentrations. Food diaries were also checked for completeness at this time by interviewing subjects. BMR was estimated using Schofield's equations (Schofield, 1985). Thirty-six subjects completed the study.

Dietary assessment

Folate intakes were estimated from the dietary data (both FFQ and 7d-WR) using the IDA package (IDA Publications Ltd, 7 Rennie Court, 20 Stamford Street, London SE1 9LP), which is based on McCance and Widdowson's Food Composition Tables (5th edition) and all available supplements.

Dietary supplement use was also recorded: type of supplement, brand name, frequency of intake. Subjects were asked to bring supplement containers on their second visit, but were only defined as supplement users if their supplement contained folic acid. Folate intake was defined as the sum of food folate and supplement folic acid intake. Average daily intakes of supplemental folic acid were added to both the FFQ and 7d-WR estimates.

Biochemical assessment

Blood samples for serum folate measurement were collected in vacutainers containing no anticoagulant, left to clot for 1 h at room temperature and then centrifuged at 1500 ***g*** for 10 min. Serum was transferred to plastic vials and stored at −80°C until the end of the study, when vials were taken to the Department of Clinical Biochemistry at King's College Hospital for measurement. Blood samples for determining erythrocyte folate concentrations were collected in vacutainers containing EDTA and taken to the Department of Clinical Biochemistry at King's College Hospital within 24 h of collection for folate and full blood count analysis. Both serum and erythrocyte folate were measured using an ion capture assay (Abbott IMx folate assay; Abbott Laboratories, Chicago, IL, USA).

Statistics

Analyses were performed using SPSS (version 8.0 for Windows; SPSS Inc., Chicago, IL, USA). Serum folate, erythrocyte folate and folate intakes assessed by 7d-WR and FFQ were normally distributed. 7d-WR were regarded as under-reported if energy intake:predicted BMR was less than 1·1 (Goldberg *et al.* 1991). Folate intakes estimated by 7d-WR were adjusted for energy intake using the method of residuals (Willett & Stampfer, 1986). This involved computing residuals from a regression model with energy intake as the independent variable and folate intake as the dependent variable and adding the residuals to the mean folate intake for the group. For the FFQ data, it was not possible to exclude under-reporters or adjust folate values for energy intake because the FFQ listed only foods rich in folate, excluding energy-rich food sources such as fats.

Reproducibility of the FFQ was assessed from the results of the FFQ administered on two occasions using the paired *t* test, Pearson's correlation coefficient and the ability to classify subjects into the same third of the distribution.

All other comparisons were done using the results of the FFQ administered on the first occasion. Validity of the FFQ was assessed by comparing results from the FFQ with the 7d-WR using a paired *t* test and classification into thirds, and by calculating correlation coefficients between FFQ and 7d-WR folate intakes and biochemical indices. Repeated measures ANOVA was used to determine the effect of gender on folate intakes reported by FFQ and 7d-WR. Because a significant effect of gender was detected separate correlation coefficients were calculated for each gender, and a partial correlation coefficient after controlling for gender was used to examine the strength of the associations in the group as a whole.

The validity of the three different methods in assessing nutritional status (FFQ, 7d-WR and biochemical indices) was compared using the method of triads (Ocké & Kaaks, 1997), which involves computing a validity coefficient, or correlation of each method with the truth from the correlations between each pair of the three methods.

Results

The characteristics of participants in the study are shown in Table 1. Men and women did not differ significantly with respect to mean age and mean BMI.

Food frequency questionnaire repeatability

The mean estimates of folate intake by repeat FFQ were not significantly different for either men (368 (SD 38) μg/d on the first occasion *v.* 352 (SD 38) μg/d on the second) or women (366 (SD 24) μg/d on the first occasion *v.* 376 (SD 31) μg/d on the second). There was a strong correlation between estimated individual intakes on the two occasions (r 0·77 and r 0·72, $P<0·001$, for men and women, respectively). The FFQ classified twelve men (75 %) and ten women (50 %) in the same thirds on both occasions. There was no misclassification into opposite thirds.

Food frequency questionnaire v. *7-d weighed food intake record*

A comparison of the folate intake estimated by FFQ with the folate intake estimated by 7d-WR revealed a significant gender by method interaction ($P<0·01$). Consequently the data for men and women are reported separately (see Table 2). In men, folate intakes reported by FFQ compared with the 7d-WR were not significantly different, but in women folate intakes were greater on the FFQ than on the 7d-WR ($P<0·05$), even after excluding subjects who under-reported on the 7d-WR. For men, the correlation coefficient between the two methods was statistically significant whereas for women it was not (Fig. 1). Excluding

Table 1. Characteristics of study participants

	Men		Women	
	n	%	*n*	%
Gender	16	44	20	56
Ethnicity				
Caucasian	15	94	18	90
Asian	0	0	2	10
Afro-Caribbean	1	6	0	0
Mean age (years)	36		37	
Range	26–64		22–65	
Smoking				
Current	0	0	3	15
Past	2	6	0	0
Use of folate-containing supplements	3	19	1	5
Mean weight (kg)	76·7		59·9	
Range	63·0–88·8		50·0–86·4	
Mean height (m)	1·78		1·62	
Range	1·66–1·90		1·52–1·78	
Mean BMI (kg/m^2)	24·2		22·8	
Range	21·0–29·8		18·0–32·1	

Table 2. Estimates of folate intake by 7-d weighed food intake record (7d-WR) and food frequency questionnaire (FFQ) in thirty-six volunteers*

(Mean values, standard errors of measurement and ranges)

		Folate intake (μg/d)			
		All subjects		Excluding under-reporters†	
		7d-WR	FFQ	7d-WR	FFQ
All subjects	Mean	316	366‡	338	373‡
(*n* 36)	SEM	17	21	19	25
	Range	142–577	173–645	161–577	173–645
Men	Mean	371	368	392	390
(*n* 16)	SEM	26	38	25	40
	Range	183–577	176–645	257–577	176–645
Women	Mean	272	366‡	289	357‡
(*n* 20)	SEM	18	24	20	32
	Range	142–422	173–542	161–422	173–542

* For details of volunteers and procedures, see Table 1 and p. 384.
† Under-reporters were individuals whose energy intake (estimated by 7d-WR):BMR was less than 1·1 (two men and five women).
‡ Mean values were significantly different from the corresponding 7d-WR values ($P<0·05$).

under-reporters did not affect the statistical significance of the correlation coefficients. The partial correlation coefficient (controlling for gender) for the two methods of estimating folate intake was r 0·53 ($P=0·001$). The FFQ classified eleven men (69 %) and nine women (45 %) in the same thirds as the 7d-WR, and only one subject in each group (6 % of men and 5 % of women) was misclassified into opposite thirds (see Fig. 2).

Table 3 shows the average amounts of folate contributed by each of the main food groups, as measured by the two methods. Vegetables were the largest source of folate in the diet followed by cereals (bread and breakfast cereals). Beverages, particularly beer, made a significant contribution to intake in men. In women the difference in estimated intake between the two methods was almost entirely due to a difference in the estimated consumption of vegetables, with habitual intake estimated by the FFQ amounting to twice as much as was actually recorded by the 7d-WR. There was also a considerable difference in the estimates of vegetable intake in the men, but this was offset by opposite differences in the estimated consumption of beverages and breakfast cereals.

Dietary methods v. *biochemical indices*

Serum and erythrocyte folate concentrations were correlated (r 0·41, $P=0·01$), as has been found in other studies

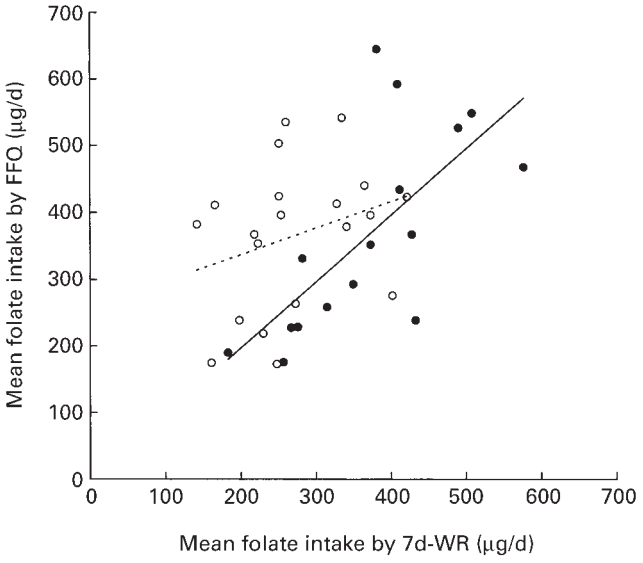

Fig. 1. Correlations of mean folate intake in sixteen men (●, —) and twenty women (○, - - -) estimated by food frequency questionnaire (FFQ) and 7-d weighed food intake record (7d-WR). For men, the correlation coefficient between the two methods was significant (r 0·69, $P=0·003$); for women it was not (r 0·30, $P=0·21$).

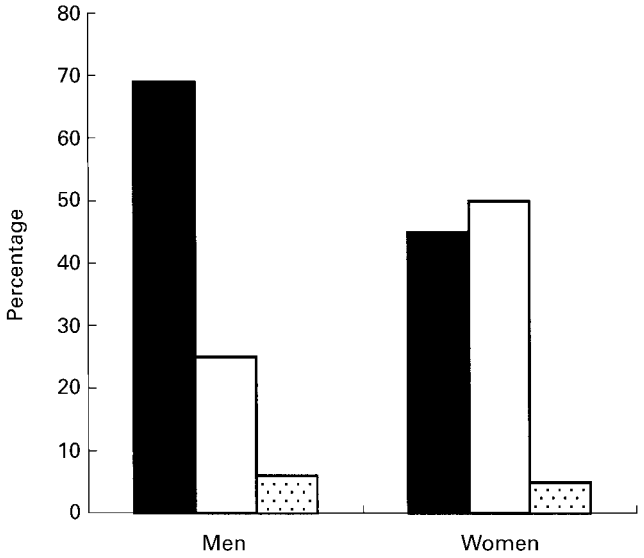

Fig. 2. Classification of sixteen men and twenty women into thirds according to folate intake by food frequency questionnaire (FFQ) and 7-d weighed food intake record (7d-WR). (■), Same thirds; (□), adjacent thirds; (▦), opposite thirds.

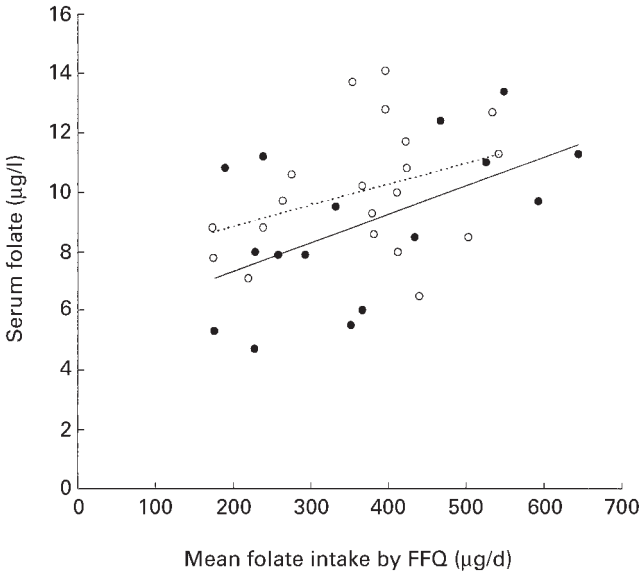

Fig. 3. Correlations of mean daily folate intake in sixteen men (●, —) and twenty women (○, - - -) estimated by food frequency questionnaire (FFQ) *v.* serum folate concentration. For men, the correlation coefficient was significant (r 0·55, P=0·03); for women it was not (r 0·36, P=0·12).

(Phekoo *et al.* 1997). In the whole group, partial correlation coefficients (controlling for gender) between folate intake estimated by 7d-WR and both biomarkers were significant, although the correlation with erythrocyte folate became non-significant after adjusting the 7d-WR values for energy intake (Table 4). Folate intakes estimated by FFQ were significantly correlated with serum but not with erythrocyte folate. When supplement users were excluded from the analysis, correlation coefficients decreased but remained significant for FFQ and 7d-WR (adjusted for energy intake) and serum folate.

In men, there were statistically significant correlations between folate intake estimated by both dietary methods and serum folate (Fig. 3). When 7d-WR folate values were adjusted for energy intake the correlation coefficient increased, but when the three supplement users were excluded the correlation coefficients for both dietary methods became non-significant. In women, the correlation coefficients between the two dietary methods and serum folate were non-significant. When erythrocyte folate was used as the biomarker the correlation coefficients were weaker than those for serum folate, especially in women.

The method of triads was used to calculate validity coefficients of the two measures of folate intake with each of the two biomarkers in turn. For both men and women, validity coefficients (ρ) for the FFQ were higher than for the 7d-WR when serum folate was the biomarker (FFQ: $\rho = 0{\cdot}85$ for men and $\rho = 0{\cdot}69$ for women; 7d-WR: $\rho = 0{\cdot}81$ for men and $\rho = 0{\cdot}44$ for women) but lower when erythrocyte folate was the biomarker (FFQ: $\rho = 0{\cdot}69$ for men and ($\rho = 0{\cdot}41$ for women; 7d-WR: $\rho = 1{\cdot}00$ for men and $\rho = 0{\cdot}72$ for women). Generally, the validity coefficients for the two dietary methods were higher than for either of the biomarkers.

Discussion

The purpose of the present study was to compare folate intake estimated by a short quantitative FFQ with intake assessed by a 7d-WR in a group of healthy men and women. The results indicate that folate intake estimated from the FFQ was highly reproducible in both men and women, and was in good agreement with folate intake assessed by 7d-WR. When data were analysed separately

Table 3. Average contribution to folate intake (μg/d) by various food groups in sixteen men and twenty women assessed by 7-d weighed food record (7d-WR) and food frequency questionnaire (FFQ)*

(Mean values and percentages of the total)

	7d-WR				FFQ			
	Men		Women		Men		Women	
Food groups	Mean	% of total	Mean	% of total	Mean	% of total	Mean	% of total
Vegetables	86	23	75	26	138	36	157	40
Bread	58	16	41	14	63	16	48	12
Breakfast cereals	46	12	44	15	30	8	45	11
Beverages (fruit juice, beers and lagers)	89	24	44	15	62	16	47	12
Dairy products	33	9	31	11	40	10	39	10
Meat and fish	16	4	10	3	12	3	7	2
Fruit	12	3	21	7	13	3	28	7
Cakes and fruitbreads	13	3	8	3	6	2	2	1
Rice and pasta	8	2	3	1	9	2	5	1
Snacks (biscuits, nuts and other savoury snacks)	8	2	7	2	4	1	5	1
High folate foods (offal, Bovril and Marmite)	4	1	5	2	7	2	9	2
Total folate intake from food	297	80	230	80	331	86	329	84
Total folic acid intake from fortified foods and supplements	75	20	59	20	52	14	64	16

* For details of volunteers and procedures, see Table 1 and p. 384.

Table 4. Correlations between folate intakes estimated by 7-d weighed food intake record (7d-WR) or food frequency questionnaire (FFQ) and biochemical indices of folate status in thirty-six men and women and after excluding supplement users*

	n		FFQ	7d-WR	
				Unadjusted for total energy intake	Adjusted for total energy intake
All subjects†	36	Serum folate	0·47‡‡	0·39‡	0·46‡‡
		Erythrocyte folate	0·25	0·38‡	0·27
Excluding supplement users†	32	Serum folate	0·42‡	0·27	0·38‡
		Erythrocyte folate	0·20	0·11	0·17
Men	16	Serum folate	0·55‡	0·52‡	0·64‡‡
		Erythrocyte folate	0·36	0·52‡	0·41
Excluding supplement users	13	Serum folate	0·44	0·34	0·44
		Erythrocyte folate	0·34	0·06	0·39
Women	20	Serum folate	0·36	0·23	0·26
		Erythrocyte folate	0·08	0·14	0·13
Excluding supplement users	19	Serum folate	0·39	0·27	0·37
		Erythrocyte folate	0·08	0·15	0·15

* For details of volunteers and procedures, see Table 1 and p. 384.
† Partial correlation coefficients controlling for gender.
‡$P<0·05$, ‡‡$P<0·01$.

by gender, agreement between the two dietary methods was stronger in men than in women. The FFQ gave higher values than the 7d-WR in women, and this appeared to be largely due to a discrepancy in the estimated consumption of vegetables. It is well documented that FFQ tend to overestimate both macro- and micronutrient intake (Margetts & Nelson, 1997), including folate (Thompson & Margetts, 1993; Robinson *et al.* 1996; Friis *et al.* 1997; Riboli *et al.* 1997). Fruit and vegetables are most often over-reported in FFQ (Salvini *et al.* 1989; Feskanich *et al.* 1993), perhaps partly as a result of social desirability or approval, which has been shown to bias self-reported dietary intake (Hebert *et al.* 1995). Such biases are more likely to occur in FFQ, which are easier to manipulate than weighed food records. It is also possible that the long list of vegetables in the FFQ contributed to the discrepancy in intake between the two methods, since estimates of fruit and vegetable intake have been shown to be related to the number of questions asked (Krebs-Smith *et al.* 1995). One way of minimising this problem would be to arrange the list of fruits and vegetables into a smaller number of groups in the FFQ. On the other hand it is known that women tend to under-report weighed food records (Martin *et al.* 1996). Although we were able to exclude data from subjects who had clearly grossly under-reported their 7d-WR, it is quite possible that some of the remaining 7d-WR values were also underestimates, and that this also contributed to the discrepancy between FFQ and 7d-WR results.

We used serum and erythrocyte folate concentrations to validate the dietary estimates of folate intake. Folate intake estimated by FFQ correlated significantly with serum folate concentration. For erythrocyte folate concentration there did appear to be a positive relationship but the correlation coefficient was not statistically significant. This may have been because erythrocyte folate is a less sensitive biomarker of intake than serum folate. Serum folate is thought to reflect recent folate intake while erythrocyte folate reflects long-term intake (Bailey, 1990). Additionally, although the FFQ asked about consumption over the previous 12 months, it may in reality have reflected more recent consumption. Supplement use may be partially responsible for the higher correlation coefficients observed in men, because it extends the range of folate intake and is easier to quantify, allowing subjects to be classified more accurately according to intake.

Folic acid, whether from supplements or from fortified foods is almost entirely bioavailable, compared with the 50 % estimated bioavailability of dietary folates (Sauberlich *et al.* 1987; Cuskelly *et al.* 1996; Pfeiffer *et al.* 1997). In the present study, bioavailability from different food sources was not taken into account. Further revisions to the nutrient database are clearly necessary as fortification of food becomes more widespread.

The validity coefficient of the FFQ was determined using the method of triads (Ocké & Kaaks, 1997). Using the data for FFQ, 7d-WR and serum folate we found that the validity coefficient of the FFQ was the highest of all three measures of folate status in both men and women. It should be noted that the method of triads is based on an assumption that the errors associated with each of the three methods of assessing nutritional status are independent. This may not be entirely valid as although the errors associated with biochemical indices are likely to be independent of those associated with dietary measures, the FFQ and the 7d-WR may have some sources of error in common.

In the present study, vegetables were the major source of folate in the diet. As there is some seasonal variation in the intake of vegetables it may be necessary to obtain estimates of intake throughout the year in order to obtain a true estimate of habitual intake. Breakfast cereals also made a significant contribution to intake and since conducting the present study a large number of breakfast cereals have been fortified with folic acid. Consequently, it is necessary to obtain information on brands of breakfast cereals used when assessing folate intake. Of course, both these points apply equally to the FFQ and the 7d-WR method. Other

limitations of the present study include the relatively small sample size and heterogeneity of the population, particularly with respect to age, BMI and supplement use.

In conclusion, the study suggests that this short FFQ is a useful method for assessing folate intake, particularly in men. It correlated better with biochemical methods in men than in women and it performed better than the weighed record in many cases. Given the increasing recognition of the role of folate in several diseases, it should provide a useful tool for assessing folate intake in future studies.

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