Use of biological markers to validate self-reported dietary intake in a random sample of the European Prospective Investigation into Cancer United Kingdom Norfolk cohort[1–3]

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ABSTRACT

Background: The validity of dietary assessment methods should be established before diet-disease associations are reported.

Objective: Our objective was to validate a 7-d food diary and a food-frequency questionnaire (FFQ) against independent biomarkers of intake in urine (nitrogen, potassium, and sodium) and blood (plasma ascorbic acid).

Design: A total of 146 healthy middle-aged men and women were recruited from the European Prospective Investigation into Cancer UK Norfolk cohort, a free-living cohort of ≈25 000 persons. Over a 9-mo period, urinary nitrogen, potassium, and sodium were estimated from 2–6 complete 24-h urine collections in 134 subjects and plasma ascorbic acid was estimated from 2–3 fasting blood samples in 118 subjects. Subjects completed 2 FFQs and two 7-d food diaries.

Results: In men and women combined, correlations between 24-h urinary nitrogen excretion and dietary intake from the 7-d food diary were high (r = 0.57–0.67) compared with those for the FFQ (r = 0.21–0.29). Similarly, correlations between urinary potassium and dietary potassium were higher for the 7-d food diary (r = 0.51–0.55) than for the FFQ (r = 0.32–0.34). There was no overall difference in correlations between plasma ascorbic acid and dietary vitamin C between the 7-d food diary (r = 0.40–0.52) and the FFQ (r = 0.44–0.45).

Conclusions: These data indicate that, despite increased subject burden, the 7-d food diary provided a better estimate of nitrogen and potassium intakes than did the FFQ in this study population. However, with respect to plasma ascorbic acid, both the FFQ and 7-d food diary provided a similar ranking of subjects according to vitamin C intake. *Am J Clin Nutr* 2001;74:188–96.

KEY WORDS Validity, biomarkers, urinary nitrogen, urinary potassium, food-frequency questionnaires, 7-d food diaries, reproducibility, FFQ, plasma ascorbic acid, urinary sodium, European Prospective Investigation into Cancer

INTRODUCTION

In epidemiologic studies, self-reported dietary intake is often used in establishing diet-disease associations. To date, the food-frequency questionnaire (FFQ) has been the dietary assessment method used most frequently in large-scale studies, primarily because it is easy to administer, is less expensive than other dietary assessment methods, and provides a rapid estimate of usual intake (1). Often the accuracy of an FFQ is evaluated by comparing its performance with more intensive recording reference methods, such as weighed-food records, food diaries, or repeat 24-h recalls (2–9). The correlation coefficients derived from such relative validity studies may be biased and, in the absence of information on true dietary intake, the magnitude of this bias cannot be evaluated (10).

Biomarkers do not rely on self-reports of food intake and thus random measurement errors of the biomarker are not likely to be correlated with those of the dietary assessment method (10). The underlying assumption of a biomarker applied to validate a measure of intake is that it responds to intake dose-dependently (1). Both urinary nitrogen and potassium can be translated into absolute estimates of nitrogen and potassium intake on the basis of several complete 24-h urine collections. However, relatively few methodologic studies have used these biomarkers to evaluate the validity of a dietary instrument (11–16). Circulating concentrations of nutrients in blood, such as plasma ascorbic acid, represent another class of biomarkers used to evaluate the performance of a dietary assessment method (17). Because these biomarkers are influenced by intervening factors such as smoking status and use of supplements, they do not reflect absolute dietary intakes (18–21). Biomarkers belonging to this category can be used only to interpret the lower limit of the true validity of the dietary assessment method (22).

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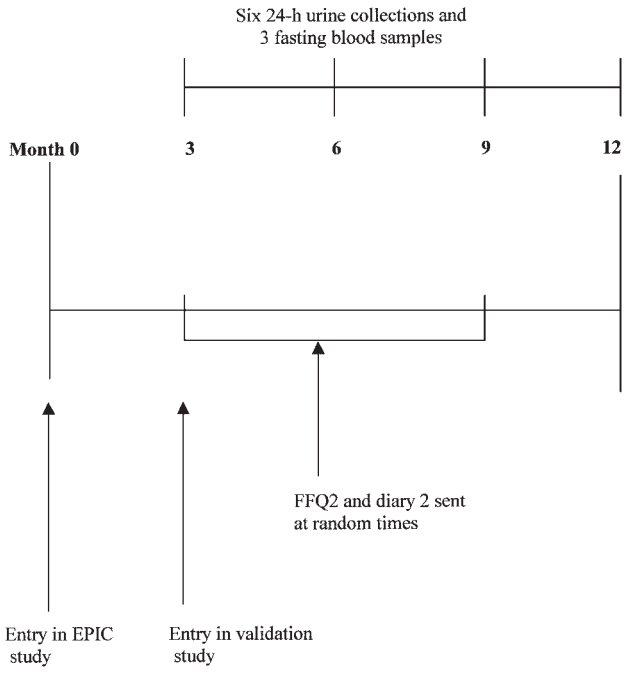

FIGURE 1. The timing of the validation study protocol in relation to the main European Prospective Investigation into Cancer (EPIC) cohort. FFQ, food-frequency questionnaire.

Our validation study was conducted during the collection of dietary information for the European Prospective Investigation into Cancer (EPIC) UK Norfolk cohort. Before starting the EPIC-Norfolk cohort, the validity of several dietary assessment methods was evaluated by comparison with biomarkers in a Cambridgeshire population (13). However, since then, the dietary assessment methods were modified and so ideally their validity should be assessed in a representative subgroup of the cohort in which the methods are being administered (23). The purpose of our study was to evaluate the performance of the dietary assessment methods being used in EPIC in terms of their reproducibility and validity in comparison with independent biomarkers in urine (nitrogen, potassium, and sodium) and blood (plasma ascorbic acid).

SUBJECTS AND METHODS

Subjects

The validation subjects were recruited from the Norfolk component of the EPIC study, a multicenter, prospective cohort study designed to examine the relation between diet and cancer (24). Healthy men and women aged 45–74 y who had participated in the main EPIC study were invited to take part in a more detailed study of diet. As part of the main EPIC study, participants provided a nonfasting blood sample and completed a detailed health and lifestyle questionnaire and 3 dietary assessment methods: a self-reported 24-h recall, an FFQ (FFQ1), and a 7-d food diary (diary 1). The validity of the 24-h recall is not addressed in this study. The design of the study is summarized in **Figure 1**.

Of the 276 EPIC participants approached to take part in the validation study (114 men and 162 women), 195 (70%) agreed to participate (84 men and 111 women). Of these, 146 (75%) completed all dietary assessment methods and 134 (69%) provided repeat urine and blood samples. Characteristics of the subjects participating in the validation study were compared with those from a random sample of 204 men and women taken from the first 2000 subjects recruited into the main EPIC-Norfolk cohort. The Norwich District Ethics Committee gave ethical permission for the main EPIC study in 1992 and for the subsequent study reported here in 1996.

Study design

Each participant was visited at home on 3 occasions over 9 mo. During each visit, subjects were asked to provide two 24-h urine collections and a fasting blood sample. Each subject was weighed on a spring balance, without shoes and in light clothing. Height and weight were used to calculate body mass index [weight (kg)/height (m^2)]. The participants were informed that they would receive a second 7-d food diary (diary 2), an FFQ (FFQ2), and a 24-h recall in the mail during the course of the validation study. The FFQ2 and 24-h recall were sent together and diary 2 was sent separately. The dietary assessment methods were sent at random times by mail and not in concordance with the collection of the biomarkers. Because of the randomization by which the dietary assessment methods were sent to the participants, the time frame covered by the second FFQ included the second food diary for some but not all participants.

Dietary assessment

Seven-day food diary

The participants were asked to record, in as much detail as possible, all food and beverages consumed over a 7-d period. The 7-d food diary included color photographs of 17 foods, each with 3 different portion sizes. Participants could choose which photographs represented their portion size or could indicate whether they consumed more or less than the amount shown in the photograph. Participants could also describe their portion size in other measures when appropriate, such as weights or household units; detailed instruction on food quantification and description was given at the front of the food diary.

Food-frequency questionnaire

The self-administered FFQ was designed to measure an individual's habitual food and nutrient intake during the past year. The questionnaire was a modified version of the FFQ used in the US Nurses' Health Study (25, 26), with a food list that was adapted to include foods commonly consumed in the United Kingdom. The FFQ consisted of 130 food items compiled from national dietary intake data. The FFQ in our study was a revised version of the questionnaire previously used in validation studies (5, 13). The main change between the previous version of the FFQ (5) and the one used in our study was the inclusion of separate questions regarding type and amount of milk, type of fat used for cooking, brand name of breakfast cereals eaten, cooking method of meat, the amount of salt used in cooking and at the table, and the use of vitamin supplements.

For each food item, participants were asked to indicate their usual consumption from 9 frequency categories, ranging from never or <1 time per month to ≥6 times per day. The FFQ did not include specific questions on portion size but rather specified medium servings, defined by natural (eg, apple, slice of

bread) or household units (eg, glass, cup, or spoon). Blank spaces were available for recording any foods that were consumed more than once a week but were not listed as a food item on the questionnaire. For seasonal fruits, such as strawberries, the participants were asked to estimate their average intake (frequency) when the fruit was in season. The gram weights of medium servings were obtained from estimates of mean values derived from the previous validation study (5) and from other published values (27). An FFQ was considered incomplete if the frequency of ≥10 food items was missing and was therefore excluded from further dietary analysis. In addition, an FFQ was excluded if a subject's nutrient intake was <2.51 MJ/d (600 kcal/d) or >16.74 MJ/d (4000 kcal/d). On the basis of these exclusion criteria, one FFQ2 was removed for one woman who reported consuming >16.74 MJ/d.

Nutrient database

Two computer programs were specifically developed for analyzing the dietary information collected in the EPIC-Norfolk cohort. The food databases associated with each program were based on published food-composition tables (28), which are frequently updated with nutrient data on new food items (29–37). Nutrient intakes for the FFQ were calculated by multiplying the frequency of consumption of food items by standard portion weights to derive a total estimate of grams of food consumed per day (5). The food items (g/d) were subsequently converted into nutrients by using the appropriate food table codes. Dietary intakes recorded in the 7-d food diaries were analyzed by using the program DINER (Diet Into Nutrients for Epidemiologic Research; AA Welch, A McTaggart, AA Mulligan, et al, unpublished observations, 2001).

Twenty-four–hour urine collections

Participants received written and verbal instructions on the technique of collecting 24-h urine samples and on the use of *para*-aminobenzoic acid (PABA) tablets (PABAcheck; Laboratories for Applied Biology, London). On the first morning of the urine collection, participants were asked to discard their first urine specimen and from then on to collect all specimens for the next 24 h, up to and including the first urine specimen of the next day. They were given three 80-mg PABA tablets and instructed to take one at each main meal on the day of the urine collection to verify completeness of the 24-h urine collection. During each collection period, the participants were asked to complete a questionnaire in which they recorded *1*) the time of start and finish of each urine collection, *2*) the time at which the PABA tablets were taken, *3*) any lost specimens, and *4*) any medication or supplements taken during the urine collection. The participants were asked to eat and drink as they would under normal living conditions during the days of urine collection. All 24-h urine samples were mixed and the volume was determined before the total sample was divided into two 25-mL glass bottles. All urine collections were divided within 1 d of the participants' completing the collection. Samples were stored until further analysis at −20°C.

Biomarkers in urine

PABA concentrations in urine were measured in triplicate by the colorimetric technique as described by Bingham and Cummings (38). Urine collections with <85% PABA recovery were considered incomplete and removed from further analyses (38). Total urinary nitrogen was measured by the Kjeldahl technique (Tecator 1015 digestor; Foss, United Kingdom; and Kjeltec 1035 analyser; Didcot, United Kingdom). Urinary potassium and sodium were measured by flame photometry (IL 943; Instrumentation Lab, Warrington, United Kingdom). Only data from those subjects who provided ≥2 complete 24-h urine collections (n = 134) were used to validate the dietary assessment methods.

Blood collection

A research nurse visited the participants at home within ≈2 wk of the time they provided a 24-h urine collection. A 20-mL blood sample was taken by venipuncture after the subjects had fasted overnight. Blood was drawn into 2 safety-monovette syringes: 10 mL containing lithium heparin for plasma and 10 mL without anticoagulant for serum. Blood samples were taken to the laboratory in an insulated box within 3–4 h of the blood draw and were stored in a refrigerator at 4–7°C until further preparation of the blood. Plasma ascorbic acid was stabilized in a standardized volume of metaphosphoric acid and analyzed by using a fluorometric assay within 1 wk of sampling (39). Only data from participants who provided ≥2 fasting blood samples (n = 118) were used in the reported analyses.

Statistical analysis

Statistical analyses were performed for men and women separately with use of the SAS program (version 6.12; SAS Institute Inc, Cary, NC). Most nutrient distributions were skewed toward higher mean values and all nutrients were $\log_e$ (natural) transformed to improve their distribution toward normality. In the case of no reported alcohol consumption, 1 g was entered before log transforming the data. Mean (±SD) nutrient intakes were calculated for each of the 7-d food diaries (diary 1 and diary 2) and FFQs (FFQ1 and FFQ2). Paired t tests were used to test the differences in reported mean values between dietary assessment methods administered in EPIC and the validation study. Pearson's correlation coefficients were used to test the reproducibility of the FFQs and the relative validity of FFQ2 against mean intake from the two 7-d food diaries. The relative validity of FFQ2 was selected because this questionnaire covered approximately the same time period as that covered by the 2 food diaries. We made 3 comparisons: FFQ1 compared with FFQ2, diary 1 compared with diary 2, and FFQ2 compared with the mean of the 2 food diaries. We therefore made the usual Bonferroni adjustment by multiplying each nominal P value by 3. Statistical significance was assessed after the Bonferroni adjustment. No formal allowance was made for the number of nutrients compared because there is substantial dependence among the comparisons.

Energy-adjusted nutrient intakes were calculated by the regression method, with energy intake as the independent variable and the nutrient intake in question as the dependent variable (40). To correct for the variability in the week-to-week variation in the food diary, correlation coefficients were deattenuated by multiplying them as follows:

$$\{1 + [(\sigma^2_w/\sigma^2_b)/n]\}^{0.5} \quad (1)$$

where n = 2 and (σ^2_w/σ^2_b) is the within-person variance divided by the between-person variance for each nutrient. Furthermore, cross-classification of nutrient scores was estimated by examining the proportion of subjects classified by the diary method that was classified into the same or extreme quartile by the FFQ2.

TABLE 1
Anthropometric characteristics and dietary intakes of the validation study participants compared with a random sample of the main cohort[1]

	Men		Women	
	Validation participants (n = 58)	Random sample (n = 102)	Validation participants (n = 88)	Random sample (n = 102)
Age (y)	60 ± 10	60 ± 9	58 ± 10	60 ± 9
Weight (kg)	83 ± 10	81 ± 11	67 ± 10	69 ± 12
Height (cm)	175 ± 6	174 ± 7	163 ± 7	160 ± 6
BMI (kg/m^2)	27 ± 3	27 ± 3	25 ± 4	27 ± 4[2]
Energy (MJ/d)	9.36 ± 2.74	9.63 ± 2.87	7.78 ± 2.65	7.86 ± 2.14
Protein (g/d)	89 ± 18	85 ± 22	80 ± 24	82 ± 21
Fat (g/d)	87 ± 38	88 ± 36	70 ± 34	69 ± 25
Carbohydrate (g/d)	266 ± 79	288 ± 90	232 ± 76	235 ± 74
NSP (g/d)	18 ± 6	19 ± 6	18 ± 6	19 ± 7
Vitamin C (mg/d)	118 ± 53	109 ± 57	136 ± 57	124 ± 65
Calcium (mg/d)	1108 ± 279	1089 ± 320	988 ± 307	1015 ± 296
Iron (mg/d)	13 ± 4	13 ± 4	12 ± 5	11 ± 5

[1] $\bar{x}$ ± SD. NSP, nonstarch polysaccharides.
[2] Significantly different from validation participants, $P < 0.05$.

The biomarker concentration for each participant was based on the mean of repeat blood or urine samples. The relation between biomarkers and reported intake was expressed as Spearman's correlation coefficients.

RESULTS

Characteristics of the 146 validation participants are compared with those of a random sample of 204 individuals from the main EPIC-Norfolk cohort in **Table 1**. Overall, there were no significant differences between the 2 groups, with the exception that the women who took part in the validation study had a significantly lower body mass index than did the women in the random sample.

The daily mean nutrient intakes assessed by each of the 7-d food diaries and the 2 FFQs, the mean of the 2 food diaries, and the reproducibility of the FFQ are presented for men and women in **Table 2**. Compared with the results for the 7-d food diary administered in the EPIC study (diary 1), results for the repeat 7-d food diary (diary 2) were not significantly different. Intakes from the 2 FFQs were also not significantly different. Compared with the mean of the 2 food dairies, the FFQ2 gave significantly higher estimates of most nutrients, with the exception of starch and alcohol intakes in women and carbohydrate, starch, sodium, iron, and alcohol intakes in men. Of those nutrients, reported intakes of starch, sodium, and iron were significantly lower in the FFQ2 for men and reported alcohol intake was significantly lower for women.

The reproducibility of the FFQ for men and women is also shown in Table 2. The crude correlation between the 2 FFQs ranged from 0.48 for β-carotene to 0.70 for energy in men and from 0.63 for starch to 0.82 for nonstarch polysaccharides in women. The most notable discrepancies in the reproducibility of nutrient intake between men and women, as expressed by the crude correlation coefficient, were for protein (0.57 and 0.70, respectively), β-carotene (0.48 and 0.78), nonstarch polysaccharides (0.58 and 0.82), and potassium (0.60 and 0.76). The mean reproducibility of the FFQ was 0.64 in men and 0.74 in women. Energy adjustment did not consistently affect the magnitude of the correlation coefficient between nutrient intakes in men (mean r = 0.63) or women (mean r = 0.73).

The crude, energy-adjusted, and deattenuated correlation coefficients between the FFQ2 and the mean of the 2 food diaries are shown for men and women in **Table 3**. Crude Pearson's correlation coefficients ranged from 0.30 for β-carotene to 0.59 for fat in men (excluding alcohol, r = 0.88) and from 0.31 for protein to 0.68 for fat in women (excluding alcohol, r = 0.94). The observed mean crude correlation was 0.51 in men and 0.54 in women. The relative validity of the second FFQ was not higher that that of the first FFQ: the mean crude correlation was 0.48 in men and 0.53 in women (data not shown). After energy adjustment, the mean correlation improved to 0.56 in men and 0.62 in women. Correlation coefficients were deattenuated to correct for week-to-week variation in the 7-d food diary. As expected, deattenuation improved the Pearson's correlation coefficient between nutrient intakes estimated by the FFQ2 and the mean of the 2 food diaries. The deattenuated and energy-adjusted correlation coefficients were >0.50 for all nutrients with the exception of protein (0.49), starch (0.44), β-carotene (0.39), and vitamin E (0.46) in men.

Classification of nutrient intake into the same and extreme quartiles of intake, derived from the FFQ2 and the mean of the 2 food diaries, was evaluated for men and women separately. Cross-classification into the same quartile ranged from 28% for β-carotene to 69% for alcohol in men and from 32% for protein to 79% for alcohol in women. Extreme misclassification (>5%) was observed for energy and starch in men and for protein, sugar, sodium, and vitamin E in women.

The number of subjects who provided ≥2 complete urine collections and blood samples and the corresponding mean (±SD) concentration and range for each biochemical measure are shown in **Table 4**. Spearman's correlation coefficients between concentrations of biomarkers in urine and blood and nutrient intakes as derived from each of the dietary assessment methods are shown in **Table 5**. Correlation coefficients between dietary nitrogen and urinary nitrogen were highest for the 7-d food diary (r = 0.67 and 0.57 for diaries 1 and 2, respectively). Correlation coefficients between urinary nitrogen and dietary nitrogen from the FFQ were much lower: 0.29 (FFQ1) and 0.21 (FFQ2). The correlation coefficients between urinary potassium and dietary potassium estimated by the FFQ were also lower

TABLE 2
Energy and nutrient intakes in men and women as estimated from 7-d food diaries and two food-frequency questionnaires (FFQs), along with Pearson's correlation coefficients between the repeat FFQs[1]

Nutrient	Diary 1	Diary 2	Mean of diary 1 and 2	FFQ1	FFQ2	*r*	
						Crude	Adjusted[2]
Men (*n* = 58)							
Energy (MJ)	9.25 ± 1.75	8.89 ± 2.01	9.07 ± 1.72	9.36 ± 2.74	9.10 ± 2.86	0.70	—
Protein (g)	82 ± 14	82 ± 16	82 ± 21	89 ± 18	88 ± 24	0.57	0.62
Carbohydrate (g)	269 ± 63	260 ± 62	265 ± 57	266 ± 79	259 ± 78	0.70	0.71
Fat (g)	83 ± 22	79 ± 24	80 ± 20	87 ± 38	84 ± 39	0.69	0.41
Sugars (g)	118 ± 40	112 ± 38	115 ± 35	137 ± 49	138 ± 43[3]	0.64	0.62
Starch (g)	148 ± 37	146 ± 36	147 ± 34	124 ± 42	115 ± 44[3]	0.69	0.69
NSP (g)	15 ± 5	15 ± 4	15 ± 4	18 ± 6	17 ± 5[4]	0.58	0.67
Potassium (g)	3.41 ± 0.7	3.46 ± 0.7	3.43 ± 0.6	3.98 ± 0.8	3.94 ± 0.8[3]	0.60	0.64
Sodium (g)	3.37 ± 0.8	3.14 ± 0.8	3.26 ± 0.8	3.07 ± 0.8	2.92 ± 0.9[5]	0.65	0.76
Calcium (mg)	931 ± 248	949 ± 253	940 ± 217	1108 ± 279	1071 ± 272[5]	0.64	0.61
Iron (mg)	14 ± 6	13 ± 4	13 ± 4	13 ± 4	12 ± 4[4]	0.69	0.78
β-Carotene (μg)	1880 ± 967	1853 ± 946	1867 ± 822	3056 ± 1259	2780 ± 1170[3]	0.48	0.47
Vitamin C (mg)	81 ± 43	74 ± 36	77 ± 34	118 ± 53	111 ± 41[3]	0.66	0.69
Vitamin E (mg)	8 ± 4	8 ± 4	8 ± 4	12 ± 6	11 ± 7[3]	0.59	0.47
Alcohol (g)	17 ± 18	14 ± 19	15 ± 8	11 ± 13	11 ± 12	0.69	0.71
Women (*n* = 88)							
Energy (MJ)	7.09 ± 1.62	7.11 ± 1.67	7.11 ± 1.54	7.78 ± 2.65	7.74 ± 2.52	0.73	—
Protein (g)	65 ± 14	66 ± 15	66 ± 13	80 ± 24	78 ± 22[3]	0.70	0.68
Carbohydrate (g)	215 ± 55	216 ± 58	215 ± 53	232 ± 76	232 ± 82	0.74	0.75
Fat (g)	63 ± 19	63 ± 20	63 ± 18	70 ± 34	69 ± 30	0.76	0.82
Sugars (g)	103 ± 37	102 ± 37	101 ± 34	123 ± 44	121 ± 47[3]	0.78	0.73
Starch (g)	109 ± 30	111 ± 31	110 ± 28	104 ± 39	106 ± 46	0.63	0.60
NSP (g)	14 ± 5	14 ± 4	14 ± 4	18 ± 6	18 ± 7[3]	0.82	0.85
Potassium (g)	3.01 ± 0.7	3.09 ± 0.8	3.05 ± 0.7	3.75 ± 0.9	3.71 ± 0.9[3]	0.76	0.77
Sodium (g)	2.42 ± 0.6	2.47 ± 0.7	2.45 ± 0.6	2.63 ± 1.0	2.75 ± 1.3	0.68	0.70
Calcium (mg)	785 ± 275	834 ± 297	811 ± 257	988 ± 307	993 ± 339[3]	0.75	0.59
Iron (mg)	11 ± 4	11 ± 5	11 ± 4	12 ± 4	12 ± 5	0.78	0.85
β-Carotene (μg)	2061 ± 1656	2178 ± 1847	2099 ± 1570	2978 ± 1322	2887 ± 1438[3]	0.78	0.75
Vitamin C (mg)	100 ± 55	90 ± 54	95 ± 48	136 ± 57	132 ± 65[3]	0.73	0.70
Vitamin E (mg)	7 ± 3	7 ± 4	7 ± 3	10 ± 6	10 ± 6[3]	0.66	0.68
Alcohol (g)	7 ± 10	7 ± 10	7 ± 10	5 ± 7	5 ± 8[3]	0.79	0.79

[1] $\bar{x}$ ± SD. NSP, nonstarch polysaccharides.
[2] Adjusted for energy intake.
[3–5] Significantly different from the mean of diary 1 and 2 after Bonferroni adjustment for 3 comparisons: [3] $P < 0.001$, [4] $P < 0.05$, [5] $P < 0.01$.

than those for the food diary in men (r = 0.24 and 0.26) and women (r = 0.28 and 0.29). The results were less consistent between dietary sodium and urinary sodium and correlations varied considerably for the same method. For instance, diary 2 was moderately correlated with sodium intake in men (r = 0.43) but not in women (r = 0.11).

The crude Spearman's correlation coefficients between plasma ascorbic acid and vitamin C intake are also shown in Table 5. For both the FFQ and 7-d food diary, higher correlations were observed in women than in men. In women, correlations were slightly higher for the 7-d food diaries (r = 0.56 and 0.48 for diaries 1 and 2, respectively) than for the FFQ (r = 0.41 and 0.39 for FFQ1 and FFQ2, respectively). In men, the magnitude of the correlation coefficient between plasma ascorbic acid and vitamin C intake was similar for the FFQs (r = 0.32 and 0.34 for FFQ1 and FFQ2, respectively) and diaries 1 and 2 (r = 0.42 and r = 0.35, respectively). Overall, exclusion of supplement users had little effect on the relation between plasma ascorbic acid and vitamin C intake.

DISCUSSION

In this validation study, we evaluated the performance of the dietary assessment methods currently being used in the EPIC-Norfolk cohort. In concordance with most methodologic studies (9, 11, 25, 41–44), the reproducibility of the FFQ in our validation study was moderate to high, with correlation coefficients on the order of 0.50–0.80. However, whereas true changes in dietary intake can reduce the correlation coefficient, high correlations of within-person measurement errors can produce artificially high correlation coefficients. In fact, one study found that when identical FFQs were repeated within a year, the within-person correlations of measurement error were as high as 53% for nitrogen intake (45).

To further evaluate the performance of the questionnaire, nutrient intake measured by FFQ2 was compared with the mean nutrient intake derived from the two 7-d food diaries. Although it could be argued that the expected relative validity of FFQ2 would be higher because of a learning effect, this was not observed in our study. Comparable with the findings of our study, Bingham et al (5) evaluated the relative validity of a sim-

TABLE 3
Pearson's correlation coefficients and percentage of classification of nutrient intake from the second food-frequency questionnaire (FFQ2) in the same and extreme quartiles of nutrient intake compared with the 2 food diaries[1]

	Men					Women				
	r			Classification		*r*			Classification	
Nutrient	Crude	Energy adjusted	Adjusted and deattenuated[2]	Same quartiles	Extreme quartiles	Crude	Energy adjusted	Adjusted and deattenuated[2]	Same quartiles	Extreme quartiles
				%					%	
Energy	0.48	—	—	38	5	0.50	—	—	41	3
Protein	0.45	0.39	0.49	36	3	0.31	0.45	0.51	32	5
Carbohydrates	0.44	0.63	0.70	38	3	0.51	0.63	0.70	41	2
Fat	0.59	0.42	0.61	52	2	0.68	0.65	0.71	43	2
Sugars	0.48	0.60	0.72	36	3	0.54	0.63	0.70	49	5
Starch	0.39	0.41	0.44	31	5	0.53	0.59	0.68	40	3
NSP	0.57	0.70	0.77	45	2	0.56	0.72	0.78	34	2
Potassium	0.54	0.67	0.74	40	3	0.37	0.62	0.70	39	1
Sodium	0.47	0.52	0.65	26	3	0.45	0.44	0.52	34	6
Calcium	0.52	0.63	0.78	47	2	0.51	0.59	0.70	40	3
Iron	0.55	0.72	0.87	40	3	0.64	0.78	0.87	40	1
β-Carotene	0.30	0.32	0.39	28	3	0.60	0.62	0.79	43	3
Vitamin C	0.45	0.57	0.70	36	2	0.53	0.51	0.59	41	1
Vitamin E	0.53	0.40	0.46	40	0	0.44	0.49	0.60	33	6
Alcohol	0.88	0.82	0.89	69	0	0.94	0.93	0.98	79	0

[1] $r > 0.30$ is significant, $P < 0.05$. NSP, nonstarch polysaccharides.
[2] Deattenuation of the correlation coefficients.

ilar FFQ among middle-aged women and reported correlations ranging from 0.39 for potassium to 0.57 for nonstarch polysaccharides (excluding alcohol), with a mean crude correlation of 0.53. However, person-specific bias associated with both dietary assessment methods could lead to a substantial underestimation of the correlation coefficient (46).

Energy adjustment has varying effects on the extent to which 2 different methods of measuring dietary intake agree, as judged by the magnitude of the correlation coefficient. Some relative-validity studies report higher correlation coefficients after energy adjustment of nutrients in both the test and reference methods (9, 12, 26, 47), yet others find little or no improvement in the correlation coefficient (6–8, 11, 44, 48). In our study, energy adjustment improved the mean correlation coefficient to 0.56 in men and 0.62 in women, with most values falling between 0.50 and 0.65.

Because the random measurement errors of urinary nitrogen and potassium are unlikely to be correlated with random errors of the dietary assessment methods, these biomarkers were used to independently determine the validity of both the test and reference methods. In the design of previous validation studies (13–15), urinary nitrogen was measured in 24-h urine samples that were collected during the same time frame as subjects were recording their diet. Because the recording of food intake is subject to behavioral modification, subjects may reduce their dietary intake while keeping a food diary (49). Therefore, it could be argued that the corresponding urinary nitrogen excretion would be lower than normal and that correlated error would exist between the biomarkers and intake. In our study, 24-h urine samples were not collected during the time subjects were recording their dietary intake, making it more likely that any errors between the dietary assessment method and biomarker were completely independent.

Both the FFQ and the food diary are prone to measurement error. In this study, the results calculated from the 7-d food diary were much closer to estimates of output from urinary biomarkers than those calculated from the FFQ. Bingham et al (13) also found that the 7-d food diary correlated well with urinary nitrogen ($r = 0.70$), whereas the FFQ was poorly correlated ($r = 0.24$) with urinary nitrogen. Despite increased subject burden, other studies have found that more labor-intensive dietary assessment methods, such as the weighed-food record or food diary, corre-

TABLE 4
Mean concentration of urinary and plasma biomarkers in men and women[1]

Biochemical marker	Men ($n = 57$)	Women ($n = 77$)
Urine		
Nitrogen (g/d)	12.8 ± 2.0 (8.6–16.8)	9.9 ± 1.9 (6.7–16.0)
Potassium (mmol/d)	82 ± 16 (41–124)	69 ± 16 (37–131)
Sodium (mmol/d)	173 ± 43 (101–303)	131 ± 33 (73–264)
Blood		
Plasma ascorbic acid (μmol/L)		
All subjects[2]	58 ± 17.5 (20–89.5)	65.2 ± 20 (15.5–104)
Excluding supplement users[3]	55 ± 16.3 (20–87)	60.4 ± 19 (15.5–104)

[1] $\bar{x}$ ± SD; range in parentheses.
[2] $n = 48$ for men and 70 for women.
[3] $n = 39$ for men and 50 for women.

TABLE 5
Spearman's correlation coefficients between nutrient intake and biomarkers[1]

	Diary 1	Diary 2	Mean of diary 1 and 2	FFQ1	FFQ2
Men					
Urinary nitrogen	0.48	0.42	0.51	0.13	0.12
Urinary potassium	0.61	0.55	0.61	0.24	0.26
Urinary sodium	0.27	0.43	0.39	0.04	0.04
Plasma ascorbic acid					
All subjects[2]	0.42	0.35	0.41	0.32	0.34
Excluding supplement users[3]	0.41	0.35	0.41	0.33	0.43
Women					
Urinary nitrogen	0.51	0.37	0.48	0.20	0.10
Urinary potassium	0.41	0.42	0.48	0.28	0.29
Urinary sodium	0.23	0.11	0.17	0.08	0.13
Plasma ascorbic acid					
All subjects[2]	0.56	0.48	0.56	0.41	0.39
Excluding supplement users[3]	0.54	0.42	0.53	0.46	0.45
Total					
Urinary nitrogen	0.67	0.57	0.66	0.29	0.21
Urinary potassium	0.55	0.51	0.58	0.32	0.34
Urinary sodium	0.48	0.48	0.48	0.20	0.18
Plasma ascorbic acid					
All subjects[2]	0.54	0.44	0.53	0.42	0.39
Excluding supplement users[3]	0.52	0.40	0.49	0.44	0.45

[1] n = 57 for men and 77 for women. FFQ, food-frequency questionnaire.
[2] n = 48 for men and 70 for women.
[3] n = 39 for men and 50 for women.

late with urinary biomarkers better than does the FFQ (13–15). In this study, the relation between urinary potassium and dietary intake was lower for the FFQ than for the 7-d food diary. However, given the large day-to-day variation in urinary nitrogen and potassium (50), inclusion of those subjects with only 2–3 complete urine collections may have attenuated the correlation coefficients reported in this study.

In our study, the second food diary was less correlated with the urinary biomarkers than was the first, perhaps suggesting that the recording of intake changed during the validation study. Underreporting of food intake may in part be explained by undereating (49), a problem encountered in both obese (49) and lean (51) subjects. Despite restricted food intake, a recent study found that the accuracy of reported intake among women tended to be better when a 7-d weighed-diet record was used to measure intake rather than an FFQ (52).

It is widely recognized that the observed weak relation between dietary and urinary sodium is attributed to the poor assessment of salt intake by dietary assessment methods, the lack of inclusion of foods prepared with salt in food-composition tables, and the high within-person variability of urinary sodium (50, 53). In the present study, a moderately high correlation was observed between sodium intake derived from the mean of 2 food diaries in men but not women. It is therefore no surprise that the association between sodium intake and excretion is so weak given that ≥8 urine collections are needed to gain precision in the estimate of an individual's mean sodium intake (54). Even when the mean of 6 urine collections and 16 d of weighed records were used to estimate sodium intake, the relation between the 2 measures was only moderate (13). Urinary sodium may be a marker of sodium intake provided sufficient urine collections are collected, but for validation purposes it is of limited value.

Plasma ascorbic acid reflects vitamin C intake and can provide evidence of the accuracy of a dietary assessment method (55–57). In this study, intakes of vitamin C calculated from both the food diary and the FFQ were correlated with plasma vitamin C. The association between self-reported vitamin C intake and plasma vitamin C remained strong after exclusion of supplement users. In a random sample of the EPIC-Norfolk cohort, correlations between dietary vitamin C (excluding supplement users) and plasma ascorbic acid measured in a single nonfasting plasma sample were of similar magnitude to those reported in the present study (r = 0.36 for both the 7-d food diary and FFQ) (58). Other studies found that the FFQ provides valid information on micronutrient intake (19, 56), particularly vitamin C intake (19, 56, 57). In contrast, one study reported no association between a single fasting plasma ascorbic acid measurement and vitamin C intake derived from the FFQ, whereas the 7-d food diary was moderately correlated (14).

In summary, we found biomarkers to be useful in determining the accuracy of dietary assessment methods. Within the EPIC-Norfolk cohort, the superior ability of a 7-d food diary to document food intake was confirmed.

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