Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers

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Results from analysis of 24 h urine collections, verified for completeness with para-amino benzoic acid, and blood samples collected over 1 year were compared with 16 d weighed records of all food consumed collected over the year, and with results from 24 h recalls, food-frequency questionnaires and estimated food records in 160 women. Using the weighed records, individuals were sorted into quintiles of the distribution of the urine N excretion:dietary N intake ratio (UN:DN). UN exceeded DN in the top quintile of this ratio; mean ratio UN:DN = 1·13. Individuals in this top quintile were heavier, had significantly greater body mass indices, were reportedly more restrained eaters, had significantly lower energy intake:basal metabolic rate ratios (EI:BMR), and had correlated ratios of UN:DN and EI:BMR (r −0·62). Those in the top quintile reported lower intakes of energy and energy-yielding nutrients, Ca, fats, cakes, breakfast cereals, milk and sugars than individuals in the other quintiles but not lower intakes of non-starch polysaccharides, vitamin C, vegetables, potatoes or meat. Correlations between dietary intake from weighed records and 24 h urine K were 0·74 and 0·82, and between dietary vitamin C and β-carotene and plasma vitamin C and β-carotene 0·86 and 0·48. Correlations between dietary N intake from weighed records and 24 h urine excretion were high (0·78–0·87). Those between N from estimated food records and urine N were r 0·60–0·70. Correlations between urine N and 24 h recalls and food-frequency questionnaires were in the order of 0·01 to 0·5. Despite problems of underreporting in overweight individuals in 20% of this sample, weighed records remained the most accurate method of dietary assessment, and only an estimated 7 d diary was able to approach this accuracy.

Dietary assessment methods: Urine nitrogen: Dietary nitrogen

The validity of measurements of dietary intake in free-living individuals is difficult to assess because all methods rely on information given by the subjects themselves, which may not be correct. In an attempt to determine objective measures for validating dietary assessments, the search has begun for analytes of biological specimens that closely reflect dietary intake, but which do not rely on reports of food consumption (Isaksson, 1980; Bingham, 1987). In individuals in energy and N balance, urine N as assessed from 8 d of complete 24 h urine

collections is an independent measure of protein intake (Bingham & Cummings, 1985). We have therefore used this method to assess (1) the validity of estimations of habitual diet from 16 d weighed records kept by 160 women aged 50–65 years over the course of 1 year, and (2) the validity of estimates of diet from 24 h recalls, diet diaries and food-frequency questionnaires. The relationship between weighed records and other potential biomarkers of dietary intake including blood levels of some vitamins was also investigated.

METHODS
Volunteers

Volunteers for the present study were obtained from the lists of two general practitioners in Cambridge in 1988 and 1989. Seventy-nine female volunteers aged 50–65 years completed the study in year one (1989–1990; group 1), and eighty-one completed the study in year two (1990–1991; group 2). Full details of the recruitment procedure and of the study participants are given elsewhere (Bingham et al. 1994). The protocol was approved by the Dunn Nutrition Unit Ethical Committee in October 1988.

Protocol

Each individual was studied at home on four occasions (seasons) over the course of 1 year, and at each season was asked to complete 4 d weighed food records, using the PETRA (portable electronic tape recorded automatic) scales (Cherlyn Electronics Ltd, Cambridge). Full details of the weighing procedure are given elsewhere (Bingham et al. 1994). The volunteers were also asked to provide two 24 h urine collections on each occasion, so that over the year each individual provided 16 d weighed dietary records, and eight 24 h urine collections. Overnight fasting blood samples were also obtained, together with body weight and fasting breath samples at each occasion. Seven other dietary methods were investigated over the course of 1 year (two different food-frequency questionnaires, two variants of the 24 h recall, and three types of food diary) and these are fully described elsewhere (Bingham et al. 1994). Volunteers in group 2 were weighed immediately before beginning and after completing the last occasion of 4 d weighed intake, and were assessed for their tendency towards restrained eating at the end of the study.

Urine collections

Subjects were instructed on the technique of 24 h urine collections on the first day of weighing their food, and asked to make the first collection on the third or fourth day. They were given a canvas bag containing two 2-litre containers, each with 2 g boric acid as preservative, a jug, funnel, safety pin to pin to their underclothes as a reminder, and a set of instructions. They were asked to discard the first specimen of the day, for example at 07.00 hours, and from then on to collect all specimens for 24 h up to and including 07.00 hours the next day. They were given three 80 mg tablets of p-amino benzoic acid (PABA; PABAcheck, Laboratories for Applied Biology, London) to take with meals as a marker for completeness of 24 h urine collections (Bingham & Cummings, 1983), and asked to fill in a questionnaire with details of any medications, the time of the beginning and end of the collections, and whether or not they had any problems or missed specimens. The collection was returned with the weighing equipment on the day after completing the fourth day of weighed records, when a further set of instructions and equipment was given for the second 24 h urine collection, to be made usually 3–4 d later. On return to the laboratory, the volume of the 24 h collection was recorded, and portions stored at −20° before analysis.

In urine, total N was determined by the Kjeldahl technique (Tecator 1002; Perstorp Analytical, Bristol, Avon) and Na and K by flame photometry. PABA was measured colorimetrically (Bingham *et al.* 1988).

Fasting blood and breath collections, anthropometry and bowel habit diary

Subjects were visited by the study nurse within 7 d of completing the 4 d diet record by prior arrangement at home between 06.00 and 09.00 hours after overnight fasting and before breakfast. The second 24 h urine collection was picked up, and subjects were weighed on a spring balance, without shoes and in light clothing. Height without shoes was also measured using a Nivotoise portable height measure. Group 2 subjects were also weighed when they were not fasting, in light clothes and without shoes the day before they were due to start weighing their food for the last time (season 4) and the day after the 4 d period. Basal metabolic rate (BMR) was calculated from the equations of Schofield *et al.* (1985).

Duplicate end-expiratory fasting breath samples were collected using a party blower into 20 ml plastic syringes fitted with three-way stopcocks for CH_4 and H_2 determination. Samples of room air were also taken and the value subtracted from the breath sample (Gibson *et al.* 1990). H_2 was measured with an exhaled H_2 monitor (Gas Measurement Instruments Ltd, Renfrew, Scotland) and CH_4 by gas chromatography using a Pye Unicam PU 4500 with flame ionization detector. Subjects were asked to keep a 7 d record of the number of stool specimens per week within 7 d of completing the weighed records.

A 30 ml sample of blood was taken by venepuncture, 20 ml in lithium heparin, and 10 ml without anticoagulant. The samples were returned to the laboratory within 3–4 h in an insulated picnic box, when 0·5 ml whole blood was retained for packed cell volume analysis, and 0·2 ml whole blood was added to 1·8 ml 22·7 mM-ascorbate at pH 4·6 before storage at $-40°$ for pteroylglutamate (folate) analysis. The remainder of the whole blood was centrifuged at 3000 rev./min for 10 min at 10°, and 0·2 ml plasma was added to 0·4 ml 1·25 M-metaphosphoric acid and stored at $-40°$ before analysis for vitamin C. Remaining plasma was stored at $-40°$ before analysis for carotenoids. After centrifugation, serum was separated from the 10 ml sample containing no anticoagulant, and stored at $-20°$ for later cholesterol analysis using the Roche cobas-bio method.

Plasma vitamin C was measured by liquid chromatography with electrochemical detection in both groups (Bates & Cowen, 1988; Margolis & Davis, 1988). In group 1 only, carotenes, retinol and tocopherols were measured using absorptiometric detection (Thurnham *et al.* 1988). The intrabatch precision of these methods is 5–10 % coefficient of variation. Erythrocyte folate in both groups was measured by radioassay (Fuller *et al.* 1983).

Food restraint score

At the end of the last season, season 4, participants of group 2 were asked to fill in questionnaires designed to assess their tendency to restrain food consumption. The first ten general questions of the Dutch eating behaviour questionnaire (DEBQ) (van Strein *et al.* 1986) on food restraint were asked and scores of 0 to 5 assigned to the answers 'never' ranging through to 'very often'. All three factors, restrained eating (factor 1), disinhibition (factor 2), and hunger (factor 3) were included from the Stunkard & Messick (1985) questionnaire (SMQ). In addition, volunteers were asked to record their maximum and minimum adult weight in order to calculate maximum change of weight over a lifetime.

Table 1. *Average values for some blood and urine analytes from subjects in the present study compared with those obtained from a randomly selected population group of women aged 50–64 years* (*Gregory* et al. *1990*)

(Mean values with their standard errors)

	Present study		Gregory *et al.* (1990)	
Analyte	Mean	SE	Mean	SE
Serum				
Cholesterol (mmol/l)	5·9	0·09	6·8	0·08
Plasma				
Retinol (μmol/l)	2·01	0·05	2·10	0·04
β-Carotene (μmol/l)	0·50	0·03	0·44	0·02
β-Cryptoxanthin (μmol/l)	0·27	0·02	0·23	0·01
Lycopene (μmol/l)	0·30	0·01	0·23	0·01
α-Carotene (μmol/l)	0·11	0·01	0·10	0·01
α-Tocopherol (μmol/l)	26·7	0·71	31·6	0·62
Erythrocyte folate (mmol/l)	714	32	503*	17
24 h urine				
Sodium (mmol)	114	2·3	124	3·0
Potassium (mmol)	73	1·5	58	1·3
Volume (ml)	1858	25	1537	37

* All age values.

Statistics

Unless otherwise stated, results are given for the mean and standard deviation or standard error, and for Pearson correlation coefficients between variables. Results are given for energy, macronutrients, non-starch polysaccharides (NSP), K, Ca, Fe, carotene, retinol and vitamin C, all of which had less than 1 % 'missing values' (numbers of foods with no entry out of the total consumed) in the database. Results are also shown for 'dietary fibre', although the database contained 9 % missing values. Cross correlations between dietary cholesterol, total folate and erythrocyte folate were also made, although the dietary data had 8 % missing values for folate. The cholesterol database was complete.

RESULTS

Dietary intakes

Group mean results of the weighed dietary intake data and that from other methods have been fully discussed elsewhere (Bingham *et al.* 1994). One hundred and fifty-six subjects completed all four seasons, and a further four subjects completed three seasons of the study, but had moved out of the area by season 4. The results from these subjects are also included (total 160 subjects).

Urine and blood results

Table 1 shows some urine and blood analytes of the group as a whole, compared with those obtained from a randomly selected group of British women of this age group (Gregory *et*

Table 2. *Comparison of complete and incomplete 24 h urine samples collected from 160 women during 1989–1990 (group 1) and 1990–1991 (group 2)**

(Mean values with their standard deviations)

	Group 1		Group 2		Average			
	Mean	SD	Mean	SD	Mean	SD	Range	P†
Complete								
n	430		506		936			
PABA (% recovery)	95	6	94	6	94	6	85–128	—
Nitrogen (g)	10·1	2·2	9·9	2·3	10·1	2·2	5·0–17·7	0·001
Potassium (mmol)	72	21	75	23	74	22	27–198	0·001
Sodium (mmol)	110	36	117	42	114	39	14–328	0·001
Volume (ml)	1934	648	1989	679	1958	664	660–5440	0·001
Duration of collection (h)	24·1	0·7	24·1	0·9	24·1	0·8	21·0–29·2	0·001
% reporting losses	11	31	15	35	13	33		0·01‡
Incomplete								
n	177		142		319			
PABA (% recovery)	71	15	72	12	72	14	16–84	
Nitrogen (g)	9·0	2·3	8·7	2·2	8·8	2·3	2·4–15·6	
Potassium (mmol)	58	19	62	20	60	20	16–134	
Sodium (mmol)	100	33	102	39	101	36	25–217	
Volume (ml)	1563	612	1576	622	1568	617	340–3850	
Duration of collection (h)	23·9	0·9	23·9	0·9	23·9	0·9	19·2–28·0	
% reporting losses	25	45	16	37	21	41		

PABA, para-aminobenzoic acid.
* For details of subjects and procedures, see p. 532.
† Incomplete v. complete averages compared.
‡ Group 1, t 3·78; group 2, not significant.

al. 1990). Values for plasma carotenes, and erythrocyte folate tended to be higher and values for plasma tocopherol and retinol lower than those found in the randomly selected group. However, dissimilar methods of analysis, likely to produce discrepancies of this order, were used for folate in the two studies. Plasma cholesterol values were markedly lower in the present study, as were urine Na values. Urine K values were higher, as was 24 h urine volume.

Urine collections

In sixteen of the seventy-nine subjects in group 1 and twenty-two of the eighty-one subjects in group 2 all eight 24 h collections contained more than 85% of the PABA marker, and were designated complete. Completeness could not be assessed in all eight of one subject's collections, and in a further four isolated samples, due to interference from a paracetamol-containing medication. These samples were not included in the analysis. Three subjects submitted only one complete collection, five subjects two, and eight subjects three collections that were designated complete. The remaining 105 subjects submitted between four and seven complete collections. Table 2 shows analytes in the collections designated complete compared with those that were incomplete by PABA marker technique. There were no significant differences between groups 1 and 2 in analytes, nor in reported hours of collection. There were, however, highly significant differences in total N, K, Na, and volume between the complete and incomplete samples. Samples designated complete were

of slightly (0·2 h) longer duration than those that were incomplete, and there was a smaller proportion of reported one or more missed specimens over the 24 h period in the complete sample in group 1. However, there were no differences in group 2.

Comparison of 24 h urine nitrogen output with recorded nitrogen intake from weighed records and grouping into quintiles

Comparisons between urine N and dietary N intake were not possible for the three individuals who submitted only a single complete urine collection, and the individual who had been taking paracetamol medication. In the remaining 156 individuals, average N intake from the 16 d records was 11·2 (SD 2·3) g/d, and that from N excretion in the complete 24 h urine 9·84 (SD 1·78) g/d, so that the average urine N:dietary N ratio was 0·91 (SD 0·09). The distribution of the urine N:dietary N ratio was not normal and is shown in Fig. 1; coefficients of skewness and kurtosis were 1·21 and 2·00. In the absence of a clear bimodal distribution, results were sorted and examined as quintiles of the distribution of the urine N:dietary N ratio. Means of this ratio varied from 0·76 in the lower quintile of the distribution to 1·13 in the upper quintile (Table 3), and these differences between means were significant ($P < 0.001$) for all quintiles. Correlations between urine N and dietary N were 0·98 to 0·99 in the bottom four quintiles of the distribution, but lower, 0·78, in the top quintile.

Energy intakes, calculated BMR and the calculated energy intake:BMR ratio are also shown in Table 3. The average energy intake:BMR ratio of 1·15 in the top quintile was significantly different ($P < 0.05$) from that of the fourth quintile, but there was no significant difference in means between the fourth and third quintiles ($P > 0.05$). The energy intake:BMR ratio was not correlated with the urinary N:dietary N ratio in the lower four quintiles of the distribution (r 0·03 to 0·12) but there was a significant inverse correlation in the top quintile (r −0·62). The thirteen subjects who submitted only two or three complete collections were fairly evenly distributed throughout the quintiles of the distribution and their energy intake:BMR ratios showed the same trends as the complete quintiles, with average ratios of 1·39, 1·58, 1·48, 1·23, and 0·98 (results not shown).

Table 3 shows means and standard errors of anthropometric and other data from 156 individuals grouped into quintiles. There were clear trends in the urine K:dietary K ratio associated with the quintiles of urine N:dietary N ratio and correlations of 0·74 to 0·90 between urine K and dietary K within quintiles. Individuals in the top quintile had a significantly greater BMI and weight than those in the fourth quintile ($P < 0.05$), but there were no significant differences between the average BMI and weight in individuals in the fourth compared with those in the third quintile ($P > 0.05$). Individuals in the lower, second and fourth quintiles put on 0·4 to 1·2 kg over the year, but those in the top quintile did not gain or lose weight and those in the third quintile lost 0·3 kg weight. There was no consistent trend with height, although individuals in the lower quintiles of the distribution were 20 and 50 mm shorter on average than those in the other quintiles. No marked trends were evident in serum cholesterol or in plasma vitamins C and E across the quintiles of the distribution, although values for some carotenoids were significantly lower in the top quintile compared with the bottom quintile.

In group 2 there was a clear trend towards more restrained eating across quintiles as judged by the SMQ, although this was less obvious from the DEBQ results (Fig. 2). The differences between the top and bottom quintiles were significant ($P < 0.05$). All quintiles in group 2 lost weight whilst weighing food, and all reported large fluctuations in weight throughout life; however, the mean differences between the highest and lowest quintiles were not significant. On average, urine N exceeded dietary N by 0·7 g, and dietary K
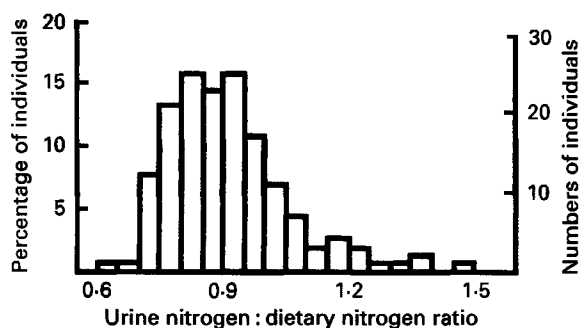
Fig. 1. The distribution of 24 h urine nitrogen excretion:dietary nitrogen intake ratio obtained from 16 d weighed records in 156 women. For details of subjects and procedures, see pp. 532–534.

exceeded urine K by 2 mmol in the top quintile (results not shown). There was no significant difference in social class index (assigning 1 to social class 1 and 5 to social class 5) between the upper and lower quintiles, upper quintile 2·51 (SD 0·18), lower quintile 2·35 (SD 0·16) (results not shown).

Table 4 shows intakes of nutrients by quintile of the distribution in urine N:dietary N ratio. Intakes were highest in the second quintile and lowest in the top quintile for energy and energy-yielding nutrients, Ca, retinol, NSP and fibre. Mean intakes of energy, fat protein and Ca were significantly lower in the top quintile compared with the fourth ($P <$ 0·05) but there were no significant differences between the mean reported intakes of individuals of the fourth compared with those of the third quintile.

Intakes of cereals, milk, eggs, fats, fish and potatoes, and sugars were highest in the second quintile and lowest in the top quintile, but there was no such trend seen in consumption of meat, other vegetables and fruit. There were significant differences between reported consumption of milk, milk products and butter between the top and fourth quintiles ($P <$ 0·05) but there were no significant differences in average reported values of the fourth and third quintiles (results not shown). Since these and the data shown in Tables 3 and 4 indicated that mean values from individuals assigned to the top quintile were different from the others, data were recalculated and Table 5 shows differences in reported food and some nutrient intakes for the top quintile compared with those of the other four quintiles of the distribution in urine N:dietary N ratio. Mean consumption of cakes, breakfast cereals, milk, eggs, fats, and sugars was significantly lower in those individuals classified in the top quintile of the distribution, and intakes of energy and all energy-yielding nutrients significantly lower. On average there was an 18 g difference in reported fat consumption, and a 27 g difference in reported sugars consumption between the average values reported in the top and the other four quintiles according to the urine N:dietary N ratio.

Assessments using different dietary methods

Table 6 shows reported mean nutrient intake of the same individuals classified into the lower four and the top quintiles of the urine N:dietary N ratio according to the weighed records, using three other methods of dietary assessment described elsewhere (Bingham et al. 1994). Using the questionnaire, individuals in the top quintile reported significantly lower mean intakes of energy, protein, starch, sugars, Ca and K than those in the other quintiles. Apart from a lower intake of sugars, there were no significant differences between quintiles with results obtained from the 24 h recall, and there were no differences at all between quintiles when results from the estimated food record (7 d diary) were compared.

Table 3. *Mean values with their standard errors and correlation coefficients (r) for anthropometric and other variables from 156 individuals grouped into quintiles of the distribution according to their urine nitrogen:dietary nitrogen (UN:DN) ratio from 16 d weighed records*§

	Lower quintile		2nd quintile		3rd quintile		4th quintile		Top quintile	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	31		31		32		31		31	
UN:DN ratio	0·76***	0·005	0·83***	0·003	0·89***	0·003	0·96***	0·004	1·13***	0·022
Range	0·69–0·79		0·79–0·86		0·86–0·93		0·93–1·00		1·00–1·47	
Urine N (g)	8·76	0·23	9·74	0·36	10·34	0·27	10·56	0·35	10·89	0·30
Dietary N (g)	11·55	0·29	11·78	0·43	11·25	0·30	11·00	0·36	9·69	0·28
r UN v. DN	0·976		0·994		0·989		0·994		0·778	
EI (MJ)	8·35	0·17	8·84	0·30	7·87	0·18	7·50	0·22	6·65†	0·23
EI:BMR ratio	1·54	0·03	1·60	0·05	1·40	0·03	1·32	0·04	1·15	0·04
r UN:DN v. EI:BMR	0·20		−0·13		0·07		0·03		−0·62	
Urine K (mmol)	65·55	2·11	74·06	3·20	75·09	3·25	72·97	3·53	75·37	4·09
Diet K (mmol)	82·45	2·15	88·59	3·67	84·36	3·08	77·67	3·31	73·82	2·97
UK:DK ratio	0·80	0·016	0·85	0·023	0·89	0·021	0·94	0·020	1·03	0·037
r UK v. DK	0·768		0·903		0·799		0·888		0·740	
Weight (kg)	61·54	1·44	63·44	1·69	65·76	1·33	65·65	1·58	71·99†	2·67
Height (m)	1·60	0·02	1·62	0·01	1·65	0·01	1·65	0·01	1·64	0·01
BMI (kg/m²)	23·48	0·58	23·88	0·65	23·94	0·50	24·40	0·50	26·59†	0·97
Δ Wt/year (kg)	0·37	0·35	0·72	0·51	−0·27	0·40	1·18	0·40	0·02	0·45
Serum cholesterol (mmol/l)	5·81	0·18	6·20	0·18	6·00	0·23	6·03	0·19	5·69	0·20

Plasma vitamins and carotenoids (μmol/l)‖

Vitamin C	69	5	59	4	62	4	60	4	61	5
Retinol	2·0	0·1	2·0	3·8	2·0	0·1	2·0	0·1	2·0	0·1
α-Tocopherol	27	2	26	1	26	1	28	2	26	1
γ-Tocopherol	1·9	0·2	2·4	0·3	2·1	1·2	2·4	0·2	2·4	0·3
Lutein	0·45	0·03	0·47	0·04	0·39	0·03	0·44	0·05	0·32‡	0·03
β-Cryptoxanthine	0·26	0·02	0·28	0·04	0·29	0·03	0·30	0·06	0·24	0·04
Lycopene	0·33	0·02	0·36	0·04	0·28	0·03	0·30	0·06	0·24	0·04
α-Carotene	0·12	0·02	0·13	0·02	0·11	0·01	0·11	0·02	0·07‡	0·01
β-Carotene	0·57	0·06	0·62	0·07	0·50	0·07	0·49	0·07	0·35‡	0·04
cis-Carotene	0·05	0·005	0·05	0·004	0·05	0·006	0·04	0·005	0·04	0·003
Food restraint measures¶										
Restraint DEBQ	5·08	0·84	8·41	0·92	6·69	0·76	7·25	1·00	8·57‡	1·22
Restraint SMQ	6·50	1·11	8·88	0·87	9·33	1·18	9·73	1·36	11·71‡	1·11
Disinhibition SMQ	5·30	1·26	5·11	0·85	5·40	0·77	4·60	0·72	5·71	0·96
Hunger SMQ	4·30	0·84	3·00	0·63	4·27	0·73	3·33	0·63	3·43	0·81
Δ Wt S4 (kg)	-0·47	0·18	-0·23	0·15	-0·56	0·17	-0·16	0·18	-0·36	0·29
Δ Wt life (kg)	10·09	2·15	13·23	1·57	15·86	2·31	13·46	2·38	17·46	3·37

EI, energy intake; BMR, basal metabolic rate; UK, urine potassium; DK, dietary potassium; BMI, body mass index; DEBQ, Dutch eating behaviour questionnaire; SMQ, Stunkard & Messick (1985) questionnaire; S4, season 4.

*** Significantly different from all other quintiles, $P < 0.001$.

† Significantly different from 4th quintile, $P < 0.05$.

‡ Significantly different from lower quintile, $P < 0.05$.

§ For details of subjects and procedures, see pp. 532–534.

‖ For items other than vitamin C, the data are for group 1.
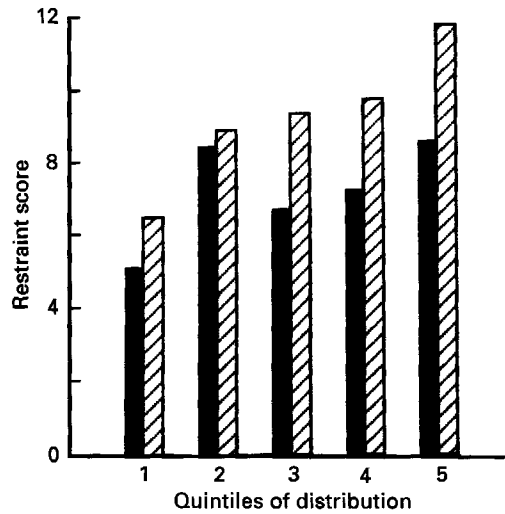
¶ Group 2 only.

Fig. 2. Relation of two measures of restrained eating, Stunkard & Messick (1985) questionnaire (▨) and Dutch eating behaviour questionnaire (■), to quintiles of the urinary nitrogen:dietary nitrogen ratio established from weighed records and 24 h urine collections in eighty women volunteers. For details of subjects and procedures, see pp. 532–534.

Table 4. *Mean values with their standard errors of daily nutrient intake from 156 individuals grouped into quintiles of the distribution according to their urine nitrogen:dietary nitrogen ratio from 16 d weighed records†*

	Lower quintile		2nd quintile		3rd quintile		4th quintile		Top quintile	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	31		31		32		31		31	
Energy (MJ)	8·35	0·17	8·84	0·30	7·87	0·18	7·50	0·22	6·65*	0·23
Protein (g)	72	2	73	3	70	2	68	2	60*	2
Fat (g)	82	3	87	3	77	3	74	3	62*	2
Starch (g)	115	5	121	6	110	5	108	5	100	5
Sugars (g)	122	6	132	7	107	6	98	5	88	2
% E protein	14	0·3	14	0·4	15	0·4	15	0·6	16	0·5
% E fat	37	0·8	37	0·9	37	0·9	37	1·0	35	0·5
% E carbohydrate	45	0·9	45	1·6	44	1·1	44	1·1	45	1·0
% E alcohol	3·0	0·6	2·8	0·8	3·5	0·8	3·4	0·6	3·6	0·9
Iron (mg)	12·7	0·6	13·3	0·7	14·0	0·7	12·5	0·7	11·9	0·7
Calcium (mg)	1027	35	1075	48	988	41	908	39	785*	36
NSP (g)	15·7	0·7	16·8	1·1	16·4	1·0	15·5	0·7	14·6	0·9
Fibre (g)	22·7	0·9	23·4	1·5	22·5	1·4	21·5	1·2	20·4	1·3
Alcohol (g)	8·5	1·8	9·2	3·3	8·9	1·9	9·0	1·7	8·6	2·1
Retinol (µg)	805	108	990	184	736	131	759	135	762	129
Carotene (g)	3·5	0·3	3·7	0·4	3·1	0·3	3·7	0·4	3·5	0·4
Vitamin C (mg)	94	5	108	8	104	8	92	6	95	8
Dietary vitamin C including supplements (mg)‡	98		101		99		109		101	

% E, percentage of dietary energy; NSP, non-starch polysaccharides.
* Mean values were significantly different from those for the 4th quintile, $P < 0.05$.
† For details of subjects and procedures, see pp. 532–534.
‡ Median values.

Table 5. *Food and nutrient intakes (g/d) from 156 individuals grouped into the top v. the other four quintiles of the distribution according to their urine nitrogen:dietary nitrogen ratio from 16 d weighed records†*

(Mean values with their standard errors)

	Lower four quintiles (*n*125)		Top quintile (*n*31)		P (top v. lower four quintiles)
	Mean	SE	Mean	SE	
Total cereals	258	3	212	13	< 0·05
Bread	83	1	82	6	NS
Breakfast cereals	23	0·1	14	2·6	< 0·01
Cakes	34	0·4	25	3·0	< 0·01
Milk and milk products	325	5	203*	19	< 0·001
Milk	256	5	150*	20	< 0·001
Eggs	24	0·3	18	2·0	< 0·01
Fats (including butter)	16	0·2	11*	1·0	< 0·001
Meat	95	0·5	96	6·4	NS
Fish	32	0·4	30	4·4	NS
Total vegetables	271	3	269	19	NS
Leafy vegetables	46	0·4	53	5·2	NS
Potatoes	82	1	83	8	NS
Fruit	202	4	212	27	NS
Nuts	3	1·0	2	1·6	NS
Sugars and confectionery	29	0·8	20	3·4	< 0·01
Beverages	938	11	971	70	NS
Alcoholic beverages	94	5	94	22	NS
Energy (MJ/d)	8·14	0·04	6·65	0·23	< 0·001
Protein	71	0·2	60	1·7	< 0·001
Fat	80	0·3	62	2·5	< 0·001
Starch	113	0·9	100	4·8	< 0·05
Sugars	115	0·1	88	5·2	< 0·001
NSP	16·1	0·03	14·6	0·85	NS

NS, not significant; NSP, non-starch polysaccharides.
* Mean values were significantly different from those for the 4th quintile, $P < 0.05$.
† For details of subjects and procedures, see pp. 532–534.

Correlations between diet and biological indices of diet

Table 7 shows averages of dietary N intake and of urine N from different methods of dietary assessment in the 156 individuals grouped into the lower four *v.* the top quintiles of the distribution according to the urine N:dietary N ratio from the 16 d weighed records. As for the weighed records, significant differences in mean intakes between the top and the other four quintiles were found for the Oxford food-frequency questionnaire, the structured 24 h recall, and the 7 d checklist with portions. No significant differences in mean intakes were apparent for results obtained from the Cambridge food-frequency questionnaire, the unstructured 24 h recall, the estimated food record (7 d diary) and 7 d checklist.

Correlations between dietary N intake and dietary assessment methods were highest for the weighed records (0·87 lowest four quintiles, 0·78 top quintile, 0·69 all values combined). The regression coefficient of the slope of the relationship between urine N (independent variable) and dietary N (dependent variable) was 0·724 (SE 0·107) in the upper quintile, which was not significantly different from that in the lower four quintiles combined (0·933 (SE 0·047), t 1·79, $P > 0.05$; Fig. 3).

Table 7 shows that correlations between urine N and dietary N from food-frequency

Table 6. *Daily nutrient consumption from food-frequency questionnaires, 24 h recalls and estimated menu records (7 d diaries) from 156 individuals grouped into the top v. the lower four quintiles of the distribution according to their urine nitrogen: dietary nitrogen ratio from 16 d weighed records†*

(Mean values with their standard errors)

	Lower four quintiles		Top quintile		P (top v. lower four quintiles)
	Mean	SE	Mean	SE	
Food-frequency questionnaire (Oxford)					
n	114		22		
Energy (MJ)	9·07	0·23	7·56	0·37	< 0·01
Protein (g)	84	2	74*	3	< 0·01
Fat (g)	90	3	74	4	NS
Starch (g)	103	3	87	6	< 0·02
Sugars (g)	140	5	113	8	< 0·01
Potassium (g)	4·1	0·1	3·6	0·2	< 0·05
Calcium (mg)	1370	20	1033*	58	< 0·001
NSP (g)	19·6	0·7	18·6	1·2	NS
Alcohol (g)	8·5	1·0	7·3	2·0	NS
Retinol (μg)	1029	95	852	110	NS
Carotene (g)	5·2	0·3	5·3	0·5	NS
Vitamin C (mg)	123	2	121	11	NS
24 h recall (Unstructured)					
n	122		31		
Energy (MJ)	7·65	0·19	7·21	0·45	NS
Protein (g)	70	2	74	7	NS
Fat (g)	77	3	76	9	NS
Starch (g)	103	5	92	5	NS
Sugars (g)	106	4	87	7	< 0·01
Potassium (g)	3·1	0·1	3·0	0·2	NS
Calcium (mg)	904	30	812	83	NS
NSP (g)	15·6	0·6	15·3	1·0	NS
Alcohol (g)	7	1	6	2	NS
Retinol (μg)	1170	419	795	392	NS
Carotene (g)	3·5	0·3	3·7	0·7	NS
Vitamin C (mg)	85	5	89	10	NS
Estimated food record (7 d diary)					
n	64		14		
Energy (MJ)	8·01	0·19	7·38	0·37	NS
Protein (g)	69	2	67	4	NS
Fat (g)	82	3	70	5	NS
Starch (g)	104	3	102	6	NS
Sugars (g)	108	4	101	8	NS
Potassium (g)	3·2	0·1	3·2	0·2	NS
Calcium (mg)	886	29	823	77	NS
NSP (g)	15·2	0·6	15·5	1·1	NS
Alcohol (g)	8	2	12	5	NS
Retinol (μg)	760	112	542	112	NS
Carotene (g)	3·2	0·2	3·8	0·6	NS
Vitamin C (mg)	104	6	115	14	NS

NS, not significant; NSP, non-starch polysaccharides.

* Mean values were significantly different from those for the 4th quintile, $P < 0.05$.

† For details of subjects and procedures, see pp. 532–534 and Bingham *et al.* 1994.

Table 7. *Comparisons between dietary nitrogen intake from different methods of dietary assessment and 24 h urine nitrogen in 156 individuals grouped into the top v. the lower four quintiles of the distribution according to their urine nitrogen:dietary nitrogen ratio from 16 d weighed records†*

	Lower four quintiles		Top quintile		P (top v. lower four quintiles)
	Mean	SE	Mean	SE	
Averages of N intake (g) from:					
16 d weighed records	11·4	0·17	9·7	0·28	< 0·001
Oxford FFQ‡	13·5	0·35	11·7	0·51	< 0·01
Cambridge FFQ	10·6	0·31	10·4	0·55	NS
Unstructured 24 h recall	11·2	0·27	11·9	1·07	NS
Structured 24 h recall	12·7	0·43	10·8	0·77	< 0·05
7 d checklist	11·1	0·20	10·3	0·38	NS
7 d checklist with portions	12·7	0·27	11·6	0·48	< 0·05
16 d weighed records G2	11·2	0·25	9·9	0·47	< 0·05
Estimated food records (7 d diary)‡	11·2	0·25	10·8	0·55	NS

	Lower four quintiles	Top quintile	All values
Correlations between 24 h urine N v.:			
N from 16 d weighed record	0·87	0·78	0·69
N from Oxford FFQ‡	0·27	0·50	0·24
N from Cambridge FFQ	0·15	0·19	0·15
N from unstructured 24 h recall (S1)	0·26	−0·31	0·10
N from structured 24 h recall (S2)	0·25	0·01	0·21
N from 7 d checklist	0·45	0·32	0·38
N from 7 d checklist with portions	0·43	0·26	0·38
N from 16 d weighed records G2	0·88	0·94	0·79
N from estimated food record (7 d diary)‡ G2	0·70	0·60	0·65

FFQ, food-frequency questionnaire; G, group; S, season; NS, not significant.
† For details of subjects and procedures, see pp. 532–534 and Bingham *et al.* 1994.
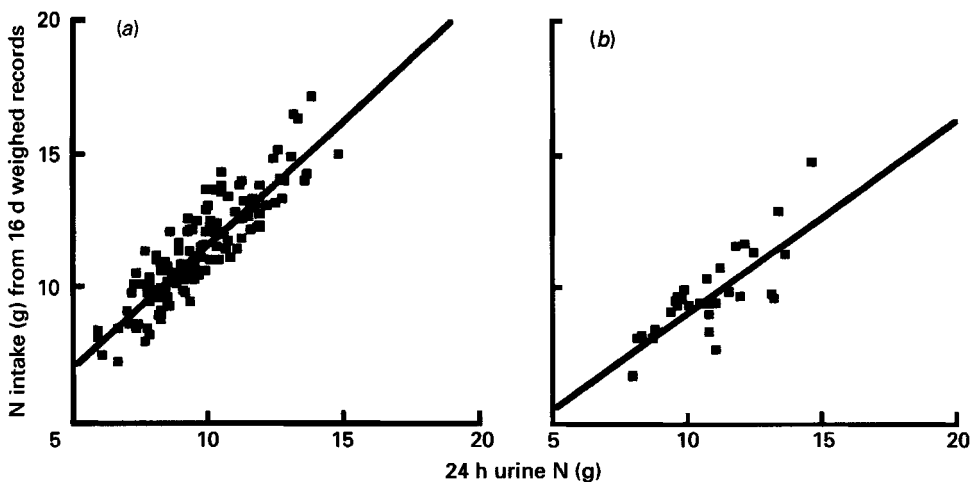‡ For Oxford FFQ, *n* 136; for 7 d diary, *n* 63.

Fig. 3. The relationship between 24 h urine nitrogen and dietary nitrogen from 16 d weighed records in individuals from (*a*) the lower four quintiles and (*b*) the top quintile of the urinary nitrogen:dietary nitrogen ratio distribution. For details of subjects and procedures, see pp. 532–534.
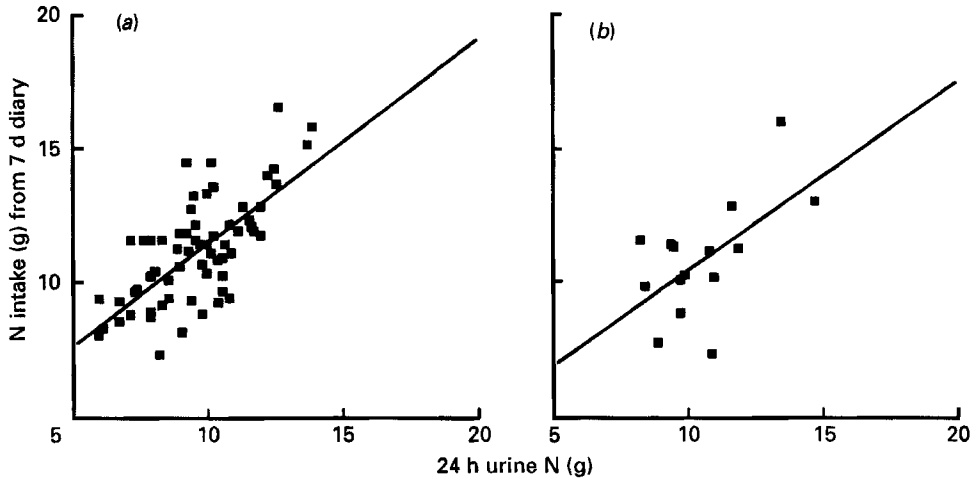
Fig. 4. The relationship between 24 h urine nitrogen and dietary nitrogen established from a 7 d estimated food record (diary) in individuals from (a) the lower four quintiles and (b) the top quintile of the urinary nitrogen:dietary nitrogen ratio distribution established from weighed records. For details of subjects and procedures, see pp. 532–534.

questionnaires and 24 h recalls were much lower, 0·1 to 0·2. There did not appear to be differences between groups of individuals classified into the top v. the other four quintiles, apart from a negative correlation (−0·31) between urine N and dietary N in the top quintile of 24 h recall data and a positive correlation (0·50) in the top quintile of Oxford food-frequency questionnaire data. Correlations were in the order of 0·2 to 0·4 for checklist methods.

When group 2 was considered separately the mean value for dietary N intake in the top quintile was significantly lower than in the other four quintiles (Table 7), as before. Correlations between urine N and dietary N in the lower four quintiles were the same (0·88) as in group 1 and 2 combined data. However, the correlation was rather higher in the top quintile, 0·94. The correlations between urine N and dietary N from the 7 d diary were 0·60 and 0·70, which compared well with coefficients of 0·88 and 0·94 obtained with weighed intake data. Fig. 4 shows 24 h urine N values in relation to dietary N values from the 7 d estimated food record. Regression coefficients were not significantly different (0·701 (SE 0·262) upper quintile, lower four quintiles 0·757 (SE 0·098), t 0·02, $P > 0·05$).

Table 8 shows correlations between dietary variables from the 16 d weighed record averages and other analytes in urine, blood and breath. There was a high correlation (0·73) between 24 h urine K excretion and dietary K intake. Fig. 5 shows the relationship between these variables for individuals in the top and lower four quintiles of the distribution of urine N:dietary N (r 0·78 top quintile, r 0·87 other four quintiles). The correlation between plasma vitamin C and dietary vitamin C with values from supplements added to values obtained from dietary records, was 0·86 (Spearman rank coefficient). The maximum plasma vitamin C value was 151 μmol/l and the minimum 6 μmol/l, with evidence of a plateau in values between 100 and 150 μmol/l even with very high supplementation levels (Fig. 6). Correlations between dietary β-carotene equivalents and α- and β-carotene in plasma were 0·62 and 0·48; all other correlations between dietary variables and analytes were less than 0·4. Correlations in the order of 0·2 were obtained between stool frequency and starch, NSP and dietary fibre intake. These correlations were generally of greater magnitude than those obtained by Gregory et al. (1990) from 7 d weighed records and single 24 h urine collections (Table 8).

Table 8. *Comparison of average results from biological markers in urine and other specimens with results from averages of 16 d weighed records in 160 women*

Dietary item	Biological marker	Correlation coefficients	
		Present study	Gregory *et al.* (1990)
Nitrogen	24 h urine nitrogen	0·69	—
Potassium	24 h urine potassium	0·73	0·36
Sodium	24 h urine sodium	0·30	0·26
Vitamin C	Plasma vitamin C	0·32	—
Vitamin C including supplements	Plasma vitamin C	0·86†	—
β-Carotene equivalents	Plasma α-carotene	0·62	0·43
	Plasma β-carotene	0·48	0·38
	Plasma cis-carotene	0·35	—
	Plasma lutein	0·36	—
	Plasma lycopene	0·33	—
	Plasma β-cryptoxanthin	0·20	—
Retinol	Plasma retinol	−0·01	0·01
Folate	Erythrocyte folate	0·10	0·18
Cholesterol	Serum cholesterol	0·10	0·09
Total fat	Serum cholesterol	0·03	−0·02
Starch	Breath hydrogen	0·11	—
	Breath methane	0·04	—
	Stool frequency	0·24	—
Non-starch polysaccharides	Breath hydrogen	0·00	—
	Breath methane	0·00	—
	Stool frequency	0·16	—
Fibre	Breath hydrogen	−0·02	—
	Breath methane	0·02	—
	Stool frequency	0·16	—

† Spearman rank correlation coefficient.
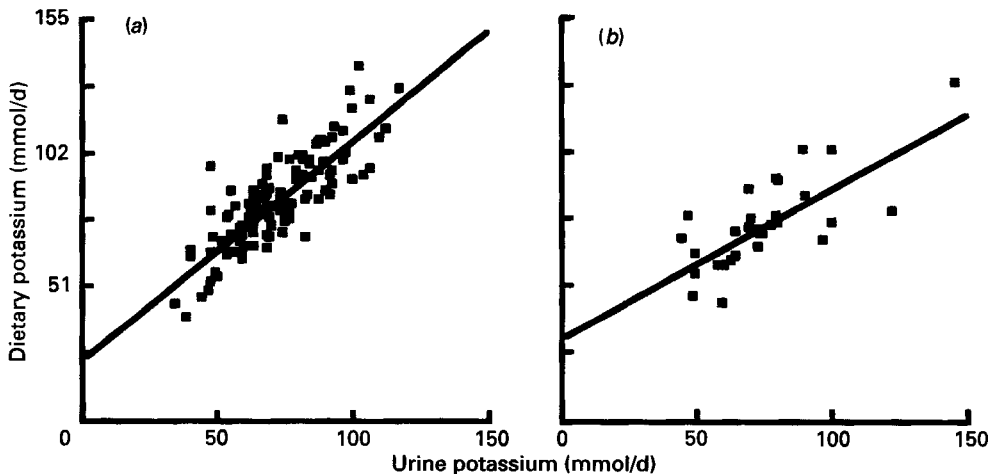
Fig. 5. Dietary potassium intake from 16 d weighed records in relation to average urine potassium excretion from 24 h urine collections in individuals from (*a*) the lower four quintiles and (*b*) the top quintile of the urinary nitrogen:dietary nitrogen ratio distribution established from weighed records. For details of subjects and procedures, see pp. 532–534.
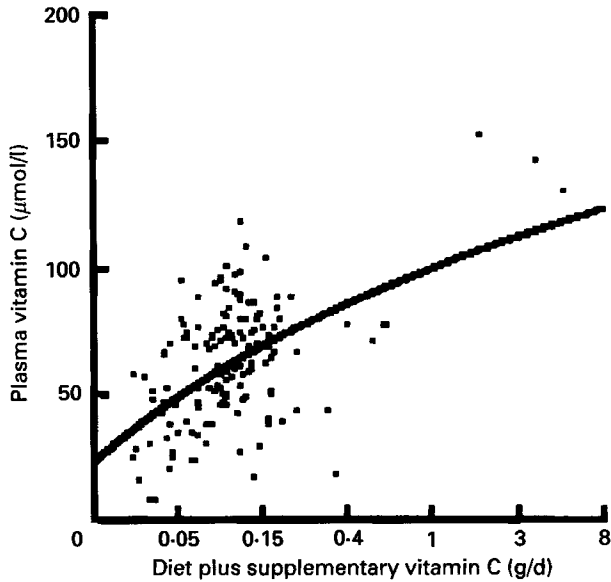
Fig. 6. Logarithmic plot of plasma vitamin C (μmol/l) in relation to log e vitamin C intake (g/d) from weighed records and records of vitamin supplements in 156 women. For details of subjects and procedures, see pp. 532–534.

DISCUSSION

The assessment of habitual food intake is one of the most problematic tasks in human nutrition, and even weighed records, regarded in the past as 'gold standards', have been criticized (Bingham, 1987). However, despite evidence of underrecording in one quintile of this volunteer group, and often-expressed doubts about the accuracy of the weighed intake technique, it was in remarkable agreement with two biological markers of food intake, the 24 h urine N and K (Table 3). Table 7 shows that it easily outperformed other simpler epidemiological methods in correlation analyses when compared with 24 h urine N.

The next best method was the 7 d estimated food diary or record (Table 7). This method also gave results for foods and nutrients that were closest to those of the 16 d weighed records (Bingham *et al.* 1994). Some confidence can therefore be placed in the results obtained from the 7 d diary. Problems with the food-frequency questionnaires used here have been described elsewhere (Bingham *et al.* 1994) and the low correlations with single 24 h recalls are to be expected from the known effect of attenuation due to day-to-day variation.

Repeat measures are required in recording methods of dietary assessment, so that attenuation of correlation from daily variation can be minimized (Bingham, 1987). In order that the task of weighing food could be simplified, and also to avoid weights of food consumed becoming too familiar to volunteers, the PETRA system was developed. This automatically records a verbal description and the weight of food consumed on a dual track cassette. The periods of observation were split into comparatively short periods of time, and spread out over a year at 3-monthly intervals. It was hoped that the validity of the observations from these records would thus be assured by the use of a system which introduces minimal interference into the life of volunteers, whilst at the same time gaining sufficient numbers of observations on each individual to minimize random error from daily variation in nutrient and food intake. The high correlations between urine N and dietary N in the majority of the distribution of urine N:dietary N would support this supposition.

When using 24 h urine collections to validate dietary assessments, the importance of including a 24 h urine collection marker to check compliance is shown in the present study, which found highly significant differences in excretion of 24 h urine analytes and volume between samples designated complete by the PABAcheck method, and those that were incomplete. Samples designated incomplete were of shorter duration and contained a larger proportion of reported missed samples (Table 2). Only samples that were complete were used for comparison with dietary intake data, so that the baseline for each individual varied from two to eight complete collections. The precision of estimates of urine N and other analyte excretion in those individuals who submitted less than four complete collections would have been reduced, but in the thirteen individuals who collected only two to three complete 24 h samples the energy intake:BMR ratio was similar to that obtained from individuals who did comply with the urine collection protocol to a greater extent and submitted four or more complete collections.

The urine N:dietary N ratio was 91 %, rather greater than the expected ratio of 80 % for a group established elsewhere (Bingham & Cummings, 1985), despite the fact that the group as a whole increased their body weight by an average of 0·40 kg over the year. These findings suggested bias in the estimation of dietary intake, either in the group as a whole or in a proportion of individuals within the group. There was no evidence of a bimodal distribution in the urine N:dietary N ratio (Fig. 1), which would have allowed a clear cut-off point between those individuals whose dietary N intake was within the expected physiological limits of 80 (SD 5)% urine N excretion (Bingham & Cummings, 1985). However, when sorted into quintiles of the distribution in urine N:dietary N ratio, the top quintile was clearly different from the rest, with a lower correlation between urine N and dietary N (0·78), an inverse association between the energy intake:BMR ratio (r −0·61), not apparent in the other 80 % of the distribution, and a significantly greater average BMI of 26·6 kg/m² than that of the adjacent quintile of the distribution. The average energy intake:BMR ratio of 1·15 was below the physiological limits that would match energy expenditure with no gains or losses of weight (Goldberg et al. 1991). Since, over the year, this group neither gained nor lost weight, it has to be concluded that individuals in this 20 % of the distribution were not reporting their usual diet.

To investigate this further, group 2 volunteers were weighed immediately before and after weighing their food over the last 4 d of the study, and assessed for their tendency to restrain their food consumption. The 0·36 kg lost over 4 d by individuals in the top quintile would have been equivalent to a daily loss of 90 g whole-body tissue, or 25 g fat-free mass. This however would contain only 0·7 g N and 1·5 mmol K (Garrow, 1993), compared with the average negative N balance of 2·7 g, and negative K balance of 10 mmol found after comparisons of dietary and urine data and allowance for faecal losses. It is probable therefore that the failure of the dietary records to match the urine output and BMR data was mainly caused by errors and omissions, rather than changes in habitual dietary habits in an attempt to slim or simplify the procedure whilst weighing food.

Questions from both the restrained-eating questionnaires indicated that individuals in the top quintile of the distribution were more restrained eaters than others (Fig. 2). Black et al. (1994) have also shown that successful slimmers who underreport their food intake are more likely to be restrained eaters. The relationship between urine N:dietary N index and restrained-eating score, though significant, (r 0·29) was not strong in this group of relatively unselected individuals. There was no relationship between hunger and disinhibition scores and underreporting, nor between gain or loss of weight over the year and restrained eating. Individuals in the fourth quintile gained on average the most weight (1·2 kg), yet had a higher score than those in the third quintile, who lost on average 0·27 kg over the year. The greater lifetime weight change suggested as a possible identifying factor

in underreporting (Black et al. 1991) recorded by individuals in the top quintile failed to reach significance when compared with the average reported by subjects in the lower quintile. There was no discernible difference in social class index across quintiles of the distribution.

Underreporting by obese individuals and patients has been documented for some time (Steen et al. 1977; Prentice et al. 1985; Lichtman et al. 1992; Hallfrisch, 1994). Total energy expenditure assessed from the doubly-labelled water technique has shown evidence of underreporting of energy intake in overweight and normal weight individuals (Prentice et al. 1985; Livingstone et al. 1990; Schoeller, 1990). However, this technique is expensive and the energy intake:BMR ratio has been suggested as a means of assessing the likely validity of group estimates of energy intake, since in the absence of gain or loss of weight it must equal the total energy expenditure:BMR ratio (Bingham, 1987; Goldberg et al. 1991). The average values obtained from 80% of volunteers were 1·3 to 1·6, and the overall average of 1·47 was close to the value of 1·50 suggested as an acceptable value for the validity of a dietary survey of 14 d observations on 100 subjects (Black et al. 1991). The coefficient of variation between measured and calculated BMR for individuals (World Health Organization, 1985) is in the order of 10% and hence, when coupled with uncertain estimates of total energy expenditure to metabolic rate, the precision of this ratio for individual estimates of validity is low (Goldberg et al. 1991). There are also limits of precision of the urine N:dietary N ratio. Table 2 shows there was no association between these two variables in subjects with normal records, but a strong association in the overweight underreporters in the top quintile of the distribution in urine N:dietary N ratio (r −0·62).

According to their records, underreporters in the top quintile of the distribution of dietary N from weighed records consumed significantly less milk than other subjects, and significantly less cakes, sugars, fats, and breakfast cereals (Table 5). As a result, differences in nutrient intake were not confined to protein but intakes of fat, sugars, starch and energy were also significantly lower, as were intakes of K, Ca and Fe (Table 5). There were no discernible differences in bread, potatoes, meat or vegetable and fruit consumption between underreporters and other subjects, so that intakes of NSP, fibre, carotene and vitamin C were the same in all groups. The validity of this finding is supported by the fact that there were no significant differences between quintiles in the blood analytes erythrocyte folate and plasma vitamin C, of which the major dietary sources are fruit and vegetables (Table 2). Plasma levels of the carotenoids tended to be higher in the group as a whole than those of a national sample reported by Gregory et al. (1990; Table 1), as were intakes of fruit and vegetables (Table 5 and Gregory et al. 1990). The differences in folate levels between the national group and this group are more likely to be attributable to difficulties with analytical methods than to differences in vegetable consumption. Methods for the analysis of carotenes were compatible, and as in the study of Gregory et al. (1990), dietary β-carotene equivalents correlated well with α-carotene (Table 8, r 0·62). However a single food, carrot, is the main dietary source of α-carotene. The correlations were less good with the major source in food, β-carotene (r 0·48) which is derived from several foods.

Of the other dietary variables measured, the correlation between dietary and 24 h urine K approached the magnitude of that for N, r 0·73 (Table 7, Fig. 5). Although the day-to-day variation in urine K excretion is greater than that in urine N (Bingham et al. 1988) it is found to a greater extent in some different foods, for example vegetables, and therefore is a useful additional validation marker in dietary surveys. K excretion was higher than in the national sample (Table 1), possibly due to greater recorded vegetable intake or to the fact that only samples of urine known to be complete were included in the present study.

No values were obtained for plasma vitamin C in the national survey but in the present study dietary and plasma vitamin C levels were highly correlated, especially when dietary intakes were corrected for high intakes occurring as a result of supplements of vitamin C (Fig. 6). Serum cholesterol was lower, as was fat as a percentage of energy intake, 36 % energy in the present survey (Bingham *et al.* 1994), 38 % energy in the survey of Gregory *et al.* (1990). The poor relationship between dietary cholesterol and serum cholesterol seen in Table 8 is well known (Willett, 1990). Saturated fat would be expected to correlate to some degree with serum cholesterol, but only values for total fat were available for analysis in the present study. Stool frequency was related weakly to starch, NSP and fibre intake, but there was no relationship of stool frequency with fasting levels of breath H_2 and CH_4. Subsequent studies have established that evening rather than early morning samples of breath H_2 and CH_4 are better predictors of dietary intake of fermentable carbohydrate (Le Marchand *et al.* 1992; McKeown, 1992).

Vitamin C, N and K apart, the weak relationships between analytes and dietary intake would suggest that their scope in validating or even replacing dietary assessments is limited, although some are a useful adjunct to dietary records. This is despite the fact that the correlations between dietary and serum and urine analytes found here were in general somewhat greater than those found in the national sample of adults (Table 8) probably due to reduction of attenuation by a greater number of observations on each individual.

Overall, the use of biological markers, especially the 24 h urine N and K output, has been shown to be a useful adjunct to detailed weighed records kept by a relatively large sample of volunteers over one year. Simple comparisons between methods of dietary assessment are unlikely to show that results from one method agree with another (Bingham, 1987), but confidence in a particular method is forthcoming if the results from it agree with an external marker, in this case the 24 h urine N and K. Weighed records remain the most accurate measure of dietary intake, but for epidemiological and surveillance purposes it would seem that estimated food records are able to approach this accuracy.

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