

# Validation with biological markers for food intake of a dietary assessment method used by Swedish women with three different dietary preferences

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## Abstract

**Objectives:** To validate a dietary assessment method, a 4-day food record together with a duplicate portion technique, with biological markers for food intake.

**Design:** Four days of duplicate portions were collected in parallel with food recording. A 24-h urine sample and the faeces corresponding to the food intake (using a coloured marker) were collected. Completeness of urine and faeces collections was assessed using *para*-aminobenzoic acid (PABA) in urine and cadmium in faeces, respectively. Biomarkers of food intake (energy, protein, fibre, sodium, potassium, calcium) were measured in urine and faeces.

**Setting:** Swedish west coast.

**Subjects:** Non-smoking Swedish women, 20–50 years of age, consuming a mixed diet ( $n = 34$ ), a mixed diet rich in shellfish ( $n = 17$ ) or a vegetarian/high-fibre diet ( $n = 23$ ).

**Results:** The average ratio (food intake according to the dietary assessment methods/biological marker) for protein, sodium, potassium and calcium was 0.86. This indicates an underestimation of the food intake by approximately 15%. The ratio of stated fibre intake to biological marker was 1.20 for the mixed diet and the vegetarian diet group, indicating an overestimation by approximately 20%.

**Conclusions:** The underestimation of the intake of protein, sodium, potassium and calcium by all three groups and the overestimation of the fibre intake by two groups indicate that underreporting is selective to certain nutrients and foods and to various groups of people. The two dependent dietary assessment methods were equally good in measuring protein intake, which indicates that the women recorded what they actually duplicated.

**Keywords**  
Validation  
Dietary assessment  
Food record  
Duplicate diet  
Biomarkers of food intake

The validity of a dietary assessment method may be defined as the extent to which the method estimates the true dietary intake. Validation of the method is essential, as invalid information may lead to incorrect associations between dietary factors and the potential effect that the diet may have on, for instance, various diseases and disease-related markers. There are major problems involved in the assessment of the true habitual dietary intake. When using prospective methods the dietary measurements may interfere with the subject's everyday life and cause a change in the food choice and food intake. In recalling past diets, ingested food items may easily be forgotten, or the amounts misjudged.

In order to evaluate the accuracy of an estimated dietary intake, it is necessary to use a method that is independent of the subject's reported dietary intake. For this purpose biomarkers of food intake, e.g. biochemical indicators of nutrient intake, have been

developed<sup>1–3</sup>. Various biological media, such as urine, faeces, blood, plasma, red blood cells, hair and nails can be used for the analysis of markers reflecting nutrient intake.

This study forms part of a project on exposure to cadmium in subjects consuming three various diets, known to contain marked differences in cadmium<sup>4,5</sup>. The three diets were: a mixed diet, a diet high in shellfish and a vegetarian diet. The purpose of the present study was to validate on a group level the dietary assessment method used, a 4-day record in combination with a 4-day duplicate portion technique, by biomarkers of food intake, analysed in urine and faeces.

## Subjects and methods

### Subjects

Non-smoking women, 20–50 years of age, were recruited via the local radio and press in two towns in

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the western part of Sweden in order to study the dietary intake of cadmium, lead and various nutrients in Swedish women with different dietary preferences<sup>4,5</sup>. The first selection of women was carried out by telephone interviews, using a questionnaire on personal characteristics such as age, weight, height, general dietary habits, former smoking habits and medication. Women without gastrointestinal disorders or any other illnesses requiring medication and with a body mass index (BMI) between 20 and 30 kg m<sup>-2</sup> were asked to participate in the study.

For evaluation of their habitual diet, the selected women were asked to complete a self-administered food frequency questionnaire (FFQ) with questions concerning current food choice, food consumption frequencies and the use of dietary supplements. Based on this information the women were categorized into three diet groups: (a) a mixed diet ( $n = 34$ ); (b) a mixed diet including shellfish at least once a week ( $n = 17$ ); and (c) a lacto-vegetarian diet or lacto-ovo-vegetarian diet, characterized by large amounts of wholemeal products, vegetables and fruits ( $n = 23$ ). Hereafter the diet groups will be referred to as mixed diet, shellfish diet and vegetarian diet. There were no differences in age, height or weight between the diet groups<sup>4,5</sup>.

This study was approved by the Ethics Committee of Karolinska Institutet, Stockholm.

### **Dietary assessment methods**

During 4 consecutive days a combined weighed and estimated food record was carried out in parallel with the collection of duplicate diets. Most women agreed to use an electronic balance to weigh all food items consumed. Foods consumed away from home were often estimated rather than weighed. A nutritionist visited the women, at home or at work, every day of the dietary study period to provide guidance about sampling and documentation, to interview the women about their previous 24-h diet record, and to help identifying any food forgotten. Duplicate portions of all foods and beverages consumed, including drinking water, were collected in acid washed plastic containers as 2–3 samples per day, and kept at  $-4^{\circ}\text{C}$  until sample preparation and analysis. To cover both inter-seasonal and intra-weekly variations in food consumption, samples were collected during four study periods between December 1991 and October 1992. During each study period, half of the subjects started the collection on a Sunday and the other half on a Wednesday. The food records were coded by the nutritionist and converted into amounts of various foods and nutrients using the national food database at the National Food Administration, Uppsala, Sweden.

### **Faeces collection**

All faeces corresponding to the diet ingested during the duplicate diet period were collected, using plastic bags that were placed in the toilet seat. The plastic bags were sealed and put into plastic containers with a tight lid. The samples were stored at  $-20^{\circ}\text{C}$  until sample preparation and analysis. A coloured marker (0.6–1.0 g carmine red) was ingested to identify the start and the end of the duplicate diet collection period. As only a few per cent of ingested cadmium is absorbed in the gastrointestinal tract, and no other significant sources of oral exposure to cadmium could be expected, cadmium in faeces was compared with cadmium in the duplicate diets in order to validate the faeces collection completeness.

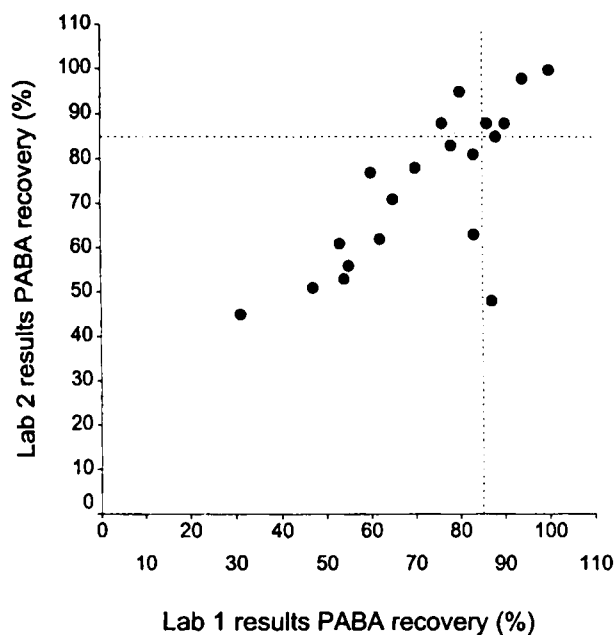
### **Urine collection**

Twenty-four-hour urine samples were collected in acid washed plastic containers. The start (after the first morning urine) and the end (right after the morning urine the next day) of the urine collection, as well as any lost specimens, were reported. Three 80 mg tablets of *para*-aminobenzoic acid (PABA check; Laboratories for Applied Biology, London, UK and the Karolinska Hospital, Stockholm, Sweden) were ingested with morning, mid-day and evening meals for evaluation of urine collection completeness<sup>6</sup>. The weight and specific weight, as well as creatinine<sup>7</sup> of the 24-h collections were determined, and 10 ml aliquots were stored at  $-20^{\circ}\text{C}$  until analysis of PABA and biomarkers. PABA was determined using a colorimetric method<sup>6</sup>. For quality control, duplicates of 20 of the urine samples were analysed for PABA at a reference laboratory, using the same analytical method. The results of the inter-laboratory comparison of PABA recovery was evaluated using linear regression analysis:  $y = 21 + 0.73X$ ,  $R = 0.75$  (Fig. 1). Three samples (15%) were misclassified, i.e. were on either side of the cut-off level of 85% PABA recovery, which according to Bingham and Cummings may be defined as incomplete collections<sup>6</sup>. The between-laboratories differences varied from 0 to 39%.

The urinary samples with a PABA recovery below 85% were adjusted to 93%, as Bingham and Cummings found a mean PABA recovery of 93% of their subjects when they proposed the PABA check method<sup>6</sup>. The authors proposed a cut-off level of 85% for complete urine collections, which is 2 SD below 93%<sup>6</sup>. We have used a linear regression analysis to adjust urine collections below 85%, using data on PABA recovery in relation to nitrogen, sodium and potassium, obtained in several studies<sup>8</sup>.

### **Biomarkers of food intake**

The estimated basal metabolic rate ( $\text{BMR}_{\text{est}}$ ) was calculated according to the Department of Health,



**Fig. 1** The results of the inter-laboratory comparison of PABA recovery (percentage of administered dose) in 20 urine samples, measured by the same method at two different laboratories. The lines inside the graph represent the cut-off point of 85% PABA recovery in urine, which may be defined as incomplete urine collections<sup>6</sup>

UK<sup>9</sup>, and was compared with the energy intake calculated from the food records.

Total nitrogen was determined in duplicate diets and urine by the Kjeldahl technique (Tecator 1002, Perstorp Analytical, Bristol)<sup>10</sup>. Nitrogen in urine was used as a biomarker of protein intake<sup>10,11</sup>. The protein intake was calculated as  $6.25 \times (\text{nitrogen in urine} + 2)$ <sup>11</sup>, where the factor 2 is for extrarenal excretion of nitrogen, and 6.25 is the constant conversion factor for average nitrogen content of protein. It was compared with the protein intake calculated from food records and duplicate diets (nitrogen in duplicate diets  $\times 6.25$ ).

Sodium in urine was used as a biomarker of the sodium (salt) intake<sup>12</sup> and was compared with the sodium intake calculated from food records. Potassium in urine and faeces was used as a biomarker of the potassium intake<sup>3</sup> and was compared with the potassium intake calculated from food records. Calcium in urine and faeces was used as a biomarker of the calcium intake<sup>13</sup> and was compared with the calcium intake calculated from food records. Sodium, potassium and calcium concentrations in urine were determined by flame atomic absorption spectrophotometry (Perkin Elmer model 5000) according to routine clinical laboratory procedures. Potassium and calcium concentrations in faeces were determined by flame atomic absorption spectrophotometry using a three-slot burner head and background correction, after dry-ashing the faecal samples overnight at 470°C in a

furnace, dissolving the ash in 1 M HNO<sub>3</sub>, and making the final solutions 0.1% for sodium and lanthanum. Standard reference materials (SRM 2670 low and elevated, NIST, USA, and Seronorm Trace Elements Urine, Nycomed, Norway) were used for quality control purposes with satisfactory results.

Three different methods were used to validate fibre intake<sup>14–17</sup>. The first method is based on stool weight, and the second method is based on the faecal water content. The faecal water content, which is related to the intake of mainly non-soluble fibre, was estimated by subtracting the faecal dry weight from the faecal total weight. The third method<sup>14</sup> estimates the intake of fibre from the stool weight according to the relationship: stool weight =  $3.5 \times$  fibre intake (based on the British food tables) + 40. A comparison of 25 food items in the British and Swedish food tables (all of which were apparently the same in the two food tables) showed that the Swedish fibre values were on average 86% of the British values ( $P < 0.05$ )<sup>13</sup>. Therefore the calculated fibre values in the present study were multiplied by 0.86.

### Statistical analysis

Statistical analyses were made using SPSS software (Chicago, IL, USA) for Windows. The Kruskal–Wallis test and Wilcoxon–Mann–Whitney test were used to detect differences between groups.

### Results

The calculated protein intake, based on duplicate diets (DD) and food records (FR), were the same, with a ratio of DD/FR close to 1, in all three diet groups (Table 1).

All the women reported complete faeces collection. On a group level, the cadmium content in faeces was approximately 100% of the cadmium content in the 4 days DDs, indicating essentially complete faeces collection.

All women reported complete urine collection, however, some repeated sampling after due to losses during the first sampling period<sup>4</sup>. The average recovery of PABA in urine was 73%, with no differences between the mixed, shellfish and vegetarian groups (71, 71 and 78%, respectively). In order to evaluate possible

**Table 1** Comparison of the dietary intake of protein estimated from the duplicate diets (DD) and the food records (FR) for the three diet groups. The results are presented as mean values (SD)

	Mixed diet	Shellfish diet	Vegetarian diet
Number	34	17	23
DD (g day <sup>-1</sup> )	64 (12)	71 (15)	58 (14)
FR (g day <sup>-1</sup> )	65 (11)	72 (14)	59 (16)
DD/FR	0.98	0.98	0.98

**Table 2** Comparison of the average daily urinary volume and urinary excretion of creatinine, protein (nitrogen), sodium, potassium and calcium in three urinary PABA recovery groups (mean values of 55, 79 and 90%, respectively). The results are presented as median; mean value (SD)

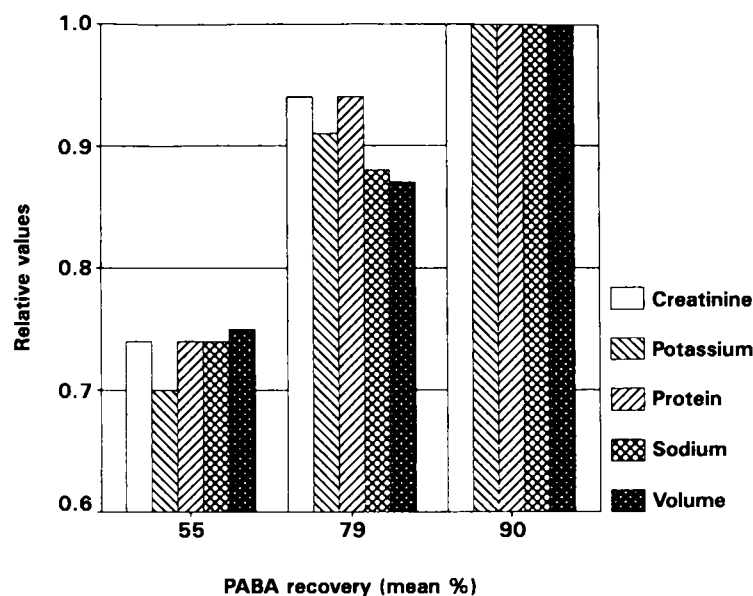
	PABA < 70%	70% < PABA < 85%	PABA ≥ 85%
Number	28	24	21
Age (years)	38; 38 (7)	41; 38 (8)	34; 34 (8)
Height (cm)	166; 167 (6)	166; 166 (5)	165; 167 (8)
Weight (kg)	62; 66 (10)	60; 62 (8)	61; 62 (8)
BMI (kg m <sup>-2</sup> )	22.8; 23.6 (3.2)	22.1; 22.5 (2.6)	21.9; 22.1 (2.2)
Volume (ml)	1328; 1469 (706)	1450; 1689 (872)	1801; 1951 (648)
Creatinine (mg)	804; 839 (245)	1060; 1062 (207)	1184; 1127 (200)
Protein (g)	52; 55 (15)	67; 70 (16)	73; 74 (16)
Sodium (mg)	2032; 2123 (879)	2473; 2541 (1057)	2798; 2881 (1001)
Potassium (mg)	2041; 2100 (786)	2616; 2741 (993)	3004; 3011 (913)

reasons for the low PABA recoveries, the women were regrouped according to PABA recoveries <70, 70–84 and ≥85%, respectively (Table 2). There were no differences in age, height, weight or BMI between the three PABA recovery groups. However, the urinary variables creatinine, volume, protein, sodium and potassium increased with increasing PABA recovery (Table 2 and Fig. 2). This indicates that a low PABA recovery is associated with an incomplete 24-h urine collection.

The dietary intakes of energy, protein, sodium, potassium, calcium and fibre, measured by the FR and DD techniques were compared with the various biomarkers for validation of the two dietary methods (Table 3). The validation was performed on a group level as neither the 24-h urine sample, nor the 4-day FR and DD collection or the faeces sampling corresponding to 4 days diet, are sufficient for estimations on an individual level. There was no statistically significant

difference in the ratios for energy (diet (FR)/BMR<sub>est</sub>) between the diet groups. The ratios for protein (diet (FR)/urine and diet (DD)/urine), sodium (diet (FR)/urine), potassium (diet (FR)/urine + faeces) and calcium (diet (FR)/urine + faeces) varied between 0.74 and 0.93, with an overall mean value of 0.86 (SD 0.06), indicating an underestimation of the dietary intake of approximately 15%. There was no statistically significant difference between the three diet groups.

According to all three fibre validation methods the lowest ratio of 'reported intake/biomarker' was found for the shellfish group (see Table 3). The fibre validation method 3 indicated an underestimation for the shellfish group of 13% (ratio of 0.87), which is in agreement with the overall mean value of under-reporting of about 15%. The mixed and vegetarian diet groups, however, overestimated their fibre intake by about 20% (ratio of 1.2) according to fibre method 3.



**Fig. 2** The relationship of urinary PABA recovery and urinary creatinine, potassium, protein (derived from nitrogen), sodium and volume for the three groups in Table 2. The urinary variables are expressed in relative terms in relation to the highest PABA recovery group, which is set to 1.0.

**Table 3** Comparison of the dietary intakes of energy, protein, sodium, potassium, calcium and fibre, measured by food record (FR), duplicate diet technique (DD), and various biomarkers for food intake in the three diet groups

Variable	Mixed diet (n = 34)	Shellfish diet (n = 17)	Vegetarian diet (n = 23)
<i>Energy</i>			
Diet (FR) (kJ)	7672 (502)	7811 (141)	7766 (180)
BMR <sub>est</sub> (kJ)	5717 (273)	5826 (376)	5664 (350)
Diet/BMR <sub>est</sub>	1.34	1.34	1.37
<i>Protein</i>			
Diet (FR) (g day <sup>-1</sup> )	65 (11)	72 (14)	59 (16)
Urine <sup>1</sup> (g day <sup>-1</sup> )	81 (15)	79 (17)	68 (12)
Diet/urine <sup>1</sup>	0.80	0.91	0.87
<i>Protein</i>			
Diet (DD) (g day <sup>-1</sup> )	64 (12)	71 (15)	58 (14)
Urine <sup>1</sup> (g day <sup>-1</sup> )	81 (15)	79 (17)	68 (12)
Diet/urine <sup>1</sup>	0.78	0.90	0.86
<i>Sodium</i>			
Diet (FR) (mg day <sup>-1</sup> )	2579 (497)	2507 (720)	2146 (717)
Urine (mg day <sup>-1</sup> )	2771 (791)	2934 (963)	2887 (1199)
Diet/urine	0.93	0.85	0.74
<i>Potassium</i>			
Diet (FR) (mg day <sup>-1</sup> )	2855 (712)	3175 (499)	3394 (622)
Urine (mg day <sup>-1</sup> )	2874 (735)	2783 (772)	3411 (1007)
Faeces (mg day <sup>-1</sup> )	584 (321)	682 (265)	854 (280)
Urine + faeces	3428 (892)	3465 (867)	4265 (1095)
Urine/(urine + faeces)	0.83	0.80	0.80
Faeces/(urine + faeces)	0.17	0.20	0.20
Diet/(urine + faeces)	0.83	0.92	0.80
<i>Calcium</i>			
Diet (FR) (mg day <sup>-1</sup> )	904 (263)	891 (256)	1025 (295)
Urine (mg day <sup>-1</sup> )	126 (62)	112 (56)	111 (87)
Faeces (mg day <sup>-1</sup> )	878 (382)	915 (415)	1062 (399)
Urine + faeces (mg day <sup>-1</sup> )	1004 (381)	1028 (423)	1173 (405)
Urine/(urine + faeces)	0.13	0.11	0.09
Faeces/(urine + faeces)	0.87	0.89	0.91
Diet/(urine + faeces)	0.90	0.87	0.87
<i>Fibre, method 1</i>			
Diet (FR) (g day <sup>-1</sup> )	19 (5.8)	19 (4.1)	30 (7.2)
Faecal wet weight (g day <sup>-1</sup> )	107 (39)	127 (59)	142 (50)
Diet/faecal wet weight	0.18 <sup>2</sup>	0.15 <sup>3</sup>	0.21 <sup>2,3</sup>
<i>Fibre, method 2</i>			
Diet (FR) (g day <sup>-1</sup> )	19 (5.8)	19 (4.1)	30 (7.2)
Water in faeces (g day <sup>-1</sup> )	81 (30)	96 (51)	105 (43)
Diet/water in faeces	0.24 <sup>3</sup>	0.20 <sup>4</sup>	0.28 <sup>2,4</sup>
<i>Fibre, method 3</i>			
Diet (FR) (g day <sup>-1</sup> )	19 (5.8)	19 (4.1)	30 (7.2)
Calculated intake (g day <sup>-1</sup> )	16 (9.6)	21 (15)	25 (12)
Diet/calculated intake	1.2	0.87	1.2

<sup>1</sup>6.25 × (nitrogen in urine + 2)<sup>10</sup><sup>2</sup>P < 0.05 between the mixed and the vegetarian diet groups.<sup>3</sup>P < 0.01 between the shellfish and the vegetarian diet groups.<sup>4</sup>P < 0.05 between the shellfish and the vegetarian diet groups.

## Discussion

### *Usefulness of urine and faeces as media for biomarker analysis*

The assessment of dietary intakes based on measurements of biochemical indicators in urine or faeces samples may be seriously affected by incomplete

collection as well as overcollection, problems that seldom have been considered and adjusted for. Though it is difficult, it is, however, crucial to obtain complete collections. Moreover, it is difficult to know when you have a complete collection. In the present study, errors due to overcollection of urine, i.e. sampling for a longer period than 24 h, was revealed

by checking the participant's protocols of start and end times of the collection periods. Incomplete collections were revealed by the PABA method, which presently is the only available test to check the completeness of urine collections<sup>6</sup>. Overcollection would result in low ratios, and incomplete collections would result in high ratios of dietary intake/biomarker, since the denominator (the biomarker) will be erroneously high or low. Incomplete urine collections would mask underreporting of the food intake. In the present study, many subjects (52 out of 73) had a PABA recovery below 85%. On an individual level there may be several reasons for a low PABA recovery, e.g. loss of specimens, PABA tablets not taken or taken too late or errors in PABA determination (see Fig. 1). However, on a group level, the urine volume and urinary excretion of creatinine, nitrogen, sodium and potassium increased with increasing PABA recovery, which indicates that low PABA recoveries were associated with incomplete urine collections (see Table 2 and Fig. 2)<sup>8</sup>.

Many minerals are partly excreted via the faeces, such as calcium (about 90%) and potassium (about 20%). Because faeces sampling is considered to be associated with major practical problems, and difficult to verify, the completeness of markers in faeces are seldom validated. In order to validate the completeness of the faeces sampling in the present study, we compared the content of cadmium in the faeces with that in the corresponding DDs, as cadmium in the diet is almost totally excreted via the faeces<sup>9</sup>. We found essentially the same content in the faeces as in the DDs, indicating that the faeces collections were complete. Our experience from this and other studies is that faeces collection is relatively easy and straightforward if the participants are thoroughly informed prior to the study start. It is rather the sample preparation and analysis that are troublesome, and thus more of a problem to the laboratory personnel than to the participants.

### **Comparison of the FR and the DD techniques**

There was no difference in calculated protein intake based on DDs and FRs. This indicates that on a group level the women were successful in recording what they actually duplicated, in all three diet groups. In general, studies on the DD technique demonstrate a 15% underestimation of the total food intake, compared to other dietary assessment methods and especially compared to biomarkers of food intake<sup>18–20</sup>. However, in the present study, the ratios 'protein intake/biomarker' were about the same for the two dependent methods used, indicating that the two methods were equally good in measuring protein intake. It also shows the impact of thorough quality assurance and quality control.

### **Estimation of the degree of underreporting and validation of the protein, sodium, potassium and calcium intake**

The validation of energy is the most crucial one, as underreporting of a single food component is often related to a general underreporting of the total food intake<sup>21</sup>. The energy intake can be validated with the doubly labelled water technique, which is reliable but very expensive. Another method used to detect underreporting of average dietary intake is based on a comparison of the calculated energy intake with the estimated basal metabolic rate<sup>3,22</sup>. The ratio 'calculated energy intake/BMR<sub>est</sub>' may be referred to as the food intake level (FIL) value. The FIL value may be compared with a physical activity level (PAL) and the ratio FIL/PAL should equal 1.0 if there is a true estimate of the energy intake and the energy expenditure. In this way the energy validation may be comparable with the validation of the other food components (protein, sodium, potassium and fibre), as a true estimate of these food components will yield a ratio value of 1.0, as would a true FIL/PAL value. One drawback with this study is that we have no measurements of the energy expenditure or the PAL. In order to make a comparison with the FIL, we have made an estimation of the PAL to be between 1.6 and 1.7, based on the observed lifestyles of the women. If in the present study we divide the calculated FIL values of 1.34, 1.34 and 1.37, respectively, by our assumed PAL of 1.6 and 1.7, the FIL/PAL values would in the first case be 0.84, 0.84 and 0.86 for the mixed, shellfish and vegetarian diet groups, respectively, and in the second case 0.79, 0.79 and 0.81, respectively. This would indicate an underestimation of the total food intake of about 10–20% for the three diet groups. If we reverse this process to estimate the PAL, and make the assumption that underreporting of 14% indicated by the biological markers (except fibre) indicate a general underreporting of the total food intake, the PAL would be approximately 1.6 (1.34/0.86, 1.34/0.86 and 1.37/0.86).

A third method of energy validation is based on the protein validation<sup>11</sup>. In the equation of Isaksson<sup>11</sup> there is a factor of 6.25 for the conversion of nitrogen to protein that is based on a mixed diet. In our study there were relatively small differences in dietary intake between the three groups in foods that varied in the conversion factor of nitrogen to protein, except that the vegetarian group and the shellfish group consumed more cereals and less milk products compared to the mixed diet group. This affects the conversion factor since cereals have a conversion factor of approximately 5.8 and milk products a factor of 6.38. Conversely, the factor 2 in Isaksson's equation is probably too low for the vegetarian group. This counteracts the effect of the lower conversion factor for cereals.

Overall, we estimate that this difference would not make a significant difference in our conclusions and that this method indicated an underestimation of the true habitual food intake of approximately 10–20% for both the FR and DD technique in all three diet groups. This is in agreement with the energy validation, indicating that the protein validation could be used for energy validation<sup>10,11,13,22,23</sup>. However, this is not always possible<sup>24</sup>.

The ratio values of 'dietary intake/biomarker' for potassium and calcium, with no significant difference between diet groups, were in agreement with the ratio values for energy and protein, indicating that these nutrients were related to the total food (energy) intake, and that most foods were underestimated to about the same extent.

Previous studies have shown a close agreement between sodium (salt) intake and sodium urinary excretion<sup>25</sup>. The faecal loss of sodium is very low, and in temperate climates the excretion via sweat is also minimal<sup>3</sup>. The use of table and cooking salt, and the variation in the sodium content of manufactured foods, make it difficult to rely on food tables in estimating sodium intake. There was no difference between the diet groups in the ratio 'dietary intake/biomarker for sodium'. In the vegetarian group, a low intake according to the food record compared to the urine marker indicating that the vegetarians add more salt to the food than is recommended in the recipes and is used in the food tables, compared to the other two diet groups.

### Validation of fibre intake

The fibre intake is seldom validated since, like minerals, the validation test is based on faecal collections. In the present study, three different methods for fibre validations were used, all based on faeces. All three methods demonstrated the lowest ratio 'fibre intake/biomarker' for the shellfish group, and they underestimated their fibre intake to the same extent as their intake of energy and other nutrients (of about 15%). The mixed diet group had the lowest fibre intake according to all three biomarkers for fibre. The potassium excretion in faeces was also the lowest in the mixed diet group, which is in line with a low fibre intake<sup>13,25,26</sup>. The vegetarian diet group had the highest fibre intake according to all three biomarkers for fibre. However, both the vegetarian and the mixed diet group overestimated their fibre intake to the same extent, of about 20%. An overestimation of the fibre intake may partly be explained by difficulties in classifying bread and breakfast cereals, because of poor labelling of fibre content or ignorance over classifying these products with respect to fibre content. It may also be explained by a greater awareness of high-fibre foods and their significance, making these

foods important to remember, perhaps resulting in an overestimation of the level of intake. Fibre-rich foods are regarded as healthy and therefore more likely to be remembered, especially when people are confronted with dietary studies and nutritionists<sup>13</sup>. There might have been a tendency to classify some food items as wholemeal and high-fibre products, even though they were not. This will lead to an overestimation of the fibre intake.

To conclude, the ratio of food intake according to the dietary assessment methods and the biological markers for food intake for protein, sodium, potassium and calcium reached a mean value of 0.86 (under-reporting by 14%). The mixed and vegetarian diet groups overestimated their fibre intake by 20% while the shellfish group underestimated the fibre intake by 13%. This indicates that underreporting and over-reporting is selective to certain foods and nutrients and varies between groups of people. This has implications for how we deal with the underreporting often seen in nutritional epidemiological studies.

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### References

- 1 Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences and National Research Council. Methodological considerations in evaluating the evidence. In: *Diet and Health: Implications for Reducing Chronic Disease Risk*. Washington DC: National Academy Press, 1989: 23–40.
- 2 Hunter D. Biochemical indicators of dietary intake. In: *Nutritional Epidemiology*, Willett W, ed. New York: Oxford University Press, 1990: 143–216.
- 3 Bingham S. The dietary assessment of individuals: methods, accuracy, new techniques and recommendations. *Nutr. Abstr. Rev.* 1987; **57**: 705–42.
- 4 Berglund M, Åkesson A, Nermell B, Vahter M. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ. Health Perspect.* 1994; **102**: 1058–66.
- 5 Vahter M, Berglund M, Nermell B, Åkesson A. Bioavailability of cadmium from shellfish and mixed diet in women. *Toxicol. Appl. Pharmacol.* 1996; **136**: 332–41.
- 6 Bingham S, Cummings JH. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clin. Sci.* 1983; **64**: 629–35.
- 7 Hare R. Endogenous creatinine serum and urine. *Proc. Soc. Exp. Biol. Med.* 1950; **74**: 148–51.
- 8 Johansson G, Bingham SA, Vahter M. A method to compensate for incomplete 24-hour urine collections in

- nutritional epidemiological studies. *Eur. J. Clin. Nutr.* (in press).
- 9 Department of Health. *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects.* London: HMSO, 1991.
  - 10 Bingham S, Cummings JH. Urine nitrogen as an independent validity measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. *Am. J. Clin. Nutr.* 1985; **42**: 1276–89.
  - 11 Isaksson B. Urinary nitrogen output as a validity test in dietary surveys. *Am. J. Clin. Nutr.* 1980; **33**: 4–5.
  - 12 Fregly MJ. Attempts to estimate sodium intake in humans. In: Horan MJ, Blaustein M, Dunbar JB, Dachadorian W, Kaplan NB, Simopoulos AP, eds. *NIH Workshop on Nutrition and Hypertension.* New York: Biomedical Information, 1985: 93–112.
  - 13 Johansson G, Callmer E, Gustafsson J-Å. Validity of repeated dietary measurements in a dietary intervention study. *Eur. J. Clin. Nutr.* 1992; **46**: 717–28.
  - 14 Bingham S. Low-residue diets: a reappraisal of their meaning and content. *J. Hum. Nutr.* 1979; **33**: 5–16.
  - 15 Jacobsen AE, Newmark HL, Bright-See E, McKeown-Eyssen G, Bruce R. Biochemical changes as a result of increased fiber consumption. *Nutr. Rep. Int.* 1984; **30**: 1049–59.
  - 16 Cummings JH, Bingham SA, Heaton KW, Eastwood MA. Fecal weight, colon cancer risk and dietary intake of non-starch polysaccharides (dietary fiber). *Gastroenterology* 1992; **103**: 1783–9.
  - 17 Wrick KL, Robertson JB, van Soest PJ, *et al.* The influence of dietary fibre source on human intestinal transit and stool output. *J. Nutr.* 1983; **113**: 1464–79.
  - 18 Bingham S, Wiggins HS, Englyst H, *et al.* Methods and validity of dietary assessments in four Scandinavian populations. *Nutr. Cancer* 1982; **4**: 23–33.
  - 19 Bro S, Sandström B, Heydorn K. Intake of essential and toxic trace elements in a random sample of Danish men as determined by the duplicate portion sampling technique. *J. Trace Elem. Electol. Health Dis.* 1990; **4**: 147–55.
  - 20 Isaksson B. A critical evaluation of the duplicate-portion technique in dietary surveys. *Eur. J. Clin. Nutr.* 1993; **47**: 457–60.
  - 21 Willet W. Implications of total energy intake for epidemiological analyses. In: Willet W, ed. *Nutritional Epidemiology.* Oxford: Oxford University Press, 1990: 245–71.
  - 22 Bingham SA. The use of 24-h urine samples and energy expenditure to validate dietary assessments. *Am. J. Clin. Nutr.* 1994; **59** (suppl): 227S–31S.
  - 23 Black AE, Jebb SA, Bingham SA. Validation of energy and protein intakes assessed by diet history and weighed records against energy expenditure and 24-h urinary nitrogen excretion. *Proc. Nutr. Soc.* 1991; **50**: 108A.
  - 24 Black AE, Bingham SA, Johansson G, Coward WA. Validation of dietary intakes of protein and energy against 24 hour urinary N and DLW energy expenditure in middle-aged women, retired men and post-obese subjects: comparison with validation against presumed energy requirements. *Eur. J. Clin. Nutr.* 1997; **51**: 405–13.
  - 25 Schachter J, Harper PH, Radin ME, Caggiula AW, McDonald RH, Diven WF. Comparison of sodium and potassium intake with excretion. *Hypertension* 1980; **2**: 695–9.
  - 26 Cummings JH, Hill MJ, Jenkins DJA, Pearson JR, Wiggins HS. Changes in fecal composition and colonic function due to cereal fibre. *Am. J. Clin. Nutr.* 1976; **29**: 1468–73.