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Two non-consecutive 24 h recalls using EPIC-Soft software are sufficiently valid for comparing protein and potassium intake between five European centres – results from the European Food Consumption Validation (EFCOVAL) study

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Abstract

The use of two non-consecutive 24 h recalls using EPIC-Soft for standardised dietary monitoring in European countries has previously been proposed in the European Food Consumption Survey Method consortium. Whether this methodology is sufficiently valid to assess nutrient intake in a comparable way, among populations with different food patterns in Europe, is the subject of study in the European Food Consumption Validation consortium. The objective of the study was to compare the validity of usual protein and K intake estimated from two non-consecutive standardised 24 h recalls using EPIC-Soft between five selected centres in Europe. A total of 600 adults, aged 45–65 years, were recruited in Belgium, the Czech Republic, France, The Netherlands and Norway. From each participant, two 24 h recalls and two 24 h urines were collected. The mean and distribution of usual protein and K intake, as well as the ranking of intake, were compared with protein and K excretions within and between centres. Underestimation of protein (range 2–13 %) and K (range 4–17 %) intake was seen in all centres, except in the Czech Republic. We found a fair agreement between prevalences estimated based on the intake and excretion data at the lower end of the usual intake distribution (< 10 % difference), but larger differences at other points. Protein and K intake was moderately correlated with excretion within the centres (ranges = 0·39–0·67 and 0·37–0·69, respectively). These were comparable across centres. In conclusion, two standardised 24 h recalls (EPIC-Soft) appear to be sufficiently valid for assessing and comparing the mean and distribution of protein and K intake across five centres in Europe as well as for ranking individuals.

Key words: **Nutrient intake: Diet: Protein: Biomarkers: Validity: Dietary recalls**

National food consumption surveys aim to provide information on the mean and distribution of food and nutrient intakes of the population and related subgroups, in order to develop and evaluate nutrition policies. In addition, national food consumption surveys are essential to provide data for risk assessment work, as conducted

Abbreviations: EFCOVAL, European Food Consumption Validation; EPIC, European Prospective Investigation into Cancer and Nutrition; FCT, food composition tables; MSM, multiple source method; PABA, *para*-aminobenzoic acid.

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by the European Food Safety Authority[1]. In Europe, food consumption data originating from national surveys are not always comparable because they differ in a number of aspects, such as the choice of the dietary assessment method and the reference period of the data collection[2–4]. Furthermore, some countries do not have national food consumption surveys in place[4].

The European Food Consumption Survey Method consortium has acknowledged the need for policy-relevant dietary indicators that are comparable among European countries, which could contribute to the establishment of a Community Health Monitoring System[5]. They recommended two non-consecutive days of 24 h recall using EPIC-Soft software (Lyon, Rhone Alpes, France) as the preferred method to assess the dietary intake in future pan-European monitoring surveys in adults. In addition, they specified total fat, SFA and ethanol as the components of most relevance in this assessment[6–8].

The 24 h recall is a commonly used dietary assessment method in food consumption surveys in Europe[4] and is also being used in surveys in the USA[9], Canada[10], Australia[11] and New Zealand[12]. A major advantage of using 24 h recalls in (inter)national surveys is that the method is useful for comparison of heterogeneous populations with different ethnicity and literacy[6]. In addition, a computerised version of 24 h recalls seems to be the best means of standardising and controlling for sources of error attributable to 24 h recall interviews[6,13]. Nevertheless, computerised 24 h recalls need to be tailor-made to every included country and/or study, e.g. by adaptations of the food and recipe list. Therefore, whether this methodology performs in a comparable way across countries with different food consumption patterns in Europe deserves further exploration, as validity of the 24 h recall depends on both the characteristics of the method and the study population.

Biological markers offer an important opportunity to evaluate the dietary assessment methods since errors are likely to be truly independent between the measurements of biomarker and dietary intake[14]. Urinary N and K are two of the few available recovery biomarkers to assess the nutrient intakes[15,16]. With the use of these two biomarkers, a single 24 h recall using EPIC-Soft has been previously validated for assessing the group mean intakes of protein of twelve centres in six countries within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study[17]. Yet, the accuracy of this methodology needs to be determined when aiming at estimating usual dietary intake among different European populations by collecting two independent 24 h recalls. Hence, following the path of the European Food Consumption Survey Method (EFCOSUM), the European Food Consumption Validation (EFCOVAL) consortium aimed to further develop and validate a European food consumption method using EPIC-Soft software for assessing the food and nutrient intakes within European countries and for comparisons between them. In the present paper, we aim to compare the validity of usual protein and K intake estimated from two non-consecutive standardised 24 h recalls using EPIC-Soft between five selected centres in Europe. This was done by addressing the bias present in the estimation of each centre's mean and distribution of intake as well as the ranking of individuals within and between centres according to their intake.

Subjects and methods

Subjects

Data were collected in five European countries: Belgium, the Czech Republic, France (Southern part), The Netherlands and Norway. These countries were selected to represent a large variety in food patterns across Europe. Data were collected in the South of France to include the characteristics of the Mediterranean diet. A food pattern from Central/Eastern Europe was represented by the Czech Republic, from the Scandinavian countries by Norway and from the western part of Europe by Belgium and The Netherlands. Another reason for their selection was their experience in performing nutrition monitoring surveys. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by ethical committees in each centre involved in the data collection.

We recruited subjects by convenience sampling through advertisements (newspaper and websites), mailing lists, among others. Recruitment of institutionalised subjects was not allowed, nor included more than one member of a household. Subjects were informed about the study through information meetings at the institutions/universities in the Czech Republic, France and The Netherlands, and by phone, letter and personally in Belgium and Norway. At these occasions, a screening questionnaire was filled in to confirm the subjects' eligibility in the study. Subsequently, the eligible participants gave written informed consent, and appointments for later visits were scheduled. Exclusion criteria were currently taking diuretics, following prescribed dietary therapy, being enrolled in another study in the same period, not being able to read or speak the national language, being pregnant, lactating, having diabetes mellitus or kidney disease and donating blood or plasma during or <4 weeks before the study. *para*-Aminobenzoic acid (PABA) was used to check the completeness of urine collections; therefore, subjects hypersensitive to PABA or taking antibiotics containing sulphonamides, which are PABA-antagonistic, were not eligible for the study.

Taking into account an anticipated dropout percentage of 20 % and aiming at a net sample of fifty per stratum, a total of sixty men and sixty women were recruited per centre (n 600). The age range of subjects was 45–65 years, which was chosen to limit the heterogeneity of the

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sample. Furthermore, we aimed to include at least ten men and ten women in each of the three predetermined categories of education level (low, intermediate and high) per centre. We used country-specific classifications to define each category level.

We excluded one subject because no data for recall and biomarker collected on the same day were available. Therefore, the study population comprised 599 subjects (296 men and 303 women).

Study design

Wageningen University (The Netherlands) was, as the coordinating centre, responsible for the overall logistics of the validation study in the EFCOVAL consortium. For standardisation, all study procedures, i.e. on recruitment and fieldwork conditions, data processing formats, quality-control aspects and specimen collection, storage and transport details, were described in protocols. The recruitment of subjects and data collection in The Netherlands were performed from April to July 2007, 6 months before the other four centres, in order to test all the procedures of the fieldwork beforehand and to be able to refine the protocols. The other centres started the fieldwork in October or November 2007, with the last centre finalising the collection by April 2008.

At the beginning of the study, subjects had their body weight and height measured in the study centres. Then, a 24 h recall and a 24 h urine collection were obtained covering the same reference day. Subjects were aware of the days of data collection but not of the purpose of the interviews. The second recall and urine collection were obtained at least 1 month after the first one.

Dietary data

The two 24 h recalls were collected using two modes of administration: one by phone and one face-to-face at the centre since it is likely that future food consumption monitoring surveys will be conducted in both ways across European countries. The order of the two modes of administration was randomly allocated among the subjects.

Furthermore, the appointments for the dietary recalls followed a randomised schedule, which included all days of the week. This randomisation allowed the same person to have the same recalled weekday for both interviews by chance. Interviewers in each centre were nutritionists or dietitians who were trained in interviewing skills and working with EPIC-Soft in the context of the validation study. They were guided by qualified local trainers who were previously trained by staff from the Wageningen coordination centre and the National Institute for Public Health and the Environment in The Netherlands. Interviewers were aware of the objectives of the study. The centres were allowed to organise their data collection in the same way they would do in a future performance of their nutritional surveillance system. An example is that interviewees were permitted to check food packages and household measures in their home for more detailed information during the phone interview while this was not possible during the face-to-face interview at the study centre. Another example is that dietary recalls in Belgium, the Czech Republic and The Netherlands were not conducted on Sundays. Therefore, Saturday's intake was recalled 2 d later, on Mondays.

The two 24 h recalls were collected using EPIC-Soft (version 9.16). The structure and standardisation procedure of EPIC-Soft have been described elsewhere[18,19]. Briefly, EPIC-Soft is a computer-assisted 24 h dietary recall that follows standardised steps when describing, quantifying, probing and calculating the food intakes[18]. All the participating countries had an existing version of EPIC-Soft available, except the Czech Republic for which a new country-specific version was developed. In addition, EPIC-Soft databases were adapted for each centre in terms of some common specifications for the EFCOVAL study (e.g. soups were treated as recipes rather than food items). Furthermore, the centres generated or updated a list of the single food items and recipes expected to be consumed by their participants. Modifications of such lists were needed afterwards based on notes made during the interview. The methods of estimation of portion size included household measures, weight/volume, standard units and portions, bread shapes and photographs. The set of photographs was developed in the context of the EPIC study[20]. Each centre chose from the EPIC portfolio of photographs the pictures that best represented their national food habits.

In the absence of harmonised recent food composition tables (FCT) including all countries of our assessment, protein and K contents in foods were calculated using country-specific FCT[21–24]. Carbohydrates, total fat, saturated fat, alcohol and dietary fibre intake as well as energy content were also calculated. We calculated energy values by summing the contributions from protein, carbohydrates, fat and alcohol and using related Atwater factors (17, 17, 37 and 29 kJ/g, respectively). In the Czech Republic, the national FCT was published about 20 years ago. Therefore, a FCT was compiled for EFCOVAL purposes in the Czech Republic with composition of most foods based on the Slovakian tables[25]. In all the centres, missing nutrient data for a food was imputed from a similar food or another FCT, based on country-specific decisions; but in a few cases, this was not possible for K, saturated fat, dietary fibre and alcohol. The percentage of missing values was $<6\%$ of all reported foods for all nutrients.

Twenty-four hour urine collections and recovery biomarkers

The subjects were instructed not to make use of acetaminophen painkillers, such as paracetamol, and sulphonamide drugs, during the days of urine collection. To check the

completeness of urinary collections, one tablet of 80 mg PABA (PABAcheck; Laboratories for Applied Biology, London, UK) had to be taken three times on the day of the urine collection: with the morning, midday and evening meals. Hence, we expected that 240 mg of PABA would be almost completely excreted within 24 h[26,27]. The collection of the 24 h urine started with voiding and discarding the first urine in the morning after waking up. Subsequently, the urine excreted during the next 24 h, up to and including the first voiding of the following day, was collected. For this purpose, each subject received labelled containers (at least two), one funnel to help the collection, one safety pin to be fixed in the underwear as a reminder for collection and a diary scheme booklet to register the timing, observations (e.g. use of medication and supplements) and possible deviations (e.g. missing urine) of the urine collection protocol. Boric acid (3 g/2 litre bottle) was used as preservative. The subjects provided their urine samples to the dietitians at the study centre when a face-to-face dietary recall was scheduled. If the 24 h recall interview was by phone, urine samples were collected at the subject's home or delivered to the study centre. When a long period was anticipated between the end of the collection and the receiving of samples, subjects were instructed to keep the urine samples at approximately 4°C, which in most cases was not more than 12 h. To verify the stability of PABA in urine, a pooled urine sample of three participants from The Netherlands were kept at four different temperatures (−20, 6, 20 and 30°C) for 8 d. At five moments (days 0, 1, 2, 4 and 7), PABA concentrations were measured. No significant changes in PABA concentrations were observed during the storage period at each temperature. The regression equation for PABA content as a function of time during storage at 20°C (assumed to be the most common storage temperature) was as follows: PABA (mg/l) = 140·2, −0·8 (time in days) with the 95% CI for the time coefficient being −2·5, 0·8.

At the laboratory of the local centres, urine was mixed, weighed and aliquoted. Then, the specimens were stored at −20°C until shipment on dry ice to the central laboratory at Wageningen University, where they were kept at the same temperature.

Chemical analysis

On the day of chemical analysis, aliquots were rapidly thawed at room temperature. Urinary N was determined colorimetrically by the Kjeldahl technique on a Kjeltec 2300 analyser (Foss, Hilleroed, Denmark) after destruction of the sample with concentrated sulphuric acid. Urinary K was measured by an ion-selective electrode on a Synchron LX20 analyzer (Beckman Coulter, Mijdrecht, The Netherlands). PABA was measured by colorimetry[28]. The intra-assay precision, expressed as CV, of these three analyses was <2%. Taking into account the extra-renal losses (approximately 19%) and the fact that protein on average contains 16% N, urinary protein was calculated as (6·25 × (urinary N/0·81))[15,29]. Urinary K was estimated by dividing the measured value by 0·77, assuming that 77% of K intake is excreted through the urine when considering faecal excretion[16,30].

Urine samples with PABA recoveries <50% were treated as incomplete and excluded from the data analysis (n 14). Additionally, the subjects who took drugs containing sulphonamides or acetaminophen or one who took less than three PABA tablets had their urine diaries checked for other deviations in the urine collection. In cases where other deviations were observed, namely urine loss during the collection or absent registration of collection time, samples were excluded from the analysis (n 4). Otherwise, samples were included (n 13) as we did not want to exclude potentially complete urines. Results of the present paper did not change by excluding these subjects. As described before[31], specimens containing between 50 and 85% of PABA recovery (n 105) had their urinary concentrations proportionally adjusted to 93% of PABA recovery. Recoveries >85% were included in data analyses without adjustments (n 1062).

Data analysis

The analyses were performed using SAS statistical package, version 9.1 (SAS Institute, Inc., Cary, NC, USA). The statistical analyses were stratified by sex and using the average of 2 d of intake and excretion, except for eighteen subjects who only had 1 d of 24 h recall and biomarker. For these subjects, the 24 h recall matched with the day of the urine collection. To assess the presence of bias (systematic errors), the mean difference between nutrient intake and excretion was calculated. ANCOVA followed by the Tukey *post hoc* test was used for testing whether biases differed between the centres. The ANCOVA model included age (continuous), education level (three categories) and BMI (continuous), given that stratified analysis of these variables showed us differential performance of the method within and between the centres. To estimate and compare the distribution of usual intake and excretion of protein and K between the centres, the multiple source method (MSM) was used as the measurement error model[32]. This model removes the effect of day-to-day variability and random error in the two 24 h recalls and biomarker estimates. The MSM was developed in the framework of the EFCOVAL study and enabled us to estimate individual usual intake. We decided not to use covariates in the calculation of usual intakes with the MSM. Plots of usual intake distributions based on the 24 h recall and biomarker were created using R software, version 2.8.1 (http://CRAN.R-project.org). The percentages of subjects consuming above certain cut-off points for each distribution curve were calculated. For both sexes, we specified eleven cut-off points to cover the whole range of protein

and K intake among the five centres. For the evaluation of ranking of individuals, we computed Pearson's correlation coefficients. For adjusted correlations, we used usual intake and excretion data corrected for within-person variability, as estimated by the MSM, and further corrected for age, BMI and education level by using partial Pearson correlations. CI of the correlations were obtained using the Fisher *Z*-transformation[33]. Energy-adjusted correlations were calculated using the residual method[34]. To test the equality of correlations, pairwise comparisons were made using Fisher *Z*-transformation[33]. Pooled correlations of the five centres were calculated by first converting the correlations into a standard normal metric (Fisher's *r*-to-*Z* transformation). Next, the pooled average was calculated, in which each transformed correlation coefficient was weighted by its inverse variance, followed by the back transformation[33]. The Cochrane *Q* test was used for testing the heterogeneity of the pooled correlation[35].

Results

The mean age of the subjects was similar in the five centres (Table 1). In both sexes, mean BMI was comparable across the centres (ranges 23·2–25·5 kg/m^2 in women and 25·5–27·9 kg/m^2 in men). Subjects with moderate and high education levels were over-represented in the study compared with individuals with a low education level, especially men in Norway. The variations in energy intake across the centres were less pronounced than in macronutrients, especially for carbohydrates.

A degree of underestimation was seen in the assessment of protein intake in all the centres. Underestimation varied from 2·7 % (Norway) to 12·4 % (The Netherlands) in men and from 2·3 % (Norway) to 12·8 % (France) in women, based on the crude differences between intake and excretion (Table 2). After adjusting for age, BMI and education level, the bias did not differ between the centres for women. However, men in the Czech Republic had a significantly smaller bias compared with those in France and The Netherlands. For K, the underestimation varied from 1·7 % in Norway to 17·1 % in France for men and from 6·6 % in The Netherlands to 13 % in France for women. An overestimation of 5·9 % for men and 1·6 % for women was found in the Czech Republic. A statistically significant difference in the adjusted bias was seen in men between France and three other centres: Belgium, the Czech Republic, The Netherlands. In women, differences were statistically significant only between France and the Czech Republic. BMI was the only factor influencing the differences between the countries at a significant level ($P<0·01$ for all analyses, except for K in women; $P=0·16$). Upon inclusion of energy intake into the ANCOVA model, the conclusion about the differences between the centres changed only for protein results in men, which lost statistical significance ($P=0·08$).

Table 1. Characteristics of five European centres in the European Food Consumption Validation Study*
(Mean values with their standard errors)

	Men										Women									
	BE (*n* 63)		CZ (*n* 58)		FR (*n* 54)		NL (*n* 59)		NO (*n* 62)		BE (*n* 60)		CZ (*n* 60)		FR (*n* 59)		NL (*n* 62)		NO (*n* 62)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	54	5·5	55	6·9	56	5·4	57	4·3	55	6·0	55	5·0	55	6·1	55	6·0	55	5·6	54	6·0
Wt (kg)	81·1	13·3	85·7	13·2	78·1	9·7	83·8	14·4	85·7	9·9	67·6	12·5	66·8	9·8	60·6	8·6	71·4	13·8	68·4	11·4
Ht (cm)	175·6	7·1	175·4	6·4	174·8	7·0	177·5	8·8	179·9	7·2	163·6	6·8	163·8	6·1	161·6	6·7	167·6	8·8	166·0	6·8
BMI (kg/m^2)	27·2	3·6	27·9	4·2	25·5	2·7	26·5	3·8	26·4	2·5	25·2	4·2	25·0	3·9	23·2	3·0	25·5	5·0	24·8	3·7
Energy (MJ/d)	11·0	0·3	12·1	0·5	10·4	0·3	11·2	0·4	11·8	0·4	8·4	0·3	8·4	0·2	8·1	0·2	8·6	0·3	8·4	0·3
Energy (% protein)	16·0	0·4	14·5	0·3	15·9	0·4	15·8	0·4	17·2	0·5	16·1	0·4	14·8	0·4	16·0	0·3	15·4	0·4	17·9	0·5
Energy (% total fat)	35·2	0·8	34·7	0·8	35·8	0·8	34·1	0·8	36·0	1·1	33·8	0·8	34·0	1·0	39·3	0·9	34·6	0·9	38·6	1·0
Energy (% carbohydrates)	41·6	0·9	47·0	1·1	44·0	1·0	43·1	1·0	42·8	1·1	44·8	1·0	49·1	1·1	42·4	1·0	46·0	0·9	40·0	1·1
Energy (% saturated fat)	13·7	0·4	12·7	0·3	13·7	0·4	13·0	0·4	13·9	0·6	13·7	0·4	12·8	0·4	14·0	0·5	12·5	0·4	14·8	0·5
Alcohol (g/d)	30·2	4·2	17·8	3·4	15·1	2·5	27·6	3·4	16·5	2·8	17·3	2·7	6·3	1·3	6·9	1·3	12·3	2·0	10·7	2·1
Dietary fibre (g/MJ per d)	2·3	0·1	2·5	0·1	2·2	0·1	2·4	0·1	2·5	0·1	2·7	0·1	3·1	0·1	2·7	0·1	3·0	0·1	2·7	0·1
Education (% of total)																				
Low	15·9		20·7		25·9		20·3		3·2		16·7		16·6		35·6		24·2		16·1	
Intermediate	23·8		24·1		24·1		20·3		30·7		25·0		46·7		27·1		40·3		19·4	
High	60·3		55·2		50·0		59·4		66·1		58·3		36·7		37·3		35·5		64·5	

BE, Belgium; CZ, Czech Republic; FR, France; NL, The Netherlands; NO, Norway.
* Dietary intake based on 2 × 24 h recalls.

Table 2. Protein and potassium intake and excretion based on 2 × 24 h recalls and 2 × 24 h urinary biomarkers for five European centres in the European Food Consumption Validation Study (Mean values with their standard errors)

	Men										
	BE (*n* 63)		CZ (*n* 58)		FR (*n* 54)		NL (*n* 59)		NO (*n* 62)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P**
Protein (g)											
Intake	101·7	3·3	100·4	4·2	95·9	3·4	101·5	3·5	115·2	3·8	
Excretion†	110·8	3·2	104·1	3·0	109·1	2·8	115·9	3·6	118·4	3·1	
% Crude difference	−8·2		−3·5		−12·1		−12·4		−2·7		
Adjusted difference	−7·5[a,b]	3·4	−1·4[a]	3·6	−14·7[b]	3·6	−14·1[b]	3·6	−2·3[a,b]	3·6	0·02
K (mg)											
Intake	4024	131	3726	164	3464	138	4326	139	4847	182	
Excretion‡	4301	148	3517	143	4180	141	4491	157	4935	138	
% Crude difference	−6·4		+5·9		−17·1		−3·7		−1·7		
Adjusted difference	−230[a,b]	144	282[a]	150	−759[b]	153	−123[a]	150	−66[a]	151	<0·01

	Women										
	BE (*n* 60)		CZ (*n* 60)		FR (*n* 59)		NL (*n* 62)		NO (*n* 62)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Protein (g)											
Intake	79·0	2·5	70·8	2·1	74·7	1·9	78·2	3·3	85·5	2·6	
Excretion†	87·5	2·6	78·8	2·2	85·7	2·0	85·1	2·9	87·5	2·1	
% Crude difference	−9·7		−2·7		−12·8		−8·2		−2·3		
Adjusted difference	−7·9	2·5	−7·9	2·5	−12·2	2·5	−6·3	2·4	−1·8	2·5	0·07
K (mg)											
Intake	3513	148	3155	143	3146	141	3618	157	3630	138	
Excretion‡	3928	138	3150	111	3617	124	3871	142	3899	102	
% Crude difference	−10·5		+1·6		−13·0		−6·6		−6·9		
Adjusted difference	−414[a,b]	115	9[a]	113	−503[b]	114	−224[a,b]	110	−274[a,b]	114	0·02

BE, Belgium; CZ, Czech Republic; FR, France; NL, The Netherlands; NO, Norway.

[a,b] Mean values with unlike superscript letters were significantly different between the countries ($P<0·05$).

* One-way ANCOVA (general linear model) based on mean difference between intake and excretion. Tukey's *post hoc* test was used for pairwise comparison between the countries. ANCOVA model included age, BMI and educational level.

† Urinary protein = (urinary N/0·81) × 6·25[15].

‡ Urinary K = (urinary K/0·77)[16].

Additionally, when we pooled the data from all the countries, no consistent trend in mean protein and K biases was observed across the different education levels and modes of administration (data not shown).

The bias in mean intake can also be observed when comparing the distributions of usual intake based on food consumption data with those obtained from excretion data (the supplementary material for this article can be found at http://www.journals.cambridge.org/bjn). The intake data curve shifted somewhat to the left (underestimation of intake) for almost all the centres compared with the excretion data. Since the prevalence of subjects consuming below or above a certain cut-off point is an important indicator for a population's nutritional status, we assessed and compared the prevalence of subjects consuming above specific cut-off points for both usual intake and usual excretion distributions (see Fig. 1 for results of protein in males and the supplementary material 'for results in females and results of K in both sexes' can be found at http://www.journals.cambridge.org/bjn). Overall, we found a fair agreement between prevalences estimated based on the intake and excretion data at the lower end of the usual protein and K intake distribution, but larger differences at middle cut-off levels. For protein in men, the smallest differences in prevalence between intake and excretion were seen in Norway (up to 15%) and the largest ones in France (up to 46%) and The Netherlands (up to 41%). For women, the smallest differences were seen in Norway (up to 11%) and the largest ones in the Czech Republic (up to 38%) and France (up to 55%). The smallest difference between K intake and excretion distribution in males was observed in The Netherlands (up to 7%) while the larger differences were seen in the Czech Republic and France (up to 21 and 40%, respectively). In women, France was the centre with the largest difference (up to 29%) between K usual intake and excretion, and The Netherlands the smallest (up to 17%).

Unadjusted Pearson correlation coefficients between average protein intake and its biomarker within centres ranged between 0·42 and 0·65 in men and between 0·46

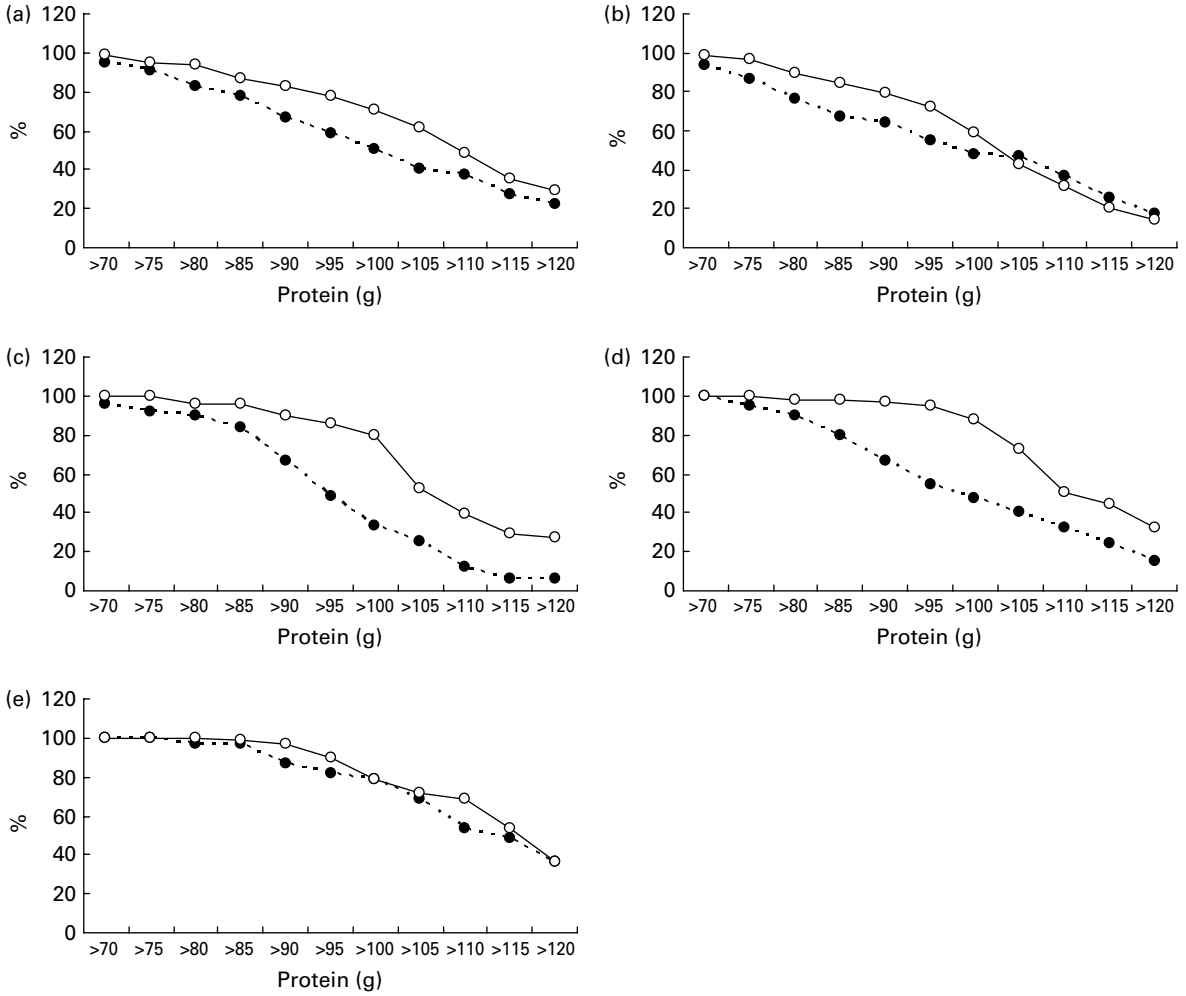

Fig. 1. Prevalence of men consuming above specific amounts of protein as estimated by usual intake distributions (an usual intake/excretion distribution estimated by the multiple source method (see 'Methods' section)) from dietary recalls (intake) and biomarkers (excretion) for five European centres in the European Food Consumption Validation Study. (a) Belgium, (b) Czech Republic, (c) France, (d) The Netherlands, (e) Norway. --●--, Intake; –○–, excretion.

Table 3. Pearson coefficients of correlation between protein intake and urinary excretion* for five European centres in the European Food Consumption Validation Study†
(Mean values and 95 % confidence intervals)

	Men							Women						
		Unadjusted		Adjusted‡		Energy-adjusted§			Unadjusted		Adjusted		Energy-adjusted	
Centres	*n*	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*n*	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
Protein intake														
Belgium	58	0·48	0·27, 0·65	0·49	0·27, 0·67	0·48	0·26, 0·66	62	0·57	0·37, 0·72	0·57	0·35, 0·72	0·35	0·13, 0·59
Czech Republic	58	0·50	0·28, 0·67	0·43	0·18, 0·62	0·25	−0·01, 0·49	58	0·56	0·35, 0·71	0·57	0·35, 0·72	0·49	0·29, 0·69
France	55	0·65	0·46, 0·78	0·67	0·47, 0·81	0·65	0·44, 0·79	48	0·46	0·23, 0·64	0·39	0·13, 0·60	0·51	0·27, 0·69
The Netherlands	58	0·42	0·18, 0·61	0·51	0·29, 0·68	0·47	0·24, 0·65	59	0·51	0·29, 0·67	0·63	0·44, 0·77	0·34	0·15, 0·60
Norway	61	0·52	0·32, 0·69	0·47	0·24, 0·65	0·50	0·27, 0·67	60	0·53	0·33, 0·69	0·52	0·30, 0·68	0·41	0·20, 0·62
Pooled‖	290	0·52	0·40, 0·63	0·51	0·39, 0·63	0·50	0·38, 0·62	287	0·53	0·41, 0·64	0·60	0·42, 0·66	0·45	0·33, 0·57
K intake														
Belgium	58	0·54	0·33, 0·69	0·53	0·32, 0·69	0·42	0·18, 0·61	62	0·69	0·53, 0·81	0·68	0·51, 0·80	0·60	0·40, 0·75
Czech Republic	58	0·45	0·21, 0·63	0·40	0·15, 0·60	0·37	0·12, 0·58	58	0·31	0·01, 0·52	0·37	0·12, 0·58	0·36	0·11, 0·57
France	55	0·62	0·42, 0·76	0·64	0·42, 0·78	0·63	0·42, 0·78	48	0·61	0·42, 0·75	0·63	0·43, 0·77	0·62	0·41, 0·76
The Netherlands	58	0·65	0·47, 0·76	0·69	0·52, 0·80	0·66	0·48, 0·79	59	0·61	0·42, 0·74	0·60	0·40, 0·75	0·36	0·10, 0·57
Norway	61	0·50	0·28, 0·67	0·50	0·28, 0·68	0·62	0·43, 0·76	60	0·49	0·28, 0·66	0·51	0·29, 0·68	0·49	0·26, 0·66
Pooled	290	0·55	0·44, 0·62	0·56	0·44, 0·68	0·56	0·44, 0·68	287	0·55	0·44, 0·67	0·57	0·45, 0·68	0·51	0·40, 0·63

* Average intake and excretion based on 2 d of collection.
† Pairwise comparisons between countries (by Fisher *Z* transformation) suggested differences for unadjusted correlations between Belgium and the Czech Republic in females and between France and the Czech Republic for energy-adjusted correlations in males.
‡ Adjusted for the within-person variability using the usual intake/excretion data as estimated by the multiple source method (see 'Methods' section) and adjusted for age, BMI and educational level using partial Pearson correlations.
§ Same adjustments as previous correlation plus energy-adjustment by the residual method.
‖ Mean values for heterogeneity were not significant for all the analyses ($P > 0{\cdot}05$).

and 0·57 in women (Table 3). After adjusting for within-person variability, age, BMI and education level, correlations ranged between 0·43 and 0·67 in men and between 0·39 and 0·63 in women. For K, unadjusted correlations ranged between 0·45 and 0·65 in men and between 0·31 and 0·69 in women. Adjusted correlations ranged between 0·40 and 0·69 in men and between 0·37 and 0·68 in women. For both protein and K, adjusting only for the within-person variability slightly increased the correlations between intake and excretion (data not shown). Statistically significant differences between correlation coefficients were only found between Belgium and the Czech Republic ($P=0·04$) for unadjusted correlations of K in women. However, after adjusting the correlations for energy, we found a significant difference between the Czech Republic (r 0·25) and France (r 0·65) for protein intake in men ($P=0·01$).

The pooled adjusted correlations in males and females were 0·51 and 0·60 for protein and 0·56 and 0·57 for K intake, respectively.

Discussion

In the present study, we compared the validity of usual protein and K intake estimated from two non-consecutive standardised 24 h recalls between five selected centres in Europe. On average, men and women under-reported protein intake from the two 24 h recalls by 8 %. For K intake, average underestimation was 7 % for men and 4 % for women.

Protein intake was markedly underestimated (approximately 12 %) in French and Dutch men, especially when compared with Czech Republic men. The same is true for K intake in French men. In women, underestimation of mean protein intake was present in all the centres and appeared to be comparable across the centres. For K intake, however, the underestimation observed in the French centre was not comparable to that of the other centres, particularly to the overestimation observed in the Czech Republic. Furthermore, we assessed the agreement between the percentage of subjects above a certain cut-off point based on 24 h recall and biomarker data. We found a fair agreement for cut-off points at the lower end of the distribution (< 10 % difference), but larger differences at other points of the intake distribution (up to 55 % difference for protein in French females). Finally, we observed moderate correlations for the ranking of individuals, which were likely to be comparable across the centres.

The results from the EPIC study, using EPIC-Soft in different centres, revealed a similar or even higher underestimation of protein intake collected from a single day (average of 13 % in men and 19 % in women)[17]. The OPEN study in the United States, which assessed the structure of dietary measurement error in 24 h recalls collected twice, has also shown a similar underestimation of protein intake (11–15 %)[36]. A few other studies indicated overestimation of protein (about 7 % for the whole population)[37]. For K, studies indicated overestimation of intake up to 20 %[38–40], similar to what we observed in the Czech Republic. Nevertheless, because of methodological differences, the comparison of bias estimates between the present study and other studies is not straightforward. For example, adjustment of N and K excretions to extra-renal losses was not consistently performed among the studies. In addition, the completeness of 24 h urine collections was not always assessed. Although we acknowledge the differences in methodology between the studies, the performance of these two standardised 24 h recalls on assessing the mean protein and K intake appeared to provide alike or even more accurate results than what have been presented in the literature so far.

In terms of assessing the whole distribution of intake, two 24 h recalls used in the study by Freedman *et al.*[39] underestimated the usual protein intake in all points of the distribution, especially at the lower end. Moreover, they found a good agreement between K intake and excretion in the whole range of percentiles. In contrast, moderate to large discrepancies were found between 24 h recall and biomarker data distributions in the present study, but not at the lower end of the distribution. The present results suggest that the assessment of protein and K inadequacy at the population level by two non-consecutive 24 h recalls in healthy European populations is, therefore, appropriate.

Independent of the size of the bias, the correct classification of individuals according to their intake is also informative on the quality of the dietary assessment. The correlations presented in the present paper are considerably higher compared with many other studies[36,41–43]. Based on this, we conclude that the method performed sufficiently for the ranking of individuals, adding evidence to the use of this standardised 24 h recall. When we adjusted the nutrient values for energy intake, this changed the correlations in both directions and resulted in more noticeable differences across the centres. We doubt, however, whether energy-adjusted values will be our main exposure of interest in future monitoring surveys and whether individual energy intake was correctly estimated using only 2 d of 24 h recall. Therefore, we do not base the conclusions of the present paper on the energy-adjusted results.

We suppose that the differences found in the size and direction of the bias (i.e. overestimation of K intake in the Czech Republic and underestimation of both K and protein in the other centres) between the centres may be explained by reasons related to characteristics of the population and of the method itself. We have controlled our statistical analyses for the influence of age, education level and BMI. As a result, BMI was the only factor significantly influencing the differences between the countries. This is in accordance with our expectations since other studies have revealed a differential under-reporting of dietary intake by subgroups of BMI[38,44]. Nevertheless, other aspects of the population

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could have affected the validity of the method between the centres in a different manner, i.e. factors related to the food pattern of the centres. Due to cultural differences in food pattern, it is expected that predominant food items contributing to protein and K intake across European countries will be different[45,46]. For example, the food group 'dairy products' was one of the major contributors (>22 %) to the protein intake in The Netherlands and Norway (in males only), whereas in the other three centres, 'meat products' was distinctly the major contributor (>30 %). Knowing that the errors in the assessment of different food groups differ, as for instance in the portion size estimation[47], differences in validity between the centres could be expected. Likewise, differences in the consumption of composite foods could have had an effect since it is more difficult to recall all ingredients of composite foods than a single food item[48,49].

Another important factor that could explain the differences between countries is the use of not harmonised FCT across the centres. Use of different conversion factors as well as distinct laboratory analyses to produce food nutrient contents across the tables is just an example which could have caused biases not to be comparable. For instance, for three of the FCT used in EFCOVAL, protein figures were calculated from N contents using the so-called 'Jones conversion factors'[50] or slight modifications of them. However, in the Dutch tables, only two of these factors were used (6·38 for milk products and 6·25 for all other foods), and in the compiled Czech table, only one factor (6·25) was applied (Slovakian tables). Since errors attributed to these differences can be proportional to the level of intake, it is impossible to conclude on the influence of using different conversion factors in the comparison between the countries. Nevertheless, further investigation about the use of these conversion factors in FCT for comparisons of nutrient intake between countries is warranted.

The present study adds value to the present knowledge of collecting dietary information using standardised 24 h recalls for possible use in national monitoring surveys. An important strength of the present study was the collection of 2 d of both dietary intake and biomarkers allowing the quantification of within-person variability and to estimate the usual intake distributions. A potential limitation of the present study is that a health-conscious sample may have been included, hampering the extrapolation of the results to the general population. However, the present results suggested that extrapolation to other populations could be done irrespective of their education level. In addition, the generalisability of protein and K results to other nutrients of interest should be done with care. Although we might want to assume that the validation results of a single nutrient can be used as a proxy to other nutrients, there is evidence nowadays that some foods and consequently related nutrients might be selectively misreported[47,51]. Besides, only 2 d of 24 h recall were used in our assessment while the inclusion of more than 2 d may be necessary to improve the use of this 24 h recall in the assessment of other nutrient intake distributions, particularly the infrequently consumed ones[52]. The statistical adjustments performed with the MSM intended to remove the day-to-day variation in intakes and assess the usual distributions of intake. But, if the variance of the nutrient intake is not reliably estimated from 2 d of intake, then the observed intake may shrink too much or too little toward the group mean intake, resulting in an inaccurate usual intake distribution[53]. The use of FFQ combined with 24 h recalls may be an option in future monitoring surveys for the calculation of usual intakes of infrequently consumed nutrients, as more days of 24 h recalls are demanding and expensive. Furthermore, the reliability of the conversion factors used to adjust urinary protein and K in our analyses can be questioned. With the assumption that the subjects were in N balance, these factors have been based on rigorously controlled feeding studies[15,16] and in the case of protein confirmed by Kipnis *et al.*[54]. Lastly, we have collected data in The Netherlands 6 months before the other centres and this may have influenced the results. Nevertheless, while the data for The Netherlands were collected in spring/summer, the data for other four countries were collected in the winter/spring. However, since minor adjustments were done in the study protocols and the differences in seasonality were small for protein and K intake, it is unlikely that a different period influences the present results.

To conclude, first, the ability of the two non-consecutive standardised 24 h recalls using EPIC-Soft software appears to be sufficiently valid for assessing and comparing the mean protein and K intake across the centres. When comparing populations in a future nutrition monitoring system, the variability in the nutrient biases of 4–7 % across the centres needs to be considered. Second, the method seems to be sufficiently valid for assessing and comparing the protein and K inadequacy of healthy populations across the centres and less appropriate to assess other points of the intake distribution. Third, the ability to rank the individuals according to protein and K intake within the centres is comparable between them, which substantiates the validity of the method. Therefore, this standardised two non-consecutive 24 h recalls, further adapted and validated in the EFCOVAL project, appear appropriate to be used in the context of a future pan-European dietary monitoring system. Built on EFCOVAL and EPIC experiences, improvements may be possible for the employment of this methodology by an even higher standardisation setting (e.g. conversion factors), which could result in an enhanced validity of the method, and thus comparability between the countries.

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