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Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population

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Abstract

Objective: The aim of the current study was to evaluate the accuracy of the new software eAT24 used to assess dietary intake in the National Food, Nutrition and Physical Activity Survey (IAN-AF) against urinary biomarkers: N (nitrogen), K (potassium) and Na (sodium).

Design: We conducted a cross-sectional study. Two non-consecutive 24-h dietary recalls (24-HDR) were applied, and a 24-h urine sample was collected. We examined differences between estimates from dietary and urine measures, Pearson correlation coefficients were calculated and the Bland–Altman plots were drawn. Multiple linear regression was used to evaluate the factors associated with the difference between estimates.

Setting: Sub-sample from the Portuguese IAN-AF sampling frame.

Participants: Ninety-five adults (men and women) aged 18–84 years.

Results: The estimated intake calculated using the dietary recall data was lower than that estimated from urinary excretion for the three biomarkers studied (protein 94·3 *v.* 100·4 g/d, K 3212 *v.* 3416 mg/d and Na 3489 *v.* 4003 mg/d). Considering 2 d of recall, the deattenuated correlation coefficients were 0·33, 0·64 and 0·26 for protein, K and Na, respectively. For protein, differences between dietary and urinary estimates varied according to BMI ($\beta = -1{\cdot}96$, $P = 0{\cdot}017$). The energy intake and 24-h urine volume were significantly associated with the difference between estimates for protein ($\beta = 0{\cdot}03$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}02$, $P = 0{\cdot}002$, respectively), K ($\beta = 0{\cdot}71$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}42$, $P = 0{\cdot}040$, respectively) and Na ($\beta = 1{\cdot}55$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}81$, $P = 0{\cdot}011$, respectively).

Conclusions: The new software eAT24 performed well in estimating protein and K intakes, but lesser so in estimating Na intake, using two non-consecutive 24-HDR.

Keywords
Urinary biomarkers
Nitrogen
Potassium
Sodium
24-h dietary recall
Validation
Portuguese dietary survey

Over the past decades, a potential interactive association between diet, lifestyle and genetics and the risk of many chronic diseases has been suggested. Accurate dietary intake assessment is important not only to find real associations between diet and health-related outcomes but also for nutritional monitoring/surveillance and for assessing compliance to dietary guidelines or to a dietary intervention in clinical/food intervention trials[1,2].

Overall, the methods and procedures used in national dietary surveys have been developed with the main aims of monitoring the nutritional status of a population, that is, analyses focusing on the intake of energy, macronutrients and micronutrients and developing tools and methods to obtain valid estimates of these intakes. The availability of detailed, harmonised and high-quality food consumption data from all European Union Member States had been recognised as a long-term objective of the European Food Safety Authority[3]. In 2014, the 'Guidance on the EU Menu methodology' was published[4], aiming to update the previously published guidance on 'General principles for the collection of national food consumption data in the view of a pan-European dietary

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survey'[3] and to cover the pan-European food consumption survey ('EU Menu') methodology. This guidance provides recommendations for the collection of food consumption, including the principal characteristics of the dietary software used to collect all the dietary data. In Portugal, a new electronic dietary assessment tool (eAT24) was specifically developed for the National Food, Nutrition and Physical Activity Survey (IAN-AF) conducted during 2015 and 2016[5], but validation studies of its application are still missing.

Regarding food intake assessment, the 24-h dietary recall (24-HDR) method, carried out on two non-consecutive days, was recommended for the adult population as it was considered the most cost-effective method[3]. Given the multifactorial complexity of diet, it is well recognised that all dietary assessment instruments are associated, with random and systematic measurement errors, although with different magnitudes, which affect dietary estimates and may obscure disease risk associations[2]. Assessing the validity of dietary self-report instruments is therefore needed to reliably interpret the results. The underlying assumption of this validation approach requires that the measurement error of the test method will be independent of the measurement error of the reference method[1]. The measurement of nutritional biomarkers in human biofluids has become increasingly used as reference instruments in dietary validation studies, since errors for biochemical markers and reporting methods appear to be independent[6–9]. Recovery biomarkers, which are a subclass of nutrition biomarkers, can provide accurate assessments of short-term food exposure and are not substantially affected by determinants other than intake.

However, recovery biomarkers are currently only available for four dietary components: energy, protein, K and Na, providing information on limited aspects of diet[1,10,11]. Urinary N can be used to estimate absolute protein intakes and is the most widely used recovery biomarker. When there is no gain or loss in body protein, the body is in N balance and dietary N intake is highly correlated with N loss[7]. A meta-analysis of metabolic studies estimated that approximately 80 % of N atoms from ingested protein are excreted in urine[12].

Although K is more widespread in food than N, urinary K excretion can be used as a recovery biomarker with accuracy similar to that of urinary N. In a metabolic study with thirteen volunteers, during 30 d, high correlation between urinary K and intake was observed (0·89). Dietary potassium was mostly recovered in urine (77 %) and stools (17·5 %)[13]. High correlations between estimated K intakes from diet records and 24-h urine samples over the same period have also been described under free-living conditions[14,15].

Urinary Na is a good measure of short-term intake since sodium excretion is determined by recent intake and is almost as variable as intake itself[1]. The reproducibility of 24-h urinary Na has consistently been lower than for urinary K, probably reflecting greater within-person variation in Na intake as compared with K intake[16].

The aim of the current study was to assess the accuracy of the software eAT24, used in the IAN-AF, against three independent urinary biomarkers of intake: N, K and Na.

Subjects and methods

Study design and participant recruitment

Participants (n 95) were a sub-sample from the same IAN-AF sampling frame, whose aims and methods had been described in detail previously[5,17]. The sample size was calculated to detect correlation coefficients ≥0·3 at the 5 % significance level and with 80 % power, being sixty-six participants needed. Considering a non-compliance rate for 24-h urine completeness of 30 %, ninety-five eligible individuals were needed. Between May and December 2016, healthy men and women aged 18–84 years from the same IAN-AF sampling frame were invited, by telephone or during the first face-to-face interview, to take part in a more detailed validation study. They were assessed for suitability by a short screening questionnaire which included the following exclusion criteria: taking diuretics; being pregnant or lactating; having diabetes or kidney disease, haemophilia or any condition requiring supplemental O_2; donating blood or plasma during or <4 weeks before the study; following prescribed dietary therapy and/or having had a urinary tract infection within 1 month of commencing the study.

Briefly, data were collected during two interviews, separated by between 8 and 15 d and conducted by trained nutritionists. At the first interview, dietary intake and physical activity questionnaires were applied and the participants received detailed written and oral instructions on the technique of collecting urine samples. Anthropometric assessment, including body weight and height, was also performed according to the standard procedures, previously described[5]. At the second interview, participants were asked to bring the urine samples and a second 24-HDR was applied. This second 24-HDR is referred as '1-d recall' in the following sections.

Dietary assessment

The dietary intake data were obtained using the eAT24 software, a new electronic dietary assessment tool, based on a client–server architecture, specifically developed for the IAN-AF, which allowed the collection and description of food consumption data by 24-h recalls, according to a procedure based on the automated multiple-pass method for 24-HDR[18], as described elsewhere[17].

All foods, including beverages and dietary supplements, consumed were recorded per eating occasion and quantified and described as eaten. This description required the utilisation of several facets and respective descriptors, through the FoodEx2 classification system[19] The place

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and time of meal consumption were also recorded for each eating occasion.

The software allowed subsequent conversion of foods into nutrients, using by default the Portuguese food composition table[20], which was continuously adapted and updated, ending with 2037 food items. A recipe module was also created, in which the recipes were disaggregated into raw ingredients allowing the description and quantification of each item.

Additionally, the software was able to include new food items or new recipes during the data collection process. For quantification, different methods were available: (i) weight or volume, (ii) standard units, (iii) photographs (food picture book including a 186 food photograph series (with six portions/food per recipe) and a household measures photograph series[21]), (iv) household measures and (v) default portions[5]. For quality control, the software provided, at the end of the interview, the individual energy and macronutrient intakes for the corresponding evaluated day.

Urine collection and processing

Participants were asked to collect urine samples on the day before the second interview. Urine samples were collected in two separate containers. The first one (a 2700-ml container identified as container A) was used to collect all urine passed during the day before the interview, except the first void of that morning. A second one (a 500-ml container identified as container B) was used to collect only the first void urine of the day of the second interview (urine sample identified as 'first morning void'). No preservatives were added to the urine containers, and the participants were asked to keep the samples refrigerated (4°C) throughout the collection period.

Participants were asked to fill in a questionnaire during the day of urine collection that included information on the time of the beginning and the end of collection, details of any medication, and whether or not they had any problems or missed urine collection.

At the laboratory, urine samples were weighed and mixed. The weights of urine from containers A and B were quantified separately, and a proportionally pooled 24-h urine sample (identified as '24-h urine') was prepared by using samples A and B.

From each participant, both urine samples ('first morning void' and '24-h urine') were aliquoted: 1×45 ml (in 50-ml Falcon pre-labelled tube) + $10 \times 1{\cdot}5$ ml (in 2 ml-pre-labelled microtubes). These aliquots were refrigerated immediately before being moved to −80°C storage, within 24 h, for further analysis. In the current study, only the analysis of '24-h urine' samples was presented.

Chemical analysis

Quantification of total N in urine samples was performed using the Kjeldahl method (Foss Tecator). To estimate protein intake from urine analysis, it was assumed that excreted nitrogen accounts for 81 % of the ingested protein due to extra-renal nitrogen losses[11]. Thus, urinary N concentration was converted to protein intake according to the following expression: protein intake (g/d) = (N concentration in urine (g/l) × 24-h urine volume (l) × 6·25)/0·81[22].

Na and K excretions were assessed using an ion-selective electrode Na^+ and K^+ assay (Beckman Coulter). Further adjustments were made to reflect extra-renal losses of Na and K estimated at 0·86 and 0·80, respectively[10].

The 24-h urine volume as adjusted for self-reported collection time according to the expression: 24-h urine volume (ml) = total volume collected (ml)/self-reported collection time (h) × 24[23]. Urine density was assumed to be approximately 1·0 g/ml. Urinary creatinine was measured by the Jaffe method (Beckman Coulter). The completeness of the 24-h urine was assessed through a combination of two criteria: the 24-h urinary creatinine excretion (mg/d) in relation to body weight (kg) (creatinine coefficients between 14·4–33·6 in men and 10·8–25·2 in women were considered sufficient to ensure that the samples corresponded to a 24-h period as recommended)[24] and total 24-h urine volume (≥500 ml)[23], and only data from complete collections were used for analysis[25,26].

Statistics

Means and SD or frequencies and percentages were used to describe the study sample.

Mean dietary intake estimated from 24-HDR was compared with mean dietary intake estimated from urinary biomarkers using a pairwise *t* test. Mean differences and SD were calculated to allow conclusions on a group level about the absolute extent of under or overestimation of intake by 24-HDR.

Crude Pearson correlation coefficient between dietary intake, using either 1-d recall or the mean of 2-d recall, and urinary biomarkers was estimated. The deattenuated correlations were also calculated to remove within-subject variance[27]. The within-subject variance estimated from 2 d of dietary recall was also used to deattenuate the correlation between the 1-d recall and urinary biomarkers.

By means of cross-classification, participants were classified into either the same tertile of intake by both methods or misclassified into the opposite tertile. Linear weighted Kappa coefficient was computed to assess the strength of agreement. The Bland–Altman plots were used to illustrate the difference between the two methods against the mean of the two methods.

Multiple linear regression was used to evaluate associations between the difference in protein, K and Na measures and covariates (gender, age (years) and BMI (kg/m^2), 24-h urine volume (ml) and energy intake (kJ)).

The significance level was fixed in 0·05. All statistical analyses were carried out using software R version 3.5.0.

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Results

From a total of ninety-five urinary specimens, nine were determined to be incomplete according to the previously mentioned coefficient creatinine-based criteria, resulting in eighty-six complete samples. Demographic and anthropometric characteristics and the energy intake of the participants are presented in Table 1. Half of the participants (50 %) were women and 12 % aged 65 years or more. Approximately, 72 % of men and 61 % of women were overweight or obese.

The geometric means and SD for protein, K and Na intakes as estimated by urinary biomarkers and as self-reported from the 24-HDR are shown in Table 2.

Mean protein intake, estimated from N urinary excretion (100·4 g/d), was significantly higher than the protein intake calculated using the single-day 24-HDR data (89·5 g/d) ($P = 0·032$). When considering the mean of 2 d of recall (94·3 g/d), mean protein intake was also higher but not significantly ($P = 0·164$). For K, mean intake estimated from urinary excretion (3416 mg/d) was slightly lower than the mean estimated from 1 d (3421 mg/d) and higher considering two 24-HDR (3212 mg/d); however, the differences were not significant ($P = 0·973$ and $P = 0·672$, respectively). The mean Na intake estimated from urinary excretion (4003 mg/d) was higher than the mean intake calculated from one (3611 mg/d) or two 24-HDR (3489 mg/d), but significant only when 2 d were considered ($P = 0·027$).

Cumulative percentiles for urinary excretion and estimated dietary intake of protein, K and Na are shown in Fig. 1. For protein, a clear tendency to underestimate the reported intake in relation to urinary excretion was observed, except for larger intake above 130 g (approximately). Below 3000 mg, urinary K excretion was lower than the reported intake, suggesting that individuals who consumed less food sources containing K were more prone to overestimate its dietary intake. Above that value, the differences between reported intake and excretion were less evident. For Na, a tendency to underestimate the reported intake in relation to urinary excretion was observed for values above 2500 mg, suggesting that individuals who consumed more Na-high products were more likely to underestimate the dietary intake.

Raw and deattenuated correlations between intake estimated from urinary biomarkers and from 1- or 2-d dietary recalls are reported in Table 3. For protein, K and Na, the deattenuated correlations between intake estimated from urinary biomarkers and from a single day dietary recall were 0·27, 0·57 and 0·26, respectively. Considering the mean intakes calculated using the 2 d of recall, both crude and deattenuated correlation coefficients improved, except for Na. For protein and K, the deattenuated correlations increased to 0·33 and 0·64, respectively. A similar trend was observed on cross-classification results (Table 4). The agreement (k coefficient) between protein or K intake estimated from urinary biomarkers and from dietary recall slightly increased when the mean of 2 d of recall was used.

The Bland–Altman graphs for assessing bias between dietary intake and urinary biomarkers are presented in Fig. 2(a)–(c) for protein, K and Na, respectively. The protein plot indicated systematic underestimation of intake and a large scatter of the differences. Nevertheless, the measurement error seems to be similar across all the observed intake levels, indicating an acceptable estimation. In relation to K, there is almost no systematic bias between dietary intake and urinary excretion. In contrast, Fig. 2(c) shows a systematic underestimation of Na intakes and a larger scatter of the differences due to wider confidence limits. The scattering range of differences tends to be higher at higher Na intake.

Demographic and anthropometric characteristics (gender, age and BMI), energy intake and 24-h urine volume associations with the difference between nutrient intakes estimated from self-reported dietary data and from urinary

Table 1 Demographic and anthropometric characteristics and energy intake of the analytic sample by gender (n 86)

	Women (n 43)		Men (n 43)		Total (n 86)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	46·2	14·9	50·3	15·3	48·3	15·2
Weight (kg)	67·1	11·6	80·5	11·5	73·8	13·3
Energy intake (1 d of recall) (kJ)	7004	2180	10 514	4138	8761	3732
Energy intake (mean of 2 d of recall) (kJ)	7037	1941	10 213	3590	8627	3284
BMI category*						
Normal weight						
n	17		12		29	
%	39·5		27·9		33·7	
Overweight						
n	19		25		44	
%	44·2		58·1		51·2	
Obesity						
n	7		6		13	
%	16·3		14·0		15·1	

*BMI category (kg/m^2): normal weight (18·5 to <25·0), overweight (25·0 to <30·0) and obesity (≥30·0).

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Table 2 Protein, K and Na reported dietary intake and urinary biomarkers for all participants (mean values with their standard errors)

	Mean	SD	*P*
Protein			
Dietary intake (1 d of recall) (g/d)	89·5	39·9	0·032*
Dietary intake (mean of 2 d of recall) (g/d)	94·3	35·1	0·164†
Estimated intake from 24-h urinary N excretion (g/d)	100·4	31·1	
Reporting accuracy‡	0·94		
K			
Dietary intake (1 d of recall) (mg/d)	3421	1363	0·973*
Dietary intake (mean of 2 d of recall) mg/d	3212	1156	0·672†
Estimated intake (mg/d) from 24-h urinary K excretion (mg/d)	3416	1332	
Reporting accuracy‡	0·94		
Na			
Dietary intake (1 day of recall) (mg/d)	3611	1832	0·112*
Dietary intake (mean of 2 d of recall) (mg/d)	3489	1616	0·027†
Estimated intake from 24-h urinary Na excretion (mg/d)	4003	1760	
Reporting accuracy‡	0·87		
Creatinine (mmol/l)	10·66	5·31	
24-h urine volume (ml)	1406	614	

*Derived by pairwise *t* test for differences in means between dietary intake estimated from 1 d of dietary recall and dietary intake estimated from urinary biomarker.
†Derived by pairwise *t* test for differences in means between dietary intake estimated from 2 d of dietary recall and dietary intake estimated from urinary biomarker.
‡Reporting accuracy: ratio of mean reported intake (estimated from 2-d dietary recalls) to that estimated from urinary biomarkers.

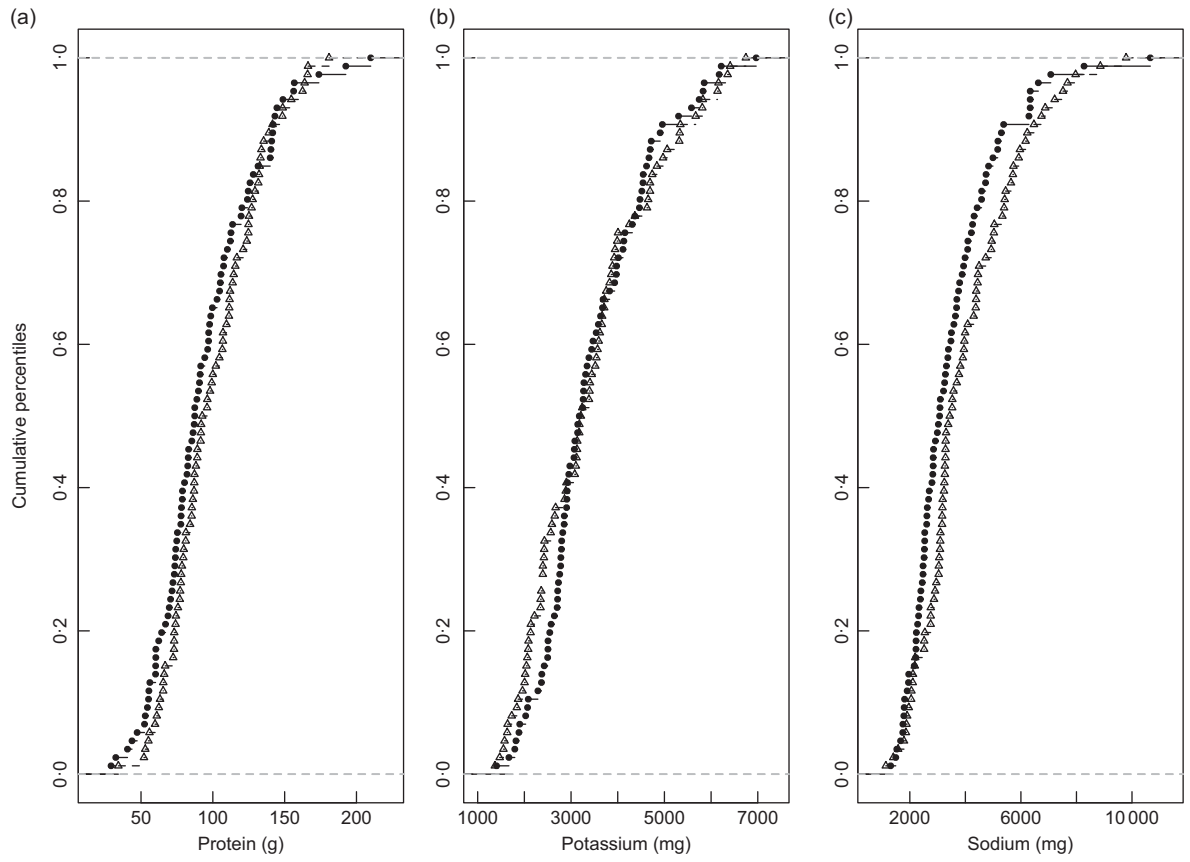

Fig. 1 Cumulative percentiles of estimates of dietary intake from 2 d of dietary recall (•) and urinary excretion (▵) for (a) protein, (b) K and (c) Na

biomarkers are presented in Table 5. For protein, differences varied according to BMI ($\beta = -1{\cdot}96$, $P = 0{\cdot}017$) but not by gender or age. No significant differences according to BMI, gender or age were observed for K and Na. The energy intake was positively associated, and 24-h urine volume was negatively associated with the difference

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Table 3 Pearson's correlation coefficient (*r*) between dietary intake and urinary excretion

	One recall day		Mean of two recall days	
	Crude	Deattenuated	Crude	Deattenuated
Protein (g/d)	0·17	0·21	0·27	0·33
K (mg/d)	0·47	0·57	0·53	0·64
Na (mg/d)	0·21	0·26	0·21	0·26

between self-reported dietary intake and intake estimated from urinary biomarkers for protein ($\beta = 0{\cdot}007$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}02$, $P = 0{\cdot}002$, respectively), K ($\beta = 0{\cdot}170$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}42$, $P = 0{\cdot}040$, respectively) and Na ($\beta = 0{\cdot}371$, $P < 0{\cdot}001$ and $\beta = -0{\cdot}81$, $P = 0{\cdot}011$, respectively).

Discussion

In the current study, we assessed the validity of protein, K and Na intakes estimated from two non-consecutive 24-HDR against 1-d urinary excretion, where the day of urine collection corresponded to the second day of dietary recall.

When compared with the estimated protein intake from 24-h N excretion, the estimated intake of protein (recorded by 24-HDR) was considerably underestimated, which has been observed in other studies[28–37]. Considering just 1 d of recall, we found an under-reporting around 10%, which was similar to values found in other studies, such as the European Food Consumption Validation Project (12·1 % in men and 12·8% in women)[28] and the American Observing Protein and Energy Nutrition Study (11–12 %)[29]. However, when the mean of 2 d of recall was considered, protein under-reporting decreased to 6 %. Correlation coefficients between protein intake estimated from self-reported data and from urinary N were low but comparable with values reported in other validation studies including short-term instruments (24-HDR), such as the American Observing Protein and Energy Nutrition Study ($r = 0{\cdot}41$ for men and $r = 0{\cdot}26$ for women)[29], the Dietary Evaluation and Attenuation of Relative Risk study ($r = 0{\cdot}29$)[38] and the UK arm of European Prospective Investigation into Cancer and Nutrition ($r = 0{\cdot}10$ for one 24-HDR)[39]. However, our results are slightly lower than those found in the European Food Consumption Validation Study ($r = 0{\cdot}65$ in men and $r = 0{\cdot}46$ in women)[28] and in a recent study which evaluate the relative validity of multiple self-reported dietary assessment methods over a 15-month period ($r = 0{\cdot}67$ for 7-d dietary records)[40].

A meta-analysis of a large set of data has confirmed that urine N should be approximately 80 % of dietary intake on average[12]; however, it could be argued that the use of inappropriate assumptions for protein extra-renal losses may have contributed to a different validity for assessment of protein intake.

For K and Na, in line with some other studies, intake estimates from a 24-HDR were expected to be lower than from urine biomarkers due to the well-described under-reporting bias[41,42]. Considering 2 d of dietary recalls, a significant under-reporting was observed for Na (3489 *v.* 4003 mg/d, $P = 0{\cdot}027$) but not for K (3212 *v.* 3416 mg/d, $P = 0{\cdot}672$). Correlation coefficients between K intake estimated from self-reported data and from urinary excretion were similar or even higher than those reported by other authors[10,28,41,43]. For Na, correlations were low, but comparable with those reported by other authors[10,41,44]. McKeown *et al.*[43] reported correlation coefficients in women similar to those we indicated in the current study but higher in men. This weak association between Na intake and excretion is not surprising, assuming that some authors suggest that a minimum of eight urine collections is needed to gain precision in the estimate of individuals' mean Na intake[45]. Bingham *et al.*[46] showed that even when the mean of six urine collections and 16 d of weighed records were used to estimate Na intake, the correlation was only moderate.

Correlation and agreement values for K were higher than those observed for Na and protein. As suggested by other authors, this differential reporting of nutrients is possibly related to differential reporting of its food sources[10,11,37,41,47]. The main food sources for K from diet, such as fruits and vegetables, seem to be less susceptible to under-reporting than the food sources for protein or Na[48]. It is widely recognised that the observed weak relation between dietary and urinary Na is attributed to the poor assessment of salt intake by dietary assessment methods, the lack of inclusion of foods prepared with salt in food composition tables and the high within-person variability of

Table 4 Cross-classifications into tertiles for agreement

	One recall day			Mean of two recall days		
	Agreement (%)	*k*	95 % CI	Agreement (%)	*k*	95 % CI
Protein	43·0	0·17	0·00, 0·34	39·5	0·19	0·03, 0·36
K	44·1	0·27	0·03, 0·36	45·3	0·27	0·11, 0·43
Na	43·0	0·17	0·00, 0·34	30·2	0·01	−0·15, 0·17

k, Kappa coefficient.

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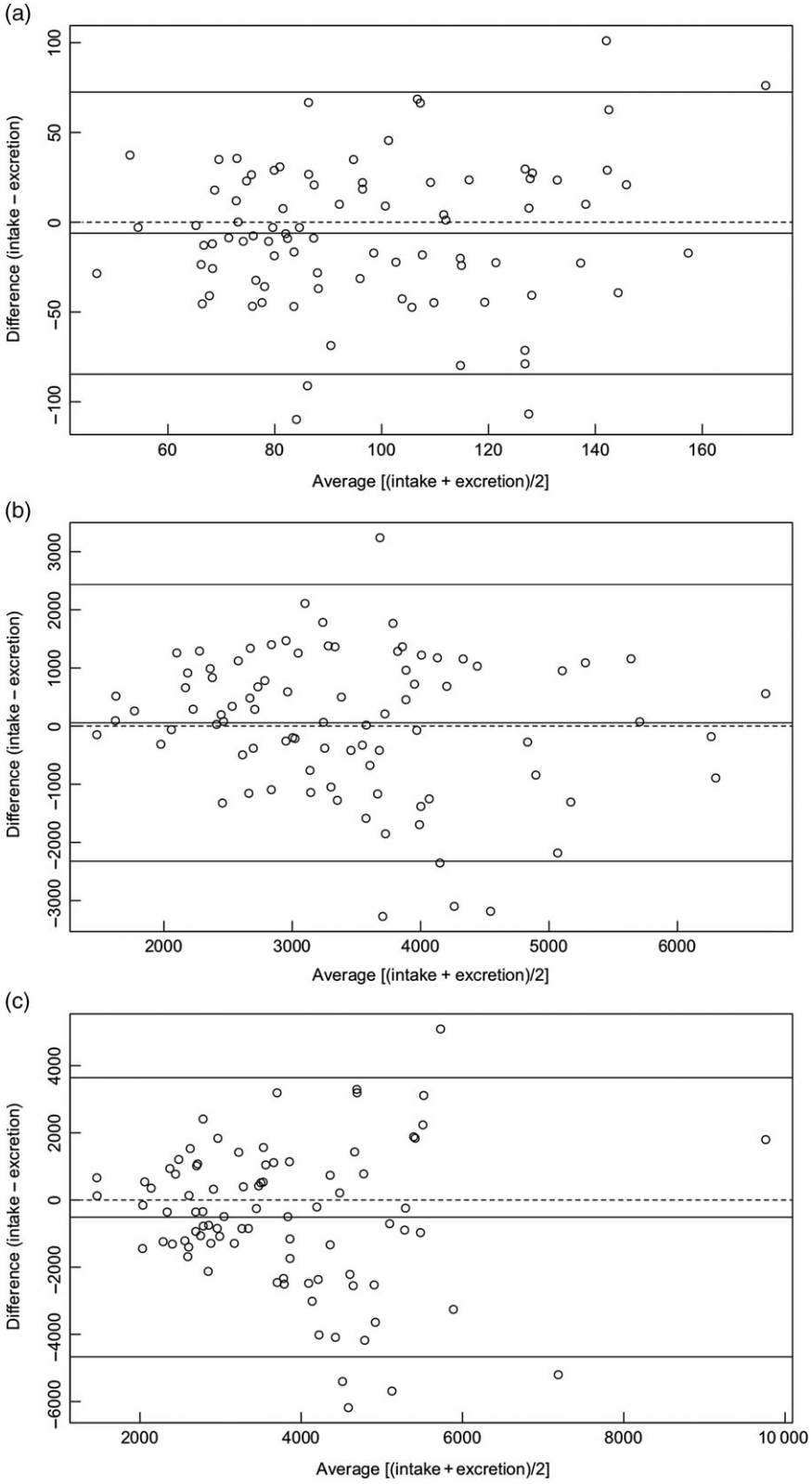

Fig. 2 The Bland–Altman graphs for assessing bias between nutrient estimation by self-reported dietary intake (mean of 2 d) and nutrient estimation by nutritional biomarkers for protein (a), K (b) and Na (c). The horizontal dashed line indicates the mean of the differences. The upper and lower dotted lines represent the upper and lower 95 % CI of agreement, which should comprise 95 % of the values in the range of the 2-fold SD ($d \pm 1{\cdot}96 \times$ SD) of the mean differences

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Table 5 Association between nutrient estimation by dietary intake from 2 d of dietary recall and nutrient estimation by nutritional urinary biomarkers according to sociodemographic, dietary and urinary covariates

Difference* as dependent variable	β	95 % CI	P
Protein (g)			
Gender†	−7·59	−22·87, 7·69	0·326
Age‡ (years)	0·15	−0·31, 0·61	0·512
BMI‡ (kg/m^2)	−1·96	−3·56, −0·36	0·017
24-h urine volume (ml)	−0·02	−0·03, −0·01	0·002
Energy intake (kJ/d)	0·007	0·005, 0·010	<0·001
K (mg)			
Gender†	−287·90	−822·91, 247·12	0·287
Age‡ (years)	−7·72	−23·90, 8·47	0·345
BMI‡ (kg/m^2)	−19·07	−75·07, 36·93	0·499
24-h urine volume (ml)	−0·42	−0·81, −0·02	0·040
Energy intake (kJ/d)	0·170	0·089, 0·251	<0·001
Na (mg)			
Gender†	−252·27	−1087·56, 583·02	0·550
Age‡ (years)	2·25	−23·02, 27·52	0·860
BMI‡ (Kg/m^2)	−73·431	−160·86, 14·00	0·099
24-h urine volume (ml)	−0·81	−1·43, −0·19	0·011
Energy intake (kJ/d)	0·371	0·242, 0·489	<0·001

*The multivariate analysis model was one linear regression model (difference between estimates = dietary intake − urinary excretion as dependent variable) with all covariates included.
†The gender variable was categorised with male being the referent group.
‡Continuous forms of age and BMI were used in the regression analysis.

urinary Na[43]. Additionally, the use of table and cooking salt and the variation in the Na content of manufactured foods make it difficult to rely on food tables in estimating Na intake. It should also be noted that the food composition data used for calculating nutrient intakes might also introduce bias. In fact, the constant changes in food marketplace and the dynamic nature of the food supply present issues and challenges for maintain food composition databases completed and updated[49].

Overall, we observed that correlation coefficients (crude or deattenuated) and agreement (*k* coefficient) between self-reported data and urinary biomarkers were higher when calculated, considering the mean of 2 d of recall than when only 1 d of recall was considered for N and K, but not for Na. For Na, in which half-life is approximately 24 h, these findings may be explained since we expect Na in the 24-h urine (comprising all excretions until the following morning) to reflect mainly the Na ingested during that day[41]. The higher K half-life in the body (approximately 16 d) may explain better correlations when 2 d of recall were considered[50].

Reporting error has been attributed to both physical (e.g. BMI) and sociodemographic characteristics, such as gender and socio-economic status[10,11,29,51]. In the current study, BMI significantly influenced the differences in protein measures but not in K and Na. Freedman *et al.*[11] pooled data from five large validation studies of dietary self-reported instruments and also showed that a higher BMI was consistently associated with under-reporting of both energy and protein intakes, using both FFQ and 24-HDR. Other studies reported this BMI effect[11,28,30,36,48,52,53]. For K and Na, BMI did not seem to influence misreporting, which is in line with a recent study[37,54]. Interestingly, Freedman *et al.*[10] reported that under-reporting for Na was strongly associated with higher BMI, being black, being male and having a high school education, but no associations were consistently reported for K intake.

Gender appeared to have no impact on the differences between measures in any of the three studied biomarkers. Several authors had studied the effect of gender on under-reporting of energy consumption and suggested that women are more likely, consciously or unconsciously, to under-report their diet than men[32,53,55]. However, when considering Na and K estimates, our findings are in line with Espeland *et al.*[16], who reported no gender effect on the relative bias of diet- *v.* urine-based measures.

Our results suggest that as the energy intake increased, greater were the differences found between the reported intake and the urinary excretion. Subar *et al.*[29] found that under-reporting tends to increase with increased exposure. The authors suggested that as the intake increased, the greater was the difficulty in reporting consumption accurately, perhaps because remembering more foods or bigger portion sizes is challenging and/or because of societal pressure to consume less[29]. These results are in accordance with our expectations since another recent study had revealed that total energy intake was independently associated with the difference between diet and urine estimates of Na and K intakes[41].

We found the difference between protein, K and Na estimates to be lower as 24-h urine volume increased. In spite of our efforts to assess the completeness of 24-h urine samples, we cannot exclude the possibility of considering incomplete samples as valid. We suggest that this may be a reason to observe higher differences between estimates for lower volumes of urine collected.

The main strength of the current study is the use of objective nutritional biomarkers, namely urinary N, K and Na, collected on one of the days of dietary recall, allowing to understand how well the urinary excretion reflects the acute intake.

Regarding the data collection, the protocol deserves to be mentioned. Interviews were carried out by highly trained nutritionists, according to the standardised procedures. Objectively measured anthropometry was performed using the same equipment model submitted to regular calibration procedures. Data inclusion was easier and accurate due to the use of the e-platform, specifically designed for this project. The multiple-pass dietary interviews minimised the omission of possible forgotten foods. Also, this method standardised the level of detail for describing foods including the portion size estimation by photographs of different portions.

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To our knowledge, the current study is the first to validate protein, K and Na intakes reported from 24-HDR against N, K and Na urinary excretion, in a sub-sample of healthy Portuguese adults. Additionally, the current study is the first one to validate the new software eAT24, supporting its suitability in further food intake assessment studies.

Among the limitations of the current study are that our sub-sample was not recruited from all geographic areas of the IAN-AF. Thus, caution should be taken in the extrapolation of the data to the general population. However, it is not expected a different performance of the tool in the IAN-AF general sample. The small sample size (n 86) has also to be mentioned, but it seems to be sufficient to show the differences when they exist.

Despite our efforts to select only valid/complete 24-h urinary samples, the application of other criteria deserves further investigation, in order to evaluate results with or without the exclusion of potential incomplete urine collections and determine its impact on validity outcomes. The use of 24-h urine sampling has been considered the gold standard and the most reliable method to estimate N, K and Na excretions. Although urinary measures are inherently more objective than measures based on self-reported dietary intake, the applicability of 24-h urine samples for validity has been questioned because there is no procedure to estimate with certainly the completeness of each 24-h urine collection[26,56]. It has been recognised that no single method, or even a combination of methods, accurately identifies incomplete urine collections[26]. Urinary para-aminobenzoic acid, used in some national surveys, has been widely recommended as an objective and exogenous marker to assess completeness of 24-h urine collection because, in theory, it seems less affected by participants' characteristics and diet[26,57]. However, even this method has its limitations, namely related to an increased burden on participants, and it has been suggested that para-aminobenzoic acid check may not be required in large population-based biomarker studies[58]. In our study, in the absence of the para-aminobenzoic acid method, urine completeness was assessed using a widely used criteria based on creatinine excretion[24] in combination with total urine volume[23,25].

We share the general limitation of one single 24-h urine collection while measuring the daily N, K and Na excretions. Only a single 24-h urine sample may not be optimal for characterising individual habitual dietary intake and may introduce random errors. Additional 24-h urine collections may be needed for individual assessment due to day-to-day within-person variability[22,59]. In addition, we should reflect about the precision of the correction factors used for estimating dietary intake from 24-h urine. Many factors may influence the percentage of N, K and Na excreted in the urine, such as absolute level of dietary intake, the seasons during which urine samples are collected and race[60]. We used the factors observed in previous studies[10,11]; however, the use of other correction factors, or no correction factors, may influence the magnitude of the error[54,61,62].

Conclusion

We assessed the validity of a dietary self-report method, using a new software – eAT24, with biomarker measurements. At a population level, we showed that two non-consecutive 24-HDR performed well in estimating protein and K intakes but lesser so in estimating Na intake. Our results confirmed that misreporting is selective to certain nutrients, which may have implications for how we deal with the misreporting often seen in nutritional epidemiological studies. Research to develop further intake biomarkers should be strongly encouraged.

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