

Challenges in estimating the validity of dietary acrylamide measurements

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Abstract

Background Acrylamide is a chemical compound present in tobacco smoke and food, classified as a probable human carcinogen and a known human neurotoxin. Acrylamide is

formed in foods, typically carbohydrate-rich and protein-poor plant foods, during high-temperature cooking or other thermal processing. The objectives of this study were to compare dietary estimates of acrylamide from questionnaires (DQ) and 24-h recalls (R) with levels of acrylamide adduct (AA) in haemoglobin.

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Methods In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, acrylamide exposure

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was assessed in 510 participants from 9 European countries, randomly selected and stratified by age, sex, with equal numbers of never and current smokers. After adjusting for country, alcohol intake, smoking status, number of cigarettes and energy intake, correlation coefficients between various acrylamide measurements were computed, both at the individual and at the aggregate (centre) level.

Results Individual level correlation coefficient between DQ and R measurements ($r_{DQ,R}$) was 0.17, while $r_{DQ,AA}$ and $r_{R,AA}$ were 0.08 and 0.06, respectively. In never smokers, $r_{DQ,R}$, $r_{DQ,AA}$ and $r_{R,AA}$ were 0.19, 0.09 and 0.02, respectively. The correlation coefficients between means of DQ, R and AA measurements at the centre level were larger ($r > 0.4$).

Conclusions These findings suggest that estimates of total acrylamide intake based on self-reported diet correlate weakly with biomarker AA Hb levels. Possible explanations are the lack of AA levels to capture dietary acrylamide due to individual differences in the absorption and metabolism of acrylamide, and/or measurement errors in acrylamide from self-reported dietary assessments, thus limiting the possibility to validate acrylamide DQ measurements.

Keywords Acrylamide · Dietary questionnaires · Haemoglobin adducts · Biomarkers · Smoking · Measurement errors

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Introduction

Acrylamide is a chemical compound found in tobacco smoke [1] and food [2] and other sources such as occupational exposure, consumer products and textiles [3]. It is a potential human carcinogen and a known human neurotoxin [4]. Acrylamide is formed in foods, typically carbohydrate-rich and protein-poor plant commodities, during cooking or other thermal processing such as frying, baking or roasting at temperatures of 120 °C or higher. Acrylamide's presence in foods may pose a public health concern.

Exposure to dietary acrylamide is usually assessed through estimates based on self-reported dietary assessments of known acrylamide-containing foods, typically by means of dietary questionnaires (DQ), weighted records or 24-h dietary recalls (R). Assessing dietary exposure to acrylamide is challenging because exposure levels depend heavily on the nature and extent of heat treatment of specific foods. In addition, preparation methods vary between different populations and are particularly difficult to capture with DQs, as these instruments are generally not conceived to collect information about the processing and the cooking methods of consumed foods. In parallel, haemoglobin (Hb) adducts of acrylamide and its primary metabolite glycidamide have been considered biomarkers of acrylamide exposure. These measurements provide information about the amount of acrylamide exposure accumulated during the last 4 months [5].

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Although recent evidence suggests that a dose–response relationship exists between dietary acrylamide exposures and Hb adduct levels [6], a number of correlation studies reported mixed results on the relationship between self-reported DQ acrylamide intake and measured Hb adduct levels in humans, the magnitude of correlation coefficients ranging from moderate [7–9] to trivial values [3, 10].

Results of epidemiological studies on the association between dietary acrylamide exposure and risk of various cancer have been reported, with inconclusive findings, often differential to the type of assessments (dietary vs. biomarkers) and the type of cancer investigated [11, 12]. As it is not clear whether the inconsistency of findings are the result of methodological limitations and/or a lack of variability in the exposure or a true lack of association, further epidemiological studies of dietary acrylamide exposure and cancer risk have been recommended [11].

In a subgroup of the European Prospective Investigation into Cancer and Nutrition (EPIC) study [13], Hb adducts of acrylamide and glycidamide were measured [14]. In the current study, acrylamide and glycidamide Hb measurements were compared to dietary levels of acrylamide assessed in DQ and R measurements. Correlation coefficients between different acrylamide measurements were computed, both at the individual and at the aggregate levels, and the main sources of variation were determined and evaluated.

Methods

EPIC is a multicentre cohort study investigating the relation between diet, nutritional and metabolic characteristics,

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various lifestyle and environmental factors, and the risk of cancer and other chronic diseases among over 500,000 study subjects [13]. Twenty-three research centres in 10 European countries participate in the study. Collection of data and blood samples started in 1992. A subsample of 37,000 participants also provided dietary information by means of a single standardized 24-h dietary recall for calibration purposes [15].

Study population

In this work, the study population, described in detail elsewhere [14], was a random sample of 510 persons from 9 European countries, that is, Denmark (Aarhus and Copenhagen), France, Germany (Heidelberg and Potsdam), Greece, Italy (Northern and Southern centres), the Netherlands, Spain, Sweden (Malmö and Umeå), United Kingdom (Cambridge and Oxford health conscious), as shown in Online Resource 1. The sample was evenly represented by men and women, with the exception of France where women only were selected. Within the groups of men and women, an equal number of smokers and never smokers, based on self-reported questionnaire data, were sampled. Study subjects' age ranges were 41–60 years in men and 43–60 years in women. The IARC and CDC ethics committees approved the study, and the study was conducted according to the guidelines of the Helsinki declaration. Hb acrylamide and glycidamide were measured in 300 µL of hemolysed erythrocytes and analysed by HPLC/tandem mass spectrometry (HPLC/MS/MS) as described previously [14, 16]. In brief, the N-terminal valines of haemoglobin with acrylamide and glycidamide attached were cleaved from the protein chain using modified Edman reaction with pentafluorophenyl isothiocyanate as Edman reagent [17]. The resulting Edman products (Acrylamide-Val-PFPTH and Glycidamide-Val-PFPTH) were extracted using liquid–liquid extraction and analysed by HPLC/MS/MS. Calibrators, reagent blanks, and quality-control materials were processed the same way as the samples. Two independent measurements per sample were performed. Haemoglobin adduct concentrations were reported relative to the amount of haemoglobin used in the analysis. The detection limits for this method were 3 and 4 pmol/g of Hb for acrylamide and glycidamide, respectively. Blood samples were analysed in a randomized manner to minimize systematic analytical differences between country groups.

Dietary data

Information on habitual dietary intakes was assessed at baseline with different dietary questionnaires (France, the Netherlands, Germany, Greece, and Italy, except Naples), a

diet history questionnaire (Spain), a modified diet history methodology combining a quantified dietary frequency questionnaire, a 7-day menu book and a 1-h interview (Malmö, Sweden), or a semi-quantitative food frequency questionnaire (United Kingdom and Naples) developed and validated in each participating country [13]. In addition, a single 24-h dietary recall measurement per subject was collected in EPIC as a reference measurement from a subsample ($\sim 8\%$) of each cohort [15]. In contrast to baseline questionnaires, 24-h dietary recall interviews were standardized across countries using the same structure and interview procedure and a common computer program (EPIC-Soft[®], IARC, Lyon, France). More details on the concept of standardization and the structure of EPIC-Soft are described in detail elsewhere [18].

In order to compile a harmonized acrylamide database, values from the EU monitoring database of acrylamide levels in food maintained by the Institute for Reference Materials and Measurements (IRMM) (<http://irmm.jrc.ec.europa.eu/activities/acrylamide/Pages/index.aspx>) were used. The data included in the IRMM database cover a broad range of food commodities. The data are mainly from official and private food laboratories, including food industry, of Germany, the Netherlands, Ireland, Greece, Italy, Spain and United Kingdom. Acrylamide values for foods not available in this database were completed with analysed data from the US FDA database (e.g. peanut butter) (US Food and Drug Administration 2004) and country-specific databases (Swedish, Dutch, Norwegian, French and German national databases). Details of national contact persons for acrylamide content in food items are available upon request to the Authors. An inventory of all food items occurring in the IRMM database and the additional sources for the 10 countries included in the present study was made and classified according to the EPIC-Soft classification [18]. The DQ items and, when available, its specific description (e.g. ‘potatoes baked’) were matched with the respective foods in the AA database. In general, DQ consumption factors on the proportion of type of cooking methods of a given food (e.g. potatoes) were extrapolated from the distribution of specific food items (e.g. boiled, fried, or roasted potatoes) using nationally available food consumption data. If an exact match was not possible, the item was matched to a mean of all foods of the food group in the AA database. Relevant information on food preparation was available for potatoes (except in Italy), bread, and breaded meat, fish and processed meat. For all countries, the same mean value of acrylamide content was assigned to the corresponding food.

Statistical analyses

Geometric means and inter-decile range (10–90th percentiles) of DQ and R measurements, of Hb acrylamide and

glycidamide adduct levels, as well as their ratio (GA/AA), were computed. In an exploratory phase, multivariable regression analyses were used to assess the contribution of a list of factors to the variability of dietary measurements, and of Hb adduct levels (acrylamide, glycidamide and their ratio). Each type of acrylamide measurement was modelled as a function of smoking status (never/current), number of cigarettes, country, age at recruitment, sex, physical activity, weight, level of schooling (None/Primary school/Technical school/Secondary school/Longer education/Not specified), alcohol (g/day), and energy intake (Kcal/day), overall and separately in smokers and never smokers. Models for Hb adducts included the effect of batch. The partial R^2 (R^2_{partial}) was used to assess, conditional on other independent variables in the model, the relative amount of variability (expressed as a percentage) that each independent variable contributed to explain [19] in acrylamide measurements of, in turn, DQ, R and Hb adduct levels. In addition, R^2_{total} was also determined, as a measure of total variability explained by the ensemble of independent factors on the variability of each acrylamide measurement.

Correlation coefficients between dietary and Hb adduct levels of acrylamide were computed. Crude coefficients were primarily computed, and then adjustment was performed by country, alcohol intake, smoking status, number of cigarettes and energy intake. Adjustment was performed using the residual method, that is, by regressing each type of acrylamide measurement on the specific list of adjustment factors and computing residuals. Correlation coefficients were computed for the whole sample, by smoking status and by sex. Country-specific correlation coefficients were also computed. In addition, means of Hb adduct levels were compared with DQ and R measurements at the aggregate level by computing correlation coefficients using arithmetic centre-specific means. Aggregate-level correlation coefficients were adjusted by the mean of centre-specific energy intake, alcohol intake and number of cigarettes, and weighed by centre-specific sample size.

Consistently in this work, Pearson’s correlation coefficients were computed. The 95 % confidence intervals (CIs) were computed by applying Fisher’s z transformation [20]. In all statistical analyses, acrylamide measurements were log-transformed (natural logarithm). Regression and correlation analyses were conducted using the MIXED and CORR procedures, respectively, of the SAS software, version 9.3 [21].

Results

The design of the EPIC acrylamide measurements validation study is presented in Online Resource 1 by sex and participating country; a total of 510 subjects (50 % never

smokers, 50 % smokers) were selected. While self-reported DQ and R means were invariant with respect to smoking status, Hb adduct levels were between two- to threefold higher in smokers, with geometric means equal to 116.5 and 86.7 pmol/g in AA and GA, respectively, compared to never smoker subjects, with means equal to 44.4 and 39.0 pmol/g (Table 1). These large differences are also reflected in the total variation observed in Hb adduct levels, where 10 and 90th percentile values were ranging between 28.1 and 75.4 pmol/g in never smokers, and between 53.8 and 243.8 in smokers. Conversely, the GA/AA ratio showed a similar pattern in never and current smokers.

The analysis of the determinants of self-reported dietary intake and Hb adduct levels of acrylamide (Table 2) indicated that country and energy intake were the most important determinants of dietary acrylamide, in both DQ and R measurements, overall and by smoking status. Statistical models suggested that the ensemble of predictors explained some 66 % of total variability in acrylamide DQ measurements and 28 % in R values. On the other hand, smoking status, and the number of cigarettes smoked in smokers, explained a large proportion of AA and GA–Hb adduct variability, conditional on all other variables in the model. Country was still an important determinant of Hb adduct level. Alcohol intake displayed a value of R^2_{partial} equal to 6.9 and 23.5 % in never smokers and smokers, respectively, for the ratio GA/AA. Large R^2_{partial} values related to alcohol intake were also observed for GA measurements. The total amount of variability explained by the set of independent

variables (total R^2) ranged between 29 and 47 % for Hb adduct levels, in smokers or never smokers.

Table 3 shows correlation coefficients between different pair-wise combinations of acrylamide measurements, crude and adjusted. Consistently in almost all comparisons, as expected, adjusted correlation coefficients displayed lower values compared to crude ones. Adjustment for energy intake had an overall small impact on correlation values. The coefficients between DQ and R ($r_{\text{DQ,R}}$) were equal to 0.35 (95 %CI: 0.27, 0.42) and to 0.17 (0.08, 0.25), for crude and adjusted estimates, respectively. Lower correlations were observed between dietary measurements and Hb adduct levels, with values equal to 0.08 (−0.01, 0.17) for $r_{\text{DQ,AA}}$, to 0.06 (−0.03, 0.15) for $r_{\text{R,AA}}$ and 0.09 (0.01, 0.18) for $r_{\text{DQ,GA}}$. Overall, correlations displayed similar values according to smoking status, with the exception of $r_{\text{DQ,GA}}$, which was equal to 0.03 (−0.09, 0.15) and 0.14 (0.02, 0.26) in never smokers and smokers, respectively. Values of $r_{\text{DQ,GA/AA}}$ displayed rather poor agreement, with values close to zero if not negative. Overall, correlation coefficients were slightly higher in women compared with men. Correlations by country are available as Online Resource 2, overall and by smoking status. Values for $r_{\text{DQ,R}}$ and $r_{\text{DQ,AA}}$ ranged from −0.02 to 0.28, and from −0.16 to 0.26, respectively. In never smokers, $r_{\text{DQ,R}}$ and $r_{\text{DQ,AA}}$ ranged from −0.07 to 0.49, and from −0.16 to 0.40, respectively, with a sample size of 30 study subjects in each country.

Figure 1 shows geometric means of AA Hb adduct levels according to deciles of DQ acrylamide intake after

Table 1 Geometric mean (GM) and inter-decile range (10–90th percentiles) of dietary questionnaire, 24-h dietary recall (24-HDR) measurements^a and haemoglobin adducts of acrylamide (AA) and glycidamide (GA), overall and by smoking status

Measurements	Unit of measure	GM	10–90th
All (<i>n</i> = 510)			
Dietary questionnaire (DQ)	µg/day	24.7	11.6–50.4
24 HDR (R)	µg/day	21.8	7.1–59.5
Acrylamide adducts (AA)	pmol/g of Hb	71.9	33.2–195.9
Glycidamide adducts (GA)	pmol/g of Hb	58.2	26.6–142.4
Ratio ^b (GA/AA)		0.95	0.87–1.03
Never smokers (<i>n</i> = 255)			
Dietary questionnaire (DQ)	µg/day	23.4	11.4–48.0
24-HDR (R)	µg/day	19.9	6.5–53.2
Acrylamide Adducts (AA)	pmol/g of Hb	44.4	28.1–75.4
Glycidamide Adducts (GA)	pmol/g of Hb	39.0	22.1–69.8
Ratio ^b (GA/AA)		0.97	0.88–1.04
Smokers (<i>n</i> = 255)			
Dietary questionnaire (DQ)	µg/day	26.1	11.8–51.8
24-HDR (R)	µg/day	23.9	8.5–64.8
Acrylamide adducts (AA)	pmol/g of Hb	116.5	53.8–243.8
Glycidamide adducts (GA)	pmol/g of Hb	86.7	41.9–179.2
Ratio ^b (GA/AA)		0.94	0.86–1.01

^a A single replicate of 24-HDR was available in this study

^b Arithmetic mean, based on the ratio of log-transformed GA over log-transformed AA

Table 2 Values of R^2_{partial} (%) for non-dietary variables (with the exception of alcohol intake) in relation to log-transformed acrylamide levels based on self-reported dietary questionnaire (DQ) and 24-h

dietary recall measurements (24-HDR), and Hb adducts of acrylamide and glycidamide measurements, together with their ratio (GA/AA)

	DQ			24-HDR		
	Overall	Never smk	Smokers	Overall	Never smk	Smokers
Smoking status	0.8	–	–	1.4	–	–
Country	58.8	58.7	60.4	14.2	13.5	16.6
Age at recruitment	<0.1	0.9	0.8	0.2	0.8	<0.1
Sex	0.4	0.9	0.1	0.8	<0.1	2.4
Physical activity	1.5	0.6	3.0	0.9	1.2	1.4
Weight	0.1	0.1	<0.1	0.1	0.1	0.1
Level of schooling	0.3	1.9	0.2	0.3	1.3	<0.1
Alcohol intake ^a	2.4	1.9	2.7	0.4	0.7	<0.1
Number cigarettes	0.1	–	<0.1	<0.1	–	0.2
Energy intake ^a	30.6	27.0	33.9	36.1	34.5	36.9
R^2_{total}	66.0	65.8	67.6	28.0	28.5	30.3

	Acrylamide			Glycidamide			GA/AA		
	Overall	Never smk	Smokers	Overall	Never smk	Smokers	Overall	Never smk	Smokers
Smoking status	17.0	–	–	11.9	–	–	0.7	–	–
Country	10.0	21.7	16.5	10.8	23.6	14.8	4.7	9.8	5.1
Age at recruitment	<0.1	0.7	0.1	<0.1	<0.1	<0.1	0.2	0.7	0.4
Sex	0.3	0.9	<0.1	0.6	0.3	0.7	5.8	7.4	3.9
Physical activity	0.9	2.6	1.3	0.5	1.1	2.5	0.6	0.7	3.2
Weight	1.5	<0.1	6.0	<0.1	1.7	2.5	2.6	3.4	2.0
Level of schooling	1.2	<0.1	3.5	1.4	0.1	2.9	0.1	0.5	<0.1
Alcohol intake ^b	<0.1	0.3	<0.1	4.8	4.4	7.1	12.3	6.9	23.5
Batch	3.6	1.9	9.3	1.3	1.5	3.6	3.4	4.0	5.4
Number cigarettes	19.5	–	29.4	16.6	–	25.3	<0.1	–	<0.1
Energy intake ^b	<0.1	2.2	2.4	1.5	0.6	7.9	0.1	<0.1	0.1
R^2_{total}	64.4	29.0	46.8	56.3	32.2	43.5	32.4	29.4	41.8

^a Dietary variables from DQ and 24-HDR were used to adjust DQ and 24-HDR measurements, respectively^b Alcohol and energy intakes according to dietary questionnaire

adjustment for country, energy intake, alcohol intake, number of cigarettes, separately by smoking status. Both groups did not show linear positive relationships.

Estimates of the coefficients obtained at the aggregate level, reported in Table 4, were in general higher than values at the individual level, particularly for values involving R measurements. DQ measurements displayed correlations with R, AA and GA equal to 0.65 (0.18, 0.88), 0.42 (–0.14, 0.78) and 0.32 (–0.25, 0.73), respectively. Aggregate correlations between acrylamide levels based on self-reported assessments, and Hb adducts were rather similar according to smoking status and sex, with the exception of $r_{R,GA}$, with values larger in smokers than never smokers. Conversely, higher values of $r_{DQ,R}$ were observed in never smokers (0.78: 0.43, 0.93) than smokers (0.48: –0.07, 0.81), and in women (0.88: 0.66, 0.96) compared to men (0.21: –0.36, 0.67).

Discussion

In this study, estimates of acrylamide based on self-reported dietary measurements (i.e. DQ and R) were compared to Hb adduct levels of acrylamide using data collected from 510 participants from 9 countries in the EPIC study. After controlling for a list of covariables, individual level correlation coefficients between DQ and R measurements and Hb adduct levels of acrylamide, and glycidamide, were overall low, both in smokers and never smokers, with correlation values consistently lower than 0.20. Unlike previous observations [9], correlations were of similar magnitude between DQ and the sum of acrylamide and glycidamide Hb levels (data not shown).

Consistent with previous evidence on Hb adducts in the EPIC study [14], dietary measurements of acrylamide varied considerably by country, particularly in DQ. This

Table 3 Individual-level correlation coefficients (95 % CI) between log-transformed values of dietary questionnaire (DQ), 24-h dietary recall (R) measurements and Hb adducts of acrylamide (AA), glycidamide (GA) and their ratio (GA/AA), crude and adjusted

	$r_{DQ,R}$	$r_{DQ,AA}$	$r_{R,AA}$	$r_{DQ,GA}$	$r_{R,GA}$	$r_{DQ,GA/AA}$
All subjects ($n = 510$)						
Crude	0.35 (0.27, 0.42)	0.17 (0.08, 0.25)	0.15 (0.06, 0.23)	0.14 (0.05, 0.22)	0.14 (0.05, 0.22)	-0.06 (-0.15, 0.03)
Adjusted ^a	0.20 (0.12, 0.28)	0.07 (-0.02, 0.16)	0.05 (-0.04, 0.14)	0.12 (0.03, 0.20)	0.07 (-0.02, 0.16)	0.08 (-0.01, 0.17)
+EI ^b	0.17 (0.08, 0.25)	0.08 (-0.01, 0.17)	0.06 (-0.03, 0.15)	0.09 (0.01, 0.18)	0.07 (-0.02, 0.16)	0.01 (-0.08, 0.09)
Never smokers ($n = 255$)						
Crude	0.36 (0.25, 0.46)	0.11 (-0.01, 0.23)	0.07 (-0.05, 0.19)	0.04 (-0.08, 0.16)	0.02 (-0.10, 0.14)	-0.10 (-0.22, 0.02)
Adjusted ^c	0.19 (0.07, 0.31)	0.03 (-0.09, 0.15)	0.01 (-0.11, 0.13)	0.01 (-0.11, 0.31)	-0.01 (-0.13, 0.11)	0.02 (-0.10, 0.14)
+EI ^d	0.19 (0.07, 0.31)	0.09 (-0.03, 0.21)	0.02 (-0.10, 0.14)	0.03 (-0.09, 0.15)	-0.01 (-0.13, 0.11)	-0.09 (-0.21, 0.03)
Smokers ($n = 255$)						
Crude	0.32 (0.21, 0.43)	0.17 (0.05, 0.29)	0.12 (-0.01, 0.24)	0.17 (0.05, 0.29)	0.15 (0.03, 0.27)	0.01 (-0.11, 0.13)
Adjusted ^c	0.21 (0.09, 0.32)	0.09 (-0.03, 0.21)	0.06 (-0.06, 0.18)	0.20 (0.08, 0.32)	0.11 (-0.01, 0.23)	0.21 (0.09, 0.32)
+EI ^d	0.16 (0.04, 0.28)	0.08 (-0.04, 0.20)	0.09 (-0.03, 0.21)	0.14 (0.02, 0.26)	0.13 (0.01, 0.25)	0.11 (-0.01, 0.23)
Men ($n = 240$)						
Adjusted ^c	0.14 (0.01, 0.26)	0.10 (0.03, 0.22)	-0.01 (-0.14, 0.12)	0.07 (-0.06, 0.20)	0.07 (-0.06, 0.20)	-0.04 (-0.17, 0.09)
Women ($n = 270$)						
Adjusted ^c	0.21 (0.09, 0.32)	0.06 (-0.06, 0.18)	0.13 (0.01, 0.25)	0.11 (-0.01, 0.23)	0.06 (-0.06, 0.18)	0.07 (-0.05, 0.19)

^a Using the residual method, data were adjusted by country, alcohol intake, smoking status, number of cigarettes

^b As in (a) with additional adjustment by energy intake

^c Using smoking status-specific residual models, data were adjusted by country, alcohol intake, number of cigarettes (in smokers only)

^d As in (c) with adjustment by energy intake

^e Using sex-specific residual models, data were adjusted by country, energy intake, alcohol intake, smoking status, number of cigarettes

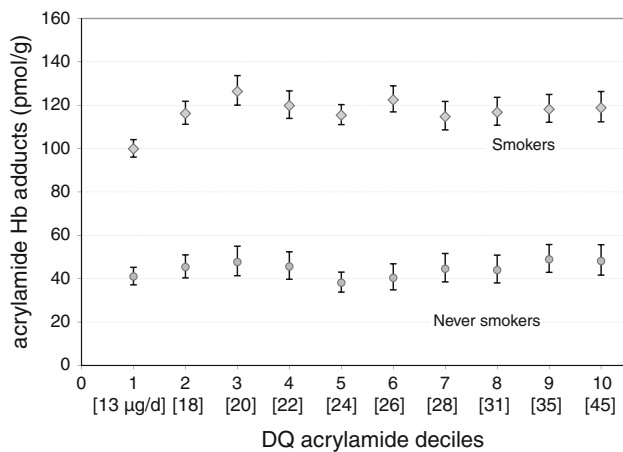


Fig. 1 Geometric means^a (95 % CI)^b of acrylamide Hb adducts by decile of DQ acrylamide intake, separately in smokers and never smokers. ^a Using smoking status-specific residual models, data were adjusted by country, energy intake, alcohol intake and number of cigarettes (in smokers only); ^b Confidence intervals determined as $\exp[\text{mean}[\log(x)] \pm 1.96 \text{ SE}[\text{mean}]]$, where x expresses individual Hb values and $\exp[\text{mean}[\log(x)]]$ is the geometric mean

variation was less apparent in R measurements, possibly because a common standardized methodology using EPIC-Soft across the participating countries was used to collect 24-h dietary recall measurements [18]. Energy intake

appeared to be a relevant factor in explaining the observed variability in acrylamide intake, thus suggesting that dietary patterns with acrylamide-rich foods tend to be characterized by large caloric contributions. An evaluation of the major food sources of dietary acrylamide intake in the EPIC study is ongoing [33].

As previously noted, smoking status and alcohol intake are the factors more strongly associated with Hb adducts variability, particularly in glycidamide [14]. While smoking is an important source of overall acrylamide exposure, lower GA-Hb adducts and GA/AA ratios were observed with increasing alcohol intake, which competitively inhibits CYP2E1-mediated metabolism of AA to GA [22]. Glycidamide is an important exposure because, unlike acrylamide, it possesses mutagenic properties [12]. In particular, in evaluating the potential health effects of acrylamide, the exposure balance between acrylamide and glycidamide in the body is of relevance.

One important limitation of haemoglobin adducts of acrylamide as a biomarker of dietary acrylamide exposure is that it reflects a fairly recent period of exposure (up to 3–4 months) and were shown to display large intra-individual variability [23], likely to reflect variations in intake of acrylamide-rich foods, whereas DQ measurements typically assess study participants’ diet over the past year.

Table 4 Aggregate-level correlation coefficients^a (95 % CI) using centre-specific means of log-transformed values of dietary questionnaire (DQ), 24-h dietary recall (R) measurements and Hb adducts of acrylamide (AA), glycidamide (GA)

	$r_{DQ,R}$	$r_{DQ,AA}$	$r_{R,AA}$	$r_{DQ,GA}$	$r_{R,GA}$
All subjects ($n = 14$)	0.65 (0.18, 0.88)	0.42 (−0.14, 0.78)	0.58 (0.07, 0.85)	0.32 (−0.25, 0.73)	0.47 (−0.08, 0.80)
Never smokers ($n = 14$)	0.78 (0.43, 0.93)	0.34 (−0.23, 0.74)	0.49 (−0.05, 0.81)	0.21 (−0.36, 0.67)	0.23 (−0.34, 0.68)
Smokers ($n = 14$)	0.48 (−0.07, 0.81)	0.25 (−0.32, 0.69)	0.70 (0.27, 0.90)	0.23 (−0.34, 0.68)	0.77 (0.40, 0.92)
Men ($n = 13$)	0.21 (−0.36, 0.67)	0.43 (−0.13, 0.78)	0.69 (0.25, 0.89)	0.19 (−0.38, 0.66)	0.55 (0.38, 0.93)
Women ($n = 14$)	0.88 (0.66, 0.96)	0.39 (−0.18, 0.76)	0.51 (−0.03, 0.82)	0.34 (−0.23, 0.74)	0.42 (−0.14, 0.78)

^a Coefficients were adjusted by centre-specific means of energy intake, alcohol intake, number of cigarettes (in smokers) and weighted by the centre-specific sample size

Given that long-term dietary exposure is more relevant with respect to development of cancer, a single measurement of blood adduct levels may not be sufficient. Furthermore, adducts capture more than one source of acrylamide exposure, not just the dietary component, and they also reflect individual differences in absorption and metabolism. Among others, the extent of passive smoking may well affect the concentration of adduct levels. In general, non-dietary sources of acrylamide Hb adducts seem to be more relevant than previously thought. In this study, correlations were consistently adjusted by smoking status, number of cigarettes smoked and alcohol intake, while no information was available on other potentially relevant factors, including the extent of individuals' exposure to passive smoking.

Recently published results from an intervention study in humans confirmed that a dose–response relationship exists between dietary acrylamide exposures and Hb adduct levels [6]. It is worthwhile mentioning that acrylamide intake in this intervention study was much higher than intakes observed in usual diets (~5 to 50-fold higher). Furthermore, the acrylamide content of duplicates of foods consumed by participants in the intervention groups were precisely analysed [6], thus the evaluation was carried out in controlled settings. A number of correlation studies reported mixed results on the relationship between dietary acrylamide intake based on self-reported measurements and measured Hb adduct levels in humans. In a small study of 50 Norwegian adults, no correlation between FFQ acrylamide intake and Hb adduct level was observed [10]. In the Malmo Diet and Cancer study, correlation coefficients between dietary acrylamide assessed with a modified diet history and adduct levels from all food sources were 0.43 in non-smokers and 0.36 in smokers [7], although correlations were probably overestimated because participants were enrolled on the basis of high and low intake of acrylamide-containing foods. Weaker correlations of the order of 0.18 and 0.17 in women and men, respectively, were reported in a sample of non-smokers [8], using FFQ and 24-h dietary recall measurements. Slightly larger correlation between FFQ acrylamide intake and Hb adducts were observed in the

Nurses' Health Study II [9], with correlations equal to 0.29 and 0.35 for AA and GA–Hb levels, respectively. In a representative study of the United States with over 3,700 non-smoking subjects (three years of age and older), a low correlation between dietary acrylamide intake estimates and AA and GA–Hb adducts was found ($r = 0.10$) [3].

Among the possible explanations for the low correlation observed between dietary acrylamide and Hb in this and other published studies, it could be worth to mention the following, in addition to the already mentioned limitations of Hb measurements. Dietary assessment instruments group multiple foods with varying acrylamide content into single items, whereas acrylamide content could vary substantially even within single foods, with respect to cooking method, cooking time, brand and others aspects of food preparation practices, for whom information is seldom available. Moreover, important information on the extent of cooking or heat treatment of both at home and commercially prepared foods was lacking in our study as in many other observational studies. Further, the acrylamide database was compiled assembling data from European sources as well as from the US FDA database, thus reducing the specificity of country-specific acrylamide estimates. Among other limitations, it is noteworthy to stress that, with few exceptions, DQs are not designed to capture information of food processing, such as cooking methods, extent of cooking and/or brands. Last but not least, it is well accepted that dietary self-reported measurements suffer from bias due to measurement errors, and thus do not provide accurate measures of intake in general.

One additional limitation in our study was the availability of only one replicate of 24-h dietary recall measurement. The sporadic nature of consumption of some acrylamide-rich food items (crisps, fried potatoes, etc.) resulted in large amounts of *day-to-day* variability in R measurements, likely to be one of the main reasons for the absence of correlation with acrylamide Hb adduct levels, with coefficients equal to 0.09 and 0.02 in smokers and never smokers, respectively.

In most recent dietary measurement validation studies, fairly basic comparisons of questionnaire measurements

with short-term assessments (food diaries or 24-HDRs) moved to more complex comparisons of self-reported assessments with objective measurements, such as dietary biomarkers [24–26]. Based on the assumption that Hb adducts could be used as objective measurements to integrate dietary estimates of acrylamide exposure, the use of measurement error model was originally planned in this study to appropriately account for the error structure of the different types of measurement involved. However, based on the limited amount of information available at the individual level in our data, even among never smoker participants, estimates were very unstable and the exercise was not implemented further, thus limiting the possibility to carry out a proper validation study.

In our study, crude and adjusted correlation coefficients were computed. Among other relevant factors, adjustment for country was undertaken to account for systematic differences across centres in questionnaire design, biological sample treatment and other unmeasured group effects. Adjustment for country attenuated the magnitude of correlation coefficients, while the impact of adjusting for energy intake was overall limited. Larger correlation coefficients were observed at the aggregate levels focusing on centre-specific means. All pair-wise comparisons displayed larger correlations than coefficients computed using the individual level component. Unlike aggregate measures, individual level measurements are affected by within-person variation, which is likely the result of random measurement errors and *day-to-day* variation in dietary exposure [27, 28]. This observation is in line with previous evidence produced in the EPIC study, where the agreement between self-reported and objective measurements was larger at the aggregate level [29, 30]. Given the multicentre nature of the EPIC study, these comparisons are a natural way to evaluate any level of evidence contained in the data, although in most epidemiological investigations, it is mostly the individual level component that is exploited [31]. In the EPIC study, the definition of statistical models that entail individual and aggregate level components into the same statistical model was used in the past [32], and it is currently the object of ongoing research.

In summary, in a subsample of the EPIC study, sources of variability of dietary acrylamide from DQ and R measurements, as well as of Hb adducts levels, were investigated. A comparison of correlation coefficients between the different type of measurements revealed that, after adjustment for relevant covariables, the agreement between estimates of acrylamide based on self-reported and objective measurements is rather poor, with larger values of correlation coefficients at the aggregate level. Values of individual level correlations were poor even among never smokers. These results indicate that in the EPIC study, the assessment of validity of dietary acrylamide measurements

is limited by a combination of relevant key factors: (1) the presence of measurement errors in dietary estimates of acrylamide, (2) the availability of one single replicate of R measurements per study subject in the present design, (3) the challenge of compiling a comprehensive database of acrylamide content in foods, (4) the lack of (over time replicates of) an objective biomarker measurement, specific enough to capture dietary exposure of acrylamide. In this context, ongoing comparisons of food sources of acrylamide as estimated by DQ and R measurements in the EPIC study might well reveal interesting features of the patterns of acrylamide consumption across EPIC centres.

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