International Journal of Hygiene and Environmental Health 217 (2014) 751–757

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Urinary concentrations of acrylamide (AA) and *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine (AAMA) and associations with demographic factors in the South Korean population

Jin Heon Lee [a,*], Kee Jae Lee [b], Ryoungme Ahn [c], Hee Sook Kang [d]

[a] Department of Environmental Education, College of Education, Kongju National University, Gongju, South Korea
[b] Department of Information and Statistics, College of Natural Science, Korea National Open University, Seoul, South Korea
[c] Department of Health Science, School of Natural Science, Dongduk Women's University, Seoul, South Korea
[d] Department of Social Welfare, College of Humanities and Social Sciences, Kongju National University, Gongju, South Korea

ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form 15 March 2014
Accepted 16 March 2014

Keywords:
Korean
Biomonitoring
Acrylamide
N-acetyl-*S*-(2-carbamoylethyl)-cysteine
Urine
Demographic characteristics

ABSTRACT

Acrylamide (AA) and *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine (AAMA) are important urinary biomarkers of acrylamide exposure in human biomonitoring, because AA is classified as a probable carcinogen in humans.

In this study, urinary AA and AAMA were assessed in the South Korean adult population aged 18–69, based on the Korean National Human Biomonitoring Survey conducted in 2009. Urinary metabolites in samples were analyzed with LC–MS/MS system.

Relying on data from 1873 representative South Korean adults, the population-weighted geometric means of urinary AA and AAMA concentrations were 6.8 ng/ml (95% CI: 6.4–7.3), and 30.0 ng/ml (95% confidence interval (CI): 28.2–31.8), respectively. The creatinine-adjusted geometric means of AA and AAMA were 6.2 μg/g creatinine (95% CI: 5.8–6.7) and 26.4 μg/g creatinine (95% CI: 24.9–28.0), respectively. When covariates for predictors of urinary metabolites were adjusted simultaneously in a log-linear multiple regressions, the strongest predictors of urinary AA were education (OR = 1.08–1.28; 95% CI: 1.11–1.48; $p = 0.0024$) and age (OR = 0.66–0.84; 95% CI: 0.54–0.97; $p = 0.0003$), and those of urinary AAMA were smoking status (OR = 1.16–2.63; 95% CI: 0.98–3.08; $p = 0.001$) and education (OR = 1.12–1.19; 95% CI: 1.02–1.38; $p = 0.0425$). The ratio of current/never smokers for urinary AA was 1.3, whereas the same ratio for urinary AAMA was 3.0.

These findings suggested that most South Koreans had detectable levels of AA and AAMA (98.7% and 99.4%, respectively) in their urine and that the body burden of AA and AAMA varied according to demographic, geographic, and lifestyle (smoking) factors.

Introduction

Acrylamide (AA), a common industrial and laboratory chemical, is present in high concentrations in carbohydrate-rich foods that are prepared at high temperatures, especially fried and baked starch-enriched food, such as rice, French fries, potato chips, and coffee (Friedman, 2003; Dybing et al., 2005; Mucci and Wilson, 2008). The consumption of these foods may result in significant human exposure to AA (Tareke et al., 2002). Based on food contents, the average daily intake of AA for adults in western countries has been estimated to be in the range of 0.2–1.4 μg/kg body weight. However, depending on diet in younger age groups, a higher exposure is assumed in children and adolescents, who reached up to 3.4 μg/kg body weight diet (95th percentile) in Berlin, Germany (Friedman, 2003; Dybing et al., 2005; Fuhr et al., 2006).

Dietary exposure to AA affects all nutritional patterns with different exposure levels across all age groups, countries, and composition of diets. The toxicokinetics of AA have been revealed through studies of rats and mice (Dybing et al., 2005; Fennell et al., 2005; Shipp et al., 2006), and AA in the human body is readily absorbed and widely distributed to tissues, crosses the human placenta, and is transferred into breast milk (Sorgel et al., 2002; Schettgen et al., 2004; Fuhr et al., 2006). AA is neurotoxic (LoPachin et al., 2003), clastogenic (Ghanayem et al., 2005), and mutagenic in somatic and germ cells in rodents (Mucci and Wilson, 2008; Klaunig, 2008), and is considered as a probable

* Corresponding author. Tel.: +82 41 850 8814.
E-mail address: ejhl@kongju.ac.kr (J.H. Lee).

http://dx.doi.org/10.1016/j.ijheh.2014.03.005
1438-4639/

carcinogen in humans (IARC, 1994). Human biomonitoring using a good biomarker could protect people exposed to AA from these dangers. *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine (AAMA) in urine is an excellent AA biomarker for biomonitoring national scale general populations, because AA that is taken up through the diet is able to form glutathione conjugates that are converted to the mercapturic acid metabolite, *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine (AAMA), which has been detected in human urine (Fennell et al., 2005, 2006; Boettcher et al., 2005, 2006). Also, the absorbed AA may be largely converted to AAMA and excreted in urine. Fuhr et al. (2006) said that, overall, 60.3% of the human dose was recovered in the urine, and unchanged AA and AAMA accounted for urinary excretion of 4.4% and 50.0% of the dose, respectively. The apparent terminal elimination half-lives for unchanged AA and AAMA were 2.4 h and 17.4 h, respectively (Fuhr et al., 2006).

Population-based surveillance of exposure to toxic substances is essential for determining the mean exposure level of a population, identifying high-risk groups, describing geographical differences, and predicting adverse health effects. Several countries, including the United States (Vesper et al., 2010), Germany (Boettcher et al., 2005; Heudorf et al., 2009) and Norway (Dybing et al., 2005), have conducted general population-based biomonitoring surveys that have included analyses of AA metabolites. In 2009 South Korea conducted a human biomonitoring survey for hazardous materials, which included urinary concentrations of AA and AAMA, using a representative sample of South Korean adults aged 18–69. Biomonitoring studies have indicated that urinary chemical levels, including those of AA and AAMA, vary significantly according to race/ethnicity in the population studied (Vesper et al., 2010). Furthermore, epidemiological studies have revealed many contributing factors to the body burdens of AA and AAMA, including sex, age, household income, and smoking status (Vesper et al., 2010; Hagmar et al., 2005). The present report describes urinary AA and AAMA concentrations according to demographic factors, and attempts to elucidate the demographic characteristics that are potentially influencing these concentrations, based on the South Korean national survey data.

Materials and methods

Study population

This study was conducted between July and October 2009 in South Korea, and was based on a cross-sectional survey representing the adult population (18–69 years of age) residing in South Korea. Participants were recruited from 100 census blocks selected by stratified two-stage cluster random sampling design, based on the National Census Registry. Of the selected subjects in the census blocks, 1870 individuals completed interviews and provided urine samples, and the overall response rate was 87.4%. The Korean Food and Drug Administration supervised this study, while the Asan Medical Center Institutional Review Board approved the study protocol in accordance with the ethical principles for medical research involving human subjects, as defined by the Helsinki Declaration. Study participants provided written, informed consent.

Data collection

Selected subjects were invited to a public health center in the designated census block for an interview and collection of urine sample. Demographic data, including sex, age, education, income, smoking status, and current residence were gathered through face-to-face interviews. Height and weight were measured while subjects were wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (in meters squared). Spot urine samples were collected at different times throughout the day, because of the easy of collection of large population samples, and the wide use of matrixes for the biomonitoring of nonpersistent chemicals, such as acrylamide that has a biologic half-life of 2.5 h (Barr et al., 2005; Bjellaas et al., 2007). The variability of volume of spot urine samples was adjusted with urinary creatinine concentrations (Barr et al., 2005). For purposes of analysis, education was categorized as less than a high school diploma, high school diploma, and college or higher. Income was categorized by monthly household income [≤$880, $880–2649, $2650–4410, and ≥$4410]. Subjects were categorized as underweight (BMI < 18.5), normal (18.5≤BMI≤23.0), overweight (23.0≤BMI < 25.0), or obese (BMI≤25.0) according to WHO definitions for Asian populations. The sample was also divided into groups according to smoking history (i.e., never, former, or current) and current residence (i.e., rural or urban).

Analysis of AA and AAMA

Stock solutions (1.0 mg/ml) of each standard were made by dissolving individual standard AA (Fluka Co., Buchs, Switzerland) and AAMA (C/C/N Co., Quebec, Canada) in water filtered through Milli-Q system (Millipore, Milford, MA, USA). Each standard solution (10 μg/ml) was prepared by diluting it 100-fold with the same water. Likewise, stock recovery standard solutions of AA (10, 30, 100 ng/ml) and AAMA (50, 200 ng/ml) were prepared by dissolving them with water through the M-Q system. Internal standard solutions (10 μg/ml) were prepared by diluting 1000 μg/ml of acryamide-d3 and AAMA-d4 (C/D/N Co., Quebec, Canada) by diluting them 100-fold with water filtered the M-Q system.

The AA and AAMA in the samples were analyzed with a LC–MS/MS system, which was composed of a HPLC system (Varian, Palo Alto, CA, USA) and triple-quadrupole tandem mass spectrometry coupled with electrospray ionization (Varian, Palo Alto, CA, USA). The column used was a Waters (Milford, MA USA) Atlantis T3-C18 (2.1 × 100 mm, 3um). The flow rate was 200 μl/min and was split by 50% prior to MS/MS detection. Acrylamide and AAMA were eluted with 0.1% formic acid in water. After 25 min, the column was washed with 95% acetonitrile, and 5% of 0.1% formic acid in water for 5 min. A 5-min gradient was run to return the column to its starting conditions, and reequilibration was conducted for 10 min (Fennell et al., 2006).

Urine samples were thawed to room temperature before further treatment and 50 μl was taken for analysis. Each internal standard solution (5 μl of each) was added to each sample and standard sample curve sample to give a final volume of 100 μl. Each sample was filtered by using the Ultrafree-MC Durapore (PVDH, 0.1 μm × 0.5 ml Millipore) and Vacuum Manifold 20-hole (Milford, MA, USA), and was then vortexed and centrifuged at 12000 rpm for 2 min, before being transferred to a vial tube. The sample injection volume of the LC–MS/MS system was 7 μl. Elution was monitored by selected reaction monitoring (SRM) in the positive ion mode. The ion source was electrospray, the nebulizer gas was set at 55 psi, and the curtain gas was set at 20 psi. The ionization voltage was set at 5400 V. The calibration samples, quality control samples, and urine samples were all subjected to the same solid-phase extraction method as described elsewhere (Bjellaas et al., 2005, 2007; Fennell et al., 2006).

The linearity in these analytes was determined from 1.0 and 10.0 (AA and AAMA, respectively) to 1,000 ng/ml, and correlation coefficients of 0.998 and 0.999 were calculated for AA and AAMA, respectively. Recovery was performed with fresh urine samples which had concentrations that similar to field samples due to the addition of standard solutions of AA and AAMA. Recovery of AA was ranged from 86.7 to 92.5% and AAMA was ranged from 92.9 to 101.0%. Intra- and inter-day accuracy and precision were