Acrylamide Exposure and Oxidative DNA Damage, Lipid Peroxidation, and Fasting Plasma Glucose Alteration: Association and Mediation Analyses in Chinese Urban Adults

Bin Wang,[1,2] Weihong Qiu,[1,2] Shijie Yang,[1,2] Limin Cao,[1,2] Chunmei Zhu,[1,2] Jixuan Ma,[1,2] Wei Li,[1,2] Zhuang Zhang,[1,2] Tao Xu,[1,2] Xing Wang,[1,2] Man Cheng,[1,2] Ge Mu,[1,2] Dongming Wang,[1,2] Yun Zhou,[1,2] Jing Yuan,[1,2] and Weihong Chen[1,2]

Diabetes Care 2020;43:1479–1486 | https://doi.org/10.2337/dc19-2603

OBJECTIVE

Acrylamide exposure from daily-consumed food has raised global concern. We aimed to assess the exposure-response relationships of internal acrylamide exposure with oxidative DNA damage, lipid peroxidation, and fasting plasma glucose (FPG) alteration and investigate the mediating role of oxidative DNA damage and lipid peroxidation in the association of internal acrylamide exposure with FPG.

RESEARCH DESIGN AND METHODS

FPG and urinary biomarkers of oxidative DNA damage (8-hydroxy-deoxyguanosine [8-OHdG]), lipid peroxidation (8-iso-prostaglandin-F2α [8-iso-PGF2α]), and acrylamide exposure (N-acetyl-S-[2-carbamoylethyl]-L-cysteine [AAMA], N-acetyl-S-[2-carbamoyl-2-hydroxyethyl]-L-cysteine [GAMA]) were measured for 3,270 general adults from the Wuhan-Zhuhai cohort. The associations of urinary acrylamide metabolites with 8-OHdG, 8-iso-PGF2α, and FPG were assessed by linear mixed models. The mediating roles of 8-OHdG and 8-iso-PGF2α were evaluated by mediation analysis.

RESULTS

We found significant linear positive dose-response relationships of urinary acrylamide metabolites with 8-OHdG, 8-iso-PGF2α, and FPG (except GAMA with FPG) and 8-iso-PGF2α with FPG. Each 1-unit increase in log-transformed level of AAMA, AAMA + GAMA (ΣUAAM), or 8-iso-PGF2α was associated with a 0.17, 0.15, or 0.23 mmol/L increase in FPG, respectively (*P* and/or *P* trend < 0.05). Each 1% increase in AAMA, GAMA, or ΣUAAM was associated with a 0.19%, 0.27%, or 0.22% increase in 8-OHdG, respectively, and a 0.40%, 0.48%, or 0.44% increase in 8-iso-PGF2α, respectively (*P* and *P* trend < 0.05). Increased 8-iso-PGF2α rather than 8-OHdG significantly mediated 64.29% and 76.92% of the AAMA- and ΣUAAM-associated FPG increases, respectively.

CONCLUSIONS

Exposure of the general adult population to acrylamide was associated with FPG elevation, oxidative DNA damage, and lipid peroxidation, which in turn partly mediated acrylamide-associated FPG elevation.

[1]Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[2]Key Laboratory of Environment and Health, Ministry of Education and Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Corresponding author: Weihong Chen, wchen@mails.tjmu.edu.cn

Received 28 December 2019 and accepted 31 March 2020

This article contains supplementary material online at https://doi.org/10.2337/figshare.12076134.

Acrylamide is an α,β-unsaturated carbonyl-containing volatile organic compound with high reactivity and water solubility that is extensively used in gel experiments, water purification, oil and sugar refining, and production of toiletries, varnishes, and plastics (1,2). In addition, acrylamide is present in cigarette mainstream smoke (3) and various commonly consumed heated foods and beverages, particularly breads, potato crisps, and coffee, attracting global concern (2,4). In addition to occupational activities, smoking and diet are potential sources of acrylamide exposure for humans.

As a proven animal carcinogen and human neurotoxicant with ubiquitous presence in daily life, acrylamide has raised worldwide concern for its potential adverse health effect on the public (2). The World Health Organization has called for further urgent research on the health hazards of acrylamide, sounding an international health alarm for its health risk (5). Studies in zebrafish and rodents have shown a significant dose-dependent increase in blood glucose levels after acrylamide exposure (6–8). High fasting plasma glucose (FPG) is an important indicator for diabetes and the third leading risk factor for global disease burden, accounting for 6.53 million deaths (9). However, epidemiological study of the effect of acrylamide exposure on FPG is limited. A pilot study conducted among 14 healthy volunteers reported an FPG increase after a 4-week intake of acrylamide-rich food but a lack of statistical significance (10). Studies with larger sample sizes are warranted to assess the association of acrylamide exposure with FPG alteration.

Acrylamide has been listed as a probable human carcinogen (group 2A) by the International Agency for Research on Cancer (IARC) on the basis of the sufficient evidence for carcinogenicity in animals and mechanistic considerations, while the epidemiological evidence is inadequate (1). High priority has been given by the IARC to assess acrylamide as a human carcinogen in future IARC monographs (11). The unifying factor available for determining the toxicity and carcinogenicity of chemicals is oxidative stress, which then causes oxidative DNA damage and lipid peroxidation (12). Nevertheless, the associations of acrylamide exposure with oxidative DNA damage and lipid peroxidation have been rarely investigated in epidemiological studies. Urinary 8-hydroxy-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin-F2α (8-iso-PGF2α) are separate critical biomarkers of oxidative DNA damage and lipid peroxidation that reflect the global oxidative stress status of the body and play important roles in carcinogenesis and glucose dyshomeostasis (13–15). Thus, assessment of the relationships between acrylamide exposure and 8-OHdG and 8-iso-PGF2α in a general population would help to understand the carcinogenic and hyperglycemic potentials of acrylamide to humans and the potential mechanism of acrylamide causing oxidative stress and affecting blood glucose levels in humans (16).

In the current study, we measured FPG and urinary 8-OHdG and 8-iso-PGF2α for 3,270 general Chinese adults from the Wuhan-Zhuhai cohort. To evaluate acrylamide exposure, we comprehensively measured urinary metabolites of both acrylamide (N-acetyl-S-[2-carbamoylethyl]-L-cysteine [AAMA]) and glycidamide (N-acetyl-S-[2-carbamoyl-2-hydroxyethyl]-L-cysteine [GAMA]) (17), a more reactive electrophilic and genotoxic epoxide biotransformed from acrylamide by cytochrome P450 2E1 (18). Urinary AAMA and GAMA are widely accepted internal biomarkers of acrylamide exposure from various sources (19,20). We aimed to estimate the associations of urinary acrylamide metabolites with FPG, urinary 8-OHdG, and 8-iso-PGF2α. We also investigated the mediating roles of 8-OHdG and 8-iso-PGF2α in the relationships between urinary acrylamide metabolites and FPG alteration to identify the potential mechanistic link.

RESEARCH DESIGN AND METHODS

Study Population

Participants were drawn from the Wuhan-Zhuhai cohort, a Chinese community-based prospective cohort that was established in 2012 and comprised 4,812 adults (18–80 years of age) who had dwelled in Wuhan or Zhuhai city for at least 5 years, as described previously (21). Our current analyses were restricted to 3,491 participants who had sufficient urine samples for measurements of acrylamide metabolites, creatinine (Cr), 8-OHdG, and 8-iso-PGF2α. Of the 3,491 participants, 3,270 without missing data and not taking hypoglycemic drugs and free of kidney disease were included in our final cross-sectional analyses. There were no significant differences between excluded and included participants in basic characteristics, such as BMI, smoking status, drinking status, and physical activity (all $P > 0.05$). The research protocol was evaluated and approved by the Ethics and Human Subject Committee of Tongji Medical College, Huazhong University of Science and Technology. All participants gave written informed consent for participation and storage and use of biospecimens.

Data and Biospecimen Collection

Standardized questionnaires and physical examinations were carried out to collect information on demographics, socioeconomics, lifestyles, anthropometrics, medical care, diseases, and biochemical indexes of urine and blood, including FPG and fasting lipids. FPG was immediately measured by an enzymatic colorimetric method with a fully automated biochemical analyzer (RX daytona; Randox Laboratories, Crumlin, U.K.). Biospecimens, including fasting blood and morning urine, were collected on the same day and separately stored at −80°C and −20°C, respectively, for further analyses. Mean arterial pressure (MAP) was computed as [(diastolic pressure × 2 + systolic pressure) / 3]. Participants who had smoked ≥1 cigarette/day or who had drunk one or more times per week for >6 months were defined as smokers and drinkers, respectively; otherwise, they were separately identified as nonsmokers and nondrinkers. Physical activity was defined as taking regular exercise two or more times per week and each time for ≥20 min for >6 months. Education degree was categorized as low (≤9 years) and high (>9 years) education levels. Family income was divided into low income (<40,000 yuan/year) and high income (≥40,000 yuan/year).

Urinary Acrylamide Metabolites Determination

Determination of urinary acrylamide metabolites, including AAMA and GAMA, was performed by an ultra-high-performance liquid chromatography system (Agilent 1290 Infinity II; Agilent Technologies, Santa Clara, CA) coupled with electrospray tandem mass spectrometry (SCIEX Triple Quad 6500; Applied Biosystems,

Foster City, CA) following a previously published method (22) with minor modifications. A 100-μL thawed aliquot of urine from each participant was diluted with mixed internal standards (AAMA-d3 and GAMA-d3) and ammonium acetate to 1 mL for measurements of AAMA and GAMA, which were accurately and precisely quantified with R^2 of the standard calibration curve >0.998, the recoveries of labeled internal standards in a range of 80–120%, the relative recoveries of target compounds in a range of 85–111%, and the coefficients of variation <10%. The limits of detection (LODs) were 0.6 ng/mL for AAMA and 1.5 ng/mL for GAMA, and concentrations below LOD were set at LOD/$\sqrt{2}$. Total urinary acrylamide metabolites (ΣUAAM) was defined as the sum of AAMA and GAMA. Valid concentrations of urinary acrylamide metabolites were corrected for urinary Cr and finally expressed as μg/mmol Cr.

Urinary 8-OHdG Determination

Urinary 8-OHdG was measured using solid phase extraction followed by high-performance liquid chromatography with electrochemical detection (Waters 2465; Waters, Milford, MA) according to a previously developed method (23) with minor modification. The detailed procedure has been described in our previously published literature (24). Valid 8-OHdG concentration was calibrated by urinary Cr and finally presented as μmol/mol Cr.

Urinary 8-Iso-PGF2α Determination

Urinary 8-iso-PGF2α was detected by a commercially available ELISA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. The detection range was 0.8–500 pg/mL, and the sensitivity was 3 pg/mL. The intra- and interassay coefficients of variance were <5% and <10%, respectively. Valid 8-iso-PGF2α level was adjusted by urinary Cr and finally shown as ng/mmol Cr.

Statistical Analyses

Concentrations of urinary acrylamide metabolites, 8-OHdG, and 8-iso-PGF2α were $\log_{10}$-transformed because of their right-skewed distributions (examined by Kolmogorov-Smirnov test). Basic characteristics by quartiles of ΣUAAM were analyzed by Cochran-Armitage trend test for dichotomous variables and variance analysis for continuous variables. We conducted both continuous (each 1-unit increase) and categorical (across quartiles) analyses to assess the relationships among urinary acrylamide metabolites, biomarkers of oxidative stress (8-OHdG and 8-iso-PGF2α), and FPG by using linear mixed models with adjustment for age, sex, BMI, smoking status, drinking status, physical activity, education level, family income, family history of diabetes, total cholesterol, triglycerides, and MAP. City (Wuhan/Zhuhai) was simultaneously included as a random effect in the models. We also fitted restricted cubic spline (RCS) models to graphically characterize their dose-response relationships. Stratified analyses were conducted by major characteristics, including age, sex, BMI, smoking status, drinking status, and physical activity. Effect modification by each stratified characteristic was estimated by adding an interaction term in the linear mixed models.

We further performed mediation analysis to evaluate the mediating role of 8-OHdG or 8-iso-PGF2α in the associations of urinary metabolites with FPG using a method described by MacKinnon et al. (25). The following two linear mixed models were fitted to estimate the direct and indirect (mediated) effects:

$$M = \varphi_0 + \varphi_{UAAM}X_{UAAM} + \varphi_C X_C + \omega_1$$

$$Y = \zeta_0 + \zeta_{UAAM}X_{UAAM} + \zeta_{OS}X_{OS} + \zeta_C X_C + \omega_2$$

where Y signifies outcome (FPG), M signifies mediator (8-OHdG or 8-iso-PGF2α), X_{UAAM} signifies exposure (urinary acrylamide metabolite), X_C signifies confounder, ζ_{UAAM} denotes direct effect, and $\varphi_{UAAM} \times \zeta_{OS}$ denotes mediated effect by the mediator. We further computed the CI of the mediated effect with the PRODCLIN program (25), and the proportion mediated by the mediator with the equation $[\varphi_{UAAM} \times \zeta_{OS} / (\zeta_{UAAM} + \varphi_{UAAM} \times \zeta_{OS}) \times 100\%]$. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC).

RESULTS

Characteristics of the Study Population

The median levels of urinary acrylamide metabolites with interquartile range (μg/mmol Cr) of the 3,270 participants were 3.28 (1.96–5.36), 0.50 (0.32–0.75), and 3.79 (2.32–6.11) for AAMA, GAMA, and ΣUAAM, respectively. As summarized in Table 1, the mean age of the study population (29.88% male) was 53.02 years. As the ΣUAAM levels gradually increased, the concentrations of FPG, 8-iso-PGF2α, and 8-OHdG and the proportions of males, smokers, drinkers, and individuals with a family history of diabetes were increased, while the MAP levels and the percentages of participants with physical activity or high family income were decreased. No trend was observed in age, BMI, education level, total cholesterol, and triglycerides.

Associations of Urinary Acrylamide Metabolites With FPG, 8-Iso-PGF2α, and 8-OHdG

As shown in Table 2, significant associations of urinary acrylamide metabolites with increased FPG (except GAMA with FPG), 8-iso-PGF2α, and 8-OHdG were found after adjusting for potential confounders (P and/or P trend < 0.05). Each 1-unit increase in log-transformed concentration of AAMA or ΣUAAM was associated with a 0.17 mmol/L or 0.15 mmol/L increase in FPG, respectively. Each 1% increase in AAMA, GAMA, or ΣUAAM was associated with a 0.40%, 0.48%, or 0.44% increase in 8-iso-PGF2α, respectively, and 0.19%, 0.27% or 0.22% increase in 8-OHdG, respectively. Besides, significantly (all $P < 0.05$) linear positive dose-response relationships of urinary acrylamide metabolites with FPG (except GAMA with FPG), 8-iso-PGF2α, and 8-OHdG were found by categorical analyses and were visually shown by RCS models (Supplementary Fig. 1). The positive association of GAMA with FPG was not found to be statistically significant (Table 2 and Supplementary Fig. 1).

Stratified analyses showed that sex (male/female), age (<55/≥55 years), BMI (<24/≥24 kg/m^2), smoking status (smokers/nonsmokers), drinking status (drinkers/nondrinkers), and physical activity (active/inactive) did not modify the significant associations of urinary acrylamide metabolites (except GAMA) with FPG, and these associations were persistent in subgroups of females, participants age <55 years, those with BMI <24 kg/m^2, the physically inactive, nonsmokers, and both drinkers and nondrinkers (P and/or P trend < 0.05) (Table 3 and

Table 1—Characteristics of study participants by quartiles of ΣUAAM and in all participants (*N* = 3,270)

Characteristic	All participants	Quartiles of ΣUAAM (μg/mmol Cr)				*P* for trend
		Q1 (≤2.32)	Q2 (2.33–3.79)	Q3 (3.80–6.11)	Q4 (>6.11)	
Participants, *n*	3,270	817	818	818	817	
Male sex	977 (29.88)	223 (27.29)	195 (23.84)	247 (30.20)	312 (38.19)	<0.001
Age (years)	53.02 ± 12.82	53.22 ± 12.61	52.50 ± 13.37	52.79 ± 12.5	53.57 ± 12.76	0.500
BMI (kg/m^2)	24.00 ± 3.53	24.02 ± 3.41	24.01 ± 3.55	24.01 ± 3.45	23.97 ± 3.72	0.781
Smokers	485 (14.83)	56 (6.85)	60 (7.33)	129 (15.77)	240 (29.38)	<0.001
Drinkers	418 (12.78)	82 (10.04)	80 (9.78)	113 (13.81)	143 (17.50)	<0.001
Physical activity	1,595 (48.78)	433 (53.00)	394 (48.17)	403 (49.27)	365 (44.68)	0.002
Education levels						0.299
Low (≤9 years)	1,957 (59.85)	479 (58.63)	510 (62.35)	511 (62.47)	457 (55.94)	
High (>9 years)	1,313 (40.15)	338 (41.37)	308 (37.65)	307 (37.53)	360 (44.06)	
Family income, yuan/year						0.015
Low (<40,000)	1,822 (55.72)	452 (55.32)	421 (51.47)	458 (55.99)	491 (60.10)	
High (≥40,000)	1,448 (44.28)	365 (44.68)	397 (48.53)	360 (44.01)	326 (39.90)	
Family history of diabetes	209 (6.39)	40 (4.90)	56 (6.85)	49 (5.99)	64 (7.83)	0.037
MAP (mmHg)	95.57 ± 13.26	96.99 ± 14.12	95.10 ± 12.59	95.49 ± 13.18	94.70 ± 13.03	0.001
Total cholesterol (mmol/L)	5.12 ± 1.33	5.18 ± 1.16	5.07 ± 1.19	5.18 ± 1.76	5.07 ± 1.10	0.298
Triglycerides (mmol/L)	1.51 ± 1.23	1.54 ± 1.41	1.46 ± 1.07	1.49 ± 1.16	1.55 ± 1.25	0.772
FPG (mmol/L)	4.85 ± 1.57	4.71 ± 1.48	4.76 ± 1.54	4.92 ± 1.5	5.02 ± 1.74	<0.001
8-iso-PGF2α (ng/mmol Cr)	61.46 (38.06–108.26)	39.20 (25.77–62.55)	54.93 (36.58–87.09)	69.00 (45.46–111.46)	98.20 (60.38–174.36)	<0.001
8-OHdG (μmol/mol Cr)	62.12 (28.24–122.51)	51.92 (23.05–102.88)	62.86 (28.18–116.92)	66.13 (31.34–126.00)	72.21 (31.04–157.08)	<0.001

Data are *n* (%) or mean ± SD or median (interquartile range).

Table 2—Associations of urinary acrylamide metabolites with FPG, 8-iso-PGF2α, and 8-OHdG (N = 3,270)

Variable	Effect estimates by continuous metabolites	Effect estimates (95% CI) by quartiles of metabolites				P for trend*
		Q1	Q2	Q3	Q4	
AAMA (μg/mmol Cr)		≤1.96	1.97–3.28	3.29–5.36	>5.36	
Estimated change (mmol/L) for FPG	0.17 (0.01, 0.33)	0 (Ref)	0.11 (−0.03, 0.26)	0.17 (0.02, 0.31)	0.20 (0.05, 0.35)	0.008
Adjusted β for 8-iso-PGF2α	0.40 (0.37, 0.44)	0 (Ref)	0.14 (0.11, 0.18)	0.21 (0.18, 0.25)	0.34 (0.31, 0.37)	<0.001
Adjusted β for 8-OHdG	0.19 (0.13, 0.26)	0 (Ref)	0.10 (0.05, 0.16)	0.15 (0.09, 0.21)	0.19 (0.13, 0.25)	<0.001
GAMA (μg/mmol Cr)		≤0.32	0.33–0.50	0.51–0.75	>0.75	
Estimated change (mmol/L) for FPG	0.03 (−0.15, 0.21)	0 (Ref)	0.01 (−0.14, 0.15)	0.01 (−0.14, 0.15)	0.05 (−0.10, 0.19)	0.581
Adjusted β for 8-iso-PGF2α	0.48 (0.44, 0.52)	0 (Ref)	0.14 (0.11, 0.17)	0.22 (0.19, 0.25)	0.33 (0.30, 0.37)	<0.001
Adjusted β for 8-OHdG	0.27 (0.20, 0.34)	0 (Ref)	0.12 (0.06, 0.18)	0.15 (0.09, 0.20)	0.18 (0.12, 0.24)	<0.001
∑UAAM (μg/mmol Cr)		≤2.32	2.33–3.79	3.80–6.11	>6.11	
Estimated change (mmol/L) for FPG	0.15 (−0.01, 0.32)	0 (Ref)	0.06 (−0.09, 0.20)	0.15 (0.01, 0.30)	0.17 (0.02, 0.32)	0.014
Adjusted β for 8-iso-PGF2α	0.44 (0.40, 0.47)	0 (Ref)	0.14 (0.11, 0.17)	0.22 (0.19, 0.25)	0.34 (0.31, 0.38)	<0.001
Adjusted β for 8-OHdG	0.22 (0.15, 0.28)	0 (Ref)	0.09 (0.04, 0.15)	0.14 (0.09, 0.20)	0.19 (0.13, 0.24)	<0.001

Adjusted for age, sex (male/female), BMI, smoking status (smokers/nonsmokers), drinking status (drinkers/nondrinkers), physical activity (active/inactive), education level (low/high), family income (low/high), family history of diabetes (yes/no), total cholesterol, triglycerides, and MAP and included city (Wuhan/Zhuhai) as a random effect in the linear mixed models. Ref, reference. *P for trend across quartiles of urinary acrylamide metabolites was tested by including the median of each quartile of urinary acrylamide metabolites as a continuous variable in the linear mixed models.

Supplementary Tables 1 and 2). Besides, the significant associations of ΣUAAM with 8-iso-PGF2α and 8-OHdG were not modified by the stratified characteristics and were persistent in almost all subgroups (P and/or P trend < 0.05), except that the relationship between ΣUAAM and 8-OHdG in the small-sample-sized subgroups of smokers or drinkers was not significant (Table 3). Stratified analyses for the associations of 8-iso-PGF2α and 8-OHdG with AAMA and GAMA (Supplementary Tables 1 and 2) showed results similar to those with ΣUAAM.

Associations of 8-Iso-PGF2α and 8-OHdG With FPG

Supplementary Table 3 presents the association of 8-iso-PGF2α with FPG in all participants and stratified by major characteristics. After adjusting for potential confounders, each 1-unit increase in log-transformed concentration of 8-iso-PGF2α was associated with a 0.23 mmol/L increase in FPG in all participants ($P < 0.05$). We also found a significant (all $P < 0.05$) linear positive dose-response relationship between 8-iso-PGF2α and FPG by categorical analyses and RCS regressions (Supplementary Fig. 2). In addition, stratified analyses found that such association was not modified by major characteristics and was persistent (P and/or P trend < 0.05) in almost all subgroups except in the small-sample-sized subgroups of males and smokers (Supplementary Table 3). We did not observe a significant relationship between 8-OHdG and FPG (data not shown).

Mediation Analysis

As presented in Table 4, the associations of FPG with AAMA and ΣUAAM rather than GAMA were significantly mediated by elevated 8-iso-PGF2α, which mediated 64.29% and 76.92% of the AAMA- and ΣUAAM-associated FPG increases, respectively. Unfortunately, the associations of urinary acrylamide metabolites with FPG were not found to be mediated by 8-OHdG (data not shown). In addition, we found no interaction effects between urinary acrylamide metabolites and 8-iso-PGF2α or 8-OHdG on FPG (all $P > 0.05$).

CONCLUSIONS

In this study, we found that urinary acrylamide metabolites were positively associated, in a dose-dependent manner, with FPG (except GAMA with FPG) and urinary 8-iso-PGF2α and 8-OHdG in a general urban adult population. In addition, 8-iso-PGF2α was dose-dependently associated with FPG increase and significantly mediated AAMA- and ΣUAAM-associated FPG elevations. Our findings indicate that acrylamide exposure is associated with FPG elevation and increased oxidative DNA damage and lipid peroxidation. Moreover, lipid peroxidation may be further involved in the mechanism underlying acrylamide-associated FPG elevation.

Our findings have substantial public health implications. Acrylamide exposure along with a resultant potential health hazard is a major public health issue that has attracted worldwide attention. Our findings highlight the public health concern regarding the carcinogenic and hyperglycemic effects on the general population from widespread acrylamide exposure and provide vital clues for illuminating the mechanisms whereby acrylamide raises blood glucose. Policies or advice from authorities are warranted to restrict or control acrylamide exposure of the public from various sources, particularly daily-consumed acrylamide-containing foods, such as potato crisps (average content 752 μg/kg), coffee (average content 509 μg/kg), and toasts (average content 446 μg/kg) (2), which are estimated to account for one-third of the calories consumed by U.S. and European populations (26).

The hyperglycemic effect of acrylamide has been documented in experimental studies in zebrafish and rodents, where a significant dose-dependent increase in blood glucose was shown after acrylamide exposure (6–8). However, epidemiological evidence was limited. Naruszewicz et al. (10) recruited 14 Polish adults to chronically ingest acrylamide-containing potato chips and found that FPG was increased after 28 days of ingestion, although no statistical significance was detected because of the small study sample size. Also, Lin et al. (27) cross-sectionally evaluated but failed to find a statistically significant association between urinary AAMA and serum glucose among 675 Taipei residents aged 12–30 years. Our present

Table 3—Stratified analysis for the associations of ΣUAAM with FPG, 8-iso-PGF2α, and 8-OHdG (N = 3,270)

	FPG			8-iso-PGF2α			8-OHdG		
Stratification characteristic	Estimated change (mmol/L) (95% CI)*	*P* for trend†	*P* for modification‡	Adjusted β (95% CI)*	*P* for trend†	*P* for modification‡	Adjusted β (95% CI)*	*P* for trend†	*P* for modification‡
Sex			0.519			0.173			0.066
Male (n = 977)	0.25 (−0.11, 0.61)	0.710		0.41 (0.34, 0.47)	<0.001		0.11 (−0.01, 0.23)	0.043	
Female (n = 2,293)	0.12 (−0.06, 0.31)	0.037		0.44 (0.40, 0.48)	<0.001		0.25 (0.17, 0.32)	<0.001	
Age (years)			0.323			0.387			0.704
<55 (n = 1,702)	0.25 (0.04, 0.46)	0.048		0.46 (0.41, 0.51)	<0.001		0.19 (0.09, 0.28)	<0.001	
≥55 (n = 1,568)	0.09 (−0.17, 0.35)	0.112		0.42 (0.37, 0.47)	<0.001		0.24 (0.15, 0.33)	<0.001	
BMI (kg/m^2)			0.426			0.133			0.343
<24 (n = 1,722)	0.12 (−0.11, 0.34)	0.022		0.46 (0.41, 0.51)	<0.001		0.24 (0.16, 0.33)	<0.001	
≥24 (n = 1,548)	0.18 (−0.07, 0.42)	0.201		0.41 (0.36, 0.46)	<0.001		0.19 (0.09, 0.29)	<0.001	
Smoking status			0.370			0.540			0.059
Smokers (n = 485)	0.01 (−0.53, 0.54)	0.871		0.46 (0.37, 0.56)	<0.001		0.04 (−0.13, 0.21)	0.588	
Nonsmokers (n = 2,785)	0.18 (0.01, 0.35)	0.009		0.43 (0.40, 0.47)	<0.001		0.24 (0.17, 0.31)	<0.001	
Drinking status			0.097			0.510			0.058
Drinkers (n = 418)	0.57 (0.04, 1.11)	0.302		0.41 (0.31, 0.51)	<0.001		0.03 (−0.16, 0.21)	0.697	
Nondrinkers (n = 2,852)	0.11 (−0.07, 0.28)	0.038		0.44 (0.40, 0.47)	<0.001		0.24 (0.18, 0.31)	<0.001	
Physical activity			0.594			0.632			0.667
Active (n = 1,595)	0.07 (−0.19, 0.33)	0.240		0.43 (0.38, 0.48)	<0.001		0.21 (0.12, 0.30)	<0.001	
Inactive (n = 1,675)	0.24 (0.04, 0.45)	0.010		0.45 (0.40, 0.49)	<0.001		0.21 (0.12, 0.30)	<0.001	

*Adjusted for age, sex (male/female), BMI, smoking status (smokers/nonsmokers), drinking status (drinkers/nondrinkers), physical activity (active/inactive), education level (low/high), family income (low/high), family history of diabetes (yes/no), total cholesterol, triglycerides, and MAP and included city (Wuhan/Zhuhai) as a random effect in the linear mixed models. †*P* for trend across quartiles of ΣUAAM in each subgroup was tested by including the median of each ΣUAAM quartile as a continuous variable in the linear mixed models. ‡*P* for modification of each characteristic was calculated by including a product term of ΣUAAM with each stratified characteristic in the linear mixed models.

Table 4—Mediated effects by 8-iso-PGF2α on associations between urinary acrylamide metabolites and FPG (*N* = 3,270)

Urinary metabolite	Direct effects (95% CI)*	Mediated effects (95% CI)†	Proportion mediated by 8-iso-PGF2α, %
AAMA	0.05 (−0.12, 0.21)	0.09 (0.02, 0.15)	64.29
GAMA	−0.07 (−0.27, 0.13)	0.10 (0.02, 0.18)	—‡
ΣUAAM	0.03 (−0.16, 0.20)	0.10 (0.03, 0.17)	76.92

AAMA, GAMA, ΣUAAM, FPG, and 8-iso-PGF2α were modeled as continuous variables with $\log_{10}$ transformations. *Adjusted for age, sex (male/female), BMI, smoking status (smokers/nonsmokers), drinking status (drinkers/nondrinkers), physical activity (active/inactive), education level (low/high), family income (low/high), family history of diabetes (yes/no), total cholesterol, triglycerides, MAP, and 8-iso-prostaglandin-F2α and included city (Wuhan/Zhuhai) as a random effect in the linear mixed models. †Tested by the PRODCLIN program; CIs not containing a 0 value denote statistical significance. ‡Proportion mediated by 8-iso-PGF2α was not calculated because of the insignificant total effect.

large general adult population–based study clearly demonstrated a significant positive dose-response relationship between AAMA and FPG. Such discrepancy is probably due to the differences in age, ethnicity, genetics, lifestyle, and sample size of the study population. Noteworthy, the association of GAMA with FPG was not observed in our study. Unlike AAMA, a direct urinary metabolite of acrylamide, GAMA is a direct urinary metabolite of glycidamide biotransformed from acrylamide. It seems likely that acrylamide rather than glycidamide may be the substantial contributor to FPG elevation.

The capacity of acrylamide in inducing oxidative damage to DNA and lipid has been identified in in vitro and in vivo studies (28–30) but assessed in few epidemiological studies. Similar to our results, a cross-sectional study conducted in 800 adolescents and young adults in Taiwan found a significant positive association between urinary AAMA and 8-OHdG (27), a biomarker of oxidative DNA damage. Unfortunately, investigators of that study (27) did not consider or measure GAMA, another important urinary biomarker of acrylamide exposure directly metabolized from glycidamide, which is much more reactive and largely responsible for the genotoxicity and carcinogenicity of acrylamide (18). In our study, we found that both AAMA and GAMA were positively associated with urinary 8-OHdG and urinary 8-iso-PGF2α, a biomarker of lipid peroxidation. Furthermore, the associations of 8-OHdG and 8-iso-PGF2α with GAMA were found to be stronger than with AAMA, suggesting a probably stronger oxidative damage potency of glycidamide toward DNA and lipid than acrylamide.

Several epidemiological studies with relatively small sample sizes have evaluated the associations of urinary 8-iso-PGF2α and 8-OHdG with blood glucose. A cross-sectional study conducted on 76 Indian Mauritians showed a positive relationship between 8-iso-PGF2α and FPG (31), and similar results were also found by Brinkmann et al. (32) and Altomare et al. (33). Also, Rytter et al. (34) investigated 56 Swedish patients with diabetes and found that urinary 8-iso-PGF2α, but not 8-OHdG, was positively associated with blood glucose. These studies support our findings of a significant relationship between FPG and 8-iso-PGF2α rather than 8-OHdG. However, a lack of statistical relationship between 8-iso-PGF2α and FPG was reported in a cross-sectional study conducted in a 77-year-old Swedish population (n = 765), where such an association was assumed to be concealed by the advanced age and age-related complications (35). Our present study provides clear evidence for the positive dose-response relationship between 8-iso-PGF2α and FPG in a large general population after adjusting for potential confounders.

Further mediation analysis found that urinary 8-iso-PGF2α rather than 8-OHdG significantly mediated the positive associations of AAMA and ΣUAAM with FPG, indicating the involvement of lipid peroxidation rather than oxidative DNA damage in the mechanisms whereby acrylamide increases FPG. Acrylamide-induced lipid peroxidation could cause adipose inflammatory changes and insulin signaling abnormalities, which could lead to insulin resistance and finally FPG elevation (36,37). Besides, acrylamide-induced lipid peroxidation could raise FPG by causing β-cell dysfunction and apoptosis (38,39). The unobserved mediating role of 8-OHdG might be interpreted by its novel biological functions in anti-inflammation and amelioration of insulin resistance (40,41). More studies are warranted to elucidate the mechanisms underlying acrylamide-induced glucose elevation.

Our study has some vital strengths. First, we provide unequivocal epidemiological evidence for the significant associations of acrylamide exposure with oxidative DNA damage, lipid peroxidation, and FPG elevation. Second, we further assessed the mediating role of oxidative DNA damage and lipid peroxidation and found for the first time that lipid peroxidation significantly mediated acrylamide-associated FPG elevation, providing an important clue for a further mechanism of study. Third, our present study was conducted in a large and representative Chinese general adult population from central and southern China, making our results better representative. Fourth, urinary metabolites of both acrylamide and its epoxide glycidamide (i.e., AAMA, GAMA) were accurately measured to comprehensively estimate the individual acrylamide exposure levels and their associations with 8-iso-PGF2α, 8-OHdG, and FPG, making our findings more convincing. The limitations that should be noted for our present study included a lack of external acrylamide exposure data (e.g., diet), which should be taken into consideration to be collected in our future studies. Besides, the cross-sectional design limits inference on temporality. Additionally, we used spot morning rather than 24-h urine samples to measure acrylamide metabolite levels, which may be subject to measurement error. However, because of the stable lifestyles and dietary patterns and nonexistent occupational exposure of our study population, participants' exposure levels of acrylamide was supposed to be relatively stable.

In conclusion, our study results constitute an unequivocal demonstration that exposure of the general population to acrylamide is significantly associated with FPG elevation, oxidative DNA damage, and lipid peroxidation, which in turn significantly mediates acrylamide-associated

FPG elevation. Our study findings support the hypothesis of an environmental etiology of FPG elevation (adverse outcome) with acrylamide exposure inducing lipid peroxidation (a mechanistic link). Further research is warranted to corroborate our findings and illuminate the underlying mechanisms.

Acknowledgments. All participants included in the study and all members of the study team are greatly acknowledged.

Funding. This study was supported by the Key Program of the National Natural Science Foundation of China (91543207) and the Major Research Program of the National Natural Science Foundation of China (91843302).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. B.W. and W.C. conceived and designed this study. W.C. was responsible for the design of the Wuhan-Zhuhai cohort study and obtaining funding. B.W. performed the statistical analyses, interpreted the data, and drafted the manuscript. B.W., W.Q., S.Y., L.C., C.Z., J.M., W.L., Z.Z., T.X., X.W., M.C., G.M., D.W., Y.Z., J.Y., and W.C. contributed to the data acquisition, manuscript revision, review for important intellectual content, and final version approval. W.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Acrylamide. IARC Monogr Eval Carcinog Risks Hum 1994;60:389–433
2. Joint FAO/WHO Expert Committee on Food Additives & World Health Organization. Evaluation of certain food contaminants: sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives [Internet], 2006. Geneva, World Health Organization. Available from http://www.who.int/iris/handle/10665/43258. Accessed 28 November 2019
3. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol 2000;38:371–383
4. Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. Nature 2002;419:448–449
5. Kapp C. WHO urges more research into acrylamide in food. Lancet 2002;360:64
6. Ghorbel I, Elwej A, Chaabene M, et al. Effects of acrylamide graded doses on metallothioneins I and II induction and DNA fragmentation: biochemical and histomorphological changes in the liver of adult rats. Toxicol Ind Health 2017;33:611–622
7. Kim SM, Baek JM, Lim SM, et al. Modified lipoproteins by acrylamide showed more atherogenic properties and exposure of acrylamide induces acute hyperlipidemia and fatty liver changes in zebrafish. Cardiovasc Toxicol 2015;15:300–308
8. Lee HW, Pyo S. Acrylamide induces adipocyte differentiation and obesity in mice. Chem Biol Interact 2019;298:24–34
9. GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017 [published corrections appear in Lancet 2019;393:132; Lancet 2019;393:e44]. Lancet 2018;392:1923–1994
10. Naruszewicz M, Zapolska-Downar D, Kośmider A, et al. Chronic intake of potato chips in humans increases the production of reactive oxygen radicals by leukocytes and increases plasma C-reactive protein: a pilot study. Am J Clin Nutr 2009;89:773–777
11. Cogliano V, Baan R, Straif K, et al. Future priorities for IARC monographs. Lancet Oncol 2008;9:708
12. Wang S, Bai Y, Deng Q, et al. Polycyclic aromatic hydrocarbons exposure and lung function decline among coke-oven workers: a four-year follow-up study. Environ Res 2016;150:14–22
13. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 2004;339:1–9
14. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004;44:239–267
15. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601–623
16. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes 2015;6:456–480
17. Boettcher MI, Bolt HM, Drexler H, Angerer J. Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. Arch Toxicol 2006;80:55–61
18. Besaratinia A, Pfeifer GP. Genotoxicity of acrylamide and glycidamide. J Natl Cancer Inst 2004;96:1023–1029
19. Boettcher MI, Schettgen T, Kütting B, Pischetsrieder M, Angerer J. Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. Mutat Res 2005;580:167–176
20. Sams C, Jones K, Warren N, et al. Towards a biological monitoring guidance value for acrylamide. Toxicol Lett 2015;237:30–37
21. Song Y, Hou J, Huang X, et al. The Wuhan-Zhuhai (WHZH) cohort study of environmental air particulate matter and the pathogenesis of cardiopulmonary diseases: study design, methods and baseline characteristics of the cohort. BMC Public Health 2014;14:994
22. Alwis KU, Blount BC, Britt AS, Patel D, Ashley DL. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). Anal Chim Acta 2012;750:152–160
23. Yuan J, Chen L, Chen D, et al. Elevated serum polybrominated diphenyl ethers and thyroid-stimulating hormone associated with lymphocytic micronuclei in Chinese workers from an E-waste dismantling site. Environ Sci Technol 2008;42:2195–2200
24. Xiao L, Zhou Y, Ma J, et al. Oxidative DNA damage mediates the association between urinary metals and prevalence of type 2 diabetes mellitus in Chinese adults. Sci Total Environ 2018;627:1327–1333
25. MacKinnon DP, Fritz MS, Williams J, Lockwood CM. Distribution of the product confidence limits for the indirect effect: program PRODCLIN. Behav Res Methods 2007;39:384–389
26. Mucci LA, Wilson KM. Acrylamide intake through diet and human cancer risk. J Agric Food Chem 2008;56:6013–6019
27. Lin CY, Lee HL, Chen YC, et al. Positive association between urinary levels of 8-hydroxydeoxyguanosine and the acrylamide metabolite N-acetyl-S-(propionamide)-cysteine in adolescents and young adults. J Hazard Mater 2013;261:372–377
28. Pan X, Zhu L, Lu H, Wang D, Lu Q, Yan H. Melatonin attenuates oxidative damage induced by acrylamide in vitro and in vivo. Oxid Med Cell Longev 2015;2015:703709
29. Abdel-Daim MM, Abd Eldaim MA, Hassan AG. Trigonella foenum-graecum ameliorates acrylamide-induced toxicity in rats: roles of oxidative stress, proinflammatory cytokines, and DNA damage. Biochem Cell Biol 2015;93:192–198
30. Alturfan AA, Tozan-Beceren A, Sehirli AO, Demiralp E, Sener G, Omurtag GZ. Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. Mol Biol Rep 2012;39:4589–4596
31. Gopaul NK, Manraj MD, Hébé A, et al. Oxidative stress could precede endothelial dysfunction and insulin resistance in Indian Mauritians with impaired glucose metabolism. Diabetologia 2001;44:706–712
32. Brinkmann C, Neumann E, Blossfeld J, et al. Influence of glycemic status and physical fitness on oxidative stress and the peroxiredoxin system in the erythrocytes of non-insulin-dependent type 2 diabetic men. Exp Clin Endocrinol Diabetes 2011;119:559–564
33. Altomare E, Vendemiale G, Chicco D, Procacci V, Cirelli F. Increased lipid peroxidation in type 2 poorly controlled diabetic patients. Diabete Metab 1992;18:264–271
34. Rytter E, Vessby B, Asgård R, et al. Glycaemic status in relation to oxidative stress and inflammation in well-controlled type 2 diabetes subjects. Br J Nutr 2009;101:1423–1426
35. Helmersson J, Vessby B, Larsson A, Basu S. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. Circulation 2004;109:1729–1734
36. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911–919; quiz 920
37. Ruskovska T, Bernlohr DA. Oxidative stress and protein carbonylation in adipose tissue - implications for insulin resistance and diabetes mellitus. J Proteomics 2013;92:323–334
38. Sasson S. Nutrient overload, lipid peroxidation and pancreatic beta cell function. Free Radic Biol Med 2017;111:102–109
39. Maulucci G, Daniel B, Cohen O, Avrahami Y, Sasson S. Hormetic and regulatory effects of lipid peroxidation mediators in pancreatic beta cells. Mol Aspects Med 2016;49:49–77
40. Choi S, Choi HH, Lee SH, et al. Anti-inflammatory effects of 8-hydroxy-2′-deoxyguanosine on lipopolysaccharide-induced inflammation via Rac suppression in Balb/c mice. Free Radic Biol Med 2007;43:1594–1603
41. Huh JY, Jung I, Piao L, Ha H, Chung MH. 8-Hydroxy-2-deoxyguanosine ameliorates high-fat diet-induced insulin resistance and adipocyte dysfunction in mice. Biochem Biophys Res Commun 2017;491:890–896