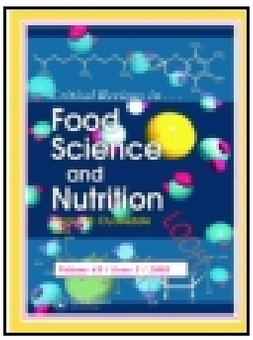

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The influence of demographic, physical, behavioral, and dietary factors on hemoglobin adduct levels of acrylamide and glycidamide in the general U.S. population

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ABSTRACT

Purpose: This study aims to better understand the individual characteristics and dietary factors that affect the relationship between estimated consumption of acrylamide and measured acrylamide hemoglobin adduct levels (HbAA) and glycidamide hemoglobin adduct levels (HbGA). *Methods*: Acrylamide levels in individual food items, estimated by the U.S. Food and Drug Administration, were linked to data collected in the 2003–2004 National Health and Nutrition Examination Survey. Multivariable linear regression was used to evaluate the relationship between estimated consumption of acrylamide and HbAA. *Results*: A significant association between acrylamide intake and HbAA was observed, after adjustment for gender, race/ethnicity, smoking status, age, and BMI ($R^2 = 0.34$). Across quartiles of acrylamide consumption, HbAA and HbGA levels increased monotonically. Among nonsmokers, an evaluation of three heavily consumed, high AA concentration foods showed a positive trend between the consumed amount of fried potatoes and HbAA in children, adolescents, and adults. A significant positive trend between the consumed amount of potato chips or coffee was indicated in adolescents, adults, and seniors. *Conclusions*: Consumption of some individual foods affects HbAA concentrations more strongly and in an age-dependent manner. Our results suggest that effective dietary guidelines for controlling acrylamide intake should be subpopulation specific.

KEYWORDS

Acrylamide; glycidamide; dietary exposure; NHANES; hemoglobin adducts

Introduction

Acrylamide (AA) is a small organic molecule existing as a white crystalline powder in its pure state (JIFSAN 2002; CDC 2013). AA has several industrial applications, as a coagulant aid for drinking water and waste water treatment processes, as a flocculent in coal preparation, in cosmetics, in sewage treatment, in the production of polyacrylamide gels used in research labs, as a grouting agent in construction, as a paper production additive, and as a soil stabilizer (Mulloy, 1996; Bergmark, 1997; Anonymous, 2005; Arvanitoyannis and Dionisopoulou, 2014; FSA, 2015). In addition, employment in occupational sectors that utilize AA in the processing and manufacturing of products is a source of exposure (Myers et al., 1991; Calleman et al., 1994; Hagmar et al., 2001). An analysis of cigarette smoke has identified AA as one of its components (Smith et al., 2000). For the general public, the diet is considered the main source of exposure to AA, and tobacco that is smoked or breathed second-hand also serves as a major contributor (ATSDR, 2012; CDC, 2013). A 2002 research study found that carbohydrate-rich foods prepared at high temperatures through baking and frying were potentially high sources of AA (Tareke et al., 2002). Several studies have identified major food sources of AA, which include fried potatoes, caffeinated coffee, breakfast cereal, breads and bakery products, and potato chips (Petersen and Tran, 2005; Wilson et al., 2009). Additional data suggest that high moisture and low-temperature conditions, such as those used for the creation of prune juice and canned ripe black olives are important to evaluate; the U.S. Food and Drug Administration (FDA) detected AA in these products during the Total Diet Study (TDS) program (Robin, 2007).

Exposure to AA has been associated with neurological impairments, upper airway irritation, ocular irritation, and dermal irritation in humans (Myers and Macun, 1991; JIFSAN, 2002; CDC, 2013). In 1994, the International Agency for Research on Cancer (IARC) classified AA as a probable human carcinogen, carcinogenicity Group 2A (IARC, 1994). Some epidemiological studies have attempted to evaluate the potential association of exposure to AA and various cancers. For instance, in 2015, Pelucchi et al. updated their 2011 review and meta-analysis of relevant literature that spanned 16 different cancer sites (Pelucchi et al., 2015). Similar to the initial review, the available data indicated that most sites lacked sufficient evidence of any increased risk associated with dietary AA, though an association with kidney cancer could not be ruled out. It was also reported that women who were never smokers showed a borderline significant increase in risk of endometrial and ovarian cancer when comparing high versus low dietary acrylamide intake. Occupational exposures to AA far exceed those expected from dietary sources. A mortality study evaluating a

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large occupational cohort of 8,508 workers with potential exposure to acrylamide in industrial plants reported findings on mortality associated with various cancer sites (Marsh et al., 2007). Cumulative exposure to acrylamide, duration of employment, time since first employment, duration of exposure, and mean intensity of exposure were evaluated and SMRs were estimated for each cancer site; there was no excess mortality observed for any cancer sites among acrylamide-exposed workers across all U.S. plants combined. Sobel et al. evaluated acrylamide exposure and mortality among an occupational cohort of workers who were assigned to acrylamide monomer and polymerization operations at the Dow Chemical Company (Sobel et al., 1986). A nonsignificant excess in mortality from all malignancies was observed in the cohort (11 observed vs. 7.9 expected). After excluding individuals with prior exposure to organic dyes, there were four deaths compared to 6.5 expected.

The metabolism of acrylamide after oral ingestion suggests that there could be potential health risks associated with it due to the formation of DNA adducts, which have been found to be carcinogenic and mutagenic in animal studies (Kutting, 2008). Acrylamide can either be detoxified by binding to glutathione prior to elimination from the body or can alternatively be activated through metabolism to glycidamide (FSA, 2015). Glycidamide has the ability to bind to DNA and lead to mutations, which is believed to be the potential mechanism for the cancers that have been observed in animals (Calleman, 1996).

For more than a decade, the scientific community has worked to understand the health implications of dietary acrylamide exposure, and regulatory agencies have been challenged with questions related to risk as well as decisions regarding the mandatory labeling of products that may contain AA. The potential for regular dietary exposure and the uncertainty surrounding health effects have led to several investigations into the relationship between hemoglobin adduct acrylamide biomarkers and self-reported dietary consumption (Wirfalt et al., 2008; Wilson et al., 2009; N.L. Tran, 2010; Vesper et al., 2010). Like these previous studies, the current study aims to evaluate the association between biomarkers of acrylamide and dietary consumption of acrylamide, along with the demographic and behavioral factors that influence this relationship. However, within this context, we further characterize how individual foods known to contain acrylamide impact body burden in the U.S. population.

Methods

Data set

National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey of the civilian, non-institutionalized U.S. population administered by the National Center for Health Statistics of the Centers for Disease Control and Prevention. NHANES uses a complex, multistage, probability design to select a representative sample of approximately 5,000 people in 15 counties across the U.S. annually (CDC, 2013). In 1999, the survey began to be administered in two-year cycles and became a continuous program that has a dynamic focus on emerging issues related to health and nutrition. Although the general sampling strategy has remained relatively constant, sampling rates for subgroups of the population have been adjusted over time in order to better represent and characterize the health and nutritional status of underrepresented communities (Curtin et al., 2012).

In an effort to increase the precision for analyses of specific subdomains (combined classifications by race and origin, income, sex, and age) within the population, the 1999–2006 survey cycles oversampled adolescents (aged 12–19), older Americans (aged 60 and more), Mexican-Americans, and the black population (Curtin et al., 2012). In addition, from 2000 to 2006, pregnant women and all others at or below the poverty line were oversampled (Curtin et al., 2012). NHANES III developers specified criteria related to the "utility of a sample for analysis purposes"; an estimated prevalence statistic on the order of 10% in a sex–age domain was required to have a relative standard error of 30% or less and the estimated absolute differences between domains of at least 10% should be detectable with a type I error rate (α) of $\leq$0.05 and a type II error rate (β) of $\leq$0.10. Oversampling in specific subgroups helped to meet the required sample size of examined persons (Curtin et al., 2012).

Study population

The sample used in this study consisted of individuals aged 3 and older whose parent or guardian completed a 24-hour dietary survey during the 2003–2004 NHANES surveillance period and who were assigned valid HbAA and HbGA biomonitoring values in the data set. The 2003–2004 NHANES survey cycle was selected for this study because it was the first and only one to collect biomonitoring data related to acrylamide exposure. A total of 9,033 individuals completed dietary surveys and, of those individuals, 6,769 had a measured HbAA value and 6,939 had a measured HbGA value. The 6,528 with both valid HbAA and HbGA concentration values were included in the final study sample. Overall, the distribution of demographic factors for the 2,505 individuals who were excluded from the study were fairly similar to those for the individuals included in the study population. Characteristics of the eligible participants are shown in Table 1 along with characteristics of the ineligible participants. The age distribution among eligible participants and ineligible participants differed due to the fact that children ages 0–2 were not eligible for blood draws. The distribution of body mass index (BMI) classifications differed among eligible and ineligible participants, with underweight individuals more likely to be ineligible for the study. NHANES does not perform blood draws on hemophiliacs or individuals undergoing chemotherapy, both of whom could be underweight and may account for the differences. In addition, a smaller proportion of participants in the eligible group did not report intake of alcohol in the previous 24 hours, again likely due to the exclusion of very young children.

Dietary consumption data

The NHANES (2003–2004) 24-hour dietary recall interview collected self-reported information on each participant's consumption in the past 24 hours. Participants completed a survey that required them to recall the foods eaten in the 24 hours prior to the survey being administered. They were provided

Table 1. Characteristics of the study population.

Characteristic	Eligible[a] %[b]	Ineligible %[b]	*P*-value[c]
Gender			
Male	49.5	47.5	0.145
Female	50.5	52.5	
Age			
0–2	0.0	21.1	
3–12	12.1	19.4	<0.001
13–19	10.5	9.4	
20–59	59.2	36.4	
60+	18.2	13.7	
Ethnicity			
Non-Hispanic white	70.8	67.4	0.079
Non-Hispanic black	11.5	15.1	
Hispanic	12.5	12.4	
Other	5.2	5.0	
Income			
Below poverty	16.4	20.3	0.010
Above poverty	83.6	79.7	
Physical activity[d]			
MET-min/wk < 500	34.6	34.0	0.788
MET-min/wk ≥ 500	65.4	66.0	
Body mass index			
Underweight	10.4	26.1	<0.001
Normal weight	33.8	28.0	
Overweight	29.3	21.3	
Obese	26.5	24.6	
Alcohol use[e]			
0 drinks/day	78.9	86.1	0.001
> 0 to < 1 drinks/day	5.6	3.6	
1 to < 2 drinks/day	5.3	4.3	
≥ 2 drinks/day	10.2	6.1	
Smoking status[f]			
Smoker	25.5	22.5	0.256
Nonsmoker	74.5	77.5	

[a]Participants who completed a 24-hour dietary survey were eligible if they had valid HbAA and HbGA measurements.
[b]Calculated from survey weighted frequencies.
[c]Rao-Scott Chi-square test.
[d]Physical activity MET-min/wk available for respondents 12 years and older only.
[e]As reported in the NHANES 24-hour dietary interview; 1 drink is approximately 15 g ethanol.
[f]Smoker defined as having a serum cotinine concentration >10 μg/L.

with visual aids that allowed for the estimation of portion sizes for each food that was reported. Children less than six years old and other sampled persons who were unable to report their dietary consumption for themselves due to age or disability used a proxy for the dietary interview. The preferred proxy was the individual responsible for food preparation. Children ages 6–11 years old were required to provide their own data with the assistance of an adult household member, whereas participants ages 12 and older were required to independently complete their dietary interview. Individuals who completed a 24-hour dietary recall interview were eligible to receive a food frequency questionnaire (FFQ), which asked participants to recall information related to their dietary habits in the past 12 months. Although the FFQ data provided potential insight into participant habits, information related to portion size was not collected. The dietary interview component of NHANES is called What We Eat in America (WWEIA) and is operated through a partnership between the U.S. Department of Agriculture (USDA) and the Department of Health and Human Services (DHHS; CDC, 2006). DHHS designs the studies and collects the data; however, the USDA maintains, codes, processes, and reviews the data. Two data files resulted from the information collected from day 1 of the 24-hour recall; one contained information on individual foods and the other contained information on total nutrient intakes for each participant. This study utilized the individual food files that provided information on the types and amounts of foods reportedly consumed by participants. The USDA's Food and Nutrient Database for Dietary Studies, 2.0 (FNDDS 2.0) contains information for the coding of individual foods and portion sizes, it was used with the individual food files to translate the food intake data into a format that was suitable for analysis (USDA, 2006).

Acrylamide concentration data

The FDA commenced the TDS in 1961; the study involved purchasing and preparing foods throughout the United States in order to analyze them for nutrient and contaminant levels (FDA). Alterations in the U.S. population's eating patterns and emerging scientific issues drive the TDS list of foods to be sampled. The study is conducted with the purchase, preparation, and analysis of approximately 280 kinds of foods and beverages from grocery stores and fast food chains four times per year, once from each geographic region in the country. The food collection or "market basket" is conducted in the Western U.S. in the winter, the North Central U.S. in the autumn, the Southern U.S. in the spring, and the Northeastern U.S. in the summer. A food sample is obtained from three cities in each region and combined to form a composite sample representing the food item in that region. Acrylamide was measured in foods for each market basket from 2003 to 2006, and the data derived from those time periods is publicly available and indicates the acrylamide levels in each table-ready food that was surveyed. The FDA also surveyed individual food products (IFP) between 2002 and 2004. A variety of food products were analyzed for acrylamide, including processed foods, prepare-at-home foods and restaurant foods (FDA). Alternatively to the composite samples collected in the TDS, the IFP tests generally did not address unit to unit variation or lot to lot variation; however, they did assess a wide range of foodstuffs to expand the list of foods with known acrylamide concentrations. The IFP tests also measured AA levels in foods "as purchased," rather than table ready; examples include ground coffee and certain cereals (FDA)

Estimating dietary intake

Unlike other studies that focused on longer-term dietary intake using FFQs, we opted to estimate the dietary intake of AA using only the 24-hour recall data. This is because it has been reported that 24-hour recalls have potential cognitive advantages over the FFQ, which asks respondents to provide "typical" intake frequencies over the course of the last year, which requires mental averaging across varying intake seasons (Schatzkin et al., 2009). In the Observing Protein and Energy Nutrition (OPEN) study, which compared the performance of an FFQ with a 24-hour recall, considerably less measurement error was observed in the 24 hour recall than in the FFQ; the task of remembering the past 24 hours may be easier than forming the complex judgments needed for reporting on the past year (Schatzkin et al., 2003, 2009). In addition, according

to the CDC's National Center for Health Statistics (NCHS), when estimating the mean of the population distribution of usual dietary intakes from 24-hour recalls, single day data are sufficient, although an assumption that that recalls are not biased (i.e., that no underreporting occurs) is required (CDC, 2013). Several of the foods of concern in this analysis, such as snacks or coffee, were items that we expected participants to consume regularly, which made the assumption of nonbias acceptable.

To ensure proper linkage of the FDA data with the NHANES dietary recall survey information, the foods tested by the FDA were coded in accordance with the USDA FNDDS 2.0 guidelines. We manually assigned a USDA code (or group of codes for very similar food items) to each food tested by the FDA. The coding was performed by members of the study team (TD, PR), and in instances of uncertainty, both members convened to come to a joint consensus. The food-specific geometric mean (GM) AA concentrations (ng/g) of the samples measured by the FDA were then assigned to each food reported to be consumed in the NHANES sample. A GM concentration was additionally calculated for each of 89 food categories. The GM concentration for each food was multiplied by the reported amount consumed (grams). Total amount of acrylamide ingested from known dietary sources was estimated as the sum of the acrylamide content of all of these foods consumed. It is important to note that this estimate represents the prior day's consumption of known sources of AA only (as defined by the FDA), to be used strictly as a relative measure of dietary exposure.

Biomonitoring data

Biomonitoring was conducted for the 2003–2004 NHANES cycle on all study participants ages three and older; biomarkers for acrylamide, glycidamide, and cotinine were collected. Concentrations of HbAA and HbGA were measured in the red blood cells of participants, providing information about both AA dose itself and its metabolism in the body. Hemoglobin adducts represent the concentration of AA and GA in the body during the life of the erythrocytes, a concentration that is proportional to the internal dose for approximately two to four months (Bjellaas et al., 2007; CDC, 2012). The glycidamide-to-acrylamide hemoglobin adduct ratio can be used as an indicator of the extent to which acrylamide is metabolized to the genotoxic metabolite glycidamide (CDC, 2012). Serum cotinine levels were measured in participants to assess smoking status. Cotinine is the primary metabolite of nicotine and is accepted as the best biomarker of tobacco smoke exposure; serum levels reflect recent exposure to nicotine in tobacco smoke. Smokers were defined as individuals with a serum cotinine level of 10 ng/mL or greater (Pirkle et al., 1996)

Data analysis

Each participant in the NHANES 2003–2004 dataset was assigned a sample weight to account for unequal probability of selection, adjustment for nonresponse, and adjustment to independent population control (CDC, 2013). To account for the survey design, these weights were applied in all subsequent analyses. The distributions of consumed AA, HbAA, and HbGA in the study sample were highly skewed. As a result, these measures were summarized as geometric means in stratified analyses and $\log_e$-transformed before entry into statistical models.

The relationship between HbAA and HbGA was evaluated visually using simple linear regression techniques. Wald F tests obtained from simple linear regression analyses were employed to assess whether the biomarker variables (HbAA, HbGA, and the HbAA:HbGA ratio) were associated with AA consumed and potentially confounding covariates, and GMs were compared across groups. The measure of AA consumed was categorized into quartiles in this crude analysis. The covariates were selected based on previously reported associations with AA exposure (Tran, 2010; Vesper et al., 2013). They included gender, age, race/ethnicity, income, physical activity, BMI, alcohol use, and smoking status. Race/ethnicity consisted of those of non-Hispanic white (treated as the reference group in statistical models), non-Hispanic black, Hispanic, or other ethnicities. Poverty was defined as having a family poverty–income ratio (PIR) less than 1.0. Physical activity surveys were used to calculate the total minutes per week for each metabolic equivalent task (MET) performed (MET-min/wk), as described on the NHANES website (CDC, 2013); these surveys were only completed by participants 12 years and older. BMI was categorized as underweight (<18.5 kg/m^2), normal weight (18.5 to <25.0 kg/m^2), overweight (25.0 to <30.0 kg/m^2), and obese (≥ 30.0 kg/m^2; WHO, 2000). Alcohol use was defined by the amount of alcohol consumed in the previous 24 hours, according to responses provided during the 24-hour dietary interview. Categories of alcohol use considered were 0 drinks per day, >0 to <1 drink per day, 1 to <2 drinks per day, and ≥ 2 drinks per day, with 1 drink equal to approximately 15 g of alcohol.

Multivariable linear regression analyses were performed to investigate the dependence of ln-transformed HbAA and HbGA on the quartiles of AA consumption with adjustment for potentially confounding covariates. Covariates were entered into the multivariable models in a forward stepwise fashion to further assess their effect on the association of interest. Product terms were included to test for significant effect modifiers. The significance of each main effect and interaction term was evaluated with a t-statistic, and all tests were conducted at the 0.05 significance level. Covariates were included in the final model if they were found to be significantly associated with the biomarker measures.

Because of the potential uncertainty in the estimated amount of acrylamide consumed, given that the FDA did not necessarily test all items within each food group, we further considered the association between HbAA levels and reported consumption of foods identified as major contributors to AA intake (OEHHA, 2005). Those who reported any consumption of the food were compared to those who did not report any consumption of the food. Smoking status and age have previously been shown to have a strong effect on HbAA concentration (Vesper et al., 2007, 2010, 2013). Consequently, GM HbAA concentrations were stratified by age categories (3–12, 13–19, 20–59, and 60+ years of age) and smoking status (current smoker vs. nonsmoker); children aged 3–12 with serum cotinine >10 ng/mL ($n = 8$) were excluded. Multivariable linear regression models of ln-transformed HbAA that included

all of the major contributor foods were fit within each stratum. Exposure-response trends were further assessed using multivariable linear regression for the foods found to be most highly associated with HbAA concentration. For this analysis, participants who reported consuming the food were grouped by quartile of consumption amount in grams.

All statistical analyses were performed using SAS 9.3 (Cary, NC, USA).

Results

Among the foods consumed by the NHANES sample, AA levels varied widely within and between food categories. Table 2 reports the geometric mean concentration of AA for foods with the highest measured concentrations by food category. The category containing fats, oils, and salad dressings did not have any foods with measurable concentrations, according to data from the FDA. Based on *a priori* knowledge, we expected high levels among processed foods in the grain products category and potato products in the vegetables category. Bread, crackers and salty snacks, brewed coffee, fried potatoes, and potato chips had the highest number of consumers reported and can be considered important dietary sources of AA. The FDA results showed a substantial mean AA concentration for unbrewed coffee of 182 ppb, although no study participants reported consuming it. The value for brewed coffee, which was heavily consumed, was only 5% of the concentration reported for unbrewed coffee at 9.9 ppb. Prune juice and olives had large mean concentrations of AA at 226.2 and 294.8 ppb, respectively; however, fewer than 2% of consumers reported eating olives and less than 0.5% reported drinking prune juice.

Table 2. Measured acrylamide levels of select high concentration foods.

Food group[a]	Geometric mean[b]	Range[b]	Number of consumers[c]
Milk and milk products			
Cocoa	56.0	5.0–909.0	38
Fried cheese	20.0	20.0–20.0	49
Ice cream	18.0	18.0–18.0	32
Meat, poultry, fish, and mixtures			
Chili	120.0	55.0–201.0	145
Breaded finfish	20.0	12.0–93.0	105
Breaded chicken	18.7	10.0–35.0	574
Eggs			
Egg, cheese, and ham muffin	13.3	12.3–15.3	17
Dry beans, peas, other legumes, nuts, and seeds			
Nuts	88.8	28.0–457.0	63
Nut butter	71.1	54.0–125.0	590
Seeds	40.6	33.0–49.5	38
Grain products			
Crackers and salty snacks	146.3	17.0–1540.0	3068
Cookies	91.4	34.0–955.0	1597
Toasted bread	66.6	23.0–364.0	658
Cereals	65.0	11.0–534.0	1654
Bread	28.0	5.0–130.0	3124
Fruits			
Prune juice	226.2	138.0–326.0	21
Vegetables			
Potato chips	425.1	108.0–2510.0	1318
Fried potatoes	333.2	20.0–1325.0	1823
Olives	294.8	19.0–1925.0	112
Sugars, sweets, and beverages			
Unbrewed coffee	182.0	27.0–609.0	0
Candy	33.9	14.8–104.0	563
Chocolate syrup	29.1	25.3–34.8	105
Brewed coffee	9.9	3.0–19.5	2367

[a]Food groups defined as per the USDA Food Surveys Research Group (FSRG).
[b]Based on samples collected as part of the FDA's Total Diet Study and analysis of individual food products, measured in parts per billion (ppb); only includes samples that exceeded the limit of detection (LOD).
[c]Number of consumers who reported consuming foods in each good category on the one-day dietary survey of the NHANES 2003–2004 cycles.

The crude association between the biomarker variables (HbAA, HbGA, HbGA:HbAA) and selected demographic, physical, behavioral, and dietary characteristics are shown in Table 3. Men had significantly higher HbAA levels compared to women, although HbGA levels were similar across genders. The HbGA:HbAA ratio was significantly higher for women (0.99) compared to men (0.91; P-value < 0.001).

Children in our study had the second highest HbAA level (59.8 pmol/g), although they had a significantly higher HbGA level (72.6 pmol/g) when compared to adolescents (55.3 pmol/g), adults (63.0 pmol/g), and seniors (45.4 pmol/g; P value < 0.001). Similarly, the HbGA:HbAA ratio for children (1.22) was significantly higher (P-value < 0.001) than that for adolescents (0.94), adults (0.93), and seniors (0.90).

Among ethnic groups, the differences in HbAA were borderline significant ($P = 0.09$); however, non-Hispanic blacks had the highest HbAA at 64.9 pmol/g of hemoglobin, followed by non-Hispanic whites, Hispanics, and those of other ethnicities at 63.6, 58.0, and 55.1 pmol/g, respectively. The Hispanic population had the highest HbGA value and the highest HbGA:HbAA ratio (1.07) which was statistically significantly different than other ethnicities. Participants who fell below the federal poverty line had significantly higher HbAA and HbGA levels than those who were above the poverty line; however, the HbGA:HbAA ratio was similar for both groups.

Categories of BMI were significantly associated with all biomarker measures. Notably, HbGA levels in underweight individuals (67.5 pmol/g) were higher than in normal weight, overweight, and obese individuals (59.6, 57.5, and 59.1 pmol/g, respectively). The HbGA:HbAA ratio was higher among underweight (1.10) and obese (1.00) participants than among normal weight (0.90) or overweight (0.92) participants. Physical activity, measured in MET-min/wk, was not significantly associated with any of the biomarker measures. The difference in measured HbAA and HbGA between smokers and nonsmokers was striking, with smokers having much higher levels. However, nonsmokers had a significantly higher HbGA:HbAA ratio (1.00) compared to smokers (0.82) (P-value < 0.001). Alcohol use was significantly associated with HbAA and the HbGA:HbAA ratio. Those who consumed ≥ 2 drinks per day had the highest HbAA levels and the lowest HbGA:HbAA ratio.

Across the acrylamide consumption quartiles, HbAA and HbGA levels increased monotonically; those who consumed the least had the lowest levels and those who consumed the most had the highest levels for both biomarkers. Both the HbAA and HbGA levels were statistically associated with acrylamide consumption categories (P-value < 0.001 for each). The HbGA:HbAA ratios were also significantly associated with acrylamide consumption quartiles (P-value $= 0.025$). Although those with the lowest and highest consumption levels had the lowest and highest ratios, respectively, the second quartile of consumption had a similar confidence interval (0.92–1.01) to that of the third quartile (0.89–1.00).

Table 3. Measured HbAA and HbGA by population characteristics.

Characteristic	N[a]	HbAA (95% CI)[b]	P-value[c]	HbGA (95% CI)[b]	P-value[c]	Ratio (95% CI)[d]	p-value[c]
Demographic							
Gender							
Male	3,229	65.4 (61.6–69.4)	< 0.001	59.7 (56.9–62.6)	0.871	0.91 (0.88–0.95)	< 0.001
Female	3,299	59.9 (56.6–63.4)		59.4 (55.7–63.4)		0.99 (0.95–1.04)	
Age							
3–12	1,216	59.8 (57.0–62.6)	< 0.001	72.6 (69.0–76.4)	< 0.001	1.22 (1.16–1.27)	< 0.001
13–19	1,553	59.0 (55.7–62.4)		55.3 (51.2–59.7)		0.94 (0.88–1.00)	
20–59	2,353	68.1 (63.8–72.7)		63.0 (59.5–66.6)		0.93 (0.89–0.96)	
60–85	1,406	50.7 (48.1–53.4)		45.4 (41.8–49.3)		0.90 (0.84–0.95)	
Ethnicity							
Non-Hispanic white	2,759	63.6 (59.7–67.7)	0.088	61.0 (56.7–65.6)	0.005	0.96 (0.92–1.00)	< 0.001
Non-Hispanic black	1,642	64.9 (56.9–73.9)		53.2 (50.3–56.4)		0.82 (0.74–0.91)	
Hispanic	1,854	58.0 (54.5–61.8)		62.1 (59.4–65.0)		1.07 (1.00–1.15)	
Other	273	55.1 (48.4–62.7)		49.7 (42.1–58.7)		0.90 (0.83–0.98)	
Income							
Below poverty	1,621	69.1 (62.5–76.5)	0.006	65.9 (60.1–72.2)	0.008	0.95 (0.90–1.01)	0.887
Above poverty	4,599	61.0 (58.4–63.8)		58.4 (55.7–61.2)		0.96 (0.92–1.00)	
Physical							
Body mass index							
Underweight	933	61.5 (56.8–66.6)	0.003	67.5 (61.7–73.8)	0.025	1.10 (1.02–1.18)	0.001
Normal weight	2,292	66.1 (61.6–70.9)		59.6 (55.6–63.9)		0.90 (0.85–0.95)	
Overweight	1,698	62.2 (58.8–65.9)		57.5 (53.2–62.1)		0.92 (0.88–0.97)	
Obese	1,512	59.4 (55.4–63.7)		59.1 (55.4–63.1)		1.00 (0.96–1.04)	
Physical activity[e]							
MET-min/wk < 500	1,986	62.7 (58.6–67.1)	0.892	58.2 (54.7–61.8)	0.910	0.93 (0.89–0.96)	0.759
MET-min/wk ≥ 500	3,423	62.9 (59.5–66.5)		58.0 (54.3–61.8)		0.92 (0.88–0.97)	
Behavioral							
Smoking status[f]							
Smoker	1,221	114.3 (103.1–126.7)	< 0.001	93.6 (84.5–103.5)	< 0.001	0.82 (0.79–0.85)	< 0.001
Nonsmoker	5,212	60.0 (49.4–52.6)		51.1 (48.8–53.5)		1.00 (0.96–1.05)	
Alcohol use[g]							
0 drinks/day	5,504	60.4 (57.3–63.6)	< 0.001	60.4 (58.0–62.8)	0.147	1.00 (0.96–1.04)	< 0.001
> 0 to < 1 drinks/day	332	62.2 (57.2–67.5)		56.5 (51.5–61.9)		0.91 (0.86–0.96)	
1 to < 2 drinks/day	229	64.6 (56.9–73.4)		54.9 (47.4–63.6)		0.85 (0.78–0.92)	
≥ 2 drinks/day	463	90.0 (71.5–91.7)		57.5 (50.5–65.6)		0.71 (0.64–0.79)	
Dietary							
Acrylamide consumed[h]							
≤ 5.17 μg/day	1,634	55.9 (51.4–60.8)	< 0.001	51.4 (46.9–56.3)	< 0.001	0.92 (0.88–0.97)	0.025
> 5.17–11.87 μg/day	1,630	58.1 (54.5–61.9)		55.8 (51.8–60.0)		0.96 (0.92–1.01)	
>11.87–24.58 μg/day	1,632	65.0 (61.9–68.2)		61.3 (57.1–65.8)		0.94 (0.89–1.00)	
> 24.58 μg/day	1,632	71.2 (65.9–77.0)		70.0 (66.2–73.9)		0.98 (0.94–1.03)	

[a]Unweighted sample size (NHANES 2003–2004); includes those with measured HbAA and HbGA values who completed the 1-day dietary survey.
[b]Geometric mean concentration, measured in pmol/g Hb.
[c]*P*-values based on Wald *F* test, which tests whether at least 1 category has a significantly different HbAA, HbGA, or HbGA/HbAA measure.
[d]Geometric mean ratio of glycidamide to acrylamide concentration.
[e]Physical activity MET-min/wk available for respondents 12 years and older only.
[f]Smoker defined as having a serum cotinine concentration >10 μg/L.
[g]As reported in the NHANES 24-hour dietary interview; 1 drink is approximately 15 g ethanol.
[h]Quartiles of estimated acrylamide consumption, measured in nanograms per day.

The best-fitting multivariable linear regression models demonstrated significant associations between acrylamide consumption and the blood biomarker levels after adjustment for potentially confounding covariates (Table 4). Covariates retained included age, gender, ethnicity, BMI (WHO-defined categories), alcohol use, and serum cotinine concentration. None of the tested interaction terms were found to be statistically significant. The model parameters explained approximately 34% of the variation in ln-transformed HbAA and 19% of the variation in ln-transformed HbGA. Exponentiation of the ln-transformed model estimates indicated that HbAA levels would increase by a factor of 1.07 (95% CI, 1.05–1.09), and HbGA levels would increase by a factor of 1.09 (95% CI, 1.07–1.12), with each quartile increase in estimated acrylamide consumption. For instance, those in the highest quartile of acrylamide consumption would be expected to have an average HbAA level 1.07-times higher than those in the third quartile of acrylamide consumption. Furthermore, as shown in the table, adjustment for the other covariates did not drastically change the estimates of the association between acrylamide consumed and biomarker levels.

Mean HbAA levels for those who reported consuming and for those who reported not consuming each of the foods previously found to contribute the most to AA intake, stratified by smoking status and age category, are shown in Table 5. Children ages 3–12 are reported separately in the table, and children with serum cotinine >10 ng/mL ($n = 8$) were excluded. When comparing consumers to nonconsumers of fried potatoes, there was a significant difference in the measured HbAA among children, nonsmoking adolescents and nonsmoking adults, after adjustment for reported consumption of other high-risk foods. In these same groups, there

Table 4. Results of multivariable regression of acrylamide and glycidamide concentrations on acrylamide intake.

	HbAA			HbGA		
	Mean ratio[c]	95% CI	R^2	Mean ratio[c]	95% CI	R^2
Acrylamide consumed						
Crude model[a]	1.09	(1.06–1.12)	0.03	1.11	(1.07–1.15)	0.03
Adjusted model[b]	1.07	(1.05–1.09)	0.34	1.09	(1.07–1.12)	0.19

[a]Represents the crude association between estimated acrylamide consumption (quartiles) and biomarker levels.
[b]Represents the association between estimated acrylamide consumption (quartiles) and biomarker levels, adjusted for age, gender, ethnicity, body mass index, serum cotinine concentration, and alcohol use.
[c]Mean ratio, or exponentiated estimate of the ln-transformed biomarker measure, represents the multiplicative increase in mean biomarker levels for each quartile increase in acrylamide consumption.

was a significant difference in HbAA between those who reported consuming any acrylamide-containing foods versus those who reported no consumption. Coffee-consuming adolescents and nonsmoking elderly participants had statistically elevated HbAA levels compared to those who did not report consuming coffee, after adjustment for consumption of other high-risk foods. The relationship between consumption of known dietary acrylamide sources and HbAA levels was not as clear among smokers, as the results may have been confounded by lack of information on frequency and duration of smoking. The results examining consumption of the individual core foods and HbGA were similar (results not reported).

Approximately 97.6% of children were reported to have consumed foods known to contain AA. Among children, the geometric mean amount of dietary AA (from specific foods known to contain acrylamide, as defined by the FDA) consumed was 0.38 μg/kg of body weight. A simple linear regression model of ln-transformed AA consumed per kg of body weight demonstrated that the geometric mean in children was significantly higher than that in adolescents (0.18 μg/kg), adults (0.15 μg/kg), and seniors (0.14 μg/kg; Table 6).

We further selected three heavily consumed foods that ranked among the highest concentrations of AA and evaluated HbAA levels in nonsmoking consumers by quartile of consumption amount in grams (Table 6). A statistically significant positive trend between the consumed amount of fried potatoes and HbAA was observed in children, adolescents, and adults. A significant positive trend between the consumed amount of

Table 5. Acrylamide hemoglobin adduct (HbAA) levels among smokers and non-smokers by food consumption.

		Hemoglobin adducts of acrylamide (pmol/g)[a]						
			Nonsmoker			Smoker		
Food consumption		3–12 years	13–19 years	20–59 years	60+ years	13–19 years	20–59 years	60+ years
Potato chips	Yes	61.5	59.6[b]	53.9	49.4[b]	93.8	126.7	84.1
	No	59.4	51.6	50.2	44.3	106.3	117.5	94.5
Fried potatoes	Yes	64.6[b]	56.2[b]	54.6[b]	46.8	112.0	132.2[b]	105.0
	No	58.3	51.7	49.8	44.5	101.2	116.1	92.2
Olives	Yes	—	52.3	57.1	48.6	—	113.6	—
	No	59.7	52.7	50.5	44.7	103.0	119.3	93.2
Prune juice	Yes	—	—	—	77.3[b]	—	—	—
	No	59.7	52.7	50.7	44.5	103.6	119.1	93.3
Crackers	Yes	64.2	55.0	52.2	48.3[b]	128.0	120.3	85.0
	No	58.7	52.4	50.5	43.7	102.7	119.0	94.4
Salty snacks	Yes	61.7	53.7	54.0[b]	47.3	117.5	124.8	115.3
	No	58.8	52.2	49.7	44.3	99.4	117.6	90.7
Chili	Yes	63.9	49.8	52.2	45.4	76.7[b]	115.4	99.5
	No	59.7	52.7	50.6	44.7	104.0	119.2	93.1
Cookies	Yes	61.4	53.5	53.2[b]	46.5	88.9	113.7	86.9
	No	59.1	52.5	49.9	44.1	105.8	120.3	95.5
Nuts	Yes	49.8[b]	55.0	48.3	46.2	140.6	109.0	142.0[b]
	No	60.2	52.6	51.0	44.5	102.0	119.7	91.1
Nut butters	Yes	60.7	54.7	53.1	46.1	129.4[b]	95.5[b]	86.0
	No	59.7	52.6	50.5	44.6	102.4	121.5	94.0
Toasted breads	Yes	59.0	64.7	51.3	44.5	108.7	121.0	97.8
	No	59.9	52.0	50.6	44.9	103.4	118.9	92.3
Cereals	Yes	61.1	51.7	50.1	45.6	91.7	128.0	91.2
	No	58.7	53.1	50.8	44.4	106.7	117.8	93.9
Coffee	Yes	68.3	47.7[b]	52.6	46.1[b]	88.5	124.4	98.1
	No	59.7	53.0	49.3	41.6	107.3	113.5	71.9
Any Food	Yes	60.5[c]	53.5[c]	51.4[c]	45.1	104.5	121.6	96.0
	No	52.1	48.9	46.0	39.3	98.6	103.9	65.7

[a]Results shown for categories with at least five consumers only.
[b]$P < 0.05$, based on multivariable linear regression including all 13 food categories.
[c]$P < 0.05$, based on two-sided t-test.

Table 6. Age-specific trends in measured acrylamide hemoglobin (HbAA) concentrations among children and non-smoking adults.

					Nonsmokers					
			Children 3–12 years		Adolescents 13–19 years		Adults 20–59 years		Elderly 60+ years	
	Percent reporting AA consumption (%)		97.6		94.6		95.4		97.3	
	Consumed AA per unit body weight (μg/kg)[b]		0.38 (ref)		0.18 ($P < 0.001$)		0.15 ($P < 0.001$)		0.14 ($P < 0.001$)	
			[Conc][c]	P[d]	[Conc][c]	P[d]	[Conc][c]	P[d]	[Conc][c]	P[d]
Serum AA (pmol/g Hb)	Potato chips[e]	None	59.4	0.202	51.6	0.002	50.2	0.014	44.3	0.006
		Q1	53.7		53.3		48.4		46.5	
		Q2	60.2		58.3		55.1		52.1	
		Q3	79.9		61.5		56.3		52.3	
		Q4	59.4		63.2		58.1		50.8	
	Fried potatoes[e]	None	58.3	0.007	51.7	0.010	49.8	0.002	44.5	0.703
		Q1	57.4		54.2		49.6		50.3	
		Q2	65.6		52.1		50.5		48.8	
		Q3	68.3		58.3		55.7		45.0	
		Q4	70.6		59.3		59.8		44.3	
	Coffee[e]	None	59.7	0.390	53.0	0.009	49.3	0.056	41.6	< 0.001
		Q1	71.0		52.9		51.1		45.0	
		Q2	—		41.3		53.7		43.9	
		Q3	—		49.5		52.6		45.7	
		Q4	—		54.8		52.7		49.3	

[a]Results shown for categories with at least five consumers only.
[b]P-value for comparison with children aged 3–12 obtained from two-sided t-test.
[c]Geometric mean HbAA concentration in pmol/g.
[d]P for trend obtained from stratum-specific simple linear regression models of ln-transformed HbAA concentration.
[e]Quartiles of amount (grams) of food item eaten in the last 24 hours.

potato chips or coffee was estimated for adolescents, adults, and seniors.

Discussion

In recent years there has been a significant amount of research examining dietary AA, much of it focused on confirming the results of the 2002 Swedish studies about the general presence of AA in foods and understanding if hemoglobin adducts are the correct biomarker. This study went further by seeking to evaluate the contribution of individual foods that have consistently been measured as high concentration and high consumption AA sources in the diet of the U.S. population. In addition, the study attempted to understand better which human demographic or behavioral characteristics are influential in the relationship between reported AA consumption and HbAA, as well as the association between HbAA and HbGA.

Although our study estimated intake of foods known to contain acrylamide (as measured by the FDA) and accounted for smoking status in our study participants, we did not have information on other potential exposure sources that could have collectively contributed to the HbAA levels. Hemoglobin adducts are not specific with regard to the source of acrylamide intake or exposure (CDC, 2012); whereas we controlled for smoking, we were not able to control for other unmeasured confounding that the population may have experienced, whether related to occupational exposures, drinking water, or residues in commercial products.

The relationship between AA consumption and HbAA varied by age, gender, ethnicity, and smoking status. Children in our study population consumed approximately two times the dietary AA as adolescents, adults, and seniors per kg of body weight (Table 4). The unadjusted HbAA levels in adults aged 20–59 were significantly higher than those in all other age groups, which is notable because the differences in reported dietary consumption across all age groups was not significant. This potentially provides further evidence regarding the influence of smoking behavior on measured HbAA and HbGA. Smoking status and age seemed to be the most influential factors affecting the association between AA consumption and biomarkers of AA in the multivariable model. Although it did not strongly influence the relationship between AA consumption and biomarkers of AA, alcohol use was positively associated with HbAA and negatively associated with HbGA. The competition of alcohol and AA as a substrate for CYP2E1 has been cited as a possible reason for the negative association with HbGA (Vesper et al., 2013).

Interestingly, the youngest members of the study population had significantly higher unadjusted mean HbGA concentrations, largely exceeding mean concentrations of HbAA and raising additional questions about how it was produced in the body. The glycidamide-to-acrylamide hemoglobin adduct ratio is important due to its use as an indicator of the extent of acrylamide metabolism and therefore as an indicator of the formation of the metabolite glycidamide, which is genotoxic. Further research is necessary in order to better understand the biological factors by which the metabolic process differs between children and adults. Chemical alterations in the body or metabolism can occur to make chemicals become more or less toxic. Xenobiotic substances can enter the body as dangerous and need to be detoxified through metabolism or are not dangerous at ingestion, but become dangerous when metabolized (WHO, 2008). AA is metabolized by cytochrome P-450 into a

mutagenic epoxide, glycidamide (Walker et al., 2007). Researchers have attempted to evaluate the pharmacokinetic differences between children and adults by leveraging pharmaceutical data to evaluate the body's handling of xenobiotics (Ginsberg et al., 2002). Generally speaking, infants eliminated many of the examined pharmaceuticals more slowly than adults, and older children eliminate some more rapidly than adults. However, there is a large amount of variability between closely related drugs that share the same metabolic pathway (Ginsberg et al., 2002). In addition, the maturation of the pathways that guide these chemical processes are different depending on the age and development of the individual; most available research is focused on animals or modeling in adults.

To our knowledge, no other study has characterized the impact of individual acrylamide containing foods on body burden within the U.S. population. Our assessment of individual foods showed that by controlling for smoking across age categories we could see some statistically significant differences in measured HbAA between those who reported consumption versus those who did not. Three major foods of concern have been potato chips, fried potatoes, and coffee (Bjellaas et al., 2007; Wilson et al., 2009; EFSA, 2015). Among nonsmokers in most age groups, we observed a statistically significant positive trend between consumption of these foods and HbAA. Although this study supports previous evidence that smoking is in fact the most important determinant in measured HbAA, our results additionally suggest how elimination of individual acrylamide-containing foods from the diet may affect HbAA in individuals of different ages. These results are notable, highlighting the foods that drive HbAA in the absence of cigarette smoking.

In 2013, the FDA Center for Food Safety and Nutrition released a draft guidance document for the food industry that included recommendations about the storage, handling, and preparation or cooking of potato-based foods, cereal-based foods, and coffees to assist growers, manufacturers, and food service operators to decrease potential acrylamide production (FDA, 2013). Several variations in the growth and manufacture process have made it complicated for the development of hard and fast rules. However, the guidance that has been provided can lead to reductions in the concentrations of AA in end food products. As researchers continue to identify the foods that contribute most heavily to blood concentrations observed in humans, more targeted research and guidance can be provided.

Proposition 65 is a "right to know" law that was approved by voters in California in 1986; it requires the state to maintain a list of chemicals that cause cancer or reproductive toxicity (OEHHA). There are approximately 800 chemicals on the Proposition 65 list, and AA was added in 1990 based on research studies that indicated it caused cancer in animals. In addition, it was discovered in 2011 that AA has the potential to cause adverse reproductive effects in animals. The FDA has not advised the general U.S. population to avoid foods that contain AA. Instead, they have recommended that individuals eat a healthy and balanced diet. According to the 2015–2020 Dietary Guidelines for Americans, individuals should consume a variety of vegetables, fruits, grains (at least half should be whole grains), fat-free or low-fat dairy products, a variety of protein foods and healthy oils. Our study highlights the individual foods that contribute relatively more to HbAA concentrations by age group. Such information may be practical and useful to consumers who wish to minimize their exposure to acrylamide. As regulatory agencies continue to develop research protocols and revise their guidance documents, our study findings related to individual foods can be a valuable resource.

Limitations

The data on food consumption for this study was meant to capture the food that was eaten in the 24 hours prior to the dietary interview; with any recall survey there is potential for misreporting. In our study we assessed population averages, with an assumption of no bias. However, any study that utilizes participant recall is subject to some level of reporting error. Although the TDS has measured levels of AA in a large group of core foods, thought to be representative of dietary trends in the U.S. population, it is also possible that participants consumed foods that did not have measured concentrations of AA reported, and those dietary contributions would not have been captured in our analysis. In addition, the assignment of food codes was performed manually by the study authors, and in some instances we were required to use professional judgment and limited food descriptions to determine food classifications. During the coding of foods, staple foods were more easily coded with values from similar foodstuffs, such as varieties of breads; however, more complex food items or mixtures, such as restaurant or specialty foods were not assigned a proxy value. For biomarkers, we relied on hemoglobin adducts that represent chemical concentrations in the body for the preceding two to four months; to more accurately measure the association between food intake and the adducts, we would need dietary data for several days during that time period rather than a single day.

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