Association between aflatoxin-albumin adduct levels and tortilla consumption in Guatemalan adults

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ABSTRACT

Aflatoxin B1 (AFB1) is a known human hepatocarcinogen and a recent study reported elevated AFB1 levels, measured by serum albumin biomarkers, among Guatemalan adults. While AFB1 can contaminate a variety of foodstuffs, including maize, Guatemala’s main dietary staple, the relationship of maize intake to serum AFB1-albumin adducts levels in Guatemala has not been previously examined. As a result, a cross-sectional study was conducted among 461 Guatemalan adults living in five geographically distinct departments of the country. Participants provided a serum sample and completed a semi-quantitative food frequency questionnaire and a sociodemographic questionnaire. Multiple linear regression analysis was used to estimate the least square means (LSM) and 95% confidence intervals (95% CI) of log-transformed AFB1-albumin adducts by quintiles of maize consumption in crude and adjusted models. Additionally, analyses of tortilla consumption and levels of maize processing were conducted. The median maize intake was 344.3 g per day [Interquartile Range (IQR): 252.2, 500.8], and the median serum AFB1-albumin adduct level was 8.4 pg/mg albumin (IQR: 3.8, 22.3). In adjusted analyses, there was no association between overall maize consumption and serum AFB1-albumin levels. However, there was a statistically significant association between tortilla consumption and AFB1-albumin levels (ptrend = 0.01). The LSM of AFB1-albumin was higher in the highest quintile of tortilla consumption compared to the lowest quintile [LSM: 9.03 95%CI: 7.03, 11.70 vs 6.23, 95%CI: 4.95, 8.17, respectively]. These findings indicate that tortilla may be an important source of AFB1 exposure in the Guatemalan population. Therefore, efforts to control or mitigate AFB1 in contaminated maize used for tortillas may reduce overall exposure in this population.

1. Introduction

Aflatoxins are secondary metabolites produced by fungi of the Aspergillus species (A. flavus and A. parasiticus) with aflatoxin B1 (AFB1) being the most toxicologically potent [1]. Growth is common in hot, humid areas on agricultural crops, whether in field, during harvest and/or during storage, in maize, groundnuts, and cottonseed [2]. In 1993, the International Agency for Research on Cancer (IARC) determined there was sufficient evidence to classify AFB1 as a group 1 human carcinogen [3,4]. Further, recent and ongoing risk assessments have confirmed to impact of AFB1 in human health and as an ongoing exposure problem in people as well as in plant and animal foods for human consumption [5–8]. Covalent adduction of AFB1 to lysine residues in serum albumin (AFB1-lys) represents a biomarker of internal...
dose, which reflects recent dietary exposure (up to ~3 months) and has been strongly associated with several health outcomes such as growth stunting in children and liver cancer [8–11]. Since aflatoxin has been demonstrated in experimental models to cause many non-cancer toxicities that have direct concerns for public health including oxidative damage, protein synthesis dysregulation and lipid dysfunction, these studies point to a need to characterize if these effects occur in at-risk human populations [8,12,13].

In Guatemala, the most populous country in Central America, more than 50% of the population live in rural areas [14] and engage in small-scale, mixed farming where maize is the main crop produced. At harvest, farmers store most of their maize for household consumption and sell the balance to meet other household needs. When household maize stores are exhausted, farmers purchase maize from market vendors [15]. The traditional Guatemalan dietary pattern is dominated by maize-based components including traditional tortillas and other traditionally processed maize-based dishes, such as tamales [16]. Tortillas, the main foodstuff present in the Guatemalan diet, is composed of maize (up to 60 g maize/100 g total weight) and water [17]. Tortillas are produced from a dough ( massa) that is made by boiling maize meal with lime, which is then washed out in an alkaline-cooking process known as nixtamalization. Nixtamalization might reduce exposure to aflatoxins; however, its effectiveness in common practice remains inconclusive [18,19]. Other traditional foodstuffs such as tamales, chuchitos and tamalitos are produced from nixtamalized dough with the addition of fat and meat.

In previous work, we found high levels of serum AFB<sub>1</sub>-lys adducts in a contemporary sample of Guatemalan adults. Levels were higher (p < 0.05) among males, rural residents, low-income individuals, and participants reporting less than elementary education [20]. Previous studies across the country had measured aflatoxin in maize samples at levels that are harmful for humans [21,22]; however, to date, no studies have investigated the association of dietary intake of maize products with serum AFB<sub>1</sub>-lys adducts levels in Guatemala. Thus, our main objective was to examine whether maize consumption and tortilla consumption in particular, was associated with AFB<sub>1</sub>-lys adduct levels among adults, in a country that is estimated to have the highest incidence of liver cancer and stunting in the western hemisphere [14,23].

2. Materials and methods

2.1. Participants and study design

A cross-sectional study was conducted between May and October 2016 among 461 Guatemalan adults residing in five departments (2 urban, 3 rural) located in southern and western Guatemala: Chichicastenango (Quiché department), Escuintla (Escuintla), Mixco (Guatemala), San Lucas Tolimán (Sololá), and San Pablo Jocotílles (Suchitepéquez). The communities were nonrandomly selected based on a combination of representativeness including, accessibility, and feasibility of conducting clinic-based visits. Study recruitment was based on household visits using maps of the community when available. In each community, a study clinic was established through alliances with local non-governmental organization or within health centers of the Guatemalan Ministry of Health. In Mixco, households were selected at random from a formal sampling frame. In the other sites, we identified households in the community using a non-random-based sampling method. The study inclusion criteria were: adults 40 years old or older, no history of liver cancer, and an ability to provide informed consent. Exclusion criteria were: pregnancy and blood relationship with another participant. All study measures were performed in two visits, one at the participant’s household and one at the study clinic by trained staff. After providing consent to participate, a socio-demographic questionnaire and a 12-month semi-quantitative food frequency questionnaire (FFQ) were administered during the first visit at the household. Additionally, blood samples were collected, and anthropometry measures were recorded at the study clinic. Most samples were obtained less than a week after the initial home visit. Among the 461 subjects enrolled, 444 provided serum samples.

Details on enrollment and response rates have been previously described [20]. The study protocol and questionnaires were approved by the Institutional Review Boards (IRB) of the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD, USA (IRB #6877) and the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, Guatemala (IRB #053-2015). Written informed consent was obtained in Spanish; translation services in Mayan languages (Kaqchikel and Quiche) were available upon request.

2.2. Sociodemographic characteristics

Sociodemographic characteristics such as sex, age, residency, education, income and ethnicity of participants were obtained during the initial home visit. Participants were coded as indigenous if they self-identified as indigenous and reported speaking an indigenous language. Urban/rural residence was a dichotomous variable pre-defined by the study site. Education was defined as highest grade attained and categorized as less than an elementary education (0–5 years) and more than an elementary education (> = 6 years). Income was then grouped into a 2-category variable and a cut off of USD 400 per month was established to define income above or below the national minimum salary according with the Ministry of Labor [24].

2.3. Anthropometry

Standard procedures were used to obtain duplicate measures of height and weight from all participants. If the difference between the measures exceeded 500 g (g) per weight and 0.5 cm (cm) for height, a third measure was obtained, and the average was calculated with the two closest measures. Height was measured to the nearest 0.1 cm and weight to the nearest 100 g [25]. Body mass index (BMI) was calculated as the weight in kg divided by height in meters squared.

2.4. Total calorie intake, grams of tortillas and maize-based food products processing

A 12-month semi-quantitative food frequency questionnaire (FFQ), validated for Guatemalan adults, was used to estimate intake of maize, maize-based products, and total calorie intake [26]. Maize-based products were added to the original FFQ based on a 18-item FFQ to assess maize consumption in Guatemala [27]. We also added questions to ascertain whether the participants grew their own maize, consumed their own maize and length of maize storage. Questions were asked using examples of cups, spoons and three portion sizes of tortillas using models with known weights. After obtaining total grams of maize, total grams of tortillas and each food item consumed per day, the energy contribution of each food item and total daily energy intake was calculated. Individuals with energy intakes greater than 5 standard deviations above or below the mean, or with less than 25% of the calories relative to the total calorie recommendation for age and sex, were excluded from the analysis as the intakes; in total 5 participants were excluded. Corn consumption into five groups; tortillas, which includes tortillas made from corn and corn flour and the other groups were categorized by levels of processing, as described in the following.

Manufacturing of industrialized and non-industrialized maize-based products vary in terms of sources of maize and automated processing that might lower mycotoxin content [28,29]. To account for these differences, items were categorized into unprocessed/minimally processed, processed, and ultra-processed maize-based food using the NOVA classification [30]. Unprocessed or minimally processed maize are items that include boiled or roasted ears of maize and fortified beverages for public health purposes such as Incaparina<sup>®</sup> and Vita-cereal<sup>®</sup>. Processed maize includes food products in which salt, sugar, or
other ingredients were added as preservatives or to increase palatability as well as products that have undergone nonalcoholic fermentation. Ultra-processed maize was defined as those items that have undergone several industrial processes such as baking, extruding, molding, re-shaping, hydrogenation and hydrolysis (i.e. to produce maize-based salty snacks). To differentiate industrialized processed maize from non-industrialized processed maize we used a fifth category named "traditionally processed" to classify home-made maize-based dishes made from raw ingredients or ingredients with minimal processing, as well as traditional street food.

2.5. Aflatoxin exposure levels

Blood samples were stored at −80 °C until analysis. Determination of AFB1-lys adducts levels was performed by isotope-dilution mass spectrometry and adduct concentrations (pg AFB1-lys/mL serum) were normalized to total serum albumin (determined by ELISA) and expressed as pg AFB1-lys adducts/mg albumin. The limit of quantification was 0.2 pg AFB1-lys/mg serum albumin. Details of the laboratory procedures have been previously reported [20].

2.6. Statistical analysis

In total, 439 individuals were included in the analytical sample. Descriptive statistics were used to characterize the sample overall and stratified by sex. In addition, we computed unadjusted geometric means and 95% confidence interval (95% CI) for AFB1-lys, total maize consumption and tortilla consumption by other covariates (e.g. age, sex, residence, indigenous ethnicity, education, family income and total calorie intake). To examine the association between AFB1-lys adduct levels and maize consumption, AFB1-lys adduct levels were log-transformed due to skewness, and maize consumption was categorized into quintiles. Multiple linear regression analysis was used to estimate the least square means (LSM) and 95% confidence interval (CI) of AFB1-lys adducts. Analysis of trends in AFB1-lys adduct levels by quintile of maize consumption was examined by adding a score (1–5) to the quintiles and include the quintiles as a continuous variable in un-adjusted (without covariates) and adjusted models for sex, income, education, residence and total calorie-intake. Covariates were selected using a stepwise regression and the change-in-estimate approach (with cutoff of 10%). Two-sided p-values are reported and p-values < 0.05 are considered statistically significant without adjustment for multiple comparisons.

Additionally, analyses were also conducted after stratification of maize intake by level of maize processing (minimally processed, traditionally processed, processed, ultra-processed) and by tortilla consumption only. All statistical analysis was conducted in SAS software v 9.4 (SAS Institute, Cary, NC).

3. Results

Of the 439 individuals in the study, 57% percent were women. The median age of the participants was 52 years (IQR: 46, 60) among women and 56 years (IQR: 49, 66) among men (Table 1). Overall, 61% percent of the individuals resided in rural areas and 54% self-identified as indigenous. In addition, two-thirds of the study participants (67%) had not completed elementary education, and over three-quarters (approximately, 76%) reported a monthly income of less than USD 400. The median BMI was significantly higher among women 28.6 kg/m² (IQR: 25.4, 32.5) than men 24.9 kg/m² (IQR: 22.9, 28.5), (p < 0.001). The median maize consumption among study participants was 344.3 g per day (IQR: 252.2, 500.8), accounting for 20% of the total daily energy intake. The median tortilla consumption was 180.0 g per day (IQR: 150.4, 180.5), thus constituting more than half (52%) of the total daily maize consumption. All the study participants had detectable AFB1-lys adduct levels, with a median of 8.4 pg/mg (IQR: 3.8, 22.3).

Table 2 depicts the geometric means of AFB1-lys adducts, maize consumption and tortilla consumption by sociodemographic and other characteristics among the study participants. Men had significantly higher AFB1-lys adduct levels than women (11.0 vs 7.8 pg/mg, respectively). Further, rural residents (12.8 pg/mg), indigenous persons (13.2 pg/mg), those with less than elementary education (11.0 pg/mg) and those with an income < USD 400 per month (10.4 pg/mg) had significantly higher levels of AFB1-lys adducts. Women had a significantly higher level of total maize consumption than men (391.4 vs 354.7 g/day, p = 0.05), but men had a significantly higher level of tortilla consumption (168.3 vs 156.9 g/day, p < 0.001). Rural residents (429.7 g/day), indigenous persons (438.5 g/day), and persons with less than a primary education (397.2 g/day) also had significantly higher geometric means of maize consumption than their urban, non-indigenous and elementary school-educated counterparts. Similar patterns were observed between tortilla consumption and these covariates.

In the regression analysis, there was a positive linear trend between maize consumption and AFB1-lys adduct levels in the unadjusted model (Table 3). The level of AFB1-lys adducts significantly increased by quintile of maize consumption (p trend = 0.01). With an increase of approximately 50% in the levels of AFB1-lys adducts between the lowest quintile (LSM: 7.08; 95% CI: 5.44, 9.21) and the highest quintile (LSM: 11.70; 95% CI: 9.0, 15.20) of maize consumption. However, the association was no longer significant after adjustment for sex, income, education, residence and total calorie intake (p = 0.97) (Table 3). Similar results were observed when maize consumption was stratified by varying levels of maize processing. We found no significant linear trend between level of maize processing (minimally processed, traditional processed, processed and ultra-processed maize) and AFB1-lys in the fully adjusted models (Supplementary Table 1).

In contrast, the analysis of AFB1-lys adduct level and quintiles of tortilla consumption revealed a statistically significant association in both the crude (p trend < 0.001) and adjusted models (p trend = 0.01) (Table 3). After covariate-adjustment, AFB1-lys levels remained marginally significantly higher (LSM: 9.03, 95% CI: 7.03, 11.70) in the highest quintile of tortilla consumption than in the lowest quintile (LSM: 6.23, 95% CI: 4.95, 8.17), p = 0.06.

Using a 5-knot restricted cubic regression spline to estimate the relationship between continuous maize consumption and log transformed aflatoxin, there was no evidence of nonlinearity in the relationship. Also, we considered interactions between maize consumption and the covariates in the final model (sex, income, education, residence and total calorie intake), and the interactions were not statistically significant.

4. Discussion

A positive linear trend between serum AFB1-lys adduct levels and quintiles of tortilla consumption was found among Guatemalan adults. Further, there was a positive association between AFB1-albumin adducts and increasing total maize and traditional processed maize consumption; however, this association was not significant after adjustment for covariates. The mean level of AFB1-lys found in this study (8.4 pg/mg) is comparable with the levels found in some parts of China before the transition from a maize-based diet to a rice-based diet [31]. Results from the current analysis; however, are difficult to compare with other populations with high consumption of tortillas or maize-based foods due to differences in the estimates of aflatoxin exposure. A previous study in Mexico found that women consumed 6 tortillas a day on average and those with high intake of tortillas had 3-fold higher average urinary fumonisin B1 (another mycotoxin that contaminates maize) levels compared with women who consumed fewer tortillas (p = 0.002) [32]. A study of Tanzanian children, reported a positive correlation between maize intake and plasma AFB1 (r = 0.267, p = 0.001) and higher levels of AFB1 were associated with higher maize intake (β = 0.049, p = 0.01) [33].
Table 1
General characteristics of study participants overall and by sex.

| Characteristics                      | Total (N = 439) | Men n = 187 (43%) | Women n = 252 (57%) |
|---------------------------------------|----------------|-------------------|---------------------|
| Age, y, median (IQR)                  | 54 (47.63)     | 56 (49.66)        | 52 (46.60)          |
| Residence, rural, n (%)               | 268 (61.1)     | 116 (62.3)        | 152 (60.3)          |
| Indigenous ethnicity, yes n (%)       | 236 (53.8)     | 102 (54.6)        | 134 (53.1)          |
| Study site, n (%)                     |                |                   |                     |
| Mixco                                 | 82 (18.7)      | 29 (15.5)         | 53 (21.0)           |
| Suchitepéquez                         | 81 (18.5)      | 38 (20.3)         | 43 (17.1)           |
| Solañol                               | 89 (20.3)      | 40 (21.4)         | 49 (19.4)           |
| Ecuáná                               | 89 (20.3)      | 42 (22.5)         | 47 (18.7)           |
| Quiché                                | 98 (22.3)      | 38 (20.3)         | 60 (23.8)           |
| Education, elementary incomplete < 6 years, n (%) | 292 (66.5) | 114 (61.0) | 178 (70.6) |
| Monthly family income, < $400 USD, n (%) | 329 (75.5) | 137 (73.7) | 192 (76.8) |
| Body mass index, kg/m², median (IQR) | 27.1 (23.9, 30.7) | 24.9 (22.9, 28.5) | 28.6 (25.4, 32.5) |
| Maize growth, yes, n (%)              | 125 (28.5)     | 60 (32.1)         | 65 (25.8)           |
| Consumption of own production,¹ yes n (%) | 124 (99.2) | 59 (98.3) | 65 (100) |
| Time of maize storage, n, (%)         |                |                   |                     |
| 1-2 months                            | 39 (33.1)      | 18 (31.0)         | 21 (35.0)           |
| 3-6 months                            | 66 (55.9)      | 33 (56.9)         | 33 (55.0)           |
| > 6 months                            | 13 (10.2)      | 7 (12.1)          | 6 (10.0)            |
| Total calorie intake, kcal/day, median (IQR) | 2034.5 (1538.2, 2607.6) | 2123.6 (1566.2, 2659.2) | 1973.0 (1486.6, 2548.4) |
| Energy density relative to maize, (%),² median (IQR) | 19.0 (13.7, 24.9) | 16.8 (12.6, 22.1) | 20.5 (14.7, 27.0) |
| Tortilla consumption per/day, grams, median (IQR) | 343.4 (252.2, 500.8) | 329.7 (258.9, 468.2) | 368.2 (245.9, 550.0) |
| AFB1-lys adducts (pg/mg),² median (IQR) | 180 (150.4, 180.5) | 180 (150.4, 180.5) | 180 (150.4, 180.5) |

¹ Consumption of maize production and time of maize storage were asked just to those who respond yes to production of maize. Sample sizes are n = 128 and n = 121 for men and women respectively.

² Energy density relative to maize was calculated as: (calories from maize/total calorie intake) x100.

AFB1-lys: Covalent adduction of Aflatoxins B1 to lysine residues in serum albumin. CI: confidence interval. IQR: interquartile range.

Table 2
Unadjusted geometric means of AFB1-lys adducts, total maize and tortilla, by general characteristics of study participants.

| Characteristics                      | AFB1-lys adducts (pg/mg) | p-value¹ | Total maize consumption grams/day | p-value¹ | Tortilla consumption grams/day | p-value¹ |
|---------------------------------------|--------------------------|----------|-----------------------------------|----------|-------------------------------|----------|
|                                       | Unadjusted geometric means (95% CI) |          | Unadjusted geometric means (95% CI) |          | Unadjusted geometric means (95% CI) |          |
| Age:                                  |                          | 0.19     | 0.003                             | 0.17     |                                |          |
| 40-49                                 | 9.7 (8.1, 11.7)          | < 0.001  | 426.5 (392.9, 463.1)              | < 0.001  | 168.5 (164.3, 172.7)           |          |
| 50-59                                 | 8.5 (6.9, 10.4)          |          | 362.3 (331.6, 395.7)              |          | 161.1 (155.3, 167.1)           | < 0.001  |
| > 60                                  | 9.0 (7.2, 11.2)          | < 0.001  | 339.0 (314.0, 365.9)              | < 0.001  | 159.1 (153.5, 164.9)           |          |
| Sex:                                  |                          |          |                                   |          |                               |          |
| Women                                 | 7.8 (6.8, 9.0)           | 0.05     | 391.4 (365.5, 419.2)              | 0.05     | 156.9 (152.0, 162.0)           | < 0.001  |
| Men                                   | 11.0 (9.0, 13.4)         | < 0.001  | 354.7 (332.2, 378.6)              | < 0.001  | 168.3 (164.2, 172.6)           |          |
| Residence:                            |                          |          |                                   |          |                               |          |
| Rural                                 | 12.8 (10.9, 14.9)        | < 0.001  | 429.7 (404.1, 456.8)              | < 0.001  | 168.2 (165.2, 171.2)           |          |
| Urban                                 | 5.3 (4.6, 6.1)           | < 0.001  | 303.2 (283.5, 324.3)              | < 0.001  | 152.2 (145.5, 159.3)           |          |
| Indigenous ethnicity:                 |                          | < 0.001  |                                   | < 0.001  |                               |          |
| Yes                                   | 13.2 (11.1, 15.7)        | < 0.001  | 438.5 (411.6, 467.2)              | < 0.001  | 168.8 (165.9, 171.7)           |          |
| No                                    | 5.8 (5.1, 6.7)           | < 0.001  | 312.7 (292.7, 334.0)              | < 0.001  | 153.9 (147.8, 160.3)           |          |
| Education:                            |                          |          |                                   |          |                               |          |
| Elementary incomplete                 | 11.0 (9.5, 12.7)         | < 0.001  | 397.2 (374.1, 421.7)              | < 0.001  | 166.1 (162.8, 169.5)           |          |
| Elementary completed                  | 6.2 (5.1, 7.5)           | < 0.001  | 335.4 (310.0, 362.8)              | < 0.001  | 153.6 (146.5, 161.2)           |          |
| Monthly family income:                |                          |          |                                   |          |                               |          |
| < USD 400                             | 10.4 (9.1, 12.0)         | 0.06     | 376.6 (356.1, 398.2)              |          | 163.7 (160.1, 167.9)           |          |
| ≥ USD 400                             | 5.7 (4.7, 7.0)           | 0.09     | 373.0 (338.2, 411.2)              |          | 155.8 (148.0, 164.1)           |          |
| Total calorie intake:                 | 0.19                     |          |                                   |          |                               | 0.03     |
| Below median                          | 9.42 (7.94, 11.18)       | < 0.001  | 344.8 (324.6, 366.26)             | < 0.001  | 159.98 (156.2, 164.0)          |          |
| Above or equal median kcal/day        | 8.71 (7.40, 10.25)       |          |                                   | 0.06     |                               |          |

¹ p values were obtained from t-test for continuous predictor or Anova F-test for categorical predictors.
In a survey conducted in 2012, levels of the parent AFB1 were measured in 640 maize samples purchased from local markets across Guatemala. The mean of AFB1 in maize samples was 179 μg/kg ± 30, which was well above 20 μg/kg, the FDA regulatory level for aflatoxin in maize destined for use in human food and many samples contained levels known to be harmful to humans [22,34]. Suchitepéquez and Quiché, both locations included in our study, ranked 2nd and 4th among departments with high amounts of AFB1 detected in the maize samples (178 ± 48 μg/kg and 108 ± 51 μg/kg, respectively) [22]. Since all departments do not produce sufficient maize to meet the demand, importation of maize is common in many departments. Guatemala department, mostly urban, is the greatest maize importer of all departments [35], hence, contamination may not be localized to the department where the maize was grown.

We did not observe an association of AFB1-lys adduct levels with increasing consumption of processed and ultra-processed maize foods such as snacks, breakfast cereals, and processed beverages. We also found no association with minimally processed foods. This may be due to mycotoxin levels being lowered by food processing, such as sorting, milling, steeping and extrusion [28,29]. Alternatively, maize selected for processing may be less likely to be contaminated with AFB1. However, these findings need careful interpretation as consumption of processed and minimally processed maize was low in the current study.

Most of the maize consumed by the study population was from tortillas, accounting for 52% of the total maize consumption. In previous work, we showed that sociodemographic characteristics associated with poverty (indigenous ethnicity, low educational attainment, low income and rural residence) were associated with levels of serum AFB1-lys adducts [20]. After adjustment for these variables we no longer observed significant associations between overall maize intake and AFB1-lys adducts. In Guatemala, a diet that is limited to maize-based foods is associated with poverty-related characteristics such as poor educational attainment and rural residence [16]. Nonetheless, we observed an association between AFB1 and tortilla consumption, as there was little variability (<1 tortilla/day) in consumption by socioeconomic status. Our results suggest than independently of socioeconomic position or location the tortillas may be the most important foodstuff in determining the serum level of AFB1-lys adducts among the Guatemalan population.

Preliminary evidence suggests an interaction between chronic aflatoxin exposure and malnutrition, as reduced uptake of nutrients from the diet may result in growth retardation. In a study conducted in the western highlands of Guatemala, tortilla consumption among children was reported to average 5 tortillas per day. Stunting prevalence was 70% and only 60% of mothers carried out adequate nixtamalization practices [36]. Moreover, a recent study in Guatemala reported a negative association between AFB1 exposure, based on food contamination surveys, and height for age-z scores in children under 5 years of age [37].

Our results have major implications for public health given the strong relationship between AFB1, and health outcomes such as immunosuppression, growth retardation and liver cancer [4,9,11,38–40]. Poor post-harvest practices such as mishandling of grain storage, leading to insect and fungal infestation in maize has been previously shown in Guatemala [15]. Good pre- and post-harvest agricultural practices, plant disease management, adequate storage and promotion of adequate nixtamalization practices are effective interventions that could reduce aflatoxin exposure in this population [11; Wild et al., 2015a].

Strengths of the present study include the availability of a robust biomarker of exposure such as AFB1-lys adducts. To date, most studies in Latin America have been food-based exposure estimates, which are not as reliable as biomarker-based exposure estimates. To our knowledge this is the first study addressing maize and tortilla consumption and serum AFB1-lys in adults in the western hemisphere. Another strength is that we were able to adjust for total calorie intake, derived from a food frequency questionnaire validated for Guatemalan adults. Total calorie intake is a well-known confounder in epidemiologic studies of diet and disease.

Limitations of the study include its cross-sectional design which precludes the ability to examine a temporal relationship between maize consumption and AFB1 levels. In addition, persons were sampled at a single point in time, and the modest sample size precluded stratification on any of covariates. Another limitation is that we could not determine the exact number of tortillas consumed every day with the FFQ. Previous studies have shown even higher intakes of tortillas in Guatemalan populations; hence, this could lead to an underestimation of tortilla consumption. For most study sites, we did not use a probability-based sampling method and the geographic areas selected were not necessarily representative of the whole country.
5. Conclusions

In conclusion, this is the first study to reveal an association between AFB1 exposure in Guatemalan adults assessed with a validated, quantitative biomarker – and consumption of specific dietary components. Moreover, the identification of tortillas as a significant source of AFB1 exposure validates previous findings of widespread aflatoxin contamination in Guatemalan maize. Effective public health interventions that could be integrated with current agricultural approaches to reduce morbidity and mortality associated with the consumption of aflatoxin-contaminated food in Guatemala are urgently needed.

Contributors

MRZ, EG, JDG, KAM, ML, OT and NDF conceived and designed the study. MRZ, EG, JDG, ML and KAM supervised the study. MKF and ARA coordinated field work, recruited subjects and collected data. JWS and PE performed laboratory analyses. CSA analyzed the data. BG made substantial contribution to statistical analysis. MKF led the manuscript writing. MKF and CSA wrote the manuscript. All authors edited, read, and approved the final draft.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.toxrep.2019.05.009.

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