Dietary determinants of aflatoxin B$_1$-lysine adduct in pregnant women consuming a rice-dominated diet in Nepal

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Abstract

Background Aflatoxins are found in diverse foods widely consumed worldwide. This study investigated the association between aflatoxin exposure and (a) consumption of specific foods, (b) dietary diversity (DD), and (c) seasonality.

Methods Women enrolled in the AflaCohort Study in Banke, Nepal ($n=1648$) were asked how often they ate certain food items in the past 7 days and 24 h. Serum aflatoxin B$_1$-lysine (AFB$_1$-lys) adduct levels, measured during pregnancy, were determined using high-performance liquid chromatography. Multivariable ordinary least squares and quantile regression models were used to examine incremental increases in AFB$_1$-lys adduct levels per frequency of food consumption and the relationship between DD, seasonality, and increases in AFB$_1$-lys adduct.

Results Roughly 94% of women were exposed to aflatoxin (geometric mean 1.37 pg/mg). Women in the 30th, 50th, and 70th quantiles of aflatoxin exposure who reported one more occasion of maize consumption in the past week showed increases in AFB$_1$-lys adduct levels: 0.094, 0.112, and 0.109 pg/mg ($p<0.05$, all). Women in the 30th, 50th, 70th, and 90th quantiles of exposure who reported one more occasion of groundnut consumption in the past week also showed increases in AFB$_1$-lys adduct levels: 0.058 ($p<0.001$), 0.085 ($p<0.01$), 0.133 ($p<0.001$), and 0.133 ($p<0.001$) pg/mg. Winter month recruitment was positively associated with AFB$_1$-lys adduct levels at all quantiles of aflatoxin exposure (range: 0.313–1.101 pg/mg, $p<0.001$). DD was not predictive of aflatoxin exposure.

Conclusions Our findings justify integrated approaches to aflatoxin reduction, including regulatory, agricultural, and food safety interventions across the value chain and at the household level.

Introduction

In South Asia, women and young children are at risk of exposure to aflatoxin, a naturally occurring toxin produced by Aspergillus fungi [1, 2]. Acute aflatoxicosis can cause coma or death. Chronic, low level exposure to aflatoxin is harmful to human health [3]. Evidence shows placental transfer of aflatoxin from mother to fetus [4] and linkages to impaired linear growth in childhood [4, 5].

Exposure occurs primarily through the consumption of contaminated foods. Maize, chilies, spices, oilseeds, and nuts are especially susceptible to aflatoxin contamination [6–8]. When ruminants ingest feed contaminated with aflatoxin they metabolize and excrete the metabolite, aflatoxin M$_1$ (AFM$_1$), in milk [9]. Aflatoxins are difficult to detect and remove because they are unobservable to the consumer and relatively resistant to thermal inactivation [10, 11].

Populations at particularly high risk of chronic aflatoxin exposure are resource-scarce, have limited dietary variety, store foods for long periods, and rely on highly susceptible foods including maize and groundnuts [12, 13]. Access to improved dietary diversity (DD) may lower aflatoxin

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exposure by lessening the dependence on aflatoxin-prone foods and counteracting the toxicity [14, 15], particularly in those consuming monotonous diets [16, 17].

This study was conducted to determine: (a) if the frequency of consumption of susceptible agricultural commodities was associated with aflatoxin exposure in pregnancy in Nepali women, (b) if increased DD was associated with lower levels of aflatoxin exposure, and (c) whether aflatoxin exposure levels vary seasonally.

**Methods**

**Study population**

The AflaCohort Birth Cohort Study (2015–2019) was conducted in Banke, a tropical district (Province 5) in the southern plains of Nepal. A rolling recruitment strategy was used to enroll 1675 healthy pregnant women. The sample size was calculated assuming an alpha of 0.05, power of 80%, attrition of 20%, and design effect of 1.5. This allowed the detection of a −0.207 standard deviations (SD) difference in postnatal height-for-age Z-score for every 1-unit increase in log average maternal AFB1-lys adducts.

Eligibility criteria included: <30 weeks pregnant, age 16–49, singleton pregnancy, living, and planning to give birth in the study area. This analysis used data collected during pregnancy (July 2015–August 2016); nation-wide strikes interrupted data collection for 3 months and resumed in December 2015.

The women (or their legal guardians) gave verbal and written consent prior to participation. The Nepal Health Research Council (295/2014), and the Tufts Institutional Review Board (11535) approved this study.

**Data collection**

Trained interviewers administered electronic surveys. Surveys included a single qualitative 7 and 24 h food frequency questionnaire (FFQ) [18] to determine the frequency of consumption of 49 predetermined food items. The food items included were based on previous dietary assessments in this population [19]. Consumption data were also collected for the past year.

Upon survey completion, interviewers measured height, weight and mid-upper arm circumference (MUAC) to the nearest 0.1 cm and 0.1 kg using ShorrBoard® Measuring Boards, 874 Seca Scales, and 65 cm adult measuring tapes, respectively.

Within a week of survey completion, nurses visited the women and collected 3–5 mL antecubital vein blood sample. Blood samples were transported on wet ice to a local laboratory for processing. Samples were air-shipped on wet ice to the Patan Academy of Health Sciences to be stored at −80 °C until they were ready to be air-shipped on dry ice to the Wang laboratory at the University of Georgia.

**Data analysis**

A total of 1650 gestational serum samples were analyzed for AFB1-lys adducts, an established biomarker of dietary aflatoxin exposure over the previous 2–3 months [20]. The levels of AFB1-lys adducts were measured using a validated high-performance liquid chromatography (HPLC) with fluorescence detection method [21].

After deactivation in 56 °C water bath for 30 min, −150 μL of each sample were digested by pronase (pronase: total protein, 1:4, w/w) at 37 °C for 3 h to release adducts. The digests were extracted and purified by passing through a Waters MAX SPE cartridge, eluted with 2% formic acid in methanol, vacuum-dried with a Labconco Centrivap concentrator (Kansas City, MO), and reconstituted with 25% methanol water for HPLC-fluorescence detection.

An Agilent 1200 HPLC-fluorescence system (Santa Clara, CA) was used to quantify AFB1-lys adducts. The mobile phases consisted of buffer A (20 mM NH₄H₂PO₄, pH 7.2) and buffer B (100% Methanol), running at a gradient to allow separation within 25 min of injection, with a typical retention time for AFB1-lys adduct at ~13 min. Separation was achieved using Zorbax Eclipse XDB-C18 reverse phase column (5 micron, 4.6 × 250 mm) equipped with a guard column, maintained at 25 °C and a flow rate of 1 mL/min during analysis. Sample injection volume was 100 μL. Excitation and emission wavelengths for detection were 405 and 470 nm, respectively. Calibration curves of authentic standard were generated weekly. Quality assurance and quality control procedures included simultaneous analysis of one authentic standard for every ten samples, and two daily quality control samples. The average recovery rate was 90% for the report, the AFB1-lys concentration was adjusted by albumin concentration, measured via UV/Visible spectrophotometry. Samples below the limit of detection (LOD) (0.4 pg AFB1-lysine/mg albumin) were substituted with a constant value of half the LOD for statistical analysis [22].

Minimum DD scores were computed using Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [23]. Food items from the 24-h FFQ were categorized into one of ten food groups: [1] grains/roots/tubers, [2] pulses, [3] nuts and seeds, [4] dairy, [5] meats, [6] eggs, [7] dark green leafy vegetables (DGLV), [8] other vitamin A sources, [9] other vegetables, [10] other fruits. A dichotomous MDD indicator was created to calculate whether women achieved MDD (consuming ≥ 5 of the 10 food groups in the previous 24 h).

Covariates analyzed included age, education, wealth status, MUAC, season, and Village Development Committee
with those included in the models being selected based on their potential for confounding.

Seasonal variations in aflatoxin exposure were examined for autumn, prewinter, winter, spring, summer, and monsoon seasons. Autumn is characterized by wet, cool weather, while the prewinter and winter are cooler and drier [24]. Spring and summer are warm and dry. The summer weather, while the prewinter and winter are cooler and drier soon seasons. Autumn is characterized by wet, cool

Fig. 1 AFB$_1$-lysine adducts in serum of pregnant women.

Fig. 2 Percent of pregnant women consuming foods from various food groups in the past 24 h.

Results

AFB$_1$-lys adducts (range: 0.4–147 pg/mg albumin) were found in 94% of samples (Fig. 1; mean concentration 3.2 ± 8.3 pg/mg albumin, geometric mean 1.37 pg/mg albumin, CI: 1.3–1.4).

In the past 24 h, 100% women consumed rice, 62% consumed pulses, while 12% consumed nuts/seeds (Fig. 2). Dairy was more commonly consumed than meat (43% versus 25%) in the past 24 h. While over 80% reported consuming vegetables, the reported 24-h consumption of fruit, eggs, DGLV, and vitamin A-rich sources was low (33%, 12%, 28%, and 20%, respectively). Only 39% achieved MDD.

Age and MUAC were significantly negatively associated with maternal AFB$_1$-lys adduct concentrations in bivariate analyses. Hemoglobin level and winter season were significantly positively associated with aflatoxin exposure (Table 1).

Weekly consumption frequencies included 31% reporting consumption of groundnuts, 3% reporting maize, and 2% reporting both groundnuts and maize (Table 2). The weekly mean frequency of maize and groundnuts consumption was 2.4 ± 2.2 and 2.8 ± 2.2, respectively. AFB$_1$-lys adduct levels were significantly higher in maize and groundnut consumers (7.9 pg/mg albumin adducts) compared with those who did not eat maize or groundnuts (2.4 pg/mg albumin adducts, $p < 0.0001$) in the past week.

Weekly maize consumption did not vary across wealth quintiles and was positively associated with maternal education ($p < 0.01$) (Table 3). Weekly groundnut consumption, in contrast, was positively associated with wealth status ($p < 0.01$) but not with maternal education. Frequency of maize ($p < 0.05$) and groundnut ($p < 0.001$) consumption in the past week were significantly higher for women recruited in the winter.

Annual consumption frequencies included 83% reporting maize, 97% groundnut, 100% chilies, and 93% reporting consuming milk (Table 2). No association was detected between aflatoxin exposure and annual consumption of
Table 1 Serum aflatoxin B1-lysine adduct levels by sociodemographic and health characteristics of pregnant Nepalese women enrolled in the AflaCohort Study.a.

| Characteristic                  | n   | n % | Mean AFB 1 | SD  | Geo mean AFB 1 | 95% CI       | Highest AFB 1 quintileb | n  | %   |
|--------------------------------|-----|-----|------------|-----|----------------|-------------|-------------------------|----|-----|
| **Age category**               |     |     |            |     |                |             |                         |    |     |
| <20                            | 347 | 21.1| 4.4        | 11.2| 1.6            | 1.4–1.9     | 77                      | 22.2|     |
| 21–24                          | 627 | 38.1| 3.3        | 7.6 | 1.4            | 1.3–1.5     | 117                     | 18.7|     |
| 25–29                          | 470 | 28.5| 2.4        | 4.4 | 1.2            | 1.1–1.4     | 93                      | 19.8|     |
| 30–34                          | 135 | 8.2 | 3.0        | 12.9| 1.2            | 1.0–1.4     | 26                      | 19.3|     |
| 35+                            | 69  | 4.2 | 2.2        | 2.6 | 1.3            | 1.0–1.6     | 16                      | 23.2|     |
| **Schooling**                  |     |     |            |     |                |             |                         |    |     |
| None                           | 606 | 36.8| 2.5        | 5.1 | 1.3            | 1.2–1.4     | 121                     | 19.9|     |
| Some primary (1–5)             | 321 | 19.5| 3.1        | 5.6 | 1.0            | 1.3–1.6     | 68                      | 21.2|     |
| Some secondary (6–10)          | 577 | 35.0| 4.2        | 12.0| 1.4            | 1.3–1.6     | 117                     | 20.3|     |
| More than secondary (10+)      | 144 | 8.7 | 2.6        | 5.1 | 1.3            | 1.1–1.5     | 23                      | 16.0|     |
| **Wealth Index Quintile**      |     |     |            |     |                |             |                         |    |     |
| Poorest                        | 328 | 19.9| 3.5        | 9.3 | 1.4            | 1.3–1.6     | 73                      | 22.3|     |
| Poor                           | 331 | 20.1| 2.8        | 6.4 | 1.2            | 1.1–1.4     | 60                      | 18.1|     |
| Middle                         | 329 | 20.0| 2.7        | 6.4 | 1.3            | 1.1–1.4     | 55                      | 16.7|     |
| Rich                           | 328 | 19.9| 4.0        | 9.4 | 1.5            | 1.4–1.8     | 80                      | 24.4|     |
| Richest                        | 332 | 20.2| 3.1        | 9.3 | 1.4            | 1.2–1.5     | 61                      | 18.4|     |
| **Religion**                   |     |     |            |     |                |             |                         |    |     |
| Hindu                          | 1258| 76.3| 3.2        | 8.5 | 1.3            | 1.3–1.4     | 244                     | 19.4|     |
| Buddhist                       | 5   | 0.3 | 3.4        | 5.3 | 1.2            | 0.0–9.3     | 1                       | 20.0|     |
| Muslim                         | 362 | 22.0| 3.1        | 7.2 | 1.4            | 1.2–1.6     | 78                      | 21.6|     |
| Christian                      | 23  | 1.4 | 5.4        | 11.2| 1.8            | 1.0–3.2     | 6                       | 26.1|     |
| **Ethnicity**                  |     |     |            |     |                |             |                         |    |     |
| Brahmin                        | 77  | 4.7 | 3.5        | 9.1 | 1.5            | 1.2–2.0     | 21                      | 27.7|     |
| Chhetri                        | 299 | 18.1| 3.7        | 9.2 | 1.4            | 1.3–1.6     | 59                      | 19.7|     |
| Tharu                          | 168 | 10.2| 2.6        | 7.1 | 1.0            | 0.8–1.2     | 27                      | 16.1|     |
| Muslim                         | 359 | 21.8| 3.0        | 7.1 | 1.4            | 1.3–1.6     | 76                      | 21.2|     |
| Dalit                          | 380 | 23.1| 2.9        | 8.6 | 1.3            | 1.2–1.5     | 73                      | 19.2|     |
| Other                          | 365 | 22.2| 3.5        | 8.5 | 1.5            | 1.4–1.7     | 73                      | 20.0|     |
| **Anemia (hemoglobin < 11 g/dL)** | |     |            |     |                |             |                         |    |     |
| No                             | 976 | 59.3| 3.5        | 9.4 | 1.4            | 1.3–1.5     | 207                     | 21.2|     |
| Yes                            | 670 | 40.7| 2.7        | 6.4 | 1.3            | 1.2–1.4     | 121                     | 18.1|     |
| **Maternal stature**           |     |     |            |     |                |             |                         |    |     |
| Short/average (>145 cm)        | 1422| 86.4| 3.2        | 8.3 | 1.4            | 1.3–1.4     | 284                     | 20.0|     |
| Very short (<145 cm)           | 224 | 13.6| 3.2        | 8.3 | 1.5            | 1.3–1.7     | 45                      | 20.1|     |
| **MUACc**                      |     |     |            |     |                |             |                         |    |     |
| Average (>23 cm)               | 1099| 66.7| 3.2        | 8.5 | 1.4            | 1.3–1.4     | 216                     | 19.7|     |
| Low (<23 cm)                   | 549 | 33.3| 3.3        | 7.9 | 1.4            | 1.3–1.5     | 113                     | 20.6|     |
| **Minimum Dietary Diversityd** |     |     |            |     |                |             |                         |    |     |
| No                             | 1003| 60.9| 3.0        | 7.3 | 1.3            | 1.2–1.4     | 200                     | 19.9|     |
| Yes                            | 645 | 39.1| 3.6        | 9.6 | 1.4            | 1.3–1.6     | 129                     | 20.0|     |
| **Season of measurement**      |     |     |            |     |                |             |                         |    |     |
| Spring                         | 514 | 31.2| 2.2        | 5.1 | 1.2            | 1.1–1.3     | 73                      | 14.2|     |
| Summer                         | 391 | 23.7| 1.3        | 2.8 | 0.8            | 0.8–0.9     | 31                      | 7.9 |     |
| Rainy/Monsoon                  | 32  | 1.9 | 1.2        | 1.3 | 0.8            | 0.6–1.1     | 2                       | 6.3 |     |
| Autumn                         | 0   | 0   | n/a        | n/a | n/a            | n/a         | n/a                     | n/a |     |
| Prewinter                      | 238 | 14.4| 6.6        | 14.1| 2.6            | 2.2–3.1     | 92                      | 38.7|     |
| Winter                         | 473 | 28.7| 4.2        | 9.6 | 1.8            | 1.6–2.0     | 131                     | 27.7|     |

*Geo geometric, MDD-W minimum dietary diversity score for women, MUAC mid-upper arm circumference

**p < 0.01; ***p < 0.001

aNumbers do not always add up due to missing responses
bHighest quintile > 2.9 pg/mg. AFB1 values were log-transformed before analysis
cMean hemoglobin of 11.2 ± 1.2 g/dL
dMean MUAC of 24.1 ± 2.5 cm
eFood and Agriculture Organization minimum dietary diversity defined as consuming ≥5 of the ten food groups in the previous 24 h, mean dietary diversity score of 4.2 ± 1.5
maize or groundnuts. Low variability in annual chili consumption limited our ability to test the association with aflatoxin exposure. Annual milk consumption was positively associated with AFB1-lys adduct concentrations ($p < 0.05$). Neither annual wheat nor rice consumption was associated with maternal aflatoxin exposure (data not shown).

In the adjusted OLS model, groundnut consumption in the past week (0.730, $p < 0.001$) and the winter season (2.339, $p < 0.001$) were significant predictors of maternal AFB1-lys adduct levels (Table 4). In the QR models, maize and groundnut consumption were heterogeneously positively associated with higher aflatoxin. Every additional occasion of reported weekly maize consumption was associated with higher AFB1-lys adduct concentrations in the 30th (0.094, $p < 0.05$), 50th (0.112, $p < 0.05$), and 70th quantiles (0.109, $p < 0.05$) of exposure. In contrast, reported weekly maize consumption was not associated with aflatoxin exposure in the OLS regression or in the QR model in the 10th and 90th quantiles of exposure.

Women in the 30th, 50th, and 70th quantiles of exposure who reported one more occasion of weekly groundnut consumption experienced significantly higher aflatoxin levels: 0.058 ($p < 0.001$), 0.085 ($p < 0.01$), and 0.133 ($p < 0.001$) pg AFB1-lys adducts per mg of albumin. Similarly, women in the 90th quantile of exposure reporting one more occasion of weekly groundnut consumption showed significantly higher concentrations of AFB1-lys adduct (0.133, $p < 0.001$). However, weekly groundnut consumption was not associated with aflatoxin for women in the 10th quantile of exposure; this may be a function of lower groundnut consumption in women with the lowest aflatoxin levels. Restricted cubic spline analyses found no evidence of a threshold effect between either weekly maize or groundnut consumption and exposure. This suggests that a linear relationship hypothesis between weekly consumption and exposure cannot be rejected for this sample, i.e., frequent consumption results in higher values in blood.

Women in the 10th and 50th quantiles of exposure who reported milk consumption had higher aflatoxin exposure (0.63 ($p < 0.01$) and 0.23 ($p < 0.05$), respectively) than those who did not consume milk in the past year. DD was not associated with maternal aflatoxin exposure in the OLS or at most quantiles in the QR models. DD scores were significantly positively associated with maternal aflatoxin in the 10th quantile of exposure (0.064, $p < 0.05$). The association between winter season and AFB1-lys adduct concentration was positive across all quantiles.

**Discussion**

Biomarker data show that the majority of the women were exposed to aflatoxin during pregnancy. Diet-associated aflatoxin exposure in these women seems to be driven by groundnut and maize consumption and is highly variable by season of measurement. Contrary to expectations, results showed no association between DD and maternal aflatoxin levels.

The geometric mean of serum maternal AFB1-lysine adduct concentration of 1.37 pg/mg albumin (95% CI: 1.30, 1.44 pg/mg albumin) in this cohort was lower than average concentrations found in similar studies. One Nepali study [1] reported 3.62 pg AFB1-lysine/mg albumin (geometric mean) in children ages 15–36 months, while two other studies in African children [32, 33] reported levels ranging 4.5–8.3 pg/mg.

The positive associations between weekly maize and groundnut consumption and serum AFB1-lys adduct concentrations are consistent with previous research as contamination is common in these commodities [34–37]. Maize and groundnut products have been known to commonly exceed the permissible limit for aflatoxin [34, 38–40]. While maize and groundnut production is low in the Banke area these two foods seem to be important sources of aflatoxin exposure.

Groundnuts are a nutrient-dense food, high in protein, fats, fiber, and multiple micronutrients and are a common snack in Nepal. They have recently gained popularity through government promotion programs [41]. Commercialization of groundnut products and market trends present an opportunity for spreading awareness and targeted measures to improve the quality of groundnut and groundnut products. Awareness campaigns and aflatoxin reduction interventions can help reduce consumption of aflatoxin-contaminated foods without compromising demand for nutrient-dense food items.

Aflatoxin M1, a hydroxylated metabolite of AFB1, can be found in milk or milk products from livestock that have ingested contaminated feed. Although it was beyond the scope of this study to measure AFM1, our study did examine the association between consuming milk and serum AFB1-lysine adduct concentration levels. Our findings showing positive associations between milk consumption during the past year and increased aflatoxin levels are in line with Kafle et al. [42] showing 44% of milk samples contaminated with aflatoxin M1. Indirect sources of contamination such as milk should not be overlooked when designing aflatoxin reduction interventions.

Although this study did not find an association between rice consumption and aflatoxin levels, rice cannot be disregarded as it is a fundamental component of the Nepali diet and can harbor low levels of aflatoxin [43]. Future work should also examine other commonly contaminated, ubiquitous foods and spices, such as black pepper, nutmeg, cumin, coriander, garlic, and dairy products (e.g., curd) [44].
Previous research suggests that DD reduces the amount of aflatoxin-prone foods consumed and counteracts adverse effects of aflatoxin [16]. Our study, with a population reliant on rice, found no association between higher DD and lower aflatoxin exposure. Findings suggest that those who diversified their diets with groundnuts or maize increased their exposure to aflatoxins. Nevertheless, DD promotion, which brings important benefits, should continue in nutrition interventions. Focused actions to lower contamination risk in these two foods should be prioritized in nutrition strategies designed to promote DD.

Seasonal variations in serum aflatoxin levels were apparent in this study, with the highest levels of exposure seen during the dry, cool winter. This strong association between AFB1-lysine adduct concentrations and winter season is consistent with the previous literature [2, 45–47]. Higher consumption of contaminated foods can come from either increased quantity consumed after harvest and/or consumption of lower quality, more contaminated foods that had been stored for long periods of time in either the household or market. Maize and groundnuts are typically harvested between August and September when optimum conditions are optimal for growth.

| Table 2 | Maize, groundnut, and chili consumption in the past week and year. |
|---|---|
| | n | % or mean ± SD | p value log AFB1 | Mean AFB1 | SD | Geo mean AFB1 | 95% CI | p value log AFB1 | Highest AFB1 quintileb |
| Maize and/or groundnut consumption | | | | | | | | | |
| Consumption in past week | | | | | | | | | |
| None (Ref) | 1050 | 63.7 | 2.4 | 6.0 | 1.1 | 1.1–1.2 | 156 | 14.9 |
| Maize only | 49 | 3.0 | 5.7 | 13.3 | 1.8 | 1.2–2.6 ** | 14 | 28.6 |
| Groundnuts only | 518 | 31.4 | 4.4 | 10.6 | 1.8 | 1.7–2.0 *** | 147 | 28.4 |
| Both | 31 | 1.9 | 7.9 | 13.3 | 3.2 | 2.0–5.1 *** | 12 | 38.7 |
| Maize | | | | | | | | | |
| Consumption in past week | | | | | | | | | |
| No | 1568 | 95.1 | 3 | 7.9 | 1.3 | 1.3–1.4 | 303 | 19.3 |
| Yes | 80 | 4.9 | 6.5 | 13.3 | 2.2 | 1.7–3.0 *** | 26 | 32.5 |
| Frequency of consumption (times/week) | 2.4 ± 2.2 | *** |
| Consumption in past year | | | | | | | | | |
| No | 282 | 17.2 | 3.2 | 7.4 | 1.5 | 1.3–1.7 | 62 | 22.0 |
| Yes | 1362 | 82.9 | 3.2 | 8.5 | 1.4 | 1.3–1.4 | 267 | 19.6 |
| Groundnut | | | | | | | | | |
| Consumption in past week | | | | | | | | | |
| No | 1099 | 66.7 | 2.5 | 6.6 | 1.2 | 1.1–1.2 | 170 | 15.5 |
| Yes | 549 | 33.3 | 4.5 | 10.8 | 1.9 | 1.7–2.1 *** | 159 | 29.0 |
| Frequency of consumption (times/week) | 2.8 ± 2.2 | *** |
| Consumption in past year | | | | | | | | | |
| No | 45 | 2.7 | 2.3 | 4.0 | 1.2 | 0.9–1.7 | 6 | 13.3 |
| Yes | 1601 | 97.3 | 3.2 | 8.3 | 1.4 | 1.3–1.4 | 323 | 20.2 |
| Chili | | | | | | | | | |
| Consumption in past year | | | | | | | | | |
| No | 4 | 0.2 | 0.7 | 0.3 | 0.6 | 0.3–1.3 | 0 | 0.0 |
| Yes | 1642 | 99.8 | 3.2 | 8.3 | 1.4 | 1.3–1.4 | 329 | 20.0 |
| Milk | | | | | | | | | |
| Consumption in past week | | | | | | | | | |
| No | 983 | 59.7 | 3.3 | 8.9 | 1.4 | 1.3–1.4 | 189 | 19.2 |
| Yes | 665 | 40.4 | 3 | 7.2 | 1.4 | 1.3–1.5 | 21 | 20.0 |
| Frequency of consumption (times/week) | 5.5 ± 4.0 |
| Consumption in past year | | | | | | | | | |
| No | 117 | 7.1 | 2.2 | 3.5 | 1.1 | 0.9–1.3 | 19 | 16.2 |
| Yes | 1531 | 92.9 | 3.3 | 8.5 | 1.4 | 1.1–1.5* | 310 | 20.3 |

AFB1 aflatoxin B1, SD standard deviation, Geo geometric, CI confidence interval, Ref reference category

*p < 0.05; **p < 0.01; ***p < 0.001

bHighest quintile > 2.9 pg/mg. AFB1 (pg/mg) values were log-transformed before analyses

cData on weekly consumption of chilies were not available
conditions for *Aspergillus* growth prevail. Prolonged, multimonth postharvest storage and suboptimal drying and storage conditions in hot, humid areas can lead to increased aflatoxin production during winter.

This study was the first to measure the association of maize and groundnut consumption and DD with maternal aflatoxin levels in pregnant women in Nepal. Findings can be used to plan interventions aimed at lowering exposure to aflatoxin, particularly in vulnerable populations. The results are generalizable because the large sample size reflected the communities represented and women were sampled from varied sociodemographic and economic circumstances. Furthermore, the outcome variable, maternal AFB1-lys adduct concentration, was objectively measured using HPLC. The use of QR in the analysis was an important methodological contribution not found in previous research, which has mostly relied on OLS and logistic regression. Unlike OLS, QR does not assume normality or homoscedasticity of errors and is much less influenced by extreme values of serum AFB1-lys. QR produced a more nuanced picture of the effects of maize and groundnut consumption patterns and DD on maternal aflatoxin exposure.

Our study has limitations. Some of the variation observed in AFB1-lys may be explained by factors we did not account for (e.g., quantities consumed, quality of the aflatoxin-prone foods consumed, food preparation methods, or individual variation in overall xenobiotic loads) [48]. Second, the study did not measure consumption over the previous 2–3 month period that is characteristic of AFB1-lys adduct half-life in the body. Finally, due to the rolling nature of the recruitment process aflatoxin data during autumn months were not available.

Results confirmed widespread aflatoxin exposure in pregnancy and showed that consumption of maize and/or groundnut consumption are dietary contributors of aflatoxin even in areas with rice-based diets. Our findings strongly support further consideration of targeted regulatory, agricultural, and food safety interventions across the value chain and at the household level to reduce aflatoxin exposure. Aflatoxin reduction campaigns should inform pregnant women and their families of both the nutritional value of consuming maize and groundnuts and of the special precautions that should be taken when purchasing, storing, and consuming agricultural food items susceptible to aflatoxin contamination. A combination of proven practical, low-cost aflatoxin reduction techniques (e.g., removal of contaminated kernels) at the household level and market level regulation of aflatoxin-prone foods could help reduce exposure to aflatoxin in vulnerable populations.

### Table 3 Average frequencies of maize and groundnut consumption in the previous week by education, wealth, and season of measurement.

|                | n  | %  | Frequency SD | Frequency SD |
|----------------|----|----|--------------|--------------|
|                |    |    | Maize        | Groundnut    |
| **Schooling**  |    |    |              |              |
| None           | 606| 36.8| 0.1          | 0.5** 0.9 | 1.8 |
| Some primary (1–5) | 321| 19.5| 0.1          | 1.0 1.2 | 2.0 |
| Some secondary (6–10) | 577| 35.0| 0.2          | 1.0 0.8 | 1.8 |
| More than secondary (10+) | 144| 8.7 | 0.1          | 0.7 0.9 | 1.7 |
| **Wealth Index** |    |    |              |              |
| Poorest        | 328| 19.9| 0.1          | 0.4 0.7 | 1.4** |
| Poor           | 331| 20.1| 0.1          | 0.8 0.8 | 1.8 |
| Middle         | 329| 20.0| 0.1          | 0.5 1.1 | 2.0 |
| Rich           | 328| 20.0| 0.1          | 0.9 1.0 | 1.7 |
| Richest        | 332| 20.1| 0.2          | 0.9 1.1 | 2.0 |
| **Season**     |    |    |              |              |
| Nonwinter      | 937| 56.9| 0.1          | 0.6** 0.4 | 1.1*** |
| Winter         | 711| 43.1| 0.2          | 1.0 1.6 | 2.3 |

SD standard deviation

**p < 0.01; ***p < 0.001

### Table 4 Multivariate ordinary least squares and quantile regression analysis of the association between weekly maize and groundnut consumption and maternal serum aflatoxin B1-lysine adduct levels.

|                | OLS | Q10 | Q30 | Q50 | Q70 | Q90 |
|----------------|-----|-----|-----|-----|-----|-----|
| Maize consumptionb | 0.549 (0.281) | 0.091 (0.054) | 0.094 (0.041)* | 0.112 (0.051)* | 0.109 (0.048)* | 0.147 (0.111) |
| Groundnut consumptionb | 0.730 (0.121)*** | 0.037 (0.027) | 0.058 (0.016)*** | 0.085 (0.026)*** | 0.133 (0.026)*** | 0.133 (0.030)*** |
| Milk consumptionc | 0.906 (0.799) | 0.630 (0.221)** | 0.194 (0.108) | 0.230 (0.106)* | 0.173 (0.128) | 0.066 (0.244) |
| Dietary diversity score | -0.229 (0.149) | 0.064 (0.029)* | 0.004 (0.020) | 0.008 (0.018) | -0.012 (0.026) | -0.057 (0.053) |
| Winter season | 2.339 (0.430)*** | 0.313 (0.091)** | 0.460 (0.059)*** | 0.552 (0.066)*** | 0.623 (0.085)*** | 1.101 (0.130)*** |
| Model Adjusted $R^2$ | 0.0639 | 0.0539 | 0.0698 | 0.0801 | 0.1010 | 0.1367 |

Standard errors in parentheses; n = 1648

**MUAC mid-upper arm circumference, OLS ordinary least squares, Q quantile

aOLS regression

bNumber of times in past week

cConsumed in past year (yes/no)

*p < 0.05; **p < 0.01; ***p < 0.001. Models adjusted for age, education, MUAC, wealth index and Village Development Committee (VDC)
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Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Mitchell NJ, Hsu HH, Chandyo RK, Shrestha B, Bodhidatta L, Tu YK, et al. Aflatoxin exposure during the first 36 months of life was not associated with impaired growth in Nepalese children: an extension of the MAL-ED study. PLoS ONE. 2017;12:e0172124.
2. Groopman JD, Egner PA, Schulze KJ, Wu LS, Merrill R, Mehra S, et al. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B(1)-lysin albumin biomarkers. Food Chem Toxicol. 2014;74:184–9.
3. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010;31:71–82.
4. Khlangwiset P, Shephard GS, Wu F. Aflatoxins and growth impairment: a review. Crit Rev Toxicol. 2011;41:740–55.
5. Gong Y, Hounsou A, Egal S, Turner PC, Sutcliffe AE, Hall AJ, et al. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ health Perspect. 2004;112:1334–8.
6. Kimanya ME, De Meulenaer B, Tisekwa B, Ndondomo-Sigonda M, Devlieghere F, Van Camp J, et al. Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. Food Addit Contam Part A Chem Anal Control Exposure Risk Assess. 2008;25:1353–64.
7. Leong YH, Rosma A, Latiff AA, Izahh AN. Associations of serum aflatoxin B1-lysin adduct level with socio-demographic factors and aflatoxins intake from nuts and related nut products in Malaysia. Int J Hyg Environ Health. 2012;215:368–72.
8. Lombard MJ. Mycotoxin exposure and infant and young child growth in Africa: what do we know? Ann Nutr Metab. 2014;64 Suppl 2 :42–52.
9. Prandini A, Tansini G, Sigolo S, Filippi L, Laporta M, Piva G. On the occurrence of aflatoxin M1 in milk and dairy products. Food Chem Toxicol. 2009;47:984–91.
10. Moser CM, Hoffmann V. Firm heterogeneity in food safety provision: evidence from aflatoxin tests in Kenya. Washington, DC: International Food Policy Research Institute (IFPRI). 2013. https://ssrn.com/abstract=2567751.
11. Mangan N, Olsen M. Mycotoxins in feed detection and control, part II controlling risks. Cambridge, United Kingdom: Woodhead Publishing; 2004.
12. Turner PC. The molecular epidemiology of chronic aflatoxin driven impaired child growth. Scientifica: Hindawi Publishing Corporation 2013;2013:1–21.
13. Turner PC, Flannery B, Isitt C, Ali M, Pestka J. The role of biomarkers in evaluating human health concerns from fungal contaminants in food. Nutr Res Rev. 2012;25:162–79.
14. Groopman JD, Kensler TW, Wild CP. Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. Annu Rev Public Health. 2008;29:187–203.
15. Chen JG, Egner PA, Ng D, Jacobson LP, Munoz A, Zhu YR, et al. Reduced aflatoxin exposure presages decline in liver cancer mortality in an endemic region of China. Cancer Prev Res. 2013;6:1038–45.
16. Wu F, Mitchell NJ, Male D, Kensler TW. Reduced foodborne toxin exposure is a benefit of improving dietary diversity. Toxicol Sci. 2014;141:329–34.
17. Schwartzbord J, Brown D, Pape J, Verdier R, Filbert M, Wang JS. Aflatoxin–lysin adducts in Haitian patients ingesting peanut and maize products. J Hunger Environ Nutr. 2014;9:244–55.
18. Shim JS, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health 2014;36:e2014009.
19. Campbell RK, Talegawkar SA, Christian P, Leclercq SC, Khatry SK, Wu LS, et al. Evaluation of a novel single-administration food frequency questionnaire for assessing seasonally varied dietary patterns among women in rural Nepal. Ecol Food Nutr. 2015;54:314–27.
20. Vettorazzi A, López de Cerain A. Chapter 17—Mycotoxins as food carcinogens. In: Carla Viegas CP, Raquel Sabino, Susana Viegas, João Brandão, Cristina Verissimo, editor. Environmental Mycology in Public Health. United States of America: Academic Press; 2016. p. 261–98.
21. Qian G, Tang L, Wang F, Guo X, Massey ME, Williams JH, et al. Physiologically based toxicokinetics of serum aflatoxin B1-lysin adduct in F344 rats. Toxicology. 2013;303:147–51.
22. Jin Y, Hein MJ, Deddens JA, Hines CJ. Analysis of lognormally distributed exposure data with repeated measures and values below the limit of detection using SAS. Ann Occup Hyg. 2011;55:97–112.
23. FAO/FHI 360. Minimum dietary diversity for women: a guide to measurement. Rome, Italy: FAO; 2016.
24. Government of Nepal. Banke district profile. Government of Nepal: 2017; Kathmandu, Nepal. http://www.namis.gov.np/downloadfile/ Banke District Profile_SKM_1433381537.pdf.
25. Nepal Government Department of Hydrology and Meteorology Monthly Rainfall Data (1999–2018). Ministry of Energy Water Resources and Irrigation. Kathmandu, Nepal. 2018. http://moeirl.gov.np/en/.
26. Rutstein SO. Steps to constructing the new DHS Wealth Index. 2016. Rockville, MD: ICF International https://dhsprogram.com/programming/wealth-index/Steps_to_constructing_the_new_DHS_Wealth_Index.pdf.
27. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plan. 2006;21:459–68.
28. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data—or tears: an application to educational enrollments in states of India. Demography. 2001;38:115–32.
29. Ministry of Health—Nepal, New Era, ICF. Nepal demographic and health survey 2011. Kathmandu, Nepal: Ministry of Health; 2012.
30. Marrie RA, Dawson NV, Garland A. Quantile regression and restricted cubic splines are useful for exploring relationships between continuous variables. J Clin Epidemiol. 2009;62:511–7 e1.
31. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. Stat Med. 2010;29:1037–57.
32. Hoffmann V, Jones K, Leroy JL. The impact of reducing dietary aflatoxin exposure on child linear growth: a cluster randomised controlled trial in Kenya. BMJ Glob Health. 2018;3:e000983.
33. Lauer JM, Duggan CP, Ausman LM, Grifﬁths JK, Webb P, Wang JS, et al. Maternal aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda. Matern Child Nutr. 2019;15:e12701.
34. Egal S, Hounsa A, Gong YY, Turner PC, Hall AJ, et al. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. Int J Food Microbiol. 2005;104:215–24.
35. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 2004;80:1106–22.
36. Koirala P, Kumar S, Yadav BK, Premarajan KC. Occurrence of aflatoxin in some of the food and feed in Nepal. Indian J Med Sci. 2005;59:331–6.
37. Shirima CP, Kimanya ME, Kinabo JL, Routledge MN, Srey C. Wild CP, et al. Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. Mol Nutr Food Res. 2013;57:1874–81.
38. Gong YY, Cardwell K, Hounsa A, Egal S, Turner PC, Hall AJ, et al. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. BMJ. 2002;325:20–1.
39. Shuaib FM, Jolly PE, Ehiri JE, Ellis WO, Yatich NJ, Funkhouser E, et al. Socio-demographic determinants of aflatoxin B1-lysine adduct levels among pregnant women in Kumasi, Ghana. Ghana Med J. 2012;46:179–88.
40. Turner PC, Collinson AC, Cheung YB, Gong Y, Hall AJ, Prentice AM, et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. Int J Epidemiol. 2007;36:1119–25.
41. Kattel S, Chhetri R, Dhakal S. Peanut cultivation and consumption in Nepal: a social cultural perspective. Kathmandu, Nepal: Tribhuwan University; 2002.
42. Kafle P, Sedai D, Rai KP, Pokharel BB. Study on the level of aflatoxin M1 contamination in raw and processed milk marketed in Kathmandu Valley. J Food Sci Technol Nepal. 2012;7:52–56.
43. Elzupir AO, Alamer AS, Dutton MF. The occurrence of aflatoxin in rice worldwide: a review. Toxin Rev. 2015;34:37–42.
44. Hammami W, Fiori S, Al Thani R, Ali Kali N, Balmas V, Miglieli Q, et al. Fungal and aflatoxin contamination of marketed spices. Food Control. 2014;37:177–81.
45. Castelino JM, Dominguez-Salas P, Routledge MN, Prentice AM, Moore SE, Hennig BJ, et al. Seasonal and gestation stage associated differences in aflatoxin exposure in pregnant Gambian women. Tropical Med Int Health. 2014;19:348–54.
46. Wild CP. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. Food Nutr Bull. 2007;28 2 Suppl:S372–80.
47. Wild CP, Yin F, Turner PC, Chemin I, Chapot B, Mendy M, et al. Environmental and genetic determinants of aflatoxin-albumin adducts in the Gambia. Int J Cancer. 2000;86:1–7.
48. Dellaﬂoria L, Dall’Asta C. Forthcoming challenges in mycotoxins toxicology research for safer food-a need for multi-omics approach. Toxins. 2017;9:1–14.