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RESEARCH ARTICLE

Associations between aflatoxin B_1-albumin adduct levels with metabolic conditions in Guatemala: A cross-sectional study

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Abstract

Background and Aims: Metabolic conditions such as obesity, type 2 diabetes, metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD) are highly prevalent in Guatemala and increase the risk for a number of disorders, including hepatocellular carcinoma (HCC). Aflatoxin B_1 (AFB_1) levels are also notably elevated in the population and are known to be associated with HCC risk. Whether AFB_1 also contributes to the high prevalence of the metabolic disorders has not been previously examined. Therefore, the purpose of this study was to assess the association between AFB_1 and the metabolic conditions.

Methods: Four-hundred twenty-three individuals were included in the study, in which AFB_1-albumin adduct levels were measured in sera. Metabolic conditions included diabetes, obesity, central obesity, metabolic syndrome, and NAFLD. Crude and adjusted prevalence odds ratios (PORs) and 95% confidence intervals (95% CI) were estimated for the associations between the metabolic conditions and AFB_1-albumin adduct levels categorized into quartiles.

Results: The study found a significant association between AFB_1-albumin adduct levels and diabetes (Q4 vs Q1 POR = 3.74, 95%CI: 1.71-8.19; *P-trend* .003).

Abbreviations: AFB_1, aflatoxin B_1; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence intervals; FLI, fatty liver index; GGT, ϒ-glutamyl transferase; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSI, hepatic steatosis index; INCAP, Institute of Nutrition of Central America and Panama; IQR, interquartile range; LMICs, low- and middle-income countries; MetSyn, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; OTA, ochratoxin A (OTA); POR, prevalence odds ratios; TE, transient elastography.

No associations were observed between AFB_1-albumin adduct levels and the other conditions.

Conclusions: As diabetes is the metabolic condition most consistently linked to HCC, the possible association between AFB_1 exposure and diabetes may be of public health importance. Further studies are warranted to replicate the findings and examine potential mechanisms.

KEYWORDS
aflatoxin, diabetes, Guatemala, metabolic syndrome, NAFLD, obesity

1 | INTRODUCTION

Rates of metabolic conditions such as diabetes, obesity, metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD) have increased worldwide.[1-4] The global prevalence of these conditions are estimated to be 9% for diabetes,[2] 12% for obesity,[5] 25% for metabolic syndrome,[3] and 25% for NAFLD,[4] and are expected to increase further, particularly in low- and middle-income countries (LMICs).[2,3,6,7] All the conditions share a number of pathophysiological mechanisms that can lead to cardiovascular complications, chronic renal and liver diseases, cancer, and death.[8] Although obesity seems to be the main driver of most metabolic conditions, there are several other factors that could explain their rising global prevalence.[9]

Aflatoxins are naturally occurring mycotoxins produced by fungi of the Aspergillus species, predominantly, *A. flavus* and *A. parasiticus*.[10] Aspergillus contaminates maize, groundnuts, and cottonseed in warm, humid environments around the world.[11] Aflatoxin B_1 (AFB_1), the most toxicologically potent of the aflatoxins, was classified as a Group 1 human carcinogen by the International Agency for Research on Cancer in 1993.[12] AFB_1-lysine adducts of serum albumin, a biomarker of internal dose, are strongly associated with the development of hepatocellular carcinoma (HCC), and other health outcomes such as childhood stunting.[11,13-15] In Guatemala, our group previously reported the finding of high levels of serum AFB_1 among adults 40 years and older,[16] a result consistent with prior evidence of high AFB_1 levels in maize samples across the country.[17] In addition, our group has reported a high prevalence of diabetes (21.6%), obesity (30.9%), central obesity (74.3%), NAFLD (60%), and metabolic syndrome (64.2%) in the same population.[18] As these metabolic conditions are associated with increased risk of HCC, and Guatemala has the highest rate of HCC in the Western Hemisphere, we speculated that AFB_1 could also be associated with the metabolic conditions. Although an examination of AFB_1 and metabolic conditions in humans has not been previously reported, a prior animal study found that administration of AFB_1 resulted in elevated glucose and cholesterol levels, serological measures associated with the metabolic disorders of interest.[19]

Therefore, the purpose of the current study was to explore the association between AFB_1 and metabolic conditions in Guatemala. Assessing the role of prevalent environmental exposures in the development of these conditions is critical to the creation of comprehensive public health measures to reduce the burden of the metabolic disorders and AFB_1 exposure in the population.

2 | METHODS

2.1 | Study population

Four hundred sixty-one individuals were recruited between May and October 2016 from five communities located in the central and western regions of Guatemala: Chichicastenango (Quiché department), Escuintla (Escuintla), Mixco (Guatemala), San Lucas Tolimán (Sololá), and San Pablo Jocopilas (Suchitepéquez). The communities were classified as either urban (Escuintla and Mixco) or rural (Chichicastenango, San Lucas Tolimán and San Pablo Jocopilas). Study recruitment consisted of household visits. In Mixco, households were selected at random from a formal sampling frame, while a nonrandom based sampling method was used in the other four sites. Detailed information on the sampling procedures for the study has previously been published.[16] Trained study staff visited selected households and invited individuals to participate. Up to two non-genetically related persons of at least 40 years of age were recruited from each household. All participants provided written informed consent. Individuals were excluded if they were pregnant or unable to provide informed consent. The institutional review boards of both the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, USA (IRB #6877) and the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, Guatemala (IRB #053-2015) approved the study.

2.2 | Data collection

Study participants were interviewed by trained staff using structured questionnaires previously validated by INCAP. The questionnaires included questions on sociodemographic characteristics, lifestyle factors, medical history, access to healthcare, and food consumption. Height, weight, and waist circumference were measured by study staff using standardized instruments and protocols as previously described.[18] Physical activity was assessed by using the International

TABLE 1 Characteristics of the study population (n = 423)

	Median	IQR[a]
Age (years)	54	(47, 62)
BMI (kg/m^2)	27.5	(24.0, 30.9)
AFB_1 (pg/mg albumin)	8.5	(3.8, 22.3)
Sex	n	%
Women	253	(59.8)
Men	170	(40.2)
Residence		
Rural	262	(61.9)
Urban	161	(38.1)
Ethnicity, self-reported		
Non-indigenous	189	(44.8)
Indigenous	233	(55.2)
Missing	1	
Education		
<6 years	287	(67.9)
≥6 years	136	(32.1)
Household income		
<400 USD/mo.	311	(74.0)
≥400 USD/mo.	109	(26.0)
Missing	3	
Alcohol intake[b]		
Never	123	(29.1)
Former	214	(50.6)
Current	86	(20.3)
Smoking		
Never	246	(58.2)
Former	144	(34.0)
Current	33	(7.8)
Physical activity		
Low	222	(52.5)
Moderate	146	(34.5)
High	55	(13.0)
Elevated fatty liver index		
No	163	(39.4)
Yes	251	(60.6)
Missing	9	
Elevated hepatic steatosis index		
No	152	(36.5)
Yes	264	(63.5)
Missing	7	
Elevated ALT or AST levels		
No	291	(68.8)
Yes	132	(31.2)
Metabolic syndrome		
No	149	(35.5)
Yes	271	(64.5)
Missing	3	
Diabetes		
No	330	(78.6)
Yes	90	(21.4)
Missing	3	
Obesity		
No	289	(69.3)
Yes	130	(30.7)
Missing	4	
Elevated waist circumference		
No	107	(25.5)
Yes	313	(74.5)
Missing	3	
Elevated waist-to-height ratio		
No	21	(5.0)
Yes	398	(95.0)
Missing	4	

[a]Interquartile range: Q1, Q3.
[b]Low-to-moderate alcohol consumption <7 drinks per week among women and <14 drinks per week among men.

Physical Activity Questionnaire short form (IPAQ 6). The methods to categorize the different levels of physical activity have been detailed elsewhere.[18]

Blood samples were obtained by trained phlebotomists using a standard protocol. An eight-hour fast was required prior to the collection of the samples. In total, 444 individuals provided serum samples.

2.3 | Laboratory assessments

Fasting glucose was measured in plasma, while total cholesterol, triglycerides, high-density lipoprotein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ϒ-glutamyl transferase (GGT) were measured in serum. All plasma and serum analytes were determined on a Cobas c111 clinical chemistry analyzer (Roche Diagnostics).

The determination of AFB_1-lys adduct levels in serum was performed by isotope-dilution mass spectrometry. Adduct concentrations (pg/AFB_1-lys/mL serum) were normalized to total serum albumin and expressed as pg AFB_1-lys adduct/mg albumin. Details of the laboratory methods have been previously described.[16] For brevity, AFB_1-lys adducts/mg albumin is denoted as AFB_1-albumin adducts throughout the manuscript. In total, 443 samples were of sufficient volume for AFB_1 assessment.

Seropositivity for hepatitis B virus (HBV) was determined by the presence of hepatitis B virus surface antigen (HBsAg) and seropositivity for hepatitis C virus (HCV) was determined by the presence of hepatitis C virus antibodies (anti-HCV). HBsAg and anti-HCV were assessed in the Hepatitis Diagnostic Laboratory of Hannover Medical School as previously described.[20]

TABLE 2 Participants' characteristics by median levels of AFB_1 adducts in pg/mg albumin

	AFB_1-albumin adduct levels	
	Median	(IQR)[a]
Sex		
Women	7.5	(3.3, 16.5)
Men	11.4	(4.4, 33.4)
Age, years		
40 to <50	10.8	(4.3, 21.2)
50 to <60	7.7	(4.0, 24.7)
60+	8.0	(3.5, 21.7)
Residence		
Rural	14.0	(5.0, 31.3)
Urban	4.9	(2.5, 10.4)
Ethnicity		
Non-indigenous	6.2	(2.8, 12.1)
Indigenous	13.7	(4.9, 32.1)
Education		
<6 years	11.7	(4.4, 26.3)
≥6 years	5.5	(2.6, 11.9)
Household income		
<400 USD/mo.	10.4	(4.4, 25.9)
≥400 USD/mo.	4.9	(3.0, 11.6)
Alcohol intake		
Never	10.3	(4.2, 24.8)
Former	8.6	(4.1, 24.5)
Current	7.5	(2.5, 15.1)
Smoking		
Never	8.7	(3.9, 21.4)
Former	7.5	(3.4, 23.7)
Current	9.1	(5.0, 30.7)
Physical activity		
Low	8.0	(3.6, 17.8)
Moderate	7.6	(3.2, 22.4)
High	20.3	(6.6, 38.6)
BMI (kg/m^2)		
<25	11.9	(4.3, 31.6)
25.0-29.9	9.1	(3.7, 21.0)
≥30	7.2	(3.3, 15.7)
Elevated fatty liver index		
No	12.2	(4.7, 31.1)
Yes	7.3	(3.3, 15.8)
Elevated hepatic steatosis index		
No	10.2	(4.2, 33.4)
Yes	7.5	(3.6, 16.7)
Elevated ALT or AST levels		
No	8.7	(3.7, 24.8)
Yes	7.5	(3.8, 18.3)
Metabolic syndrome		
No	11.6	(3.7, 31.2)
Yes	7.8	(3.8, 16.8)
Diabetes		
No	8.0	(3.6, 21.4)
Yes	9.8	(4.2, 28.4)
Obesity		
No	9.5	(4.2, 25.1)
Yes	7.2	(3.3, 15.7)
Elevated waist circumference		
No	13.1	(4.9, 35.1)
Yes	7.8	(3.4, 18.3)
Elevated waist-to-height ratio		
No	15.8	(4.8, 38.7)
Yes	8.3	(3.6, 22.2)

[a]Interquartile range: Q1, Q3.

2.4 | Metabolic conditions definitions

The focus of the investigation was on metabolic conditions; therefore, individuals were excluded from the analysis if they were HBsAg and/or anti-HCV positive (n = 6), or if they reported high alcohol consumption levels defined by drinking ≥7 drinks/week among women, and ≥14 drinks/week among men (n = 14). As HBV, HCV and excessive alcohol consumption were exclusionary criteria, the results for fatty liver disease in the current study are referred to as NAFLD. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared (kg/m^2), with BMI ≥30 kg/m^2 considered to be obese. Abdominal obesity was defined using two measures; elevated waist circumference (≥90 cm among men and ≥80 cm among women) and elevated waist-to-height ratio (>0.50).[21] Diabetes was defined by self-report of a physician's diagnosis (86%) and/or measured fasting glucose ≥126 mg/dL (14%). Metabolic syndrome was defined as elevated waist circumference plus at least two of the following factors: serum triglycerides >150 mg/dL, low HDL (<40 mg/dL in men and <50 mg/dL in women), blood pressure >130/85 mmHg, and/or serum glucose >100 mg/dL.[22] NAFLD was estimated by several methods; the fatty liver index (FLI), the hepatic steatosis index (HSI), and by the presence of elevated levels of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST). FLI was calculated as: $(e^{0.953} \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745) / (1 + e^{0.953} \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745) \times 100$.[23] An FLI ≥60 was considered elevated. HSI was calculated as: 8 × (ALT/AST ratio) + BMI (+2, if female; + 2, if

TABLE 3 Prevalence odds ratios (POR) and 95% confidence intervals (CI) for the associations between AFB_1-albumin adduct levels (pg/mg) and the metabolic conditions

		Crude model		Adjusted model[a]	
AFB_1	Non-case/case	POR	95% CI	POR	95% CI
Diabetes					
AFB-Q1	85/20	1.00	(referent)	1.00	(referent)
AFB-Q2	84/22	1.11	(0.58, 2.15)	1.18	(0.57, 2.44)
AFB-Q3	82/22	1.14	(0.57, 2.26)	1.51	(0.73, 3.11)
AFB-Q4	79/26	1.40	(0.71, 2.76)	3.74	(1.71, 8.19)
P-value for trend			.35		.003
Metabolic syndrome					
AFB-Q1	38/67	1.00	(referent)	1.00	(referent)
AFB-Q2	29/77	1.51	(0.85, 2.68)	2.30	(1.04, 5.07)
AFB-Q3	31/73	1.34	(0.74, 2.40)	1.85	(0.84, 4.09)
AFB-Q4	51/54	0.60	(0.34, 1.05)	1.74	(0.80, 3.78)
P-value for trend			.07		.25
Obesity					
AFB-Q1	67/37	1.00	(referent)	1.00	(referent)
AFB-Q2	65/40	1.11	(0.64, 1.93)	1.14	(0.61, 2.12)
AFB-Q3	74/30	0.73	(0.41, 1.28)	0.83	(0.43, 1.59)
AFB-Q4	83/23	0.51	(0.28, 0.94)	0.92	(0.44, 1.90)
P-value for trend			.01		.61
Elevated waist circumference					
AFB-Q1	20/84	1.00	(referent)	1.00	(referent)
AFB-Q2	24/82	0.85	(0.44, 1.65)	1.04	(0.45, 2.38)
AFB-Q3	21/83	0.90	(0.48, 1.71)	1.16	(0.56, 2.40)
AFB-Q4	42/64	0.38	(0.21, 0.71)	0.91	(0.44, 1.88)
P-value for trend			.002		.84
Elevated waist-to-height ratio					
AFB-Q1	3/101	1.00	(referent)	1.00	(referent)
AFB-Q2	7/99	0.42	(0.11, 1.68)	0.71	(0.15, 3.30)
AFB-Q3	4/99	0.74	(0.16, 3.42)	1.39	(0.28, 6.87)
AFB-Q4	7/99	0.42	(0.11, 1.67)	1.01	(0.25, 4.00)
P-value for trend			.36		.69
Elevated fatty liver index					
AFB-Q1	35/69	1.00	(referent)	1.00	(referent)
AFB-Q2	31/74	1.21	(0.67, 2.18)	1.70	(0.72, 3.97)
AFB-Q3	37/63	0.76	(0.44, 1.34)	1.10	(0.51, 2.40)
AFB-Q4	60/45	0.38	(0.22, 0.68)	0.72	(0.30, 1.75)
P-value for trend			<.001		.29
Elevated hepatic steatosis index					
AFB-Q1	36/68	1.00	(referent)	1.00	(referent)
AFB-Q2	32/73	1.21	(0.67, 2.17)	1.88	(0.65, 5.48)
AFB-Q3	32/70	1.16	(0.64, 2.09)	1.66	(0.59, 4.64)
AFB-Q4	52/53	0.54	(0.30, 0.97)	1.42	(0.47, 4.26)
P-value for trend			.04		.60

(Continues)

TABLE 3 (Continued)

		Crude model		Adjusted model[a]	
AFB_1	Non-case/case	POR	95% CI	POR	95% CI
Elevated ALT or AST levels					
AFB-Q1	73/32	1.00	(referent)	1.00	(referent)
AFB-Q2	68/38	1.27	(0.73, 2.24)	1.32	(0.71, 2.47)
AFB-Q3	71/35	1.12	(0.63, 2.02)	1.13	(0.60, 2.15)
AFB-Q4	79/27	0.78	(0.43, 1.41)	0.95	(0.47, 1.94)
P-value for trend			.36		.84

[a]Adjusted for age, sex, residence, indigenous, smoking, alcohol intake, physical activity, and BMI (except for obesity, waist circumference and waist-to-height ratio > 0.50). Quartile ranges (min, max) of AFB_1 adducts in pg/mg albumin; Q1: 0.19-3.68, Q2: 3.79-8.45, Q3: 8.49-22.15, Q4: 22.34-814.82.

diabetes mellitus).[24] An HSI score >36 was considered elevated. AST levels of >41 U/L among men and >33 U/L among women were considered to be elevated, while ALT levels >40 U/L among men and >32 U/L among women were considered to be elevated. Prior research in a U.S. Hispanic/Latino population has used elevated ALT/AST levels, in the absence of viral infection or excessive alcohol intake, as a proxy measure of NAFLD.[25]

2.5 | Statistical analysis

Descriptive statistics, including medians for the continuous variables and frequencies for the categorical variables, were computed. In addition, median and interquartile ranges (IQRs) of AFB_1-albumin adducts in relation to all the study characteristics were computed. The generalized estimating equation method with the mean model based on logistic regression and an independence working correlation matrix was used to estimate the prevalence odds ratios (POR) and 95% confidence intervals (CI) for the associations between the metabolic conditions and AFB_1-albumin adduct levels by quartiles. A dose-response relation between the metabolic conditions and AFB_1-albumin adducts was calculated by scoring the quartiles as 1 through 4 and including the score as a continuous variable in the models. Multivariable-adjusted models were examined using stepwise regression and included age, sex, residence (urban, rural), indigenous (self-reported indigenous vs no indigenous), smoking (never, former, current), low-to-moderate alcohol intake (never, former, current), physical activity (low, moderate, intense) and BMI for all conditions other than obesity and the measures of central obesity. Since this is an exploratory analysis, no adjustment for multiple comparisons was done.

In addition to the main analysis, PORs and 95%CIs were estimated to assess associations between total maize and tortilla consumption with each metabolic condition.[26]

All tests of significance were two-sided. *P*-values less than .05 were considered statistically significant without adjustment for multiple comparisons as the analyses were exploratory. Statistical analyses were conducted in SAS version 9.4 (SAS Institute, Cary, North Carolina).

3 | RESULTS

Table 1 shows the characteristics of the 423 study participants. The median age was 54 years (interquartile range [IQR] 47, 62 years), 61.9% resided in rural communities and 55.2% self-identified as indigenous. A majority (74.0%) of participants had a monthly income of less than US$400 and 67.9% had completed less than 6 years of schooling. In addition, 20.3% were current drinkers of low-to-moderate amounts of alcohol, while 7.8% were current smokers. The median BMI was 27.5 kg/m^2 and 52.5% of participants reported low levels of physical activity.

The prevalence of NAFLD in the population varied depending on the index used: 60.6% of the participants had an elevated FLI, 63.5% had an elevated HSI and 31.2% had an elevated ALT or AST level. The prevalence of metabolic syndrome was 64.5% while the prevalence of diabetes was 21.4%. The anthropometric measures indicated that 30.7% of the population were obese, 74.5% had an elevated waist circumference and 95.0% had an elevated waist-to-height ratio. The median AFB_1 level was 8.5 pg/mg albumin.

Table 2 displays the median AFB_1-albumin adduct levels by participants' characteristics. The median levels were higher among men (11.4 pg/mg albumin), persons less than age 50 (10.8 pg/mg albumin), residents of rural communities (14.0 pg/mg albumin), self-reported indigenous persons (13.7 pg/mg albumin), persons with less than 6 years of education (11.7 pg/mg albumin) and persons who earned less than $400 per month (10.4 pg/mg). In addition, the median AFB_1 level was higher among individuals with high levels of physical activity (20.3 pg/mg albumin). In contrast, the median level of AFB_1 (7.2 pg/mg albumin) was lower among individuals with BMI ≥30 kg/m^2. Individuals with NAFLD (determined by elevated FLI, elevated HSI, or elevated ALT/AST), metabolic syndrome, obesity, elevated waist circumference, and elevated waist-to-height ratio also had lower median AFB_1 levels than did other persons. In contrast, the median level of AFB_1 was slightly higher among individuals with diabetes.

The associations between quartiles of AFB_1-albumin adduct levels and the metabolic conditions are shown in Table 3. AFB_1-albumin adduct levels were significantly associated with diabetes in the adjusted model (Q4 vs Q1 POR: 3.74, 95%CI: 1.71-8.19; *P-trend*

.003). In contrast, there were no significant associations between AFB_1-albumin adduct levels and NAFLD, metabolic syndrome, obesity, and the measures of central obesity in the adjusted models.

Further examination of whether the relationship between AFB_1 and diabetes differed by obesity found no significant difference (P value = .94). In addition, the examination of maize and tortilla consumption in relationship to the metabolic conditions identified no significant associations (Table S1).

4 | DISCUSSION

The current study found a potential association between AFB_1-albumin adduct levels and diabetes. No significant associations were observed, however, between AFB_1-albumin adduct levels and NAFLD, obesity, central obesity, or metabolic syndrome.

To our knowledge, this is the first study to provide evidence of an association between AFB_1 and diabetes in humans. Several animal studies, however, have demonstrated that mycotoxin exposure may play a role in the development of diabetes. For example, a recent study in female rats reported that long-term exposure to Ochratoxin A (OTA), a mycotoxin similar to AFB_1, increases glucose levels and decreases insulin levels.[27] In addition, OTA may cause damage to the Langerhans islet cells of the pancreas.[27] Similarly, a study in chicks demonstrated elevated levels of glucose after administration of intraperitoneal OTA.[28] The results are consistent with a rodent study that examined penitrem A, a potent mycotoxin elaborated by *Aspergillus* and other species, that has diabetogenic effects.[27,29] Furthermore, a study that evaluated long-term exposure to AFB_1 on the development of type 1 diabetes in mice, found decreased levels of major urinary protein 1, an indicator of increased insulin sensitivity.[30] In addition, studies have reported that mycotoxins may exert a synergistic effect with other contaminants. Specifically, a study of male rats that examined the effects of OTA and the organochlorine insecticide, endosulfan, reported elevations in glucose levels and in liver enzyme (AST, ALT) levels after administration.[31]

Why mycotoxins would cause diabetes in humans is not clear as there are limited human data. It has been suggested that AFB_1, in addition to its well-known mutagen and carcinogen effects, may also act as an endocrine disruptor.[32] For example, a study reported that AFB_1 had effects on genes (eg, CYP19A1) important in endocrine regulation in placental cells.[32] Furthermore, a population study of AFB_1, −exposed workers in a flour mill reported that AFB_1 was associated with altered levels of both gonadotropins and gonadal hormones.[33] Other studies have suggested that AFB_1 interferes with endocrine function by disrupting enzymes and substrates that are responsible for the synthesis of various hormones.[34] Endocrine disruptors are known to be associated with elevated fasting insulin, increased body mass index, and reduced cognitive and neurodevelopment.[35] In addition, there is accumulating evidence that endocrine disruptors may interfere with epigenetic, structural and functional pathways that are involved in the regulation of lipid metabolism and adipogenesis,[35] which may induce insulin resistance.

Mycotoxins could also be related to the development of diabetes by causing dysbiosis of the gut microbiome, leading to dysregulation of intestinal function and impaired immune responses.[36] Gut dysbiosis may reshape intestinal barrier functions and host metabolic and signaling pathways, which are directly or indirectly related to the insulin resistance in diabetes.[37] Several studies have reported that dysbiosis of the gut microbiome plays a role in the rapid progression of insulin resistance in diabetes.[37] A recent rodent study demonstrated disruption of the gut microbial metabolism after 2-weeks of oral AFB_1 exposure.[38] A similar study among mice demonstrated that AFB_1 could alter the gut microbiome in a dose-response manner.[39] The authors suggested that AFB_1 can induce adverse changes of the community structure of gut microbiota and significant disruption of multiple metabolic pathways that play a role in gluconeogenesis, the Krebs cycle, and lactic acid production.[38]

It is possible that AFB_1 in the current analysis was simply acting as a proxy variable for maize or tortilla consumption. However, this hypothesis is not supported by the lack of significant findings in the maize-diabetes and tortilla-diabetes analyses. The results suggest that AFB_1 is potentially associated with diabetes, independent of the high level of maize consumption.

In contrast to the findings of the current study, it has been previously suggested that exposure to AFB_1 could lead to hepatic steatosis and steatohepatitis.[34] A number of animal studies have demonstrated parenchymal changes including fatty liver, as well as changes in size and morphology of the liver after consumption of AFB_1.[40,41] A study that administered aflatoxin (1 mg/kg of feed) to chickens found the characteristic effects of aflatoxicosis, including yellow liver, became evident after 56 days on study.[40] Another study that evaluated the effects of AFB_1 and AFB_2 contaminated corn on hepatic function of chickens, found the development of pathological lesions in the liver, changes in serum biochemical parameters (eg, ALT, AST, GGT), and damage to hepatic antioxidant function.[41] It is possible, of course, that the current study was unable to detect an AFB_1-NAFLD association due to the use of proxy measures of NAFLD rather than clinical measures such as imaging or liver biopsy. None of the metabolic conditions, other than diabetes, were diagnosed by a physician in this study. Moreover, mechanisms underlying conditions such as NAFLD are not fully understood, and it is likely that hidden mechanisms will be discovered in the near future.[42]

It has been proposed that the nuclear factor erythroid 2 p45-related factor 2 (NRF2) in response to oxidative stress, activates the expression and production of antioxidant enzymes leading to the reduction of aflatoxin levels.[10] Obese individuals exhibit increases in systemic oxidative stress that activate NRF2, which upregulates genes encoding important cytoprotective enzymes, including the glutathione S-transferases (GST).[43] Thus, it is possible that GSTs divert activated aflatoxin away from albumin binding. This is not implying that being obese is "protective," but it is potentially pointing to a change in the underlying metabolic condition that could affect the biomarker level. It is likely that obese middle-aged adults have been obese for a number of years, hence their obesity would have a long-term impact on metabolism whereas diabetics may be a more recent and shorter-term "exposure." Finally, while speculative,

since aflatoxins have been demonstrated to partition into fat such as found in the fat layer in milk, it is possible that obese individuals sequester some of the absorbed aflatoxin which would result in diminished bioavailability for metabolic conversion to albumin adducts.[44] This sequestration in body fat has been demonstrated with other contaminants. For example, a study that measured changes in serum concentrations of lipophilic persistent organic pollutants (POPs) in individuals who underwent bariatric surgery, found an increase in POP serum concentration of POP previously sequestered in adipose tissue. The authors highlighted the potential release and redistribution of POPs after a large and rapid weight loss to other lipid-rich organ such as the brain, kidney, and liver.[45]

It is noteworthy to mention that in risk assessment there is the assumption that there is no threshold level that constitutes a "safe" or "normal" level of exposure. AFB_1-lys adducts are biomarkers of internal dose that reflect the formation of mutagenic AFB_1-DNA adducts and the risk of liver cancer and other health outcomes increase with the level of aflatoxin exposure.[16]

The strengths of the current study include the use of a robust biomarker of AFB_1 exposure, and the use of comprehensive questionnaires that permitted the adjustment for important covariates, including sociodemographic, lifestyle, and clinical factors. The study also had some limitations. The cross-sectional design precluded the examination of a temporal relationship between AFB_1 and the metabolic conditions. In addition, AFB_1 biomarker levels were determined in a single sample, which may not have accurately reflected cumulative AFB_1 exposure over time. However, it is unlikely that AFB_1 exposure varied greatly over time, as maize is the most important dietary staple in Guatemala and is the crop most likely to be contaminated by AFB_1.[17] Another limitation is that liver fat indices, rather than clinical measures were used to estimate NAFLD, and the indices are not as specific or sensitive as other diagnostic tools such as magnetic resonance imaging, ultrasound, or transient elastography (TE). The performance of the indices, however, has been validated previously in other populations. Moreover, our group recently found good agreement between NAFLD by liver indices (FLI and HSI) and NAFLD by TE in the U.S. National Health and Nutrition Examination Survey.[46] The percent agreement for FLI and TE was 75.1% and for HSI and TE was 74.3%. In population studies where imaging of participants is not feasible, the use of FLI and HSI are reasonable substitutes to assess NAFLD, as the indices can be calculated easily and are based on affordable tests.

In conclusion, the current study found that AFB_1 was potentially associated with diabetes. Underlying mechanisms for the association are not fully elucidated, thus, further studies are warranted to confirm the findings and explain potential mechanisms.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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All authors have read and approved the final version of the manuscript.

Christian S. Alvarez, the corresponding author, had full access to all the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author: Christian S. Alvarez, affirms that this manuscript is honest, accurate, and transparent account of the study being reported; no aspects of the study have been omitted; any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Approval for the present work was obtained from both the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, USA (IRB #6877) and the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City, Guatemala (IRB #053-2015). All participants provided informed consent.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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