Published in final edited form as:

Mol Nutr Food Res. 2016 March ; 60(3): 511–518. doi:10.1002/mnfr.201500382.

Dietary exposure to aflatoxin and micronutrient status among young children from Guinea

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Abstract

Scope—Aflatoxin exposure coincides with micronutrient deficiencies in developing countries. Animal feeding studies have postulated that aflatoxin exposure may be exacerbating micronutrient deficiencies. Evidence available in human subjects is limited and inconsistent. The aim of the study was to investigate the relationship between aflatoxin exposure and micronutrient status among young Guinean children.

Method and results—A total of 305 children (28.8 ± 8.4 months) were recruited at groundnut harvest (rainy season), of which 288 were followed up 6 months later post-harvest (dry season). Blood samples were collected at each visit. Aflatoxin-albumin adduct levels were measured by ELISA. Vitamin A, vitamin E and β -carotene concentrations were measured using HPLC methods. Zinc was measured by atomic absorption spectroscopy. Aflatoxin exposure and micronutrient deficiencies were prevalent in this population and were influenced by season, with levels increasing between harvest and post-harvest. At harvest, children in the highest aflatoxin exposure group, compared to the lowest, were 1.98 (95% CI: 1.00, 3.92) and 3.56 (95% CI: 1.13, 11.15) times more likely to be zinc and vitamin A deficient.

Conclusion—Although children with high aflatoxin exposure levels were more likely to be zinc and vitamin A deficient, further research is necessary to determine a cause and effect relationship.

Keywords

Aflatoxin; biomarker; children; micronutrient deficiency; seasonal variations

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YYG contributed to the original study design and implementation. SW and GC have contributed to data analysis. All authors contributed to the manuscript writing, editing and approving the final article.

The authors declare no conflict of interest.

1. Introduction

Micronutrient deficiency is a major undernutrition issue affecting young children from developing countries. In 2011 it was estimated that 45% of global deaths of children under the age of 5 years old were attributed to micronutrient deficiency, alongside foetal growth restriction, stunting, wasting and suboptimum breastfeeding [1]. The main micronutrient deficiencies of public health concern among young children include vitamin A and zinc, which have been associated with child mortality [1], and iron and iodine, which are thought to be associated with impaired cognitive development [2-5]. Africa appears to have high prevalence of deficiencies in these main micronutrients [6-9], with the prevalence of vitamin A deficiency (serum retinol <0.70 µmol/L) in children under 5 years reported as 41.6% [6].

Micronutrient deficiency is not merely a result of inadequate dietary intake or immune dysfunction, but rather a consequence of a more complex array of social, economic and environmental underlying determinants including: poverty, food insecurity and limited accessibility to a safe and hygienic environment [1]. It is fundamental that these underlying determinants of micronutrient deficiencies and other undernutrition related issues are recognized and addressed, so that interventions targeting the immediate causes, such as dietary intake, will be more effective.

Aflatoxin is a toxic mycotoxin that frequently affects dietary staples, specifically groundnuts and maize in sub-Saharan Africa. High prevalence of aflatoxin exposure, which occurs throughout the life course [10-11], coincides with the high prevalence of micronutrient deficiencies in developing countries, specifically sub-Saharan Africa [6-9]. Chronic exposure to aflatoxin through dietary intake has been previously associated with immune suppression [12-14] and stunted child growth [15-17]. It has been postulated that aflatoxin exposure may impair gut permeability, raise the susceptibility to infection (e.g. those causing diarrhoea), lower nutrient bioavailability and subsequently be an underlying determinant of micronutrient deficiency [18].

To date, however, most of the evidence supporting the hypothesis that aflatoxin exposure is an underlying determinant of micronutrient deficiency is from animal feeding studies [19]. The limited evidence in human subjects, all being conducted in West Africa, is somewhat inconsistent. Some studies have reported an inverse relationship, in that increased aflatoxin exposure, measured by the aflatoxin-albumin adduct (AF-alb) biomarker, was associated with lower vitamin A concentrations in adult subjects [20-21]; whereas some studies in children showed no association between aflatoxin exposure and vitamin A concentrations [12-16]. Other micronutrients investigated include zinc, which showed no association with aflatoxin exposure in a cohort of young children from Benin [16] or in a young cohort of Gambian children [12]; vitamin C concentrations, which were inversely correlated with aflatoxin exposure in young children from Gambia [12] and vitamin E, which also showed an inverse relationship with aflatoxin exposure in adults from Ghana [20].

Early childhood, especially during the weaning phase, is considered to be a critical period for child growth and development with increased risk of micronutrient deficiency and aflatoxin exposure. Further exploration of the relationship between aflatoxin exposure and

micronutrient deficiency in young children is warranted to help make decisions if aflatoxin exposure should be considered in nutrition intervention programs. This study was, therefore, conducted to examine the relationship between dietary exposure to aflatoxin, measured by the AF-alb biomarker, and micronutrient deficiency (vitamin A, β -carotene, vitamin E and zinc) among young children from Guinea. In West Africa aflatoxin exposure and micronutrient concentrations are typically influenced by season; therefore, measurements were taken at harvest (the rainy season) and 6 months later at post-harvest (the dry season).

2. Methods

Participants and study surveys

Children (n=305) from subsistence farming households, aged between 10 and 46 months, were randomly recruited from 16 villages located within the region of Kindia, West Guinea, an area where groundnuts are a dietary staple and aflatoxin exposure is high [22–26]. The children were recruited at the groundnut harvest (October or November 2005), at the end of the rainy season, and were followed up approximately 6 months post-harvest towards the end of the dry season (May 2006). Ethical approval was obtained from the Guinea National Ethics Review Committee and the London School of Hygiene and Tropical Medicine (ethics no. 3093). Written informed consent was obtained from each mother to enroll her child into the study.

Trained field workers collected socio-demographic information (age, gender, ethnicity, socio-economic status) at baseline by interviewing the children's mothers in local language. To determine socioeconomic status (SES) a weighted score based on house type (materials used for floor, roof and walls) was calculated. Five groups were generated, where 1 is the lowest SES and 5 is the highest SES. A simple dietary questionnaire was distributed at each visit to the mothers to ascertain their child's groundnut consumption frequency in the previous week and current breastfeeding status (partially or fully weaned – collected at harvest only).

At each visit (harvest and post-harvest) a qualified nurse collected a 5 ml blood sample from the children in the local health center. The samples were immediately centrifuged, followed by separation into plasma, then stored as aliquots at -20°C before shipped on dry ice to the University of Leeds for laboratory analysis.

Aflatoxin-albumin adduct analysis

A competitive ELISA method, as described previously [27], was used to measure AF-alb concentrations in 250 μ l serum samples. In brief, the method involved albumin extraction and digestion, purification using Sep-Pak C18 cartridges, followed by a competitive ELISA. For the purposes of quality control, three positive and one negative control samples were analyzed along with each batch of samples. All samples were analyzed in triplicate and repeated on two separate days. The coefficient of variation had to be less than 25% between the repeats for the results to be accepted. The limit of detection of the assay was 3 pg/mg albumin. A value of 1.5 pg/mg albumin was assigned to samples with AF-alb levels below this limit.

Micronutrient analysis

Following hexane extraction of 200 µl plasma, a reversed phase HPLC method [28, 29] was used to analyze vitamins A (retinol), E (α-tocopherol) and β-carotene concentrations. Zinc was measured by atomic absorption spectroscopy and analyses were conducted in the Laboratory of the Leeds General Infirmary following standard protocols.

Statistical analysis

All analyses were performed using SPSS for Windows version 21.0 (SPSS Inc, Chicago, IL). AF-alb data was skewed and log transformed for analysis. The differences in AF-alb concentrations according to gender, breastfeeding status (at harvest only) and SES were examined using independent samples t-test or ANOVA, and results are presented as geometric means and 95% confidence intervals. Micronutrient concentrations were not normally distributed and transforming the data did not make a difference; therefore, data are presented as median and inter-quartile range (IQ). To determine the differences in micronutrient concentrations according to gender, breastfeeding status (at harvest only) and SES, non-parametric tests (Mann Whitney U test and Kruskal Wallis test) were used. To determine the quantity of children deficient in vitamin A (retinol), vitamin E (α-tocopherol) and zinc, cut offs of < 0.70 µmol/L [6], < 11.6 µmol/L [30] and < 9.9 µmol/L [31] were used, respectively. There are no recognized cut offs for β-carotene deficiency.

Paired-samples t-tests were used to investigate the seasonal differences between AF-alb concentrations measured at harvest (the rainy season) and at post-harvest (the dry season). For the seasonal differences in micronutrient concentrations, Wilcoxon signed-rank tests were used, and micronutrient deficiencies, McNemars tests were used. Only the children that had corresponding AF-alb levels and micronutrient concentrations at each time point (harvest vs. post-harvest) were included in the analyses.

Participants were subdivided according to quartiles (Q) of aflatoxin exposure at harvest and at post-harvest. The following AF-alb (pg/mg) cut-offs were used to define the quartiles at harvest: Q1, 5.26; Q2, 5.27–11.72; Q3, 11.73–25.04 and Q4, 25.05+, and at post-harvest: Q1, 10.08; Q2, 10.09–18.28; Q3, 18.29–27.60 and Q4, 27.61+. Logistic regression was undertaken and odd ratios calculated to determine the occurrence of having a micronutrient deficiency (vitamin A, vitamin E and zinc) according to the level of aflatoxin exposure (quartiles) at each time point. As there are no recognized cut offs for β-carotene deficiency, median split was used to identify a low and high group. Adjustments were made for potential confounding factors including: age, gender, SES and breastfeeding status (at harvest only).

3. Results

Participants

Table 1 summarizes the baseline characteristics of the children. A total of 305 children (50.5% males) with a mean age of 28.8 ± 8.4 months were recruited at the groundnut harvest. Six months later post-harvest, 288 of these children (49.7% males) were followed up (mean age 35.6 ± 8.2 months), equating to a 5.6% (n=17) loss to follow up rate. Absence

at blood collection as well as death were the reasons for non-participation at the 6 month time point. Forty-three percent of the sample were from medium SES households. At harvest, 22% (67/305) of the children were consuming breast milk (partially breastfed). The children consuming breast milk were significantly younger than fully weaned children (22.8 ± 9.2 vs. 30.4 ± 7.3 , $P < 0.001$). Groundnut was consumed daily by 75% (225/300) of the children at harvest, contrasting to 41% (116/286) at post-harvest.

Aflatoxin exposure

Of the blood samples collected at harvest ($n=305$) and post-harvest ($n=288$), 88.2% and 93.4% had detectable AF-alb concentrations, respectively. The geometric mean AF-alb concentration of the sample ($n=305$) at harvest (baseline) was 12.1 pg/mg (95% CI: 10.9, 14.7). Table 2 shows the seasonal differences in aflatoxin exposure concentrations including only the children that had AF-alb levels measured at both time points ($n=288$). The geometric mean AF-alb concentration at post-harvest was significantly higher than at harvest (16.3 pg/mg, 95% CI: 14.4, 18.5 vs. 12.7 pg/mg, 95% CI: 10.9, 14.7; $P = 0.009$). There were no gender differences in AF-alb levels at harvest or at post-harvest ($P > 0.05$). Fully weaned children, compared with partially breastfed children, had a significantly higher geometric mean AF-alb concentration at harvest (14.0 pg/mg, 95% CI: 11.9, 16.4 vs. 7.2 pg/mg, 95% CI: 5.3, 9.9; $P < 0.001$). AF-alb levels did not differ among the SES groups at harvest, but did differ significantly at post-harvest. Children in SES 2 had a significantly lower geometric mean AF-alb concentration than children in SES 1 and SES 5 (7.6 pg/mg, 95% CI: 3.8, 15.1 vs. 20.1 pg/mg, 95% CI: 16.3, 24.8 and 18.8 pg/mg, 95% CI: 13.6, 25.7; $P < 0.05$).

Micronutrient deficiency

Due to blood sampling difficulty for the very young children, there was not a sufficient amount of blood collected from some children to measure micronutrient concentrations. At the two time points, harvest and post-harvest, vitamin A and E concentrations were measured in 229 and 99 plasma samples; zinc concentrations were measured in 289 and 160 samples and β -carotene concentrations were measured in 217 and 90 samples, respectively. Median (IQ) concentrations of zinc ($n=289$), vitamin A ($n=229$), vitamin E ($n=229$) and β -carotene ($n=217$) at harvest (baseline) were: 9.6 $\mu\text{mol/L}$ (8.0-11.1), 0.52 $\mu\text{mol/L}$ (0.35-0.65), 9.4 $\mu\text{mol/L}$ (1.0-14.7) and 0.68 $\mu\text{mol/L}$ (0.26, 1.45), respectively. At harvest (baseline), 55% (159/289) were classified as zinc deficient, 81.2% (186/229) were deficient in vitamin A and 58.5% (134/229) were deficient in vitamin E. Table 2 shows the seasonal differences in micronutrient concentrations and status, including only the children that had measurements at both time points. Vitamin A ($n=76$), vitamin E ($n=76$) and β -carotene ($n=68$) median concentrations increased significantly over the 6 month time period from harvest to post-harvest ($P < 0.001$); whereas zinc concentrations ($n=153$) decreased ($P = 0.008$). Correspondingly, fewer children were deficient in vitamin A (60.5% vs. 78.9%, $P < 0.001$) and vitamin E (26.3% vs. 44.7%, $P = 0.001$) at post-harvest than at harvest; while more children were deficient in zinc at post-harvest than at harvest (75.2% vs. 57.5%, $P = 0.001$).

At harvest, females had a significantly higher median vitamin A concentration than males (0.57 $\mu\text{mol/L}$, IQ: 0.41-0.70 vs. 0.47 $\mu\text{mol/L}$, IQ: 0.31-0.61; $P = 0.007$). Fully weaned children had a significantly higher median vitamin A concentration than partially breastfed

children at harvest (0.53 $\mu\text{mol/L}$, IQ: 0.39-0.67 vs. 0.45 $\mu\text{mol/L}$, IQ: 0.28-0.60; $P < 0.047$). Also at harvest, children in SES 2 had a higher median vitamin E concentration than children in SES 4 (15.4 $\mu\text{mol/L}$, IQ: 7.4-20.5 vs. 4.1 $\mu\text{mol/L}$, IQ: 1.0-12.8). At post-harvest, there were no significant differences in the micronutrient concentrations according to gender or SES.

The relationship between aflatoxin exposure and micronutrient deficiency

Table 3 shows the odds ratio of having a micronutrient deficiency by increasing level of aflatoxin exposure, where Q4 is the highest exposure group and Q1 is the lowest (reference category). Geometric mean (95% CI) AF-alb concentrations (pg/mg) of each quartile group at harvest were: Q1, 2.6 (2.3, 2.9); Q2, 7.8 (7.4, 8.2); Q3, 16.2 (15.5, 16.9) and Q4, 67.3 (57.4, 79.0), and at post-harvest: Q1, 4.0 (3.4, 4.7); Q2, 14.4 (13.9, 15.0); Q3, 21.6 (20.9, 22.2) and Q4, 57.1 (48.4, 67.4). At harvest, children in the highest aflatoxin exposure group (Q4), compared to the lowest (Q1) were 1.98 (95% CI: 1.00, 3.92) and 3.56 (95% CI: 1.13, 11.15) times more likely to be zinc and vitamin A deficient, respectively, after adjusting for age, sex, SES and breastfeeding status. Participants in the second lowest aflatoxin exposed group (Q2) were 2.05 (95% CI: 1.03, 4.07) times more likely to be zinc deficient than the lowest exposed aflatoxin group (Q1). There were no associations between increasing level of aflatoxin exposure and micronutrient status at post-harvest.

4. Discussion

Aflatoxin exposure and micronutrient deficiency in young children from Guinea

Aflatoxin exposure and micronutrient deficiency are both highly prevalent in this population of young children from Guinea. Of the sample, 88.2% and 93.4% had detectable AF-alb concentrations at harvest and post-harvest, respectively. These prevalence rates, which have not been reported for Guinean children aged between 10 and 46 months, are somewhat similar to those previously reported for Guinean adults [23] and children aged 2 to 5 years [26]. Aflatoxin is typically detected in > 95% of blood samples collected from West African populations [10].

Young children from West Africa, specifically those weaning onto family foods, are seen as a vulnerable population group in terms of aflatoxin exposure [32]. For instance, young children tend to have higher metabolic rates owing to growth and development; hence, food consumption levels per kg body weight are higher relative to adults, making them more susceptible to the detrimental effects of toxin exposure [33]. Furthermore, weaning foods in West Africa are typically cereal and legume based, both of which are susceptible to aflatoxin contamination. Groundnut, a type of legume, has been previously shown to be a source of aflatoxin contamination in the Guinean diet [25], and findings from this study showed that it is a typical weaning food, as 75% of the children consumed groundnut daily at harvest.

Although breast milk is considered a pathway for aflatoxin exposure in young infants [32], breastfeeding appears to be a period when aflatoxin exposure is low [11], and this was evident in the current study. The children who were partially breastfed, compared to those fully weaned, had lower aflatoxin concentrations. This relationship, which has not been

previously investigated in Guinean children, may be attributable to the 8 month age difference observed between the breastfed and the fully weaned children (mean 22.8 ± 9.2 months vs. 30.4 ± 7.3 ; $P < 0.001$). As children get older their food intake increases, and consequently, so does their level of aflatoxin exposure [32]. The WHO recommends exclusive breastfeeding up to 6 months, with partial breastfeeding alongside complementary feeding for an additional 18 months [34]. In developing countries breastfeeding is often discontinued early, which could lead to high levels of aflatoxin exposure at a young age. Thus, following the recommendations for optimum breastfeeding is essential, not only to help prevent typical nutritional disorders, such as protein energy malnutrition and micronutrient deficiency, but also to reduce the level of aflatoxin exposure.

In the current sample over 80% of children were deficient in vitamin A, 55% were deficient in zinc and 59% were deficient in vitamin E at the groundnut harvest season. These micronutrient deficiency rates are alarmingly high, especially vitamin A deficiency that appears much higher than the WHO's [6] estimate of 45.8% for preschool children (<5 years) from Guinea, using the same cut off ($<0.70 \mu\text{mol/L}$). The dissimilarity observed, however, is likely a result of the different methods used to measure vitamin A deficiency. There was no country specific data on vitamin A deficiency for Guinea; instead the WHO used regression-based estimates, which is an arbitrary method. The current study measured retinol concentrations in plasma samples, which is considered a more objective assessment to determine nutritional status. Nevertheless, both results highlight the magnitude of vitamin A deficiency in this population group and the challenges it presents in relation to public health.

Inadequate food intake, lack of diet diversity and higher nutritional requirements during rapid growth are likely contributing factors of micronutrient deficiencies in this population group. Mass supplementation and fortification of staple foods are some of the dietary intervention methods used to help reduce the burden of micronutrient deficiencies in developing countries, and they have shown some success [35]. Micronutrient deficiency, however, is a multifactorial issue; therefore, targeting dietary intake alone without addressing the underlying determinants including poverty, infection, food insecurity and safety, will not effectively reduce prevalence and its associated health burden.

Seasonal variations in aflatoxin exposure and micronutrient concentrations

It is well established that aflatoxin contamination encompasses a seasonal pattern. Contamination levels typically increase during post-harvest storage (dry season), owing to hot and humid conditions that can promote fungal growth and toxin production [36]. Seasonal variations in aflatoxin exposure were apparent in this study, with lower concentrations observed at harvest (rainy season) compared with post-harvest (dry season). The mean seasonal difference in AF-alb concentrations, however, was marginal ($\sim 4\text{pg/mg}$) compared with seasonal differences observed in other studies conducted in West Africa, some of which have reported exposure levels twice as high in the dry season than the rainy season [25, 37-39].

Vitamin A, β -carotene and vitamin E concentrations followed a similar seasonal pattern to aflatoxin exposure; plasma levels increased modestly from harvest (rainy season) to post-

harvest (dry season). In West Africa, especially among rural populations, nutritional status is typically worse during the rainy season. A likely reason for this is that stocks of the previous year's harvest are likely to have dwindled [40]. Food is more abundant during the dry season, especially tropical fruit such as mangoes, which are good sources of vitamin A and β -carotene.

Conversely, zinc concentrations exhibited a different seasonal trend, with levels decreasing modestly over the 6 months from harvest to post-harvest. This could be reflective of the availability of leafy green vegetables during the rainy season, which is a good source of zinc. Without dietary intake data, however, it is difficult to establish the exact food sources and dietary patterns that correspond with the plasma micronutrient and AF-alb concentrations observed at the two time points (harvest and post-harvest) in this population group. Thus, further research is warranted to determine the seasonal dietary pattern of these Guinean children.

The relationship between aflatoxin exposure and micronutrient deficiency

It has been proposed that aflatoxin exposure mediates intestinal damage, reducing micronutrient absorption [18-41]; although, this has not been demonstrated empirically. Smith et al., [41] investigated the pathways by which exposure to mycotoxins can result in faltered growth in children by reviewing evidence from animal and human studies. They speculated that aflatoxin exposure may lead to inhibition of protein synthesis, which may promote intestinal damage, resulting in reduced absorption capacity of essential nutrients and impaired intestinal barrier function.

In the current study at harvest children in the highest aflatoxin exposure quartile group, compared to the lowest quartile group, were 2 times and 3.6 times more likely to be zinc and vitamin A deficient. Although these relationships are statistically significant after adjusting for age, gender, breastfeeding status (weaning) and SES, the strength of associations are weak. The results from this study, however, are comparable with studies also conducted in West Africa, but with adult subjects. For example, Tang et al. [20] found a significant negative correlation between AF-alb levels and vitamin A ($r = -0.110$, $P = 0.013$) in adults from Ghana. Another study [21] also conducted in Ghana found that adults with high aflatoxin exposure (AF-alb > 0.80 pmol/mg) compared to those with low aflatoxin exposure, were more likely to be deficient in vitamin A (OR 2.61, 95% CI: 1.03-6.58). In contrast, the current study found no association between aflatoxin exposure and vitamin E; while the aforementioned study by Tang et al. [20] found an inverse relationship ($r = -0.149$, $P < 0.001$). Furthermore, two studies of children found no correlations between aflatoxin exposure and vitamin A [12, 16] or zinc [12, 16]. Animal studies have found significant associations between aflatoxin exposure and low vitamin A concentrations, specifically in broiler chickens [42, 43], and low zinc concentrations found in piglets exposed *in utero* to aflatoxin B1 and G1 [44]. This conflicting evidence from previous research, and the fact that the strength of the significant associations observed in the current study were weak, makes it difficult to draw valid conclusions regarding this complex relationship between aflatoxin exposure and micronutrient deficiency. Thus further research is necessary to help establish if there is a cause and effect relationship.

The relationship between aflatoxin exposure and micronutrient deficiency was not significant during the post-harvest season. There were, however, fewer blood samples available for micronutrient analysis at post-harvest (the dry season), which would have reduced the power to detect significant associations. A larger sample size would be required to effectively assess the relationship between aflatoxin exposure and micronutrient deficiency at post-harvest to exclude the possibility that a type 2 error occurred in this analysis.

Strengths and limitations

The main strength of the current study is the use of objective measurements for assessing the level of aflatoxin exposure and micronutrient concentrations in plasma. Self-reported dietary intake, for instance, is subject to social desirability and recall biases that can lead to under reporting of micronutrient intake. Furthermore, aflatoxin contamination in food is heterogeneous in nature, which could result in unreliable estimates of exposure. The fact that there was an insufficient amount of blood collected from some children to measure micronutrient concentrations, owing to blood sampling difficulties in the very young children, is a major limitation of this study. A consequence of this missing micronutrient data, during the analysis, is the reduced power to detect significant associations between aflatoxin exposure and micronutrient concentrations, especially at the post-harvest visit. Another limitation is that this was an observational study and therefore the relationships observed may or may not be causal. Vitamin A, zinc, iron and iodine deficiency are the four main micronutrient deficiencies of public health concern in developing countries such as Guinea. This study, however, did not measure iron and iodine concentrations. Future research is warranted to explore the overall impact of aflatoxin exposure on these specific micronutrients.

Conclusion

Aflatoxin exposure and micronutrient deficiency were highly prevalent in this sample of young children of weaning age from Guinea, with modest seasonal variations apparent. There were significant relationships observed between aflatoxin exposure and zinc and vitamin A deficiency at harvest; although the strength of associations were weak. No relationships were observed at post-harvest; however, the study's power was reduced owing to reduced sample size at this time point. The limited and inconsistent evidence supporting the hypothesis that aflatoxin exposure is a possible contributing factor for micronutrient deficiencies warrants further investigation to determine a cause and effect relationship.

Acknowledgments

The authors acknowledge financial support from the Wellcome Trust, and from NIEHS, USA grant no. ES06052. We thank Dr Christopher Wild and Prof. Andrew Hall for their great contribution to the project design and delivery. We acknowledge the support from the field workers and the participants without whom the study would not be possible.

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Abbreviations

AF-alb	aflatoxin-albumin adducts
Q	quartile
SES	socio-economic status

Table 1
Baseline (at harvest) characteristics

	Number (%) unless other stated
N	305
Age (Mean $\pm$ SD)	28.8 $\pm$ 8.4
Gender	
Male	154 (50.5)
Female	151 (49.5)
SES^a	
1	72 (23.6)
2	17 (5.6)
3	131 (43)
4	38 (12.5)
5	47 (15.4)
Breastfeeding status	
No	238 (78)
Yes (partially) ^b	67 (22)
Groundnut consumption	
< 7 days	75 (25)
7 days	225 (75)

^aSES determined by a weighted score based on house type (material used for floor, roof and walls), 1 is the lowest SES and 5 is the highest SES.

^bChildren consuming breast milk and solid food.

Table 2
Seasonal differences in aflatoxin exposure, micronutrient concentrations and deficiency

	No. of paired samples	Harvest	Post-harvest	P value
AF-alb (pg/mg)^a	288	12.7 (10.9, 14.7)	16.3 (14.4, 18.5)	0.009
Zinc (μmol/l)^b	153	9.6 (8.2-11.0)	8.7 (7.9-9.9)	0.008
Zinc deficiency n(%)^c				
No		65 (42.5)	38 (24.8)	
Yes		88 (57.5)	115 (75.2)	0.001
Vitamin A (μmol/l)^b	76	0.58 (0.42-0.66)	0.65 (0.54-0.79)	<0.001
Vitamin A deficiency n(%)^c				
No		16 (21.1)	30 (39.5)	
Yes		60 (78.9)	46 (60.5)	<0.001
Vitamin E (μmol/l)^b	76	12.2 (8.3-15.1)	14.4 (10.9-18.4)	<0.001
Vitamin E deficiency n(%)^c				
No		42 (55.3)	56 (73.7)	
Yes		34 (44.7)	20 (26.3)	0.001
β-carotene (μmol/l)^b	68	1.79 (1.13-2.48)	2.17 (1.44-2.82)	<0.001

^a AF-alb values are presented as geometric mean (95% CI) and seasonal differences are analyzed using paired samples t-test.

^b Micronutrient concentrations are presented as median (IQ) and seasonal differences are analyzed using Wilcoxon signed rank test.

^c Micronutrient deficiencies (yes/ no) are presented as frequencies (%) and seasonal differences are analyzed using McNemers test. Zinc deficiency, <9.9 μmol/L; vitamin A deficiency, <0.70 μmol/L and vitamin E deficiency, <11.6 μmol/L.

Table 3
Cross-sectional relationships between aflatoxin exposure and micronutrient deficiency (odd ratios 95% CI)

Micronutrient deficiency	Sample size	Q1	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)
Harvest					
Zinc	289	Crude	Ref	1.90 (0.98, 3.67)	2.02 (1.04, 3.94) ^a
		Adjusted	Ref	1.74 (0.89, 3.40)	1.98 (1.00, 3.92) ^a
Vitamin A	229	Crude	Ref	1.15 (0.47, 2.83)	2.78 (0.96, 8.02)
		Adjusted	Ref	1.57 (0.60, 4.11)	3.56 (1.13, 11.15) ^a
Vitamin E	229	Crude	Ref	1.05 (0.50, 2.22)	1.66 (0.78, 3.51)
		Adjusted	Ref	1.11 (0.51, 2.43)	1.98 (0.89, 4.42)
β-Carotene	217	Crude	Ref	0.89 (0.42, 1.92)	1.21 (0.57, 2.55)
		Adjusted	Ref	0.99 (0.45, 2.19)	1.28 (0.59, 2.80)
Post-harvest					
Zinc	160	Crude	Ref	1.61 (0.62, 4.16)	1.17 (0.44, 3.09)
		Adjusted	Ref	1.79 (0.65, 4.94)	1.63 (0.56, 4.75)
Vitamin A	99	Crude	Ref	1.20 (0.37, 3.86)	1.20 (0.37, 3.86)
		Adjusted	Ref	0.99 (0.28, 3.51)	1.12 (0.32, 3.87)
Vitamin E	99	Crude	Ref	1.00 (0.27, 3.66)	1.23 (0.35, 4.37)
		Adjusted	Ref	0.80 (0.19, 3.40)	1.13 (0.29, 4.45)
β-Carotene	90	Crude	Ref	1.75 (0.52, 5.84)	2.10 (0.63, 7.03)
		Adjusted	Ref	1.36 (0.37, 5.04)	1.55 (0.42, 5.69)

Analyzed using logistic regression. Data presented as odd ratios (95% CI). Adjusted for age, gender, SES and breastfeeding (at harvest only) status. Q1 is the reference group (low exposed) and Q4 is the highest exposed group. Significantly different from Q1, reference group:

^aP 0.05.