

Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning

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Background Dietary exposure to high levels of the fungal toxin, aflatoxin, occurs in West Africa, where long-term crop storage facilitates fungal growth.

Methods We conducted a cross-sectional study in Benin and Togo to investigate aflatoxin exposure in children around the time of weaning and correlated these data with food consumption, socioeconomic status, agro-ecological zone of residence, and anthropometric measures. Blood samples from 479 children (age 9 months to 5 years) from 16 villages in four agro-ecological zones were assayed for aflatoxin-albumin adducts (AF-alb) as a measure of recent past (2–3 months) exposure.

Results Aflatoxin-albumin adducts were detected in 475/479 (99%) children (geometric mean 32.8 pg/mg, 95% CI: 25.3–42.5). Adduct levels varied markedly across agro-ecological zones with mean levels being approximately four times higher in the central than in the northern region. The AF-alb level increased with age up to 3 years, and within the 1–3 year age group was significantly ($P = 0.0001$) related to weaning status; weaned children had approximately twofold higher mean AF-alb adduct levels (38 pg AF-lysine equivalents per mg of albumin [pg/mg]) than those receiving a mixture of breast milk and solid foods after adjustment for age, sex, agro-ecological zone, and socioeconomic status. A higher frequency of maize consumption, but not groundnut consumption, by the child in the preceding week was correlated with higher AF-alb adduct level. We previously reported that the prevalence of stunted growth (height for age Z-score HAZ) and being underweight (weight for age Z-score WAZ) were 33% and 29% respectively by World Health Organization criteria. Children in these two categories had 30–40% higher mean AF-alb levels than the remainder of the children and strong dose–response relationships were observed between AF-alb levels and the extent of stunting and being underweight.

Conclusions Exposure to this common toxic contaminant of West African food increases markedly following weaning and exposure early in life is associated with reduced growth. These observations reinforce the need for aflatoxin exposure intervention strategies within high-risk countries, possibly targeted specifically at foods used in the post-weaning period.

Keywords Aflatoxin, dietary exposure, biomarkers, child growth, weaning

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Foetal and early childhood environment are considered critical for growth and disease risk in later life.¹ Nutritional status of the mother during pregnancy and that of the infant following birth are both important in this respect.² In developing countries, many individuals are not only malnourished but are also chronically exposed to high levels of toxic fungal metabolites (mycotoxins) in their diet. The heterogeneous distribution of

mycotoxins within a given food commodity hinders accurate exposure measurement and hence associations between exposure and human health have been difficult to establish.³ One family of mycotoxins, the aflatoxins produced by *Aspergillus spp.*, are proven carcinogens, immunotoxins, and cause growth retardation in animals.⁴⁻⁶ The development and use of aflatoxin-albumin (AF-alb) adducts as a biomarker of exposure revealed that over 90% of West African sera tested contained detectable AF-alb adducts, with exposure occurring throughout life, including *in utero* and *via* breast milk.⁷⁻¹² This biomarker has significantly contributed to our understanding of the role of aflatoxins in human hepatocellular carcinoma.¹³ The high prevalence and level of exposure throughout childhood suggests that child growth and development could be critically affected but to date little attention has been paid to understanding these effects. Aflatoxin exposure has been suggested as a causal or aggravating factor for Kwashiorkor in African children^{14,15} but problems in study design make interpretation difficult.⁵

In parts of West Africa, maize and groundnuts are major dietary staples. Aflatoxin exposure has been linked to the consumption of these crops with a marked seasonality in exposure,^{10,12} presumably related to crop management practices and hot, humid storage conditions, which facilitate fungal growth and mycotoxin production.^{16,17} Rural populations in Benin and Togo rely on maize as a staple whilst groundnuts are frequently included in sauces and as a snack. Both commodities are stored under conditions that can promote *Aspergillus* growth and aflatoxin contamination. The level of aflatoxin in stored maize in this region has been shown to exceed 100 ppb in 50% of tested samples.^{17,18}

This study was conducted to establish the geographical pattern and the influence of sociological factors, weaning, and patterns of weaning food consumption on aflatoxin exposure in Benin and Togo by measuring serum AF-alb in 479 children in 16 villages across four agro-ecological zones. Aflatoxin exposure has previously been shown to correlate with impaired growth in these children.¹⁹

Material and Methods

Subject recruitment

Benin and Togo are neighbouring countries that can be considered in four zones of varying ecological characteristics (from north to south): Sudan savannah (SS), Northern Guinea savannah (NGS), Southern Guinea savannah (SGS), and Coastal savannah (CS). Rainfall and humidity decrease from south to north. Consequently the southern areas (SGS and CS) have two maize growing seasons each year, while the northern areas, NGS and SS have only one season. Harvesting periods also differ somewhat by zone.¹⁷ The interviews and blood sampling in this study were conducted from January to April, timed to occur after maize had been in storage for approximately 4 months; the exception was the far north (SS) where maize had only been stored for 2 months at the time of sampling. Four villages within each of these four zones were selected on the basis of their proximity to a regional health centre to facilitate rapid processing and temporary storage of blood samples. Ethical approval was obtained from the Ministries for Health in Benin and Togo. Thirty children per village, aged 9 months to 5 years, were randomly recruited with inclusion of only one child per

household. For inclusion the household had to have maize in store at the time of the study. The head of household and the mother of the selected index child were informed about the nature of the study, and where they agreed to participate, signed a statement of informed consent. A questionnaire, administered by trained interviewers to the mothers of children recruited to the study, obtained information on the family: frequency of maize and groundnut consumption, social and economic status of the mother and household. The socioeconomic status of the mother was calculated to produce a continuous variable on the basis of an evaluation of the mother's possessions (means of transport, land, cattle, machines, and equipment), earnings, and savings. The socioeconomic status of the household was calculated as a continuous variable based on quality and ownership of housing and means of transportation of the head of the household (e.g. truck, car, bicycle). In the multivariable analysis emphasis was placed on the mother's status because it was judged to be more directly relevant to the nutritional and health status of the young children studied. Maternal education was classified into three categories: no school education, primary school or secondary school education. Information was also collected on the child, namely age, sex, sibling number, food consumption (including frequency of maize and groundnut consumption during week prior to blood sampling), weaning status, and general health status. Consumption of maize and groundnuts were dichotomized to categories of 'frequent' (>4 times per week) or 'infrequent' (≤ 4 times per week) for statistical analysis.

Aflatoxin exposure assessment

A 5-ml blood sample was obtained from each child, and serum separated. One blood sample of the 480 was missing. The levels of AF-alb adduct were determined by albumin extraction, digestion and enzyme linked immunosorbent assay (ELISA) as previously described.²⁰ The detection limit was 3 pg AF-lysine equivalents per mg of albumin (pg/mg). Controls included three positive and one negative control analysed alongside batches of samples. Samples were measured in quadruplicate on at least two occasions on separate days.

Weight and height measurement

Child body weight and height were measured using accurately calibrated instruments (electronic scales: Soehnle, max wt 20 kg, accurate to 10 g; height measurement: SCHORR, Maryland, USA). Field workers, trained to maximize repeatability, made all height and weight measurements. Weight for age Z-score (WAZ), height for age Z-score (HAZ) and weight for height Z-score (WHZ) were calculated according to the median value of the international reference population recommended by National Center for Health Statistics (NCHS)/World Health Organization (WHO).²¹ Weight for height Z-score reflects body shape, a low value indicating wasting or thinness, whilst a low HAZ indicates stunting. A low WAZ is indicative of being underweight. According to the WHO criteria, a Z-value < -2 for any of these criteria is recognized as malnutrition and < -3 as severe malnutrition.

Statistical analysis

The AF-alb adduct data were not normally distributed, and were natural log transformed for statistical analysis. The difference

between means was tested by t-test or ANOVA. Significant variables of age, agro-ecological zone, weaning status, and socioeconomic status were entered into a multivariable model to analyse effects on anthropometric data and AF-alb level. Initially, in univariate analysis both measures of socioeconomic status (mother and household) were compared with AF-alb levels but for the multivariable analysis, mother's socioeconomic status was used (see Subject recruitment). All the analysis was performed using SAS software. Geometric means for AF-alb with 95% CI are reported in the Tables and text unless otherwise stated.

Results

Aflatoxin-albumin adduct distribution

Aflatoxin-albumin adduct distribution by age, sex, agro-ecological zone, and socioeconomic status was determined (Table 1). Aflatoxin-albumin adducts were detected in 475/479 (99%) of the serum samples and the mean adduct level was 32.8 pg/mg albumin (range 5–1064 pg/mg albumin). There was no difference in AF-alb level between male and female children. The AF-alb levels were lowest in children aged <1 (geometric mean 9.5 pg/mg; 95% CI: 6.6–14.4) and increased with age up to 2–3 years old (geometric mean 42.9 pg/mg; 95% CI: 35.7–51.7), at which point adduct levels reached a plateau. However, this trend was not significant when adjusted for agro-ecological zone, weaning status (see below), and socioeconomic status. There was a marked variation in AF-alb by agro-ecological zone. The SS in the far north had the lowest mean level, whilst SGS had the highest mean adduct level (Table 1). The lowest mean village adduct level, Gbatope (12.7 pg/mg) in CS, was 14 times lower than the village with the highest mean level, Lainta (177.7 pg/mg) in SGS.

The measure of mother's socioeconomic status was compared with AF-alb levels (Table 1). Whilst there were differences in AF-alb levels across quartiles there was no consistent trend between the two measures; the group having the highest socioeconomic status did have the lowest mean AF-alb level but the next highest quartile had the highest level. Household socioeconomic status was also compared with AF-alb level and no statistically significant correlation was observed ($r = -0.048$; $P = 0.299$). This absence of an association persisted after adjustment for age, weaning, and agro-ecological zone ($P = 0.996$). The AF-alb levels were also compared with maternal education with geometric mean levels of 34.2 (95% CI: 30.4–38.5), 31.4 (95% CI: 25.8–38.1), and 20.3 pg/mg (95% CI: 12.7–32.6) in mothers with no school education ($n = 356$), primary school education ($n = 102$), or secondary school education ($n = 20$) respectively; this difference is not significant ($F = 2.23$; $p = 0.109$).

Weaning status

Children were classified as either breastfed (i.e. still partially or wholly breastfed) or fully weaned (i.e. no breast milk). Amongst the breastfed children, the majority (98%) were being weaned onto a maize-based porridge and/or the family meal. In children ≤ 3 years of age, 158/302 were still being breastfed although only one child was exclusively breastfed. In children > 3 years of age all but three children were fully weaned. The mean AF-alb level was approximately twofold higher in weaned children than in those breastfed for ≤ 3 years. After adjusting for age, socioeconomic status, and agro-ecological zone in a multivariable model there remained a highly significant ($P = 0.0001$) 1.8-fold difference in adduct levels between weaned and breastfed children (37.7 pg/mg; 95% CI: 30.8–46.1 and 21.1 pg/mg; 95% CI: 17.5–25.5, respectively) (Figure 1). When children aged 1–3 years were divided into 6-month age groups there was a

Table 1 Aflatoxin-albumin adduct (AF-alb) distribution by age, sex, agro-ecological zone, and mother's socioeconomic status

Variable/Category	No.	AF-alb geometric mean (95% CI) (pg/mg) ^a			<i>P</i> ^b
		Unadjusted mean	<i>P</i> ^b	Adjusted mean ^c	
Total	479	32.8 (25.3–42.5)			
Age (year)					
<1	14	9.5 (6.6–14.4)		17.8 (10.9–29.2)	
1–	152	21.1 (17.9–24.9)		27.7 (23.1–33.1)	
2–	136	42.9 (35.7–51.7)		31.4 (25.9–38.0)	
3–	101	44.7 (37.2–53.7)	< 0.01	30.1 (23.9–37.9)	> 0.05
4–5	52	41.3 (31.9–53.4)		30.7 (23.2–40.7)	
Sex					
Male	248	31.2 (27.0–36.0)	> 0.05	27.2 (23.6–31.5)	> 0.05
Female	231	34.8 (30.4–39.9)		26.7 (23.0–31.0)	
Zone					
Sudan Savannah	120	18.0 (15.5–20.8)		16.5 (13.6–19.9)	
Northern Guinea Savannah	120	33.8 (28.7–39.8)		26.7 (22.1–32.3)	
Southern Guinea Savannah	119	75.9 (62.8–91.8)	< 0.01	57.7 (47.6–70.0)	< 0.01
Coastal Savannah	120	25.3 (20.9–30.6)		20.9 (17.2–25.4)	
Socioeconomic status (quartiles)					
1 (low)	129	30.0 (25.0–35.7)		27.7 (23.0–33.4)	
2	121	31.5 (25.8–38.4)		26.3 (21.9–31.6)	
3	112	46.5 (37.3–58.0)	0.0012	32.4 (26.7–39.4)	0.03
4 (high)	117	27.1 (22.5–32.7)		22.4 (18.3–27.4)	

^a pg AF-lysine equivalents per mg of albumin.

^b *P*-value for F-test or t-test.

^c Adjusted for all the other factors included in the model.

significant linear trend in the mean AF-alb level with age (P for trend < 0.001) (Figure 2). Within a given age group there were marked differences in mean adduct levels by weaning status. For example, in age group 1.5–2.0 years the mean AF-alb level was 39.8 pg/mg for weaned (95% CI: 27.3–58.0) and 25.0 pg/mg for breastfed (95% CI: 20.1–31.0) children.

Maize and groundnut consumption

Maize was consumed all year by 98% of families in the SS, SGS, and CS, and 78% of families in the NGS. Groundnut consumption varied more by zone, with families from SGS reporting 'all year' consumption most frequently (87%) compared with 7%, 58%, and 58% in SS, NGS and CS respectively. For consideration of frequency of maize and groundnut consumption, children were classified into two groups for each commodity: those consuming the commodity ≤ 4 times in the week prior to the survey and those consuming it > 4 times in that week (Table 2). The mean AF-alb level was higher in the group consuming maize more frequently ($P < 0.01$). After adjusting for age, socioeconomic status, agro-ecological zone, and weaning status, the AF-alb level was significantly ($P = 0.049$) higher for more frequent maize consumers compared with less frequent consumers. The AF-alb levels were not associated with frequency of groundnut consumption.

Anthropometry

In general the children in this study were growth impaired when compared with an international reference population. The mean values for each of the three growth parameters WAZ, HAZ, and WHZ were below zero (mean = -1.5 , -1.56 , and -0.67 respectively) with particularly low values for WAZ and HAZ. The prevalence rate for being underweight (WAZ < -2) was 29%, for stunting (HAZ < -2) it was 33%, and for wasting (WHZ < -2) 6% (Table 3). A total of 43 (9.5%) children had a Z-score

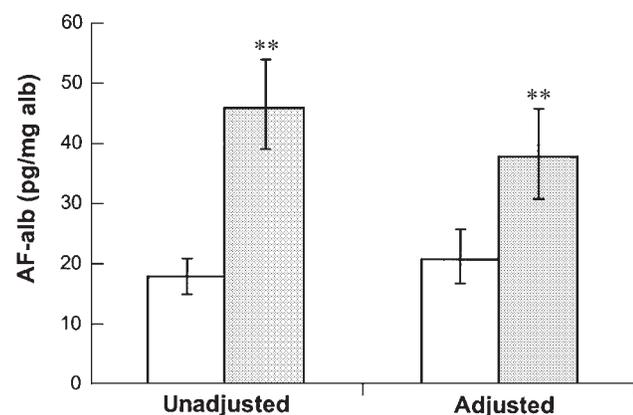


Figure 1 Aflatoxin-albumin (AF-alb) adduct level and weaning status in children. Aflatoxin-albumin adducts levels in: □ breastfed (n = 158) and ■ weaned (n = 144) children aged ≤ 3 years. Unadjusted geometric means were 45.6 (95% CI: 38.8–53.7) and 18.0 (95% CI: 15.2–21.3) for weaned and breastfed group respectively. After adjusting for age, socioeconomic status, and agro-ecological zone in a multivariable model there was a highly significant difference between adduct levels in weaned and breastfed children ($P = 0.0001$). The adjusted geometric means were 37.7 (95% CI: 30.8–46.1) and 21.1 (95% CI: 17.5–25.5) for the weaned and breastfed groups respectively. $**P < 0.0001$.

of ≤ -3 in at least one parameter, a situation classed as extreme malnutrition by WHO criteria. As reported previously,¹⁹ after adjusting for age, sex, socioeconomic status, weaning status, and agro-ecological zone, there was an extremely strong association between increased AF-alb levels and stunted growth in children (HAZ; trend test: $F = 15.19$, $P = 0.0001$, $r^2 = 0.3766$). There was also a significant association with being underweight (WAZ; trend test, $F = 8.48$, $P = 0.0038$, $r^2 = 0.3680$) but not with weight for height Z-score (data not shown).

Discussion

In West Africa, rural populations are chronically exposed to high levels of aflatoxin with exposure occurring *in utero* and early in childhood.^{7–12} The high levels in Benin and Togo are consistent with those observed elsewhere in West Africa. In fact, some of the highest levels of AF-alb ever measured were found in this group of children (5.4% had levels > 200 pg/mg with a maximum level in one child of 1064 pg/mg). The AF-alb levels varied in the four agro-ecological zones, with SGS having the highest AF-alb level (Table 1). This broadly reflects aflatoxin contamination levels reported previously in maize within Benin.^{17,18}

The AF-alb level increased with age for children < 3 years old, after which the level reached a plateau. It is important to understand whether this is due to age *per se* or a change in diet from breast milk to solid food. In a multivariable model including children aged ≤ 3 years, weaning status was significantly associated with AF-alb with an approximately twofold higher mean adduct level in those no longer being breastfed. In this model, age was not significantly associated with AF-alb and the original association probably reflected the changing pattern of weaning status with age. When children, aged 1–3 years, were divided into 6-month age groups (1.0–1.5, 1.5–2.0, 2.0–2.5, 2.5–3.0 years) AF-alb levels were higher in weaned than breastfed children within each group where the comparison could be made (Figure 2). This again suggests weaning status, rather than age, to be the major determinant of aflatoxin exposure level in young children. The lower AF-alb levels associated with breastfeeding almost certainly reflect lower levels of aflatoxin in breast milk than weaning and family foods and even lower levels of exposure might be expected in wholly breastfed infants. In addition, the majority of aflatoxins in milk are in the form of the less toxic and carcinogenic AFM₁ hydroxylated metabolite rather than the highly toxic AFB₁ predominantly found in foods.^{4,11,22}

Both maize and groundnuts are susceptible to *Aspergillus spp.* growth and aflatoxin contamination. This is particularly problematic in countries such as Benin and Togo because maize is the principal staple food in the area and groundnuts are frequently consumed in snacks and sauces. The frequency of maize consumption by the child showed only a relatively weak positive correlation with the AF-alb level. This probably reflects a number of factors including: the absence of an estimate of quantity of maize consumption; the effects of sorting and food preparation on aflatoxin levels; the contribution of aflatoxin from other dietary components; and the fact that the AF-alb marker integrates exposure over a period longer than the one week to which the questionnaire relates. There was no significant association between reported groundnut consumption and

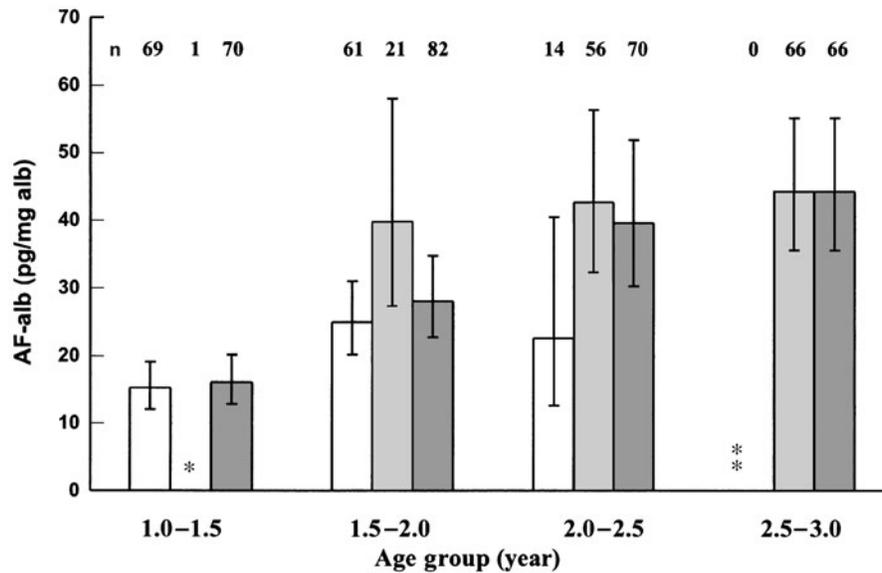


Figure 2 Aflatoxin-albumin (AF-alb) adduct level and weaning status in children with age. Aflatoxin-albumin adducts levels in 6-month age groups for children from 1–3 years after adjusting for agro-ecological zone and socioeconomic status. Geometric mean adjusted adduct levels are indicated with 95% CI. There was a significant ($P < 0.0001$) increasing trend in the mean AF-alb level for all children (weaned and breastfed) but not when weaned and breastfed children were considered separately. For the two age groups with sufficient numbers of both weaned and breastfed children, weaned children had significantly higher adduct levels (group 1.5–2.0, $P = 0.037$; group 2.0–2.5, $P = 0.05$). *one child only in this group, adduct level 133.8 pg/mg (pg AF-lysine equivalents per mg of albumin); **no breastfed children were found in the 2.5–3.0 age group. The 14 children aged <1 year were not included in this analysis.

Breastfed: □ Weaned: ■ Total: ■.

Table 2 Maize and groundnut consumption and Aflatoxin-albumin adduct (AF-alb)

	Consumption frequency (group)	No.	AF-alb (pg/mg alb) ^a
			Mean (95% CI)
Maize	≤4 times/week (0)	81	24.5 (19.5–30.8)
	>4 times/week (1)	398	34.8 (31.2–38.9) ^b
Groundnut	≤4 times/week (0)	310	31.8 (28.1–36.0)
	>4 times/week (1)	169	34.5 (29.3–40.6)

^a pg AF-lysine equivalents per mg of albumin.

^b Compared to group (0), t-test, $P < 0.01$.

Table 3 Prevalence of malnutrition in children

Criteria ^a	Total ^b	Prevalence rate (no. of cases)	
		Malnutrition ^c	Extreme malnutrition ^d
Wasting	454	6% (27)	0.2% (1)
Underweight	454	25% (113)	4% (17)
Stunting	455	24% (109)	9% (40)

Malnutrition status for children based on World Health Organization guidelines.

^a Diagnosis of wasting based on weight for height Z-score (WHZ); underweight based on weight for age Z-score (WAZ); stunting based on height for age Z-score (HAZ).

^b Malnutrition is defined as less than –2 for any of the three parameters.

^c Extreme malnutrition is defined as less than –3 for any of the three parameters.

^d Accurate ages were missing for 24 children and weight for one child.

AF-alb. Groundnuts are often consumed as a snack and children may have consumed these away from the home unbeknown to the mother, leading to underreporting. However, despite the lack of association, subjects from SGS had the highest reported frequency of groundnut consumption and the highest AF-alb levels. In addition, when comparing Lainta and Gbatope, the two villages with the extremes of aflatoxin exposure, the most notable difference is the frequency of groundnut consumption; Lainta having the higher consumption and the highest mean AF-alb level. Thus it is possible that an effect of groundnut consumption on AF-alb level may have been masked by the more frequent consumption of aflatoxin-contaminated maize or missed due to underreporting and the other limitations discussed above for maize.

We have previously reported the striking associations between aflatoxin and impaired growth in these children.¹⁹ In animal models, aflatoxin is reported to reduce growth rates,⁶ but this had not been previously studied in human populations. These adverse growth effects are strongly correlated with the change from breastfeeding to solid foods, including maize, which is used in ground form as the basis for porridge for weaning purposes. Whether the effects of weaning foods and associated reduced growth are a direct result of aflatoxin exposure cannot be confirmed from the current cross-sectional study. For example, the AF-alb biomarker may be reflecting an infant's consumption of fungus-affected food, which as a consequence is of poor nutritional quality. However, as aflatoxins are secondary fungal metabolites and different species of *Aspergillus* produce different amounts of aflatoxin, toxin level is not always correlated with

the fungal load. In this context, socioeconomic status might also be expected to correlate with poor food quality and higher AF-alb levels but no strong effect was observed. This absence of effect notwithstanding, the difficulties in assessing socioeconomic status in these populations should be noted. An alternative explanation of our observations could be that malnutrition affects albumin level or turnover and consequently disrupts the relationship between aflatoxin exposure and AF-alb. However, serum albumin levels were measured in this study and were within the normal physiological range (data not shown).

This study suggests that extended breastfeeding results in a period of lower aflatoxin exposure, in a population whose primary weaning foods are at high-risk of aflatoxin contamination. Weaning results in a marked increase in exposure, as aflatoxin-contaminated household foods begin to be consumed, and this change in diet is associated with growth faltering, particularly stunting. The aflatoxin contamination of maize used in weaning foods may be a major source of exposure but a contribution from groundnuts cannot be ruled out from this

study. Future work is needed, first to confirm this effect in longitudinal studies in children; second, to understand the mechanism by which aflatoxin affects growth; and third, to address the possible associated health consequences. The observations also emphasize the need for aflatoxin exposure intervention strategies within high-risk countries, possibly targeted specifically at the post-weaning period.¹⁶

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KEY MESSAGES

- Aflatoxins are carcinogenic and immunosuppressive fungal toxins contaminating staple foods at high levels in West Africa.
- Children in Benin and Togo had a marked increase in aflatoxin exposure when weaned onto solid foods, particularly maize.
- There was a strong association between the high levels of aflatoxin exposure upon weaning and impaired growth in these children.
- These observations reinforce the need for aflatoxin exposure intervention strategies, possibly targeted specifically at the post-weaning period, in West Africa.

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