CHAPTER 4.

Effects of aflatoxins and fumonisins on child growth

Although animal studies over the past 50 years have repeatedly shown an association between aflatoxin exposure and growth impairment in many species, the evidence has been lacking in humans.

Child growth faltering in low-income countries usually begins in utero and continues for about 2 years postnatally. Therefore, the current analysis is focused on studies of exposures to aflatoxins and/or fumonisins during pregnancy in relation to birth outcomes (e.g. low birth weight) as well as growth outcomes in early childhood. The bulk of the literature relating child growth impairment to mycotoxin exposure focuses on aflatoxin-related stunting. Khlangwiset et al. (2011) summarized the animal and epidemiological studies that showed an association between child growth impairment and aflatoxin exposure. Here, the human studies are critiqued in greater depth in relation to the results obtained and aspects of study design, such as control of important confounding factors and cofactors.

Six studies were deemed to be of high quality, with well-defined sample sizes, exposure or dose assessments, outcome measures, and appropriate multivariate analyses. These are summarized in Table 4.1 and are categorized by toxin (aflatoxin vs fumonisin) and by the timing of the exposure and outcome measurement (pre- vs postnatal).

Eight additional studies did not meet these quality criteria and are therefore not included here (De Vries et al., 1989; Abdulrazzaq et al., 2002; Turner et al., 2003; Abdulrazzaq et al., 2004; Okoth and Ohingo, 2004; Sadeghi et al., 2009; Mahdavi et al., 2010; Shouman et al., 2012).

Studies of pre- or postnatal aflatoxin exposure and postnatal growth

Two studies were published involving a total of 680 children living in four agro-ecological zones of Benin and Togo in West Africa (Gong et al., 2002, 2004). In the cross-sectional study, height-for-age and weight-for-age were lower in a dose-dependent fashion for increasing aflatoxin exposures as measured by aflatoxin–albumin adducts (AF–alb) in serum (Gong et al., 2002). Separately, in a multivariate analysis controlling for these factors as well as age and sex, it was determined that AF–alb levels in children's serum were significantly associated with weaning status: the earlier the weaning, the higher the aflatoxin exposure (Gong et al., 2003). In the longitudinal study, over

Table 4.1. Summary of evidence reviewed on the effects of aflatoxin and fumonisin on child growth

Reference	Study site; context	Study sample	Study design	Exposure measurement and characterization	Outcome measurement and reporting	Handling of covariates and confounders	Findings; inference
				AFLATOXIN STUDIES			
Postnatal exposure							
Gong et al. (2002)	Benin and Togo; rural, 16 villages selected to include high exposures; 33% stunted, 29% underweight, 6% wasted	Community-based, 479 children, 9 months to 5 years	Cross-sectional	AF–alb by ELISA; geometric mean, 32.8 pg/mg (range, 5–1064 pg/mg)	Measured height, weight, age; reported HAZ, WAZ	Age, sex, SES (undefined), agro-ecological zone, weaning status	Dose–response relationship with HAZ and WAZ; overall adjusted negative correlation with HAZ ($P = 0.001$) and WAZ and WHZ ($P = 0.047$)
Gong et al. (2004)	Benin; rural, 4 villages selected to include variable exposures; maize and groundnuts	Community-based samples, 200 children (50 per village), 16–37 months at baseline; 181 effectively analysed	Prospective, 8-month FU; samples collected at baseline, middle, and end	AF–alb by ELISA; mean exposures by village: 11.8, 31.1, 45.9, and 119.3 pg/mg	Measured height, weight, age; reported absolute change in height and weight	Age, sex, baseline height, weaning status, mother's SES, village	Height increment by AF–alb quartile, with adjustment, $P < 0.001$ by trend test; 8-months height increment regressed average AF–alb over 3 time points; no Z-scores reported; weight increment not associated with AF–alb
Shirima et al. (2015)	Three districts in northern and central United Republic of Tanzania; documented co-exposure with fumonisin; high maize and groundnuts	Community-based sample, 166 children recruited at age 6–14 months; 44% stunted and < 3% wasted at baseline	Prospective, 12-month FU; samples collected at baseline, 6 months, and 12 months	AF–alb by ELISA; geometric mean, 4.7, 12.9, and 23.5 pg/mg at baseline, 6 months, and 12 months, respectively (fumonisin also assessed; see below)	Measured height, weight, age; reported absolute change in height and weight	Sex, age, baseline length, village, breastfeeding, maternal education, SES, protein and energy intakes	AF–alb not associated with growth
Pre- or postnatal exposure and postnatal growth							
Turner et al. (2007)	The Gambia; rural	Pregnancy cohort; 138 singleton infants followed for 14 months; 107 analysed	Prospective, 14-month FU; samples collected twice during pregnancy (4.5 months, 8 months) and cord blood and infant at age 16 wk; monthly postnatal FU	AF–alb by ELISA; median values (% detectable): pregnancy average, 38.9 pg/mg (100%); cord blood, 2.5 pg/mg (48.5%); infant at 16 wk, 2.5 pg/mg (11.0%)	Measured birth weight, length, postnatal height, weight, age; reported WAZ and HAZ in mixed longitudinal model using GEE	Sex, age, placental weight, maternal weight, gestation duration, season	Pregnancy AF–alb associated with rate of HAZ and WAZ decline ($P < 0.001$); effects on WHZ not reported

Table 4.1. Summary of evidence reviewed on the effects of aflatoxin and fumonisin on child growth (continued)

Reference	Study site; context	Study sample	Study design	Exposure measurement and characterization	Outcome measurement and reporting	Handling of covariates and confounders	Findings; inference
				AFLATOXIN STUDIES (continued)			
Prenatal exposure and birth outcomes							
Turner et al. (2007)	The Gambia; rural	Mean birth weight, 2.9 kg					Pregnancy AF–alb not associated with birth weight or length
Shuaib et al. (2010)	Kumasi, Ghana	785 women presenting for delivery, singleton uncomplicated pregnancy; 20.3% LBW, 19.1% preterm, 13.6% SGA	Cross-sectional between mother and baby at birth	AF–alb by HPLC; mean, 10.9 pg/mg	Preterm birth (< 37 wk GA; method unclear); SGA (< 10th percentile of a reference; reference unclear); stillbirth (> 20 wk GA); LBW (< 2.5 kg)	Baby's sex, number of children, maternal education, maternal income, malaria exposure, anaemia, helminths, *Strongyloides stercoralis*	Rates of all outcomes except preterm highest in Q4 of AFB_1, but only LBW significant, Q4 vs Q1 AOR, 2.09 (95% CI, 1.19–3.68); NS for SGA or stillbirth
				FUMONISIN STUDIES			
Postnatal exposure							
Kimanya et al. (2010)	Rural northern United Republic of Tanzania; high maize and groundnuts	Community-based sample, 215 infants aged 6 months at baseline; stunting prevalence at baseline not reported, but LAZ appears to be < −1	Prospective, 6-month FU; samples collected at baseline and 6 months	Dietary fumonisin intake estimated when infants were aged 6–8 months by maize intake from two consecutive 24-hour recalls and HPLC analysis of FB_1, FB_2, and FB_3 in maize samples collected from the home on the days of the recall; 26 infants had intakes > 2 µg/kg bw/day (JECFA PMTDI)	Measured weight, height, age, sex; only absolute height and weight at 12 months were analysed as outcome	Total energy and protein intakes from complementary foods, village, sex, WHZ at baseline	Primary analysis was by high vs low intake of fumonisin (cut-off, 2 µg/kg bw/day); high-intake children were already significantly shorter at baseline; at age 12 months, high-intake infants were on average 1.3 cm shorter and 328 g lighter than low-intake infants
Shirima et al. (2015)	Three districts in northern and central United Republic of Tanzania; documented co-exposure with aflatoxin; high maize and groundnuts	Community-based sample, 166 children recruited at age 6–14 months; 44% stunted and < 3% wasted at baseline	Prospective, 12-month FU, samples collected at baseline, 6 months, and 12 months	Free UFB_1 in urine samples collected on 2 days was measured by HPLC-MS after solid-phase extraction	Measured height, weight, age; reported absolute change in height and weight	Sex, age, baseline length, village, breastfeeding, maternal education, SES, protein and energy intakes	UFB_1 levels at baseline and 6 months were associated with LAZ at 6 months and 12 months, respectively; mean UFB_1 levels from all 3 time points were strongly inversely related to LAZ at 12 months; UFB_1 quartiles were inversely related to LAZ in a linear dose–response manner

AF–alb, aflatoxin–albumin adducts; AFB_1, aflatoxin B_1; AOR, adjusted odds ratio; bw, body weight; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FB_1, fumonisin B_1; FU, follow-up; GA, gestational age; GEE, generalized estimating equations; HAZ, height-for-age Z-score; HPLC-MS, high-performance liquid chromatography-mass spectrometry; JECFA, Joint WHO/FAO Expert Committee on Food Additives; LAZ, length-for-age Z-score; LBW, low birth weight; NS, not significant; PMTDI, provisional maximum tolerable dietary intake; Q1–Q4, quartile 1–quartile 4; SES, socioeconomic status; SGA, small for gestational age; UFB_1, urinary fumonisin B_1; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score; wk, week or weeks.

a period of 8 months children with the highest aflatoxin exposures had the smallest gains in height (Gong et al., 2004). These results were also adjusted for weaning status, agro-ecological zone, and socioeconomic status. The important contribution of this body of work is that in both a cross-sectional and a longitudinal study, higher aflatoxin exposures were shown to be correlated with children's height-for-age and also growth trajectories over a critical period of child development.

A study in The Gambia found a significant association between in utero aflatoxin exposure and growth faltering in infants (Turner et al., 2007). This longitudinal study of 138 pregnant women and their infants followed the infants for 1 year and controlled for season, sex, placental weight, maternal weight, and gestation time, with AF–alb measured by enzyme-linked immunosorbent assay (ELISA). AF–alb in maternal blood serum was a strong predictor of length/height gain and weight gain in the first year of life. It was predicted that if the maternal AF–alb levels dropped from 110 pg/mg to 10 pg/mg, the weights and heights of infants at age 1 year would increase by 0.8 kg and 2 cm, respectively (Turner et al., 2007).

In the United Republic of Tanzania, Shirima et al. (2015) studied a cohort of 166 infants aged 6–14 months at enrolment and followed them for 12 months. AF–alb was measured by ELISA at baseline and 6 and 12 months later. Anthropometric measurements were also taken at each time point. Aflatoxin levels in this study were lower than in the West African studies, rising from a geometric mean of 4.7 pg/mg at baseline to 23.5 pg/mg at the end of the study. The authors found no significant association between aflatoxin dose and stunting in this population.

No study has found an association between aflatoxin exposure and wasting, although wasting was not common in these populations.

Establishing causality of the association between aflatoxin exposure and growth faltering, as reported for studies in Benin and Togo, is uncertain due to the general difficulty of separating the effects of aflatoxin level from possible poor quality of the child's diet. However, in the longitudinal study there was no association between AF–alb and micronutrient levels, suggesting that aflatoxin exposure was not accompanied by a general micronutrient deficiency (Gong et al., 2004). Furthermore, the infant diet in The Gambia includes groundnuts, as opposed to maize in Benin and Togo, and yet results were broadly consistent across these populations. The lack of an association between aflatoxin exposure and growth impairment in the Tanzanian study suggests that there may be a threshold effect. Generalizing the evidence from these four studies is difficult because of their limited geographical distribution (three sites in West Africa) and insufficient information on the links between aflatoxin level, dietary and other cofactors, and growth outcomes.

Studies of maternal aflatoxin exposure and birth outcomes

Shuaib et al. (2010) studied mothers' AF–alb levels at delivery and birth outcomes (preterm birth, small-for-gestational-age, low birth weight, and stillbirth) in Kumasi, Ghana. In this study, AF–alb was measured using high-performance liquid chromatography (HPLC) in the blood of 785 mothers immediately after they had given birth. After adjusting for sociodemographic variables (age, education, socioeconomic status, residence, and type of toilet facilities), it was found that the mothers in the highest quartile of AF–alb levels were at significantly higher risk of having babies with low birth weight, defined as being below 2.5 kg (adjusted odds ratio, 2.09; 95% confidence interval, 1.19–3.68). None of the other birth outcomes were associated with aflatoxin measure.

For the postnatal growth outcome in the Turner et al. (2007) study in The Gambia described above, birth weight and length were measured but were not associated with maternal AF–alb concentrations in mid and late pregnancy.

The validity of the findings from these studies on aflatoxin and low birth weight is uncertain because they have small sample sizes for adverse birth outcomes, and thus may not be sufficiently powered to detect important outcomes. Furthermore, it is difficult in observational studies to separate the effects of aflatoxin dose from possible poor nutritional quality of the maternal diet (i.e. monotonous maize diet with little dietary diversity).

Studies of postnatal fumonisin exposure and infant growth

Two recent studies from the United Republic of Tanzania suggest that fumonisin exposure may also be associated with stunting in children. Kimanya et al. (2010) estimated fumonisin exposure in 215 infants by measuring fumonisin in maize flour and estimating the daily fumonisin intake of the infants based on mothers' dietary recall. In this prospective cohort study, infants were enrolled at age 6 months and followed until age 12 months. Exposure was categorized as high or low using the Joint WHO/FAO Expert Committee on Food Additives (JECFA) provisional maximum tolerable dietary intake (PMTDI) of 2 µg/kg body weight/day as the cut-off.

Even at baseline, the 26 infants in the high-exposure category were shorter than those with low exposure. By age 12 months, the highly exposed infants were significantly shorter (by 1.3 cm) and lighter (by 328 g) on average than the 105 infants with low exposure, after controlling for total energy and protein intake, sex, and village.

In the same study in the United Republic of Tanzania described above for aflatoxin exposure, Shirima et al. (2015) found that levels of urinary fumonisin B_1 (UFB_1) at recruitment were negatively associated with length-for-age Z-scores (LAZ) at both 6 months and 12 months after recruitment. Mean levels of UFB_1 from all three sampling times showed an inverse association with LAZ and length velocity at 12 months after recruitment. UFB_1 levels (averaged from two urine samples) at baseline and 6 months were associated with LAZ at 6 months and 12 months, respectively. Mean UFB_1 levels from all three time points were strongly inversely related to LAZ at 12 months.

These initial studies of fumonisin and infant growth are small and offer only limited evidence but do strongly suggest the need for further research on this relationship. The Shirima et al. (2015) study also demonstrates the co-occurrence of aflatoxin and fumonisin in maize-based diets and emphasizes the need for multiple mycotoxin assessments to make clear inferences about causal factors.

Uniting aflatoxin and fumonisin in a single framework is critical because dietary co-exposure is common in Africa and parts of Latin America (see Chapter 1). Smith et al. (2012) suggested possible mechanisms by which foodborne mycotoxin exposure, singly or in combination, may contribute to impaired growth by compromising gut health. Gut enteropathy has been associated with chronic immune stimulation, which is inversely correlated with growth during infancy (Campbell et al., 2003). Increased intestinal permeability may allow translocation of microbial products, which can stimulate a systemic inflammatory response. Smith et al. (2012) described two main pathways by which environmental enteropathy may cause growth retardation: malabsorption of nutrients in the small intestine and systemic immune activation, resulting in suppression of the insulin-like growth factor 1 (IGF-1) axis, which is strongly associated with stunting in African infants (Prendergast et al., 2014). In older children (6–17 years), there is evidence that aflatoxin modulates IGF-1 (Castelino et al., 2015).

Scientific gaps and future research needs

Taken together, the studies described above suggest that mycotoxin exposure contributes to child growth impairment independent of, and together with, other risk factors that may cause stunting.

Among the multiple potential causes of growth faltering in young children globally, dietary mycotoxin exposure emerges as a potentially important factor. The weight of evidence linking aflatoxin with growth impairment has increased over the past five decades of research – first, primarily in animal studies and, in the past decade, in the epidemiological studies reviewed above.

One critical knowledge gap is the mechanism or mechanisms by which mycotoxins may cause child growth impairment. Nor, indeed, is it known whether all mycotoxins use the same mechanism of toxicity that leads to growth impairment (and this should not be assumed). As such mechanisms are elucidated, the weight of evidence linking mycotoxins with growth impairment would become stronger. Several possible mechanisms have been proposed; certainly, one or more may be relevant to the role of mycotoxins in growth impairment.

Immune system dysfunction mediated by mycotoxin exposure (Bondy and Pestka, 2000; Turner et al., 2003) could increase risk of infections in children, which can lead to growth impairment from energy losses (e.g. diarrhoea or vomiting) and/or energy expended on recovery from illness. Also, aflatoxin/fumonisin-mediated changes in intestinal integrity could make hosts more vulnerable to intestinal pathogens (Gong et al., 2008b; Smith et al., 2012).

The IGF-1 axis may represent a common causal pathway in mycotoxin effects on hepatocellular carcinoma as well as growth retardation. Deregulation of the IGF axis has been identified in the development of hepatocellular carcinoma. An increased expression of IGF-2 and the IGF-1 receptor (IGF-1R) and associated binding proteins with degrading receptors have emerged as crucial events in malignant transformation and tumour growth, in altering cell proliferation, and in deactivation of apoptotic pathways. Aflatoxin B_1 (AFB_1) was shown to induce phosphorylation of IGF-1R and activation of the signalling cascade involving Akt (also known as protein kinase B) and Erk1/2 (extracellular signal-regulated protein kinases) in hepatoma cell lines (Ma et al., 2012). AFB_1 was also found to downregulate insulin receptor substrate 1 (IRS-1) while upregulating IRS-2 through preventing proteasomal degradation. Of interest is that the *p53* mutant *p53*-mt249 increases IGF-2 transcription, suggesting that *p53* mutation may be a link between AFB_1 and IGF-2.

Given the widespread global prevalence of aflatoxin and fumonisin exposures and the large associations observed with stunting in the seminal studies from West and East Africa, additional prospective studies are needed in a wider variety of contexts. If the associations reviewed in this chapter are established, then the global burden of disease associated with mycotoxin exposure may be far greater than that based on mycotoxin links to cancer. Future prospective studies must be designed with adequate sample size to elucidate thresholds in dose–response and rigorously control for other known causes of growth faltering, such as low nutrient intake and diarrhoea prevalence. Studies of wasting as an outcome (in addition to stunting) would be informative. Intervention studies in humans are ultimately needed to disentangle effects of toxins from effects of monotonous maize diets and associated poverty.