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Interventions targeting child undernutrition in developing countries may be undermined by dietary exposure to aflatoxin.

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Short running head: Aflatoxin exposure and child undernutrition

Abbreviations: AFB1, Aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFM1, aflatoxin M1; AF-alb, aflatoxin-albumin adduct; GM, geometric mean; HAZ, height-for-age z score; ID, Iron deficiency; IGF, insulin-like growth factor; LAZ, length-for-age z score; PEM, protein energy malnutrition; RCT, randomized control trial; SMD, standardized mean difference; VAD, vitamin A deficiency; WAZ, weight-for-age z score and WHZ, weight-for-height z score.

Abstract

Child undernutrition is a major adverse public health burden in developing countries, specifically in sub-Saharan Africa and South Asia. Nutrition interventions such as micronutrient supplementation, as well as complementary feeding targeting the major micronutrient deficiencies have only reduced the burden of child undernutrition to a certain extent, indicating that others factors may play a role. Aflatoxin exposure, which is also highly prevalent in developing countries, may be considered to be an aggravating factor for child undernutrition. Increasing evidence suggests that aflatoxin exposure can occur in any stage of life including *in utero* through a trans-placental pathway and in early childhood (through contaminated weaning food and family food). Early life exposure to aflatoxin is associated with adverse effects on low birth weight, stunting, immune function suppression, and liver function damage. The mechanisms underlying impaired growth and aflatoxin exposure are still unclear but intestinal function damage, reduced immune function and alteration in the insulin-like growth factor axis caused by liver damage, are suggested hypotheses. Given the fact that both aflatoxin and child undernutrition are common in sub-Saharan Africa, effective interventions aimed at reducing undernutrition cannot be satisfactorily achieved until the interactive relationship between aflatoxin and child undernutrition is clearly understood and an aflatoxin mitigation strategy has taken effect in those vulnerable mothers and young children.

Keywords: Aflatoxin, child undernutrition, stunting, kwashiorkor, micronutrient deficiencies

Introduction

Child undernutrition including stunting, wasting and micronutrient deficiencies is a major public health problem for low-income countries. The short- and long-term health consequences of child undernutrition can be severe and irreversible and include impaired cognitive

development, increased vulnerability to infectious diseases, and reduced educational outcomes and economic productivity in adulthood (1). Furthermore, undernutrition is responsible for approximately 3.1 million child deaths each year, with 45% of all child deaths in 2011 having been attributed to this cause (1).

It is recognised that there is a window of opportunity for reducing the burden and the lasting impact of child undernutrition, in particular impaired growth. This critical period is defined as the first 1000 days of life from conception to 24 months of age (2, 3). Bhutta et al., (4) reviewed the potential effect on child undernutrition outcomes of interventions such as breastfeeding promotion, micronutrient supplementation and diversified complementary feeding during this critical period and up to 36 months in the 36 counties with the highest burden of child stunting. By modeling the survival and linear growth status of the annual birth cohort from birth to 36 months, these authors concluded that existing interventions could potentially reduce stunting at 36 months by 36%; mortality by 25% (from birth to 36 months); and stunting, wasting, fetal growth restriction and micronutrient deficiencies disability-adjusted life-years by approximately 25%. Although, these outcomes are encouraging, there are likely to be other underlying determinants of undernutrition that need to be addressed.

There is increasing evidence that exposure to aflatoxin could be one of the underlying factors. Aflatoxin is a mycotoxin produced by *Aspergillus flavus and Aspergillus parasiticus* that contaminate staple crops in many of the countries where child stunting is also prevalent. Although *Aspergillus* molds occur in soil across a wide geographic distribution, hot and humid conditions are favourable for aflatoxin production, with stress to crops caused by drought conditions promoting the contamination of susceptible crops (such as maize and groundnuts) in the field (5). Further growth of the fungus and production of aflatoxin is enhanced by post-

harvest storage conditions that involve high humidity (6). There are four main types of aflatoxin, namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). AFB1 is the most potent toxin and is the most prevalent, accounting for an average of 70% of the total aflatoxin content in food, although this may vary depending on the strain of the fungus and local conditions. Aflatoxin M1 (AFM1) is a toxic metabolite of aflatoxin B1, which can be found in milk of lactating mothers, and milk and meat of animals exposed to aflatoxin.

Human exposure to contaminated food is highest in countries with high consumption of susceptible staple crops grown and stored under optimal fungal growth conditions. Aflatoxin exposure often causes acute outbreaks and sometimes fatal liver toxicity (7). Chronic exposure can increase the risk of liver cancer (8), in particular through an interaction with the hepatitis B virus. There is increasing evidence that aflatoxin plays a role in other health effects such as hepatomegaly (9), immune suppression (10-12) and growth faltering in children (13, 14). Chronic aflatoxin exposure is evident throughout life, including the critical first 1,000 days (15).

With the increasing evidence that aflatoxin can exacerbate the effects of undernutrition, and contribute to growth faltering, it is likely that aflatoxin exposure has inhibited the expected growth improvement predicted for nutritional intervention programs. In this review we will summarise the burden of childhood undernutrition and the current achievement of nutritional specific interventions for improving child growth, review the evidence for aflatoxin exposure exacerbating undernutrition and reflect on the necessity for considering aflatoxin exposure in nutritional intervention programs.

Child undernutrition and nutrition specific interventions in the developing world

Protein energy malnutrition

Protein energy malnutrition (PEM), considered to be the leading form of childhood malnutrition in developing countries, includes the disorders kwashiorkor, marasmus and marasmus-kwashiorkor, which are differentiated by the balance between inadequate protein intake and other energy sources (16). PEM is often a consequence of suboptimal breastfeeding, delayed and/ or inadequate supplementation of appropriate complementary foods, lack of diet diversity and infection that can lead to decreased absorption of essential nutrients.

In 2000, the WHO estimated that 26.7% of children < 5 years of age in developing countries had PEM (17). There is a lack of recently conducted population based studies that have investigated the prevalence of the different types of PEM in developing countries. Kwashiorkor, oedematous malnutrition, has been included within the estimates for the prevalence of, and deaths attributable to, severe acute malnutrition (SAM) (weight-for-height (WHZ) below -3, according to WHO standards (18)). In 2011 the global prevalence of SAM in children < 5 years was 3% (19 million) with higher percentages observed in central Africa (5.6%) and south-central Asia (5.1%) (1).

A recent systematic review evaluated the effectiveness of inpatient management for SAM using the WHO protocol, as well as community-based treatments in low- and middle-income settings (19). The authors found that case fatality rates for inpatient management of SAM, following the WHO protocol, which involves fluid management and micronutrient supplementation, ranged from 3.4% to 35%. Only two studies reported nutrition recovery rates, which were 79.7% and 83.3%. For the community-based treatment of SAM that involves the use of ready-

to-use therapeutic food (RUTF), 51% of children were more likely to achieve nutritional recovery than a standard care group. Although, this systematic review was limited in the availability of high quality studies, the nutritional recovery rates of the interventions reviewed were advantageous. The authors have concluded that future studies are warranted to compare approaches to managing SAM and this includes identifying and tackling other aggravating determinants of SAM.

Growth faltering

Stunting (height-for-age Z score (HAZ) < 2), wasting (weight-for-height Z score (WHZ) < 2) and underweight (weight-for-age Z score (WAZ) < 2) (18) are major indicators of child undernutrition. Severe undernutrition is considered when Z sores are <3. In 2011, approximately 165 million (25.7%) children under the age of five years globally had stunted growth, 52 million (8%) were classified as wasting and 100 million (16%) were underweight (20). South-central Asia (36% stunted, 15% wasting and 30% underweight) as well as East (42% stunted) and West Africa (36% stunted and 22% underweight) had the highest prevalence. Growth faltering in early life is a predisposing risk factor for poor cognitive development, reduced educational outcomes and economic productivity, as well as reduced survival in adulthood (1). Micronutrient deficiencies alongside recurring infections are some of the well-recognised causes of child growth faltering in developing countries. There are three micronutrient deficiencies of public health concern in developing countries; vitamin A, Iron and zinc deficiency. Interventions (supplementation) targeting these specific micronutrients and their impact on growth outcomes are summarised in **table 1**.

Zinc deficiency

125 A recent analysis conducted by Wessells and Brown (21) estimated the global prevalence of
126 zinc deficiency (ZD) as 17% in 188 countries, using zinc intake obtained from FAO food
127 balance sheets, with zinc and phytate contents calculated using a nutrient composition database
128 (table 1). Low-income countries such as those in sub-Saharan Africa and South Asia were most
129 at risk with a ZD prevalence of over 25% (21). ZD is primarily caused by low intake of animal
130 products and exacerbated by persistent diarrhoea (22, 23). ZD can negatively impact the
131 immune system, thereby enhancing susceptibility to infectious diseases such as diarrhoea,
132 malaria and pneumonia, especially in children (22). It may also aggravate intestinal
133 permeability and chronic inflammation, both pathways that underlie environmental
134 enteropathy, which is a sub-clinical condition involving reduced intestinal function that can
135 affect micronutrient absorption (23). Zinc has a fundamental role in cell division and growth;
136 thus, it can result in decreased concentrations of circulatory Insulin-like Growth Factor 1 (IGF-
137 1), a possible pathway for slowed child growth in Zinc deficient children (24).
138
139 ZD in developing countries coincides with the high prevalence of stunted growth in children
140 observed in these countries (1, 21). In fact, assessing the number of children < 5 years old that
141 have stunted growth has been considered to be a proxy for zinc deficiency (21, 25), although
142 this is an indirect method of measuring ZD, and consequently is subject to confounding factors.
143 It would be expected, therefore, that zinc supplementation would have a positive effect on
144 growth. Four meta-analyses (26-29) have been identified that have investigated the impact of
145 zinc supplementation on growth indices in childhood (table 1). Three meta-analyses found that
146 zinc supplementation had a significant positive effect on linear growth (26, 27, 29) and two
147 found it had a positive effect on weight gain (26, 27). In contrast, Ramakrishnan et al. (28)
148 found no effect of zinc supplementation on linear growth or weight change but did find a
149 significant positive effect on change in WHZ score. Although it is apparent from the

aforementioned evidence that zinc can have a positive impact on growth, it is important to highlight that its effect is only marginal.

Iron deficiency

Iron deficiency (ID) is the leading cause of anaemia (haemoglobin < 110g/L) and accounts for ~50% of all cases (30). For this reason anaemia is typically used as a proxy for ID. Stevens et al. (31) estimated the global prevalence of total and severe anaemia in three population groups known to be most vulnerable to these conditions; women of child bearing age (15-49 years), children (6-59 months) and pregnant women. Using representative population based data collected from 107 countries, it was evident that anaemia is of epidemic proportions worldwide (table 1). Regional analysis showed Central and West Africa as having the highest prevalence of anaemia and severe anaemia in children aged < 5 years in 1995 (80% and 9.7%) and 2011 (71% and 4.9%). The high prevalence observed in developing parts of the world is mostly likely due to diets low in iron rich foods alongside poor absorption and diets high in phytate compounds that inhibit iron absorption (32). Parasite infections as well as tuberculosis and HIV are also thought to be risk factors.

Poor growth and cognitive development during childhood have been suggested as major consequences of iron deficiency; although, the evidence supporting these suggestions is inconclusive. For example, several systematic reviews and meta-analyses of randomized controlled trials (RCTs) have failed to discover a positive effect of iron supplementation on different growth parameters in children (table 1) (33-36). However, a recent systematic review and meta-analysis (37), found a small positive effect on growth (HAZ) in children that were aged between 5 and 12 years. Likewise, systematic reviews have reported that iron supplementation can have an impact on cognitive development especially in older children (37,

38) but appears to be ineffectual in young children and infants (35, 36, 38, 39). This evidence indicates that iron supplementation may have more of an impact on growth performance and cognitive development during mid-childhood. Of course, this may challenge the view that interventions targeting growth should occur in the first 1,000 days of life (2, 3), as beyond this timeframe interventions are considered to be ineffectual. Nevertheless, it is noticed that the positive effect on growth reported in these studies (37) was only marginal, indicating that iron supplementation targeting mid childhood may only have limited success as a public health intervention.

Vitamin A deficiency

According to a WHO (40) report, vitamin A deficiency (VAD), defined as having serum (plasma) retinol concentrations less than < 0.70 μmol/l or having a history of night blindness in more severe cases, is considered a major public health problem in developing countries, specifically in Asia and sub-Saharan Africa. In that report, the global prevalence of VAD measured between 1995 and 2005 in pregnant women was 15.3% and when stratified according to WHO regions, Africa and Asia had the highest rates (14.3% and 18.4%). This trend was also observed in children under 5 years old. Global prevalence was 33.3%, with Africa (41.6%) and Asia (33.5%) having higher rates than other parts of the world.

The developing fetus and preschool aged children are considered to be at-risk populations, owing to the rapid growth and subsequent increased nutritional requirements during these stages of the life course. In developing countries these additional nutritional requirements are frequently not met owing to the lack of diet diversity, as well as the affordability of foods high in vitamin A such as animal products, citrus fruits and dark green vegetables.

200 Over the past decade, some observational studies have found that maternal VAD was associated
201 with lower birth weight (41, 42). In contrast, according to a recent systematic review and meta-
202 analysis vitamin A supplementation during pregnancy had no positive effect on birth weight
203 (43). Furthermore, vitamin A supplementation during childhood showed little or no effect on
204 growth performance in several RCTs (33, 44-47).

Table 1: Major micronutrient deficiencies of public health concern: prevalence, supplementation and growth outcomes in children

Micronutrient	Prevalence	Micronutrient supplementation on growth. Evidence from systematic reviews and meta-analyses	Effects on physical growth (95%CI)
Zinc	*Wessells and Brown (21)* Using country specific FAO food balance sheets All ages (> 6months) **Global:** 17.3 ± 11.1% **sub-Saharan Africa:** 25.6 ± 12.2% **South Asia:** 29.6 ± 3.6% Prevalence of inadequate zinc intake was correlated with the prevalence of stunting in children < 5 years (r = 0.48; p <0.001)	Brown et al. (26) Meta-analysis of RCTs Children <12 years or prepubertal Zinc supplementation ≥ 8 weeks	Zinc supplementation had a positive effect on change in height (effect size = 0.35; 95% CI: 0.19-0.51) and change in weight (effect size = 0.31; 95% CI: 0.18-0.44). There was no significant effect on WHZ.
		Brown et al. (27) Meta-analysis of RCTs Infants, pre-schooler and older pre-pubertal Zinc supplementation 2 weeks to 15 months	Zinc supplementation had a positive effect on change in height (effect size = 0.17; 95% CI: 0.08-0.26), change in weight (effect size = 0.12; 95% CI: 0.05-0.19) and a small marginal effect on change in WHZ score (effect size = 0.06; 95% CI: 0.00-0.12) compared with control groups.
		Ramakrishnan et al. (28) Meta-analysis of RCTs Children ≤5 years Zinc supplementation ≥ 8 weeks	Zinc supplementation had no significant positive effect on change height or weight gain but did have a small positive effect on WHZ score (effect size = 0.06; 95% CI: 0.01-0.11) in comparison with placebo-controlled groups.

		Imdad and Bhutta (29) Meta-analysis of RCTs Children < 5 years Zinc supplementation ≥ 8 weeks	Zinc supplementation had a positive effect on linear growth (effect size = 0.19; 95% CI: 0.08-0.30) compared to placebo-controlled group.
Iron	*Stevens et al., (31)* Iron deficiency anaemia (haemoglobin <110 g/L) Children < 5 years **Global:** 43% (95% CI: 38-47) **Central and West Africa:** 71% (95% CI: 67-74) **South Asia:** 58% (95% CI: 44-69)	Ramakrishnan et al. (33) Meta-analysis of RCTs Children < 18 years Iron supplementation ≥ 8 weeks	Iron supplementation had no significant effect on height or weight compared to a control group.
		Sachdev et al. (34) Meta-analysis of RCTs Children < 14 years Oral Iron supplementation duration 2 months to 12 months	Iron supplementation had no significant effect on WAZ, WHZ, HAZ, mid upper arm circumference, skinfold thickness or head circumference compared to control groups.
		Low et al. (37) Meta-analysis of RCTs Children 5 to 12 years Oral iron supplementation ≥ 5 days per week	Iron supplementation had no significant effect on absolute height or absolute weight or WHZ score but did have a significant but small positive effect on HAZ score compared with a control group (effect size = 0.09; 95% CI: 0.01-0.17).
		Pasricha et al. (35) Meta-analysis of RCTs Children aged 4-23 months	Iron supplementation had no significant effect ($P > 0.05$) on final weight, WAZ scores, change in

		Daily oral iron supplementation	weight, final length, HAZ scores, change in length or weight for length z score in comparison with the control group.
		Thompson et al. (36) Meta-analysis of RCTs Children 2 to 5 years Oral iron supplementation ≥ 5 days per week	Iron supplementation had no positive effect on final height, final weight, change in height and change in weight compared to a control group
Vitamin A	*WHO (40) (*Serum retinol <0.70 µmol/L) Children < 5 years **Global:** 33.3% (95% CI: 31.1-35.4) **Africa:** 44.4% (95% CI: 41.3-47.5) **South East Asia:** 49.9% (95% CI: 45.1-54.8)	Ramakrishnan et al. (33) Meta-analysis of RCTs Children < 18 years Vitamin A supplementation ≥ 8 weeks	Vitamin A supplementation had no positive effect on absolute height change or weight change

205 It is clear from the evidence above that supplementation interventions targeting the main
206 micronutrients of public health concern in developing countries are not entirely effective in
207 improving child growth. Vitamin A supplementation markedly has no impact on child growth,
208 whereas zinc and iron supplementation seem to have peripheral effects. This suggests that there
209 are other underlying determinants of child growth faltering that need to be addressed.

210

211 **Aflatoxin related undernutrition issues in the developing world**

212 *Aflatoxin exposure and its relationship with growth faltering.*

213 The development and application of the AF-alb biomarker has enabled a number of
214 epidemiology studies examining human health effects of aflatoxin exposure (48). This
215 biomarker, which is usually measured by an ELISA method (49) has shown a good correlation
216 with aflatoxin intake in adults through a groundnut based diet in The Gambia (50), as well as
217 in children through a maize-based weaning diet in Tanzania (51). Compared to other available
218 short term (for previous 1-2 days exposure) biomarkers such as the aflatoxin DNA adduct,
219 AFM1 and aflatoxin metabolites in urine, this biomarker reflects the previous 2-3 months
220 exposure at the individual level, and is therefore more appropriate for assessing chronic
221 exposure related health outcomes.

222

223 There is mounting evidence that aflatoxin exposure occurs from gestation onwards throughout
224 life (15). Exposure occurs *in utero* through the transfer of aflatoxins from the mother to the
225 foetus via the placenta. Several studies have investigated this route of exposure and have found
226 detectable levels of aflatoxin or AF-alb in cord blood samples (52-56). Only a few studies have
227 examined the impact of exposure *in utero* on birth weight (57-59). All have reported a
228 significant inverse relationship with higher exposure *in utero* corresponding to lower weight at

229 birth. A study by de Vries et al (57) conducted in rural Kenya, examined aflatoxin levels in
230 maternal and cord blood samples. Aflatoxin was detected in over half of the maternal samples
231 and 37% of the cord blood samples. Females born to aflatoxin positive mothers had a mean
232 birth weight that was 225g lower than those born to mothers free from aflatoxin exposure.
233 Similar results were observed in a study conducted in the Middle East by Abdulrazzaq et al.,
234 (58), where high aflatoxin levels in maternal and cord blood samples were significantly related
235 to lower birth weights (r = -0.654, P = 0.0001 and r = -0.565, P = 0.001, respectively). More
236 recently, a cross-sectional study of 785 pregnant Ghanaian women, after adjusting for socio-
237 demographic variables and other factors, found increased odds of delivering a baby with a low
238 birth weight in the highest quartile (59). The highest quartile represented the highest levels of
239 aflatoxin exposure measured in blood during pregnancy (OR, 2.09; 95% CI: 1.19–3.68).

240

241 Aflatoxin exposure *in utero* may also play a role in stunted growth in early childhood (up 24
242 months). Only one study to date has explored this temporal relationship (56), and found that
243 higher levels of AF-alb in maternal blood were significantly associated with lower weight (P =
244 0.012) and height (P = 0.044) gain, after adjusting for potential confounding factors.
245 Furthermore, the authors predicted that a reduction in maternal AF-alb level from 110 pg/mg
246 to 10 pg/mg would lead to a 2 cm increase in height and a 0.8 kg increase in weight within the
247 first 24 months of life.

248

249 Usually studies that have examined exposure *in utero* by measuring maternal blood only
250 obtained measurements at one point in time. A recent study conducted by Castelino et al., (60)
251 explored the effect of season and gestation stage on aflatoxin exposure in pregnant women from
252 Gambia. Results showed that mean AF-alb levels were higher during the dry season than the

253 rainy season. AF-alb levels increased marginally from early to later gestation during the dry
254 season (41.8 vs 34.5 pg/mg; $P < 0.05$). Although early pregnancy has been considered a period
255 when the foetus is most vulnerable, later pregnancy marks the fast growth period of the foetus,
256 which may exert a profound adverse impact on growth. Further research is warranted to
257 determine the longer term health effects of aflatoxin exposure during both early and late
258 pregnancy.

259

260 Weaning is the transition from breast milk to solid food, and typically commences between 3
261 and 6 months. It is often a period in developing countries when children are most susceptible
262 to PEM, specifically, kwashiorkor. Because weaning foods such as maize are prone to aflatoxin
263 contamination, there may also be high aflatoxin exposure during the weaning period. This was
264 evident in a study conducted by Gong et al., (61) in Benin and Togo, as children that were fully
265 weaned had approximately 2-fold higher mean AF-alb levels than children who were still
266 partially breastfed. Although breastfeeding is a period of lower aflatoxin exposure, there is still
267 some exposure from breast milk, with aflatoxin M1 having been found in breast milk samples
268 in many studies (62). Nevertheless, AFM1, which is the hydroxylated metabolite of aflatoxin
269 that is found in milk, is less toxic than AFB1 that is found in food; therefore extending the
270 breastfeeding period may help reduce the negative health impacts, such as growth faltering, that
271 are associated with aflatoxin exposure.

272

273 The impact of aflatoxin exposure on growth is considered the most prominent during the first
274 two years after birth. One of the first studies examining the association between aflatoxin
275 exposure and child growth performance was a cross-sectional study of 480 children from Benin
276 and Togo aged between 9-months and 5 years (13). Prevalence of aflatoxin was high in this

277 sample with 99% of the children having detectable levels and a reported geometric mean of
278 32.8 pg/mg. Undernutrition was also evident as 33%, 6 % and 29% of the children were
279 classified as having stunted growth (HAZ <-2), wasting (WHZ <-2) and being underweight
280 (WAZ <-2); respectively. Significant negative correlations between AF-alb and each of the
281 growth parameters were observed ($P = 0.001$ for stunting; $P = 0.047$ for wasting and $P = 0.005$
282 for underweight). Another cross-sectional study by Turner et al., (10) found that AF-alb levels
283 were weakly associated with wasting ($P = 0.034$) but not with stunting or underweight.

284

285 These earlier studies were the first in determining the association of aflatoxin dietary exposure
286 with growth impairment in human subjects, and generated hypotheses for further investigations.
287 Cross-sectional studies are the best way to measure prevalence (63); however, they do have
288 limitations, as they cannot be used to establish the temporal sequence of the relationship
289 observed. A subsequent study using a longitudinal design, examined the effects of aflatoxin
290 exposure on growth in a cohort of 200 children from Benin (16-37 months) followed up over
291 8-months (14). High prevalence of aflatoxin exposure was found across the cohort with almost
292 all samples being positive for aflatoxin at each time point and with mean AF-alb levels of 37.4
293 pg/mg (February), 38.7 pg/mg (June) and 86.8 pg/mg (October). Results showed that both AF-
294 alb levels measured in February and the mean AF-alb level from the three time points, were
295 inversely correlated with HAZ and WHZ growth parameters that were measured at the end of
296 the study. This relationship remained after adjusting for potential confounding factors such age,
297 sex, height, weaning status, SES and geographical location, although only for the HAZ growth
298 parameter ($P < 0.001$). Furthermore, there was a difference in height of 1.7 cm between the
299 highest and lowest AF-alb quartile over the 8 month period. This study has helped to show the
300 temporal relationship between aflatoxin exposure and impaired child growth. Although
301 additional longitudinal studies conducted in different geographical locations and populations

will strengthen the evidence on the likelihood of this effect being cause and effect. Furthermore, plausible mechanisms that link aflatoxin exposure with impaired child growth should be investigated.

Aflatoxin exposure and protein-energy malnutrition

It has been proposed that the development of kwashiorkor may be partly attributable to aflatoxin exposure, although the evidence is circumstantial. Both aflatoxin exposure and kwashiorkor are prevalent in hot and humid tropical countries where maize and rice are staples, both affect children in early life and both are associated with impaired child growth (15, 64). In addition, the clinical and metabolic manifestations of kwashiorkor are somewhat similar to those of aflatoxin exposure, such as fatty liver and immunosuppression (65).

As shown in **table 2**, the association between the exposure to aflatoxin and kwashiorkor has been investigated in a plethora of studies since the 1980's (65-78). The typical study designs employed by the majority of these studies were case-control or cross-sectional, and involved measuring the prevalence and concentration of aflatoxin in blood and urine samples. In most studies it was found that aflatoxin was detected more frequently or concentrations were higher in blood samples of children with kwashiorkor in comparison with children with marasmus, and healthy children (65, 68, 69, 76-78). Furthermore, aflatoxin was detected more often in liver specimens from children who had died from kwashiorkor compared to other diseases and other protein malnutrition disorders (66).

Although evidence suggests that aflatoxin exposure may be related to kwashiorkor prevalence, a causal relationship has not been established. Furthermore, most of the studies did not measure AF-alb levels in serum of exposed children, which has been shown to be a more reliable biomarker. A fundamental step in unravelling any link between aflatoxin and kwashiorkor is to understand the possibility that the metabolic manifestations of kwashiorkor affect the way that aflatoxins are metabolised and excreted from the body, or *vice versa*. Future studies, undertaking a longitudinal design are required to determine if aflatoxin exposure plays an aetiological role in the causation of kwashiorkor.

Table 2. The relationship between protein energy malnutrition and aflatoxin exposure

Study	Country/ study population	Aflatoxin Exposure		
		Blood – detection (%) and mean concentration	**Urine – detection (%) and mean concentration**	**Other – detection (%) and mean concentration**
Hendrickse et al. (65)	Country: Sudan 252 children K (n = 44) MK (n = 32) M (n = 70) AM controls (n = 106)	177 samples (total aflatoxin pg/ml). K (36.4%) (GM: 706) MK (21.9%) (GM: 412) M (19.3%) (GM: 211) AM controls (15.9%) (GM: 77) The difference between the groups approached significance ($P = 0.05$). Kwashiorkor group mean aflatoxin concentration was significantly higher than the control group ($P = 0.01$).	250 samples (total aflatoxin pg/ml). K (36.4%) (GM: 706) MK (21.9%) (GM: 412) M (19.3%) (GM: 211) AM controls (15.9%) (77) No significant differences between the groups identified.	
Lamplugh and Hendrickse, (66)	Country: Nigeria and South Africa 8 children (aged between 9 months and 24 months) K (n = 3) MK (n = 3) M (n = 1) Control (n = 1)			8 autopsy liver specimens. K (all three of the liver samples contained AFB1: 2000, 4900 and 1400 pg/g). MK (1 liver sample had no aflatoxins; one contained a small quantity of aflatoxin M1 (15 pg/g) and in the third sample aflatoxicol was found (8500 pg/g). M (no aflatoxins found) Control (no aflatoxin found)

Apeagyei et al, (67)	Country: Ghana 22 children (aged between 5 months and 48 months) K (n = 22)			22 autopsy liver specimens Aflatoxin B1 was detected in 20 of the samples (90.9%). The remaining 2 samples contained aflatoxicol (9.1%).
Coulter et al., (68)	Country: Sudan 584 children K (n = 141) MK(n = 152) M (n = 152) AM controls (n = 180)	457 samples (total aflatoxin pg/ml) K (37.7%) (GM:154) MK (28.6%) (GM: 82) M (26.3%) (GM: 77) AM controls (21.3%) (GM: 81) Difference between the number of positive samples found in each group was significant (P <0.05). No differences between the groups in concentrations of aflatoxin identified.	463 samples (total aflatoxin pg/ml) K (27.2%) (GM: 308) MK(39.0%) (GM: 490) M (26.1%) (GM: 438) AM controls (28.4%) (GM:258) No significant difference between the numbers of positive samples found in each group. No significant differences between the groups in concentrations of aflatoxin identified.	
deVries et al., (69)	Country: Kenya 41 children K (n = 14) MK (n = 6) M (n = 11) Controls (n = 10)	39 samples (total aflatoxin (pg/ml) K (64%) (mean: 6666) MK (50%) (mean: 386) M (36%) (mean: 3412) Controls (30%) (mean: 759)	36 samples (total aflatoxin pg/ml) K (42%) (mean: 324) MK (60%) (mean: 1294) M (45%) (mean: 261) Controls (75%) (mean: 759) No differences in detection rates.	
de Vries et al., (70)	Country: Kenya 13 children K (n = 5)		K (4 out of 5 children excreted aflatoxin via urine).	K (all 5 of the children excreted aflatoxin in their stools).

	MK (n = 7) Underweight (n = 1)		MK (5 out of 7 children excreted aflatoxin via urine). The underweight child's urine samples tested negative for aflatoxin.	Total aflatoxin excreted (urine and stools) ranged from 0.08 ug/kg to 4 ug/kg body weight). MK (3 out of 7 children excreted aflatoxin in their stools). Total aflatoxin excreted (urine and stools) ranged from nil to 1.5 ug/kg body weight). The underweight child's stools tested negative for aflatoxin.
Household and Hundt (71)	Country: South Africa 320 children (mean age of 38 months) K (n = 47) M (n = 17) Controls (n = 256)		448 urine samples Aflatoxin B1, B2, G1, G2 and aflatoxicol were not detected in any of the samples.	
Ramjee et al., (72)	Country: South Africa 109 children aged between 6 months and 2 years K (n = 45) M (n = 13 Underweight (n = 16) AM controls (n = 35)	109 samples K (56%) M (31%) Underweight (56%) AM controls (49%) No differences among the groups in the number of aflatoxin positive results.	50 samples K (16%) M (10%) Underweight (no samples tested) Age matched controls (25%) No differences among the groups in the number of aflatoxin positive results. The serum/ urine ratio was significantly higher in the kwashiorkor group than in the other groups ($P = 0.001$).	

Adhikari et al., (73)	Country: South Africa 36 children aged between 6 months and 2 years K (n=36)	36 samples Aflatoxin was detected in 21 samples (58%)		
Oyelami et al., (74)	Country: Nigeria 40 children (aged between 4 and 168 months) 20 children who died from kwashiorkor 20 children who died of other diseases			40 lung specimens K (90%) Other diseases (65%) No significant differences among the groups in the number of aflatoxin positive results.
Oyelami et al., (75)	Country: Nigeria 45 children 24 children who died from kwashiorkor (aged between 6 months and 72 months) 21 children who died of other diseases (aged between 4 months and 168 months)			45 kidney specimens (total aflatoxin pg/g) K (58%) (mean: 3851) Other diseases (62%) (mean: 1271) No significant differences among the groups in the number of aflatoxin positive results. No differences among the groups in mean concentrations of total aflatoxins.
Hatem et al., (76)	Country: Egypt 70 children (aged between 6 and 24 months) K (n = 30) M (n = 30) AM controls (n = 10)	30 samples (total aflatoxin ng/ml) K (80%) (mean: 70.58) Ma (46.7%) (mean: 25.21) AM controls (0) Aflatoxins were detected more frequently in blood samples of	30 samples (total aflatoxin ng/100ml) K (80%) (mean: M (46.7%) AM controls (0) Aflatoxins were detected more frequently in urine samples of	

		the kwashiorkor group than the marasmus group (P = 0.007). Mean serum levels of total aflatoxin were significantly higher in the kwashiorkor group relative to the marasmus group (P <0.001).	the kwashiorkor group than the marasmus group (P = 0.007). Mean levels of total aflatoxin excreted in urine were significantly higher in the kwashiorkor group relative to the marasmus group (P = 0.052).
Tchana et al., (77)	Country: Cameroon 78 children (aged between 13 months and 12 years) K (n = 31) MK (n=11) AM controls (n = 36)		42 samples (aflatoxin B1) K (35.5%) MK (45.5%) AM controls (11.1%) Detection levels of AFB1 excreted in urine were significantly higher in the kwashiorkor and marasmus kwashiorkor groups relative to the control group (P <0.05).
Onyemelukwe et al. (78)	Country: Nigeria 111 children (aged between 7 months and 60 months) K (n = 36) MK (n = 29) M (n = 13) AM controls (n = 33)	111 samples (total aflatoxin ug/L) K (88.9%) (median: 165.6) MK (93.1%) (median: 228.4) M (76.9%) (median: 234.3) AM controls (63.6%) (median: 20.7) Median serum levels of total aflatoxin were significantly higher in each protein energy malnutrition group relative to	55 samples (total aflatoxin ug/L) K (84.6%) (median: 79) M (60%) (median: 43.8) Ma (81.8%) (median: 14.4) AM controls (90.9%) (median: 42.6) No differences among the groups in the number of aflatoxin positive results.

	the control group (kwashiorkor vs. control P <0.001, marasmic kwashiorkor vs. control P <0.001, marasmus vs. control P = 0.031). There were no significant differences between the protein malnutrition groups.	Median total aflatoxin levels in urine samples were significantly higher in the kwashiorkor group relative to the marasmus group (P = 0.011). No other significant differences were identified between the groups.

Abbreviation: AM, age-matched; GM, geometric mean; K, kwashiorkor; M, marasmus; MK, Marasmic kwashiorkor

Aflatoxin exposure and micronutrient deficiencies

It has been hypothesized that aflatoxin exposure mediates intestinal damage resulting in reduced nutrient absorption and increased intestinal permeability resulting in faltered growth (79, 80). It is, therefore, possible that aflatoxin exposure exacerbates micronutrient deficiencies and by reducing aflatoxin exposure the incidence of micronutrient deficiencies may be reduced correspondingly. Previous research has established the relationship between aflatoxin exposure and the effect on these micronutrients in feeding experiments in animal studies as reviewed by Williams et al., (81). Increasing levels of aflatoxin in feed were significantly related to decreasing concentrations of vitamin A in poultry (82); vitamin D concentrations in chickens (83); vitamin A and E in swine (84) as well as zinc in piglets (85).

Owing to the species difference, it is difficult to directly apply these findings to humans. Only a few studies have been identified that have examined the relationship between micronutrient concentrations and aflatoxin exposure in humans. Two of these studies were conducted in children (10, 14). As part of their investigation into the effect of aflatoxin exposure on immune function in Gambian children aged between 6 and 9 years Turner et al. (10) investigated the correlation between vitamins A (a- and b-carotene and lycopene) and C with AF-alb levels. Vitamin C was the only micronutrient that demonstrated an inverse relationship with AF-alb ($P = 0.01$). A study conducted by Gong et al. (14) that examined the relationship between aflatoxin exposure during the post weaning period and growth faltering, measured vitamin A and zinc levels to assess if they were potential confounding factors. No significant correlations between vitamin A and zinc with AF-alb levels were observed. A more recent cross-sectional study (86) of 147 Ghanaian adults found a significant negative correlation between AF-alb levels and vitamin A concentrations in plasma samples (-0.20; $p<0.05$). Participants with high AF-alb

levels (>0.80 pmol/mg albumin) had a 2.6-fold greater risk of having lower vitamin A levels after adjusting for potential confounding factors (odds ratio = 2.61; CI = 1.03- 6.58; P = 0.04). Tang et al., (87) found similar results in another sample of 507 Ghanaian adults. A correlation analysis revealed significant negative correlations between AFB1-albumin adducts and vitamin A ($r = -0.110$; $p = 0.013$) and vitamin E ($r = -0.149$; $p < 0.001$).

It is very difficult to draw specific conclusions based on the above evidence. Firstly, only a small number of studies have been identified that have examined the relationship between aflatoxin exposure and micronutrient deficiency in human subjects. Secondly, two studies found no associations between vitamin A and AF-alb levels, whereas two studies did, indicating that this relationship is not consistent across studies. Furthermore, the temporal relationship has not yet been investigated as the above studies were cross-sectional; although Gong et al. (14) was a longitudinal study, the micronutrients measured were only considered as potential confounding factors for the relationship between aflatoxin exposure and impaired child growth, and further explorations of these variables were not carried out. It is, consequently, still unknown whether aflatoxin exposure exacerbates micronutrient deficiencies and if this contributes to impaired child growth, which previous researchers have advocated (80). Future studies opting for a longitudinal or experimental (RCT) design are warranted to help establish whether a temporal relationship exists.

Possible mechanisms for aflatoxin's effects on growth

It has been hypothesized that aflatoxin may affect child growth through one or more of three mechanisms; 1) by contributing to enteropathy, 2) immune suppression and 3) modulating the insulin-like growth factor (IGF) pathway through liver toxicity (79, 80). Enteropathy is a

frequent condition observed in babies in Africa, and may be partly attributable to aflatoxin related toxic damage to the intestine epithelium, which leads to further "leak" of nutrients, i.e. aflatoxin exacerbates the reduction of nutrient uptake in an environment where undernutrition is already rife. The immune suppression effect of aflatoxin, for which there is a lot of evidence in animal species (88), and increasing evidence in humans (10-12), could enhance susceptibility to infections such as those causing diarrhoea, which would reduce nutrient uptake. Liver toxicity due to chronic aflatoxin exposure may damage the production of Insulin like Growth Factor pathway proteins (IGFs) in the liver, leading to reduced IGFs in circulation and an adverse impact on child growth. A recent in vitro study using human liver cells demonstrated that aflatoxin down-regulated IGFs genes and protein levels in a dose-dependent manner (89). In agreement with this result, both IGF1 and IGFBP3 levels were found to be inversely correlated with AF-alb biomarker in Kenyan schoolchildren. Although the effect of aflatoxin on IGFs only explained about 16% of total effect of aflatoxin on child growth, given the complex causes of child stunting, the data provides preliminary evidence that aflatoxin-induced changes in IGFs could contribute to growth impairment where aflatoxin exposure is high (89).

Aflatoxin co-exposure with other mycotoxins on child undernutrition

Many countries in sub-Saharan Africa have a largely maize-based diet for both weaning food and family food. It has been noted that groundnuts, although often having higher incidence and levels of aflatoxin contamination than maize, rarely cause aflatoxicosis. Major aflatoxicosis often occurs in populations with high maize consumption. This is partly because maize is a major component of the diet and is consumed in much larger amounts than groundnuts. Another possibility is that another mycotoxin, fumonisin, often co-occurs with aflatoxin in maize in these regions (90-92) and it is hypothesized that the co-exposure may greatly enhance aflatoxin

toxicity, both acute (aflatoxicosis), and chronic such as the childhood hepatomegaly reported in Kenya (9).

Weaning food was found to be frequently co-contaminated with aflatoxin and fumonisin in Tanzania, and fumonisin exposure by dietary assessment has been reported to be associated with child stunting and linear growth in Tanzania (93). One hundred and sixty-six children (aged 6-14 months) from representative regions in Tanzania were studied longitudinally over one year to examine exposure to both mycotoxins and its impact on child growth. AF-alb levels tripled during the first 6 months, and further doubled during the second 6 months, with mean levels of 4.7, 12.9 and 23.5 pg/mg, respectively. Fumonisin exposure measured using urinary FB1 biomarker was exceedingly high at both maize harvest seasons but with a lower level observed at 6 months after harvest, reflecting a field mycotoxin contamination pattern (92). Urinary FB1 at recruitment were negatively associated with HAZ at both 6 months and 12 months from recruitment. Mean levels of urinary FB1 had an inverse association with HAZ at 12 months from recruitment and length velocity. The negative association between AF-alb and HAZ was not significant, possibly owing to study power limitation. These data show that fumonisin may contribute to child growth impairment and highlight the potential role of co-contamination with aflatoxin and fumonisin. More recently, Srey et al. (94) reported exposure to dietary deoxynivalenol (DON), another mycotoxin with known growth inhibition in animals, also occurs in these children, in agreement with food based exposure analysis in Tanzania (95). This suggests that the children are frequently exposed to the three mycotoxins, all of which may have an impact on growth faltering.

An increasing number of recent studies have reported multi-mycotoxin exposure in different populations including some African groups (96-98). The methodology applied in these studies

typically involves simultaneous measurement of multiple mycotoxins using advanced LC-MS/MS technique, and this offers great advantages as it gives useful data on multi-mycotoxin exposure in a population. At present validation of the approach when applied to health outcomes is in its infancy. It was evident from these studies that firstly, multiple mycotoxins co-exist in staple foods such as maize and their by-products (96) and secondly, human populations in Africa are co-exposed to proportionally high levels of multi-mycotoxins (97, 98). Ediage et al. (97) cross-sectional study found no association between stunting, wasting or underweight in children aged under five, although multiple mycotoxins were found in urine samples. Whilst the multi-mycotoxin measurements require further validation, these studies provide a preview of the co-exposure issue and with time more will be revealed, adding further complexity to the health risk studies. How to assess the health outcomes associated with multiple toxins will thus be a critical challenge ahead and this will lead to a new era of multiple toxins exposure assessment methodology development.

Conclusions

Aflatoxin exposure is highly prevalent in developing countries; often this co-exists with malnutrition, enteropathy, and infectious disease in young children. The fact that over 90% of samples from young children from West Africa had detectable AF-alb, in contrast to less than 1% in the developed world clearly demonstrates a huge public health burden associated with aflatoxin in sub-Saharan Africa. The greatest challenge ahead is not only to understand how these problems may interactively impact on child stunting, but more importantly to explore the most effective intervention method for child undernutrition, and eventually to reduce child mortality. Many supplementation trials targeting the major micronutrient deficiencies aimed at improving child growth have failed to produce a significant positive effect. We believe that the

452 high levels of aflatoxin exposure in these populations are likely to be exacerbating the problems
453 posed by child undernutrition and that future nutrition interventions should take aflatoxin
454 exposure into account. The most effective outcomes are likely to be produced by an attack on
455 two fronts- reduction of aflatoxin exposure and improvement in nutritional status.

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