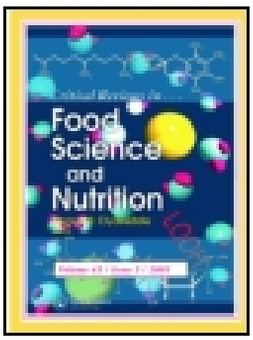

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Sinead Watson, Yun Yun Gong & Michael Routledge (2015): Interventions Targeting Child Undernutrition in Developing Countries May Be Undermined by Dietary Exposure to Aflatoxin, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1040869

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1040869

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

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Abstract

Child undernutrition, a form of malnutrition, is a major public health burden in developing countries. Supplementation interventions targeting the major micronutrient deficiencies have only reduced the burden of child undernutrition to a certain extent, indicating that there are other underlying determinants that need addressed. 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 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 children.

Keywords

Aflatoxin, child undernutrition, stunting, kwashiorkor, micronutrient deficiencies

Introduction

Malnutrition includes both overweight, which occurs when particular nutrients are over consumed, and undernutrition, which occurs when there is inadequate energy and/ or nutrient intake, poor nutrient absorption or nutrient loss due to infection (Black et al., 2008). Undernutrition, including stunting, wasting as well as micronutrient deficiencies, is a major public health problem for low-income countries, specifically among young children (Black et al., 2013). 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 (Black et al., 2013). 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 (Black et al., 2013).

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 (Shrimpton et al., 2001; Victora et al., 2010). Bhutta et al., (2008) reviewed the potential effect on child undernutrition outcomes of nutrition-specific 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, causes of undernutrition are multifactorial and are not solely related to dietary intake. Nutrition-specific interventions address the immediate determinants of child undernutrition, such as inadequate food and nutrient intake, but do not consider the underlying causes such as food insecurity, poverty and limited access to clean water, hygienic environments and health services (Black et al., 2013; Ruel et al., 2013).

There is increasing evidence that exposure to aflatoxin could be an underlying factor of child undernutrition. Aflatoxin is a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* that contaminates 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 (Pitt et al., 2013). Further growth of the fungus and production of aflatoxin is enhanced by post-harvest storage conditions that involve high humidity (Mutegi et al., 2013). There are four main types of aflatoxin, namely aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). AFB_1 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 M_1 (AFM_1) 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 (Azziz-Baumgartner et al., 2005). Chronic exposure can increase the risk of liver cancer (IARC, 2002), 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 (Gong et al., 2012), immune suppression (Turner et al., 2003; Jiang et al., 2005, 2008) and growth faltering in children (Gong et al., 2002, 2004). Chronic aflatoxin exposure is evident throughout life, including the critical first 1,000 days (Wild and Gong, 2010).

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 nutrition-specific interventions for improving child growth, review the evidence for aflatoxin exposure exacerbating undernutrition and reflect on the necessity for considering aflatoxin exposure in nutrition 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 (Ahmed et al., 2012). PEM is often a consequence of inadequate food intake; lack of diet diversity; consuming a diet of poor protein quality, typically cereal based and

low in animal products; suboptimal breastfeeding; delayed and/ or inadequate supplementation of appropriate complementary foods and infection that can lead to decreased absorption of essential nutrients (Latham, 1997).

In 2000, the WHO estimated that 26.7% of children < 5 years of age in developing countries had PEM (WHO, 2002). There is a paucity 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) (WHO, 1986). 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%) (Black et al., 2013).

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, focusing on children under the age of five years (Lenters et al., 2013). The authors found that case fatality rates for inpatient management of SAM, following the WHO protocol, which involves treating infections, 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, according to WHO standards) (WHO, 1986) 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 (UNICEF/WHO/World Bank, 2012). 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 (Black et al., 2013). 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 micronutrient deficiencies and their impact on growth outcomes are summarised in **table 1**.

Zinc deficiency

A recent analysis conducted by Wessells and Brown (2012) estimated the global prevalence of zinc deficiency (ZD) as 17% in 188 countries, using zinc intake obtained from FAO food balance sheets, with zinc and phytate contents calculated using a nutrient composition database (table 1).

Low-income countries such as those in sub-Saharan Africa and South Asia were most at risk with a ZD prevalence of over 25%. ZD is primarily caused by low intake of animal products and exacerbated by persistent diarrhoea (Ahmed et al., 2012; Lindenmayer et al., 2014). ZD can compromise aspects of immunity, thereby enhancing susceptibility to infectious diseases such as diarrhoea, malaria and pneumonia (Ahmed et al., 2012). It may also aggravate intestinal permeability and chronic inflammation, both pathways that underlie environmental enteropathy, which is a sub-clinical condition involving reduced intestinal function that can affect micronutrient absorption (Lindenmayer et al., 2014). Zinc has a fundamental role in cell division and growth; thus, it can result in decreased concentrations of circulatory Insulin-like Growth Factor 1 (IGF-1), a possible pathway for slowed child growth in ZD children (Prasad, 2013).

ZD in developing countries coincides with the high prevalence of stunted growth in children observed in these countries (Wessells and Brown, 2012). In fact, assessing the number of children under five years old that have stunted growth has been considered to be a proxy for zinc deficiency (de Benoist et al., 2007; Wessells and Brown, 2012); although, this is an indirect method of measuring ZD, and consequently is subject to confounding factors. It would be expected, therefore, that zinc supplementation would have a positive effect on growth. Four meta-analyses (Brown et al., 2002, 2009; Ramakrishnan et al., 2009; Imdad and Bhutta, 2011) have been identified that have investigated the impact of zinc supplementation on growth indices in childhood (table 1). Three meta-analyses found that zinc supplementation had a significant positive effect on linear growth (Brown et al., 2002, 2009; Imdad and Bhutta, 2011) and two found it had a positive effect on weight gain (Brown et al., 2002, 2009). In contrast, Ramakrishnan et al. (2009) found no effect of zinc supplementation on linear growth or weight

change but did find a 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 anemia (hemoglobin < 110g/L) and accounts for ~50% of all cases (UNICEF/UNU/WHO, 2001). For this reason anemia is typically used as a proxy for ID. Stevens et al. (2013) estimated the global prevalence of total and severe anemia 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 anemia is of epidemic proportions worldwide (table 1). Regional analysis showed Central and West Africa as having the highest prevalence of anemia and severe anemia 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 (Zimmermann and Hurrell, 2007). 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 randomised controlled trials (RCTs) have failed to discover a positive effect of iron supplementation on different growth parameters in children (table 1) (Ramakrishnan et al., 2004; Sachdev et al., 2006; Pasricha et al., 2013; Thompson et al., 2013). However, a recent systematic review and

meta-analysis (Low et al., 2013), 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 (Sachdev et al., 2005; Low et al., 2013) but appears to be ineffectual in young children and infants (Sachdev et al., 2005, Szajewska et al., 2010; Pasricha et al., 2013; Thompson et al., 2013). 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 (Shrimpton et al., 2001; Victoria et al., 2010), as beyond this timeframe interventions are considered to be ineffectual. Nevertheless, it is noticed that the positive effect on growth reported in these studies (Low et al., 2013) 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 (2009) 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 five 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 and dark green vegetables. Over the past decade, some observational studies have found that maternal VAD was associated with lower birth weight (Gazala et al., 2003; Tielsch et al., 2008). In contrast, according to a recent systematic review and meta-analysis vitamin A supplementation during pregnancy had no positive effect on birth weight (Thorne-Lyman and Fawzi, 2012). Furthermore, vitamin A supplementation during childhood showed little or no effect on growth in several RCTs (Rivera et al., 2003; Rmakrishnan et al., 2004; Mahawithange et al., 2007; Chhagan et al., 2010; Lima et al., 2010).

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

Aflatoxin related undernutrition issues in the developing world

Aflatoxin exposure and its relationship with growth faltering

The development and application of the AF-alb biomarker has enabled a number of epidemiology studies examining human health effects of aflatoxin exposure (Routledge and Gong, 2011). This biomarker, which is usually measured by an ELISA method (Chapot and

Wild, 1991), has shown a good correlation with aflatoxin intake in adults through a groundnut based diet in The Gambia (Wild et al., 1992), as well as in children through a maize-based weaning diet in Tanzania (Routledge et al., 2014). Compared to other available short term (for previous 1-2 days exposure) biomarkers such as the aflatoxin DNA adduct, AFM1 and aflatoxin metabolites in urine, this biomarker reflects the previous 2-3 months exposure at the individual level, and is therefore more appropriate for assessing chronic exposure related health outcomes.

There is mounting evidence that aflatoxin exposure occurs from gestation onwards throughout life (Wild and Gong, 2010; Khlangwiset et al., 2011). Exposure occurs *in utero* through the transfer of aflatoxins from the mother to the foetus via the placenta. Several studies have investigated this route of exposure and have found detectable concentrations of aflatoxin or AF-alb in cord blood samples (Maxwell et al., 1989; Dennings et al., 1990; Wild et al., 1991; Jonsyn et al., 1995; Turner et al., 2007). Only a few studies have examined the impact of exposure *in utero* on birth weight (de Vries et al., 1989; Abdulrazzaq et al., 2004; Shuaib et al., 2010). All have reported a significant inverse relationship with higher exposure *in utero* corresponding to lower weight at birth. A study by de Vries et al. (1989) conducted in rural Kenya, examined aflatoxin concentrations in maternal and cord blood samples. Aflatoxin was detected in over half of the maternal samples and 37% of the cord blood samples. Females born to aflatoxin positive mothers had a mean birth weight that was 225g lower than those born to mothers free from aflatoxin exposure. Similar results were observed in a study conducted in the Middle East by Abdulrazzaq et al., (2004), where high aflatoxin concentrations in maternal and cord blood samples were significantly related to lower birth weights ($r = -0.654$, $P < 0.0001$ and $r = -0.565$, $P < 0.001$, respectively). More recently, a cross-sectional study of 785 pregnant Ghanaian

women, after adjusting for socio-demographic variables and other factors, found increased odds of delivering a baby with a low birth weight in the highest quartile (Shuaib et al., 2010). The highest quartile represented the highest concentrations of aflatoxin exposure measured in blood during pregnancy (OR, 2.09; 95% CI: 1.19–3.68).

Aflatoxin exposure *in utero* may also play a role in stunted growth in early childhood. Only one study to date has explored this temporal relationship (Turner et al., 2007), and found that higher concentrations of AF-alb in maternal blood were significantly associated with lower WAZ (-0.25; P = 0.012) and HAZ (-0.21; P = 0.044) measurements, after adjusting for potential confounding factors. Furthermore, the authors predicted that a reduction in maternal AF-alb concentration from 110 pg/mg to 10 pg/mg would lead to a 2 cm increase in height and a 0.8 kg increase in weight within the first 12 months of life.

Usually studies that have examined exposure *in utero* by measuring maternal blood only obtained measurements at one point in time. A recent study conducted by Castelino et al., (2014) explored the effect of season and gestation stage on aflatoxin exposure in pregnant women from Gambia. Results showed that mean AF-alb concentrations were higher during the dry season than the rainy season. AF-alb concentrations increased marginally from early to later gestation (34.5 pg/mg vs. 41.8 pg/mg; $P < 0.05$). Although early pregnancy has been considered a period when the foetus is most vulnerable, later pregnancy marks the fast growth period of the foetus, which may exert a profound adverse impact on growth. Further research is warranted to determine the longer term health effects of aflatoxin exposure during both early and late pregnancy.

Weaning is the transition from breast milk to solid food, and normally begins between 3 and 6 months. It is often a period in developing countries when children are most susceptible to PEM. Because weaning foods such as maize are prone to aflatoxin contamination, there may also be high aflatoxin exposure during the weaning period. This was evident in a study conducted by Gong et al., (2003) in Benin and Togo, as children that were fully weaned had approximately 2-fold higher mean AF-alb concentrations than children who were still partially breastfed. Although breastfeeding is a period of lower aflatoxin exposure, there is still some exposure from breast milk, with AFM1 having been found in breast milk samples in many studies (Khlangwiset et al., 2011). AFM1, a hydroxylated metabolite of aflatoxin, has a hepatotoxic and a hepatocarcinogenic potential, and is recognised by the International Agency for Research on Cancer as a possible human carcinogen (Group 2B) (IARC, 2002). There is limited information, however, regarding the adverse health effects of AFM1 in humans, especially in exclusively breastfed children (IARC, 2002). Nevertheless, exclusive breastfeeding up to six months should still be encouraged, as AFM1 found in milk is less toxic and occurs at lower levels than AFB1 that is found in food. Furthermore, breast milk is abundant in immunological and nutritional components (Thibeau and D'Apolito, 2012), which are essential for proper growth and development.

The impact of aflatoxin exposure on growth is considered the most prominent during the first two years after birth. One of the first studies examining the association between aflatoxin exposure and child growth performance was a cross-sectional study of 480 children from Benin and Togo aged between nine months and five years (Gong et al., 2002). Prevalence of aflatoxin was high in this sample with 99% of the children having detectable concentrations and a reported

geometric mean of 32.8 pg/mg. Undernutrition was also evident as 33%, 6% and 29% of the children were classified as having stunted growth (HAZ <-2), wasting (WHZ <-2) and being underweight (WAZ <-2); respectively. Significant negative correlations between AF-alb and each of the growth parameters were observed (P = 0.001 for stunting; P = 0.047 for wasting and P = 0.005 for underweight). Another cross-sectional study of 472 Gambian children aged between six and nine years (Turner et al., 2003), found that AF-alb concentrations were weakly associated with wasting (P = 0.034) but not with stunting or underweight.

These earlier studies were the first in determining the association of aflatoxin dietary exposure with growth impairment in human subjects, and generated hypotheses for further investigations. Cross-sectional studies are the best way to measure prevalence (Mann, 2003); however, they do have limitations, as they cannot be used to establish the temporal sequence of the relationship observed. A subsequent study using a longitudinal design, examined the effects of aflatoxin exposure on growth in a cohort of 200 children from Benin (16-37 months) followed up over 8-months (Gong et al., 2004). High prevalence of aflatoxin exposure was found across the cohort with almost all samples being positive for aflatoxin at each time point and with mean AF-alb concentrations of 37.4 pg/mg (February), 38.7 pg/mg (June) and 86.8 pg/mg (October). Results showed that both AF-alb concentrations measured in February and the mean AF-alb concentration from the three time points, were inversely correlated with HAZ and WHZ growth parameters that were measured at the end of the study. This relationship remained after adjusting for potential confounding factors such age, sex, height, weaning status, socio-economic status (SES) and geographical location, although only for the HAZ growth parameter (P < 0.001). Furthermore, there was a difference in height of 1.7 cm between the highest and lowest AF-alb

quartile over the eight month period. This study has helped to show the temporal relationship between aflatoxin exposure and impaired child growth. Although 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 (Wild and Gong, 2010; Hendrickse, 1997). Furthermore, they have similar clinical and metabolic characteristics, such as fatty liver and immunosuppression (Hendrickse et al., 1982).

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 (Hendrickse et al., 1982; Lamplugh and Hendrickse, 1982; Apeagyei et al., 1986; Coulter et al., 1986; de Vries et al., 1987; de Vries et al., 1990; Household and Hundt, 1991; Ramjee et al., 1992; Adhikari et al., 1994; Oyelami et al., 1997; Oyelami et al., 1998; Hatem et al., 2005; Tchana et al., 2010; Onyemelukwe et al., 2012). 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. Some studies 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 (Hendrickse et al., 1982; Coulter et al., 1986; de Vries et al., 1987; Hatem et al., 2005; Onyemelukwe et al., 2012). Furthermore, it has been suggested that children with kwashiorkor have an inability to metabolise aflatoxin, as in conjunction with the high concentrations found in serum, excretion of aflatoxin seems to occur at a lesser and slower rate (Ramajee et al., 1992). This is likely owed to the impaired function of the liver; a clinical manifestation of this protein malnutrition disorder (Ramjee et al., 1992). Adhikari et al. (1994) proposed that this retention of aflatoxin may also help explain the increased number of infections observed in aflatoxin-positive children with kwashiorkor from Durban, South Africa, compared to aflatoxin-negative children with kwashiorkor.

Autopsies have also been conducted on liver (Lamplugh and Hendrickse, 1982; Apeagyei et al., 1986), lungs (Oyelami et al., 1997) and kidney (Oyelami et al., 1998) specimens of African children whom died as a result of PEM. Aflatoxin was detected more often in liver specimens from children who had died from kwashiorkor compared to other diseases and other protein malnutrition disorders (Lamplugh and Hendrickse, 1984).

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 concentrations 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.

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 (Gong et al., 2008; Smith et al., 2012). 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., (2004). Increasing concentrations of aflatoxin in feed were significantly related to decreasing concentrations of vitamin A in poultry (Pimpukdee et al., 2004); vitamin D concentrations in chickens (Glahn et al., 1991); vitamin A and E in swine (Harvey et al., 1994) as well as zinc in piglets (Mocchegiani et al., 1998).

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 human subjects (Turner et al., 2003, Gong et al., 2004; Tang et al., 2009; Obuseh et al., 2011). All the studies were conducted in West Africa. Two studies found no association between vitamin A and AF-alb concentrations in children (Turner et al., 2003; Gong et al., 2004), whereas two studies did find an inverse relationship in adult subjects (Tang et al., 2009; Obuseh et al., 2011); higher aflatoxin exposure was correlated with lower vitamin A concentration. Aflatoxin exposure was inversely correlated with vitamin C (Turner et al., 2003) and vitamin E (Tang et al., 2009) concentrations, but was not correlated with zinc (Gong et al., 2004).

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, the temporal relationship has not yet been investigated as the above studies were cross-sectional; although Gong et al. (2004) 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. Furthermore, seasonal influences were not taken into account when assessing these correlations; both aflatoxin exposure levels and micronutrient intakes can fluctuate with season. It is, consequently, still unknown whether aflatoxin exposure exacerbates micronutrient deficiencies and if this contributes to impaired child growth, which previous researchers have advocated (Smith et al., 2012). 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 (Gong et al., 2008; Smith et al., 2012). 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 (Bondy and Pestka, 2000), and increasing evidence in

humans (Turner et al., 2003; Jiang et al., 2005, 2008), 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 concentrations in a dose-dependent manner (Castelino et al., 2015). In agreement with this result, both IGF1 and IGFBP3 concentrations 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 (Castelino et al., 2015).

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 concentrations 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 (Kimanya et al., 2008; Kimanya et al., 2009; Shirima et al., 2013) 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 (Gong et al., 2012).

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 (Kimanya et al., 2010). 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 concentrations tripled during the first 6 months, and further doubled during the second 6 months, with mean concentrations 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 concentration observed at 6 months after harvest, reflecting a field mycotoxin contamination pattern (Shirima et al., 2013). Urinary FB1 at recruitment were negatively associated with HAZ at both 6 months and 12 months from recruitment. Mean concentrations 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. (2014) 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 (Kimanya et al., 2014). 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 (Abia et al., 2013; Ediage et al., 2013; Shephard et al., 2013). 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 (Abia et al., 2013) and secondly, human populations in Africa are co-exposed to proportionally high concentrations of multi-mycotoxins (Ediage et al., 2013; Shephard et al., 2013). Ediage et al. (2013) 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 undernutrition, 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 high concentrations of aflatoxin exposure in these populations are likely to be exacerbating the problems posed by child undernutrition and that future nutrition interventions should take aflatoxin exposure into account. The most effective outcomes are likely to be produced by an attack on two fronts- reduction of aflatoxin exposure and improvement in nutritional status.

Abbreviations

AFB_1, Aflatoxin B_1

AFB_2, aflatoxin B_2

AFG_1, aflatoxin G_1

AFG_2, aflatoxin G_2

AFM_1, aflatoxin M_1

AF-alb, aflatoxin-albumin adduct

DON, deoxynivalenol

FB_1, fumonisin B_1

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, randomised control trial

VAD, vitamin A deficiency

WAZ, weight-for-age Z score

WHZ, weight-for-height Z score.

Acknowledgments

The authors declare that there are no conflicts of interest. All authors were involved in the conception of the review, conducting the literature search, writing and editing the article. All authors read and approved the final article.

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Table 1: Major micronutrient deficiencies of public health concern: global prevalence and regions with the highest prevalence, micronutrient supplementation and physical growth outcomes in children

Micro-nutrient	**Prevalence**	**Micronutrient supplementation on growth. Evidence from systematic reviews and meta-analyses**	**Effects on physical growth among children who received micronutrient supplementation (95%CI)**
Zinc	Wessells and Brown, 2012	Brown et al., 2002	Change in height (effect size = 0.35; 95% CI: 0.19-0.51)
	Country specific FAO food balance sheets	Meta-analysis of RCTs	Change in weight (effect size = 0.31; 95% CI: 0.18-0.44)
	All ages (> 6 months)	Children <12 yrs or pre-pubertal	There was no significant effect on WHZ
	Global: 17.3 ± 11.1%	Zinc supplementation ≥ 8 wks	
	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., 2009	Change in height (effect size = 0.17; 95% CI: 0.08-0.26)
		Meta-analysis of RCTs	Change in weight (effect size = 0.12; 95% CI: 0.05-0.19)
		Infants, pre-schooler and pre-pubertal	Change in WHZ score (effect size = 0.06; 95% CI: 0.00-0.12)
		Zinc supplementation 2	

		wks to 15 mo	
		Ramakrishnan et al., 2009	No significant effect on change height or weight gain
		Meta-analysis of RCTs	Positive effect on WHZ score (effect size = 0.06; 95% CI: 0.01-0.11)
		Children ≤5 yrs	
		Zinc supplementation ≥ 8 wks	
		Imdad and Bhutta, 2011	Positive effect on linear growth (effect size = 0.19; 95% CI: 0.08-0.30)
		Meta-analysis of RCTs	
		Children < 5 yrs	
		Zinc supplementation ≥ 8 wks	
Iron	Stevens et al., 2013	Ramakrishnan et al., 2004	No significant effect on height or weight
	IDA (haemoglobin <110 g/L)	Meta-analysis of RCTs	
	Children < 5 years	Children < 18 yrs	
	Global: 43% (95% CI: 38-47)	Iron supplementation ≥ 8 weeks	
	Central & West Africa: 71% (95% CI: 67-74)		
	South Asia: 58% (95% CI: 44-69)		
		Sachdev et al., 2006	No significant effect on WAZ, WHZ, HAZ
		Meta-analysis of RCTs	No significant effect on mid upper arm circumference, skinfold
		Children < 14 yrs	thickness or head circumference

		Iron supplementation 2 mo to 12 mo	
		Low et al., 2013	No significant effect on absolute height, absolute weight or WHZ
		Meta-analysis of RCTs	A positive effect on HAZ (mean difference = 0.09; 95% CI: 0.01-0.17)
		Children 5 to 12 yrs	in comparison with control subjects
		Oral iron supplementation ≥ 5 days per wk	
		Pasricha et al., 2013	No significant effect on final weight, WAZ, final length or LAZ
		Meta-analysis of RCTs	Children randomised to iron supplementation groups had lesser
		Children aged 4-23 months	length (SMD −0·83, 95% CI: −1·53 to −0·12) and weight gain
		Daily oral iron supplementation	(SMD –1·12, 95%: −1·19 to −0·33) in comparison with control subjects.
		Thompson et al., 2013	No positive effect on physical growth.
		Meta-analysis of RCTs	
		Children 2 to 5 yrs	
		Oral iron supplementation ≥ 5 days per wk	
Vitamin A	WHO, 2009	Ramakrishnan et al., 2004	No positive effect on absolute height change or weight change
	(Serum retinol <0.70 µmol/L)	Meta-analysis of RCTs	
	Children < 5 years	Children < 18 yrs	
	Global: 33.3% (95% CI: 31.1-35.4)	Vitamin A supplementation ≥	

		8 wks	
	Africa: 44.4% (95% CI: 41.3-47.5)		
	South East Asia: 49.9% (95% CI: 45.1-54.8)		

IDA, Iron deficiency anaemia; SMD, standard mean difference

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

		Aflatoxin Exposure		
Study	**Country/ study population**	**Blood – detection n (%) and mean concentration**	**Urine – detection n (%) and mean concentration**	**Other – detection n (%) and mean concentration**
Hendrickse et al., 1982	Country: Sudan	177 samples tested (total aflatoxin pg/ml)	250 samples tested (total aflatoxin pg/ml)	
	252 children			
	K (n = 44)	K 16/44 (36.4%) (GM: 706)	K 14/42 (33.3%) (GM: 143)	
	MK (n = 32)	MK 7/32 (21.9%) (GM: 412)	MK 8/32 (25%) (GM: 742)	
	M (n = 70)	M 11/57 (19.3%) (GM: 211)	M 18/70 (25.7%) (GM: 508)	
	AM controls (n = 106)	AM controls 7/44 (15.9%) (GM: 77)	AM controls 21/106 (19.8%) (191)	
		The difference between the groups approached significance (*P* = 0.05)	No significant differences between the groups identified	
		Kwashiorkor group mean aflatoxin concentration was significantly higher than the control group (*P* = 0.01)		
Lamplugh & Hendrickse, 1982	Country: Nigeria and South Africa			
	8 children (aged between 9 and 24			8 autopsy liver

	months)			specimens
	K (n = 3)			K (all three of the liver samples contained AFB1: 2000, 4900 and 1400 pg/g)
	MK (n = 3)			MK (1 liver sample had no aflatoxins; one contained a small quantity of AFM1 (15 pg/g) and in the third sample aflatoxicol was found (8500 pg/g)
	M (n = 1)			M (no aflatoxins found)
	Control (n = 1)			Control (no aflatoxin found)
Apeagyei et al., 1985	Country: Ghana			22 autopsy liver specimens
	22 kwashiorkor children (aged between 5 and 48 months)			Aflatoxin B1 detected in 20 of the samples (90.9%) Remaining 2 samples contained aflatoxicol (9.1%)

Coulter et al., 1986	Country: Sudan			
	584 children	457 samples tested (total aflatoxin pg/ml)	465 samples tested (total aflatoxin pg/ml)	
	K (n=141) (median age: 18.1 months)	K 52/138 (37.7%) (GM: 154)	K 31/114 (27.2%) (GM: 308)	
	MK (n=111) (median age: 17.9 months)	MK 28/98 (28.6%) (GM: 82)	MK 30/77 (39.0%) (GM: 490)	
	M (n=152) (median age: 18.1 months)	M 31/118 (26.3%) (GM: 77)	M 31/ 119 (26.1%) (GM: 438)	
	AM controls (n = 180) (median age 17.5 months)	AM controls 22/103 (21.3%) (GM: 81)	AM controls 44/155 (28.4%) (GM: 258)	
		Significant between group differences in the number of positive samples (P <0.05)	No between group differences in the numbers of positive samples	
		No differences between the groups in concentrations of aflatoxin	No differences between the groups in concentrations of aflatoxin	
de Vries et al., 1987	Country: Kenya			
	41 children	39 samples (total aflatoxin (pg/ml)	36 samples (total aflatoxin pg/ml)	
	K (n = 14) (mean age: 54 months)	K 9/14 (64%) (mean: 6666)	K 5/12 (42%) (mean: 324)	
	MK (n = 6) (mean age: 48 months)	MK 2/4 (50%) (mean: 386)	MK 3/5 (60%) (mean: 1294)	
	M (n = 11) (mean age: 60 months)	M 4/11 (36%) (mean: 3412)	M 5/11 (45%) (mean: 261)	
	Controls (n = 10) (mean age: 43	Controls 3/10 (30%) (mean: 759)	Controls 6/8 (75%) (mean: 223)	

	months)			
			No differences in detection rates	
de Vries et al., 1990	Country: Kenya			
	13 children			
	K (n = 5)		K (4 out of 5 excreted aflatoxin via urine)	K (5 excreted aflatoxin in their stools)
				Aflatoxin excreted (urine and stools) ranged from 0.08 to 4 ug/kg body weight)
	MK (n = 7)		MK (5 out of 7 children excreted aflatoxin via urine)	MK (3 out of 7 children excreted aflatoxin in their stools)
				Aflatoxin excreted (urine and stools) ranged from nil to 1.5 ug/kg body weight).
	Underweight (n = 1)		Underweight child's urine sample tested negative for aflatoxin	Underweight child's stools tested negative for aflatoxin
Household & Hundt, 1991	Country: South Africa			
	384 children		448 urine samples	
	K (n = 47) (mean age: 18 months)		No aflatoxin or aflatoxicol detected	

	M (n = 17) (mean age: 12.8 months)		No aflatoxin or aflatoxicol detected	
	Community group (n = 320) (mean age: 38 months)		No aflatoxin or aflatoxicol detected	
Ramjee et al., 1992	Country: South Africa			
	109 children (aged between 6 months & 2 years)	109 samples	50 samples	
	K (n = 45)	K 25/45 (56%)	K 4/24 (16%)	
	M (n = 13)	M 4/13 (31%)	M 1/10 (10%)	
	Underweight (n = 16)	Underweight 9/16 (56%)	Underweight (no samples tested)	
	AM controls (n = 35)	AM controls 17/35 (49%)	AM controls 4/16 (25%)	
		No between group differences in the number of aflatoxin positive results	No between group differences in the number of aflatoxin positive results.	
			Serum/ urine ratio significantly higher in the kwashiorkor group than in the other groups (*P* = 0.001)	
Adhikari et al., 1993	Country: South Africa			
	36 children (aged between 6 months & 2 years)	36 samples		
	K (n=36)	Aflatoxin detected in 21 samples (58%)		
Oyelami et	Country: Nigeria			

al., 1996				
	40 children (aged between 4 & 168 months)			40 lung specimens
	children who died from kwashiorkor (n=20)			K 18/20 (90%)
	children who died of other diseases (n=20)			Other diseases 13/20 (65%)
				No significant differences among the groups in the number of aflatoxin positive results
Oyelami et al., 1998	Country: Nigeria			
	45 children (aged between 1.5 & 168 months)			45 kidney specimens (aflatoxin pg/g)
	children who died from kwashiorkor (n=24)			K 14/24 (58%) (mean: 3851)
	children who died of other diseases (n=21)			Other diseases 13/21 (62%) (mean: 1271)
				No between group differences in the number of aflatoxin positive results

				No between group differences in mean aflatoxin concentrations
Hatem et al., 2005	Country: Egypt			
	70 children (aged 6 to 24 months)	70 samples (total aflatoxin ng/100ml)	70 samples (total aflatoxin ng/100ml)	
	K (n = 30) (mean age: 11.9 months)	K 24/30 (80%) (mean: 70.58)	K 24/30 (80%) (mean: 14.96)	
	M (n = 30) (mean age: 9.8 months)	M 14/30 (46.7%) (mean: 25.21)	M 14/30 (46.7%) (mean: 11.14)	
	AM controls (n = 10) (mean age: 15.9 months)	AM controls (0)	AM controls (0)	
		Aflatoxin was detected more frequently in the kwashiorkor group than the marasmus group (P = 0.007).	Aflatoxin was detected more frequently in the kwashiorkor group than the marasmus group (P = 0.007).	
		Total aflatoxin mean concentration was significantly higher in the kwashiorkor group relative to the marasmus group (P <0.001)		
Tchana et al., 2010	Country: Cameroon			
	78 children (aged between13 months & 12		42 samples (aflatoxin B1)	

	years)			
	K (n = 31)		K 11/31 (35.5%)	
	MK (n=11)		MK 5/11 (45.5%)	
	AM controls (n = 36)		AM controls 4/36 (11.1%)	
			AFB1 was detected more frequently in the kwashiorkor and marasmus kwashiorkor groups relative to the control group (*P* <0.05)	
Onyemelukwe al., 2012	Country: Nigeria			
	111 children (aged between 7 & 60 months)	111 samples (total aflatoxin ug/L)	55 samples (total aflatoxin ug/L)	
	K (n = 36)	K 32/36 (88.9%) (median: 165.6)	K 11/13 (84.6%) (median: 79)	
	MK (n = 29)	MK 27/29 (93.1%) (median: 228.4)	MK 12/20 (60%) (median: 43.8)	
	M (n = 13)	M 10/13 (76.9%) (median: 234.3)	M 9/11 (81.8%) (median: 14.4)	
	AM controls (n = 33)	AM controls 21/33 (63.6%) (median: 20.7)	AM controls 10/11 (90.9%) (median: 42.6)	
		Total aflatoxin was significantly higher in each PEM group relative to the control group	No between group differences in the number of aflatoxin positive results	
		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 PEM groups	Median total aflatoxin concentration was significantly higher in the kwashiorkor group relative to the marasmus group ($P = 0.011$)	

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

