“The epidemiology of aflatoxin driven stunting in developing countries”

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Poor Infant Growth – Stunting

• **Stunting defined as**
  • Height for Age – Z-scores two standard deviations less than the WHO guidelines

• **Stunting significance**
  • Infants increased vulnerability to infectious diseases
  • Infants high mortality risk
  • Cognitive impairment risk - beyond childhood
  • Reduced life long productivity
Prevalence estimates for stunting among children < 5 years.

Countries with the highest burden of malnutrition - 90% of the global burden of malnutrition.

Bhutta et al. (2013),
Stunting – Africa prevalence 30-35%

- **Causes**
  - Poor intrauterine growth – maternal
  - Associated with intestinal enteropathy - infant
  - Under-nutrition – calories, diversity, nutrient rich
  - Timing and nutrient density of weaning foods
  - Intestinal Infection

- Diet and infections are critical components that contribute to the burden of growth faltering........but..

- Growth faltering is not fully (or even well) explained by dietary insufficiency and infection - - - mycotoxins ??
Aflatoxin - foods and occurrence

- Maize / Groundnut / Cotton Seeds
- Field / Storage

- Also - milk, cheese, nuts, figs, spices,

- HOT and Humid world regions
  - 500 million high risk of chronic exposure

- Acceptable levels 4ppb (EU), 20 ppb US
AFB₁ BIOTRANSFORMATION & EXPOSURE ASSESSMENT
AFLATOXIN

AFM1          AF-guanine            AF-albumin

Measure in Urine          Measure in Blood
Aflatoxin and Growth Faltering
Aflatoxins and Growth Faltering

• Animal
  • Data supports a role for aflatoxin in Growth faltering
  • Use of aflatoxin uptake inhibitors improves growth velocity

Evidence in Human

• What are the exposure patterns?

• Is growth faltering association with aflatoxin exposure?

• What are the possible mechanisms?
Aflatoxin-albumin bio-measures in women and infants
Turner et al., Int J Epidemiology

<table>
<thead>
<tr>
<th>Group</th>
<th>AF-Alb (pg/mg) *Mean (range)</th>
<th>Prevalence of positive samples (%)</th>
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<tbody>
<tr>
<td>Maternal during pregnancy</td>
<td>40 (5 - 261)</td>
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In USA <0.1% would have >5pg/mg, <0.001% would have > 20pg/mg
# Aflatoxin-albumin adducts in African Children

<table>
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<th>COUNTRY</th>
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<tr>
<td>The Gambia n=391</td>
<td>57 (nd-720)</td>
<td>83</td>
<td>3-8</td>
</tr>
<tr>
<td>The Gambia n=444</td>
<td>41 (3-459)</td>
<td>100</td>
<td>3-4</td>
</tr>
<tr>
<td>The Gambia n=466</td>
<td>24 (nd– 456)</td>
<td>93</td>
<td>6-9</td>
</tr>
<tr>
<td>Benin &amp; Togo n=479</td>
<td>33(nd-1064)</td>
<td>99</td>
<td>1-5</td>
</tr>
<tr>
<td>*Benin n=200</td>
<td>137 (nd-688)</td>
<td>98</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>239 (nd-744)</td>
<td>100</td>
<td></td>
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<tr>
<td></td>
<td>388 (5-1568)</td>
<td>100</td>
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<tr>
<td>Guinea n=124</td>
<td>9.2 (nd-66)</td>
<td>96</td>
<td>2-5</td>
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Infant / early childhood aflatoxin and stunting – Benin & Togo

- Gong et al BMJ

- 480 children aged 9 months/5yrs

- Cross-sectional

- AF-Alb mean 33 (range 5 – 1064 pg/mg albumin)

- Basic Anthropometric data
Exposure to Aflatoxin Associated with Reduced Growth in West African Children

(Gong et al., Brit Med Journal)
Infant / early childhood aflatoxin and growth velocity – Benin - longitudinal

Gong et al EHP
Egal et al Int J F Micro

• 200 children aged 1-2 years

• Aflatoxin-albumin adducts
  • Time 1: mean 37 pg/mg range nd – 688 pg/mg
  • Time 2: mean 39 pg/mg range nd – 744 pg/mg
  • Time 3: mean 88 pg/mg range 5 - 1568 pg/mg
AF-albumin mean (range) vs average height
n= 200 Beninese children (age 1-2 yrs)

Gong et al., 2004:

\[ p < 0.001 \]
Growth in AF exposed older children from the Gambia.

Turner et al., EHP

- 472 Gambian Children aged 6-9 years

- AF-alb - mean 22.3pg/mg (range 5-456pg/mg)

- AF-alb not significantly associate with stunting

- AF-alb modestly inversely associated WHZ (p<0.05)
  - wasting
Birth outcomes and AF-alb levels in Ghanaian women.
Shuaib, et al., 2010

• 785 pregnant women blood taken after delivery.

• AF-alb adduct maternal serum
  • mean 11pg/mg; range 1 – 269pg/mg

• Women within the highest AF-alb quartile were more likely to have low birth-weight babies (OR, 2.1; 95% CI, 1.2-3.7)

• Aflatoxin and risk for low birth-weight ($p_{trend} = 0.007$)
### Maternal aflatoxin exposure and infant growth velocity

*Turner et al., Int J Epid*

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Infant growth was measured from birth, every 4 weeks → 1 year
Growth velocity - GEE multiple regression analysis

• Maternal AF-alb associated with WAZ score $p=0.012$.

• Maternal AF-alb associated with HAZ score $p=0.044$.

• WHAT DOES THIS MEAN / PREDICT?
Growth velocity - GEE multiple regression analysis

• Maternal AF-alb associated with WAZ score p=0.012.

• Maternal AF-alb associated with HAZ score p=0.044.

**WHAT DOES THIS MEAN / PREDICT?**

• A reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg would lead
  • 0.8 kg increase in weight
  • 2 cm increase in height

• ALSO

  • **Week 16** AF-alb additionally contributed to the model for HAZ p=0.002, though not significant for WAZ (p=0.06).

All adjusted for sex, age, placental weight, maternal weight, gestation duration and season
Growth Velocity Faltering
Low birth Weight
Growth Velocity Faltering
Growth Faltering
Growth Faltering

Average maternal at birth - 2 time points

Week 16 Infant
16 – 37 mths
9 – 60 mths
6-9 years

Pregnancy begins

A – Early introduction of some infants to weaning foods
B – Most infants introduced to weaning foods
C – Most infants fully weaned
Mechanism?

- Liver Toxicity
  - liver damage/regeneration at expenditure of other homeostatic needs

- Modulation of liver produced growth factors e.g. IGF-1

- Direct Intestinal Toxicity – prolonged enteropathy

- Immune Suppression
Intestinal Enteropathy

Growth Faltering and Intestinal permeability in Gambian infants

Fig. 2. 'Mirror image' relationship between growth (g/month; ...) and intestinal permeability assessed by the dual-sugar (lactulose : mannitol; L : M; —) test in two typical Gambian children.
Growth faltering and intestinal permeability Gambian Infants

L:M ratio is a measure of intestinal leakiness

**FIGURE 1** Deterioration in mean weight- and height-for-age Z-scores with age for all infants in the study (n = 71).

**FIGURE 2** Variation in the lactulose:mannitol intestinal permeability ratio with age in Gambian infants. Values are geometric means and 95% confidence intervals for all of the children in the study (n = 71).

Campbell et al., 2003
Brain \[\text{Inflammatory Response} \downarrow \text{Appetite} \uparrow \text{Cytokines}
\]

Mycotoxins \rightarrow \text{Liver} \quad \text{Mycotoxin and Microbes} \rightarrow \text{Gut} \rightarrow \text{Brain}

Liver \downarrow \text{IGF-1} \downarrow \text{Caloric intake}

Gut \uparrow \text{endotoxin} \rightarrow \text{Intestinal Enteropathy} \rightarrow \text{Inflammatory Response} \rightarrow \text{Brain}

\text{Mycotoxins} \rightarrow \downarrow \text{Nutritional homeostasis} \downarrow \text{surface area} \downarrow \text{barrier function} \downarrow \text{nutrient transport} \rightarrow \text{prolonged infection}

Stunting

* \downarrow \text{surface area} \downarrow \text{barrier function} \downarrow \text{nutrient transport} \text{prolonged infection}
Design of the SHINE Trial

Zimbabwe
Sanitation Hygiene Infant Nutrition Efficacy Study

Principal Investigators: J Humphrey, R Stoltsfuz
Funders: DFID, NIH, Gates, UNICEF
Grantees: Zvitambo, Johns Hopkins, Cornell
Scatterplot AF-alb versus HAZ (stunting)
The study used a subset of children who were fully weaned (age 18-60 months, n=315) from 16 villages in 4 geographic zones – Turner unpublished observation from Benin

Maybe there is a threshold, and maybe it varies by population, based on other, nutritional and hygiene components
And we need to understand this on a population by population basis
Zimbabwe
Study Area: Rural, sparsely populated

Two entire districts
(180,000 K people)
Subsistence farming
High food insecurity
Very low exclusive BF
15% HIV
ONE OF THE VILLAGE SITES IN ZIMBABWE: NOVEMBER 2014
Randomized trial in rural Zimbabwe, highly food insecure

• Outcome: stunting in infants from 0-18 months

<table>
<thead>
<tr>
<th>Control</th>
<th>Infant Feeding: Education + Nutributter</th>
<th>WASH: Integrated Water, Hygiene &amp; Sanitation</th>
<th>WASH + Infant Feeding</th>
</tr>
</thead>
</table>

![Image of Nutributter](image1)

![Image of rural setting](image2)

![Image of hygiene practice](image3)
Birth outcomes:
Preterm birth, stunted-for GA, small-for-GA

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<th>Infancy</th>
</tr>
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<tr>
<td>Mo: 3</td>
<td>8</td>
</tr>
<tr>
<td>AF</td>
<td>4200</td>
</tr>
<tr>
<td>FUM</td>
<td>200</td>
</tr>
<tr>
<td>DON</td>
<td>200</td>
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Postnatal outcomes (all time points):
Length-for-age, morbidity
biomarkers of gut health, anemia
FUM/DON subsample: calories consumed
In multivariable ordinal logistic models, geographical location (p<0.001), seasonality (p<0.001), and dietary practices (p=0.011) were significant factors in the geographical distribution of aflatoxin exposure.

AFM1 nd--6046 pg AFM1/mg creatine

Subset 1580 analysed to date
**Tanzania Intervention**

- 1500 mother infant pairs
- Restrict aflatoxin exposure using densitometry sorting of grains
- Follow from 6-18 months
- Evaluate AF
- Evaluate growth

**Preventing and reducing contamination**

- Mycotoxin level
  - Size separation
  - Aspiration
  - Density separation
  - Optical sorting
- Removed affected product (%)

![Map of Tanzania](image-url)
Relative Mean AF-alb adduct level infants aged 0-12 months, The Gambia Modified from Turner et al., 2007
The Gambia - Introduction of weaning foods

% Introduced

Age / Weeks

Turner, Prentice et al., unpublished observations
The Gambia - Introduction of weaning foods

% Introduced

Age / Weeks

0 4 8 12 16 20 24 28 32 36 40 44 48 52

0 20 40 60 80 100

- Introduction pattern over time
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Protocol for Primary Prevention Study to Reduce Aflatoxin Exposure in Guinea, Conakry

Blood 1
30 subjects

Blood 2
30 subjects

Blood 3
30 subjects

Oct          Dec          Feb
Harvest
Groundnut sample

>55% REDUCTION IN EXPOSURE

Intermediate visits to villages 1) check compliance, 2) short dietary questionnaire on foods consumed in previous 24 hours, 3) check humidity in storage facilities

Turner et al Lancet
Aflatoxin Intervention Studies

Primary Prevention
- West Africa
  - Harvest
  - Storage
  - Washing

Chemoprevention
- Egypt/China
  - Probiotics
- West Africa
  - Clay
- China
  - Oltipraz
  - Chlorophyllin
  - Broccoli

Uptake Inhibitors
- Reduction in aflatoxin exposure