Tufts UNIVERSITY
School of Medicine

Why Food is Not Enough

Environmental Enteropathy, Mycotoxins, the Gut Microbiome, and Malnutrition

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Learning Objectives

- Learn about important new (and unexpected) data on the roles of the gut microbiome, aflatoxins, and sanitation in health and nutrition for low-income countries.
- Understand new integrative paradigms about how health and growth are affected by nutrition and the environment.
- Contextualize this information in terms of US (high-income country) history.

Focus of this talk

- Malnutrition and under-nutrition remain major global health issues, even as obesity and over-nutrition are on the rise.
- An operating paradigm has been that the lack of food is the key gap.
- This turns out to be too simple – new data is showing major influences of the external, and internal (microbiome), environments.

Simple Idea – Not Enough Food Leads to Malnutrition; solution= Food.

Not Enough Nutrients

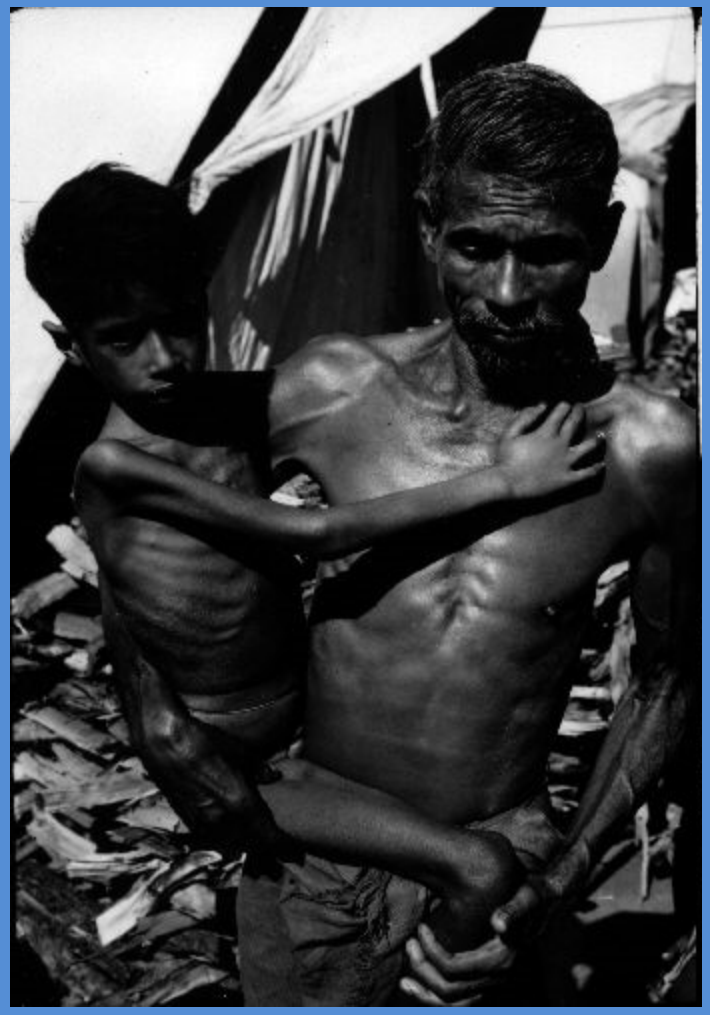
- Stunting
- Wasting
- Small for Gestational Age/Low Birth Weight
- Micronutrient Deficiency (Fe, Zn, vitamin A, Iodine)

Stunting – low *height* for age

CDC Children with Kwashiorkor – Stunting, protein deficiency

Wasting – low *weight* for age

This slide dates to a famine in South Asia
Acute Shortage of Food



CDC

Supposition:

↑food =↑income &↑nutrition
and thus to **better health**

……

<u>Higher Income</u> = Can afford more food, more diverse/healthier diet
<u>Higher production</u> of food = more food available in household

BILL & MELINDA GATES *foundation*

What We Do

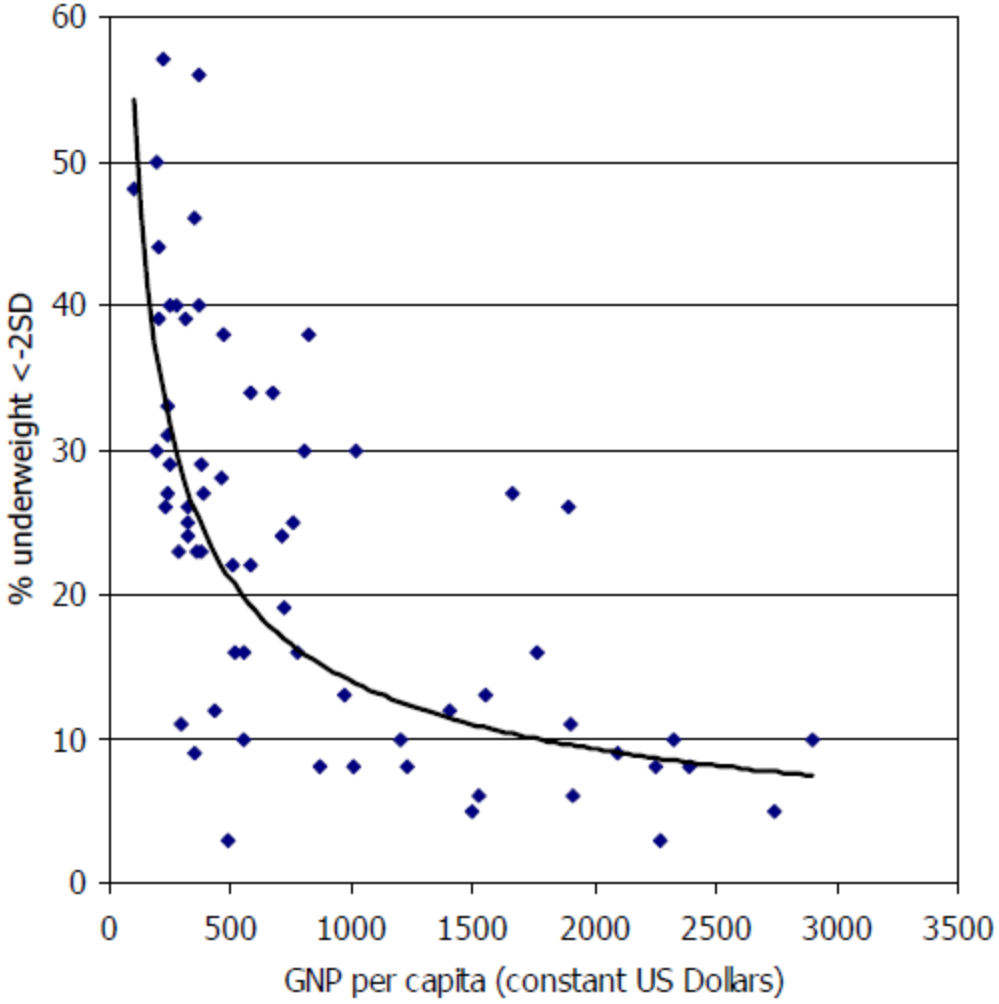
AGRICULTURAL DEVELOPMENT

STRATEGY OVERVIEW

A maize farmer in Tanzania whose crop yields have increased through the use of better seeds.

OUR GOAL: to reduce hunger and poverty for millions of farming families in Sub-Saharan Africa and South Asia by increasing agricultural productivity in a sustainable way.

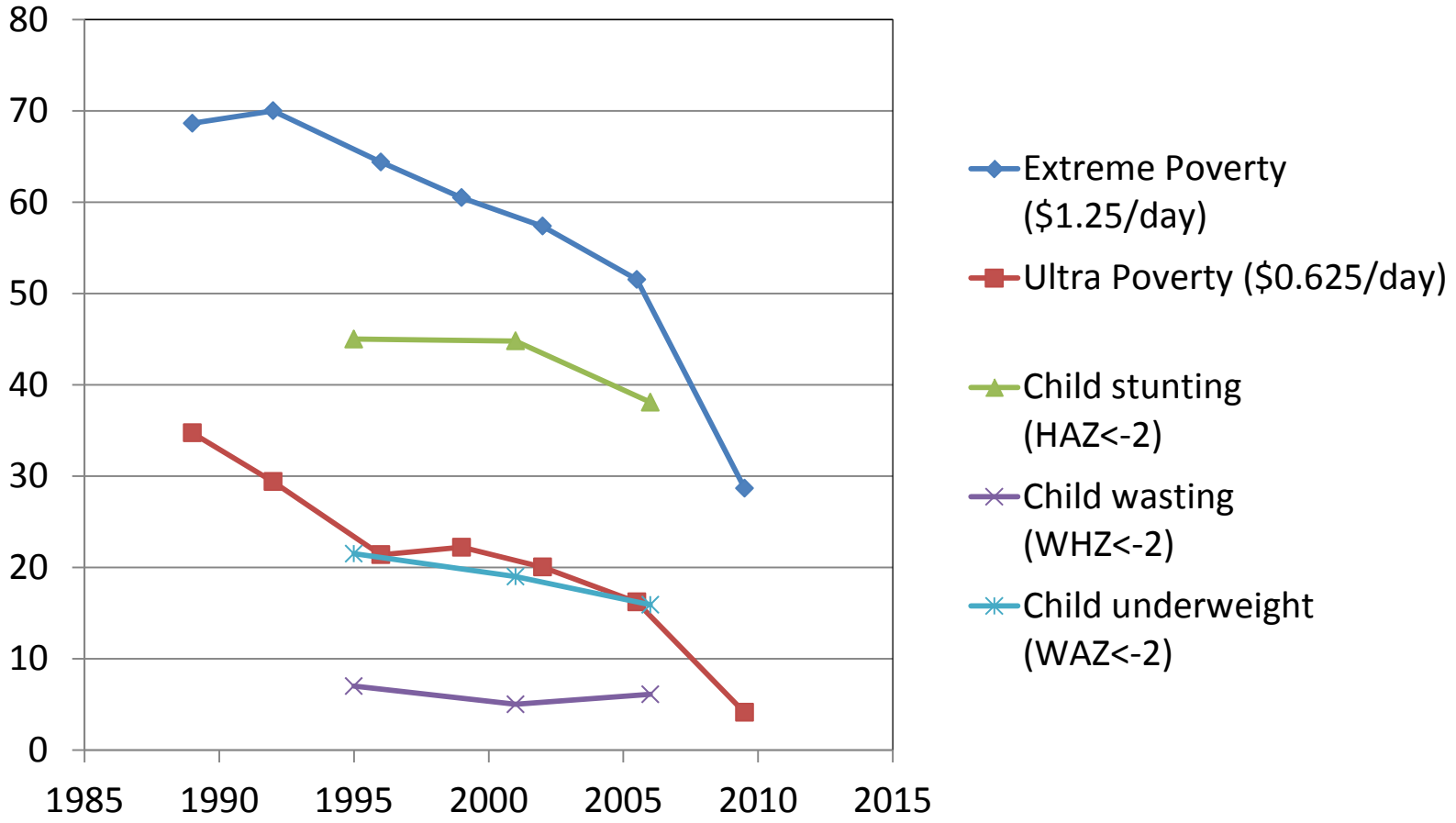
World Bank data – underweight versus GNP



Why isn't the Relationship a Straight line if More $$$ = Better Nutrition??

From presentation by Will Masters: note **steep rate of decline in poverty versus very modest rate of decline in undernutrition** -

Poverty and child undernutrition in Uganda, 1989-2009



Source: Poverty rates are calculated from World Bank (2011), PovcalNet (http://iresearch.worldbank.org/PovcalNet/), updated 11 April 2011. Estimates are based on over 700 household surveys from more than 120 countries, and refer to per-capita expenditure at purchasing-power parity prices for 2005. Undernutrition rates are from Uganda Demographic and Health Surveys 1995 (Mar.-Aug. 1995), 2000-01 (Sept. 2000-March 2001), and 2006 (May-Oct. 2006).

Lancet June 2013

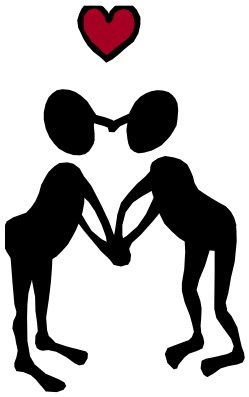
Maternal and Child Nutrition 2

Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?

Zulfiqar A Bhutta, Jai K Das, Arjumand Rizvi, Michelle F Gaffey, Neff Walker, Susan Horton, Patrick Webb, Anna Lartey, Robert E Black, The Lancet Nutrition Interventions Review Group, and the Maternal and Child Nutrition Study Group

- 800,000 neonatal / 3.1 million childhood deaths per year. 165 million stunted children.
- If top 10 nutrition interventions targeted to 34 countries with 90% of childhood deaths …
- Reduce deaths by **15%**, stunting by **20%**, acute wasting by **61**%. (For < $10 billion per year).

Bad News: Lancet review (2013) of how much "food would fix" – not much (20%).



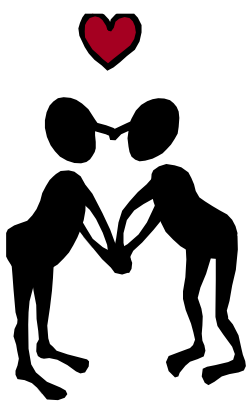
PREGNANCY

EARLY CHILDHOOD

ADOLESCENT, PRECONCEPTION, GESTATIONAL, AND MATERNAL NUTRITION
ADEQUATE CALORIES (PROTEINS, FATS, CARBOS) IN ALL LIFE STAGES
DIVERSITY OF MICRONUTRIENTS, VITAMINS, HIGH QUALITY PROTEINS
OPTIMAL BREASTFEEDING, RESPONSIVE FEEDING PRACTICES, STIMULATION
GOOD COMPLEMENTARY FEEDING 6-23 MONTHS, DIETARY DIVERSITY
WEALTH, EDUCATION – [BE SURE TO CHOOSE YOUR PARENTS WELL]
Others…..

All are *Known* Revalent Nutrition Actions

It's not just what you eat…
It's your external and internal environment
And how they are linked (water and sanitation)



PREGNANCY

EARLY CHILDHOOD

MYCOTOXINS: FUNGAL FOOD TOXINS WHICH IMPAIR GROWTH AND IMMUNITY

ENVIRONMENTAL ENTEROPATHY:

INFLAMED, LEAKY, DYSFUNCTIONAL INTESTINES

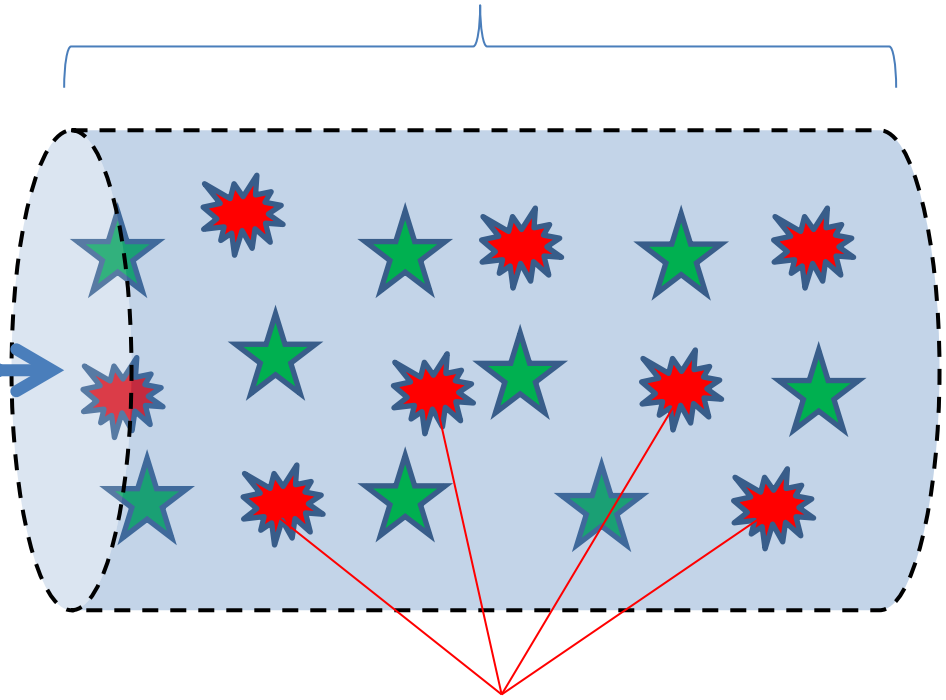
THE GUT *MICROBIOME* - *GUT BACTERIA GONE BAD*

MYCOTOXINS IN FOOD

HUMAN AND ANIMAL PATHOGENS

MICRO- AND MACRO-NUTRIENTS

PERMEABLE ("LEAKY") AND INFLAMMED GUT



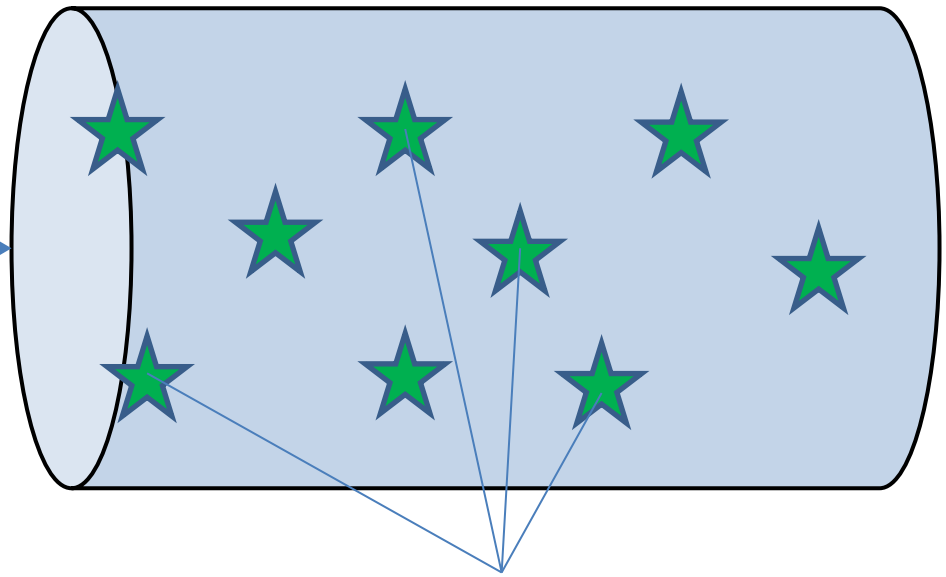
UNHEALTHY INTESTINAL MICROBIOME

MYCOTOXINS IN FOOD

HUMAN AND ANIMAL PATHOGENS

MICRO- AND MACRO-NUTRIENTS

NORMAL GUT – NOT PERMEABLE



HEALTHY INTESTINAL MICROBIOME

Agriculture in Urban Nairobi: Sewage

Left: broken sewage main in field. Right: lush fields.

Farmers work in contaminated fields; crops contaminated with human pathogens; go home to families carrying tools & wearing boots that have been in sewage…

Water needed for crop productivity, vegetable kitchen gardens and dietary diversity (animal meat protein is good), to promote income, keep farmers hydrated and fit for work, …. Promote gender equality …. Irrigation, reservoir construction help address climate change ….

What else does the water carry?

AGRICULTURAL WASTEWATER

ORGANISM	TYPICAL SOURCE
ROTAVIRUS	HUMANS; PERHAPS ANIMALS
HEPATITIS A	HUMANS
HEPATITIS E	HUMANS, **SWINE**
E. coli (bacteria)	**CATTLE,** HUMANS
Shigella species	HUMANS
Salmonella enterica (bacteria)	**CATTLE, POULTRY, SWINE, HUMANS**
Campylobacter jejuni (bacteria)	**POULTRY**
*Cryptosporidium** (protozoan)	**CATTLE,** HUMANS**, OTHER FARM ANIMALS**
*Microsporidia** (fungus)	**FARM AND DOMESTIC ANIMALS,** HUMANS
* Causes chronic diarrhea, wasting, malnutrition in people with HIV/AIDS	
Cryptosporidium – a leading cause of diarrhea children < 24 months; known to cause stunting; and children have x 4 risk of death in next year	

Pathogens in Rural and Agricultural Water and Watersheds. USDA 2010

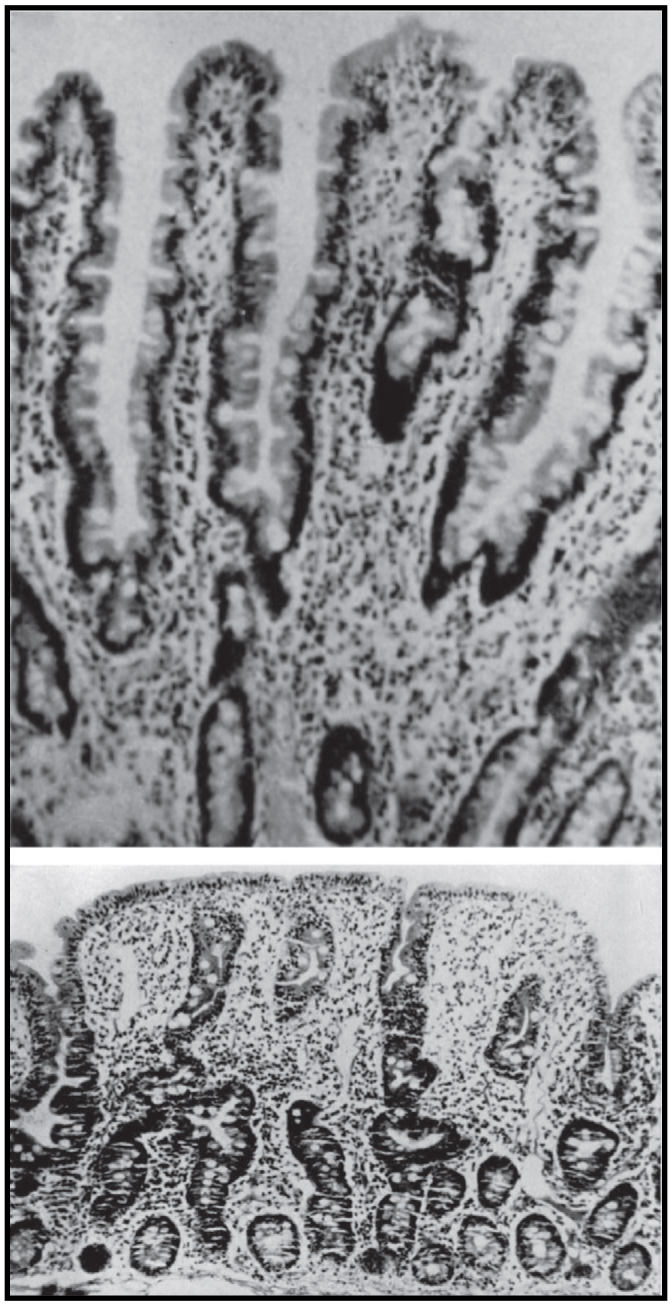
Poor Sanitation / Hygiene. Fecal Contamination of Domestic Environment

Fecal Ingestion Infants/Children and Enteric Infections

(1) Increased gut permeability (2) Bacteria (and gut contents) leak into body (3) Intestinal Inflammation

ENVIRONMENTAL ENTEROPATHY

In studies dating to 1993, 43% of stunting explained by increased gut permeability (Also called environmental enteric dysfunction, EED, and tropical enteropathy).



ENVIRONMENTAL ENTEROPATHY (EE or EED)

People living in contaminated environments have leaky, chronically inflamed intestines

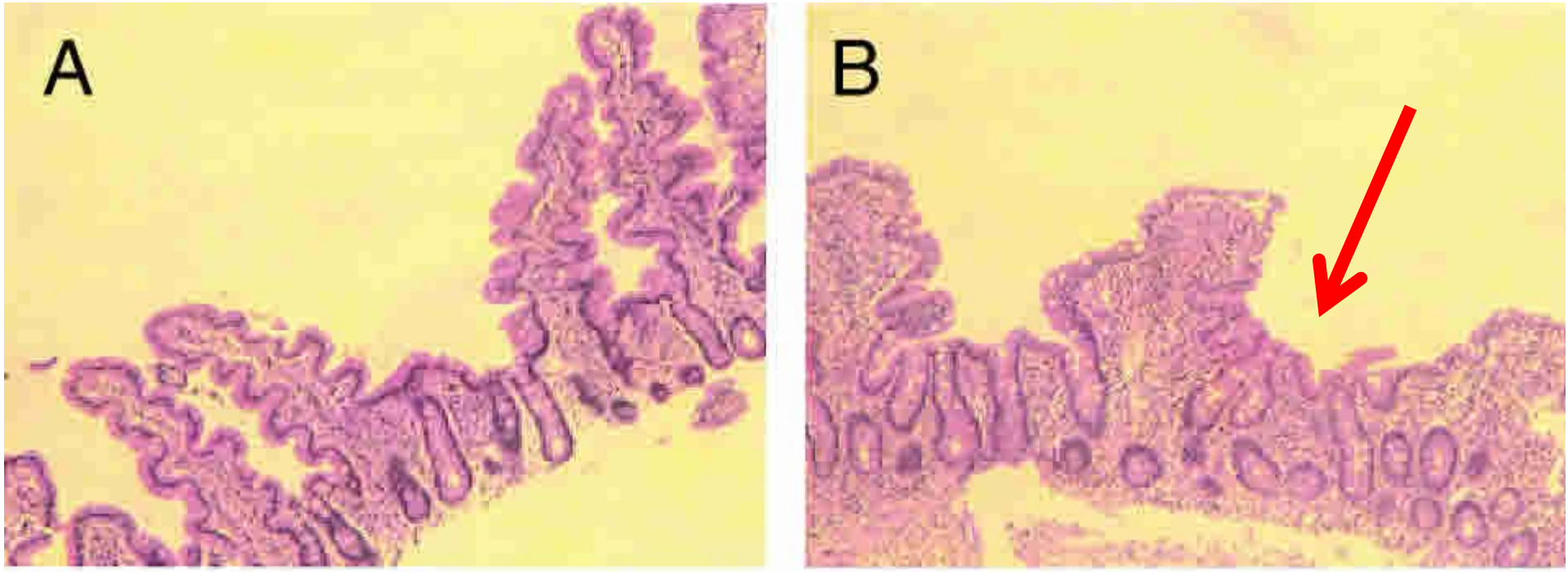
EE - Short blunted villi, tissue is infiltrated with inflammatory cells. 15% less protein and 5% less carbohydrate is absorbed. ↑ nutritional needs, bacteria leak into body, leads to anemia. **Bad bacteria** are likely cause.

Korpe & Petri, Trends in Molecular Medicine June 2012, Vol. 18, No. 6

Am. J. Trop. Med. Hyg., 70(4), 2004, pp. 412–419

RESPONSES OF SMALL INTESTINAL ARCHITECTURE AND FUNCTION OVER TIME TO ENVIRONMENTAL FACTORS IN A TROPICAL POPULATION

PAUL KELLY, IAN MENZIES, ROGER CRANE, ISAAC ZULU, CAROLE NICKOLS, ROGER FEAKINS, JAMES MWANSA, VICTOR MUDENDA, MAX KATUBULUSHI, STEVE GREENWALD, AND MICHAEL FARTHING



Mild (left) and severe (right) villus blunting

Less absorptive surface area is present

Am. J. Trop. Med. Hyg., 70(4), 2004, pp. 412–419

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TABLE 4

Intestinal infections detected in 3,260 monthly samples from asymptomatic participants*

Organism	Frequency of isolation	Frequency of isolation one month before investigation
Cryptosporidium parvum	31	1
Isospora belli	11	0
Microsporidia	5	0
Giardia intestinalis	40	5
Blastocystis hominis	236	19
Ascaris lumbricoides	489	33
Hookworm	92	13
Strongyloides stercoralis	11	0
Schistosoma mansoni	12	0
Trichuris trichiura	6	0
Taenia saginata	7	0
Iodamoeba butschlii	120	5
Entamoeba histolytica/dispar	12	0
Entamoeba hartmannii	47	4
Chilomastix mesnili	208	25
Endolimax nana	259	12
Hymenolepis nana	19	2
Salmonella spp.	44	4
Shigella spp.	2	3
Aeromonas hydrophila	13	1
Citrobacter rodentium	608	42
Vibrio cholerae	3	0

* The table shows which organisms were isolated from asymptomatic participants and which organisms were isolated from participants in the month prior to investigations carried out (jejunal biopsy and sugar testing). It is apparent that for many organisms it is not possible to determine any effect on the mucosa since there were too few isolates in the month prior to investigation.

- Water and sanitation reduce transmission of pathogens;
- Water and sanitation interventions improve nutritional status – (is it decreased diarrhea)?
- Tropical enteropathy renamed environmental enteropathy (EE) when the linkage to unsanitary environment recognized. Hallmark of EE is gut mucosal damage, permeability. (Keusch et al: Env. Enteric Dysfunction)
- Recognition that persons with EE have "asymptomatic" infections with pathogens

Lunn et al Lancet 1991: **Intestinal permeability, mucosal injury, and growth faltering in Gambian infants.**

- Infants aged 2-10 months recruited into longitudinal study (n=119 ≥ 3 observations). Infants had diarrhea 7.5%, and "growth depressing permeability" 76% of the time. **43% of stunting** explained by ↑gut permeability and ↓ absorptive capacity (differential absorption of lactulose and mannitol)

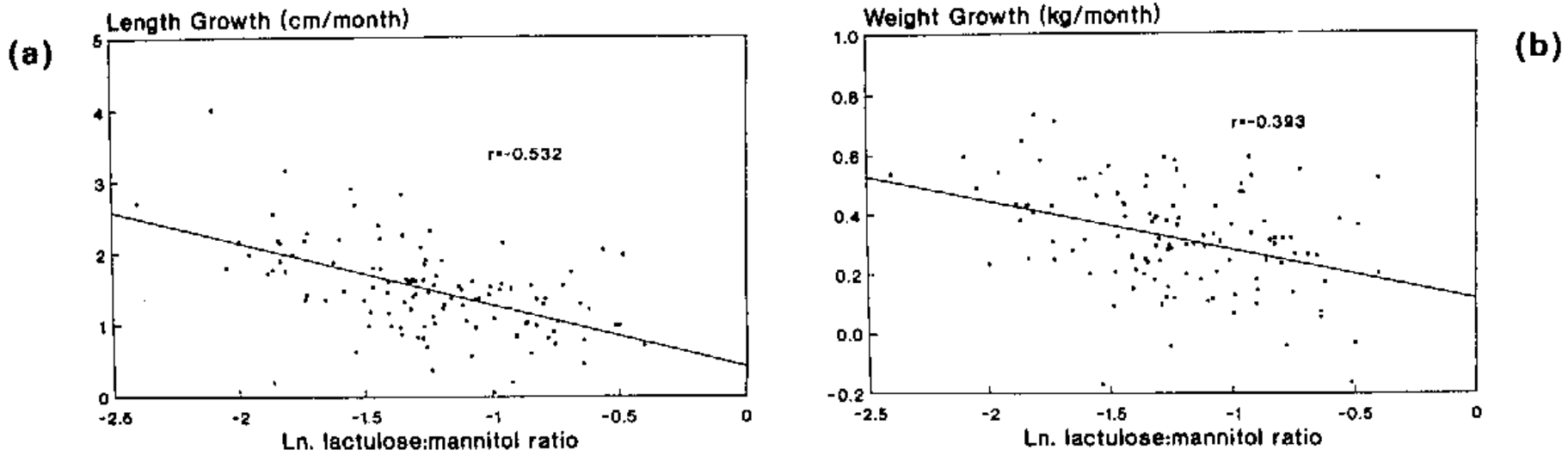
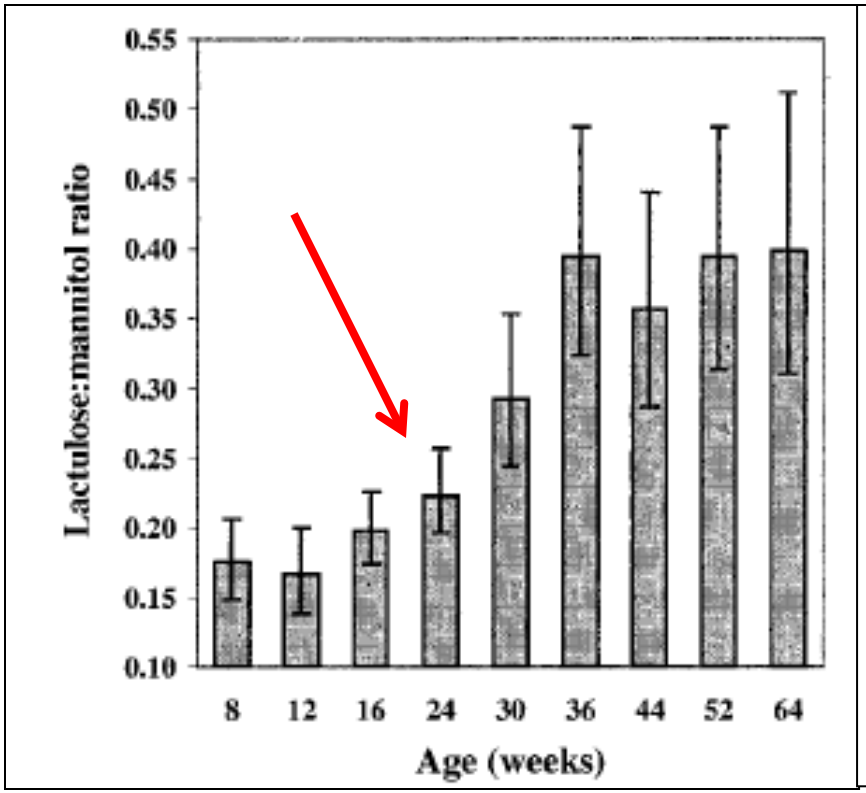


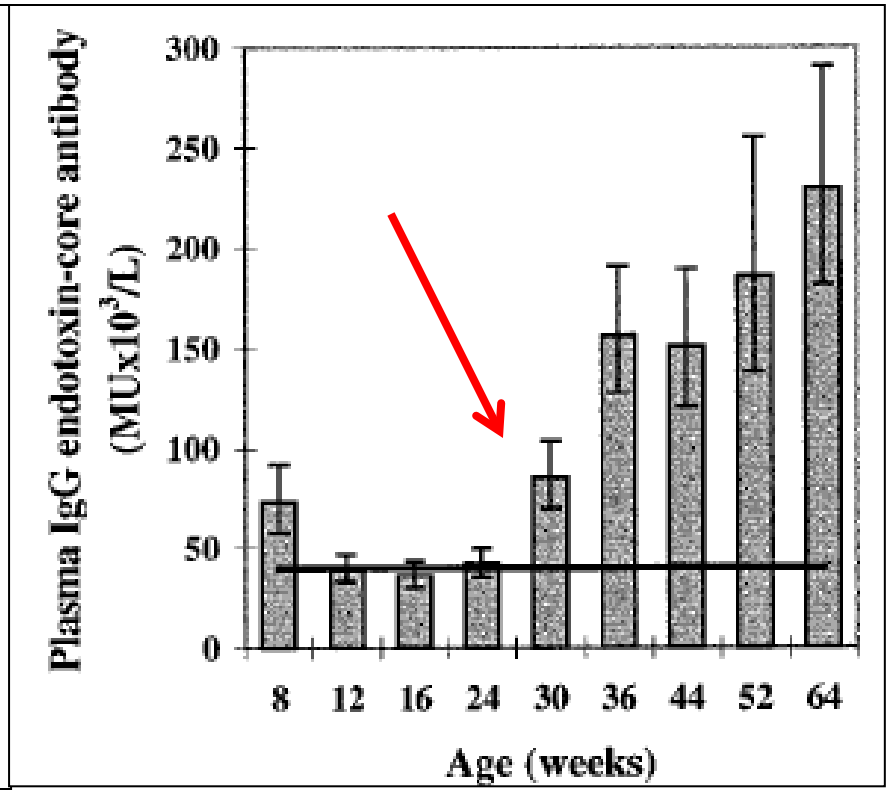
Fig 2—The relation between intestinal permeability (expressed as $\log_e$ lactulose:mannitol ratio) and mean monthly (a) length and (b) weight growth of 119 rural Gambian infants.

Significance of regression coefficients, $p < 0{\cdot}001$.

Intestinal permeability and mucosal damage (left) and antibody to bacterial endotoxin (right) rise after weaning when exposure to pathogens increases

Lunn et al *Lancet* 1991





Handwashing is "necessary but not sufficient"

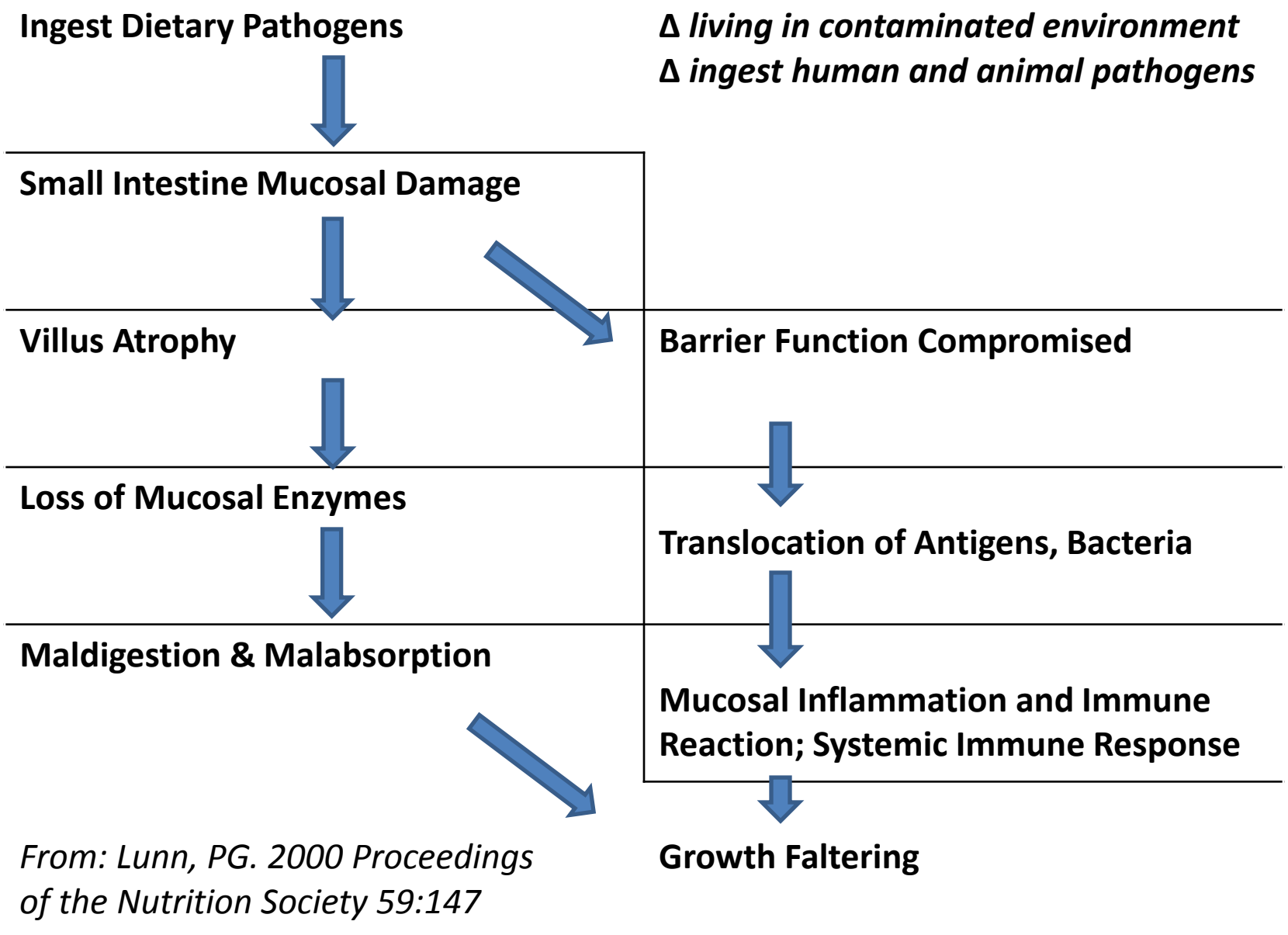
Amer J Human Biol 23:621-629 (2011)

Original Research Article

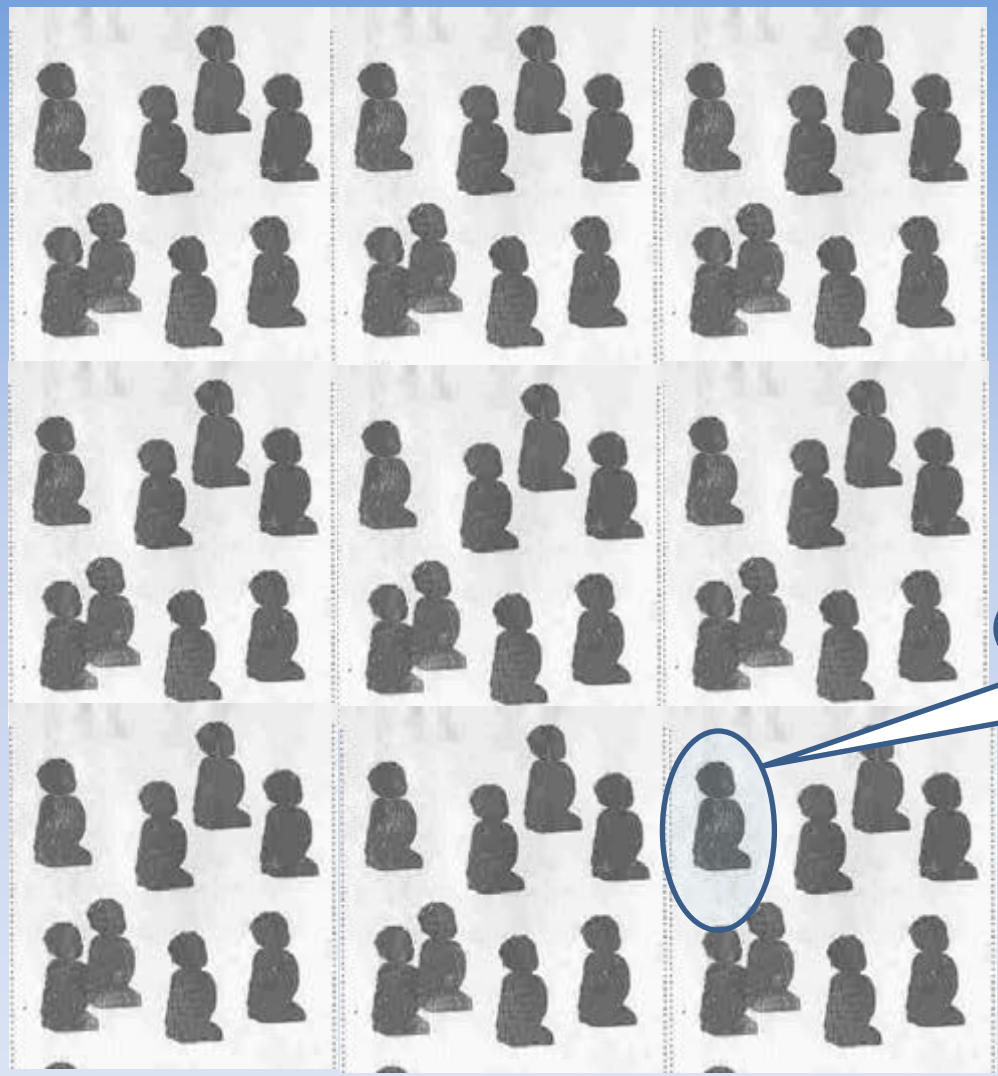
Hand-Washing, Subclinical Infections, and Growth: A Longitudinal Evaluation of an Intervention in Nepali Slums

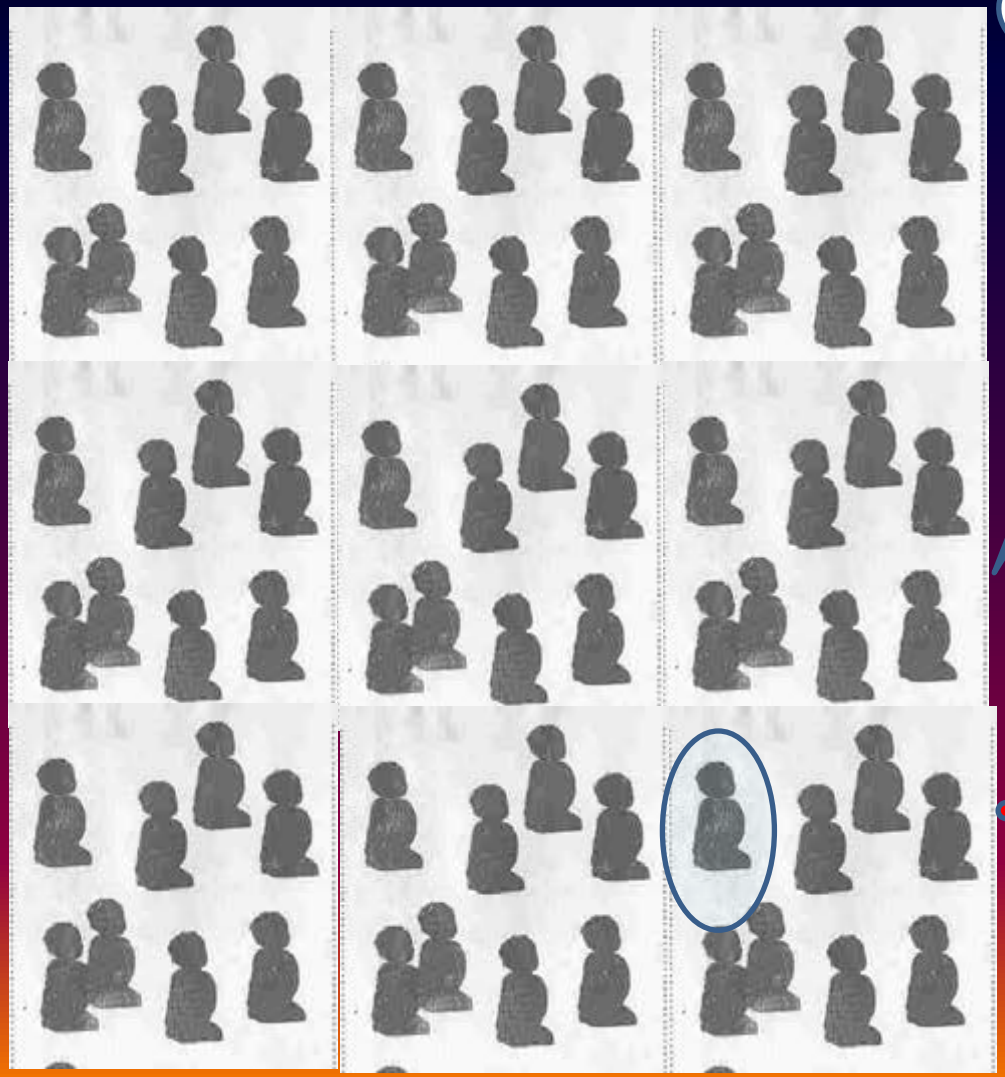
REBECCA LANGFORD,[1*] PETER LUNN,[2] AND CATHERINE PANTER-BRICK[3]
[1]*School of Social and Community Medicine, University of Bristol, Canynge Hall, Bristol, BS8 2PS, United Kingdom*
[2]*Department of Biological Anthropology, University of Cambridge, Cambridge, CB2 3DZ, United Kingdom*
[3]*Jackson Institute for Global Affairs and Department of Anthropology, Yale University, New Haven, Connecticut*

- 1st longitudinal study to assess hand-washing and enteropathy. 45 intervention, 43 control
- ↑mucosal damage = ↓ growth ($p \leq 0.01$ HAZ, WAZ)
- **Handwashing led to 41% ↓ diarrhea morbidity**
- ***No* change in markers of mucosal damage**
- *HW alone* doesn't address chronic subclinical infxn



From: Lunn, PG. 2000 Proceedings of the Nutrition Society 59:147


Has Diarrhea
Treat To Prevent
Death, Morbidity



ALL HAVE ENVIRONMENTAL ENTEROPATHY

Without any overt signs of ‘clinical’ illness

Ok, so you have a leaky, inflamed gut. What lives in it?

Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor

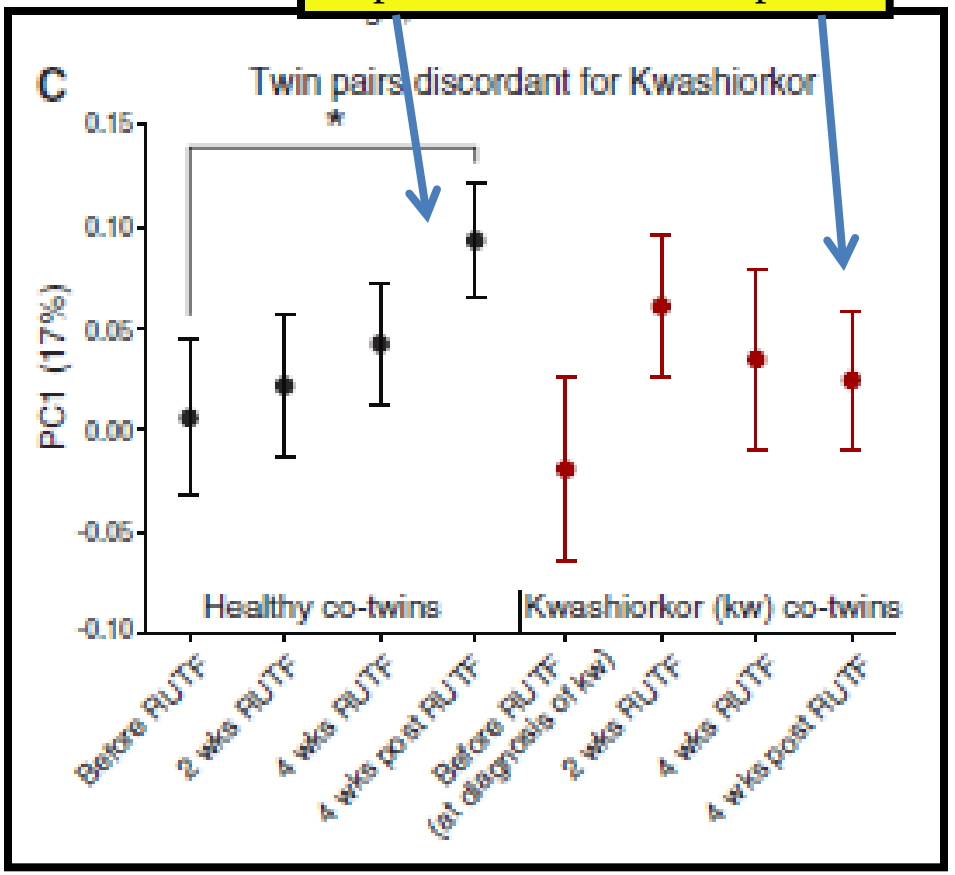
Michelle I. Smith,[1*] Tanya Yatsunenko,[1*] Mark J. Manary,[2,3,4] Indi Trehan,[2,3] Rajhab Mkakosya,[5] Jiye Cheng,[1] Andrew L. Kau,[1] Stephen S. Rich,[6] Patrick Concannon,[6] Josyf C. Mychaleckyj,[6] Jie Liu,[7] Eric Houpt,[7] Jia V. Li,[8] Elaine Holmes,[8] Jeremy Nicholson,[8] Dan Knights,[9,10]† Luke K. Ursell,[11] Rob Knight,[9,10,11,12] Jeffrey I. Gordon[1]‡

Science 339:548-554.

1 February 2013

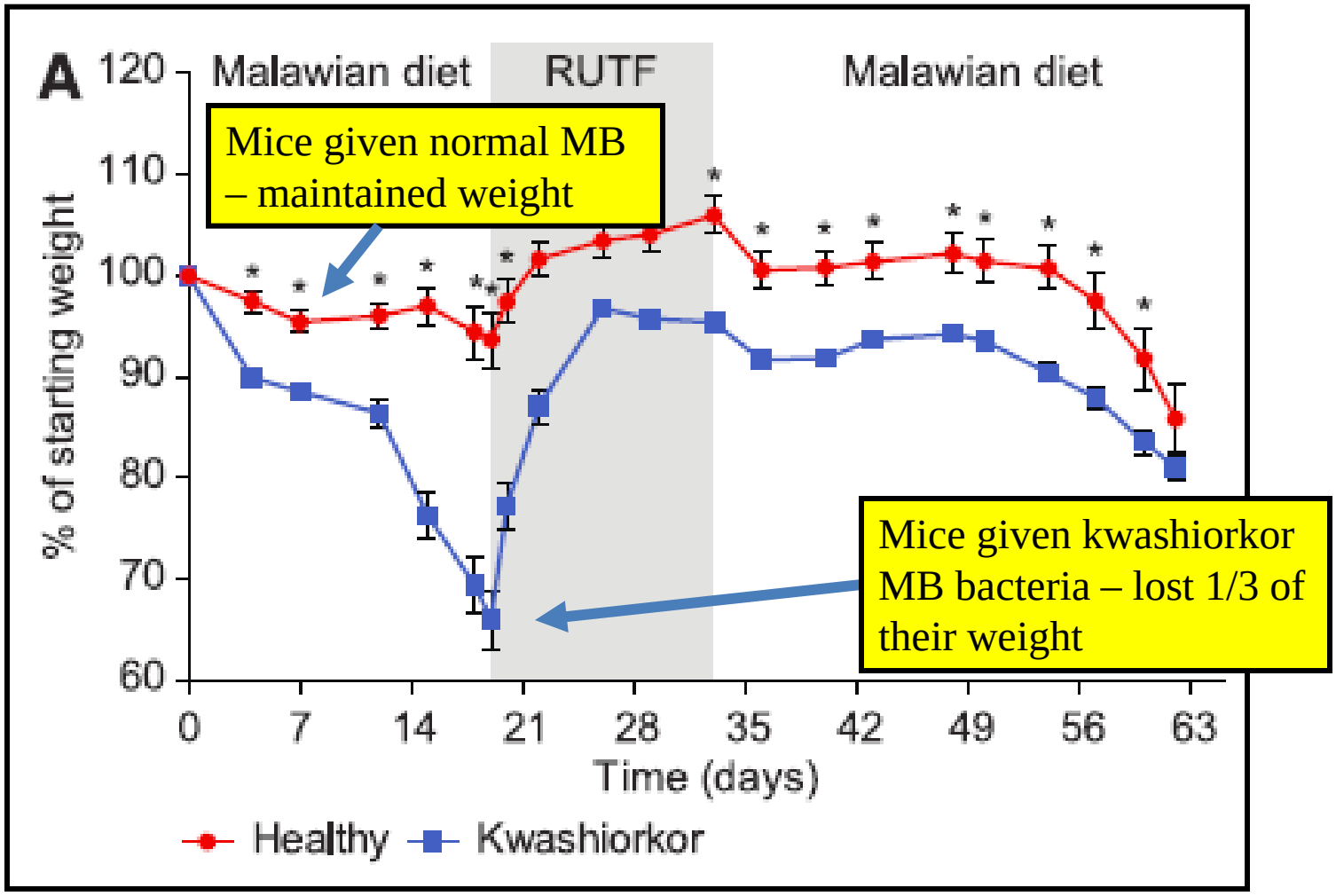
- 317 Malawian twins studied first 3 years of life
- 50% both well nourished; 43% discordant (one well, one malnourished); 7% both were malnourished.
- Both twins in discordant pairs received RUTF, a therapeutic food. Gut microbiomes (MB) studied: RUTF → transient MB improvement.

Improve Don't Improve



Then…

Gnotobiotic (sterile gut) mice – given either Normal or Kwashiorkor MB



Major adverse changes in amino acid and other gut metabolites

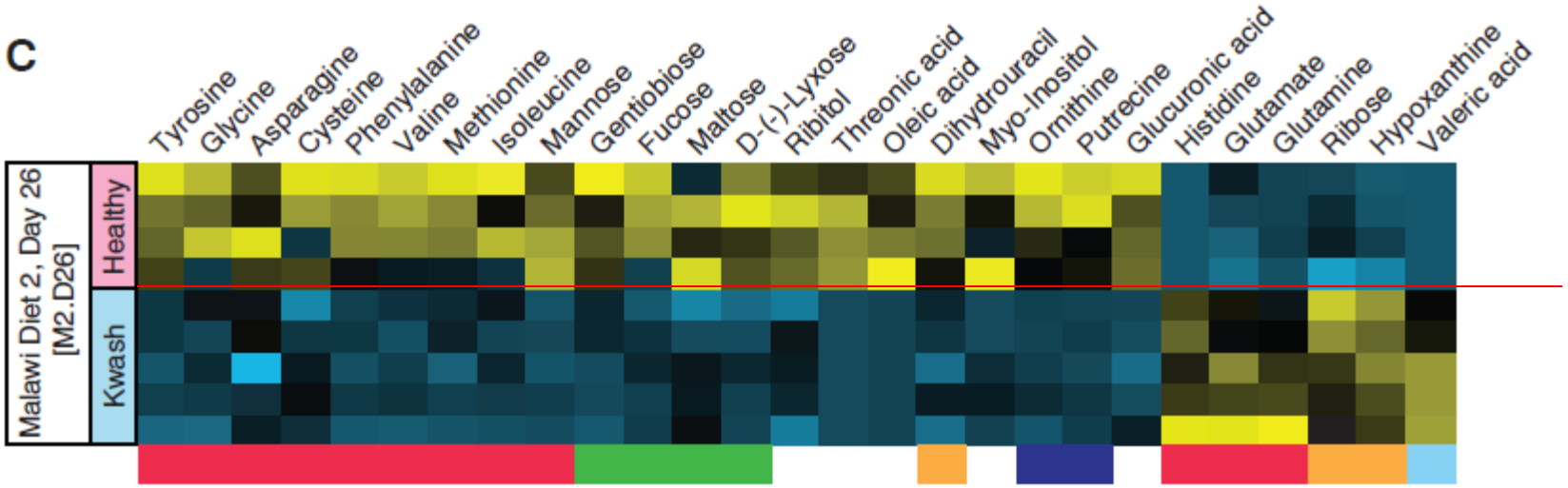
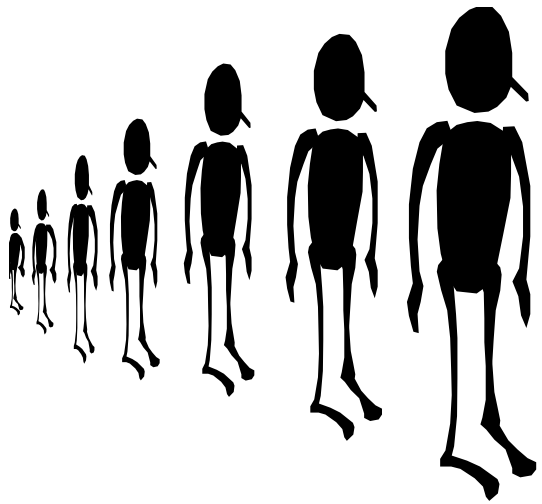


Fig. 3. Metabolites with significant differences in their fecal levels in gnotobiotic mice colonized with microbiota from discordant twin pair 196 as a function of diet. Data are from fecal samples collected 3 days before the end of (**A**) the first period of consumption of the Malawian diet (M1, day 16; abbreviated M1.D16), (**B**) RUTF treatment (RUTF.D10), and (**C**) the second period of Malawian diet consumption (M2.

Decoupled **TCA cycle** intermediates (↑ succinate) – mitochondrial metabolites – **↓energy metabolism**
Kwashiorkor microbiota + Malawi diet = **abnormal sulfur metabolism** (methionine, cysteine; protein metabolism)

Good Nutrition for Growth & Health

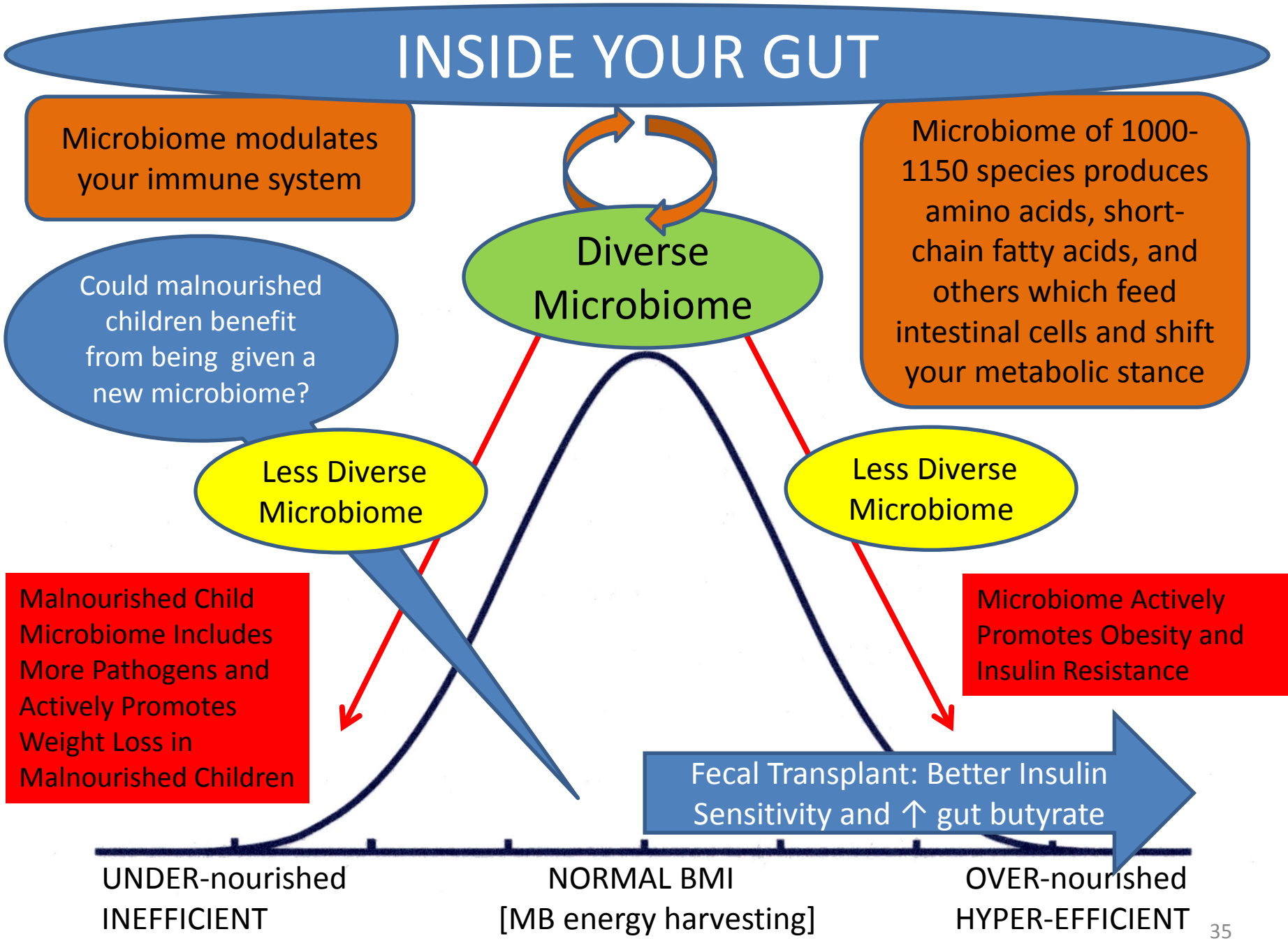


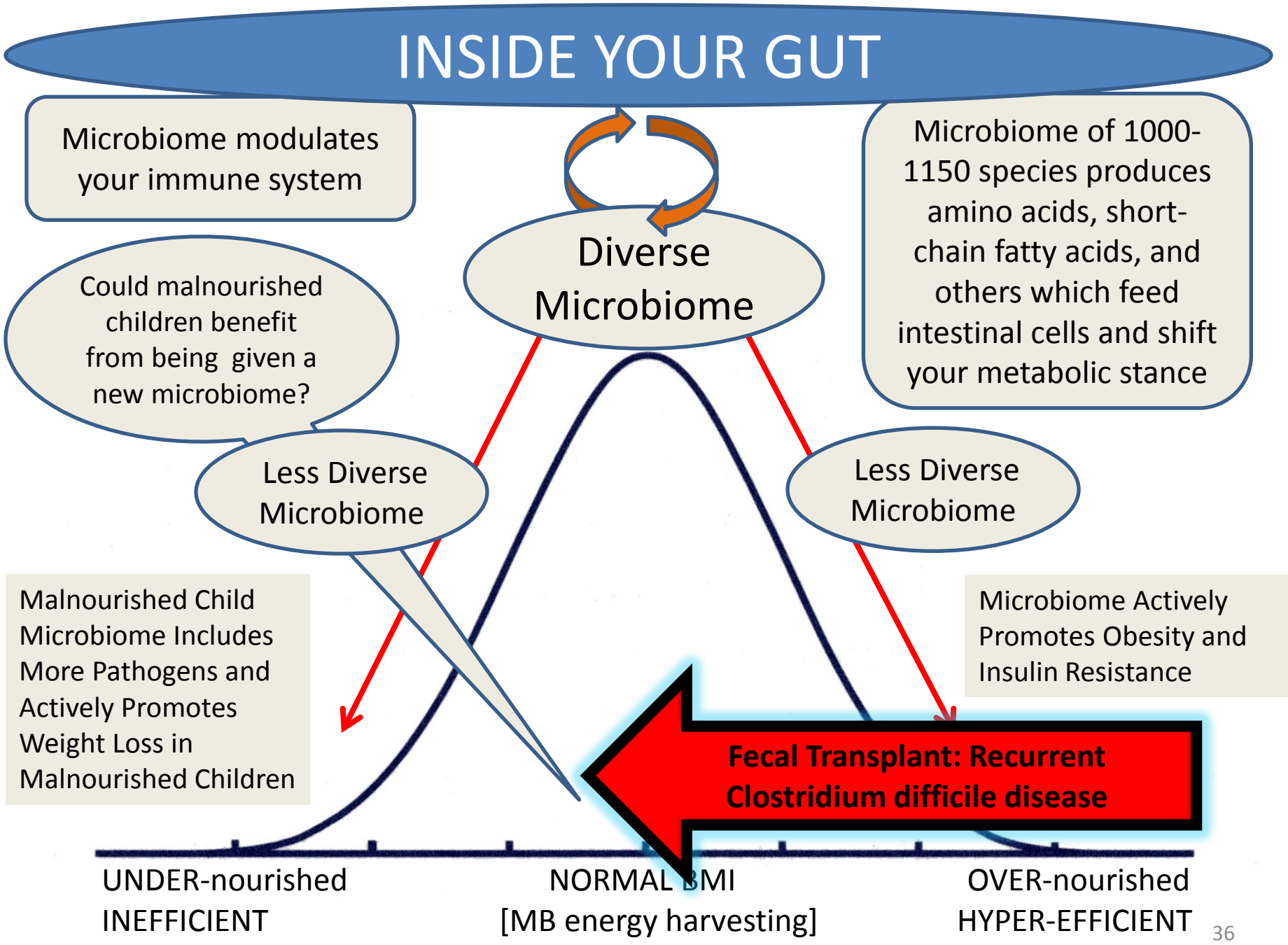
Poor populations:

- > 99% will have environmental enteropathy in the absence of good water/sanitation.

- Lacking WASH and barriers to fecal contamination, they will have a different spectrum of gut bacteria (the **gut microbiome**) than people with good WASH

- **Next: Aflatoxins**


INSIDE YOUR GUT
Microbiome modulates your immune system
Diverse Microbiome
Microbiome of 1000-1150 species produces amino acids, short-chain fatty acids, and others which feed intestinal cells and shift your metabolic stance
Could malnourished children benefit from being given a new microbiome?
Less Diverse Microbiome
Less Diverse Microbiome
Malnourished Child Microbiome Includes More Pathogens and Actively Promotes Weight Loss in Malnourished Children
Microbiome Actively Promotes Obesity and Insulin Resistance
Fecal Transplant: Better Insulin Sensitivity and ↑ gut butyrate
UNDER-nourished
INEFFICIENT
NORMAL BMI
[MB energy harvesting]
OVER-nourished
HYPER-EFFICIENT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Fecal Transplant: Recurrent Clostridium difficile disease
UNDER-nourished INEFFICIENT
NORMAL BMI [MB energy harvesting]
OVER-nourished HYPER-EFFICIENT

Environmental Enteropathy occurs when people live in contaminated environments. It is reversible. For example, US Peace Corps volunteers develop EE when they live in rural African villages. When they return to the US, their EE goes away.

The absence of fecal material – be it human or animal – in the environment both prevents and "treats" EE.

Water/sanitation is critical to this separation.

- Dean Spears has looked at open defecation as a marker of sanitation using 140 DHS data sets from 60 countries.

How much stunting is due to poor sanitation (and possibly EE?)

How much international variation in child height can sanitation explain?

Dean Spears*

First circulated: 10 December 2012
This version: 17 January 2013

Key findings Spear's analysis of 140 DHS from 65 'developing' countries

- Open defecation (certainly a marker of a "contaminated environment") is linked to a **1.24 S.D. decrease** in the height of children.
- **Sanitation alone** accounts for **54%** of the between-country height variation (next slide).
- Open defecation and a lack of sanitation in an household, along with country GDP, predict child height *more than* mother's height or education; governance; or infrastructure.

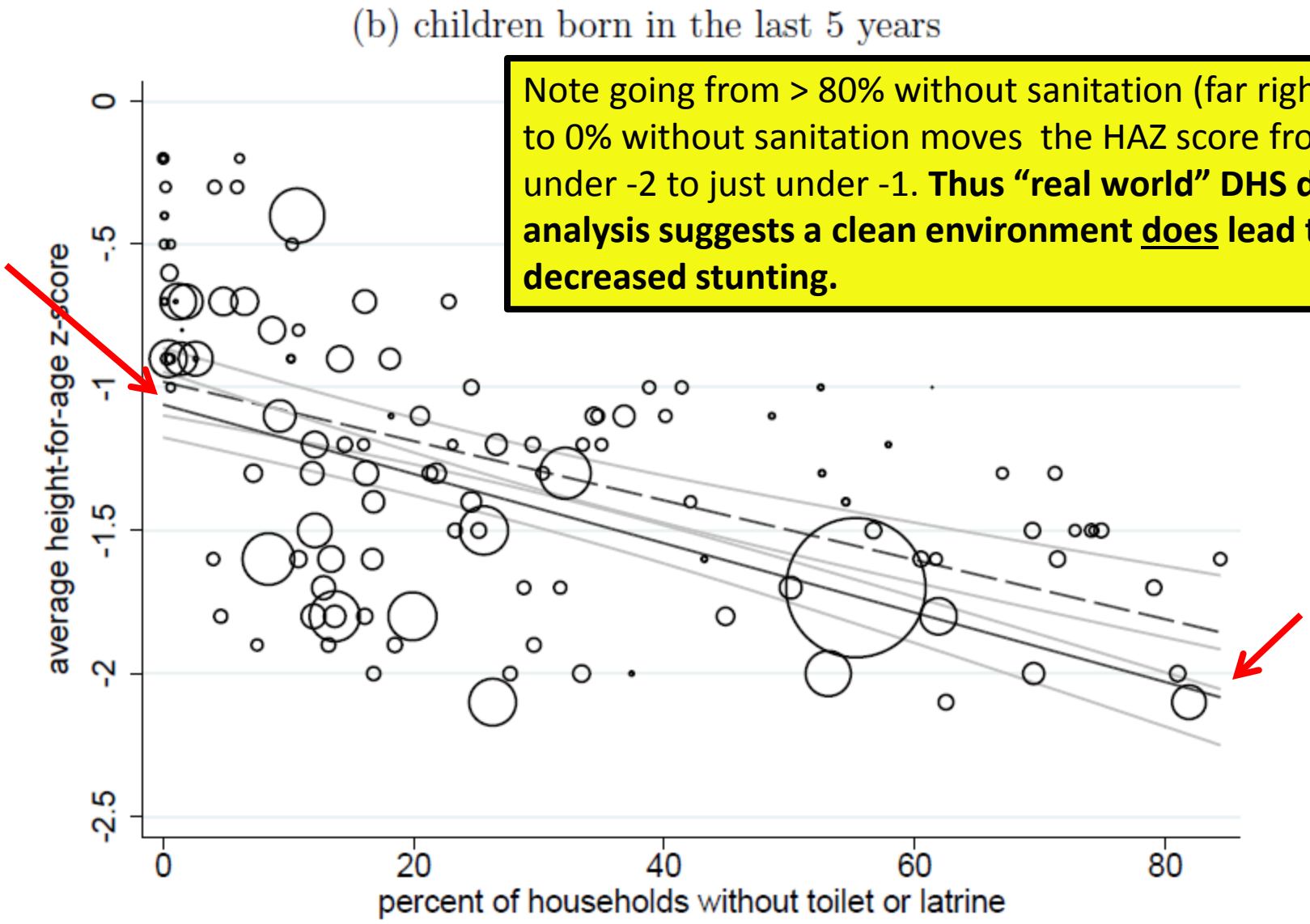


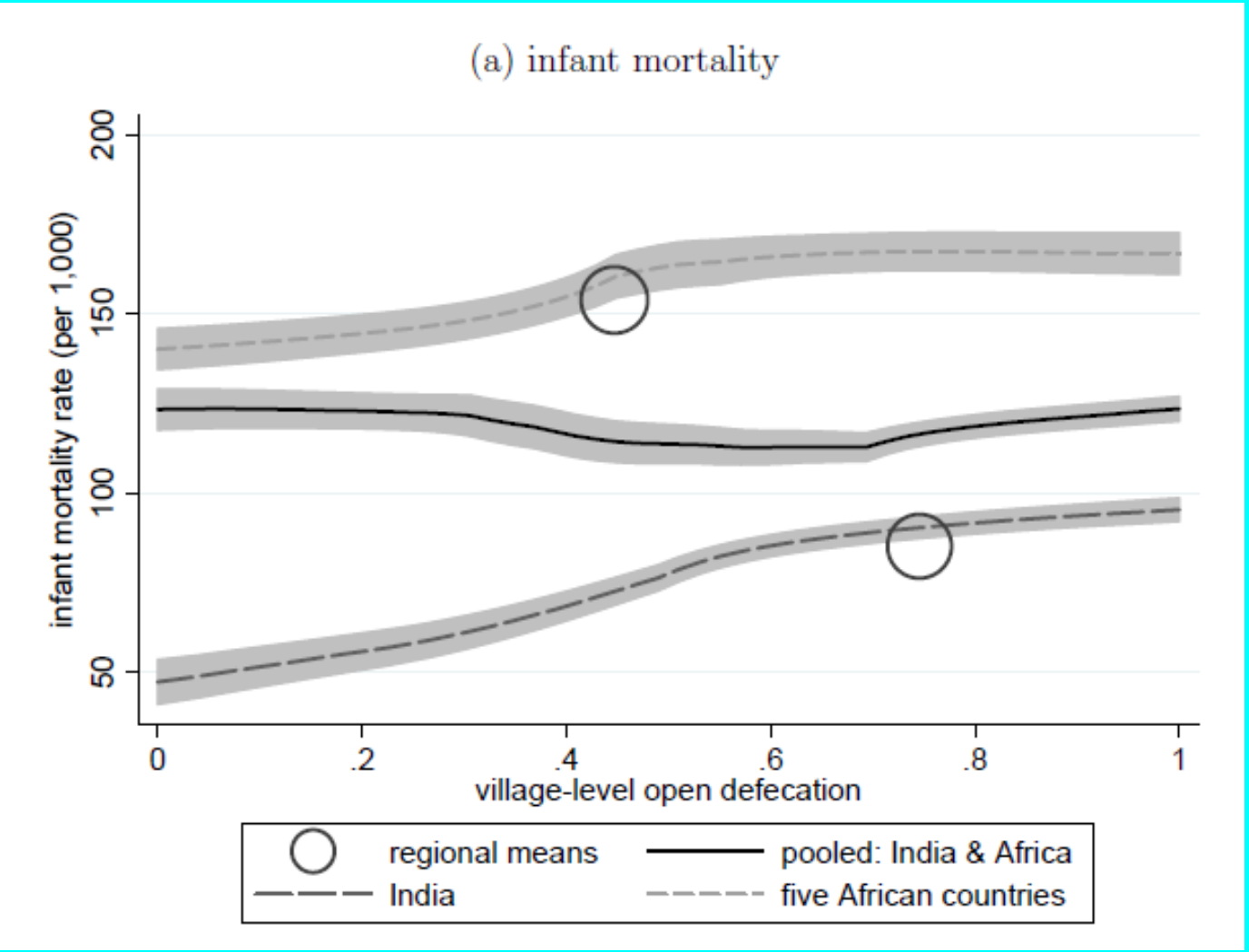
Figure 1: Open defecation predicts child height, across DHS survey round country-years

Solid OLS regression lines weight by country population; dashed lines are unweighted.

Econometric analyses Spears 2013

- Sanitation predicts stunting even when income is controlled. "…The difference between Nigeria's 26% open defecation rate and India's 55% is associated with an increase in child height approximately equivalent to quadrupling GDP per capita." **Again: India would have to quadruple national income to make up for its poor sanitation as compared to Nigeria.**
- Sanitation and population density interact, open defecation harms human capital. Open defecation (no sanitation) explains **65%** of global height. The policy case for sanitation as a public good is immense.

Mortality Rises with Bad Sanitation

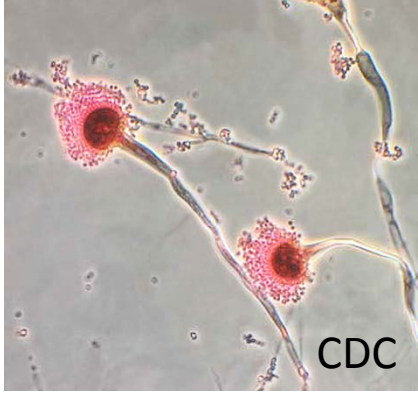


Aflatoxins and other mycotoxins

Drying Cassava, Kamwenge Uganda: note green/yellow fungal discoloration

Photo: J K Griffiths Uganda December 2012

Aflatoxins (Aflatoxins are mycotoxins)



- Produced by *Aspergillus* fungus
- Known – hepatoxic & cause liver cancer in people
- Known in mammals to cause growth faltering and ↓ *in utero* growth (e.g. low birth weight)
- Associated* with lower birth weight, growth, stunting, and wasting in children
- Associated* with lower CD4 and higher viral loads (e.g. worse immunity) in people with HIV
- Widespread exposure in sub-Saharan Africa, SE Asia; maize, peanuts, many other crops.

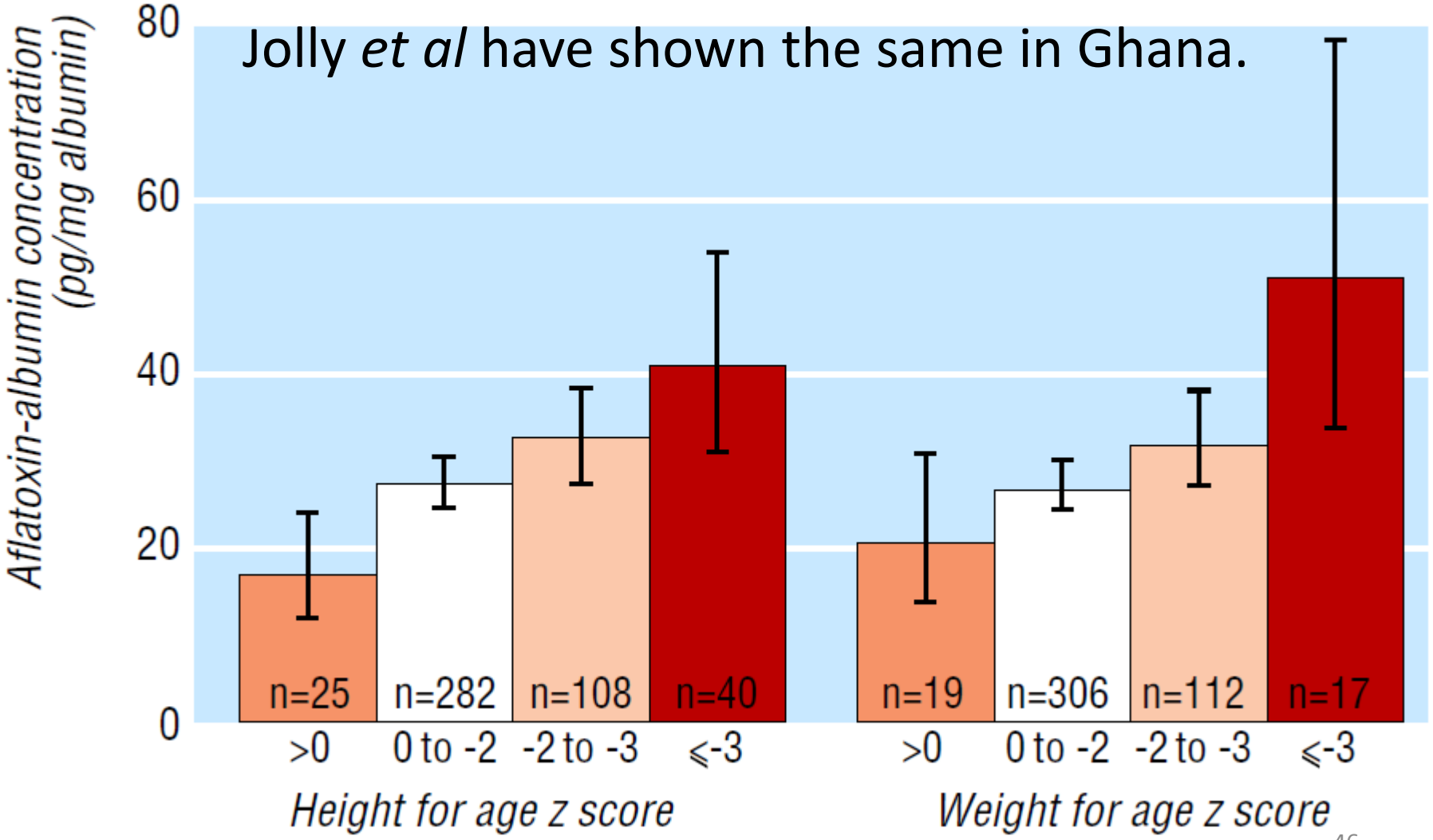
*Some criticize these studies for only being "associative" - but it is *unethical* to give aflatoxins to people. Prospective studies of exposure and outcomes are needed to show "causation."

Aflatoxins II

- Contamination occurs in the field; promoted by poor (too humid) post-harvest storage.
- Passed *in utero* and in breast milk to children
- Complementary food (e.g. porridge made from maize) is frequently contaminated – as are milk, eggs, chickens, animal meats…
- Prevention: storage without moisture/oxygen; dispersal of natural variant *Aspergillus* which lacks toxin; test and condemn crops/foods

Gong et al (BMJ, 2002) showed that **stunting** and **weight for age was inversely related to blood aflatoxin levels** in Gambia ($p < 0.001$, $R^2 = 0.37$).

Jolly *et al* have shown the same in Ghana.



PIGLET DATA
Dersjant-Li et al 2003

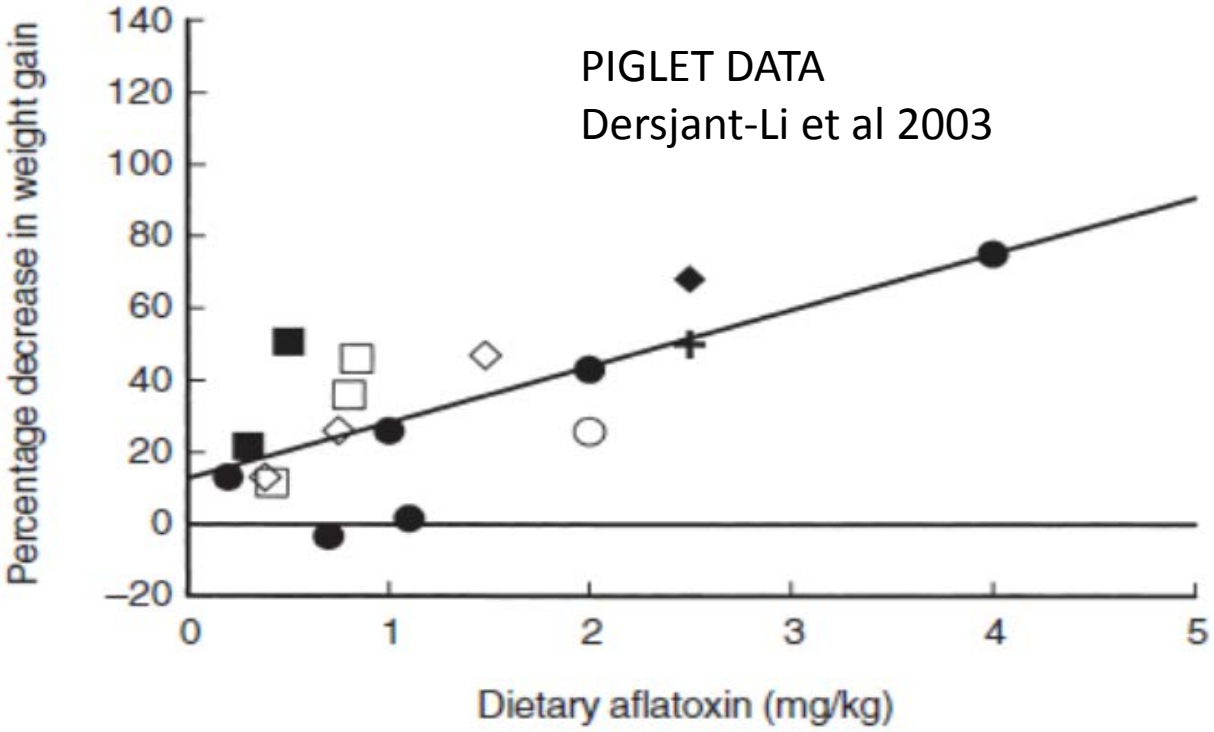
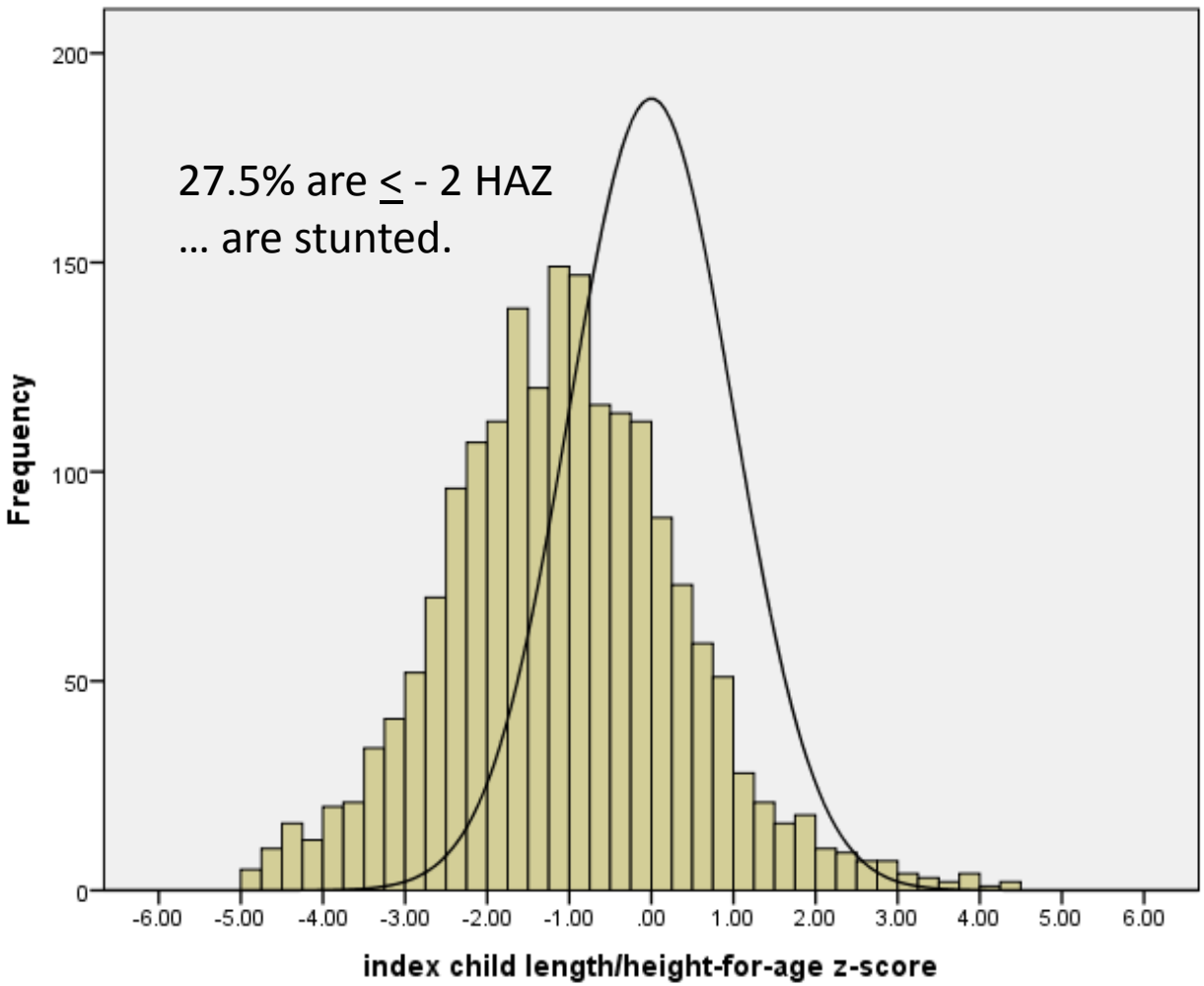
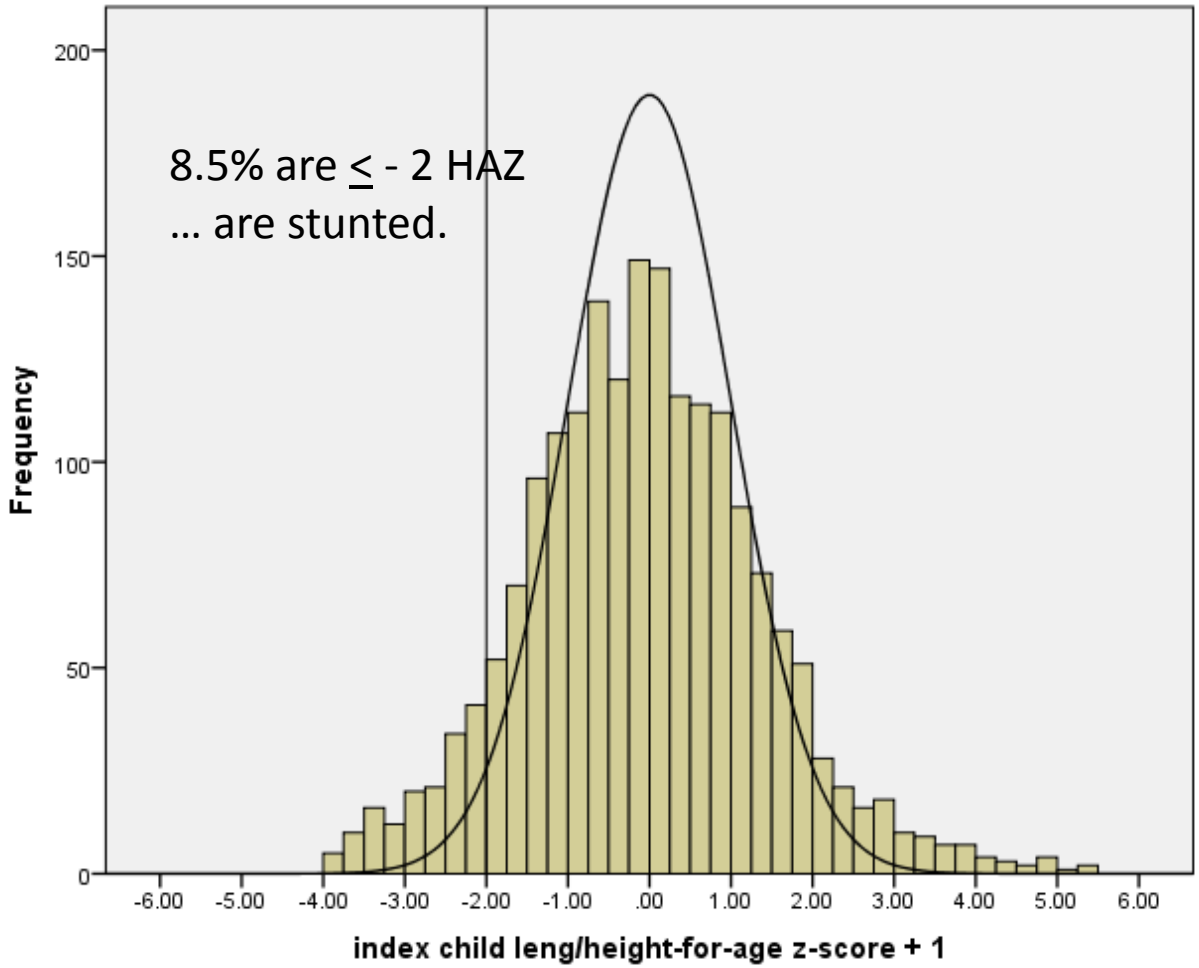


Fig. 1. The relationship between dietary aflatoxin and decrease in weight gain of pigs relative to a toxin-free control group ($Y = 12{\cdot}7 + 15{\cdot}6X$; R^2 0·52). Data were derived from Table 1: (○), Harvey *et al.* (1989; cultured material); (●), Armbrecht *et al.* (1971; cultured material); (□), Lindemann *et al.* (1993; naturally contaminated material); (■), Panangala *et al.* (1986; naturally contaminated material); (◇), Southern & Clawson (1979; naturally contaminated material); (+), Harvey *et al.* (1995*a*; naturally contaminated material); (◆), Harvey *et al.* (1995*b*; naturally contaminated material).



Nutrition Innovation Lab-Africa children 0-24 months 2012 panel survey

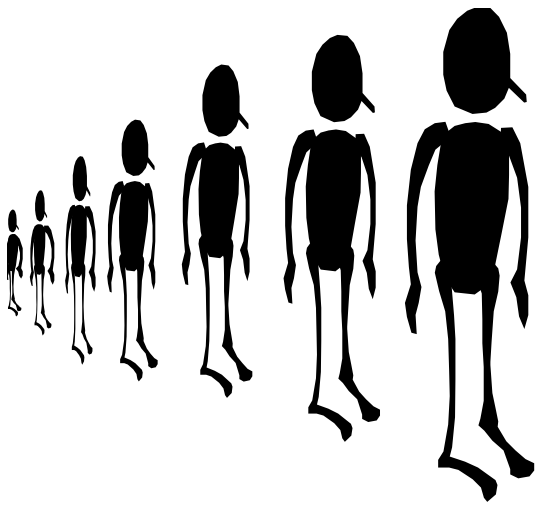
Apply + 1 HAZ unit estimate from Turner et al 2007 (benefit reducing AF by one log)



Nutrition Innovation Lab-Africa children 0-24 months 2012 panel survey

Apply + 1 HAZ unit estimate from Turner et al 2007 (benefit reducing AF by one log)

Good Nutrition for Growth & Health



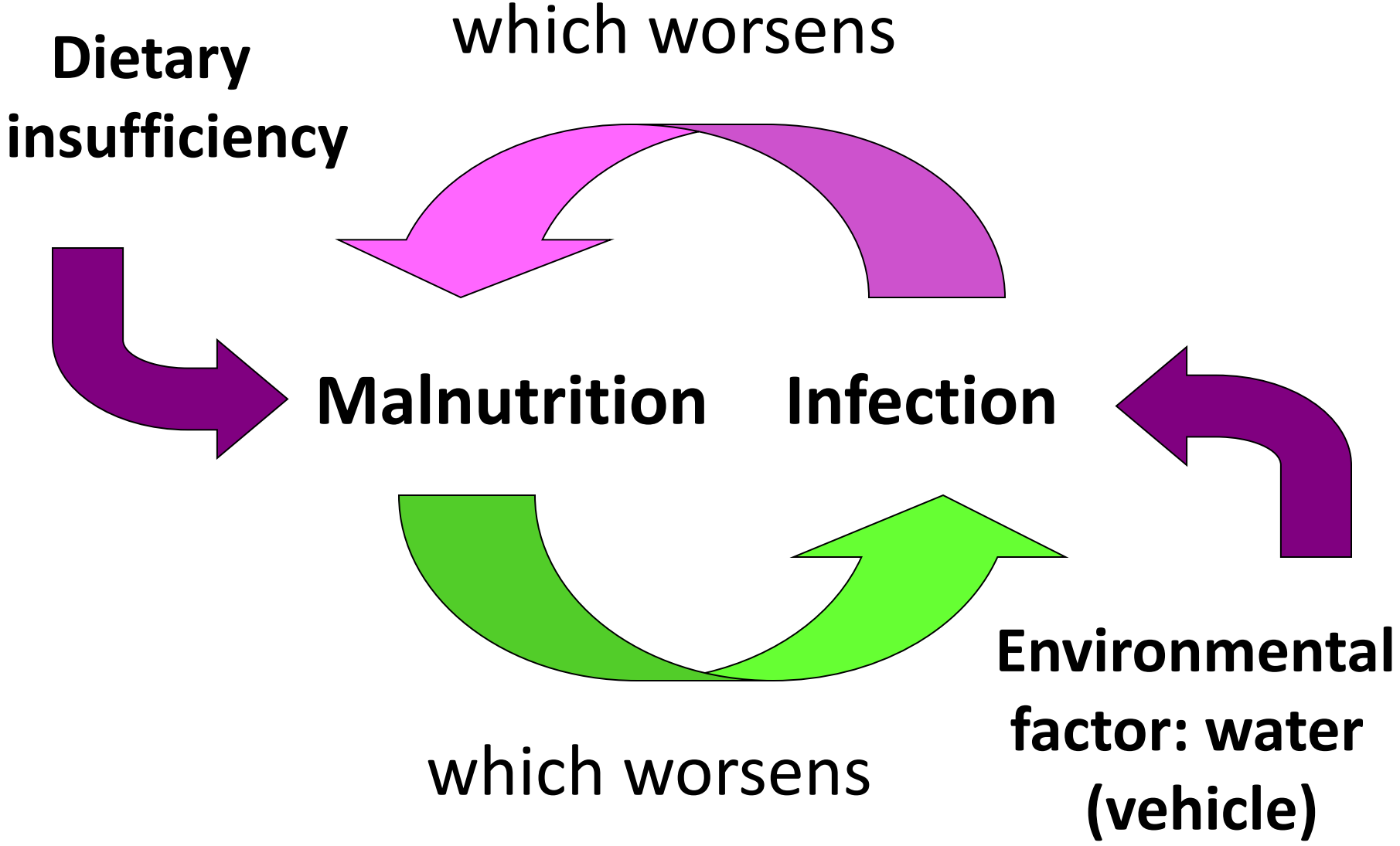
Poor populations:

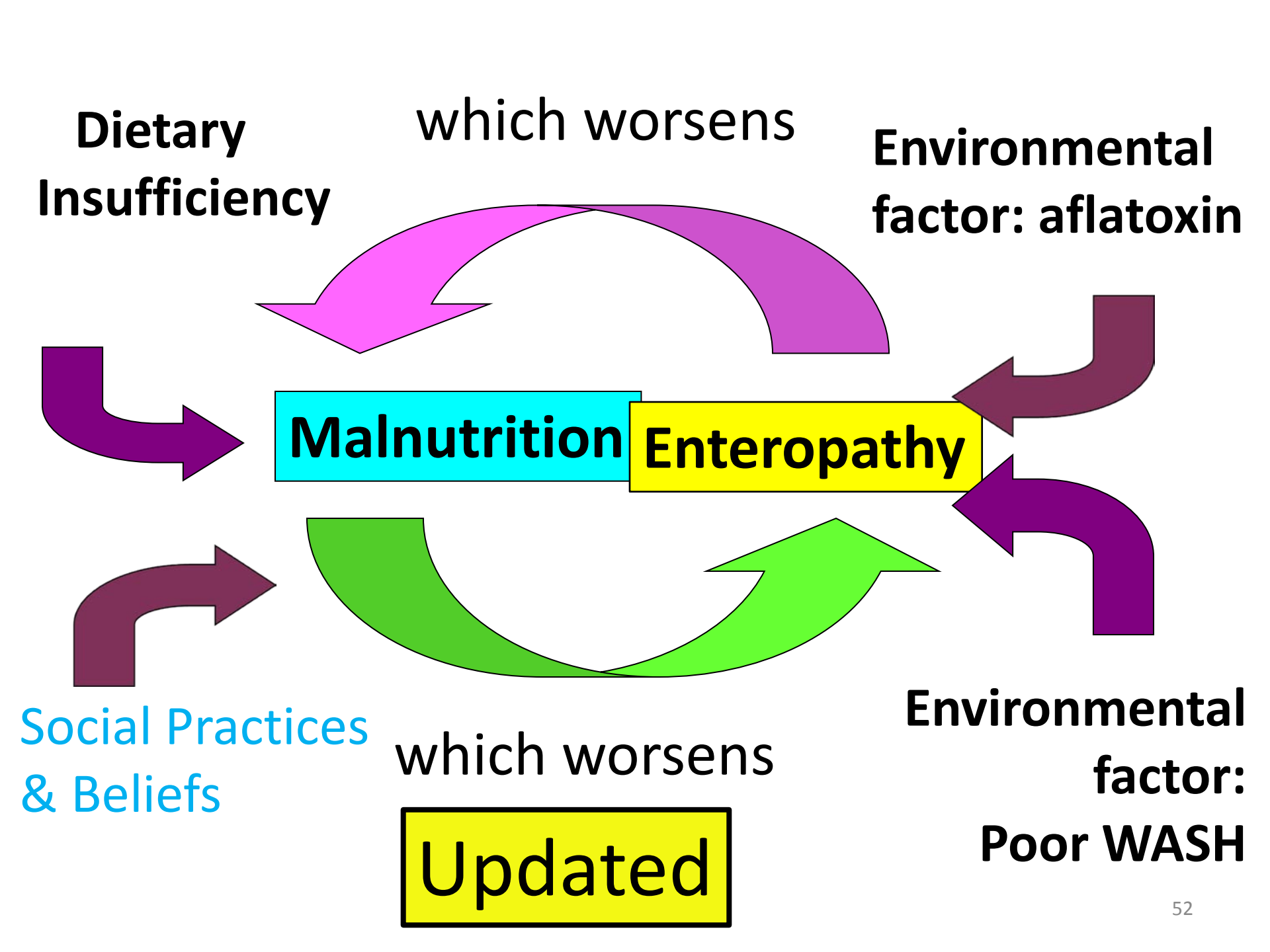
-Will have monotonous, non-diverse diets lacking key nutrients

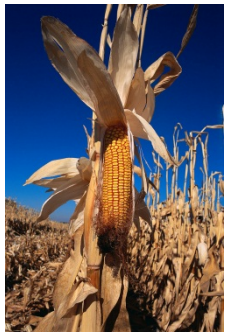
-Will likely eat aflatoxins in foods.

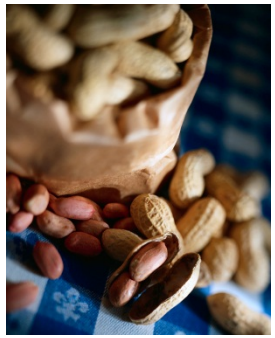
> 95% will have environmental enteropathy in the absence of good water/sanitation.

-Lacking WASH and barriers to fecal contamination, they will have a different spectrum of gut bacteria (the **gut microbiome**) than people with good WASH


which worsens
Dietary insufficiency
Malnutrition
Infection
which worsens
Environmental factor: water (vehicle)


Dietary Insufficiency
which worsens
Environmental factor: aflatoxin
Malnutrition
Enteropathy
Social Practices & Beliefs
which worsens
Environmental factor: Poor WASH
Updated





Maize, groundnuts
Key staple crops

Aspergillus spp. + moisture + warm temperature = Aflatoxin formation

Aflatoxin ingestion, duodenal uptake - Metabolites bind to DNA, proteins – can measure in blood, urine, tissues
Immunosuppression

Agricultural interventions

Enteropathy – permeable intestine with documented increased nutrient needs, state of chronic inflammation
Microbiome – less diverse, abnormal nutrient utilization by flora

Leaky Inflamed Intestine (EE)

WASH interventions

Nutrition interventions

Clinical Manifestations:
Cycle of repeated infections
Worsening nutritional status – stunting, underweight, IUGR

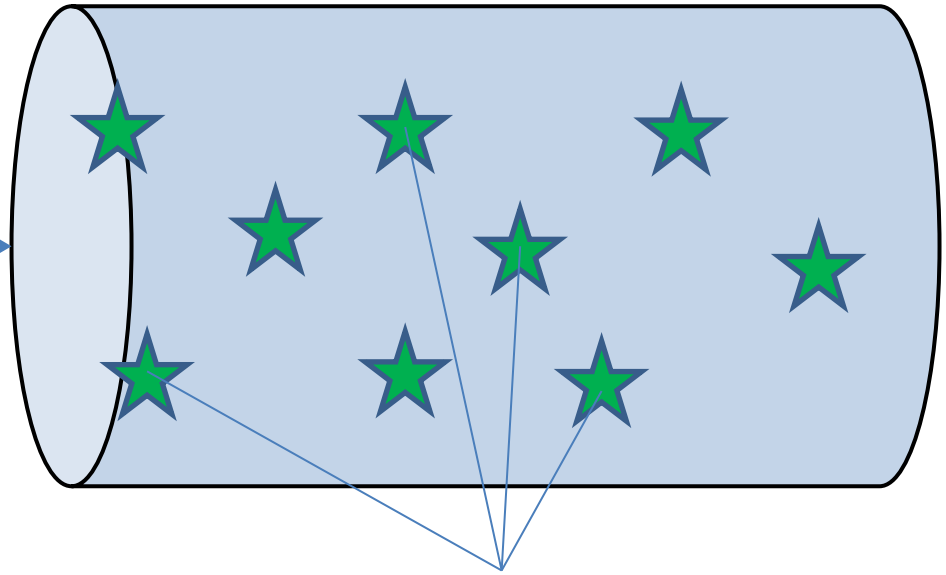
Diet, Societal Conditions
Diet: poor diversity, inadequate caloric & micronutrient intake, leading to **immunosuppression**
Pathogen exposure: Widespread food, water, environment contamination

MYCOTOXINS IN FOOD

HUMAN AND ANIMAL PATHOGENS

MICRO- AND MACRO-NUTRIENTS

NORMAL GUT – NOT PERMEABLE



HEALTHY INTESTINAL MICROBIOME

Take-Home: healthy growth requires:

- ✓ Adequate, varied nutrition with enough calories, micronutrients, and vitamins
- ✓ The absence of environmental toxins such as aflatoxin – immunosuppression, poor intra-uterine and post-natal growth, liver toxicity
- ✓ A clean environment which prevents environmental enteropathy, with its chronic inflammation and higher nutritional needs
- ✓ A normal gut microbiome which does not starve its host of nutrients and promote weight loss

Thanks!

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Photo: JK Griffiths Tanzania 2008

Talk version 3 updated Feb 19 2013