Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Biological impact of MNPs: a focus on infants and iron

Prof. Michael Zimmerman, M.D.

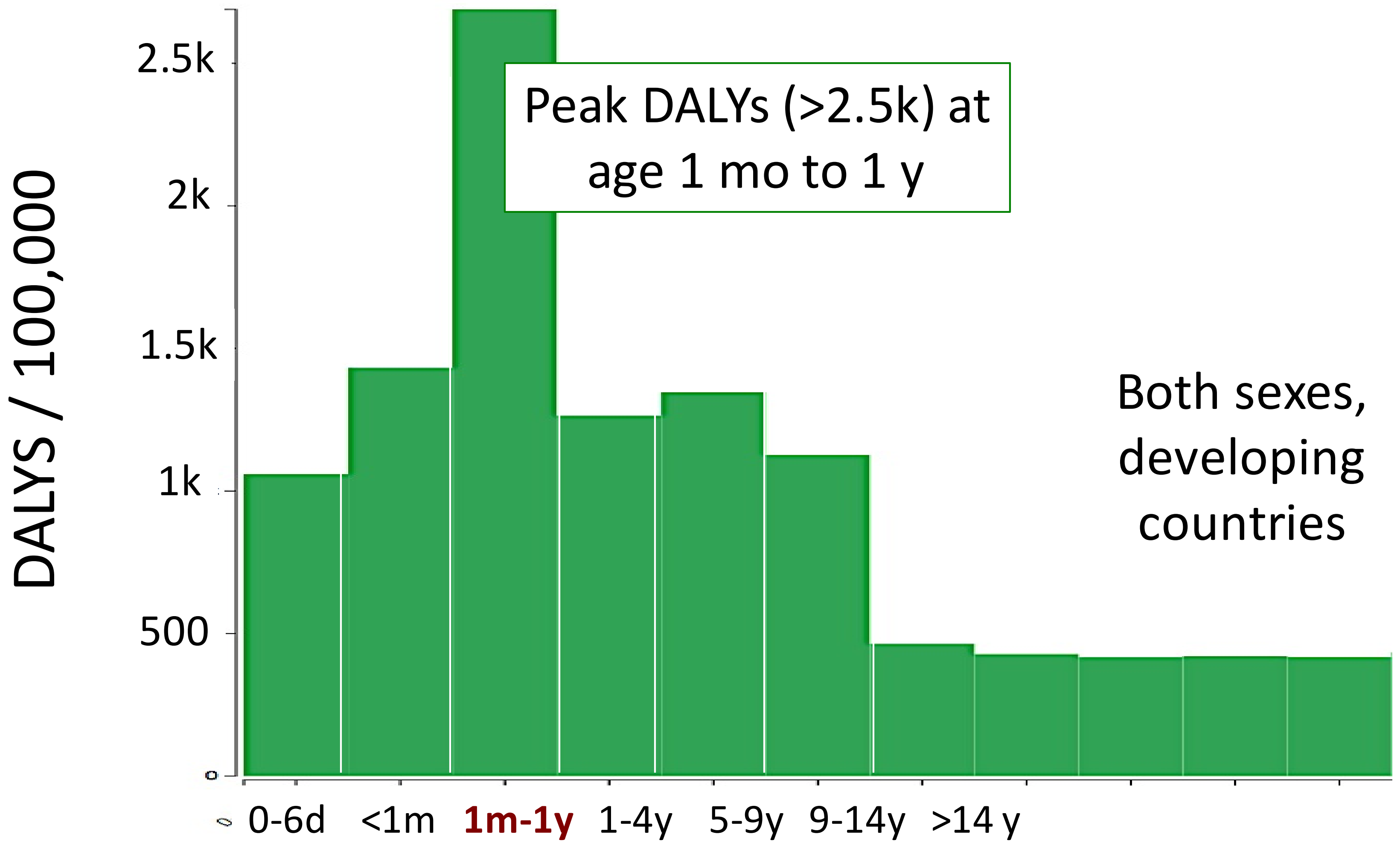
Human Nutrition, ETH, Switzerland

MNPs developed as an intervention for increasing micronutrient intake, particularly **iron**, in **children < 2 y of age**

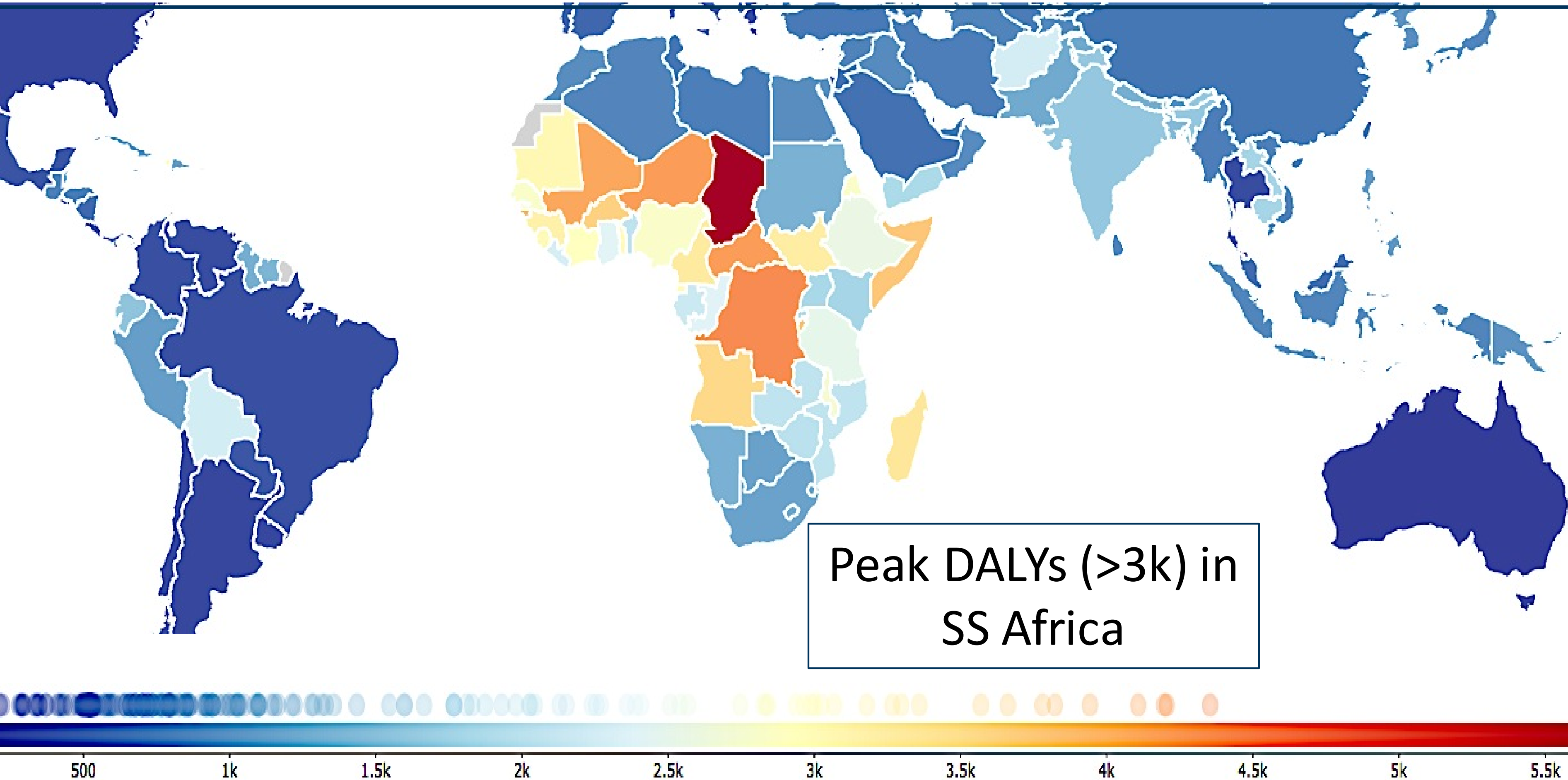
6-12 mo-old Kenyan infants: ≈70% anemia, 8-10% severe anemia

Foote et al., Am J Trop Med Hyg. 2013; Jaeggi et al., Mat Child Nutr. 2014

2013 Global burden of disease due to IDA : highest burden in 1m-1 y-old children (IHME, 2015)



2013 Global burden of disease due to IDA in <5 y-old children (IHME, 2015)



DALYS / 100,000

Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age

De-Regil LM, Suchdev PS, Vist GE, Walleser S, Peña-Rosas JP
Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

THE COCHRANE COLLABORATION®

- Assessed the effects and safety of home fortification with MNPs on nutrition, health and developmental outcomes in children <2 y
- Included studies up to Feb 2011

Selection criteria

- Included randomised and quasi-randomised trials with either individual or cluster randomization
- Participants: children <2 y, no specific health problems
- Intervention: consumption of food fortified with MNPs formulated with at least iron, zinc and vitamin A compared with placebo, no intervention or use of iron containing supplements

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Trials included

- Eight trials (3748 participants) in countries where anemia a public health problem (>40% affected)

<table>
<tr><th>Country</th><th>Nutrients</th><th>Iron and zinc</th><th>Vitamin A (µg)</th><th>Author and yr</th></tr>
<tr><td>Cambodia</td><td>6</td><td rowspan="8">12.5 mg
as Fe
Fumarate

5 mg
as Zn
gluconate</td><td>300</td><td>Giovannini 2006</td></tr>
<tr><td>Ghana</td><td>6</td><td>300</td><td>Christofides 2006</td></tr>
<tr><td>Ghana</td><td>6</td><td>300</td><td>Adu-Afarwuah 2007</td></tr>
<tr><td>Haiti</td><td>5</td><td>400</td><td>Menon 2007</td></tr>
<tr><td>India</td><td>5</td><td>300</td><td>Hirve 2007</td></tr>
<tr><td>Kenya</td><td>15</td><td>400</td><td>Suchdev 2011</td></tr>
<tr><td>Kyrzgyz Republic</td><td>5</td><td>300</td><td>Lundeen 2010</td></tr>
<tr><td>Pakistan</td><td>6</td><td>300</td><td>Sharieff 2006</td></tr>
</table>

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Trials included

- Interventions lasted 2-12 months
- 6 trials compared MNP vs no intervention or a placebo; 2 compared MNP vs daily iron drops
- Most trials assessed as at low risk of bias

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Six primary outcomes

- Anemia (defined as Hb <110 g/L)
- Iron deficiency (defined by trial)
- Hemoglobin concentration (g/L)
- Iron status (defined by trial)
- Weight-for-age (Z-scores)
- All-cause mortality

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Main results: MNP *compared with no intervention or placebo*

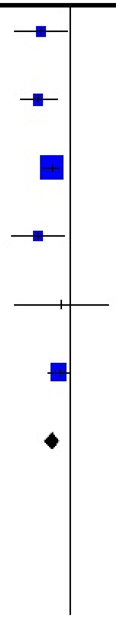
- Home fortification with MNPs reduced:
  - anemia by 31% (six trials, RR 0.69; 95% CI 0.60 to 0.78)
  - iron deficiency by 51% (four trials, RR 0.49; 95% CI 0.35 to 0.67)

- intervention appeared equally effective in:
  - populations with different baseline anemia prevalence
  - at all ages
  - at all duration of intervention (2 mo vs >6 mo)
  - in settings described as malaria-endemic vs settings where malaria sporadic

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Provision of MNP *vs no intervention or placebo*, anemia

Study or subgroup	MNP n/N	no int/placebo n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
Adu-Afarwuah 2007	18/98	31/96		6.1 %	0.57 [0.34, 0.95]
Giovannini 2006	25/65	43/60		12.1 %	0.54 [0.38, 0.76]
Lundeen 2010	148/283	206/274		45.9 %	0.70 [0.61, 0.79]
Menon 2007	18/76	22/50		6.0 %	0.54 [0.32, 0.90]
Sharieff 2006a	5/13	6/13		2.0 %	0.83 [0.34, 2.06]
Suchdev 2011	88/205	113/214		27.8 %	0.81 [0.66, 1.00]
Total (95% CI)	**740**	**707**		**100.0 %**	**0.69 [0.60, 0.78]**



Total events: 302 (MNP), 421 (no int/placebo)

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 6.17$, df = 5 ($P = 0.29$); $I^2 = 19\%$

Test for overall effect: $Z = 5.65$ ($P < 0.00001$)

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Main results: MNP *compared with no intervention or placebo*

- Home fortification with MNPs reduced:
  - anemia by 31% (six trials, RR 0.69; 95% CI 0.60 to 0.78)
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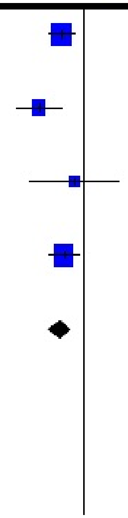
  - in settings described as malaria-endemic vs settings where malaria sporadic

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Provision of MNPs *vs no intervention or placebo*, iron deficiency

Study or subgroup	MNP n/N	no int/placebo n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
Adu-Afarwuah 2007	28/98	39/72		39.2 %	0.53 [0.36, 0.77]
Giovannini 2006	9/65	31/60		19.3 %	0.27 [0.14, 0.52]
Sharieff 2006a	3/13	4/13		6.1 %	0.75 [0.21, 2.71]
Suchdev 2011	19/73	88/192		35.4 %	0.57 [0.37, 0.86]
Total (95% CI)	**249**	**337**		**100.0 %**	**0.49 [0.35, 0.67]**



Total events: 59 (MNP), 162 (no int/placebo)

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 4.34$, df = 3 (P = 0.23); $I^2 = 31\%$

Test for overall effect: Z = 4.30 (P = 0.000017)

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Main results: MNP *compared with no intervention or placebo*

- Home fortification with MNPs reduced:
  - anemia by 31% (six trials, RR 0.69; 95% CI 0.60 to 0.78)
  - iron deficiency by 51% (four trials, RR 0.49; 95% CI 0.35 to 0.67)

- intervention appeared equally effective in:
  - populations with different baseline anemia prevalence
  - at all ages
  - at all duration of intervention (2 mo vs >6 mo)
  - in settings described as malaria-endemic vs settings where malaria sporadic

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Main results: comparison with *daily iron supplementation*

- Home fortification with MNPs produced similar results on:
  - anemia (one trial, RR 0.89; 95% CI 0.58 to 1.39)
  - Hb (two trials, MD -2.36 g/L; 95% CI -10.30 to 5.58)

- however, given the limited amount of data results should be interpreted cautiously

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

No effects on growth

- Home fortification with MNPs compared with no intervention or placebo (2 trials), no effect on:
  - weight-for-age Z-scores (MD 0.00; 95% CI -0.37 to 0.37)
  - length-for-age Z scores (MD 0.04; 95% CI -0.15 to 0.23) *(secondary outcome)*
  - weight-for-height Z scores (MD 0.44; 95% CI -0.44 to 0.52) *(secondary outcome)*

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Summary of main findings

Patient or population: children 6 to 23 months
Settings: community settings
Intervention: home fortification with multiple micronutrient powders
Comparison: placebo/no intervention

MD +6 g/L Hb
MD +20 ng/ml ferritin

Outcomes	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
Anaemia	**RR 0.69** (0.60 to 0.78)	1447 (6 studies)	**moderate**[1]
Iron deficiency	**RR 0.49** (0.35 to 0.67)	586 (4 studies)	**high**[2]
Haemoglobin (g/L)	**MD 5.87** (3.25 to 8.49)	1447 (6 studies)	**moderate**[1,3]
Iron status (ferritin concentrations in ng/mL)	**MD 20.38** (6.27 to 34.49)	264 (2 studies)	**low**[1,4]
Weight-for-age Z-score	**MD 0** (-0.37 to 0.37)	304 (2 studies)	**moderate**[1,5]
All-cause mortality	**0**	0 (0 studies)	None of the trials reported on this outcome.

CI: Confidence interval; **RR:** Risk ratio;

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Conclusions

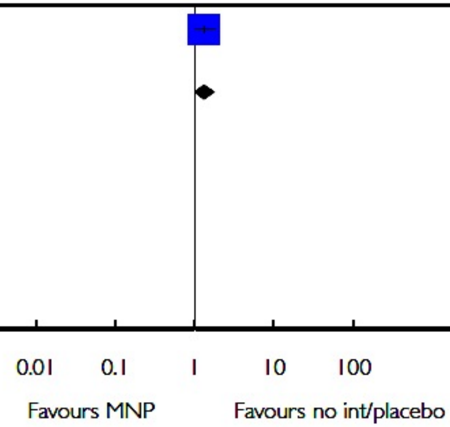
- Home fortification with MNPs an effective intervention to reduce anemia and iron deficiency in children 6-23 mo
- Effects comparable to daily iron supplementation
- MNPs well accepted but adherence variable, in some cases comparable to that achieved using standard iron supplements
- Benefits on zinc status, vitamin A status, morbidity, mortality or developmental outcomes unclear

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

- Since publication, several RCTs of MNPs have reported (Zlotkin et al 2013, Soofi 2013, Barth-Jaeggi et al 2014, Yousafzai et al. 2014) with results generally consistent with the review

MNPs vs *no intervention or placebo*: diarrhea

Study or subgroup	MNP n/N	no int/placebo n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
Menon 2007	72/125	35/81		100.0 %	1.33 [1.00, 1.78]
Total (95% CI)	**125**	**81**		**100.0 %**	**1.33 [1.00, 1.78]**



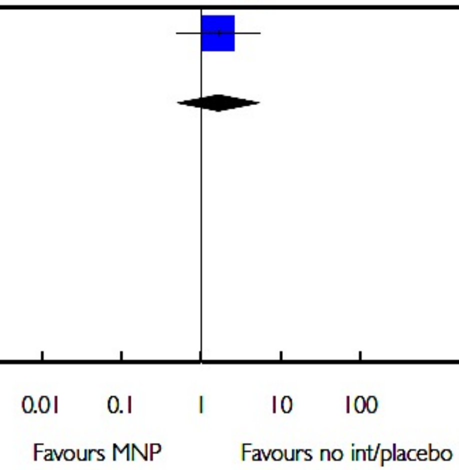
Total events: 72 (MNP), 35 (no int/placebo)

Heterogeneity: not applicable

Test for overall effect: Z = 1.93 (P = 0.053)

Test for subgroup differences: Not applicable

Study or subgroup	MNP n/N	no int/placebo n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
Giovannini 2006	7/65	4/62		100.0 %	1.67 [0.51, 5.42]
Total (95% CI)	**65**	**62**		**100.0 %**	**1.67 [0.51, 5.42]**



Total events: 7 (MNP), 4 (no int/placebo)

Heterogeneity: not applicable

Test for overall effect: Z = 0.85 (P = 0.39)

Test for subgroup differences: Not applicable

Cochrane Database Syst Rev. 2011 Sep 7;(9):CD008959

Systematic review: MNPs increase risk for diarrhea in children by 4%

REVIEW **Open Access**

Effectiveness of Micronutrient Powders (MNP) in women and children

Rehana A Salam[1], Ceilidh MacPhail[2], Jai K Das[1], Zulfiqar A Bhutta[1,3*]

Study or Subgroup	log[Risk Ratio]	SE	Experimental Total	Control Total	Weight	Risk Ratio IV, Fixed, 95% CI
Bhutta	0.0275	0.0177	750	778	48.2%	1.03 [0.99, 1.06]
Bhutta	0.0459	0.0172	746	778	51.0%	1.05 [1.01, 1.08]
Giovannini 2006	0.5124	0.6011	65	62	0.0%	1.67 [0.51, 5.42]
Menon 2007	0.2875	0.1487	125	81	0.7%	1.33 [1.00, 1.78]
Osei 2011	-0.2791	0.4822	186	201	0.1%	0.76 [0.29, 1.95]
Total (95% CI)			**1872**	**1900**	**100.0%**	**1.04 [1.01, 1.06]**

Heterogeneity: $Chi^2 = 4.43$, $df = 4$ ($P = 0.35$); $I^2 = 10\%$
Test for overall effect: $Z = 3.15$ ($P = 0.002$)

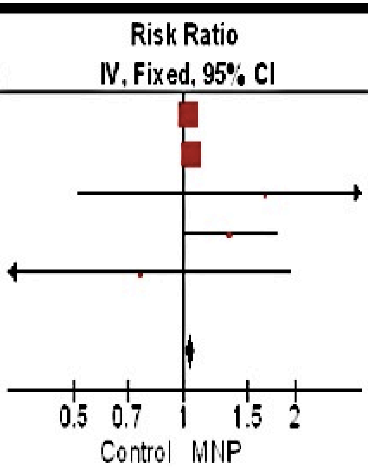
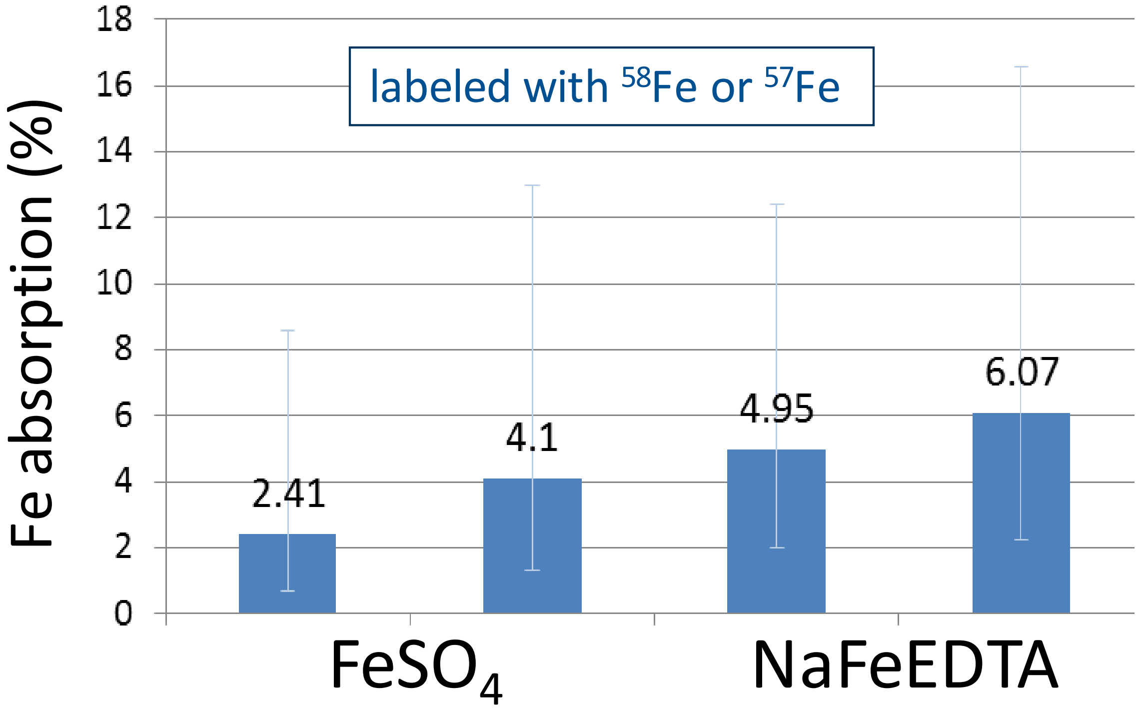


Figure 4 Forest Plot for the impact of MNPs on diarrhea in children

Iron from MNPs is poorly absorbed and produces large increases in colonic iron

- Fe absorption from MNPs added to maize high or low in PA in ID women
- Even ‘highly bioavailable’ Fe fortificants are absorbed <10%



Troesch et al. Am J Clin Nutr 2009

- Fe in the body is tightly bound, limiting supply to potential pathogens, and during infection, iron supply is sharply reduced in the extracellular compartment

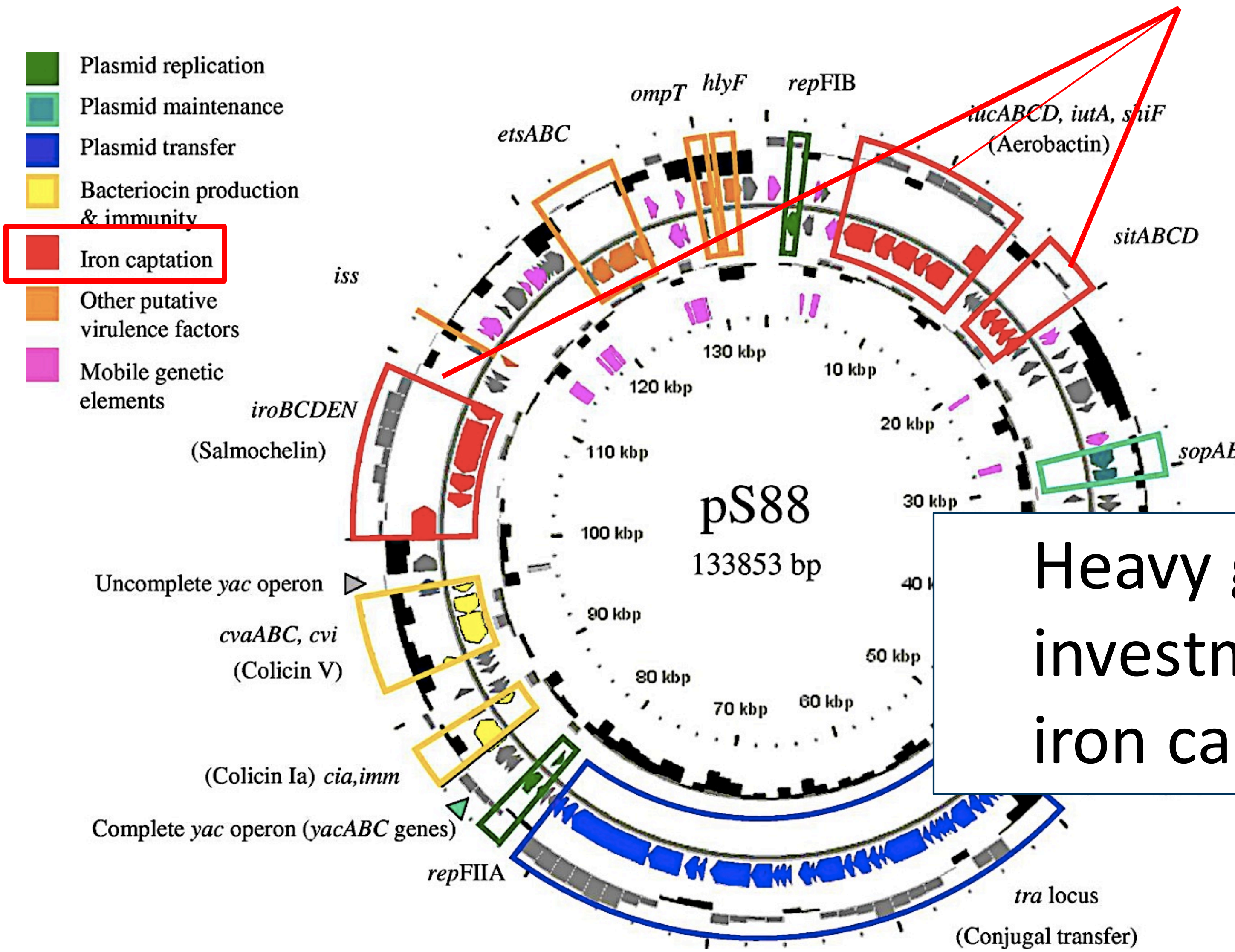
- But there is *no similar system for sequestration of dietary iron in the gut lumen*

Iron is a growth-limiting nutrient for many gut bacteria, including pathogens

- Strains vigorously compete for unabsorbed dietary iron in the colon, as colonization may depend on ability to acquire iron
- Beneficial 'barrier' bacteria, such as lactobacilli, reduce colonization by enteric pathogens, but do not require iron.
- Most gram-negative bacteria (e.g. *Salmonella*, *Shigella* or pathogenic *E. coli*) - iron acquisition plays an essential role in virulence and colonization
  - 500+ bacterial siderophores with high Fe-binding constants

Virulent *E. coli* (strain S88) plasmid:

3 different Fe uptake systems



Heavy genomic investment in iron capture

Infect Immun 2009; 77(6): 2272–2284

THE LANCET

Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial

12.5 mg

Sunil Sazawal, Robert E Black, Mahdi Ramsan, Hababu M Chwaya, Rebecca J Stoltzfus, Arup Dutta, Usha Dhingra, Ibrahim Kabole, Saikat Deb, Mashavi K Othman, Fatma M Kabole

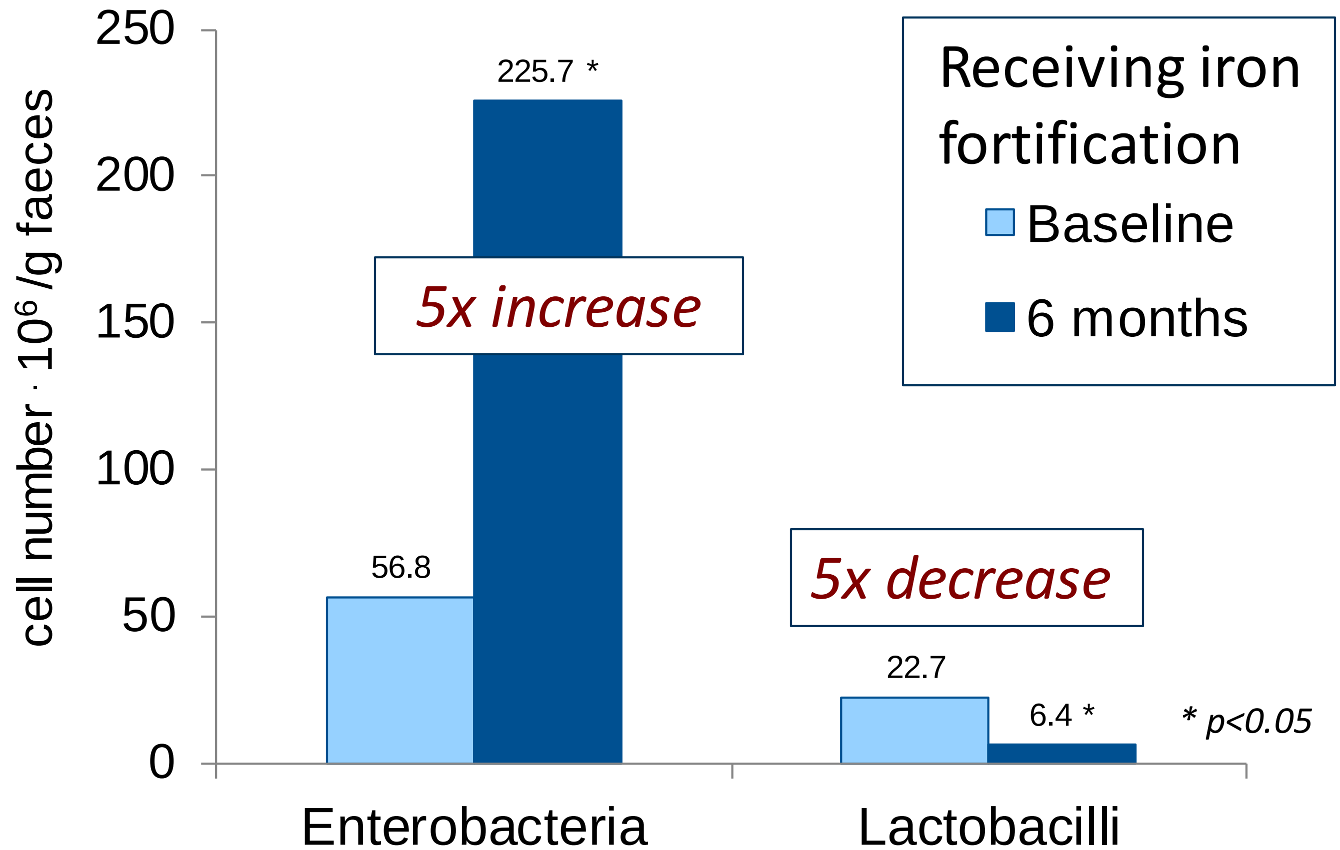
2007 WHO Consultation, interpreting the Pemba study : *'it is unclear whether the risks of iron are specific to malaria or whether they apply to other infections, including sepsis from enteric bacteria'*

Iron fortification sharply increases enterobacteria and decreases Lactobacilli numbers in African children

The American Journal of Clinical Nutrition

The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Côte d'Ivoire[1–4]

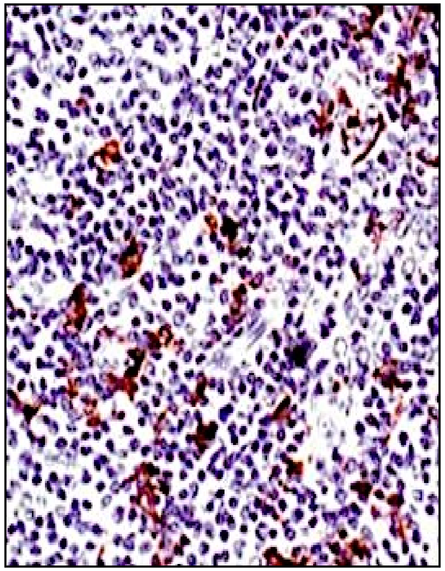
Michael B Zimmermann, Christophe Chassard, Fabian Rohner, Eliézer K N'Goran, Charlemagne Nindjin, Alexandra Dostal, Jürg Utzinger, Hala Ghattas, Christophe Lacroix, and Richard F Hurrell

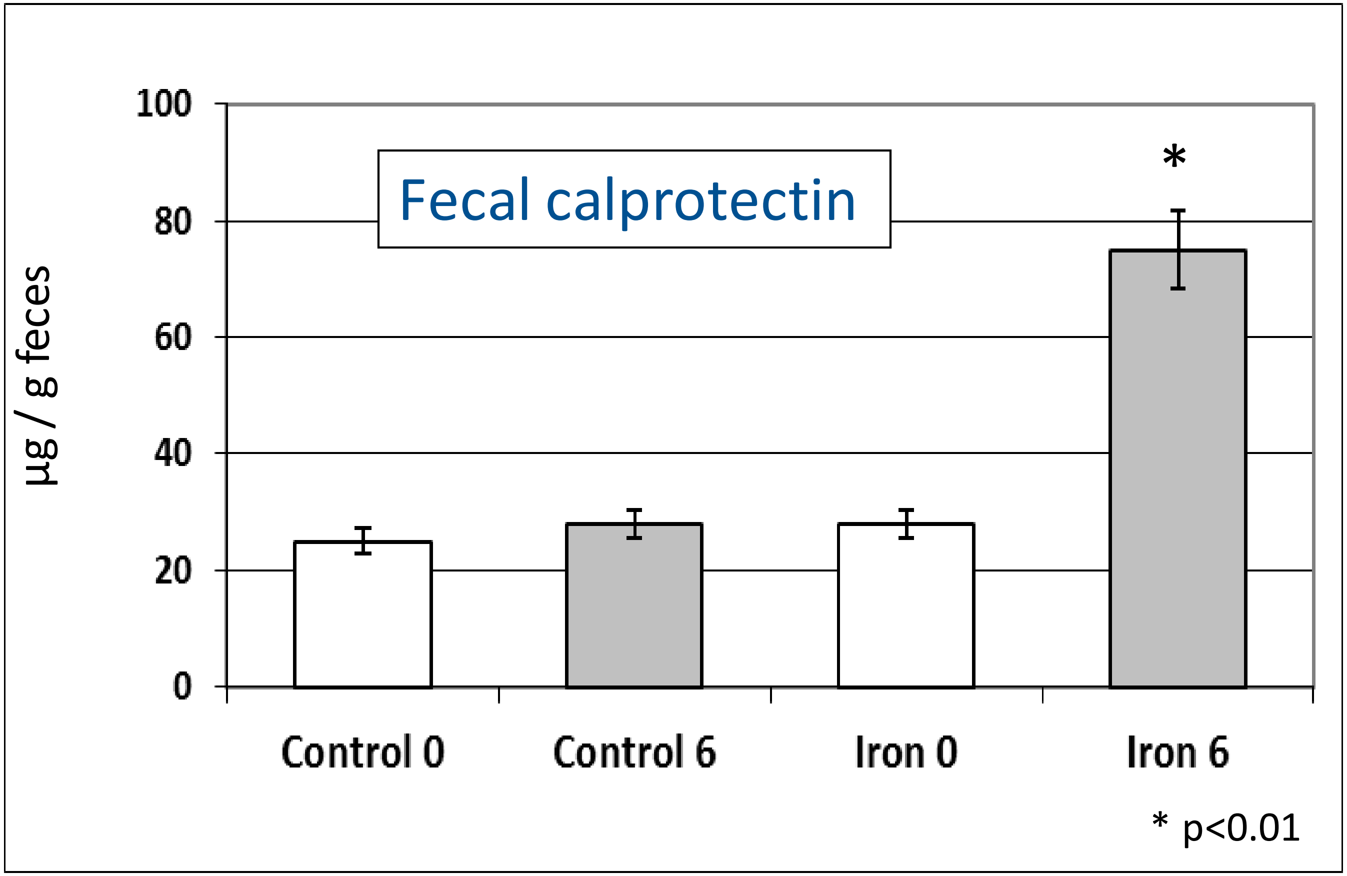


Zimmermann et al. AJCN 2010

Fortification increased gut inflammation

- Fe increased fecal calprotectin 4-fold; correlated with increase in fecal enterobacteria





Zimmermann et al. AJCN 2010

THE LANCET

Effect of provision of daily zinc and iron with several micronutrients on growth and morbidity among young children in Pakistan: a cluster-randomised trial

Sajid Soofi, Simon Cousens, Saleem P Iqbal, Tauseef Akhund, Javed Khan, Imran Ahmed, Anita K M Zaidi, Zulfiqar A Bhutta

- Cluster randomized, ca. 2700 infants at 6 mo age
- 'In-home' fortification with a micronutrient powder (MNP) 12.5 mg Fe/day, one year trial
- Not blinded, compared to unsupplemented group

Lancet 2013; 382: 29–40

	Group A: control group		Group B: MNP without zinc		Group C: MNP with zinc		p value†
	Incidence per child year	IRR	Incidence per child year	IRR	Incidence per child year	IRR	
Age 6·0–17·9 months							
Any diarrhoea	3·73 (3030)	1·0	4·16 (3229)	1·05 (0·94–1·17)	4·32 (3323)	1·12 (1·01–1·26)	0·12
Bloody diarrhoea	0·08 (69)	1·0	0·16 (124)	1·63 (1·12–2·39)	0·17 (132)	1·88 (1·29–2·74)	0·003‡
Severe diarrhoea (≥6 stools per day)	1·31 (1063)	1·0	1·94 (1503)	1·28 (1·03–1·57)	1·69 (1304)	1·17 (0·95–1·45)	0·07
Persistent diarrhoea (>14 days)	0·06 (51)	1·0	0·10 (75)	1·41 (0·87–2·28)	0·09 (68)	1·33 (0·82–2·16)	0·34
Admission to hospital with diarrhoea	0·04 (29)	1·0	0·05 (37)	1·30 (0·71–2·38)	0·04 (31)	1·01 (0·54–1·90)	0·63

Lancet 2013; 382: 29–40

In the MNP groups:

- increased days with diarrhea (p=0.001)
- increased incidence of bloody diarrhea (p=0.003) and severe diarrhea (p=0.07)

In diarrheal stools, MNP - inc
cause of diarrhea in region (5

Stool samples at baseline and endpoint currently being analyzed at ETH Zurich

Original Investigation

Effect of Iron Fortification on Malaria Incidence in Infants and Young Children in Ghana
A Randomized Trial

Stanley Zlotkin, MD, PhD; Samuel Newton, MD, PhD; Ashley M. Aimone, MSc; Irene Azindow, BSc; Seeba Amenga-Etego, MSc; Kofi Tchum, MPhil; Emmanuel Mahama, MSc; Kevin E. Thorpe, MMath; Seth Owusu-Agyei, PhD

- Double-blind, cluster randomized trial in Ghanian children (6-35 months, n = 1958)
- Received daily MNP with iron (12.5 mg/d) or without iron for 5 months followed by 1-month of further monitoring
- Insecticide-treated bed nets provided at enrollment

JAMA. 2013;310(9):938-947.

During intervention, 23% more children admitted to hospital in Fe group, nonsignificant increase in diarrhea

Table 3. Effect of Providing Micronutrient Powder With Iron at the Time of Hospital Admissions and Other Diagnoses

JAMA. 2013;310(9):938-947.	No. (%) of Children		
	Iron Group (n = 967; 444.8 Child-Years of Follow-up)	No Iron Group (n = 991; 455.8 Child-Years of Follow-up)	Risk Ratio (95% CI)
Hospital admissions			
Overall[a]	174 (18.0)	150 (15.1)	1.17 (0.98-1.40)
Intervention (wk 1-20)	156 (16.1)	128 (12.9)	1.23 (1.02-1.49)
Postintervention (wk 21-24)	18 (2)	22 (2)	0.82 (0.52-1.29)
Other diagnoses[b]			
Malaria	283 (29.3)	287 (29.0)	1.00 (0.81-1.23)
Pneumonia	13 (1)	11 (1)	1.20 (0.67-2.14)
Pneumonia and positive rapid diagnostic test[c]	6 (<1)	6 (<1)	1.02 (0.46-2.25)
Diarrhea	162 (16.8)	147 (14.8)	1.12 (0.86-1.46)
Diarrhea and positive rapid diagnostic test[c]	132 (13.7)	133 (13.4)	1.00 (0.76-1.32)
Cerebral malaria or meningitis	10 (1)	8 (1)	1.28 (0.59-2.78)
Other diagnosis and positive rapid diagnostic test[c]	10 (1)	6 (<1)	1.71 (0.68-4.31)

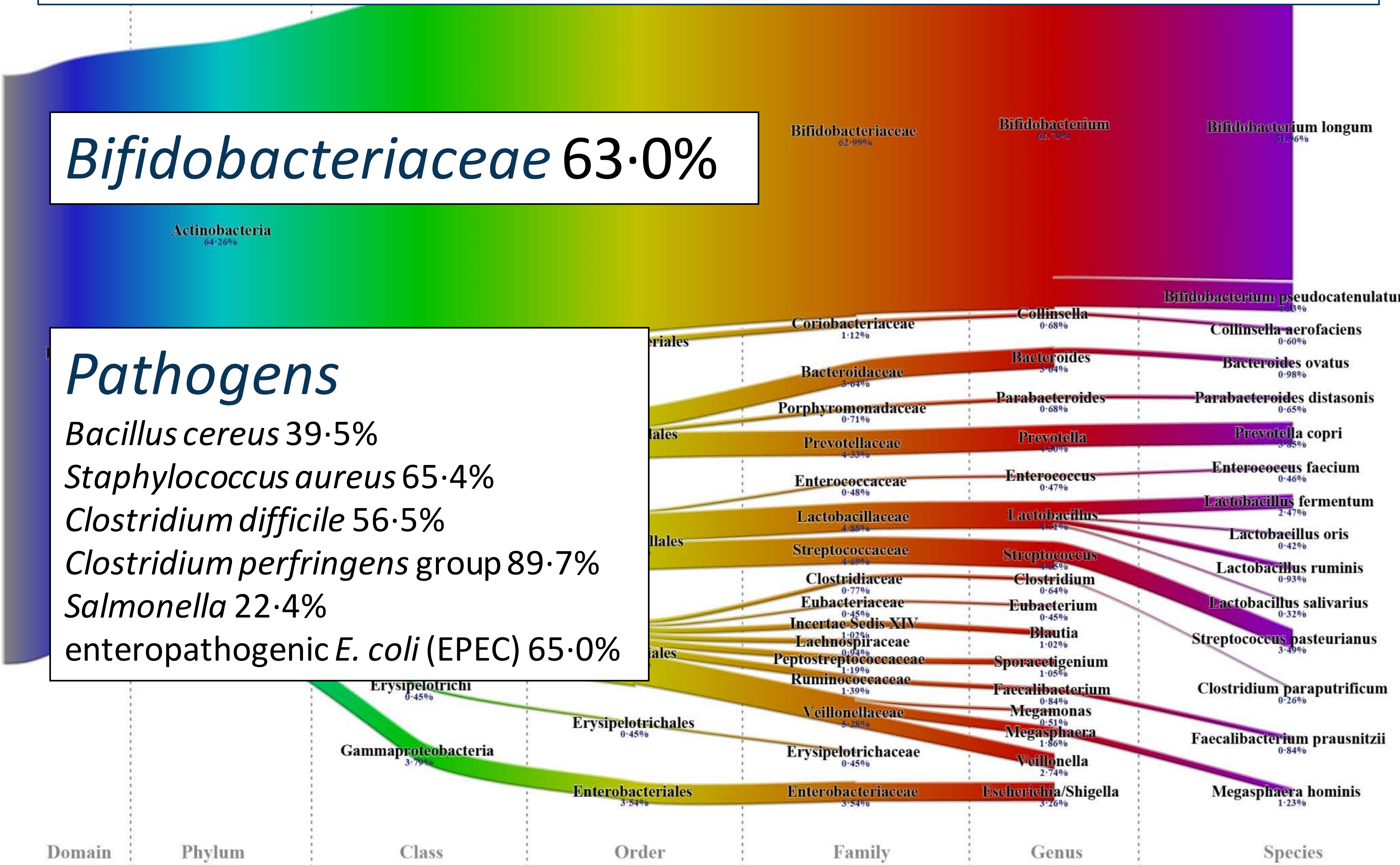
Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants

Tanja Jaeggi,[1] Guus A M Kortman,[2] Diego Moretti,[1] Christophe Chassard,[1] Penny Holding,[3] Alexandra Dostal,[1] Jos Boekhorst,[4] Harro M Timmerman,[4] Dorine W Swinkels,[2] Harold Tjalsma,[2] Jane Njenga,[5] Alice Mwangi,[5] Jane Kvalsvig,[6] Christophe Lacroix,[1] Michael B Zimmermann[1]

Gut 2015;64:5 731-742

- 6 month-old weaning Kenyan infants (n=124)
- Assessed two commonly used MNPs with and without Fe on infant gut microbiota:
  - 'MixMe' contains 2.5mg Fe as NaFeEDTA vs MNP-Fe
  - 'Sprinkles' contains 12.5mg Fe as Fe fumarate vs MNP-Fe
- Stool samples: 0, 3 wk and 4 months
- Gut analyses
  - 10 commensal / 5 pathogens (q-PCR), pyrosequencing
  - Fecal calprotectin

Baseline gut microbiome: mainly Bifidobacteria, but highly contaminated with pathogens

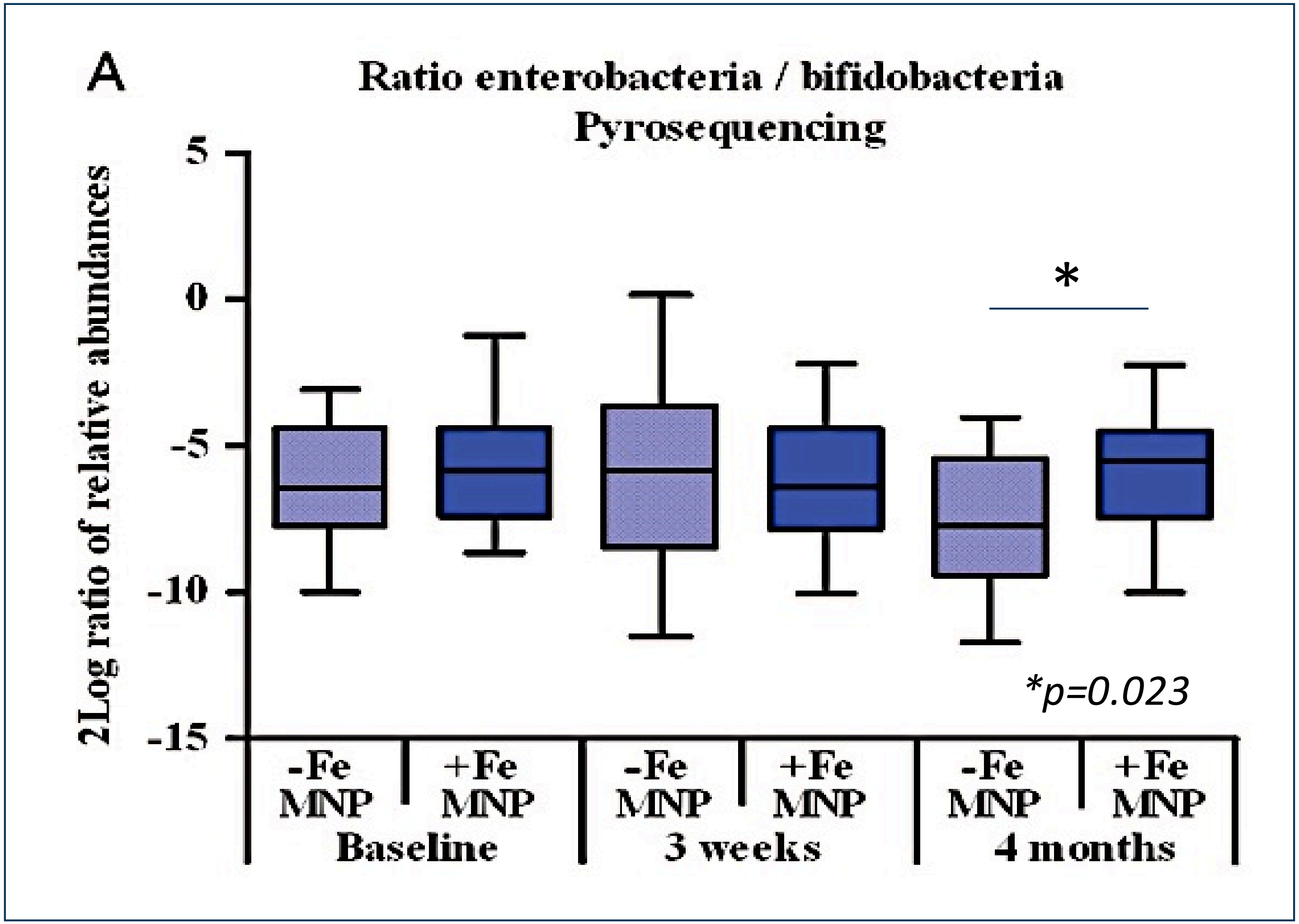


Bifidobacteriaceae 63·0%

Pathogens

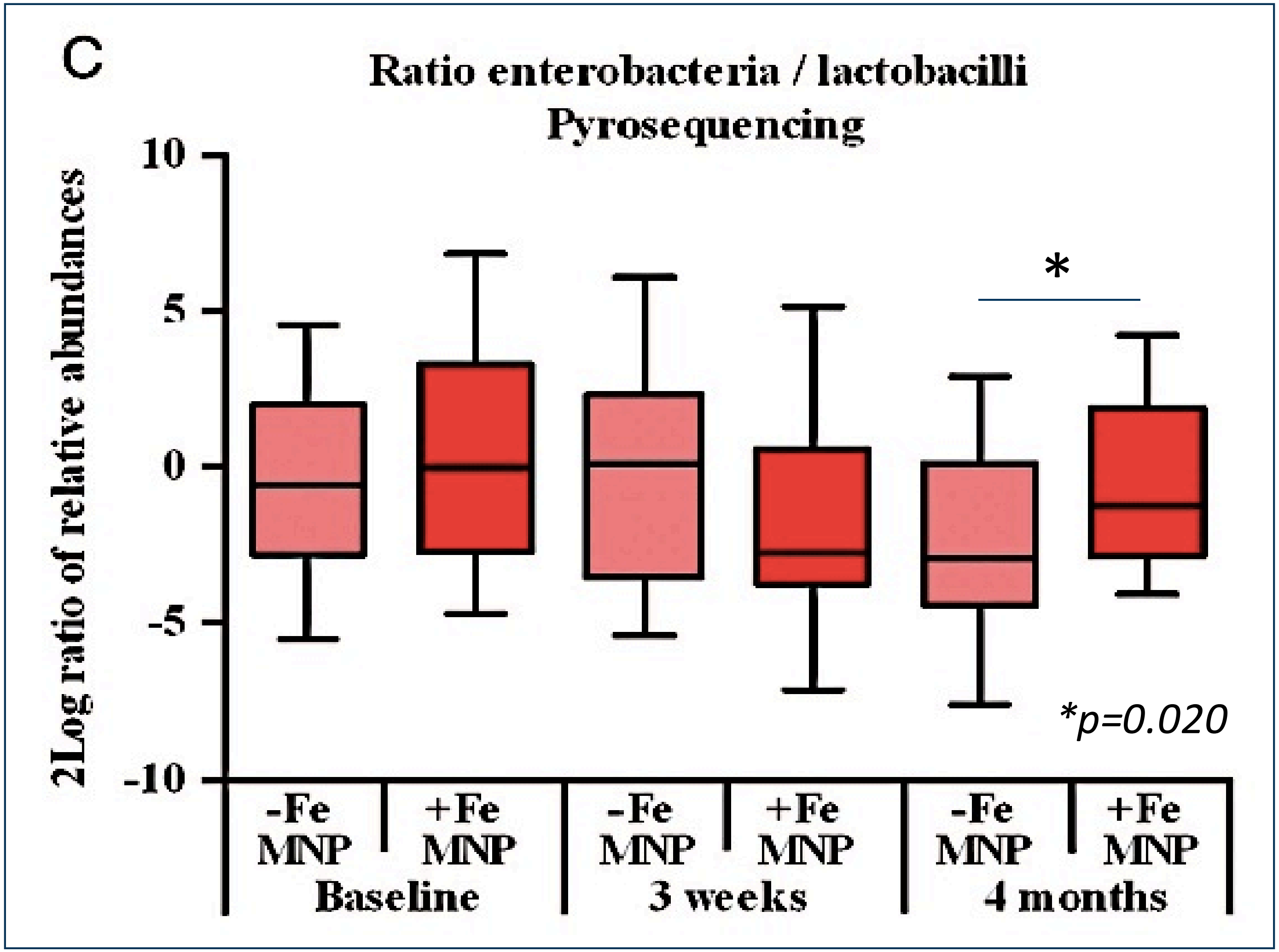
Bacillus cereus 39·5%
Staphylococcus aureus 65·4%
Clostridium difficile 56·5%
Clostridium perfringens group 89·7%
Salmonella 22·4%
enteropathogenic *E. coli* (EPEC) 65·0%

Increased ratio of enterobacteria to bifidobacteria at 4 months in +FeMNP



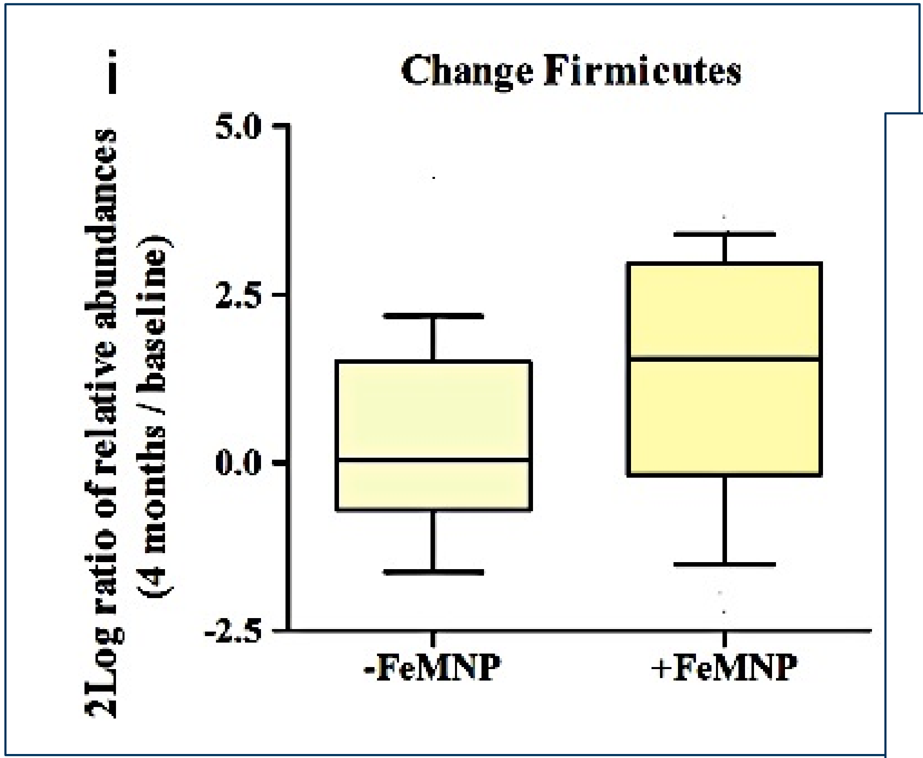
Jaeggi et al. Gut 2014

Increased ratio of enterobacteria to Lactobacilli at 4 months in +FeMNP

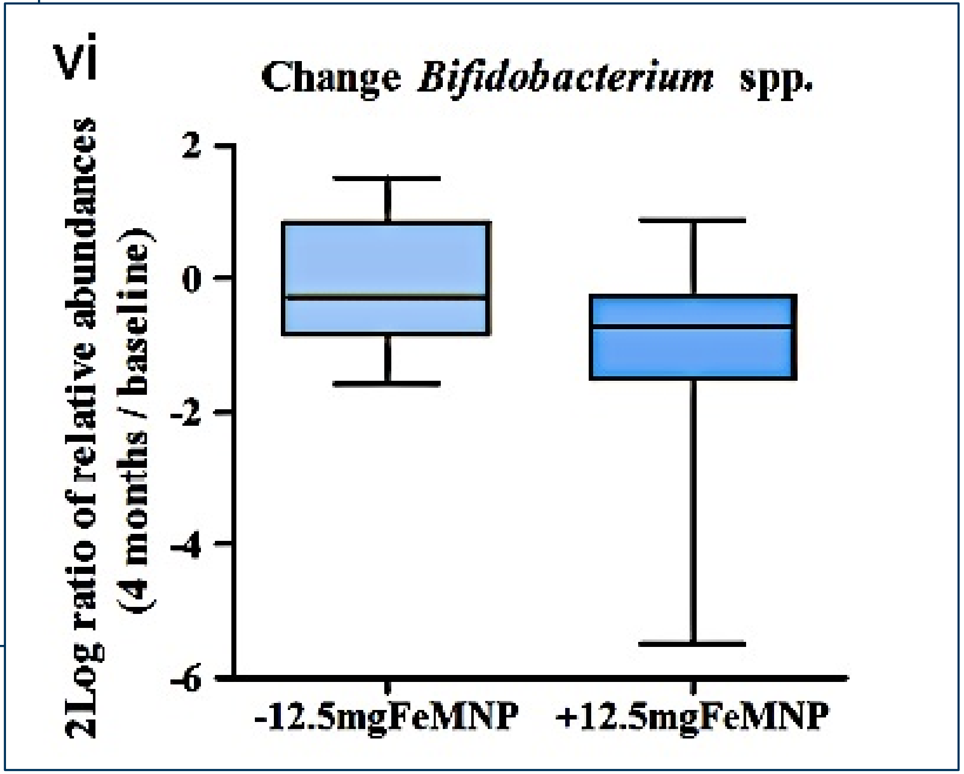


Jaeggi et al. Gut 2014

+Fe MNPs and commensals: Higher abundances of Firmicutes; lower abundances Bifidobacteria

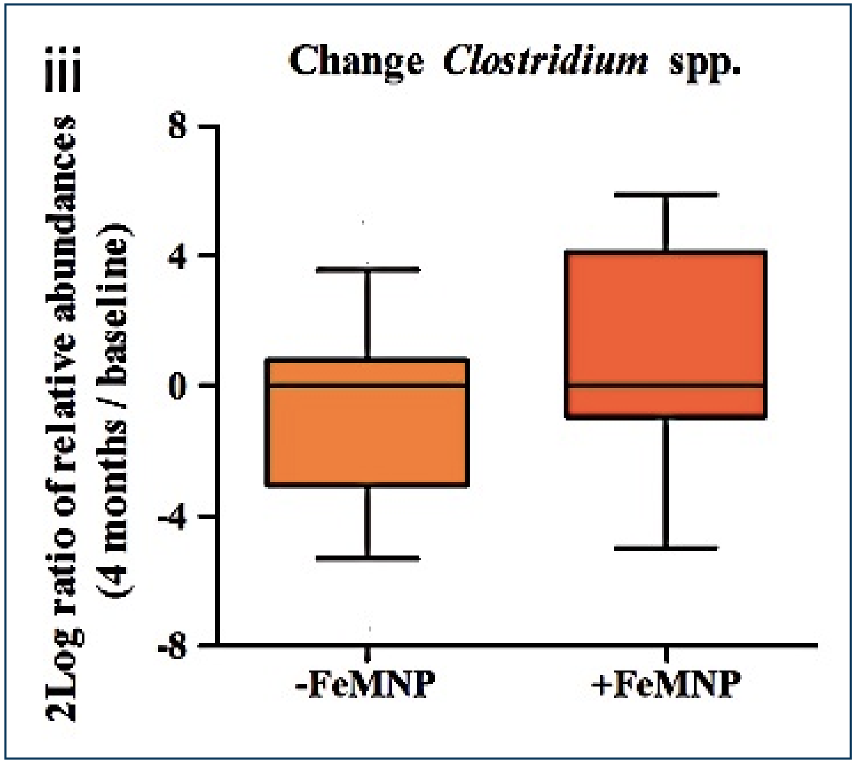


p=0.034

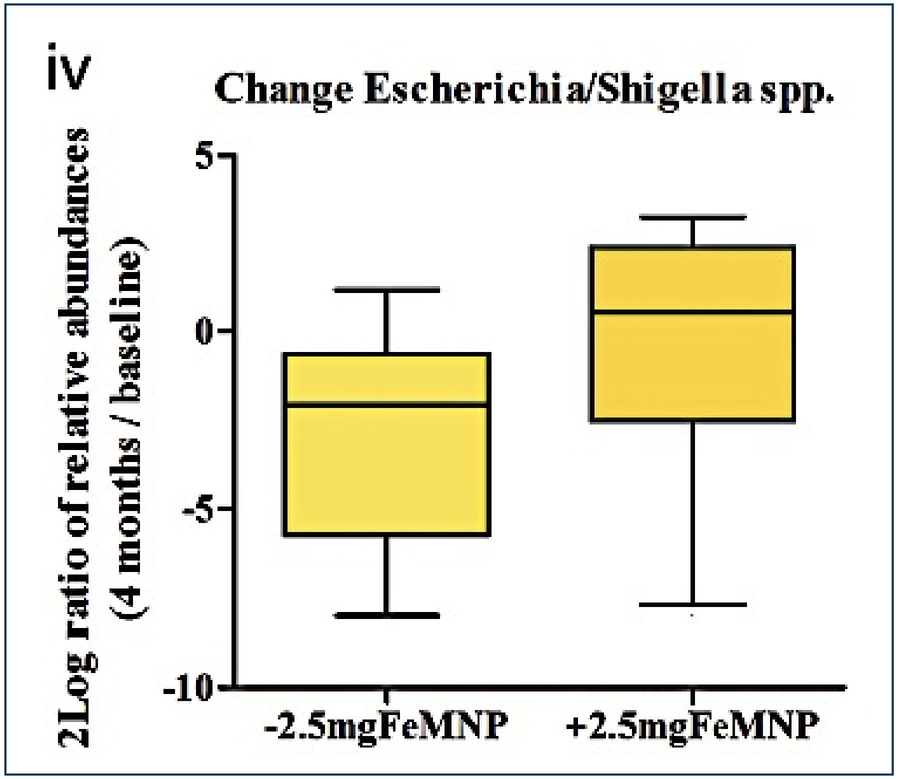


p=0.049

+Fe MNPs and pathogens: Higher abundances of *Clostridium spp*. and Escherichia/Shigella



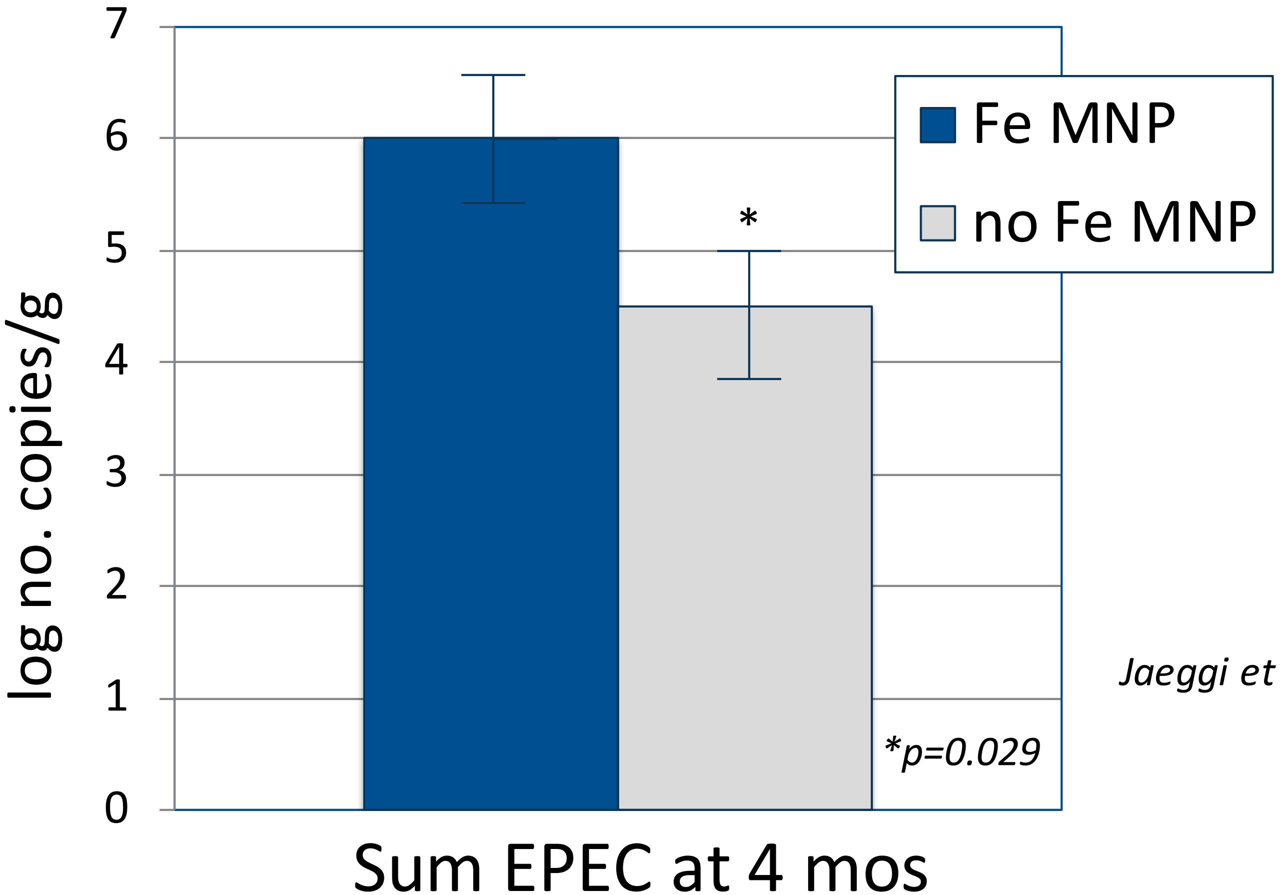
p=0.033



p=0.010

Jaeggi et al. Gut 2014

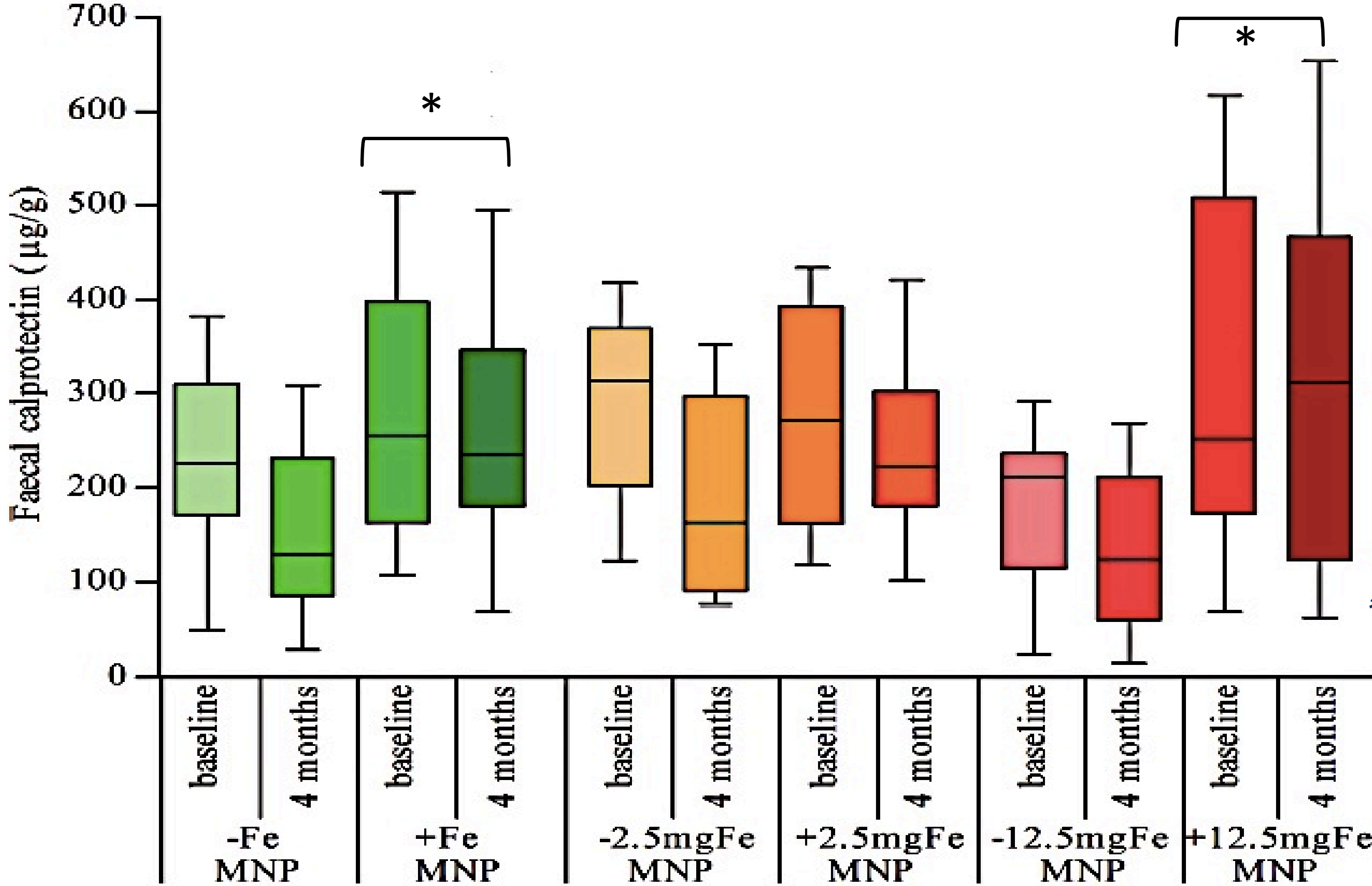
+Fe MNPs increased pathogenic *E. coli* (ETEC ST, ETEC LT, EHEC stx1)



Jaeggi et al. Gut 2015

Greater number treated episodes of diarrhea in +FeMNP: 27.3% vs. 8.3% *(p=0·092)*

+FeMNPs : 2-fold increase in fecal calprotectin, increase in +12.5 mg FeMNP



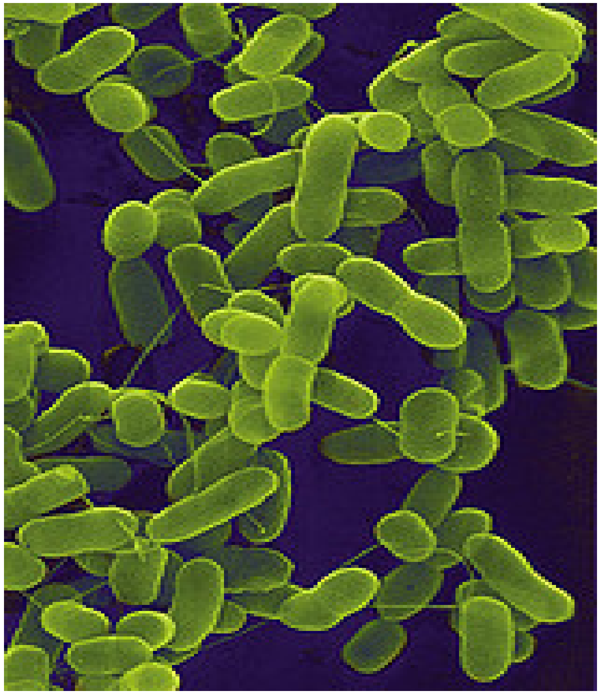
*p<0.05

Jaeggi et al. Gut 2014

Conclusions

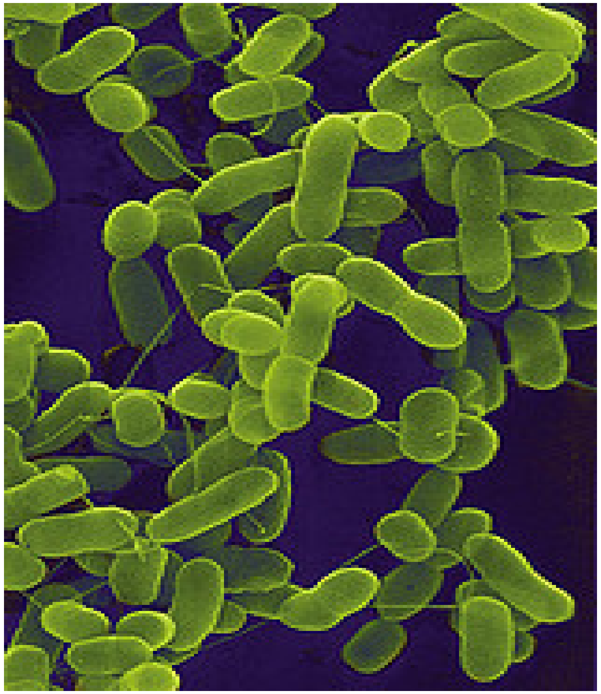
- In breastfed, 6 mo old infants in rural Africa, the gut microbiome dominated by *Bifidobacteriaceae*, but harbours many gram- and gram+ pathogens

- Iron decreases abundances of bifidobacteria and increases enterobacteria, shifting gut balance away from beneficial 'barrier' strains



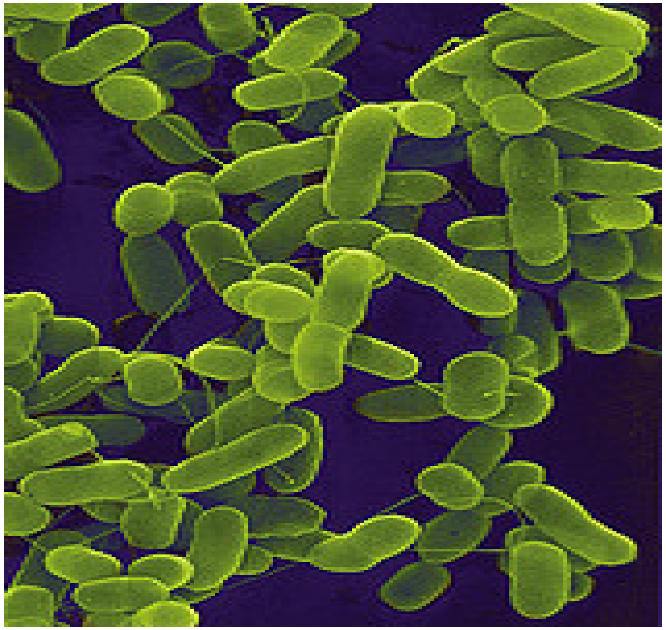
Conclusions

- Iron in MNPs increases abundances of potential pathogens, particularly Clostridium and EP *E. Coli*
- These changes in the gut microflora are accompanied by an increase in gut inflammation and, possibly, diarrhea



Summary

During infancy, MNPs can effectively deliver Fe and reduce IDA, but they may increase gut inflammation and, possibly, diarrhea



Remaining questions

Do these changes in the infant gut microbiome translate into clinical relevant diarrhea/sepsis/ other morbidity?

> Adequately powered RCTs of +FeMNPs in SubSaharan African and South Asian infants

- Primary outcomes: episodes/severity of diarrhea
- gut microbiome measurements in a subsample

Remaining questions

How can we deliver Fe more safely to infants using MNPs?

- Reduce iron dose, increase absorption (+phytase)
- Prebiotics > increase bifidobacteria and Lactobacilli and SCFA, improve barrier function (*Le Blay et al. 2010*)
- Could a prebiotic in MNP allow us to administer Fe and correct IDA, but do it safely?
- 4 month RCT in Kenyan infants: 5 mg Fe dose (2.5 mg as Fe fumarate and 2.5 mg as NaFeEDTA) + phytase +/- 7.5 g GOS
- Results by March 2016

Thank you

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