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

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

Peak DALYs (>2.5k) at age 1 mo to 1 y

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

Peak DALYs (>3k) in SS Africa

DALYS / 100,000
Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age

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

- 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
Trials included

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Nutrients</th>
<th>Iron and zinc</th>
<th>Vitamin A (μg)</th>
<th>Author and yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambodia</td>
<td>6</td>
<td></td>
<td>300</td>
<td>Giovannini 2006</td>
</tr>
<tr>
<td>Ghana</td>
<td>6</td>
<td>12.5 mg as Fe Fumarate</td>
<td>300</td>
<td>Christofides 2006</td>
</tr>
<tr>
<td>Ghana</td>
<td>6</td>
<td></td>
<td>300</td>
<td>Adu-Afarwuah 2007</td>
</tr>
<tr>
<td>Haiti</td>
<td>5</td>
<td>5 mg as Zn gluconate</td>
<td>400</td>
<td>Menon 2007</td>
</tr>
<tr>
<td>India</td>
<td>5</td>
<td></td>
<td>300</td>
<td>Hirve 2007</td>
</tr>
<tr>
<td>Kenya</td>
<td>15</td>
<td></td>
<td>400</td>
<td>Suchdev 2011</td>
</tr>
<tr>
<td>Kyrzgyz Republic</td>
<td>5</td>
<td></td>
<td>300</td>
<td>Lundeen 2010</td>
</tr>
<tr>
<td>Pakistan</td>
<td>6</td>
<td></td>
<td>300</td>
<td>Sharieff 2006</td>
</tr>
</tbody>
</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
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
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

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

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)
  - 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 MNPs vs no intervention or placebo, iron deficiency

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

Total events: 59 (MNP), 162 (no int/placebo)
Heterogeneity: Tau² = 0.03; Chi² = 4.34, df = 3 (P = 0.23); I² = 31%
Test for overall effect: Z = 4.30 (P = 0.000017)
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
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
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)
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

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect (95% CI)</th>
<th>No of Participants (studies)</th>
<th>Quality of the evidence (GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>RR 0.69 (0.60 to 0.78)</td>
<td>1447 (6 studies)</td>
<td>moderate¹</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>RR 0.49 (0.35 to 0.67)</td>
<td>586 (4 studies)</td>
<td>high²</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>MD 5.87 (3.25 to 8.49)</td>
<td>1447 (6 studies)</td>
<td>moderate¹,³</td>
</tr>
<tr>
<td>Iron status (ferritin concentra-</td>
<td>MD 20.38 (6.27 to 34.49)</td>
<td>264 (2 studies)</td>
<td>low¹,⁴</td>
</tr>
<tr>
<td>tions in ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>MD 0 (-0.37 to 0.37)</td>
<td>304 (2 studies)</td>
<td>moderate¹,⁵</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>0</td>
<td>0 (0 studies)</td>
<td>None of the trials reported on</td>
</tr>
</tbody>
</table>

CI: Confidence interval; RR: Risk ratio;
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

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- 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

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>MNP</th>
<th>no int/placebo</th>
<th>Risk Ratio M-H,Random,95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H,Random,95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menon 2007</td>
<td>72/125</td>
<td>35/81</td>
<td>1.33 [ 1.00, 1.78 ]</td>
<td>100.0 %</td>
<td>1.33 [ 1.00, 1.78 ]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>125</strong></td>
<td><strong>81</strong></td>
<td></td>
<td><strong>100.0 %</strong></td>
<td><strong>1.33 [ 1.00, 1.78 ]</strong></td>
</tr>
<tr>
<td>Total events: 72 (MNP), 35 (no int/placebo)</td>
<td></td>
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<tr>
<td>Heterogeneity: not applicable</td>
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<tr>
<td>Test for overall effect: Z = 1.93 (P = 0.053)</td>
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</tr>
<tr>
<td>Test for subgroup differences: Not applicable</td>
<td></td>
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</tbody>
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<thead>
<tr>
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<th>Risk Ratio M-H,Random,95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H,Random,95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giovannini 2006</td>
<td>7/65</td>
<td>4/62</td>
<td>1.67 [ 0.51, 5.42 ]</td>
<td>100.0 %</td>
<td>1.67 [ 0.51, 5.42 ]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>65</strong></td>
<td><strong>62</strong></td>
<td></td>
<td><strong>100.0 %</strong></td>
<td><strong>1.67 [ 0.51, 5.42 ]</strong></td>
</tr>
<tr>
<td>Total events: 7 (MNP), 4 (no int/placebo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Heterogeneity: not applicable</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.85 (P = 0.39)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test for subgroup differences: Not applicable</td>
<td></td>
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</tr>
</tbody>
</table>

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

![Fe absorption graph](image)

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

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 effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Côte d’Ivoire

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

Receiving iron fortification

- Baseline
- 6 months

**5x increase**
- Enterobacteria: Baseline 56.8, 6 months 225.7

**5x decrease**
- Lactobacilli: Baseline 22.7, 6 months 6.4

* p<0.05

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 * p<0.01
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
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 - increase in *Aeromonas spp*, common cause of diarrhea in region (5.9% control vs. 7.3–11.3% MNPs)

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

In *Lancet* 2013; 382: 29–40
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>
<thead>
<tr>
<th>Table 3. Effect of Providing Micronutrient Powder With Iron at the Time of Hospital Admissions and Other Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JAMA. 2013;310(9):938-947.</strong></td>
</tr>
<tr>
<td>Hospital admissions</td>
</tr>
<tr>
<td>Overall&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intervention (wk 1-20)</td>
</tr>
<tr>
<td>Postintervention (wk 21-24)</td>
</tr>
<tr>
<td>Other diagnoses&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malaria</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Pneumonia and positive rapid diagnostic test&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Diarrhea and positive rapid diagnostic test&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cerebral malaria or meningitis</td>
</tr>
<tr>
<td>Other diagnosis and positive rapid diagnostic test&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
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 Zimmermann1

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

* $p=0.023$

Jaeggi et al. Gut 2014
Increased ratio of enterobacteria to Lactobacilli at 4 months in +FeMNP

Jaeggi et al. Gut 2014

*p = 0.020
+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*

Jaeggi et al. Gut 2014
+Fe MNPs increased pathogenic *E. coli* (ETEC ST, ETEC LT, EHEC stx1)

Greater number treated episodes of diarrhea in +FeMNP: 27.3% vs. 8.3% (*p=0.092*)

Jaeggi et al. Gut 2015
+FeMNPs: 2-fold increase in fecal calprotectin, increase in +12.5 mg FeMNP

*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 +FeMNP in Sub-Saharan 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