Vitamins and Minerals RIS of the American Society for Nutrition

Webinar:
Iron and Vitamin D Impacts on the Microbiome

November 28, 2017
A Few Reminders

CPE Credit

• ASN designates this educational activity for a maximum of 1 CPEUs. Dietitians and Dietetic Technicians, Registered should only claim credit commensurate with the extent of their participation in the activity.

• To claim credit, please take the post webinar evaluation to be emailed after the webinar.

This webinar is being recorded. Please mute your phone and/or computer microphone.
Questions & Answers

- Please use the “questions” box on your “Go To Meetings” screen to submit questions to our presenters.
- Please submit your questions at any time during today’s webinar.
Faculty

Speakers

- Michael B. Zimmermann, MD
  Human Nutrition, ETH Zürich
  Swiss Federal Institute of Technology
  Zurich, Switzerland

- Jun Sun, PhD
  Associate Professor
  University of Illinois at Chicago

Moderators

- Nana Gletsu Miller, PhD
  Purdue University

- Lisa Tussing-Humphreys, PhD
  University of Illinois at Chicago
Learning Objective

At the end of this program, attendees will be able to:

- Explain the impacts of iron and vitamin D on the gut microbiome.
- Describe mechanisms that have been put forth to explain how iron and vitamin D supplementation affect intestinal function of the host.
- Communicate the limits of knowledge in this emerging area and the needs for future research.
Iron and the human gut microbiome

Prof. Michael Zimmermann, MD
ETH Zurich, Switzerland
## Disclosures

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</table>
Anaemia as a public health problem by country: Preschool-age children


Category of public health significance (anaemia prevalence)
- Normal (<5.0%)
- Mild (5.0-19.9%)
- Moderate (20.0-39.9%)
- Severe (≥40.0%)
- No Data

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be final agreement.

©WHO 2008. All rights reserved.
Iron forticants are poorly absorbed and produce large increases in colonic iron.

**Absorption from 12.5 mg iron as ferrous fumarate in Ghanaian infants, by iron status**

<table>
<thead>
<tr>
<th>Iron status</th>
<th>mg Fe</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency anemia (n=32)</td>
<td>3.03</td>
<td><strong>8.25</strong> (2.9–17.8)</td>
</tr>
<tr>
<td></td>
<td>(1.1–5.9)(^a)</td>
<td></td>
</tr>
<tr>
<td>Iron deficiency (n=20)</td>
<td>1.64</td>
<td><strong>4.48</strong> (1.1–10.6)</td>
</tr>
<tr>
<td></td>
<td>(0.03–4.8)</td>
<td></td>
</tr>
<tr>
<td>Iron sufficiency (n=20)</td>
<td>1.67</td>
<td><strong>4.65</strong> (1.5–12.3)</td>
</tr>
<tr>
<td></td>
<td>(0.5–4.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Am J Clin Nutr* 2004; 80(5): 1436-1444
Fe in the body is tightly bound, limiting supply to 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:
- although neutral pH and host defense molecules, such as lipocalin, may reduce iron solubility and availability to gut microbes.
Iron is a growth-limiting nutrient for many gut pathogens, but beneficial bacteria require little or no iron. 

Iron acquisition plays an essential role in virulence and colonization.

Barrier Bacteria (Bifidobacteria / Lactobacilli) 

Require little or no iron

Kortman et al. 2014  Weinberg ED 1997
Home fortification with multiple micronutrient powders sharply reduces iron-deficiency anemia

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

- 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
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)

#### Table:

<table>
<thead>
<tr>
<th>Age 6-0–17·9 months</th>
<th>Group A: control group</th>
<th>Group B: MNP without zinc</th>
<th>Group C: MNP with zinc</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence per child year</td>
<td>IRR</td>
<td>Incidence per child year</td>
<td>IRR</td>
<td>Incidence per child year</td>
</tr>
<tr>
<td>Any diarrhea</td>
<td>3.73 (3030) 1·0</td>
<td>4.16 (3229) 1.05 (0.94–1.17)</td>
<td>4.32 (3323) 1·12 (1.01–1.26)</td>
<td>0·12</td>
</tr>
<tr>
<td>Bloody diarrhea</td>
<td>0.08 (69) 1·0</td>
<td>0.16 (124) 1·63 (1·12–2·39)</td>
<td>0.17 (132) 1·88 (1·29–2·74)</td>
<td>0·003‡</td>
</tr>
<tr>
<td>Severe diarrhea (≥6 stools per day)</td>
<td>1·31 (1063) 1·0</td>
<td>1·94 (1503) 1·28 (1·03–1·57)</td>
<td>1·69 (1304) 1·17 (0·95–1·45)</td>
<td>0·07</td>
</tr>
<tr>
<td>Persistent diarrhea (&gt;14 days)</td>
<td>0·06 (51) 1·0</td>
<td>0·10 (75) 1·41 (0·87–2·28)</td>
<td>0·09 (68) 1·33 (0·82–2·16)</td>
<td>0·34</td>
</tr>
<tr>
<td>Admission to hospital with diarrhea</td>
<td>0·04 (29) 1·0</td>
<td>0·05 (37) 1·30 (0·71–2·38)</td>
<td>0·04 (31) 1·01 (0·54–1·90)</td>
<td>0·63</td>
</tr>
</tbody>
</table>

*Lancet 2013; 382: 29–40*
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

<table>
<thead>
<tr>
<th>JAMA. 2013;310(9):938-947.</th>
<th>No. (%) of Children</th>
<th>Risk Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron Group (n = 967; 444.8 Child-Years of Follow-up)</td>
<td>No Iron Group (n = 991; 455.8 Child-Years of Follow-up)</td>
</tr>
<tr>
<td>Hospital admissions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall^a</td>
<td>174 (18.0)</td>
<td>150 (15.1)</td>
</tr>
<tr>
<td>Intervention (wk 1-20)</td>
<td>156 (16.1)</td>
<td>128 (12.9)</td>
</tr>
<tr>
<td>Postintervention (wk 21-24)</td>
<td>18 (2)</td>
<td>22 (2)</td>
</tr>
<tr>
<td>Other diagnoses^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>283 (29.3)</td>
<td>287 (29.0)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>13 (1)</td>
<td>11 (1)</td>
</tr>
<tr>
<td>Pneumonia and positive rapid diagnostic test^c</td>
<td>6 (&lt;1)</td>
<td>6 (&lt;1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>162 (16.8)</td>
<td>147 (14.8)</td>
</tr>
<tr>
<td>Diarrhea and positive rapid diagnostic test^c</td>
<td>132 (13.7)</td>
<td>133 (13.4)</td>
</tr>
<tr>
<td>Cerebral malaria or meningitis</td>
<td>10 (1)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>Other diagnosis and positive rapid diagnostic test^c</td>
<td>10 (1)</td>
<td>6 (&lt;1)</td>
</tr>
</tbody>
</table>
6 month RCT rural Ivorian schoolchildren (n=139)
Fe-fortified biscuits containing 20 mg iron/d, 4 times/wk as electrolytic iron vs. control
No benefit on anemia or iron status
Stool samples collected at baseline (before intro of Fe) and after 6 months of fortification
Iron fortification increases enterobacteria and decreases Lactobacilli numbers in African children.
Fortification increased gut inflammation

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

* p<0.01

Zimmermann et al. AJCN 2010
- 6 month-old Kenyan infants (n=124)
- Assessed two commonly used MNPs with and without Fe on infant gut microbiota:
  - ‘MixMe’ 2.5mg Fe as NaFeEDTA vs MNP-Fe
  - ‘Sprinkles’ 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
+Fe MNPs and pathogens: Higher abundances of *Clostridium* spp. and *Escherichia/Shigella*

Jaeggi et al. Gut 2014

$p=0.033$

$p=0.010$
+Fe MNPs increased pathogenic *E. coli* (ETEC ST, ETEC LT, EHEC stx1)

![Graph showing comparison between Fe MNP and no Fe MNP on Sum EPEC at 4 mos.](Jaeggi et al. Gut 2015)
+FeMNPs increased gut inflammation: 2x increase in fecal calprotectin

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

* p<0.05

Jaeggi et al. Gut 2014
Safer MNP formulations are needed

2 Strategies

① Reduce the iron dose and maximize absorption to retain efficacy
   • 5 mg/day (ferrous fumarate and NaFeEDTA) + phytase

② Add a component that could mitigate the adverse effects of the iron on the gut microbiome
   • Galacto-oligosaccharides (GOS) → Prebiotic to maintain/promote beneficial barrier bacteria (e.g. Bifidobacterium spp.)
## Composition of the micronutrient powders

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount per sachet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, D, E, C, B vitamins, Cu, I, Se, Zn, Phytase</td>
<td></td>
</tr>
</tbody>
</table>

### Control group

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>Add to 11 g</td>
</tr>
</tbody>
</table>

### Fe group

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (as ferrous fumarate)</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Iron (as NaFeEDTA)</td>
<td>2.5 mg</td>
</tr>
</tbody>
</table>

### FeGOS group

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (as ferrous fumarate)</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Iron (as NaFeEDTA)</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Galacto-oligosaccharides (GOS)</td>
<td>7.5 g, as 10.5 g GOS -75</td>
</tr>
</tbody>
</table>

Vivinal GOS 75 Powder (Friesland Campina, Wageningen, The Netherlands)
1. Iron absorption study in Kenyan infants

Objective

- Determine if prebiotic (GOS) consumption daily for 3 weeks affects iron absorption from a MNP containing a mixture of ferrous fumarate and sodium iron EDTA (FeFum+NaFeEDTA) in Kenyan infants.

_Clinical Nutrition_ study:

Consumption of galacto-oligosaccharides increases iron absorption from a micronutrient powder containing ferrous fumarate and sodium iron EDTA: a stable-isotope study in Kenyan infants

Daniela Paganini,1 Mary A Uyoga,3 Colin I Cercamondi,1 Diego Moretti,1 Edith Mwasi,4 Clarissa Schwab,2 Salome Bechtler,1 Francis M Mutuku,5 Valeria Galetti,1 Christophe Lacroix,2 Simon Karanja,3 and Michael B Zimmermann1
Iron absorption using stable isotopes in Kenyan infants: prefeeding of GOS increased iron absorption 60% and absorption from the 5mg FeGOS MNP was high: 18.8%

Paganini et al. AJCN 2017
Study Aim
Evaluate the **efficacy and safety** of a new MNP formula with a low dose (5 mg) of highly bioavailable iron as **NaFeEDTA** (2.5 mg) and **ferrous fumarate** (2.5 mg) and the **prebiotic galacto-oligosaccharides (GOS)**

Hypotheses
1. A MNP formula containing 5mg of highly bioavailable iron would reduce anemia
2. The addition of GOS to the MNP would mitigate the adverse effects of iron on the infant gut microbiome and inflammation
Study overview

Included in the intervention and randomization: n= 155

Baseline: Venipuncture and fecal sampling

- **Control group:** Consumption of MNPs without iron and without GOS daily for 4mo (n=51)
  - Week 3: Fecal sampling (n=51)
  - 4 months: Venipuncture and fecal sampling (n=48)

- **Fe group:** Consumption of MNPs with FeFum + NaFeEDTA daily for 4mo (n=52)
  - Week 3: Fecal sampling (n=52)
  - 4 months: Venipuncture and fecal sampling (n=49)

- **FeGOS group:** Consumption of MNPs with FeFum + NaFeEDTA + GOS daily for 4mo (n=52)
  - Week 3: Fecal sampling (n=52)
  - 4 months: Venipuncture and fecal sampling (n=48)

Lost follow up (n=10)

- Age 6.5-9.5 months old infant

Paganini et al. *Gut* 2017
Low dose MNP (5mg) was clearly efficacious in reducing anemia and all measures of iron deficiency

<table>
<thead>
<tr>
<th></th>
<th>All infants</th>
<th>Control</th>
<th>Fe</th>
<th>FeGOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>155</td>
<td>51</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Age (mo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.4 [6.9,9.3]</td>
<td>7.5 [6.9,9.1]</td>
<td>7.5 [6.9,9.3]</td>
<td>7.3 [6.9,9.2]</td>
</tr>
<tr>
<td>Male/Female</td>
<td>77/78</td>
<td>28/23</td>
<td>27/25</td>
<td>22/30</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>104 (97,110)</td>
<td>103 (97,110)</td>
<td>103 (93,108)</td>
<td>106 (102,111)</td>
</tr>
<tr>
<td>4 months</td>
<td>109 (99,116)</td>
<td>101 (93,108)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112 (107,122)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111 (103,116)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anemia, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>115 (74%)</td>
<td>38 (75%)</td>
<td>41 (79%)</td>
<td>36 (69%)</td>
</tr>
<tr>
<td>4 months</td>
<td>76 (54%)</td>
<td>40 (83%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17 (35%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19 (42%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron deficiency anemia, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>105 (68%)</td>
<td>35 (69%)</td>
<td>38 (73%)</td>
<td>32 (62%)</td>
</tr>
<tr>
<td>4 months</td>
<td>66 (47%)</td>
<td>35 (73%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 (27%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 (40%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Anemia defined as an Hb <110 g/l. Iron deficiency anemia defined as Hb <110 g/l + PF <30 mg/L and/or sTfR > 8.3 µg/ml. Across rows, different letter superscripts indicate significant differences (p<0.05).

Paganini et al. *Gut* 2017
Differences in gut microbial composition at 4 months

A) In Fe, compared to control:
- lower *Lactobacillus* (p=0.048)
- lower *Bifidobacterium* (p=0.058)
- higher *Clostridiales* (p=0.015)
- higher *Enterobacteriaceae* (p=0.086)

B) In Fe, compared to FeGOS:
- lower *Bifidobacterium* (p=0.007)
- lower *Lactobacillus* (p=0.006)
- higher *Clostridiales* (p=0.001)

C) In FeGOS, compared to control
- no significant differences

Nodes represent taxa. Node-size corresponds to the relative abundance (in %). Fold difference calculated as $2\log$ of ratio of relative abundance between groups (0=no difference, 1=twice as abundant). Significance: $p$ value of Mann–Whitney U test.

Paganini et al. *Gut* 2017
Sum of virulence and toxin genes of 10 enteropathogens were higher in Fe compared to FeGOS at 3 weeks but not at 4 months.

- *C. perfringens* (65%)
- *C. difficile* (35%)
- EPEC (63%)
- ETEC LT (26%)
- ETEC ST (21%)
- EHEC stx1 (8%)
- EHEC stx2 (18%)
- *S. aureus* (13%)
- *B. cereus* (5%)
- *S. enterica* (4%)

Boxes show the median and 25th and 75th percentiles; whiskers show the range; points show individual values. *P<0.05; **P<0.01.

Paganini et al. *Gut* 2017
Fecal Calprotectin a marker of gut inflammation decreased by 30% in de FeGOS at 3 wks but unlike the previous used 12.5 mg iron MNP did not increase in the Fe group.

Intestinal fatty acid binding protein, a plasma biomarker of enterocyte damage, was increased with Fe, but not in FeGOS.

Boxes show the median and 25th and 75th percentiles; whiskers show the range; point show individual values. *P<0.05.

Paganini et al. Gut 2017
Infant morbidity: Respiratory tract infections (RTIs)

More infants in Fe were treated for RTIs during the intervention compared to control and FeGOS (p=0.024; p=0.098)

Over the 4 months there was a decrease in incidence of RTIs in the FeGOS group (p=0.013)

Paganini et al. *Gut* 2017
Conclusions

- A MNP containing a 5 mg daily dose of highly bioavailable iron is efficacious in reducing IDA in African infants; a 60% reduction in iron dose compared to current MNPs containing 12.5 mg iron.

- This 5 mg dose is likely safer in that it induces less adverse changes in the gut microbiome and no increase in fecal calprotectin compared to a 12.5 mg iron dose.

- Prebiotics given with iron-containing MNPs in the African setting may be beneficial to support gut health, retain natural infant gut microbiome development and to reduce the incidence of RTIs.
Vitamin D/ Vitamin D receptor and Gut Microbiome

Jun Sun, Ph.D. AGA Fellow
Associate Professor
Departments of Medicine
University of Illinois at Chicago
Chicago, IL
junsun7@uic.edu
## Disclosures

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Outlines

- Non-classical functions of vitamin D and Vitamin D receptor (VDR);
- Our recent findings on intestinal VDR regulation of microbiome in the context of health and inflammation;
- Potential therapeutic strategies to manipulate VDR and microbiota.
Vitamin D and Health

Skin

UV-B
7-dehydrocholesterol
Pre-D₃
D₃

Liver

DBP
Circulation

Intestine

Parathyroid glands

PTH

Kidney

1α,24,25(OH)₂D₃

1α-OHase

25(OH)D₃

25-OHase

24-OHase

D₃

Excretion

Intestine

Dietary sources of vitamin D

Nature Reviews | Cancer
Vitamin D and Health

Multiple sclerosis (850 publications)
Colon cancer (644 publications)
Immunity (1170 publications)
Bone (20344 publications)
Muscle (2430 publications)
Blood pressure (1518 publications)
Diabetes (3370 publications)
Tuberculosis (834 publications)

Searched on May 7, 2015

VDR action: molecular mechanism

1,25 Vitamin D3

VDR

NUCLEUS

RXR

VDR

VDRE

RNA Polymerase

DNA

GENE TRANSCRIPTION

RXR: Retinoid X receptor
VDRE: Vitamin D-response element
## Target genes of VDR signaling pathway

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>Cyp27B1</td>
<td>Enzyme that catalyzes the conversion of inactive pro-vitamin D3 hormone into the active form</td>
</tr>
<tr>
<td>Cyp24</td>
<td>VDR-specific hydroxylase</td>
</tr>
<tr>
<td>Cathelicidin</td>
<td>Antimicrobial peptide</td>
</tr>
<tr>
<td>Beta defensin-4 (DEFB4)</td>
<td>Antimicrobial peptide</td>
</tr>
</tbody>
</table>
VDR and Its affected Genes

Active VDR affects transcription of at least 913 genes and impacts processes ranging from calcium metabolism to the expression of key antimicrobial peptides.

Vitamin D/VDR deficiency is associated with various diseases

- complex immune disorders;
- cancer;
- obesity, diabetes, and metabolic syndrome;
- cardiovascular disease;
- stress/anxiety.
Pathogenesis of Inflammatory Bowl Disease (IBD)

IBD includes Crohn’s disease (CD) and Ulcerative Colitis (UC)

Interaction of various factors contributing to chronic intestinal inflammation in a genetically susceptible host

Vitamin D and Inflammatory Bowel Diseases (IBD)

- Low vitamin D status has been reported in patients with IBD \(^1\).
- Vitamin D is an environmental factor that influences IBD \(^2\).

1. Sentongo et al., 2000
2. Reviewed by Lim et al., 2005
Increasing trend of IBD in industrialized countries
Polymorphisms in the VDR gene (chromosome 12) are associated with susceptibility to Crohn’s Disease\(^1\) and UC\(^2\).

VDR IL-10 double knock out mice develop severe IBD that involved the whole intestinal tract\(^3\).

1. Simmons *et al.*, 2000
2. Dresner-Pollak *et al.*, 2004
The genetic, environmental, and microbial determinants of IBD


### Changes in the microbial profile

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roseburia hominis</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Clostridium IV</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Xivax Clostridium</td>
<td>Fusobacterium</td>
</tr>
<tr>
<td>Faecalibacterium</td>
<td>adherent-invasive</td>
</tr>
<tr>
<td>Prasuniflora</td>
<td>E. coli (AIEC)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Proteus</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td></td>
</tr>
</tbody>
</table>

### Table: Risk Factors for CD and UC

<table>
<thead>
<tr>
<th>Factor</th>
<th>CD Risk</th>
<th>UC Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>Risk in Caucasians and Middle Eastern migrants</td>
<td>Protective in Caucasians and Asians</td>
</tr>
<tr>
<td>Antibiotic use in childhood</td>
<td>Risk in Caucasians, protective in Asians/Middle Eastern migrants</td>
<td>Risk in Caucasians, protective in Asians/Middle Eastern migrants</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Protective in Asians and most studies in Caucasians</td>
<td>Protective in Asians and most studies in Caucasians</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>Risk in Caucasians</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>Risk in Caucasians</td>
<td>Protective in Caucasians</td>
</tr>
<tr>
<td>Low levels of vitamin D</td>
<td>Risk in Caucasians</td>
<td>Risk in Caucasians</td>
</tr>
<tr>
<td>Tea or coffee consumption</td>
<td>Protective in Asians</td>
<td>Protective in Asians</td>
</tr>
</tbody>
</table>

### Hygiene hypothesis:

Having pets in childhood, living on a farm, larger family size, and drinking unpasteurized milk were inversely associated with the risk of CD and UC.

### Changing diet:

Introduction of packaged food, fast food chains, increased use of antibiotics, increased fat (monounsaturated and polyunsaturated fatty acids) consumption and sugar intake, less dietary fibers is associated with risk of IBD.

### Dietary chemicals:

Food additives – saccharin, sucralose, carboxymethylcellulose and polysorbate-80, common emulsifiers (including polysorbates, sorbate esters, lecithin), might increase risk of IBD (data are derived from animal models).

### Over 200 IBD risk loci (37 specific for Crohn's disease and 32 for ulcerative colitis) have been discovered. However, modest fraction of predicted heritability can be explained by known genes or loci.
New target genes of VDR signaling pathway

<table>
<thead>
<tr>
<th>New target gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 2</td>
<td>Tissue Barriers Epithelial tight junctions</td>
</tr>
<tr>
<td>ATG16L1</td>
<td>Autophagy (self-eating), IBD risk</td>
</tr>
</tbody>
</table>

Papers from Sun lab
Wu, et al., *Gut*, 2014
The Functions of vitamin D/VDR

1,25-Dihydroxyvitamin D (1,25(OH)₂D₃), the hormonal form of vitamin D, is a multi-functional hormone

- **Calcium homeostasis and bone development**
- Immune response
- Anti-proliferation
- Anti-inflammation

**Vitamin D/VDR and gut microbiome???
The gut microbiome is a newly discovered organ

*EMBO reports* 7, 688–693 (2006)
doi:10.1038/sj.embor.7400731
Microbiome as “Human Organ”

Reasonable to view microbiome as an organ

Weighs >3 lbs (as similar as human heart)

Organized system of cells more akin to immune system than liver

Dominated by 4 large groups of bacteria or phyla: Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria

Functions: structural, protective, and metabolic functions
The Good...

Challenge

the bad...

and the ugly.
Interaction among microbiome, vitamin D, and VDR signaling is a nearly unexplored area.
VDR distribution in the normal mouse colon

DC: Distal colon
PC: Proximal colon

Fermentation in the Colon

Bengmark S, et al., 1998

Proximal colon
- High concentration of substrates
- Saccharolysis
- Acid pH (5–6)
- Rapid bacterial growth

Distal colon
- Low substrate availability
- Proteolysis
- Neutral pH
- Slow bacterial growth
VDR relocation in the mouse colon after bacterial infection colonization

Human commensal E. coli F18 regulates expression of VDR in mono-associated mice

Relocation of VDR after *Salmonella* Infection

VDR+/+ Control    WT *Salmonella*    VDR-/- control

*Wu, et al. American J. of Pathology, 2010*
VDR null mutant mice have worse outcomes with *Salmonella*-induced infection.

*Wu, et al. American J. of Pathology, 2010*
VDR expression protects against *Salmonella* colonization and mucosal invasion.
VDR expression decreased in UC patients
VDR, bacteria invasion, and intestinal inflammation

Wu, et al., 2010
LARGE INTESTINE

VDR signaling is altered by exposure to enteric bacteria

The vitamin D receptor (VDR) signaling pathway has important immunoregulatory and anti-inflammatory roles in gastrointestinal diseases, such as IBD and colorectal cancer. A mouse study published in the *American Journal of Pathology* now shows that VDR signaling protects against excessive immune responses to organisms in the intestinal lumen. “We found that VDR expression determines how intestinal epithelial cells respond to pathogenic bacterial triggers,” explains Jun Sun, the study’s corresponding author. Importantly, the effects of bacteria on VDR signaling seem to be independent of vitamin D₃, which is the VDR ligand.

Interestingly, VDR expression correlated with bacterial load, being highest in the proximal colon, where enteric bacteria grow strongly, and reduced in the distal colon, an area in which bacterial growth is limited. Furthermore, VDR-null mice exhibited a proinflammatory phenotype—indicated by increased activity of nuclear factor κB (NFκB) and high serum levels of interleukin 6—even in the absence of infection. VDR-null mice also showed a heightened response to infection with *Salmonella* compared with wild-type mice (mice lacking VDRs had greater cecal shortening, worse intestinal inflammation and increased mortality).

In wild-type mice, VDR is normally expressed by fully differentiated intestinal cells at the top of crypts. However, both VDR expression and transcriptional activity increased as a direct result of *Salmonella* infection, in conjunction with relocation of VDR expression to cells further down the crypts. “Intestinal VDR signaling responds to both commensal and pathogenic bacterial stimulation,” says Sun.

The VDR forms a complex with NFκB subunit p65 in osteoblasts, and Sun and colleagues demonstrated that this interaction also occurs in the mouse intestine *in vivo*. NFκB is an essential regulator of the innate and adaptive immune responses, but the functional relevance of this interaction has yet to be elucidated. The researchers showed that deletion of VDR completely abolished the formation of the complex with NFκB and allowed nuclear translocation of the p65 subunit, which might account for the proinflammatory features of VDR-null mice.

“VDR is an important contributor to intestinal homeostasis and host protection from bacterial invasion and infection,” conclude the researchers. “Future research could establish VDR signaling as a new target for treatment of infection and inflammatory bowel disease,” suggests Sun.

Shreya Nanda

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Established intestinal epithelial cell VDR knockout mice

Wu, et al., Gut, 2015
Alterations of the gut microbiome in the intestinal epithelial VDR deficient (VDR$^{\Delta IEC}$) mice

Wu, et al., Gut, 2015
Bacteroides fragilis, a common human commensal microbiota, has been associated with IBD and colon cancer.

Distribution and abundance of *Bacteroides fragilis* by fluorescence in situ hybridization (FISH)
Deletion of intestinal VDR leads to abnormal Paneth cells

Wu, et al., Gut, 2015
VDR deletion leads to less autophagy protein LC3-RFP activation *in vitro*

Wu, et al., Gut, 2014
Increased VDR protein enhances ATG16L1, an IBD risker gene, in the VDR^{+/+} organoids.
VDR and ATG16L1 in human colon

VDR IHC

ATG16L1
Bacterial natural product butyrate restores Paneth cells \textit{in vivo}
Working model of intestinal VDR in inflammation and dysbiosis

Wu et al., *Gut*, 2015
Probiotics LGG-CM enhances VDR protein expression *in vitro*

![Graph showing relative density of VDR over time](image)
Vitamin D receptor pathway is required for probiotic protection in colitis

Human *vrd* genetic variation shapes microbiome

*Parabacteroides* vs rs7974353 (VDR), GLM *p*=0.007

*Parabacteroides* in Vdr WT/KO mice, *p*=0.040

**Wang et al,** Genome-wide host-microbiota association analysis of 1,812 individuals identifies vitamin D receptor genetic variation and other host factors shaping the gut microbiota. *Nature Genetics.* 2016
Effects of high doses of vitamin D₃ on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract

Mina Bashir¹ · Barbara Prietl¹ · Martin Tauschmann¹ · Selma I. Mautner¹ · Patrizia K. Kump² · Gerlies Treiber¹ · Philipp Wurm³ · Gregor Gorkiewicz³ · Christoph Högenauer² · Thomas R. Pieber¹

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Abstract
Purpose Vitamin D is well known for its effects on bone mineralisation but has also been attributed immunomodulatory properties. It positively influences human health, but in vivo data describing vitamin D effects on the human gut microbiome are missing. We aimed to investigate the effects of oral vitamin D₃ supplementation on the human mucosa-associated and stool microbiome as well as CD8⁺ T cells in healthy volunteers.
Methods This was an interventional, open-label, pilot study. Sixteen healthy volunteers (7 females, 9 males) were endoscopically examined to access a total of 7 sites. We

Results Vitamin D₃ supplementation changed the gut microbiome in the upper GI tract (gastric corpus, antrum, and duodenum). We found a decreased relative abundance of Gammaproteobacteria including *Pseudomonas* spp. and *Escherichial/Shigella* spp. and increased bacterial richness. No major changes occurred in the terminal ileum, appendiceal orifice, ascending colon, and sigmoid colon or in stools, but the CD8⁺ T cell fraction was significantly increased in the terminal ileum.

Conclusion Vitamin D₃ modulates the gut microbiome of the upper GI tract which might explain its positive influence on gastrointestinal diseases, such as inflammatory bowel disease
Highlights

- Vitamin D/VDR is an important contributor to host protection and intestinal and microbial homeostasis.

- The tissue-specificity of vitamin D/VDR: reduced intestinal VDR expression in IBD

- Human vrd gene variation and genetic regulation in IBD
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