Microbially synthesized vitamers: Forgotten contributors to host folate status?

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Main research questions:

- What are the relationships between microbiome composition/activity, host nutritional status and inflammation?
- Which microbial species contribute to host nutritional status?
- How does the host nutritional status shape the composition and the activity of the gut microbiome?

Interactions between the microbiome & nutrition in chronic inflammatory diseases

Folate: function & chemical structure

- Key vitamin involved in normal cellular function, growth and development
- Produced by microorganisms and plants
- Folate-requiring reactions: One-carbon metabolism (i.e., amino acid metabolism, purine and pyrimidine synthesis)
- Formation of S-adenosylmethionine (primary methylating agent)

Folic acid

Polyglutamylated folates

\[ \text{Folate} \]

- Pterin
- pABA
- Glu

\[ \gamma\text{-Glu tail} \]

pABA: p-aminobenzoate
Glu: Glutamate

Folate: daily requirement & deficiency

- Daily folate requirement: **300 µg/d** (higher in pregnancy)
- Long-term disruption of folate metabolism:

  - Megaloblastic anemia
  - Hyperhomocysteinemia and cardiovascular diseases
  - Neurological disorder (dementia, Alzheimer’s disease)
  - Embryonic abnormalities (neural tube defect)
  - Cancer (disruption of DNA repair and DNA methylation)
Folic acid

DHF: dihydrofolate
dTMP: thymidylate
dUMP: deoxyuridine monophosphate
MS: methionine synthase
MTHFR: methylenetetrahydrofolate reductase
SAH: S-adenosilylhomocysteine
SAM: S-adenosylmethionine
THF: tetrahydrofolate
TS: thymidylate synthase
DNA methylation
Neurotransmitter synthesis
DNA synthesis
Folate absorption across the small intestine

1/6. Glutamate carboxypeptidase II
2. Reduced folate carrier (RFC)
3. Limited diffusive folate absorption
4. Protein-coupled folate transporter
5. Folate polyglutamate synthetase
6. Multidrug resistance associated protein transport

THF: tetrahydrofolate
Folate sources

- **Dietary source**
  - Absorbed in the small intestine

- **Bacterial source**
  - Absorbed in the colon
  - Production approaches/exceeds levels in diet
  - Production increased by fibers/prebiotics
Folate depoglutamylation & uptake in colon

- **Enzyme/transporter:**
  Presence of glutamate carboxypeptidase & reduced folate carrier transporter

- **Cecal folate uptake in healthy individuals (n=6)**
  Cecal infusion: 855 nmol (13C₅)-5-CHO-THF

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(13-C₅)-5-CH₃-THF

Plasma tetrahydrofolates (nmol/person)

- Time (h)
- Caplet ingestion

Abundance of main bacterial segregates 3 enterotypes

- Abundance of genes, involved in bacterially synthesized vitamins differ between enterotypes

Enterotype II: overrepresentation of enzymes involved in folate biosynthetic pathway

\[\rightarrow\text{Abundance of genes, involved in bacterially synthesized vitamins differ between enterotypes}\]
Preliminary studies: folates in *L. reuteri* strains

- *L. reuteri* ATCC 6475 (anti-inflammatory strain)
- *L. reuteri* ATCC 55730 (pro-inflammatory strain)

M = Glu

n = glutamate

M' = M + methyl modification

Preliminary studies: folates in *L. reuteri* strains
Role of microbially synthesized folates in inflammatory bowel diseases (IBD)
Inflammatory bowel diseases (IBD)

- IBD: chronic inflammation of the intestinal mucosa
- One form: Crohn’s disease (CD)
  - Characterization:
    - can affect any portion of the gastrointestinal tract (predominant: terminal ileum and/or colon)
    - inflammation: relapsing, transmural, asymmetrical

- Higher incidence of CD in industrialized countries
  Germany: 5.2 cases/100 000

- Dramatic increase since the 19th century
CD – a result of a multifactorial interplay

Infectious agents (?)

Immune-related triggers → CD

Environmental factors ← Genetic predispositions

Baumgart et al., 2007; Molodecky et al., 2010
Symptoms:
Frequent diarrhea, abdominal pain, fever, fistula, blood in stool, weight loss due to malnutrition/malabsorption

Folate deficiency: 22-35 % of CD patients

Additional causes: antifolate medications

Low folate status in CD:
1. ↑ levels of homocysteine
2. ↑ risk of developing cancer
3. Genetic abnormalities (MTHFR variant, FOHL1)

MTHFR: methylenetetrahydrofolate reductase
FOLH1: folate hydrolase (γ-glutamyl-carboxypeptidase)
“Dysbiosis”: an abnormal ratio of beneficial and aggressive bacterial species

- Altered balance of the microbiota in ileal CD:

\[ \text{Relative bacterial abundance} \]

- Depletion of specific bacterial genes involved in folate biosynthesis in IBD patient

Sartor et al., 2012; Walters et al., 2014
1. Serum folate deficiency in ileal CD patients reflects an altered folate metabolism in the gastrointestinal tract due to host and/or a gastrointestinal microbiota component.

2. Altered folate metabolism in the gastrointestinal tract contributes to microbial dysbiosis and increased inflammation in ileal CD patients.
Pilot observational study: study design

Participants

Test group:
Total CD patients with ileal involvement (n=30)

Control group:
Total healthy controls (n=30)

Sample collection

day 0

Blood
serum, plasma, whole blood
50 mL

Stool
> 20 g

Food Frequency Questionnaire

Only from CD patients:
Case report form (CRF)

day 7

Group 1
with low serum folate status (≤ 5.5 ng/mL)

Group 2
with high serum folate status (> 5.5 ng/mL)

Group 3
with low serum folate status (≤ 5.5 ng/mL)

Group 4
with high serum folate status (> 5.5 ng/mL)
Pilot observational study: analysis of blood and fecal samples

Sample collection

Blood
50 mL

Stool
> 20 g

Food Frequency Questionnaire

Case report form

Analysis

Folate

Gene variant
MTHFR

Oxidative stress
inflammatory markers

LC-MS/MS

Microbiological assay

Superoxide dismutase

Serum amyloid A

Folate

Gene expression
folh1

Bacterial composition

Inflammatory markers

LC-MS/MS

Microbiological assay

16S rRNA gene pyrosequencing

Calprotectin

Evaluation of dietary folate intake

Inclusion of disease activity, disease location, drugs, surgery

MTHFR: methylenetetrahydrofolate reductase, folh1: folate hydrolase (y-glutamyl-carboxypeptidase), LC-MS/MS: liquid chromatography-mass spectrometry
Pilot observational study: primary and secondary endpoint

Primary endpoint:

1.) Differences in fecal folate levels and folate forms between
    - low and high serum folate status groups
    - between the test and control group

Secondary and exploratory endpoints:

1.) Differences in inflammatory and oxidative stress markers between
    - low and high serum folate status groups
    - between the test and control group
2.) Correlations between microbiota composition and inflammatory markers/gene variants
Method development: Folate detection/quantification by nano-Ultra Performance Liquid Chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS)

1) Monoglutamylated folates
2) Polyglutamylated folates
1. Mefox detection in serum?

- MeFox: oxidation product of 5-CH3THF
- Same molecular mass as 5-CH0-THF (isobaric compound). Higher retention time

Example: Serum (healthy)
Spiked/unspiked with 16 ng/mL 5-CH3-THF
2. Applications: Polyglutamylated folates in bacterial cell extracts

- **Bacteroides ovatus DSM 1447**
- **Bifidobacteria infantis ATCC 15697**
- **E. coli K12 MG 1655**

Spiked folic acid (4.4 ng/mL) before extraction and analysis by mass spectrometry.

- 5-CH3-THF: 5-methyl tetrahydrofolate
- 5-CHO-THF: 5-formyl tetrahydrofolate
- 5,10-CH=THF: 5,10-methenyl-tetrahydrofolate
- 5,10-CH2-THF: 5,10-methylene-tetrahydrofolate
- THF: tetrahydrofolate
- DHF: dihydrofolate

Glun: number of glutamates (polyglutamylated tail)
Conclusion

- Better understanding:
  1. Role of bacterially synthesized folates in health and disease
  2. Contribution of the microbiota to host folate status

- New methods for the measurement of monoglutamylated and polyglutamylated folates in serum, fecal samples are being developed.

**Long term goals:**

Development of nutritional strategies or bacterially synthesized nutritional compounds aimed at restoring appropriate microbiome composition and immune homeostasis, in order to treat or manage inflammation.
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