FOLATE METABOLISM – A CHALLENGE FOR MOLECULAR NUTRITION

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Folate Challenges

- Dietary Reference Intakes, Average Requirements, Individual Requirements
- Bioavailability from natural sources
- Absorption and transport
- Analytical challenges of folates and folate metabolites in biological samples (food, tissues, blood, cells)
- Individual variation of folate metabolism
- Health consequences of individual variation of folate metabolism
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"Of the controlled metabolic studies reviewed above, greatest weight was given to the study by O'Keefe for five reasons: (1) it was designed as a maintenance study for the purpose of estimating the folate requirement; (2) although it included only five subjects, this sample size exceeds that in the Sauberlich study, which was also rigorously controlled; (3) it evaluated the metabolic response of homocysteine in addition to erythrocyte and serum folate; (4) the diet was fed for 70 days in contrast to very short repletion phases in other metabolic studies [...] and (5) it provided folate largely in the form of folic acid, thus minimizing the possibility that folate intake was underestimated. Moreover, considering the evidence that problems with methods have led to underestimates of the folate content of food, it is likely that the subjects in the Sauberlich et al. (1987) and Milne et al. (1983) studies received more folate than reported."
Dietary Reference Intakes for Folate

DRI for adults was therefore set at 400 µg/d.

Is this an acceptable evidence based value for the majority of the population?
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Bioavailability

The majority of studies on bioavailability of folates are based on balance studies with synthetic folate or on studies with stable isotope labeled folates from non-food sources. Information on true bioavailability from food is lacking. Therefore, studies with intrinsically labeled folates from foods are required to get a better picture.
Bioavailability

Production of intrinsically labeled spinach grown on $^{15}$N-labeled nitrogen sources and LC-MS/MS determination of labeled folate in human bioavailability studies.

Preliminary studies indicate an average concentration of 35-60% of all folates carrying at least one labeled nitrogen, thus allowing differentiation of labeled folates from non-labeled folates from other sources.

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Topology of the reduced folate carrier, Sadlish et al., Biochem. J. 2002; 364: 777–786
Absorption and Transport

- Reduced folate carrier hRFC:
- Folate receptors
- Human proton-coupled, high-affinity folate transporter heme carrier protein (PCFT/HCP1)

Absorption and Transport

Reduced folate carrier hRFC:
- 46-58 kDa protein
- Transmembrane anionic exchange
- Dissociation coefficient for 5-methyl-THF in µM range
- Low affinity, high capacity system
- Low affinity to folate (200-400 µM) compared to reduced folates and methotrexate (MTX) (1-10 µM)
- RFC activity depends on differentiation of the cell
- hRFC transcripts are expressed in all tissues, with highest levels in placenta, followed by liver and peripheral leukocytes, low activities in heart and skeletal muscle
- CNS and brain show relatively high RFC levels
- Differences exist for hRFC expression in fetal tissues (highest in lung and heart)
Absorption and Transport

Folate receptors FR:
- High affinity folate binding protein
- Glycopeptide bound to cell membrane by glycosylphosphatidylinositol-residues
- Bind folic acid and 5-CH₂-THF in subnanomol range
- Transport by endocytotic mechanism
- Expressed at apical membrane of epithelial cells, no contact to circulating folates
- 3 different folate receptors (FR-α, FR-β, FR-γ)
- No sequence homology to RFC
- ca. 48-50 kDa
- FR have higher affinity to folic acid and 5-methyl-THF (1-10 nM) compared to RFC, but lower affinity to other reduced folates (10-300 nM).
- Folate binding is decreased in energy deficiency and in presence of chloride

Absorption and Transport

Human proton-coupled, high-affinity folate transporter heme carrier protein (PCFT/HCP1):
- Principle folate transporter at low pH, probably main transporter in intestinal absorption
- „loss-of-function“ mutation results in inherited folate malabsorption
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BCR-intercomparison on the determination of folates in food, determination of folate in a Brussels sprouts RM using microbiological assay (MA), enzyme protein-binding assay (EPBA), radioassay kit (RIA) and liquid chromatography (LC). (Δ) chicken pancreas, (×) human plasma and (+) hog kidney deconjugase enzymes
Folate Challenges

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Folate Determination

Folates are rather instable and transform into isomeric compounds with equal mass but (luckily) different eluation properties from the LC-system. Analytical challenges are the accurate qualitative and quantitative determination of all relevant folates, preferably including polyglutamates.
Folate Determination

Degradation of tetrahydrofolate and formation of THF-fragments at m/z 265 (measured by LC-MS/MS using a microTOF-Q system)
Folate Determination

Folate determination requires fast sample preparation and analysis particularly for THF and 5-MeTHF, since those metabolites are rather unstable. Addition of antioxidants can significantly reduce degradation of folate metabolites.
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Individual Variation of Folate Metabolism

MTHFR (methylene tetrahydrofolate reductase) gene is located on chromosome 1 (1p36.3). It is a C677→T polymorphism, with a change of the triplet GCC to GCT (at position 677 of the 12.436 bp sequence)
Individual Variation of Folate Metabolism

Translation of the modified genetic code of the MTHFR gene leads to insertion of a valin residual instead of an alanin residual into the enzyme peptide.

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Specific versus residual MTHFR activity from individuals with MTHFR polymorphism

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Health Consequences
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FUTURE WORK
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- Folate metabolism on several levels within the concept cell to society:
  - Folate transport and metabolism of C. elegans using RNAi
Future Work

Folate metabolism on several levels within the concept cell to society

C. elegans

Sus scrofa
Future Work

Folate metabolism on several levels within the concept cell to society

- Folate transport and metabolism of C. elegans using RNAi
- Folate transport and metabolism at high and low dosage levels of folates and methionin in a pig model

Determination of folate transporter mRNA and folate metabolism in 4 groups of growing pigs with diet high in folate/methionin (FA+/Met+), low in folate/methionin (FA-/Met-) and variations thereof (FA+/Met-, FA-/Met+)

(in co-operation with W. Windisch, University of Natural Resources and Applied Life Sciences – Universität für Bodenkultur)
Future Work

- Folate metabolism on several levels within the concept cell to society:
  - Folate transport and metabolism of C. elegans using RNAi
  - Folate transport and metabolism at high and low dosage levels of folates and methionin in a pork model
  - Determination of folate bioavailability from foods

Preparation of intrinsically labelled folates by growing spinach on sources of 15N (K15NO3, (15NH4)2SO4) and application of this to humans for the determination of folate bioavailability from foods

\[
\text{5-Methyl-5,6,7,8-tetrahydrolfolic acid}
\]

Chemical Formula: C_{15}H_{14}N_{2}O_{7}Br
Exact Mass: 466.17
Molecular Weight: 466.41

- Generation of metabolite databases
- Mass specs of different samples for metabolic profiling

- Variation of folate metabolism in humans with different genetic background
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Epigenetic effects of folates in response to MTHFR-SNPs

It is a long and rocky path to combine all data from genomics, transcriptomics, proteomics, and metabolomics, into practical nutrition concepts in respect to folate metabolism. The question still remains, whether all this effort will result in a healthier population.
THANK YOU FOR YOUR ATTENTION!

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