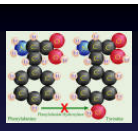

Micronutrients and Their Bioavailability in the PKU-Diet

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Institute of Nutritional Sciences
University of Vienna

18th E.S. PKU Meeting, 29th to 31st October 2004, Bled - Slovenia

Micronutrient deficiency result from:

- Inadequate dietary intake
- Impaired absorption
- Limited bioavailability
- Excessive losses
- Increased requirements

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Definition of Bioavailability

The term bioavailability refers to the extent to which a bioactive compound elicits a response in a target tissue.

Rowland et al. 2001

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Techniques for Assessment of Bioavailability

In vitro methods:

1. Uptake of isolated tissue/cultured cells.

In vivo methods:

2. Blood response/uptake by tissues.
3. Measurements of rates of absorption (radioactive or stable isotopes).
4. Assessment of body retention (balance studies, mass or isotope).
5. Response of physiologic variables: antioxidant capacity, parameter of oxidative stress, lipid peroxidation.

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Bioavailability: Influencing Factors

1. The efficiency of digestion
2. The previous intake of the nutrient
3. The body "status" of the nutrient
4. Gut transmit time
5. The presence of gastro-intestinal disorders or disease
6. Other products with which the food stuff is consumed
7. The prior-treatment (namely cooking or processing) of the product

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Bioavailability

➡ **Endogenous factors**

- gastric-pH
- cellular and tissue content
- pool size
- initial status
- interindividual variability (responders and nonresponders)
- BMI
- gender
- age

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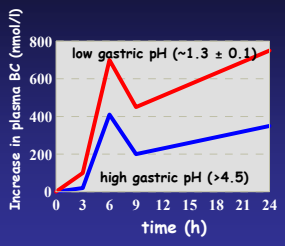
Endogenous Factors I

Gastric pH value

n = 12 healthy adults, supplemented with 120 mg BC

Plasma response with pH = 1.3 ± 0.1 higher than with pH > 4.5



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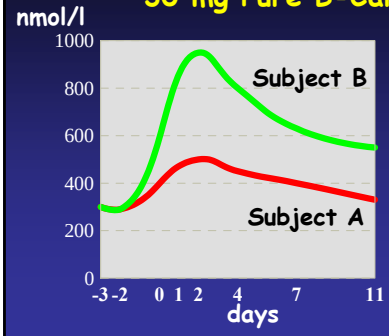
Profile of Plasma β-Carotene for Two Subjects (A and B) after Ingesting 30 mg Pure B-Carotene



After supplementation with 30 mg BC plasma BC concentrations increase in subject A about 85% and in subject B about 272%. The difference in maximal concentration increase between individuals can be as high as 10-fold.

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Bioavailability

- Exogenous Factors
  - natural / synthetic
  - dose
  - diet composition
    - fats and fatty acids
    - fibers
  - seasonal variation

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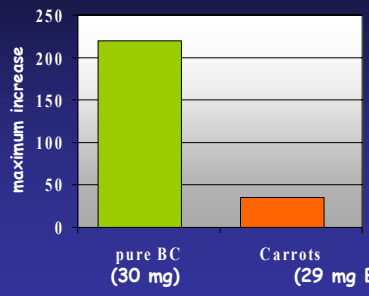
Changes in Plasma Concentration of β-Carotene after Ingesting a Single Dose of Pure β-Carotene or Vegetables



Plasma response with carrots 21% of pure beta-carotene

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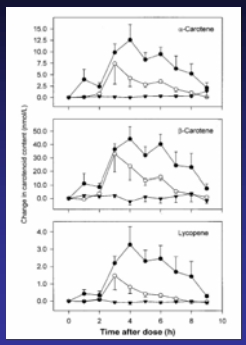
Plasma Response to a Fresh Vegetable Salad with and without Dietary Fat



The subjects had ingested a fresh vegetable salad with salad dressing containing 0 (▪, fat-free), 6 (○, reduced-fat), or 28 (•, full-fat) g canola oil; *n* = 7. The change in the chylomicron content of each carotenoid was greater after ingestion of the reduced-fat salad dressing than after ingestion of the fat-free salad dressing ($P < 0.04$). Similarly, the change in the chylomicron content of each carotenoid was greater after ingestion of the full-fat salad dressing than after ingestion of the reduced-fat salad dressing ($P < 0.02$). *(Brown et a. 2004)*

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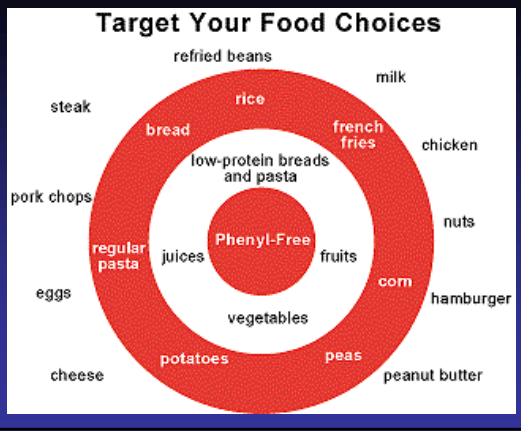
Target Your Food Choices



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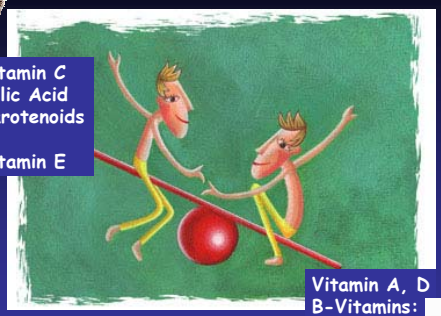
Vitamin C
Folic Acid
Carotenoids

Vitamin E



Vitamin A, D
B-Vitamins:
Vitamin B2
Vitamin B6
Vitamin B12

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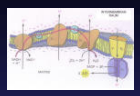
Riboflavin - Sources

IfEW

Riboflavin Functions



- Component of FMN and FAD
- FMN and FAD are coenzymes of flavoproteins that participate in tissue oxidation and respiratory processes
- FAD is component of the *glutathione reductase* (antioxidative enzyme especially in erythrocytes)

Synergism

- With folic acid, vitamin B_6, vitamin K and niacin
- Vitamin B_2-deficiency leads to complications of the functions of all of these vitamins

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Riboflavin Deficiency

Early Symptoms

- Cheilosis
- Glossitis
- Seborrheic Dermatitis (face and scrotum)



Late Symptoms

- Normocytic normochrome anemia
- Visual impairment
- Burning of the eyes

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Decreased Availability of Riboflavin

- Type of the vitamin
- Food matrix
- Dysfunction of absorption
- Alcohol abuses
- Pharmaceuticals like antidepressiva, oral contraceptive



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Intake Recommendation of Riboflavin

0.6 mg Riboflavin per 1000 kcal
Minimum: 1.2 mg/d

The supply is increased through increased metabolism (e.g. fever, physical exercise)

UL: not given

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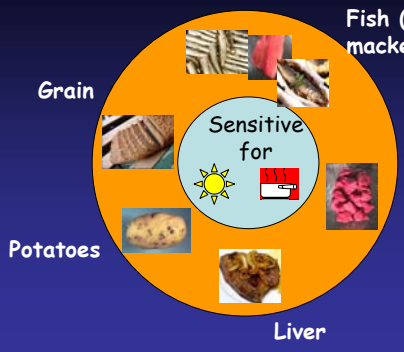
Vitamin B_6 - Sources



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Functions of Vitamin B6

- Cofactor within the protein metabolism
- Transaminations (PLP and PMP)
- Decarboxylisations: e.g. formation of biogenic amines
- Metabolism of OH- and SH-containing amino acids (threonin, serin, cystein)

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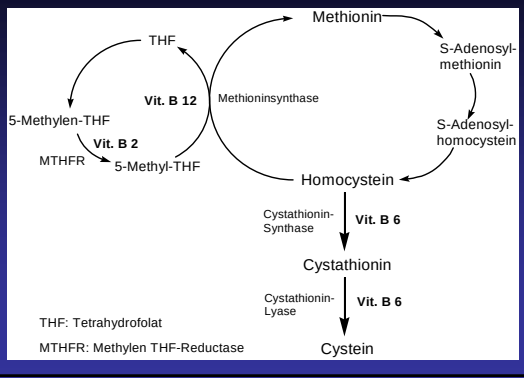
Vitamin B6, B12, Folic Acid



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Decreased Availability of Vitamin B6

- Form of dietary vitamin B6
- Food composition
- Food processing
- Drug interactions and antagonist
- Oral contraceptive agents



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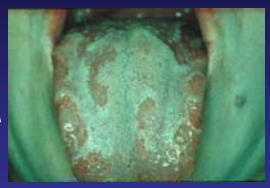
Vitamin B_6 Deficiency

Early symptoms

- unspecific

Late symptoms

- dermatitis
- glossitis („geographic tongue" reduced taste perception)
- neurological disorders



„geographic tongue"

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Intake Recommendation of Vitamin B6

Women:
0.02 mg Vitamin B_6 /1g Protein ≈ 1.2 mg/d
Men:
0.02 mg Vitamin B_6 /1g Protein ≈ 1.5 mg/d

The supply is increased with increased metabolism (e.g. growing, cold).
Increased supply in pregnancy and lactation.

UL: 25 mg/d

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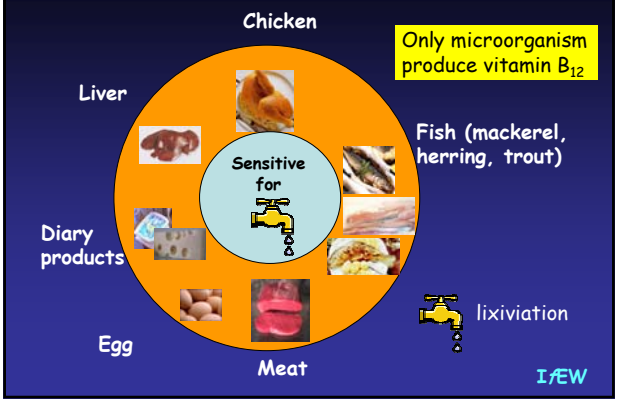
Cobalamin - Sources



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Functions of Vitamin B12

- **Homocysteine** metabolism (high homocysteine concentrations damage blood vessels and brain)
- **Methyl-Cobalamin** transfers as coenzyme Methyl-groups (-CH_3). For example: Homocysteine + CH_3 = Methionin
- **5'Desoxyadenosyl-Cobalamin** is involved in intramolecular transfer reactions. For example: metabolism of fatty acids

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Decreased Availability of Vitamin B12

- Impaired acid and pepsin secretion
- Malabsorption by pancreatic insufficiency
- Secretion of intrinsic factor
- Alcohol abuses inhibit cobalamin absorption
- High amounts of vitamin C (5 - 10 of the recommended amount) inactivate Vitamin B_{12} by reduction

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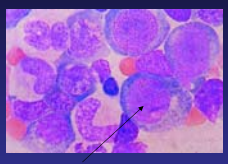
Vitamin B12 Deficiency

Early symptoms
- Disturbance of cell division
- Glossitis
- Diarrhoe

Late symptoms
- Pernicious anemia with abnormal preliminary stages of erythrocytes (so called megaloblasts)
- Neurological symptoms:



Megaloblastic anemia

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Intake Recommendation of Vitamin B12

Children < 4 years: 1 µg/d
Children < 4 to 7 years: 1.5 µg/d
Children < 7 to 10 years: 1.8 µg/d
Children < 10 to 13 years: 2 µg/d
Children > 13 and adults: 3 µg/d

UL: no available

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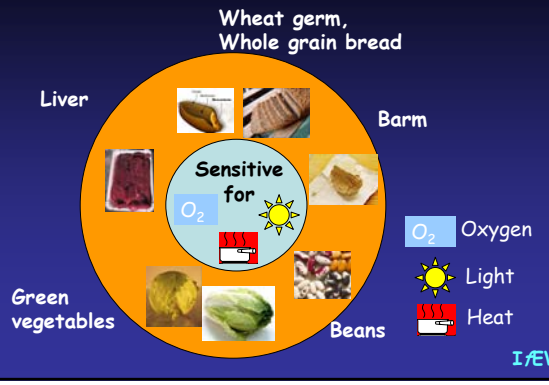
Folic Acid - Sources



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Decreased Availability of Folic Acid

- Vitamin B12 deficiency (methyl-THF can not be recycled to THF
- Dysfunction of absorption
- Alcohol abuses
- Drugs (Zytostatika, Antiepileptika, Antimalaria drugs)

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Deficiency of Folic Acid

- Increased homocysteine and decreased methionine blood levels
- In Pregnancy: increased rate of abortus, neural tube defect, deformation, growth failure because of inadequate DNA-synthesis
- Megaloblastic anemia

Spina bifida

IfEW

Intake Recommendation of Folic Acid

Adults:
400 µg folate equivalents per day

Women who wish to have children should take supplements (400µg/d) to a minimum of 1 month before and the first 3 month of pregnancy to prevent neural tube defect

UL: 1000 µg/d (Pteroylmonoglutamat)

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Trace Elements

Involvement of trace elements in biological functions

- *Transport/storage:*
  Metalloproteins (Fe, Cu, others)
- *Biochemical regulation:*
  - Enzyme activators
  - Metalloenzymes:
    free radical metabolism
    (Zn, Mn, Fe, Cu, Se)
    cell immunity (Se, Zn, Fe)

- *Bone structure*

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Trace metal involvement in producing and scavenging of free radicals

- *Metalloenzymes*
  - Superoxide dismutase
    cytoplasmatic : Cu, Zn
    mitochondrial : Mn
  - Xanthine dehydrogenase : Mo
  - Glutathione peroxidase : Se
- *Metalloproteins*
  - Transferrin : Fe
  - Caeruloplasmin : Cu
    (Ferroxidase I) : Fe
  - Metallothionein : Cu, Zn, Cd
- *Fenton chemistry:*

$2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$

$O_2^- + Fe^{3+} \rightarrow Fe^{3+} - O_2^- \rightarrow Fe^{2+} - O_2 \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$

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Influencing factors on mineral absorption

Exogenous Factors

- Binding form (e.g. Hem-Iron – free Iron)
- Amount of the mineral in the gut (high dosage lowers absorption rate)
- Compounds of the diet (e.g. dietary fibre, chelate- and complexing substances affect absorption)

Endogenous Factors

- Gastrointestinal pH (e.g. decreased gastric HCl-concentrations diminish Fe-absorption)
- Interaction with other nutrient compounds (e.g. Vit. C promotes Fe-absorption)
- Interactions with other minerals (e.g. high Zn-intake diminishes Fe- and Cu-absorption)

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Dietary factors affecting bioavailability

Trace element	Enhancers	Inhibitors
Chromium	histidine, nicotinic acid	zinc, iron
Copper	animal protein, fructose	phytate, vitamin C (?), iron, zinc
Iron	vitamin C, meat	phytate, polyphenols, calcium
Manganese		calcium, phosphorus, phytate, iron
Selenium	thiols, vitamin C	methionine, phosphorus, heavy metals
Zinc	animal protein	phytate, iron

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Clinical classification of trace element deficiency

- ***Low intake***
  - natural-isolated
  - mixed
  - therapeutic, accidental
- ***Malabsorption***
  - generalized
  - specific
- ***Intermediary metabolism***
  - transport proteins
  - receptors
- ***Increased demands***
  - growth, repair
  - external loss - catabolism
    - mucous membranes
    - skin

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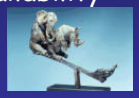
Trace element deficiency in humans Increased risk:

- Inadequate intake low bioavailability
- Systemic defects in bioavailability
- Negative balance
- Increased requirements



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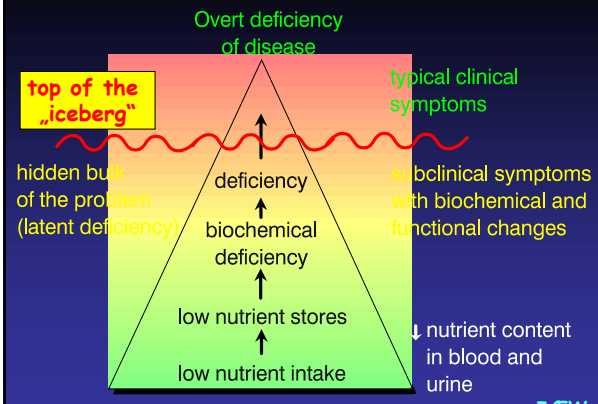

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Consequences of essential trace element deficiencies

(Human and animal studies)

trace elements	pathological effect
Se, Zn, Cu, Fe, I	↓ cell immunity
Se?, Zn, Cu, I	↓ growth
Zn, Cu, I	↓ reproduction
Zn, Cu, I, Mo	↓ nerve cell function
Cu, Fe	anemia
Zn, Cu	↓ bone health
Se, Cu, I	↓ cardiac function
Se?, Zn, Cu	↓ pancreas function

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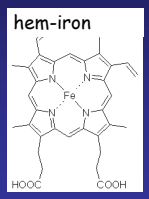
Iron – Body Storage and Sources

Pool: 5 g

More than 2/3 as haemoglobin and myoglobin

Sources
- Meat
- Liver
- Whole wheat grain
- Pulses

contain Hem-Iron



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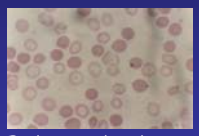
Iron – Functions and Deficiency

Functions
- Oxygen transport (Hemoglobin, Myoglobin)
- Important factor in energy metabolism (respiratory process)
- Compound of oxidising and reducing enzymes (e.g. peroxidasen)

Deficiency
- Early symptoms: mucosa lesions, weakness
- Late symptoms: hypochrome, microzytic anemia



Erythrocyts in hypochrome, microzytic anemia

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Substances affecting iron absorption

Substances inhibiting absorption	*Substances facilitating absorption*
Phytic acid	Cysteine containing proteins
Polyphenols	Ascorbic acid
Calcium	
Protein	

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Iron – Recommendation

• Children:	8-15 mg/d
• Women up to 50 years:	15 mg/d
• Men and women over 50 years:	10 mg/d
• Pregnant and lactating women:	30/20 mg/d

• *UL*: within the EU no UL defined. (In USA UL of 45 mg/d)

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Zinc – Body Pool and Sources

Pool: 1.5 – 2.5 g

70% in bone skin and hair

30 % mainly in liver, kidney, muscle

Sources
- oysters
- whole wheat grain
- liver
- nuts
- meat

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Zinc - Functions and Status

Functions
- Compound and activator of various enzymes
- Functions on DNA (transcription)
- Storage of insulin
- Immune system (growth of t-lymphocytes)
- Site specific antioxidants

Bioavailability influencing factors
- Amount of phytate
- The type and amount of protein
- Total zinc content

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General situations where zinc deficiency may occur

- ***Dietary deficiency:*** **e.g.**
  - protein energy malnutrition
  - synthetic diets
  - i.v. feeding
  - underdeveloped rural communities
- ***Malabsorption syndromes:*** **e.g.**
  - Acrodermatitis enteropathica
  - non-specific malabsorption (Coelic, Crohn's disease)
- ***Increased body losses:*** **e.g.**
  - chelating agent therapy
  - chronic diarrhea
- ***Abnormal zinc metabolism:*** **e.g.**
  - Alcoholic liver disease (?)

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Zinc – Recommendation

• Children:	3-9.5 mg/d
• Women:	7 mg/d
• Men:	10 mg/d
• Pregnant and lactating women:	10/11 mg

- *UL*: 25 mg/d

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Cooper – Body Pool and Sources

Pool: 80 – 100 mg → 1.25 mg loos per day via faeces and urine

Sources

- shellfish
- whole grain bread
- liver
- nuts
- meat

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Cooper - Functions and Deficiency

Functions

- Compound of metalloenzymes (endogen antioxidative system)
- Compound of ceruloplasmin (transport of cooper, catalysis the oxidation of Fe^{2+} to Fe^{3+})

Deficiency

- Hypochrome microcytic anemia
- Leucocytopenie, granulocytopenie
- Bone fracture
- Diminished pigmentation of hair and skin
- Neurological symptoms

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Factors affecting cooper absorption

HOST RELATED	
+	**-**
infancy	old age
Wilson's disease	disease states with malabsorption syndromes
	protein energy malnutrition
DIET REALATED	
low cooper diet	high copper diet
human milk	cow's milk/infant formulas
animal protein	vegetable protein
fructose	zinc, iron, molybdenum
	ascorbic acid
	calcium and phosphate
	penicillamine

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Cooper – Recommendation

• Children up to 7 years:	0.5-1.0 mg/d
• Children over 7 years and Adults:	1.0-1.5 mg/d

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Conclusion

Perceptions of these interactions, evaluation of nutrient intake of subjects with regard to absorption inhibiting and facilitating constituents could lead to more effective strategies to prevent micronutrient deficiency.

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