Dose Response and Bioavailability of Micronutrients Obtained from Supplements versus Food

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## Disclosures

<table>
<thead>
<tr>
<th>AFFILIATION/FINANCIAL INTERESTS (prior 12 months)</th>
<th>CORPORATE ORGANIZATION</th>
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<tbody>
<tr>
<td>Grants/Research Support:</td>
<td>None</td>
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<tr>
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<td>Almond Board of California, AuthenTechnologies, Biothera, Blytheco, Central Garden (Pet Food), Daedelus Humanitarian, McDonald’s, California Walnut Commission, Coca-Cola, Mushroom Council, ObiProbiotic, Quaker Oats, Spherix Consulting, TAAG, U.S. Pharmacopeia, Vets-Plus (Pet Food), Numerous Law Firms, Numerous PR Firms</td>
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<tr>
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<tr>
<td>Stock Shareholder:</td>
<td>None</td>
</tr>
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<td>Other</td>
<td>None</td>
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Learning Objectives

* Review the principles of pharmacodynamics relative to nutrients
* Examine the impact of food and dietary supplement matrices on nutrient bioavailability
* Describe the important differences between nutrient content and biological relevance
What is a Food?

* FD&C Act §201(f):
  (1) articles used for food or drink for man or other animals,
  (2) chewing gum, and
  (3) articles used for components of any other such article.
What is a Dietary Supplement?

FD&C Act §201(ff)(1):

* A product “intended to supplement the diet” that contains one or more of the following:
  - A vitamin, mineral,
  - Herb or Botanical,
  - Amino acid
* “a dietary substance for use by man to supplement the diet by increasing the total dietary intake” or
* “concentrate, metabolite, constituent, extract, or combination of above”
Absorption
Pharmacodynamics
**Host Factors**

* Homeostatic mechanisms that regulate absorption or excretion depending on nutrient status of host
* Variable factors include, not limited to:
  - Age
  - Sex
  - Physiologic state (e.g., pregnancy)
  - Dietary load/source

Heaney RP. J Nutr 2001;131:1344S-8S
Solomons & Slavin. J Nutr 2001;131:1392S-95S
Krebs NF. J Nutr 2001;131:1351S-4S
King JC. J Nutr 2001;131:1355S-8S
Food Factors

* Presence and content of other nutrients
  - High levels of Zn decreases Fe and Cu absorption
  - Vitamin C improves non-heme Fe absorption
  - Vitamin D improves Ca, P, and Mg absorption

* Presence of absorption inhibitors
  - Oxalate
  - Phytate
  - Polyphenols (e.g., tannins)

* Food preparation and storage
Percent Below EAR of Selected Nutrients Based on NHANES 2007-2010

Usual intakes compared to DRIs for individuals aged ≥ 4 years

* Nutrient identified by 2010 DGAC as being a nutrient of public health concern
MMVM = Multivitamin/mineral supplement

Wallace et al., J Am Coll Nutr 2014;33:94-102
## Bioequivalence & DRIs

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Bioequivalence &amp; Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>DRI expressed as μg retinol activity equivalents (RAE)</td>
</tr>
<tr>
<td></td>
<td>1 μg RAE = 1 μg <em>all-trans</em>-retinol = 2 μg supplemental <em>all-trans</em>-β-carotene = 12 μg</td>
</tr>
<tr>
<td></td>
<td>other provitamin A carotenoids</td>
</tr>
<tr>
<td>Iron</td>
<td>Algorithm for estimating dietary Fe bioavailability: 18% bioavailability based on differences</td>
</tr>
<tr>
<td></td>
<td>in absorption from heme- and nonheme-Fe sources in a mixed US diet</td>
</tr>
<tr>
<td>Niacin</td>
<td>No adjustment is made for bioavailability, but the requirement is expressed in niacin</td>
</tr>
<tr>
<td></td>
<td>equivalents (NEs) which allows for some conversion from tryptophan</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Bioavailability of 75% is assumed from a mixed diet</td>
</tr>
<tr>
<td>Folate</td>
<td>DRI expressed as dietary folate equivalents (DFEs): 1 μg DFE = 0.6 μg folic acid</td>
</tr>
<tr>
<td></td>
<td>from fortified food or as a supplement take with meals = 1 μg food folate = 0.5 μg</td>
</tr>
<tr>
<td></td>
<td>supplement taken on an empty stomach</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>An assumed absorption from foods is 50% in included in DRI; those aged ≥ 51 yo</td>
</tr>
<tr>
<td></td>
<td>should consume fortified foods or vitamin B12 supplements due to reduced absorption from food</td>
</tr>
<tr>
<td></td>
<td>forms</td>
</tr>
</tbody>
</table>

Yetley EA Am J Clin Nutr 2007;85(suppl):269S-76S
Dietary Supplements: Hostile Matrix

* Vitamin analogues develop during storage and through interactions with other nutrients in dietary supplements
* USP established Dietary Supplements Verification Program (2002) consistent with 21 CFR Part 111
* NSF established stability testing protocol for dietary supplements with expiration date (2011) consistent with 21 CFR Part 111

Takenaka et al., Biosci Biotech Biochem 1997;61:2137-39
Folic Acid Inequities

* Food forms
  - Polyglutamated (methyl or formyl)
  - Reduced
  - Labile
  - Low bioavailability (~50%) : due to food matrix / binding, GI destruction, incomplete hydrolysis

* Fortification & Supplements
  - Monoglutamate
  - Oxidized
  - Stable
  - Good bioavailability (~85% with food; ~100% sans food): already “free” from food matrix, oxidized form, less susceptible to GI destruction, in monoglutamate form

* Other factors
  - Host folate pool, presence of other nutrients (vitamin C, vitamin B12, vitamin B6, niacin, riboflavin, choline)
  - Host genetics, homeostatic mechanisms, sex, ethnicity, biological/physiological status

Caudill MA. Am J Clin Nutr 2010;91(suppl):1455S-60S
IOM, 1998
Food Dynamics of Vitamin B12

* Only synthesized by select bacteria and some algae
  - Aerobic: *Pseudomonas denitrificans*
  - Anaerobic: *Bacillus megaterium*, *Propronibacterium shermanii*, *Salmonella typhimurium*, *Lactobacillus reuteri*

* Major food sources
  - Shellfish – 52.4 μg/100 g
  - Meat – 9.4 μg/100 g
  - Fish – 8.9 μg/100 g
  - Eggs – 1.3 μg/100 g
  - Milk – 0.4 μg/100 g

* Absent from plant foods

* Algae sources
  - *Chlorella* sp. - 200 μg/100 g
  - *Prophyra* - 77 μg/100 g

Watanabe et al., J Agric Food Chem 2013;61:6769-75
Watanabe et al., J Nutr Sci Vitaminol 2002;48:325-31
Taranto et al., J Bacteriol 2003;185:5643-47
Raux et al., Biochem J 1998;335:159-66
Food Dynamics of Vitamin B12

* Bound to protein in food
  - Released upon gastric hydrolysis: HCl and protease
* Supplement
  - Free form; hydrolysis not required
  - Sublingual forms – may not be better than typical oral forms
* “Free” B12 binds with IF → absorption in distal ileum
  - Absorption ~ 56%
  - Absorption decreases with age (10-30% with atrophic gastritis) and when IF binding capacity is exceeded
  ➢ Unable to absorb food form; readily absorb supplement forms

Carmel R. Blood 2008;112:2214-21
Sharabi et al., Br J Clin Pharmacol 2003;56:635-8
Food Dynamics of Vitamin B12

* Inactive corrinoid compounds in some supplements
  - Cyanobacteria (e.g., *Spirulina*, *Aphanizomenon*, *Nostoc*)
  - Vitamin B12 content varies by analytical method
  - Most of the corrinoid compounds are biologically inactive [pseudo B12] (e.g., low absorption, poor IF binding)

Watanabe et al., J Agric Food Chem 2013;61:6769-75
Herbert & Drivas. JAMA 1982;248:3096-97
Plant-based diets (little or no animal products) →
↑ absorption inhibitors (e.g., phytate, oxylate, phenols)

- **Phytate (1-3%)**
  - Food sources: seeds, nuts, vegetables, fruit, legumes, cereal grains → ↓ *in vivo* Fe absorption 18-82%; may be counteracted by ascorbic acid, meat, fish and poultry (animal products)

- **Oxalic acid**
  - Food sources: cereal grains, vegetables, legumes → ↓ Fe and Zn absorption and possibly Ca

- **Polyphenols**
  - Food sources: tea, coffee, red wine, vegetables, grains, herbs, spices → ↓ *in vitro* Fe absorption; little *in vivo* data that are clinically significant

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Hallberg et al., Am J Clin Nutr 1989;49:140-4
Siegenberg et al., Am J Clin Nutr 1991;53:537-41
Brune et al., J Nutr 1992;122:442-9
Phytate-mineral complexes are insoluble

Absorption inhibition may be, in part, due to aggregation of insoluble complexes

Enzymatic hydrolysis via exogenous phytase reduces insoluble complexes and increases mineral availability (e.g., Fe, Zn)

Common phytase source is from Aspergillus niger

Troesch et al., Food Nutr Bull 2013;34:S90-S101
Phytase and Zn Bioavailability

Davidsson et al., Br J Nutr 2004;91:287-94
Phytase and Iron Bioavailability

Sandberg et al., J Nutr 1996;126:476-80
Iron & Food Processing

Relative Biological Value

Control Fe(SO₄)  NP w/ FOP  P w/ FOP  NP w/ CI  P w/ CI  NP w/ EI  P w/ EI

NP: Not Processed  FOP: Ferric Orthophosphate  CI: Carbonyl Iron  EI: Electrolytic Iron

P: Processed

Clemens & Mercurio, J Food Sci 1981;46:930-932,935
Iron & Food Storage

Clemens & Mercurio, J Food Sci 1981;46:930-932,935
Iron & Food Storage

Iron Valence Profile
% total iron

- Fe(0)
- Fe(+2)
- Fe(+3)

6 mos 12 mos 6 mos 12 mos 6 mos 12 mos

Ferric Orthophosphate
Carbonyl Iron
Electrolytic Iron

Clemens & Mercurio, J Food Sci 1981;46:930-932,935
Particle size: Small - 13.5 ± 10.4 μ; Large - 18.0 ± 13.6 μ

\[ \text{Ca Retention} = \text{Intake}_{\text{Ca(mg/d)}} - \left( \text{Urinary Excretion}_{\text{Ca(mg/d)}} + \text{Fecal Excretion}_{\text{Ca(mg/d)}} \right) \]
\[ \text{Net Ca Absorption} = \text{Intake}_{\text{Ca(mg/d)}} - \text{Fecal Excretion}_{\text{Ca(mg/d)}} \]
\[ \% \text{ Apparent Ca Absorption} = \left( \frac{\text{Intake}_{\text{Ca(mg/d)}} - \text{Fecal Excretion}_{\text{Ca(mg/d)}}}{\text{Intake}_{\text{Ca(mg/d)}}} \right) \times 100 \]

Elble et al., J Am Coll Nutr 2011;30:171-7
Questions

- Does mineral encapsulation influence bioavailability?
- Does the type of encapsulation material affect bioavailability?
- Does the encapsulant to nutrient ratio impact bioavailability?
Relative Bioavailability of Encapsulated FeSO$_4$

<table>
<thead>
<tr>
<th>Form</th>
<th>RBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeSO$_4$•H$_2$O (non-encapsulated)</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>FeSO$_4$ (encapsulated) in ethyl cellulose (20:80 ratio of capsule:FeSO$_4$•H$_2$O)</td>
<td>133 ± 10</td>
</tr>
<tr>
<td>In 40:60 ratio with</td>
<td></td>
</tr>
<tr>
<td>Partially hydrogenated soybean oil</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>Partially hydrogenated palm oil</td>
<td>95</td>
</tr>
<tr>
<td>Mono- and diglycerides</td>
<td>116 ± 7</td>
</tr>
<tr>
<td>Partially hydrogenated soybean and cottonseed oil</td>
<td>79</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>87</td>
</tr>
</tbody>
</table>

Plant Iron

* Plant ferritins – natural nanostructures of protein cages (10-12 nm) that include iron and oxidants (cavity of 5-8 nm)

* Molecular breeding (Quantitative Trait Locus) of corn may increase iron bioavailability
  - Identified hybrids with specific iron gene biomarkers → improved production + improved iron bioavailability (Caco2 cells)

Theil et al., J Biol Inorg Chem 2006;11:803-810
Tako et al., Nutr J 2013;12:3 http://www.nutritionj.com/content/12/1/3
Hb and $^{59}$Fe Absorption Among 15 Nonanemic, Healthy Women Receiving Low-Phosphate Mineral (animal) Ferritin or FeSO$_4$

Hb and $^{59}$Fe Absorption Among 15 Nonanemic, Healthy Women Receiving High-Phosphate Mineral (plant) Ferritin or FeSO$_4$

Kinds of Evidence - Polyphenols

* Primarily in vitro studies
* Few in vivo studies (animals, humans)
* Mechanisms of action
  - Resveratrol
    - Different between fat and muscle tissue
    - Sirt 1 activation?
    - 5’ AMP-activated protein kinase (AMPK) activation?
* Typical concentrations
  - In vitro studies: low µmol/L to mmol/L (wide range)
  - Dietary: < nmol/L (plasma)
  - Pharmacological: ~ 1 g/d ≈ >650 bottles of red wine
* Tissue distribution

Um J-H et al., Diabetes 2010;59:554-63
Park SJ et al., Cell 2012;148(3):421-33.
Visioli et al., Crit Rev Food Sci Nutr 2011;51:524-46
Pathways of Polyphenol Absorption

STOMACH
Polymers → Monomers

SMALL INTESTINE
Glycosides → Aglycones

COLON
Glycosides → Aglycones

LIVER
Conjugation (methylation, sulphation, glucuronidation)

PORTAL VEIN

Cells and tissues

Urinary excretion

Violi et al., Crit Rev Food Sci Nutr 2011;51:524-46
Bioavailability of Polyphenols

* Bioavailability quite variable (e.g., 0.3% anthocyanins; 43% for isoflavones)
* Partition coefficients based on structure (hydrophilic, sugar moiety; molecular weight)
* Increased number of sugar molecules (e.g., glucose, galactose, xylose) may improve absorption small intestine (selected enzymes, transport proteins)
* Phenolics with rhamnose are not absorbed in small intestine; must be degraded by microflora enzymes
* Acylated flavonoids absorbed without deconjugation
* Isoflavone aglycones absorbed in stomach; glycosides absorbed in duodenum

Selma et al., J Agri Food Chem 2009;57:6485-6501
Pharmanutrition

Plasma Concentration-Time Curve for Total Radioactivity After Oral 25mg (110 μmol)

Mean values ± SD; n=6

Absorption: ~ 70%
Plasma half-life: 9.2 ± 0.6 hr
Plasma detection (parent molecule): < 5 ng/mL

Urine recovery (%): 70.5 ± 10.5
Fecal recovery (%): 12.7 ± 14.9

Walle T et al., Drug Metabol Dispos 2004;32:1377-82
Prophylactic Zn Dose Response

Ecuadorian Children 12-36 mo old with Low Initial LAZ during a 6-mo Intervention with ZnSO₄

- All doses reduced incidence of diarrhea
- No adverse events observed
- IOM (2011) advises 7 mg/d in this age (1-3 yrs) group as UL for Zn

Wuehler et al., Am J Clin Nutr 2008;87:723-33
Enteral Product Implications

* Enteral products (n=20; liquid and powder) mixed with
  - Rice pudding (not heated)
  - Chocolate dessert (heated → cooled)
  - Tea (heated → cooled)
  - Banana smoothie (not heated)

* *In vitro* bioaccessibility of Fe, Zn, Ca via dialysis
  - Potential mineral supply = Mineral concentration x % dialyzed x g/serving
Enteral Product Implications

Adapted from Galán & Drago. J Sci Food Agric 2014;94:515-21
# Drug Factors

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Depletion</th>
<th>Interaction(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statins (e.g., Mevacor®)</td>
<td>Coenzyme Q10</td>
<td>Niacin $\rightarrow$ ↑ risk neuropathies</td>
</tr>
<tr>
<td>Synthetic thyroid (e.g., Synthroid®)</td>
<td>Ca</td>
<td>Fe + soy $\rightarrow$ interfere with absorption</td>
</tr>
<tr>
<td>Diuretics (a: K⁺ depleting (e.g., Lasix® or b: K⁺ sparing (Aldactone®))</td>
<td>a: e.g., Mg, K, Zn, B₁, B₆, Vitamin C b: e.g., Folate, Fe, Vitamin C</td>
<td>b: Mg preserved</td>
</tr>
<tr>
<td>Beta-blockers (e.g., Interal®)</td>
<td>Coenzyme Q10 Melatonin</td>
<td>K $\rightarrow$ ↑ plasma levels Mg (Rx for arrhythmias)</td>
</tr>
<tr>
<td>Anti-diabetics (e.g., Glucophage®)</td>
<td>Vitamin B₁₂, Folic acid, Coenzyme Q₁₀</td>
<td>May ↑ DHEA (dehydroepiandrostosterone)</td>
</tr>
</tbody>
</table>

Yetley EA. Am J Clin Nutr 2007;85(suppl):269S-76S
Sørensen JM. J Alt Compl Med 2002;8:293-308
Enteral Feeds and Meds Delivery

* Variable responses; drug dependent including drug solubility, binding cations, or enteral components

* Examples
  - Improved update if enteral feed withheld at least one hour following med oral administration: ciprofloxacin, phenytoin, warfarin
  - Absence or limited evidence of drug enteral feed interaction with many medications; some medications adhere to ng tubing

* Additional research on potential drug-nutrient interactions is needed, particularly among those with long-term continuous enteral nutrition support

Fleisher et al., J Parenter Enteral Nutr 1990;14:513-16
Wright et al., J Parenter Enteral Nutr 2000;24:42-8
Dickerson et al., Pharmacotherapy 2008;28:308-13
Which Product?
Questions

* Does food composition necessarily imply good bioavailability?
* Does food processing and storage always lead to decreased bioavailability?
* Is the bioavailability of innate nutrients in foods always better than that of the same nutrients used in dietary supplements?
* What “bioavailability” factors should be considered in determining DRIs?
* Should the nutrient facts panel indicate some form of a bioavailability index?
* What “messages” on bioavailability should be delivered to consumers?
Questions

* Does the specific diet form, macronutrient composition, or volume of a meal have an impact on bioavailability, either directly (in terms of chelation or other effects) or indirectly (in terms of changes in gastric pH)?
* Does the microflora/microbiome of the bowel have any impact on micronutrient availability? If so, what mechanisms might be involved?
* What are the mechanisms by which gastric bypass (bariatric) surgery have an impact on nutrient bioavailability?
* How might ongoing use of proton pump inhibitors (e.g., Prilosec, Prevacid, Nexium) or H2 blockers impact bioavailability?
* How may gastroparesis (gastric motility dysfunction) among diabetics affect nutrient bioavailability? Do prokinetic meds (5-HT4 promotility agents), such as metoclopramide or tegaserod (restricted usage), improve nutrient delivery?
**Summary**

* Defining nutrient bioavailability
  - Refers to the proportion of a nutrient that is absorbed from the diet and used for normal body functions.

* Components that affect nutrient bioavailability:
  - release of the nutrient from the physicochemical dietary matrix
  - effects of digestive enzymes in the intestine
  - binding and uptake by the intestinal mucosa
  - transfer across the gut wall to the blood or lymphatic circulation
  - systemic distribution
  - systemic deposition
  - metabolic and functional use
  - excretion