Mineral stable isotope studies in small children

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The role of stable isotope-based studies in evaluating mineral metabolism

- Three primary roles:
  - Assess bioavailability to develop nutritional guidelines - e.g. fortification plans
  - Evaluation of abnormalities in absorption and excretion due to disease conditions (e.g. rickets, Crohn's disease, JRA).
  - Determine physiological values, - body pool masses and turnover rates

Why stable isotopes?

What are the micronutrients?

- Five minerals typically evaluated: calcium, iron, zinc, magnesium and copper (also selenium, molybdenum)
- Vitamins A, B6, C, D, E, K also (deuterium, 13-C methods)
- Ease and range of science which can be performed is based on multiple factors
  - Number of naturally occurring isotopes
  - Presence of low-abundance isotopes
  - Cost of isotopes
  - Size of body pools and effects of age on this
  - Analytical capabilities
  - Rates of excretion and turnover of minerals

Principles of Use

- Occur naturally
- Nuclei do not decay
- Natural abundances known
- Can be purified for nutrition research
- Administer isotope with low natural abundance
- Use as "tracers"
- Measure levels in urine, blood, or feces
- Correct for amount expected to occur naturally

Minerals

- Calcium
  - 40Ca, 42Ca (0.65%), 43Ca, 44Ca (2.08%), 46Ca (0.003%), 48Ca
  - Give one isotope IV (e.g. 42Ca) and one p.o. (e.g. 46Ca OR 44Ca)
  - Collect complete 24-hour urine sample after dosing
  - Measure isotope ratios in urine sample (TIMS). Blood methods, single isotope methods also used, esp. in adults.
- Magnesium
  - 24Mg, 25Mg (10%), 26Mg (11%)
  - No low abundance isotopes makes clinical studies more difficult
  - Oral 26Mg and IV 25Mg. High doses (1 mg/kg orally) needed
  - Measure complete 72 hour urine (TIMS or ICP-MS).
Stable Isotopes Available

- **Iron**
  - 54Fe, 56Fe, 57Fe (2.1%), 58Fe (0.28%)
  - Usually administer maximum of two isotopes (57Fe and 58Fe)
  - Give oral isotopes on day-1 (with food) and 2 (ref dose)
  - Take blood sample on day-15 (some use day-28 in babies)
  - Measure isotope ratios in RBC (usually TIMS)
  - Use of IV dose can give direct absorption measurement.

- **Zinc**
  - 64Zn, 66Zn, 67Zn (4.1%), 68Zn, 70Zn (0.62%)
  - Can also use 68Zn (18%) in some circumstances as third isotope.
  - Give one isotope IV (70Zn) and one orally (67Zn). Oral isotope usually given with juice or with food.
  - Collect urine sample at least 72 hours later.
  - Measure isotope ratios in urine samples (ICP-MS, TIMS).

Basic principles of evaluation

- Methods must be safe and well-tolerated by small children.
- Methods generally must avoid fecal collections, multiple blood samples (>2).
- Multiple minerals should be assessed at the same time.
- Methods used should reasonably reflect usual dietary absorption.

Benefits to using stable isotopes to assess micronutrient status in children

- Safe for all populations
- Accurate measurement of fractional absorption without long-term dietary regulation. Easy to compare dietary sources or interventions.
- Assess absorption without fecal collections.
- Can perform multi-mineral studies.
- Obtain information about body pool masses and turnover rates
- Relatively field-friendly

What are their limitations or risks and how can they be minimized?

- Safety
  - I am unaware of any complications ever being reported to occur from the appropriate use of any mineral/stable isotope.
  - We have administered over 3000 intravenous zinc, magnesium, and calcium doses in the last 15 years without any incidents.
  - The use of intravenous iron is a special issue, but is probably safe if appropriate medical safeguards are in place.
  - Families should be aware of rare risks related to any intravenous infusion including bleeding, bruising, and infection.
  - A focused medical history and physical examination should be performed on every research subject to ensure the subject meets the study requirements and is otherwise healthy.

Ethical Considerations

- Studies generally have no direct benefit to healthy subjects
- Must ensure safety of isotopes and procedures used
  - Blood draws and urine/fecal collection done properly
  - Ensure isotopes adequately tested prior to use
  - Trained medical/nursing team
  - Appropriate skin preparation, phlebotomy technique, blood and urine handling.
- Should provide safe, fun and educational experience for children.
Toddlers: Who cares?
- Few nutritional data on children 12-48 mo.
- Most dietary recommendations either estimate upwards from infants or (usually) downward from adults or older children.
- Age of transitional diet. Adult dietary patterns not established. Inappropriate to use data from other ages.
- Mineral insufficiency pose unique risks.
- Rickets primarily reported in this age group.
- Iron deficiency may lead to lifelong learning problems if persists after age 2-3 yrs.
- Zinc may limit growth as iron supplementation is provided.
- Large gaps exist between usual intakes and recommended intakes. Usual intakes is higher than recommendations (unlike adults).

Why are there few data on toddlers?
- Near-impossibility of doing 5-7 day metabolic balances.
- Perception that slow growth and nutrition are not issues.
- Emphasis on preventing over-nutrition, not providing adequate nutrition.
- Iron is perceived as most important nutrient.
- Many years of experience with evaluation using biochemical testing.
- Diets not clearly defined - transition between infant diets and regular table food.
- Most data are from very high-risk populations, few data related to US or European groups.

What do toddlers (age 1-4 y) eat in the US?

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommended Intake</th>
<th>25th percentile</th>
<th>50th percentile</th>
<th>75th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, mg</td>
<td>500 (AI)</td>
<td>599</td>
<td>766</td>
<td>957</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>65 (EAR)</td>
<td>148</td>
<td>180</td>
<td>215</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>2.5 (EAR)</td>
<td>4.43</td>
<td>5.81</td>
<td>7.74</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>3 (EAR)</td>
<td>5.47</td>
<td>7.46</td>
<td>10.44</td>
</tr>
<tr>
<td>Copper, mcg</td>
<td>260 (EAR)</td>
<td>280</td>
<td>390</td>
<td>510</td>
</tr>
</tbody>
</table>

Note: UL for zinc is 7 mg in 1-4 y/o and 12 mg for 5-8 y/o

Median intakes for 1-3 year olds based on 1994 CSFII data

Research approaches: Overview
- Determine relationship between intake and mineral absorption over a range of typical intakes. Evaluate threshold levels, enhancers and inhibitors of absorption.
- Evaluate interrelationship of absorption and biochemical/hormonal status markers.
- Continue to develop and assess new status markers.
- Re-evaluate current dietary recommendations based on these data.
- Evaluate long-term consequences of interventions such as neuro-cognitive outcomes.
A study of toddlers and their mineral needs

- Healthy Children Ages 12-48 months
- No Chronic Illness
- No Medications
- Full Term, Birth Weight >2.5kg
- 5th to 95th percentile weight and height for age.

Dietary calcium sources

<table>
<thead>
<tr>
<th>Food</th>
<th>2-5y</th>
<th>6-11y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>52%</td>
<td>59%</td>
</tr>
<tr>
<td>Cheese</td>
<td>11%</td>
<td>11%</td>
</tr>
<tr>
<td>Yeast Bread</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>2.5%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Cakes/ cookies etc</td>
<td>1.7%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Pancakes/ waffles etc</td>
<td>1.8%</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

Subar, Pediatrics ’98; 102:913

Calcium: Fractional absorption decreases with increasing intake

Calcium: Total absorption increases with increasing intake

Conclusions: Calcium

- Absorption allows reasonably accurate calculation of retention, which in children is regulated primarily by dietary absorption.
- Tissue accretion requirement ~ 100-150 mg/d.
  - Need ~ 200 mg/d absorbed Ca.
  - This is achieved at ~ 450-500 mg/d intake
- Threshold is unclear - probably > 800 mg/d.
- Not much of any suggestion of a real intake problem in the US in this age group (<20% below 500 mg/d).
- For 4-8 y/o similar mean intakes (about 800 mg/d), but DRI AI in US is 800 mg/d.

Absorption and bone density in children

- In growing children, one can estimate rate of calcium retention over time using changes in whole body DEXA.
- Newer techniques including pQCT and bone u/s are not well established in this age group yet.
- Requires about 6 mo of intervention.
- Extremely difficult to isolate single food or nutrient effects.
- Relatively large sample size may be needed
- Combination of absorption and DEXA may be needed.
- Bone turnover markers unreliable in this age group.
**Conclusions: calcium**

- Isotope measures of absorption are useful if specific bioavailability or nutrient interaction (e.g. prebiotics) is of interest. Otherwise, may not be needed.
- Long-term studies can use DEXA as outcome.
- However, it will be difficult to find a benefit to a single supplement intervention.
- Other factors, such as season, race, gender, puberty, genetics will confound the analysis.
- Calcium is among the most-difficult minerals to assess as there is no useful biochemical measure in children.
- Small amount of fortificants/supplementation might be helpful, high doses of supplements not needed in children < 4 y. Supplements or fortificants may have more value for 4-6 y/o.

**Zinc intakes**

<table>
<thead>
<tr>
<th>Food</th>
<th>2-5y</th>
<th>6-11y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>17%</td>
<td>21%</td>
</tr>
<tr>
<td>Milk</td>
<td>21%</td>
<td>17%</td>
</tr>
<tr>
<td>Ready-to-eat cereal</td>
<td>11%</td>
<td>12%</td>
</tr>
<tr>
<td>Cheese</td>
<td>5.3%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Poultry</td>
<td>5.4%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Yeast Bread</td>
<td>5.0%</td>
<td>4.9%</td>
</tr>
</tbody>
</table>

Subar, Pediatrics '98; 102:913

**Zinc intakes in 1-4 y/o**

EAR for 4-8 y/o is 4 mg/d

**Relationship of intake and recommendations in small children**

**Zinc: Fractional absorption not related to intake**

**Zinc: Total absorption increases with increasing intake**

$R^2 = 0.67$
**Zinc**

- Zinc regulation is regulated primarily via secretory losses.
- EAR is 2.5 mg/d, 25th percentile intake is 4.4 mg and UL is 7 mg/d (~70% of usual intakes in US).
- ~1.2 mg absorbed Zn needed to provide 0.14 mg/d retention based on DRI estimates of urinary/secretory losses.
- Intake of 2.5 mg leads to ~0.9 mg/d absorbed iron. Zinc intake of ~4.0 mg/d provides the needed 1.2 mg of absorbed zinc.
- Thus, current EAR is probably too low.
- Likely that UL is too low also - but need more nutrient interaction data to evaluate this.
- In the US however, not much evidence of severe zinc deficiency and more concern, perhaps misplaced about zinc toxicity. Small amounts of fortification reasonable but UL should be changed for children < 4 y/o!

**Dietary iron requirements in children**

<table>
<thead>
<tr>
<th>Life Stage Group</th>
<th>EAR (mg/d)</th>
<th>RDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0 through 6mo</td>
<td>0.27 (AI)</td>
<td>0.27 (AI)</td>
</tr>
<tr>
<td>7 through 12 mo</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>1 through 3y</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4 through 8y</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>9 through 13y</td>
<td>5.9</td>
<td>5.7</td>
</tr>
<tr>
<td>14 through 18y</td>
<td>7.7</td>
<td>7.9</td>
</tr>
</tbody>
</table>

**Iron intakes**

Iron: Fractional absorption not related to intake

- R² = 0.038

Iron: Total absorption not related to intake

- R² = 0.02

Iron: Fractional absorption increases with low ferritin

- R² = 0.26, p = 0.003
**Intake or Status: What matters in determining dietary iron absorption?**

- **Dependent variable:** Ln% iron absorption
- **Independent variables:** Fe intake, Ln Ferritin
- No significant effects of gender, ethnicity or age

<table>
<thead>
<tr>
<th>Variable</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron intake</td>
<td>-1.2</td>
<td>0.23</td>
</tr>
<tr>
<td>Ln Ferritin</td>
<td>-3.6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Conclusions: iron in toddlers**

- Fe fractional absorption is regulated by iron status and not closely related to short-term intake.
- Multiple factors affect iron status in addition to intake. Our data do not take into account heme/non-heme iron.
- Absorbed iron requirement is ~ 0.6 mg/d for tissue accretion.
- Current EAR of 3 mg is probably adequate to achieve this. Other factors are clearly more important and we do not have data surrounding the EAR to evaluate this well. 25%ile in US of 5.5 mg intake leaves large error margin.
- No reason to change EAR or target higher values from these data, but no suggestion of harm from usual intakes. UL of 40 mg/d not approached from diet.

**Bioavailability of a multinutrient fortified beverage**

Instituto de Investigación Nutricional (Lima, Perú)
Baylor College of Medicine

**Product nutrient concentration**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Ingredient</th>
<th>Level/Serving</th>
<th>Amount</th>
<th>% Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>β-Carotene</td>
<td>2400 µg</td>
<td>600 µg</td>
<td>31%</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>Riboflavin</td>
<td>0.40 mg</td>
<td>1.9 mg</td>
<td>44%</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>Niacinamide</td>
<td>2.7 mg</td>
<td>12 mg</td>
<td>50%</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Pyridoxine HCl</td>
<td>0.5 mg</td>
<td>1.0 mg</td>
<td>50%</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>Folic Acid</td>
<td>140 µg</td>
<td>300 µg</td>
<td>47%</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Cyanocobalamin</td>
<td>1.3 µg</td>
<td>1.8 µg</td>
<td>55%</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbic Acid</td>
<td>60 mg</td>
<td>45 mg</td>
<td>130%</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>dl-α-Tocoph. Ac.</td>
<td>7.5 mg</td>
<td>11 mg</td>
<td>31%</td>
</tr>
<tr>
<td>Calcium</td>
<td>Tricalcium Phos</td>
<td>120 mg</td>
<td>1.000 mg</td>
<td>9%</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe Bisglyc. Chelate</td>
<td>7.0 mg</td>
<td>8 mg</td>
<td>80%</td>
</tr>
<tr>
<td>Iodine</td>
<td>Potassium Iodide</td>
<td>60 µg</td>
<td>120 µg</td>
<td>30%</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc Gluconate</td>
<td>3.75 mg</td>
<td>8 mg</td>
<td>47%</td>
</tr>
</tbody>
</table>

**Introduction**

- School-aged children received: Fortified beverage + meal
- n=40
- One drink daily of fortified beverage for 4 weeks
- Assess
  - Iron and zinc absorption with and without a meal

**Entry Criteria**

- Generally healthy
- No acute infections
- No chronic medications
- Age 6-10 y
- All students were from a single school in the Villa el Salvador area of Lima, Peru
Study Design

- Day 15 & 16: Iron and zinc doses administered
- Days 18-20: Urine collected for zinc absorption

Iron and zinc absorption

- Day 15
  - Given 2mg 68Zn (IV)
  - Subjects randomized in 2 groups to receive drink labeled with 58Fe/70Zn or 57Fe/67Zn
  - 58Fe/70Zn drink given with a meal; 57Fe/67Zn drink given without a meal
- Day 16
  - Groups received labeled drink that was not received on Day#15

Results: Iron Absorption Analysis

- Mean Iron absorption:
  - With meal: 9.8 ± 6.7%
  - Without meal: 11.6 ± 6.9%
- Iron absorption with/ without meal
  - With meal: Geometric mean = 7.2%
  - Without meal: Geometric mean = 9.8%
  - P-value = 0.08

Results: Zinc Absorption

- With meal:
  - 24 ± 11%
- Without meal: 23 ± 8%
- P value = N.S.

Iron requirement: Proportion met by fortified beverage

- Mean absorbed iron requirements for this age range is 0.6 to 0.8 mg/day
- Total iron absorption: 0.7 mg/day

Our group