Mineral stable isotope studies in small children

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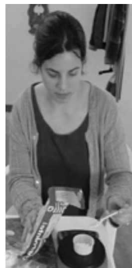
## Why stable isotopes?

## 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



## 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).

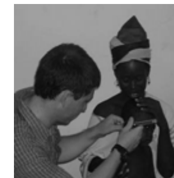
## Basic principles of evaluation

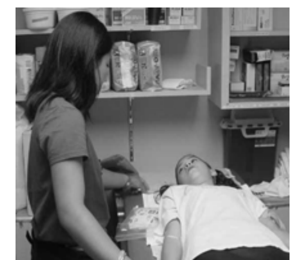
- Methods must be safe and well-tolerated by small children.
- Methods generally must avoid fecal collections, multiple blood samples (>2).
- Multiple minerals should be assessed at the same time.
- Methods used should reasonably reflect usual dietary absorption.

## 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.

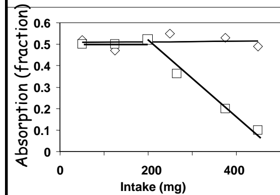
# What do toddlers (age 1-4 y) eat in the US?

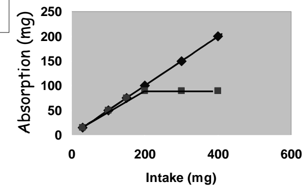
| *Nutrient* | *Recommended intake* | *25th percentile* | *50th percentile* | *75th percentile* |
|---|---|---|---|---|
| Calcium, mg | 500 (AI) | 599 | 766 | 957 |
| Magnesium, mg | 65 (EAR) | 148 | 180 | 215 |
| Zinc, mg | 2.5 (EAR) | 4.43 | 5.81 | 7.74 |
| Iron, mg | 3 (EAR) | 5.47 | 7.46 | 10.44 |
| Copper, mcg | 260 (EAR) | 280 | 390 | 510 |

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

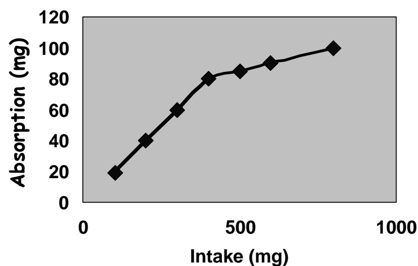
Median intakes for 1 – 3 year olds based on 1994 CSFII data

# Relationships between intake and absorption




# In real world, thresholds for minerals are unlikely to be absolute




# 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

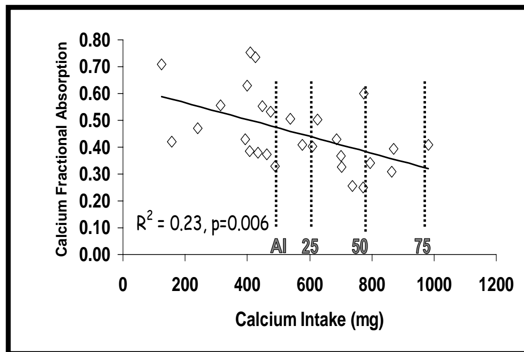
- Healthy Children Ages 12-48 months
- No Chronic Illness
- No Medications
- Full Term, Birth Weight >2.5kg
- $5^{th}$ to $95^{th}$ percentile weight and height for age.

## Dietary calcium sources

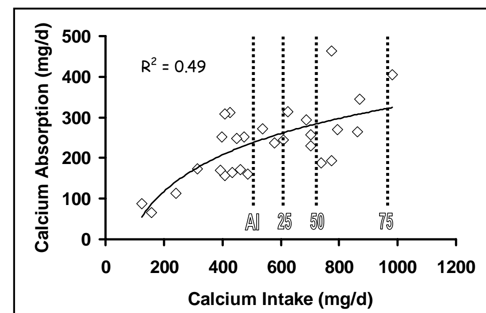
| Food | 2-5y | 6-11y |
|---|---|---|
| Milk | 52% | 59% |
| Cheese | 11% | 11% |
| Yeast Bread | 6% | 7% |
| Ice Cream | 2.5% | 2.9% |
| Cakes/ cookies etc | 1.7% | 2.2% |
| Pancakes/ waffles etc | 1.8% | 2.0% |

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

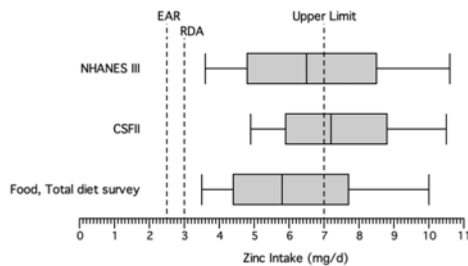
- Isotope measures of absorption are useful if specific bioavailability or nutrient interaction (e.g. prebiotics) is of interest. Otherwise, may not be needed.
- Long-term studies can use DEXA as outcome.
- However, it will be difficult to find a benefit to a single supplement intervention.
  - Other 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

| Food | 2-5y | 6-11y |
|---|---|---|
| Beef | 17% | 21% |
| Milk | 21% | 17% |
| Ready-to-eat cereal | 11% | 12% |
| Cheese | 5.3% | 5.5% |
| Poultry | 5.4% | 4.6% |
| Yeast Bread | 5.0% | 4.9% |

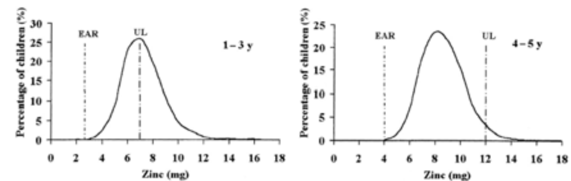
Subar, Pediatrics '98; 102:913

## Zinc intakes in 1-4 y/o

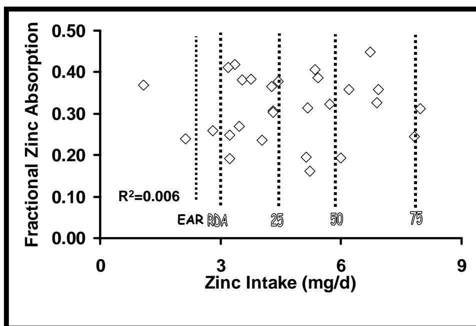



EAR for 4-8 y/o is 4 mg/d

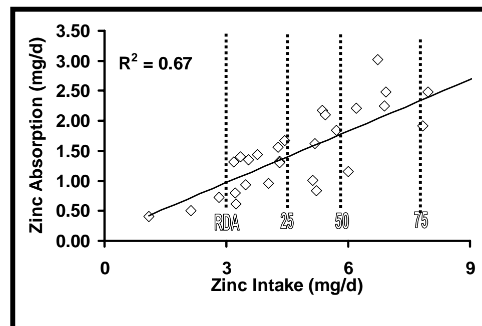
## Relationship of intake and recommendations in small children




## Zinc: Fractional absorption not related to intake




## Zinc: Total absorption increases with increasing intake



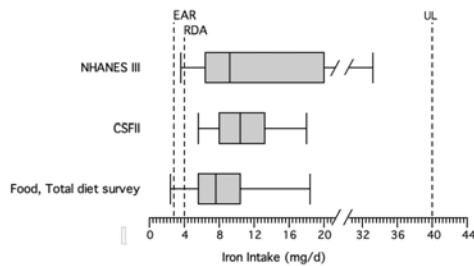
## Zinc

- Zinc regulation is regulated primarily via secretory losses.
- EAR is 2.5 mg/d, 25%ile intake is 4.4 mg and UL is 7 mg/d (~70% ile 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

| Life Stage Group | EAR (mg/d) | | RDA | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 0 through 6mo | 0.27 (AI) | 0.27 (AI) | N/A | N/A |
| 7 through 12 mo | 6.9 | 6.9 | 11 | 11 |
| 1 through 3y | 3.0 | 3.0 | 7 | 7 |
| 4 through 8y | 4.1 | 4.1 | 10 | 10 |
| 9 through 13y | 5.9 | 5.7 | 8 | 8 |
| 14 through 18y | 7.7 | 7.9 | 11 | 15 |

## Iron intakes

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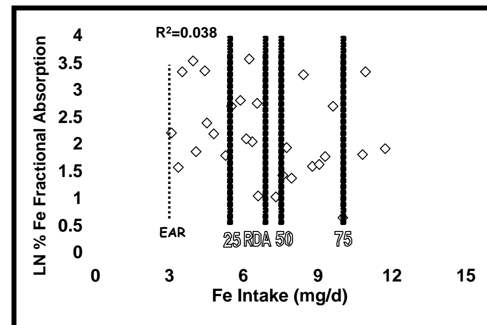
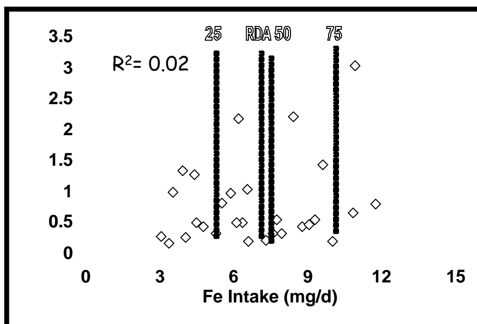
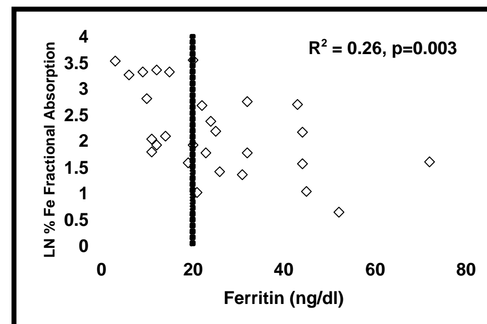



## Iron: Total absorption not related to intake




## Iron: Fractional absorption increases with low ferritin



## 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

| Variable | T-value | P-value |
|---|---|---|
| Iron intake | -1.2 | 0.23 |
| Ln Ferritin | -3.6 | 0.001 |

## 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

| Nutrient | Ingredient | Level/Serving | Children 9-13 Yrs. - U.S. RDA | |
|---|---|---|---|---|
| | | | Amount | % Level |
| Vitamin A | β-Carotene | 2400 μg | 600 μg retinol | 33% |
| Vitamin $B_2$ | Riboflavin | 0.40 mg | 0.9 mg | 44% |
| Vitamin $B_3$ | Niacinamide | 2.7 mg | 12 mg | 23% |
| Vitamin $B_6$ | Pyridoxine HCl | 0.5 mg | 1.0 mg | 50% |
| Folic Acid | Folic Acid | 140 μg | 300 μg | 47% |
| Vitamin $B_{12}$ | Cyanocobalamin | 1.0μg | 1.8 μg | 55% |
| Vitamin C | Ascorbic Acid | 60 mg | 45 mg | 133% |
| Vitamin E | dl-α-Tocoph. Ac. | 7.5 mg | 11 mg d-α-Tocoph. | 31% |
| Calcium | Tricalcium Phos | 120 mg | 1,300 mg | 9% |
| **Iron** | **Fe Bisglyc. Chelate** | **7.0 mg** | **8 mg** | **88%** |
| Iodine | Potassium Iodide | 60 μg | 120 μg | 50% |
| **Zinc** | **Zinc Gluconate** | **3.75 mg** | **8 mg** | **47%** |

## 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 1: Anthropometric measurements. Baseline labs. Daily 240 ml of fortified drink started.
- Day 15 & 16: Iron and zinc doses administered
- Days 18-20: Urine collected for zinc absorption
- Day 30: Anthropometric measurements. Endpoint labs including blood for isotope ratios.

## 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