Soy ferritin: implications for iron status of vegetarians

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Dietary iron deficiency and anemia afflict ~1.5 billion people worldwide

- **Sources of dietary iron**
  - **Heme-Fe**
    - Metalloporphyrin ring complex
    - Meat products
  - **Non-heme Fe**
    - Iron (Fe$^{+2}$ and Fe$^{+3}$) complexes
    - Lactoferrin, Transferrin
    - Ferritin
Strategies to improve Fe nutrition

• Fe supplementation
• Fe fortification
• Reduction of inhibitors of Fe absorption
• Modified dietary/food preparation practices
• Biofortification
Biofortification

- Development of plant varieties with higher contents of Fe, Zn, Se, vitamin A, carotenoids, etc.

- Sustainable
“Genetic modification”

- Conventional breeding/selection methods
  (Fe & Zn +50-100% ; Se +++ )
- Genetic engineering
  a) insertion of novel genes
  b) enhancing expression of genes
  c) depressing expression of genes, disrupting synthetic pathways, etc.
Candidate proteins for Fe biofortification

- Lactoferrin - 2 Fe atoms/mol (=1.5 mg Fe/g Lf)
- Ferritin ~4,500 Fe atoms/mol theoretically;
  500-1,000 atoms/mol practically (?)
  (=65-130 mg Fe/g Ft)
Ferritin (Ft)

- ~ 480 kDa
- Iron storage protein found in plants and animals
- 24 subunits of two types: H (heavy) & L (light)

- Apo-protein shell coordinates up to 4500 Fe atoms within its core
Earlier human studies on Fe absorption from ferritin show different results

• Some studies suggest that Fe is well absorbed from ferritin, others that Fe bioavailability is low

• Issues: extrinsic vs intrinsic labeling but, more importantly, *type* of extrinsic and intrinsic labeling
Type of extrinsic and intrinsic labeling

- **Extrinsic**: adding isotope to ferritin vs removing Fe from ferritin and adding Fe back w/ isotope

- **Intrinsic**: “conventional” intrinsic labeling (low incorporation into ferritin) vs “inflammatory response” ferritin
Iron absorption from soybeans in women with low Fe stores (Murray-Kolb et al., 2003)

- **Design:** Women with marginal Fe deficiency
  - Intrinsically labeled ($^{55}\text{Fe}$) soybeans
  - RBC incorporation
  - Reference dose ($^{59}\text{Fe}$)

- **Results:** Fe absorption from meal was 27%
  - "ref dose was 61%"

- **Conclusion:** Soybean Fe (49% in ferritin) is a good source of nutritional Fe
Iron absorption from ferritin and FeSO₄ in non-anemic women (Davila-Hicks et al., 2004)

• **Design**: Women with marginal Fe deficiency
  Extrinsically labeled (⁵⁹Fe) horse spleen ferritin
  (after Fe removal and addition of ⁵⁹Fe/Fe)
  Whole body counting + RBC incorporation
  ⁵⁹Fe-labeled Ft or FeSO₄ given in meal
  using cross-over design

• **Validity of labeling**: Reconstituted ferritin identical to native ferritin as determined by EXAFS and Mössbauer spectroscopy (E. Theil)
Fe absorption from animal-type and plant-type ferritin measured by whole body counting

Type of ferritin mineral does not affect absorption of Fe
Iron absorption from soybean ferritin and FeSO₄ in non-anemic women (Lönnerdal et al., 2006)

• Design: Women with marginal Fe deficiency
  Extrinsically labeled (⁵⁹Fe) purified soybean ferritin
  (after Fe removal and addition of ⁵⁹Fe/Fe)
  Whole body counting + RBC incorporation
  ⁵⁹Fe-labeled Ft or FeSO₄ given in meal
  using cross-over design

• Validity of labeling: Reconstituted ferritin identical to native ferritin as determined by EXAFS and Mössbauer spectroscopy (E. Theil)
Fe absorption from soybean ferritin and FeSO₄ in young women

- There was no significant difference in Fe absorption between groups
Potential mechanisms for Fe uptake from ferritin

- Release of Fe and absorption as “free” Fe via divalent metal transporter-1 (DMT-1)

- Uptake of Fe bound to ferritin (or ferritin subunits) via a receptor-mediated mechanism
Effect of dietary factors on Fe uptake from intact ferritin

- Tannic acid increased Fe uptake from ferritin, whereas ascorbic acid had no effect
In vitro digestion of ferritin

• Pepsin digestion at pH 2 (normal digestion) or at pH 4 (compromised digestion) e.g. young children, ferritin in plastids, etc.

• Pancreatin digestion after neutralization with bicarbonate to pH 7
Effect of dietary factors on Fe uptake from ferritin digested at pH 4 or pH 2

[Graphs showing the effect of dietary factors on Fe uptake from ferritin digested at pH 4 and pH 2]
Stability of ferritin against digestion

SDS-PAGE of human albumin (lanes 2-4, positive control), human lactoferrin (lane 8-10, negative control) and ferritin (lanes 5-7)
Uptake of ferritin-Fe by Caco-2 cells

![Graph showing ferritin uptake by Caco-2 cells](image-url)
Binding of ferritin to Caco-2 cells

- Binding of ferritin was saturable, indicative of a receptor-mediated process.
Effect of inhibiting endocytosis on ferritin uptake by Caco-2 cells

- Inhibition of endocytosis by sucrose effectively blocked ferritin uptake, but had no effect on ferrous iron uptake
Fe is well utilized from ferritin

- What can be done to optimize the amount of bioavailable Fe provided as phytoferritin?

  1) plant breeding/selection

  2) genetic engineering
Ferritin content of legumes and grains

- Present in soybeans, peas, lentils, beans, corn

- Reported contents:
  50-80 mg/kg (Laulhere et al., 1987)
  800-1,000 Fe atoms/mol

  assuming 50 g beans in a composite meal -
  this corresponds to 0.5 mg Fe

  if plant-breeding could increase Ft content by 100-200 % (?)

  this would still only provide 1-1.5 mg Fe
Increasing the trace element content of plants

• Insertion of genes for novel trace element containing proteins
• Over-expression of “native” genes for trace element storage proteins
• Over-expression of transporters that facilitate plant uptake of trace elements
• Increased fertilization with trace elements
The potential benefits of genetic engineering should be explored…

…but safety issues and economic/political implications should also be carefully addressed.
Expression of proteins that facilitate trace element uptake

- Lactoferrin
- Ferritin
Insertion of novel metal-binding proteins

Expression of human lactoferrin in rice

• “Gene-gun” technology

• Glutellin promoter: endosperm-specific expression

• High level of expression (5g/kg; 6% of total protein)

• Each molecule of lactoferrin binds 2 Fe\(^{3+}\)
Iron content of transgenic rice expressing human lactoferrin
Insertion of novel metal ion storage-proteins

Expression of ferritin in rice

- Soybean ferritin in rice (Goto et al. 2000)
- *Phaseolus vulgaris* ferritin in rice (Lucca et al. 2001)

*Agrobacterium*-mediated transformation, glutellin promoter
Iron content of transgenic rice expressing *Phaseolus* ferritin

![Iron Content Graph]

- **Iron Content**
- **Rice lines**

Lucca et al., 2001
How much Fe could be provided by ferritin if expression is maximized?

- Native content in plants: 50-80 mg/kg corresponds to ~ 0.5 mg Fe/ 50 g beans

- High protein expression in rice (promoter-driven): 5 g/ kg rice (i.e. 100x higher) corresponds to ~ 50 mg Fe/ 50 g beans

- Can the plant transport this much Fe into seeds?
Enhanced expression of proteins facilitating uptake of trace elements from soil

- Leghemoglobin (nodules)
- Ferric reductase
  - Phloem-mobile Fe-chelator
  - Targeting to source region
  - Increased phytoferritin, heme-containing enzymes, peptide-chelates
Increasing ferritin expression

• Will consumer accept:
  
  pink rice?
  red rice?
  brown rice?

Maybe beans, lentils will “hide” color better?

Taste?
Phytoferritin

- Bioavailable form of Fe
- Potential for increased contents in legumes and cereals
- Long-term effects of increased phytoferritin consumption on prevention of Fe deficiency?
- Combine with phytate reduction or phytase co-expression?
Acknowledgments

- Annika Bryant
- Penni Davila-Hicks
- Swati Kalgoankar
- Dr. Shannon Kelleher
- Dr. Xiaofeng Liu
- Dr. Elizabeth Theil
- NIH grant HL56169