Calcein Quenching In Vitro Method for Assessing Dietary Iron Bioavailability

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Objective & Outcome

To develop a reliable, faster, and cost effective method to assess food iron bioavailability

Outcome:

<table>
<thead>
<tr>
<th>Method</th>
<th>Time (hours)</th>
<th>Material cost (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widely used ferritin method</td>
<td>~27</td>
<td>~150</td>
</tr>
<tr>
<td>New method</td>
<td>~2 😊</td>
<td>~15 😊</td>
</tr>
</tbody>
</table>
Iron deficiency is a major nutritional problem (1).

Assessing iron bioavailability (IBA) in complex meals is difficult.

Caco-2 cells are widely used to assess food IBA (2).

Limitations of old ferritin method: non-specific response, long duration, high expense (3).

Not available: in vitro methods that are easy to perform and predict human absorption accurately.
Method: Meal Composition

Semi-purified meal based on earlier human studies (4-6).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (egg white powder), $g$</td>
<td>3.8</td>
</tr>
<tr>
<td>Carbohydrate (dextrimaltose), $g$</td>
<td>8.4</td>
</tr>
<tr>
<td>Fat (corn oil), $g$</td>
<td>4.4</td>
</tr>
<tr>
<td>Water, $mL$</td>
<td>45</td>
</tr>
<tr>
<td>Iron, $\mu g$</td>
<td>510</td>
</tr>
</tbody>
</table>

Control meal had no test factor added, but the test meals contained Ascorbic acid / Beef / Bran / Phytate / Tea.
Method: In vitro digestion

Mimic digestion in human gastrointestinal tract:

Samples $\rightarrow$ HCl $\rightarrow$ pH 2 (gastric) $\rightarrow$ Pepsin $\rightarrow$ 1 hr, 37°C Incubation $\rightarrow$ NaHCO$_3$ $\rightarrow$ pH 6 (duodenal) $\rightarrow$ Bile salts & Pancreatic enzymes

Centrifugation $\rightarrow$ Enzyme Inactivation $\rightarrow$ Ferritin method (3) $\rightarrow$ Calcein method

Supernatant $\rightarrow$ Caco-2 cell uptake
Method: Calcein Method

- Calcein AM (CAM) is a nonfluorescent cell-permeant dye
- CAM → green-fluorescent calcein
- Calcein fluorescence: quenched by iron
Calculation

- Quenching % = \((I-F)/I\) x 100

- The amount of bioavailable iron that enters into the cell is proportional to the degree of quenching.

- Higher quenching \(\rightarrow\) higher iron absorbed \(\rightarrow\) higher IBA
Results

- **Ascorbic acid** and **beef** enhanced IBA by ~8-fold.
- IBA was decreased by 66% with **bran** and completely inhibited by **phytate** and **tea**.
Comparison to old method

Correlation between ferritin and calcein quenching data

\[ R^2 = 0.99; \ p < 0.005 \]

# Normalized to control meal
Comparison to human data

Correlation between human absorption* and calcein quenching data

$R^2 = 0.81; p < 0.05$

* (4-6)

# Normalized to control meal
Summary / Conclusions

- IBA assessed with calcein method showed high correlation with IBA measured with standard ferritin method ($R^2=0.99; p<0.005$) as well as with published human absorption data (4-6) ($R^2=0.81; p<0.05$).

- Calcein method: reliably measure IBA
  - more cost effective
  - faster

- Can be useful as a high throughput method to screen a large number of samples.
1. World Health Organization (WHO).
Thank you!

Questions?