Effect of Orange and Apple Juices on Iron Absorption in Children

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Objective: To measure iron absorption in children from meals containing apple juice or orange juice so as to determine if iron absorption will be greater with orange juice because of its higher ascorbic acid content than apple juice, a noncitrus fruit juice that US children reportedly prefer.

Design: On 2 successive days, children consumed identical meals that included apple juice on one day and orange juice on the other, in random order. The meals were labeled with iron-57 on one day and iron-58 on the other. Iron absorption was measured from red blood cell incorporation of the iron stable isotopes 14 days later.

Setting: Nutrition research institute in a major metropolitan medical center.

Patients: A total of 25 healthy children, 3 to 6 years of age, were recruited, of whom 21 (11 male and 10 female) completed the study.

Intervention: Identical meals served with orange juice and apple juice were given on consecutive days, in a balanced randomized design.

Main Outcome Measures: Iron absorption measured by established stable isotope methods.

Results: Median iron absorption from the meal ingested with apple juice was 7.17% (mean±SD, 9.48%±9.68%). Median iron absorption from the meal ingested with orange juice was 7.78% (9.80%±6.66%; P=.44). Iron absorption from the meal that included apple juice was significantly correlated with serum ferritin concentration (P=.02); iron absorption from the meal that included orange juice tended to correlate with serum transferrin receptor concentration (P=.051).

Conclusions: As children absorb iron well from a meal that includes either orange or apple juice, a preference for apple juice does not pose a concern with regard to the prospect of iron-deficiency anemia, which remains a significant health problem in the United States.

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Iron-deficiency anemia remains the most prevalent nutritional deficiency in the world and affects more than 2 billion people globally. In the United States, it is estimated that 8% of children aged 1 to 5 years are iron deficient. Iron-deficiency anemia has been associated with lower scores on tests of mental and motor functioning in toddlers and with worse school performance in adolescents. In humans, iron absorption can be greatly influenced by the presence of enhancers and inhibitors of iron absorption in the rest of the diet. Ascorbic acid can significantly increase iron absorption, while phytate and calcium may inhibit it.

As children mature, their intake of foods and fruit juices becomes more diversified. A recent US Department of Agriculture study found that children are more likely to consume apple juice than orange juice. Apple juice contains far less ascorbic acid than orange juice, so this raises the question of whether such a preference adversely affects iron absorption and iron nutrition. Previous studies in children have suggested that ascorbic acid can increase iron absorption from a school breakfast in Peruvian children and from a chocolate-flavored milk drink in Jamaican children. However, apple and orange juices are complex beverages that differ in many ways. Substitution of one for the other does not, therefore, change solely ascorbic acid intake. Although one study in adults has suggested that iron absorption is lower when apple juice, vs orange juice, accompanies a meal, there are few data available on this important nutrition issue in children.
In this study, we compared the effect of apple juice, vs that of orange juice, on iron absorption in children consuming a meal. As apple juice naturally contains less ascorbic acid, we hypothesized that iron absorption would be higher with orange juice.

METHODS

STUDY POPULATION

Twenty-five children, 3 to 6 years of age, were recruited from the greater Houston, Tex, area by public advertisement. A total of 21 children completed the study (11 male and 10 female). Children were considered eligible if they were 3 to 6 years of age, between the 5th and 95th weight-for-height percentiles, had no underlying medical problems, took no medications or vitamin supplements, and would drink both apple juice and orange juice. The Baylor College of Medicine Institutional Review Board approved the protocol, and informed written consent was obtained from the parent or guardian of each child before enrollment.

STUDY DESIGN

On the morning of the study, fasted subjects were admitted to the Metabolic Research Unit of the Children’s Nutrition Research Center in Houston. Height and weight were measured by standard clinical methods. They were given a meal of toast with jelly or butter, a choice of a noncitrus fruit, and 60 mL of low-pulp, non–calcium-fortified orange juice (Tropicana Products, Inc, Bradenton, Fla; ascorbic acid content, 39 mg/100 mL) or apple juice (Tree Top, Inc, Selah, Wash; ascorbic acid content, 1 mg/100 mL), to which 5 mg of aqueous ferrous sulfate, as described previously. Stable isotopes were used. Stable isotopes are naturally occurring, nonradioactive isotopes that can be used safely in any patient population.

ISOTOPE PREPARATION

Iron-57 (95.82 atom percent) and iron-56 (93.13 atom percent) were purchased (Trace Sciences International Corp, Richmond Hill, Ontario) and converted to aqueous solutions of ferrous sulfate, as described previously. The fruit juices were labeled with 5 mg of the iron-57-enriched tracer or 1.5 mg of the iron-56-enriched tracer. In the latter case, 3.5 mg of nonenriched ferrous sulfate was added to ensure that the amount of ferrous sulfate added to the 2 juices was the same. The isotopes were drawn up into preweighed syringes and added to the juices 18 to 24 hours before use. The exact amount of isotope given was measured by the change in weight of the syringe.

SAMPLE PREPARATION

Blood samples were collected by venipuncture into an EDTA-anticoagulated tube and a plain tube (no anticoagulant). A portion of the EDTA-anticoagulated sample was used to measure a complete blood cell count. The remainder was separated by centrifugation and prepared for iron isotope ratio analysis as previously described. Iron isotope ratios were measured by thermal ionization magnetic sector mass spectrometry (Finnigan MAT 261; Thermo Finnigan, Bremen, Germany). Data were expressed as iron-56/iron-54 (86Fe) and 58Fe/56Fe ratios and corrected for differences in fractionation by means of the ratio of the 2 nonadministered isotopes, iron-54 (86Fe) and 56Fe. Three blocks of 10 scans each were made until the desired degree of precision was obtained (typically relative SD < 0.2%). This method is similar to that used for reference analysis and is capable of high precision and accuracy for iron isotope ratio measurement.

Serum was separated from the plain tube by centrifugation and stored at −20°C pending analysis. Serum ferritin concentration was measured with a solid-phase, 2-site fluoroimmunometric assay (DELFIA method; PerkinElmer Inc, Boston, Mass), and soluble serum transferrin levels were measured with an enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis, Minn).

CALCULATIONS

Iron isotope ratios were converted to tracer-tracee ratios (TTR) as described previously. Iron incorporation into red blood cells was calculated from the following equations:

\[
\text{57Fe Incorporation} = 100\% \times \left( \frac{[\text{57Fe TTR}] \times [\text{Fe}_{\text{circ}}]}{\text{Dose of 57Fe-Enriched Tracer}} \right)
\]

and

\[
\text{58Fe Incorporation} = 100\% \times \left( \frac{[\text{58Fe TTR}] \times [\text{Fe}_{\text{circ}}]}{\text{Dose of 58Fe-Enriched Tracer}} \right)
\]

where Fe_{circ} is the total amount of circulation hemoglobin iron, and is determined by the following formula:

\[
\text{Fe}_{\text{circ}} \text{ (in milligrams)} = \text{Blood Volume} \times \text{Hemoglobin Concentration} \times \text{Body Weight} \times 3.47
\]

where Blood Volume is 65 mL/kg.

Iron incorporation was converted to fractional iron absorption on the basis of the assumption that 80% of absorbed iron was incorporated into red blood cells within 14 days; therefore,

\[
\text{Iron Absorption} = \text{Iron Incorporation} / 0.8
\]

STATISTICAL ANALYSIS

The difference between iron absorption from the meal containing apple juice and that from the meal containing orange juice was examined by means of a paired t test. Serum ferritin concentrations were log-transformed (to the base 10) to normalize the distribution. Statistical analysis was carried out with StatView version 5.0.1 for Macintosh (SAS Institute Inc, Cary,
Table 1. Demographic Data for the 21 Subjects Who Completed the Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Finding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. M/F</td>
<td>11/10</td>
</tr>
<tr>
<td>Ethnicity, No.</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>14</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5</td>
</tr>
<tr>
<td>African American</td>
<td>2</td>
</tr>
<tr>
<td>Age, y</td>
<td>4.47 ± 0.88 (3.08-5.89)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>16.66 ± 1.48 (13.3-19.7)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>104.5 ± 5.1 (97.2-114.8)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36.5 ± 2 (33.4-40.4)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>12.2 ± 0.5 (11.3-13.5)</td>
</tr>
<tr>
<td>Serum ferritin, ng/mL</td>
<td>27.7 ± 15.5 (8.1-58.3)</td>
</tr>
<tr>
<td>Serum transferrin receptor, mg/L</td>
<td>6.5 ± 1.1 (4.8-8.5)</td>
</tr>
</tbody>
</table>

SI conversion factor: To convert serum ferritin to picomoles per liter, multiply by 2.247.

*Values are mean ± SD (range) where applicable.

Table 2. Nutritional Intake From the 2 Study Meals*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Orange Juice Meal</th>
<th>Apple Juice Meal</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>168 ± 50</td>
<td>177 ± 54</td>
<td>.04</td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.2 ± 1.8</td>
<td>3.3 ± 1.8</td>
<td>.65</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>33.2 ± 11.2</td>
<td>35.8 ± 12.2</td>
<td>.003</td>
</tr>
<tr>
<td>Protein, g</td>
<td>2.5 ± 0.7</td>
<td>2.1 ± 0.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>35 ± 8</td>
<td>32 ± 8</td>
<td>.88</td>
</tr>
<tr>
<td>Phosphate, mg</td>
<td>39 ± 11</td>
<td>34 ± 11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Iron, mg†</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>.18</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>0.25 ± 0.08</td>
<td>0.21 ± 0.08</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Copper, mg</td>
<td>0.13 ± 0.04</td>
<td>0.09 ± 0.04</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Phytate, mg</td>
<td>7.8 ± 2.3</td>
<td>8.0 ± 2.1</td>
<td>.43</td>
</tr>
<tr>
<td>Ascorbic acid, mg</td>
<td>35.9 ± 9.6</td>
<td>4.5 ± 3.7</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Data are mean ± SD.
†Excluding the iron tracer added to the meal.

NC). Results were considered significant at P = .05. Power calculations were carried out using DSTPLAN version 4.2 (University of Texas M. D. Anderson Cancer Center, Houston). Data for iron absorption are given as both the median and the mean ± SD. The effect of iron status on iron absorption was assessed by simple regression analysis. The proxy markers of iron status used were the hemoglobin concentration, serum ferritin concentration (before and after log transformation), the serum transferrin receptor concentration, and the serum ferritin-transferrin receptor ratio.

POWER CALCULATION

We expected an iron absorption of approximately 8% (SD, 4%). Assuming the smallest clinically significant decrease in iron absorption to be 3%, a sample of 20 was required to have an 80% power (β = .20) to detect such a difference at a P < .05 (α = .05). To allow for subject attrition, 25 subjects were recruited.

RESULTS

Twenty-five subjects were recruited, of whom 21 completed the study. An insufficient amount of blood was obtained from 2 subjects on day 14 for analysis. Another 2 subjects did not return for the day 14 visit. Demo-

graphic data representing the 21 subjects who completed the study are shown in Table 1. One subject was mildly anemic (hematocrit, 33.4%; hemoglobin level, 11.3 g/dL) and had unexpectedly high iron absorption. Results are expressed, and statistical analyses were carried out, both with and without this subject. The composition of the 2 meals, excluding the juices, was similar. However, when the juices were included, differences in ascorbic acid content and in several other nutrients were seen (Table 2).

IRON ABSORPTION

Median iron absorption from the meal with apple juice was 7.17% (mean ± SD, 9.48% ± 9.68%). Median iron absorption from the meal with orange juice was 7.78% (9.80% ± 6.66%) and was not statistically different (P = .44 after log transformation; 95% confidence interval for difference, −1.54% to 3.50%). When the single outlier was excluded, results were similar, with a median iron absorption of 6.65% (7.63% ± 4.76%) from the meal with apple juice and 7.55% (9.82% ± 6.84%) from the meal with orange juice (P = .19 after log transformation; confidence interval for difference, −3.92% to 3.29%).

EFFECT OF HEMOGLOBIN, SERUM FERRITIN, AND SERUM TRANSFERRIN RECEPTORS ON IRON ABSORPTION

Neither iron absorption from apple juice (P = .50) nor that from orange juice (P = .82) was correlated with hemoglobin concentration. Iron absorption from apple juice was significantly negatively correlated with serum ferritin level (P = .02), log serum ferritin (P = .01), and the ferritin–transferrin receptor ratio (P = .02), but was not correlated with the serum transferrin receptor concentration (P = .99). In contrast, iron absorption from orange juice tended to increase as serum transferrin receptor concentration increased (P = .051) but was not correlated with the serum ferritin concentration (P = .67), log serum ferritin (P = .65), and the ferritin–transferrin receptor ratio (P = .45).

COMMENT

In this study, we used a stable isotope–based method to compare the absorption of iron from a meal accompanied by a non–ascorbic acid–fortified apple juice vs that from a meal accompanied by orange juice containing ascorbic acid. As the orange juice had greater ascorbic acid content than apple juice, we hypothesized that iron absorption would be greater from the meal given with orange juice. Contrary to our expectations, iron absorption was similar in children consuming meals that were accompanied by either apple or orange juice.

These results differ from those of a previous study by Fairweather-Tait et al7 in which a beverage containing ascorbic acid led to a 2-fold increase in iron bioavailability. Similarly, a study by Ballot et al10 showed that juices containing citric acid and ascorbic acid significantly increase iron absorption from a rice meal.
beneficial effect on iron absorption in adults.\textsuperscript{21} Al-
amounts. Some studies have shown malic acid to have a
of juice, whereas the orange juice contained negligible
obtained approximately 400 mg of malic acid per 100 mL
ascorbic acid levels. The apple juice in our study con-
thoses between apple and orange juices than ascorbic
acid content.

In iron-deficient Mexican women, consumption
of 50 mg of ascorbic acid per day as \textit{agua de limón}
(limeade) for 2 weeks significantly increased iron
absorption.\textsuperscript{20} However, this population is very different
from the one we studied in age, iron status, and
diet. Addition of ascorbic acid to complex foods has
been shown to increase iron absorption in Peruvian
and Jamaican children.\textsuperscript{14,15} but there are more differences between apple and orange juices than ascorbic
acid content.

One could speculate, therefore, that other ingredi-
ents present in apple juice may have a beneficial effect on
iron absorption and counteract the effect of lower
ascorbic acid levels. The apple juice in our study con-
tained approximately 400 mg of malic acid per 100 mL
of juice, whereas the orange juice contained negligible
amounts. Some studies have shown malic acid to have a
beneficial effect on iron absorption in adults.\textsuperscript{21} Al-
though the effect of malic acid on nonheme iron absorp-
tion in children has not been studied, it is possible that
malic acid or some other component of the apple juice
counteracted the effect of the differing ascorbic acid
concentration. Alternatively, the higher levels of zinc and cop-
per in the orange juice meal may have impaired iron
absorption and negated any beneficial effect of ascorbic acid.
The point remains that substitution of apple juice for or-
ange juice in the diet does not only result in a change in
ascorbic acid intake.

Consumption of apple juice is increasing in the
United States. A survey conducted by the US Depart-
ment of Agriculture in 1994 found a large increase in total
beverage consumption in the United States, the greatest
of which was seen in noncitrus juices, especially apple
juice.\textsuperscript{13} Although a number of studies in developing coun-
tries have shown that iron status improves as the intake
of ascorbic acid in the diet increases, our study was on
children in a developed country with generally good iron
status, and we were not able to demonstrate a negative
effect of apple juice consumption on iron absorption in
this population.

Current dietary recommendations are that chil-
dren consume 5 servings of fruit, juices, and vegetables
daily. However, actual intakes are well below this level.
Apple juice is well accepted by children; therefore, it is
reassuring that consumption of apple juice, rather than
orange juice, does not appear to have an adverse effect
on iron absorption from the diet and can be encouraged
as a healthy addition to the diets of preschool-aged chil-
dren. However, it should be remembered that excessive
amounts of apple juice can lead to abdominal pain and
diarrhea, as its carbohydrate composition can lead to sugar
malabsorption.\textsuperscript{22}

\textbf{What This Study Adds}

Ascorbic acid enhances iron absorption. However, chil-
dren reportedly prefer noncitrus juices (eg, apple juice),
which contain little vitamin C, over citrus juices (eg, or-
ange juice) with high vitamin C content. Previous studies
have not established whether apple juice consump-
tion by children reduces iron absorption, and thus
increases the risk of pediatric iron deficiency, com-
pared with consumption of orange juice.

This study demonstrates that young children ab-
sorb iron well from a meal that includes either apple juice
or orange juice. Thus, our findings imply that a child’s preference for apple juice over orange juice with a meal
will not have an adverse effect on iron absorption or lead
to iron-deficiency anemia, a significant health problem
that persists worldwide.

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