Difructose Anhydride III Enhances Bioavailability of Water-Insoluble Iron in Anemic Vietnamese Women

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Summary  Difructose anhydride III (DF AIII) is an indigestible disaccharide and has been shown to enhance iron absorption in animal studies; however, the effect has not been investigated in anemic subjects. We investigated the efficacy of co-administration of DF AIII with water-insoluble iron in the treatment of iron deficiency anemia in Vietnamese women. One hundred sixty-eight moderately anemic women (80 g/L < hemoglobin (Hb) < 120 g/L) participated in a double-blinded, placebo-controlled study with daily supplementation of iron for 6 mo. The volunteers were randomly assigned into four groups, i.e., Group A: received 15 mg Fe as ferric pyrophosphate; Group B: received 15 mg Fe as ferric pyrophosphate and 1.25 g DF AIII; Group C: received 15 mg Fe as ferrous sulfate; Group D: received a placebo. Hb and iron status were measured at baseline and after 2, 4 and 6 mo of intervention. The ratio of transferrin receptor to ferritin was used to estimate stored and functional body iron (BI). One hundred sixteen (69.0%) women completed the trial. After 6 mo, mean (± SE) Hb concentration was higher in Group A (121.6 ± 1.7 g/L), Group B (126.4 ± 1.5 g/L) and Group C (126.8 ± 1.6 g/L) compared to Group D (107.0 ± 1.7 g/L, p < 0.0001). Mean change in BI was twofold greater in Group B (5.0 ± 0.5 mg/kg) than that in Group A (2.5 ± 0.6 mg/kg, p = 0.008). The percentage of anemia was significantly reduced in Group B (18.8%) compared to Group D (95.8%, p < 0.0001) and Group A (39.1%, p = 0.033). Co-administration of DF AIII enhances Hb concentration and iron stores more than single administration of water-insoluble iron in anemic Vietnamese women.

Key Words  difructose anhydride III, iron, anemia, randomized controlled trial

Anemia affects 1.62 billion people, which corresponds to 24.8% of the worldwide population (1). Iron deficiency (ID) is estimated to be the most common cause of anemia worldwide and is particularly prevalent in developing nations in Africa and Asia (2). In Vietnam, anemia had been widely prevalent and the prevalence of iron deficiency anemia (IDA) in reproductive-age women and children was 40.2 and 45.3%, respectively (3). Through the great efforts of the National Program to Control IDA, the prevalence of IDA in reproductive-age women was reduced to 24.3% in the year 2000 (4). However, until the present, IDA remains a public health issue in Vietnam, especially in reproductive women, children and workers in some garment factories (5). Finding an efficient strategy for controlling IDA has attracted much interest as a part of policy planning in Vietnam.

To combat iron deficiency, food fortification programs are considered the most cost-effective and sustainable approach. However, the success of an iron fortification program depends largely on the careful choice of the iron compound. A cheap and highly bioavailable iron compound that causes no organoleptic changes would be the ideal fortification compound. Ferrous sulfate (FeSO₄) which is a water-soluble iron compound is the most bioavailable iron compound, but it often causes unacceptable color or flavor changes in the food vehicle (6). Ferric pyrophosphate is a water-insoluble iron compound often used by European food companies to fortify infant cereals and chocolate drink powders and its main advantage is that it causes no adverse color and flavor changes to food vehicles (6). However ferric pyrophosphate is only poorly soluble in dilute acid, such as gastric juice, and is thus only of mediocre absorption in humans (7). Its low absorption rate needs further improvement.

Indigestible carbohydrates, such as fructooligosaccharide (FOS) (8, 9), water-soluble soybean fiber (10), and insoluble dietary fibers (11) have received attention in promoting iron absorption. Difructose anhydride III (DF AIII) is a naturally occurring indigestible disaccharide (Fig. 1) in the root of Lycoris radiate (12). This disac-
charide has recently been processed from inulin with Arthrobactor sp. H65-7 inulin fructotransferase [EC 2.4.1.93; Inulinase II (13)] for mass-production, and is stable at high temperature under acidic conditions (pH 2.0 at 100˚C for 30 min) (14). DFAIII has been shown to enhance iron absorption in animal studies (10, 15, 16); however, the effect has not been investigated in anemic subjects.

The objective of this study was to investigate the efficacy of co-administration of DFAIII with water-insoluble iron on treatment of iron deficiency anemia in Vietnamese women.

**SUBJECTS AND METHODS**

**Study design and participants.** This study was conducted from Aug 2006 to Apr 2007 with female factory workers in two garment factories in Hai Duong and Hung Yen Province in Vietnam. This study was designed with two phases. The first phase of the study involved screening of the female factory workers to identify anemic women. All the women aged 20–49 y working in the factories were recruited into the screening study, which was held independently for the study. Phase two involved a randomized, double-blind, placebo-controlled intervention where women consumed iron supplementation every day for 6 mo.

It was calculated that a minimum of 37 women with moderately anemic status would be required in each group to demonstrate a significant difference in hemoglobin (Hb) concentration of 8 g/L at 90% power and 5% significance (two side test). Power calculations were based on a standard deviation for hemoglobin of 10.3 g/L determined from a group of anemic women (17). Anticipating 16% dropouts, a sample size of at least 42 subjects per group was required at baseline.

Women aged 20–49 y with moderately anemic status (Hb concentration<120 but >80 g/L) (18) were included in this study. Subjects were excluded if they were pregnant, breastfeeding, parasite infection positive or if they had any known health problems likely to influence anemic status including a history of gastrointestinal or intestinal resection; blood transfusion; renal disease; genital disease; cancer; malaria infection or severe infection such as dysentery, hepatitis and cholera.

**Intervention.** One hundred and sixty-eight women who met the study criteria were randomly assigned into four groups. i.e., Group A: received 15 mg Fe as ferric pyrophosphate (IPP). Group B: received 15 mg Fe as IPP and 1.25 g DFAIII; Group C: received 15 mg Fe as FeSO₄ as a positive control as they experienced anemia improvement and Group D: received a placebo after being stratified by age and Hb levels.

Each supplement was taken daily twice; after breakfast and after lunch, under the monitoring of trained health staff members for 6 mo. Each participant consumed 10 tablets (2.500 mg)/d and the composition of the tablets for each group was Group A: IPP 187.5 mg (elemental iron 15 mg), lactose 2,282.5 mg, sucrose stearate 30 mg; Group B: IPP 187.5 mg (elemental iron 15 mg), DFAIII 1.250 mg, cellulose 532.5 mg, sucrose stearate 30 mg, lactose 500 mg; Group C: ferrous iron 40.8 mg (elemental iron 15 mg), lactose 2,429.2 mg, sucrose stearate 30 mg; and Group D: lactose 2,470 mg, sucrose stearate 30 mg. All four types of supplements provided were identical in appearance in the form of tablets and were manufactured by FANCL Co. (Kanagawa, Japan). The dose of DFAIII (1.25 g/d) was based on a study that determined the stimulatory effects of DFAIII on calcium (Ca) absorption in humans (19) since the dose-stimulate effect of DFAIII on iron absorption has not been determined. The efficacy of DFAIII on Ca absorption has been demonstrated in in vivo balance studies with rats (20–23) and humans (19, 24). The safety of oral ingestion of DFAIII in healthy humans was previously determined with repeated 9 g/d of DFAIII ingestion for 4 mo with incidence of diarrhea and some gastrointestinal symptoms (25). No serious adverse effects were observed in the study (25) and the dose of DFAIII in the present study was under this level. The dose of iron (15 mg/d) was decided by a factor related to production of the tablets. One tablet contained 1.5 mg iron and we supplied 10 tablets, anticipating that study participants would be apprehensive and refuse to take more than 10 tablets; therefore 15 mg iron administration was the maximum feasible dose.

**Assessments.** Blood samples were collected before breakfast in fasting condition at baseline (T0), and after 2 mo (T2), 4 mo (T4) and 6 mo (T6) intervals. Concentrations of Hb, serum ferritin (SF) and transferrin receptor (TfR) were measured at each sampling. C-reactive protein (CRP) was measured at T0 and T6. Hb was measured by the cyanmethemoglobin method within 12 h. SF was measured by a two-site enzyme-linked immunosorbent assay with monoclonal reagents for both the capture and indicator antibodies (26). TIR was measured by using an assay with double monoclonal antibodies against intact TIR purified from human placenta (27). CRP was measured by latex Immunoagglutination assay (Sekisui Medical Co. Ltd., Japan). The ratio of TIR to SF was used to estimate stored and functional body iron (BI). The following formula was used for the estimation (28):

\[
BI \ (mg/kg) = -[\log (TIR/SF) - 2.8229]/0.1207.
\]

Anemia was defined as a Hb concentration<120 g/L and ID was defined as a SF concentration <15 µg/L (18) or a TIR concentration >8.5 mg/L (27). Infectious status was measured by CRP for short-term effects and the ele-
Elevation for CRP was considered to be significant at a level of \( >3.0 \text{ mg/L} \) (29).

Body height and weight were measured at T0, T2, T4 and T6. Dietary intakes were collected by 24-h food recall for 3 non-consecutive days at T0 and T6. Interviewers were centrally trained and certified in data collection according to standardized methods (30). Conversion to nutrient intake was based on food composition tables for Vietnamese foods (31). Gastrointestinal parasites were determined at T0 and T6 from fecal specimens using standard Kato-Katz methodology (32).

Ethics: Informed written consent was obtained from all participants. The study was approved by the Ethical and Scientific Committee of Vietnam National Institute of Nutrition, and the Ethical Committee of FANCl Co. in Japan. Women with Hb less than 80 g/L at a screening of the study participants were treated with a proper treatment schedule. Women diagnosed with hookworm infection at the screening were treated...
with single dose of Albendazole. At the end of the intervention, all women who were still anemic were referred to the health center and received iron supplements.

**Statistical analysis.** Statistical analysis was performed with SPSS version 15.0 (Statistical Package for Social Science, Inc.). Normally distributed data were described as means±SDs or SEs, while non-normally distributed data were expressed as geometric means with 95% CI. Data not normally distributed (SF) were log transformed for comparisons. Characteristics of study participants such as age, anthropometrics, nutrient intakes, Hb and iron status were compared across the groups by using analysis of variance (ANOVA). Mean differences of Hb, SF, TIR and BI among groups at T2, T4 and T6 were assessed using a multivariate linear regression model to allow us to account for potential confounders due to differences between groups in their baseline concentrations. Bonferroni’s adjustment was used for the multiple comparisons. For non-normally distributed variables (SF) a comparison among groups of the absolute difference between T0 and T6 values was made by the Kruskal-Wallis test; if $p<0.05$, the Mann-Whitney U test (2 comparisons) was used for the multiple comparison. In addition, the effect of the treatments on the prevalence of anemia and ID during the intervention study was evaluated. Logistic regression analyses were performed for the binary response variables such as prevalence of anemia and ID. $p$ values $<0.05$ were considered significant.

**RESULTS**

Figure 2 shows the flow diagram of the study participants from initial screening to the data analysis in the present study. The screening study was implemented in 1,759 women. During the screening survey, seven women with a low Hb concentration (<80 g/L), 1,478 women with a Hb concentration≥120 g/L, one pregnant woman, 11 lactating women, 42 hookworm infection-positive women and 41 women with a history of disease likely to influence anemic status unrelated to iron deficiency were excluded from the study; thus 179 (10.2%) women met the inclusion criteria. Of those 179 women, 168 women were randomly selected for the intervention, all women who were still anemic were referred to the health center and received iron supplements.

| Group A (n = 23) | Group B (n = 32) | Group C (n = 26) | Group D (n = 24) | $p^1$ |
|------------------|------------------|------------------|------------------|-------|
| Age (y)          | 26.3±5.52       | 25.8±5.4         | 26.6±5.2         | 28.2±5.4 | 0.415 |
| Height (cm)      | 154.8±4.4       | 153.4±4.3        | 152.6±4.0        | 152.1±3.5 | 0.128 |
| Weight (kg)      | 46.5±5.3        | 44.5±4.9         | 46.4±6.3         | 43.8±4.7 | 0.187 |
| Body mass index (kg/m²) | 19.4±2.0       | 18.9±1.9         | 19.9±2.4         | 18.9±1.8 | 0.231 |

Table 1. Baseline characteristics of 111 moderately anemic women.

1. *p*-values of ANOVA. Bonferroni adjustment was made for multiple comparisons.
2. Mean±SD (all such values).
3. Median; 25th, 75th percentile in parentheses. For the statistical analysis, the parameter was log transformed.
4. *ab*Values in a row with different superscript letters are significantly different, $p<0.05$. 

**Baseline characteristics of participants**

Table 1 shows the characteristics of the participants (n=105). Age and anthropometrics did not differ among the four groups. The mean (±SD) iron intakes and iron intakes from animal food were 11.3 (±2.7) and 1.4 (±0.7) mg/d, respectively. There was no significant difference in the iron intake among the four groups. Nutrient intakes that may affect non-heme iron absorption such as animal protein, vitamin C and Ca were not significantly different among the four groups, either. Although phytate and polyphenol intakes are known to influence iron absorption (33), they were not assessed because of the lack of food composition data in Vietnam (31). These nutrient intakes...
Changes in hemoglobin, iron status, anemia and iron deficiency

Table 2 shows concentration of Hb and iron status at T2, T4 and T6, and the differential changes between T0 and T6. At T2, median SF in Group C was the highest among the four groups and was higher than that in Group A (p=0.004 and 0.017, respectively) and Group B (p=0.010 and 0.011, respectively) compared to those in Group D. Prevalence of anemia was 100% in all the groups. Prevalence of ID (SF < 15 μg/L or TR > 8.5 mg/L) was 64.0, 75.0, 59.3 and 51.9% in Group A, Group B, Group C and Group D, respectively; but there was no significant difference among the groups (p = 0.501).

Changes in hemoglobin, iron status, anemia and iron deficiency

Table 2. Effects of interventions on hemoglobin and iron stores.

|                      | Group A (n=23) | Group B (n=32) | Group C (n=26) | Group D (n=24) | p1  |
|----------------------|---------------|---------------|---------------|---------------|-----|
| Hemoglobin (g/L)     |               |               |               |               |     |
| T2                   | 110.3±1.5     | 111.1±1.3     | 115.8±1.4     | 111.7±1.5     | 0.026 |
| T4                   | 113.1±1.5     | 117.0±1.3     | 117.8±1.4     | 106.4±1.5     | <0.0001 |
| T6                   | 121.6±1.7     | 126.4±1.5     | 126.8±1.6     | 107.0±1.7     | <0.0001 |
| ΔT0–T6               | 14.6±1.7      | 19.3±1.5      | 19.7±1.6      | -0.1±1.7      | <0.0001 |
| Serum ferritin (μg/L)|               |               |               |               |     |
| T2                   | 17.2 (5.5, 51.9)     | 30.5 (9.4, 53.4) | 56.5 (26.6, 93.8) | 30.6 (7.2, 97.3) | <0.0001 |
| T4                   | 18.4 (10.3, 39.7)   | 28.2 (15.8, 65.6) | 55.7 (32.2, 85.4) | 22.3 (6.2, 65.9) | <0.0001 |
| T6                   | 25.4 (12.8, 42.6)   | 39.9 (18.4, 69.9) | 51.0 (33.2, 68.7) | 18.6 (4.9, 57.0) | <0.0001 |
| ΔT0–T6               | 6.3 (–1.0, 19.0)   | 14.9 (5.1, 39.0) | 22.5 (6.2, 33.9) | -5.2 (–25.1, 0.8) | <0.0001 |
| Transferrin receptor (mg/L) |           |               |               |               |     |
| T2                   | 6.6±0.4        | 6.0±0.4       | 6.1±0.4       | 7.3±0.4       | 0.067 |
| T4                   | 6.7±0.4        | 5.6±0.3       | 5.4±0.3d      | 7.3±0.4       | <0.0001 |
| T6                   | 5.8±0.6        | 4.3±0.6bc     | 5.4±0.6d      | 10.9±0.6a     | <0.0001 |
| ΔT0–T6               | -2.8±0.6bc     | -4.4±0.6b     | -3.3±0.6b     | 2.2±0.7a      | <0.0001 |
| Body iron (mg/kg)    |               |               |               |               |     |
| T2                   | 3.5±0.5c       | 4.8±0.4bc     | 6.1±0.5b      | 2.0±0.5a      | <0.0001 |
| T4                   | 3.7±0.5b       | 5.5±0.4c      | 6.6±0.5c      | 1.6±0.5b      | <0.0001 |
| T6                   | 4.7±0.6b       | 7.2±0.5c      | 6.6±0.6bc     | -1.0±0.6a     | <0.0001 |
| ΔT0–T6               | 2.5±0.6b       | 5.0±0.5c      | 4.3±0.6bc     | -3.1±0.6a     | <0.0001 |

T0, at baseline; T2, after 2 mo; T4, after 4 mo; T6, after 6 mo.
1 Linear regression was used after control for baseline values, and a Bonferroni adjustment was made for the multiple comparisons. For non-normally distributed variables (serum ferritin) a comparison among groups of the absolute difference between T0 and T6 values was made by the Kruskal-Wallis test; if p<0.05, the Mann-Whitney U test (2 comparisons) was used for the multiple comparison.
2 Adjusted β coefficient; mean±SE (all such values).
3 Δ change.
4 Median; 25th, 75th percentile in parentheses. For the statistical analysis, the parameter was log transformed.
a,b,c Values in a row with different superscript letters are significantly different, p<0.05.
higher Hb and iron stores compared to Group D (p<0.0001). The improvements of Hb and BI level in Group B compared to Group A were 132% (Hb: 19.3 g/L compared to 14.6 g/L) and 200% (BI: 5.0 mg/kg compared to 2.5 mg/kg), respectively. Compared to Group C, improvements of Hb and BI were 74% (Hb: 14.6 g/L compared to 19.7 g/L) and 58% (BI: 2.5 mg/kg compared to 4.3 mg/kg) in Group A, while these were 98% (Hb: 19.3 g/L compared to 19.7 g/L) and 116% (BI: 5.0 mg/kg compared to 4.3 mg/kg) in Group B, respectively.

At T6, percentage of anemia was significantly reduced in Group A (39.1%, p=0.001), Group B (18.8%, p<0.0001) and Group C (11.5%, p<0.0001) compared to Group D (95.8%); while percentage of ID was significantly reduced only in Group B (12.5%, p<0.0001) and Group C (3.8%, p<0.0001) but not in Group A (43.5%, p=0.113) compared to Group D (66.7%). Percentage of anemia and ID were significantly lower in Group B (p=0.033 and 0.013, respectively) and Group C (p=0.001 and 0.007, respectively) compared to Group A.

DISCUSSION

We observed administration of IPP with DAIIII in anemic subjects increased body iron by two times com-
pared to administration of IPP alone and the level of increment was similar to the administration of FeSO₄ alone after 6 mo of the supplementation. Intakes of other nutrients such as animal protein, organic acids, phytate and polyphenols are important factors for iron bioavailability (7, 33). In the present study, dietary intakes were assessed at T0 and T6. The distribution of intakes of the other nutrients that may affect non-heme iron absorption such as animal protein, vitamin C and Ca were not different among the four groups. Furthermore, cereals, legumes and vegetables that are main sources of phytate and polyphenol intakes were similar in the four groups (data not shown). These results indicated that intakes of other nutrients might not be confounding factors in the present study.

After 6 mo of iron administration, we confirmed no difference between IPP with DFAIII and FeSO₄, the positive control. However, we may not have estimated the efficacy of IPP with DFAIII on anemia or iron deficiency as well as the efficacy of FeSO₄ correctly. Relative bioavailability (RBV) and iron absorption have been reported to be variable by food matrices given with the iron components (34); furthermore, these are closely related to baseline iron status (33, 35). We used iron supplementation by tablet in the present study. Accordingly, food matrices were not a confounding factor on RBV. We analyzed blood samples with careful consideration; however, there were significant differences in TIR and body iron among the four groups at the baseline. Therefore, to assess the efficacy of IPP with DFAIII on RBV compared to FeSO₄, we need a further study with a design of stratified randomization considering TIR and body iron.

In our study, we did not determine the effect of DFAIII on the absorption of water-soluble iron; however, there have been several studies determining the effect with the aim of discovering its mechanism in animal models. By using Sham-operated and totally gastrectomized male Sprague-Dawley rats fed test diets prepared according to the AIN93G formulation, which includes water-soluble iron (36), DFAIII feeding was reported to restore gastrectomy-induced iron malabsorption, resulting in complete prevention of iron-deficiency anemia in rats (10). The study suggests that cecal fermentation of DFAIII may contribute to the improvement in these gastrectomy-induced defects. Another study using a AIN93G-based diet showed the effects of feeding different types of non-digestible disaccharides on iron absorption in comparison with fructo-oligosaccharide (FOS) in normal and ovariec-tomized rats and suggested the effects depend on the disaccharide partly associated with the cecal fermentation of these disaccharides (16). The mechanism of iron absorption by cecal fermentation of DFAIII have been suggested by the other studies as well: under the conditions of tannic acid (TA) suppression of iron absorption, the feeding of TA with DFAIII decreased pH of the cecal content and increased major organic acid pools in the study (15). Shiga et al. (37) also showed that feeding DFAIII increased short-chain fatty acid pools and decreased pH of cecal contents in gastrectomized rats. A lately published study showed another mechanism of DFAIII-induced increases in iron absorption in rats fed an AIN93G-based diet: it indicates the effect as a result of increased cecal iron absorptive capacity through expansion of the cecal mucosa maintaining divalent metal transporter-1 (DMT-1) mRNA expression (38).

In conclusion, this study indicates that co-administration of DFAIII enhances Hb concentration and iron stores more than administration of water insoluble iron alone in anemic Vietnamese women. Considering the unacceptable issues of water-soluble iron, administration of DFAIII, in conjunction with water-insoluble iron, can be reasonably relied on as a strategy to control anemia or iron deficiency.

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