Heme iron-based dietary intervention for improvement of iron status in young women

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Running title: Dietary heme iron intervention

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ABSTRACT

Background: Conventional iron deficiency treatment with pharmacological iron doses often causes side effects. Heme iron has high bioavailability and a low capacity to cause gastrointestinal side effects.

Objective: To investigate the possibility of using heme iron in the form of blood-based crispbread as a diet-based treatment programme for improving the iron status of women of reproductive age.

Research Methods & Procedures: In a 12-week intervention study, 77 women (mean age = 24 y) were assigned to four groups, which were given: blood-based crispbread (35 mg Fe, 27 mg of which was heme Fe), iron supplementation comprising 35 mg non-heme iron/day (Fe_{35mg}), iron supplementation comprising 60 mg non-heme iron/day (Fe_{60mg}), and controls (iron-free tablets).

Results: Body iron increased significantly in the crispbread group by a median of 2.7 mg/kg (interquartile range (IQR) = 3.1, n=18), in the Fe_{35mg} group by 2.7 mg/kg (IQR = 2.8, n=11), and in the Fe_{60mg} group by 4.1 mg/kg (IQR = 3.6, n=13), whereas no change was observed in the control group. No statistically significant difference in iron status increase was observed between the crispbread group when compared with either of the two iron supplement groups.

Conclusion: Dietary-based treatment containing heme iron has few side effects and can be used efficiently to improve the iron status of women of reproductive age.

KEY WORDS:

Heme iron, Iron status, Blood bread, Iron supplementation, Dietary intervention, Women, Iron deficiency, Bread, Iron status, Healthy, Women of childbearing age, Gastrointestinal side-effects, Randomized intervention study.

INTRODUCTION

Iron deficiency (ID) is the most common nutrient deficiency globally, affecting an estimated two billion people in both developed and developing countries [1, 2]. The prevalence of iron deficiency in European women of reproductive age has been estimated at between 8 and 30% [3]. However, conventional treatment comprising pharmacological doses of iron in tablet form often causes side effects such as stomach pain, constipation, diarrhea and feelings of nausea [4, 5]. An important consequence is the risk of low patient compliance in taking the conventional medication [6]. Therefore, identifying treatment options with negligible gastrointestinal side effects would be highly valuable in the battle against ID in both healthy individuals and those who are especially sensitive to such side effects, e.g. patients with short bowel syndrome [7].

Dietary iron can be described as being either heme or non-heme. Heme iron represents a relatively small part of the total dietary iron intake, but has a higher bioavailability than non-heme iron [8] and has also been demonstrated to have a low ability to cause gastrointestinal side effects [9]. In many cultures around the world, heme iron-rich blood products have been used in the diet. There are also innovative approaches to developing a heme iron concentrate and a heme iron-based supplement / fortificant, e.g. hemoglobin-based meat pigment [10-12]. The potential of microbial produced heme iron has also been studied [13]. In order to investigate the benefits of heme iron as a nutrition-based treatment for low iron reserves, the aim in this study was to explore the effectiveness of blood-based crispbread for improving iron status in young women. The main research questions in this study were; Can substitution of part of the diet with blood-based crispbread each day for a 12-week period improve the iron status of healthy non-anemic women of fertile age?

SUBJECTS AND METHODS

Study design

A controlled longitudinal intervention study of a 12-week duration was conducted in two stages. In stage one (January 2007 through June 2007), 46 female subjects were recruited. Due to a high number of withdrawals (see the “Result” section) during the intervention 2007, an additional 31 female subjects were recruited for a second stage (January 2011 through June 2011). By this all subjects underwent the intervention during the same, relative narrow, time of the year. Since there is a risk of differences in dietary habits depending on season, we consider this to be a strength. The subjects (total n = 77 women) were randomized into the following four intervention groups: blood-based crispbread, iron supplementation with 35 mg Fe (Fe_{35mg}), iron supplementation with 60 mg Fe (Fe_{60mg}), and a negative control group. Evaluation was carried out by assessment of habitual diet, height and weight, together with venous blood samples collected at baseline and after the intervention (Figure 1).

Ethics

The study protocol was in accordance with the Helsinki Declaration of 1975 as revised in Seoul 2008 and approved by the regional ethics review board in Gothenburg (reg. no. 650-06). Hence, the subjects were informed that they could withdraw from the study at any time without giving a reason.

Subjects

Voluntary female participants of reproductive age were recruited by recruitment posters at two Swedish universities (University of Gothenburg and Chalmers University of Technology). A total of 293 women contacted our research group for additional and more specific information. After getting the full study information the majority of theses 293 decided not to participate, and some additional subject were absent at start. Forty-seven females were excluded according to the exclusion criteria. Inclusion criteria were healthy non-smoking females with no anemia (hemoglobin concentration >120 g/L), not pregnant or lactating, and not exercising heavily. Exclusion criteria were blood donation less than two months before the start of the study, medications or diet supplements (incl. iron supplements), underlying malabsorption diseases, or other medical problems known to affect Fe homeostasis. Since an activated acute-phase reaction has a marked effect on iron homeostasis and iron absorption, it is of outer most importance to, as far as possible, minimizing the devastating effect of infection / inflammation. Thus, exclusion criteria also included infection / inflammation (see Anthropometric and laboratory measurements section).

Dietary assessment

Dietary assessment was performed by means of a previously used food frequency questionnaire (FFQ) elucidating habitual meal patterns [14]. In order to investigate habitual dietary patterns before and during the study, food containing dietary elements that affect iron absorption, such as coffee and tea, dairy products, citrus fruit juice, whole meal products, meat, fish, and poultry, was assessed. The weekly intake of meals (breakfast, lunch and dinner) was also evaluated. The Food Frequency Questionnaire used in this trial was divided into five sections: breakfast; lunch; dinner; in-between meals; other foods. Portion sizes of foods were described in terms of household measures, standard weights and by photographs of portions of known weights. Weekly intake of meals was evaluated in the FFQ by a checklist of foods and beverages containing dietary elements known to influence iron absorption. The checklist contained a frequency response section to report how often each food item was consumed over a specified period of time. In short, The FFQ was designed to answer three questions; What foodstuffs are habitually eaten? How much of this foodstuff is eaten at each occasion? How often is this foodstuff eaten? To answer this last question the subjects had to state frequency where ten different options were given: Rarely / never, Once a month, Twice a month, and 1, 2, 3, 4, 5, 6, or 7 times a week.

Blood-based crispbread

The dietary heme iron used in this study came from blood-based crispbread, which was baked using whole meal rye flour, sifted wheat and rye flour, water, blood from cattle and pigs, sodium chloride, and bicarbonate. Each daily ration of crispbread (75 g $\approx$ 10–11 slices) contained 1.15 MJ and 35 mg Fe, 27 mg of which was heme iron. The iron content of the crispbread was analyzed as previously described [15, 16]. Analysis of total phytate phosphorus in the bread (134 mg/100 g bread) was analyzed as previously described [17]. The subjects were encouraged to spread their intake throughout the day.

Tablets

The iron tablets used in the Fe_{35mg} group were Twinlab Iron Caps (Ideasphere Inc., American Fork, Utah, US), each containing 18 mg Fe as ferrous fumarate. On four occasions/days during the study the subjects in the Fe_{35mg} group were instructed to consume one tablet. Thus, the mean daily iron intake from the tablets during the 12-week intervention was 35 mg. The iron tablets used in the Fe_{60mg} group were Erco-Fer® (Orion Pharma, Orion Corporation, Espoo, Finland), each containing 60 mg Fe as ferrous fumarate. Placebo tablets that contained no iron but were otherwise identical to Erco-Fer® were planned for use in the control group. However, due to a sudden shutdown in the production of Erco-Fer® the company was unable to supply these placebo tablets. Thus as an ad hoc solution, tablets containing 500 mg of folic acid (Recip AB.) were used in the control group.

Compliance

Apart from the blood-based crispbread administered to the crispbread group, all subjects were asked to maintain their habitual dietary patterns for the duration of the study. Every second week they came to our laboratory to collect their two-week ration of tablets or crispbread. On these occasions compliance (i.e. how bread or tablets were taken, and if missed, when and how much) and possible side-effects or discomfort due to the bread or tablet intake were evaluated and documented by face-to-face interviews. If any problems were experienced between these occasions, the subjects were strongly advised to contact the research team.

Anthropometric and laboratory measurements

Weight and height were measured with the subjects in light clothes and no shoes. Blood samples were collected at baseline and after 12 weeks for analysis of: Hemoglobin concentration (Hb), serum iron concentration (S-Fe), total iron binding capacity (TIBC), transferrin saturation (TSAT), serum ferritin concentration (SF), soluble transferrin receptor (sTfR), and the acute-phase proteins C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP). The analyses were conducted at an accredited reference laboratory (Clinical Chemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden), according to the ISO/IEC 15 189 Standard for Medical Laboratories. In addition, since acute phase activation has such a major impact on iron homeostasis and iron absorption [18, 19], at blood sampling the subjects were asked about any infections, such as a cold, cough, sore throat, or fever during the previous weeks. Positive answers regarding infections and/or CRP > 5 mg/L and/or AGP > 1.2 g/L were exclusion criteria

Iron status outcome variables

The outcome variables evaluated were SF, sTfR, Hb, and change in the amount of body iron reserves in accordance with Cook *et al.* [20], based on the ratio of soluble transferrin receptor to serum ferritin. Body iron reserves based on Cook *et al.* [20] have been shown to be less affected by inflammation. Thus, calculating the amount of body iron in accordance with Cook has proved to be a reliable measure of the effectiveness of fortification interventions [21]. In the present study, body iron reserves were considered the main iron status outcome.

Statistics

The main hypothesis was that after 12 weeks there would be a significant increase in iron status in women who had eaten 75 g of blood-based crispbread per day.

The sample size and power calculation was based on the following assumptions: I): the subjects would be in iron balance, irrespective of the intervention; *II):* mean total absorption of Fe from the crispbread would be 12% [8, 22]; *III):* mean body weight in women of reproductive age is 65 kg. Based on these assumptions, the 12-week intervention, eating 75 g of blood-based crispbread/day would result in a total of 353 mg of absorbed iron. This represents an increase of 5.4 mg/kg in body iron reserves. In order to have an 80% probability (i.e. a power of 80%) of observing a 5.4 ± 5.4 µg/L (SD) increase in body iron reserves in the crispbread group, 16 subjects would be studied. The significance level was 0.05. Due to skewness of many of the parameters, data are presented as median and interquartile range (IQR). Normality was tested using a Shapiro-Wilk test. When comparing differences in changes over time between groups, Bonferroni-adjusted one-way repeated-measures ANOVA was used for normally distributed means and Kruskal-Wallis one-way analysis of variance for comparing means not normally distributed. Paired-sample t-tests were used when analyzing changes over time within each group. All P-values are two-tailed and considered statistically significant if <0.05. The statistics program used was SPSS for Windows version 18.0.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline anthropometric and laboratory values

Median body weight (n=77) was 62.1 kg (interquartile range=10.2 kg). Baseline BMI for the Fe_{35mg} group (median= 23.4 kg/m^2) was significantly higher compared to that in the crispbread (median= 20.9 kg/m^2, p<0.028) and the Fe_{60mg} group (median= 20.5 kg/m^2, *p*<0.006). The Hb and ferritin concentrations in the Fe_{35mg} group (median Hb = 136 and median ferritin = 30 µg/L) were also significantly higher compared to the Hb (*p*<0.047) and ferritin concentrations (*p*<0.021) in the Fe_{60mg} group (median Hb = 130 and median ferritin = 19 µg/L) (Table 1).

Withdrawals and exclusions

Flow diagram of the progress through the phases of the trial, including withdrawals (18%) and exclusions (13%), are shown in figure 1. Number of subjects included in the final analysis were 18, 11, 13, and 12 for the crispbread group, the Fe_{35mg} group, the Fe_{60mg} group, and the control group, respectively (total n=54) (figure 1).

Compliance

Compliance was controlled every second week during the intervention as well as post-intervention. Compliance among six subjects who did not eat all the administered crispbread ranged from 98.5% to 60%, with a mean of 89.6%. On a group level (n=18), the mean total compliance in the crispbread group was 96.5%. In the Fe_{35mg} group, compliance was 100%, i.e. all subjects reported taking the administered iron tables. In the Fe_{60mg} group, two subjects reported not taking ~3% of the administered tablets, giving a mean total compliance of 99.5%. In the control group, one subject reported ~10% missed tablets (i.e. mean total compliance of 99.5%).

Side effects

Four subjects in the crisp bread group reported constipation during the first two weeks of the intervention, which then disappeared. One subject described nausea and stomach pain during the first two days of the intervention, while another reported an increased degree of flatulence during the first week. Almost half of the subjects described this amount of crispbread as crumbly and that it stuck to their teeth.

In the Fe_{35mg} group one subject reported that she experienced constipation after four weeks, which continued more or less for the remainder of the intervention. Another subject felt that during the intervention her stomach functioned better than before.

In the Fe_{60mg} group five subjects reported gastrointestinal side effects. Two experienced loose stools, one of whom also felt sick when on a few occasions she took the tablet during the day instead of at night. Another two subjects reported nausea, one only occasionally and the other about a month into the 12-week study period. The latter subject also reported positive effects, such as being more alert and energetic. The fifth person also felt more energetic but had side effects such as constipation and dark stools, although these only occurred during the last two or three weeks of the study.

In the control group one subject reported side effects in the form of fatigue, nausea, stomach pain, and dizziness.

Dietary intake before intervention

Regarding habitual dietary patterns before the study, the only between-group differences were that *I):* the crispbread group had a significantly higher weekly coffee and tea intake in connection with dinner at baseline compared to the Fe_{60mg} group and the control group (1.1 times per week *vs.* 0.1 and 0.0, respectively); and *II):* the Fe_{60mg} group had a significantly lower weekly intake of fish and poultry at lunch and dinner at baseline compared to the Fe_{35mg} and the control group (2.2 times per week *vs.* 3.8 and 4.4, respectively).

Dietary intake during the intervention

There were no significant within-group changes over time in habitual dietary patterns, or in the intake of food containing dietary factors affecting iron absorption in the crispbread, Fe_{60mg} or Fe_{35mg} groups. However, in the control group there were significant within-group changes over time in; *I)*: coffee and tea intake in connection with breakfast, lunch, and dinner (median 7.0 times per week *vs.* 4.5 times per week, p <0.050); *II)*: the number of times per week that meat, fish, or poultry was eaten in connection with lunch or dinner (median 9.4 *vs.* 6.8, p <0.012), and; *III)*: the number of times per week that meat was eaten in connection with lunch or dinner (median 4.6 *vs.* 2.5, p <0.012).

Intake of blood-based crispbread during the intervention

The mean number of servings of crispbread per day was 3.8 and the mean number of slices per day in connection with breakfast, lunch, dinner, and between meals was 3, 2, 2, and 3, respectively. Thus, the total daily ration of crispbread was split into ~ 20 g servings (7.2 mg heme and 2.1 mg non-heme iron).

Post-intervention anthropometric and laboratory values

After 12 weeks, significant changes were seen in the crispbread group for ferritin (13 µg/L, p>0.001), TfR (-0.5 mg/L, p>0.013), and body iron reserves (2.7 mg/kg, p>0.001). In the Fe_{35mg} group, significant changes were seen for ferritin (19 µg/L, p>0.001), TfR (-0.2 mg/L, p>0.027), and body iron reserves (2.7 mg/kg, p>0.001). In the Fe_{60mg} group, significant over-time-changes were observed in Hb (7 g/L, p>0.001), ferritin (22 µg/L, p>0.001), TfR (-0.8,

$p>0.008$), and body iron (4.1 mg/kg, $p>0.001$). In the control group, no over-time changes were observed.

Post-intervention, the increase in Hb was significantly higher in the Fe_{60mg} group compared to the control group ($P>0.008$), which was also the case for ferritin ($P>0.001$), TfR ($P>0.004$), and body Fe ($P>0.001$).

All concluded in Table 2 and Figure 2.

DISCUSSION

The present study presents scientific evidence of the effectiveness of a blood-based heme iron rich food product as a dietary alternative for the improvement of iron status in women of reproductive age. Similar positive heme iron effects have also been observed in schoolchildren served cookies [11, 23] and biscuits [24] fortified with hemoglobin concentrate. Although after 15 months ferritin did not differ from controls, there was a small but statistically significant higher hemoglobin concentration in the group administered biscuits [24]. Heme iron-fortified cookies have even been suggested to improve the intellectual performance of low-income preschool children [25]. In hemodialysis patients, oral heme iron administration has also been found to successfully replace intravenous iron therapy [26]. A daily 3.6 mg dose of heme iron together with 24 mg iron fumarate in the second half of pregnancy prevents depletion of iron reserves after birth in most women [27]. Thus, the present study on heme-iron crispbread increases previous knowledge by applying it to a different population.

Other dietary-based interventions to improve iron status have indicated that iron supplementation in tablet form has greater potential than dietary modification (including individual dietary counseling) [28, 29]. In these cases, the better effectiveness of iron supplementation was seen in adult New Zealander and Australian women [28, 29], whose basic diet can be considered to have a relatively high baseline bioavailability. **In previous publications, it has been concluded that in cases where the diet has low iron bioavailability, it is difficult to achieve good effects on iron status by fortification with iron if bioavailability is not improved** [30, 31]. The New Zealand study by Heath *et al.* [28] concluded that although the dietary regimen improved iron status, supplementation is likely to be a more practical option. Thus, the regimen used in the present study, based on a single heme iron-rich food with high bioavailability, was expected to succeed since it combines quantity and quality.

Previous observations of heme iron absorption from one large dose of iron (43 mg) in the form of blood sausage served with 150 ml milk revealed almost the same magnitude of iron absorption (4%) [8]. However, since the milk contained 180 mg of calcium, the only known inhibitor of both heme and non-heme iron absorption [32], it most likely reduced iron absorption considerably. Furthermore, the large dose of heme iron from the blood sausage was over the saturability level (15 mg) proposed for heme iron absorption [33]. In the present study, the subjects stated that they followed the instructions regarding spreading the intake of crispbread over the day. Thus, the total daily amount of blood-based crispbread was split up into 3.8 servings (median), giving each meal a 7.2 mg heme iron and 2.1 mg non-heme iron content, which is below the saturability level.

The absorption of iron from wheat rolls containing 5 mg heme and 3.5 mg non-heme iron has been reported to be 16% and 10%, respectively, when served with and without meat [8]. Swain *et al.* [22] measured an absorption of 15% from capsules containing 5 mg heme iron. On this basis, when planning the present study it was anticipated that the mean total iron absorption from the blood-based crispbread would be 12%.

A plausible explanation for the modest effect could be that the subjects ate the bread without any meat, which has been shown to be essential for high heme iron absorption [8, 34]. However, no relationship was found between the frequency of eating meat together with blood-based crispbread and change in iron status. Although, to our knowledge, there have been no published dose-response studies on meat intake and heme iron absorption, an alternative explanation might be that the quantity of meat required to enhance heme iron absorption was not consumed. Since calcium is known to inhibit the absorption of heme iron [32], eating the blood-based crispbread with dairy products might lead to a poorer heme iron effect. The median number of times per week that dairy products were eaten in connection with breakfast, lunch, dinner, and between meals was 5, 2, 0, and 0, respectively. Thus, since the intake of crispbread was stated to be spread evenly throughout the day, only a minor part was eaten with calcium of dairy origin. Furthermore, no association was observed between changes in iron status and intake of dairy products together with blood-based crispbread. There were no significant within-group changes over time in habitual dietary patterns or intake of food containing dietary factors affecting iron absorption in any of the three intervention groups.

Randomized clinical trials in medicine *per se* have an efficacy oriented approach, i.e. studying the capacity of producing a beneficial effect under ideal conditions. However, in iron focused dietary interventions where a foodstuff is administered, this is done to a diet that already contains a multitude of other foodstuffs and dietary elements with the capacity to influence iron absorption. This provides dietary interventions with a certain element of effectiveness approach similar to the one in the field/ routine care. Especially since introducing one foodstuff to a diet inevitably leads to some degree (major or minor) of changes in the overall diet. Accordingly, the generalizability of the findings in the present study to the actual effectiveness in healthy young women of reproductive age can be considered to be high. Effectiveness means the extent to which an intervention, when applied in the field, does what it is intended to do for the defined population. By adopting an effectiveness approach on the present results, the iron absorption from the blood-based crispbread was concluded to be 6%.

It requires motivation to adhere to a diet intervention [28] and the motivation may be greater if iron deficiency is present. Differences of opinion prevail, however, about which treatment is best. It has been demonstrated that after a dietary intervention, iron status continued to improve during the follow-up period [29, 35]. According to Patterson [29], and Hallberg [35], this means that the most appropriate approach may be to use dietary interventions in mild iron deficiency and supplement the treatment with tablets in severe iron deficiency. Thus, crispbread can form part of a treatment regimen, together with a low dose of iron supplement, further minimizing the risk of gastrointestinal side effects. Alternatively, a low daily intake could be a prophylactic approach in vulnerable groups with high iron requirements.

When it comes to comparing costs, tablets are put on top of a diet. Crispbread, on the other hand, inevitably would replace something else in the overall diet. By this, calculating the cost for introducing crispbread must include an estimation of the financial value of the foodstuff(s) which is replaced by the crispbread. On the other hand, if iron tablets are not tolerable the cost aspect is irrelevant. Patients troubled with gastrointestinal side effects from conventional iron supplementation could possibly benefit from a dietary heme iron-based treatment. One example is patients who have undergone extensive bowel surgery (so-called short bowel syndrome, SBS). These patients have greatly reduced absorptive capacity, which puts them at risk of contracting multiple nutrient deficiencies, including iron deficiency [36]. In addition, the reduced absorptive capacity also makes these SBS patients vulnerable to the gastrointestinal side effects that are also often seen in enteral treatment using therapeutic doses of iron. However, any extrapolation of the results obtained in healthy females to SBS patients would have to be confirmed.

CONCLUSION

In summary, this food product with its high iron-bioavailability and iron density is a promising and easy-to-use nutritional agent for combating iron deficiency, especially as a food product rich in iron instead of iron tablets may reduce the feeling of undergoing treatment.

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FIGURE LEGENDS

***Figure 1.* Study design**

This was a 12-week intervention study. The subjects were allocated into four groups that received a daily ration of (1): 75 g blood and rye flour-based crispbread (containing 35 mg iron, 27 mg of which was heme iron), (2) iron tablets containing 35 mg iron, (3) iron tablets containing 60 mg iron, or (4) tablets without iron. Evaluation was at baseline and after 12 weeks by collection of blood samples and assessment of habitual diet, height, and weight.

***Figure 2.* Changes in hematological biomarkers in women following the 12-week intervention**

Results of post-intervention changes in (A) body iron reserves in accordance with Cook *et al*. [20], (B) serum ferritin, and (C) soluble transferrin receptor, in the blood-based crispbread group (BB), the group administered 35 mg Fe per day (Fe_{35mg}), the group that received 60 mg Fe per day (Fe_{60mg}), and the control group, illustrated as a box plot covering the lower (25^{th}) to the upper (75^{th}) quartile. The line inside the box depicts the median value (50^{th} percentile) and the whiskers depict values up to 1.5 times the interquartile range (IQR). Values greater than 1.5 times but less than 3 times the IQR (from the end of the box) are labeled outliers (O). Values greater than three times the IQR (from the end of a box) are labeled extreme (indicated by an asterisk *). The cross (X) inside the box represents the mean value. Statistically significant within-group changes over time are illustrated by means of the respective *p*-values, unless non-significant (NS).