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ORIGINAL CONTRIBUTION

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The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores

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Abstract *Background* Single-meal and short-term studies have shown an enhancing effect of meat on iron absorption, but there are few interventions of longer duration comprising measurements of biomarkers of iron status. *Aim of the study* To assess the impact of a meat-based and a vegetable-based diet on iron status of women of childbearing age. *Methods* For 20 weeks, 57 women aged 19–39 years with low iron stores (serum ferritin ≤30 μg/l and haemoglobin ≥120 g/l) consumed either a meat-based or a vegetable-based diet. Haemoglobin and serum ferritin concentrations were measured at baseline, after 10 and 20 weeks. Information about dietary intake before and during intervention, meat/fish intake, menstruation and contraceptive methods were recorded. *Results* The women who consumed the meat-based diet had a significantly ($P < 0.001$) higher intake of meat/fish, 152 (147–168) g/day (median (Q_1–Q_3)) compared to the women consuming the vegetable-based diet 31 (24–36) g/day, while the total iron intake was similar in the two groups (mean ± SE) 11.0 ± 0.5 and 12.3 ± 0.3/day mg/day, respectively. Serum ferritin remained unchanged in women on the meat-based diet ($n = 29$)(before intervention (median (Q_1–Q_3)): 16.3 (12.7–25.3) μg/l and after intervention: 16.5 (10.3–25.3) μg/l, but declined from 17.3 (10.9–23.7) to 11.2 (8.8–14.6) μg/l ($P < 0.001$) in women on the vegetable-based diet ($n = 28$). *Conclusions* Our results emphasize the importance of the delicate balance between dietary iron content and iron bioavailability for the maintenance of blood indicators of iron stores in women with initially low iron status.

Key words bioavailability – haemoglobin – serum ferritin – meat-factor

Introduction

Iron deficiency is the most common nutritional disorder in the world [30], and fertile women are particularly vulnerable because of their high physiological iron requirements [20]. In Denmark, approximately 45% of 16- to 31-year-old women have serum ferritin concentrations of ≤30 (μg/l (measured according to The World Health Organization International Ferritin Standard 80/602) [18], indicating that many fertile women are at high risk of becoming iron deficient if their dietary iron intake, iron bioavailability or iron absorption is decreased, or if their iron demands are increased, for instance because of heavy menstruation or pregnancy. To improve iron status it is important to consider both the amount of

dietary iron intake, as well as the bioavailability of dietary iron [11, 27], and iron losses.

Non-haem iron, predominantly found in vegetables, grains and fruits but also in meat, constitutes about 85–90% of the total dietary iron in a Western diet [10]. Bioavailability of non-haem iron is low and is affected by the presence of other components of the diet [7, 16]. Single meal-studies have shown that iron bioavailability is enhanced by the so-called "meat-factor" [3, 11, 24] and vitamin C [28], whereas phenolic compounds [25] and phytic acid as part of dietary fibre [2] inhibit iron absorption. The role of calcium on iron bioavailability is not conclusive [8, 9, 21]. Earlier studies with meat (beef, pork, lamb, liver, chicken, and fish) have shown that different types of meat enhance non-haem iron absorption in a similar manner [4, 6]. Haem iron present in meat constitutes approximately 40% of the total iron content in meat [26] and it is more efficiently absorbed than non-haem iron. Besides these dietary factors iron absorption also depends on the size of body iron stores [7]. Hunt showed that a diet of high- but not low-iron bioavailability promoted the adjustment of iron absorption to body iron stores in women [12].

Most studies on bioavailability of dietary iron have been conducted as single-meal or short-term studies, whereas the impact on iron status following longer-term interventions with diets of low and high iron bioavailability has received less attention. In two recent studies, iron absorption, iron balance and iron status in men and women were followed over a 12-week period on a diet with either a low or high iron bioavailability [12, 15]. Biomarkers for iron status were unaffected by both types of diets in both men and women.

Due to the vulnerability of fertile women to iron deficiency it is important to know what effect different types of diets have on iron status over longer periods of time. The aim of the present study was to address this issue by comparing the impact of a meat- or a vegetable-based diet on iron status over a 20-week period in fertile women with low iron status.

Subjects and methods

Subjects

The study was approved by the Ethical Committee of Copenhagen and Frederiksberg. The participants were recruited through newspaper advertisements and by posting on university notice boards. In order to identify young, fertile, non-anaemic women with low iron stores (serum ferritin concentrations corresponding to World Health Organization (WHO) International Ferritin Standard values ≤30 μg/l and haemoglobin ≥120 g/l) a total of 134 women were screened. Of these, 61 Caucasians women were enrolled in the study. Four women dropped out during the study for various personal reasons (lack of time, moving, etc.). In the 57 women who completed the study, median age was 26 years (range 19–39) and baseline BMI was 22.2 ± 2.6 kg/m^2 (mean ± SD). All subjects were premenopausal non-smokers and did not perform heavy exercise, and none was pregnant or lactating. The women had not donated blood or used any dietary supplements 3 months prior to and during the intervention period. Information about contraceptives as well as duration of menstruation was recorded in a questionnaire.

Study design

The 57 women were allocated to either a meat- or a vegetable-based diet. A stratified random treatment allocation was applied, using serum ferritin levels at screening and habitual meat intakes as blocking factors. For logistic reasons, the study was carried out over two different study periods. The women's diet was supplemented with either meat or vegetable products for a period of 20 weeks. Women allocated to the meat-based diet were provided 150 g meat daily, but had no further dietary restrictions. Women allocated to the vegetable-based diet were allowed to consume a maximum of 250 g meat and 120 g fish (prepared weight) per week. Body weight was monitored at baseline, and after 10 and 20 weeks intervention. The women registered daily intake of the portioned meat/vegetable products and also additional intake of meat and fish (type, amount, preparation) during the intervention period. Further, they conducted two 7-day weighed food records in order to assess their diet prior to and during the intervention. Intake of energy and nutrients was calculated from a food-composition computer program (Danish Tables of Food Composition, DANKOST 2000, version 1,40. Danish Catering Center, Herlev, Denmark).

Diets

Meat- and vegetable products were prepared at the Department of Human Nutrition or purchased as ready-to-eat, and provided to the women as frozen portions for 2 weeks consumption. Women consuming the meat-based diet received 150 g meat per day, which on average consisted of 42% lean pork, 29% lean beef and 29% chicken. Women consuming the vegetable-based diet received vegetable products with an energy- and iron content similar to that of the meat products. The vegetable products consisted of commonly consumed vegetables

(e.g. carrots, leeks, onions, potatoes, mushrooms), nuts and eggs, but contained less than 5% soy products and only a limited amount of pulses to reduce the content of phytic acid. The haem iron content of the women's diet during the intervention period was estimated from their intake of meat and fish. The estimation was based on the following assumptions: the total iron content of meat and fish is 1.6 mg iron per 100 g wet weight [23], and 40% of the iron in meat, poultry and fish is haem iron [26]. Compliance was estimated from the amount of meat and vegetable products that was returned, together with the daily registration of fish and meat intake by the subjects.

Blood samples

Changes in iron status were evaluated by measurements of serum ferritin and haemoglobin concentrations. Blood samples were drawn after 10 min rest in the sitting position from a cubital vein at baseline (week 0), and after 10 and 20 weeks of intervention. In each subject, eight blood samples of 10 ml were drawn in the fasting state between 08:00 and 10:00 h (one at screening, three on consecutive days at baseline, one after 10 weeks, and three on consecutive days after 20 weeks). No alcohol or heavy physical activity was allowed 36 h prior to blood sampling. In order to avoid inappropriately high serum ferritin values due to infection, subjects were requested to report recent signs of infection at the scheduled blood sampling and the blood sampling was postponed until 2 weeks after the infection had subsided.

Blood haemoglobin (Hb) concentration was analyzed by photometric absorptiometry on a Sysmex KX-21 (Sysmex Corporation, Kobe, Japan); the interassay variation is 0.6%. For conversion purposes, Hb in mmol/l × 16.12 = Hb in g/l. Analysis of serum ferritin was performed by a solid-phase, two-site fluoroimmunometric assay on an Auto DELFIA System (Wallac Oy, Turku, Finland) with a detection limit of 0.5 μg/l. The analysis was standardized against a ferritin standard obtained from the WHO International Laboratory for Biological Standards Ferritin, Human spleen for immunoassay (80/578) (National Institute of Biological Standards and Controls, South Mimms, UK); the interassay variation is 4.9%. Serum was stored at −20°C for analysis after the study was closed. Analysis of serum α_1-1-antichymotrypsin concentration was performed using a turbidimetric/nephelometric method (with reagents from DAKO A/S, Glostrup, Denmark) on Cobas Mira (Roche Diagnostic Systems, Basel, Switzerland); the interassay variation is 4.2%. In each subject, all samples were analyzed within the same batch.

Statistical methods

Statistical analysis was performed using the Statistical Analysis System (version 9.1, SAS Institute, Cary, USA). The significance level was set at 5%. For each subject arithmetic mean values for serum ferritin and haemoglobin concentrations were calculated from the three measurements on successive days at baseline and after 20 weeks of intervention.

Descriptive analysis of nutrient intake, serum ferritin and haemoglobin concentrations is shown as mean ± SE or median (Q_1–Q_3), where Q_1 and Q_3 are 25–75%, respectively. The median was used instead of the mean for dietary intake variables with a skewed distribution. Due to a skewed distribution for vitamin C, calcium and serum ferritin, values were ln-transformed in the statistical analyses and were presented in the descriptive analysis using median (Q_1–Q_3).

Baseline differences in nutrient intakes, serum ferritin and haemoglobin concentrations between the two intervention groups were tested using analysis of variances. Nutrient intake, serum ferritin and haemoglobin concentrations were outcome variables. Due to a skewed distribution of serum ferritin, a log-transformation was used prior to analysis. Diet treatment groups were included as a fixed effect and study period as a random effect.

Due to large differences in variation in daily consumption of meat/fish intake between the two intervention groups differences in meat/fish intake during the intervention period was tested using the non-parametric Wilcoxon rank sum test. The association between intervention groups and the use of contraceptions (yes, no) was tested using Fischer's exact test. Differences in duration of menstruation between the intervention groups were tested using an analysis of variance.

Differences in nutrient intake during the intervention were tested by analysis of variances, with dietary intervention group included as a fixed effect, baseline dietary intake as a covariate and study period as a random effect. The interaction between the covariate and dietary intervention group was included as a fixed effect and deleted from the model if non-significant.

To evaluate the effect of the two dietary intervention groups on serum ferritin and haemoglobin concentrations after 20 weeks intervention, analysis of variances were performed. Due to a skewed distribution of serum ferritin, a log-transformation was used prior to analysis. Dietary intervention groups were included as a fixed effect, baseline serum ferritin and haemoglobin concentrations were included as covariates and study period as a random effect. The interaction between the covariate and diet group was included as a fixed effect and deleted from the model

Table 1 Energy and nutrient intake at baseline and during the dietary intervention study

	Meat-based diet $n = 28$		Vegetable-based diet $n = 28$	
Dietary content[a]	Prior to intervention	During intervention	Prior to intervention	During intervention
Energy (MJ/day)	9.1 ± 0.4	8.7 ± 0.3	9.6 ± 0.3	9.5 ± 0.3
Protein (g/day)**	75.2 ± 3.4	89.3 ± 2.8	75.3 ± 2.3	68.5 ± 2.2
Fat (g/day)**	65.9 ± 3.8	62.3 ± 2.3	65.8 ± 2.7	71.4 ± 3.1
Carbohydrate (g/day)**	294 ± 15	266 ± 14	325 ± 12	319 ± 13
Dietary fibre (g/day)*, **	18.6 ± 1.6	18.6 ± 1.6	25.8 ± 1.4	25.8 ± 1.4
Vitamin C (mg/day)**	99 (76–151)	80 (57–118)	135 (81–192)	150 (104–200)
Total iron (mg/day)	10.8 ± 0.5	11.0 ± 0.5	11.7 ± 0.5	12.3 ± 0.3
Calcium (mg/day)**	1,070 (862–1,329)	900 (670–1,229)	1,070 (859–1,364)	1,095 (1,009–1,382)

* Significant difference between diet groups prior to intervention ($P = 0.001$)
** Significant differences between diet groups during intervention (protein $P < 0.001$, fat $P = 0.017$, carbohydrate $P = 0.034$, dietary fibre $P = 0.048$, vitamin C; $P = 0.008$, and calcium $P = 0.026$)
[a] mean ± SE or median (Q_1–Q_3)

if non-significant. Model assumptions were evaluated using Shapiro–Wilks test for normality and visual inspection of residual plots.

Results

In total 57 women completed the study, 29 consumed the meat-based diet and 28 the vegetable-based diet. Body weight remained stable during the study. Twelve women (43%) in the meat-based intervention group and 15 (54%) in the vegetable-based intervention group used oral contraceptives, this difference was statistically insignificant. None of the women used intrauterine contraceptive devices. The duration of menstruation was (mean ± SE) 4.8 ± 0.8 days ($n = 56$). No differences were seen with respect to the duration of menstruation between users and non-users of oral contraceptives.

One woman in the meat-based group did not fill-in the food record and was excluded from the dietary calculations. The daily consumption of meat and fish (median (Q_1–Q_3)) during the intervention period was 152 (147–168) g/day for women consuming the meat-based diet and 31 (24–36) g/day consumed by women on the vegetable-based diet ($P < 0.001$). Daily intakes of energy and selected nutrients prior to and during the intervention are shown in Table 1. No differences were observed between the two dietary intervention groups with respect to energy intake. Prior to and during the intervention, women consuming the meat-based diet had a significantly lower intake of dietary fiber ($P = 0.001$ and $P = 0.048$, respectively) than women consuming the vegetable-based diet. During the intervention, women in the meat-based group had a significantly lower intake of total fat ($P = 0.017$), carbohydrate ($P = 0.034$), and vitamin C ($P = 0.008$) compared to women in the vegetable-based group, while their intake of protein was significantly higher compared to the women consuming the vegetable-based diet ($P < 0.001$) (Table 1). Changes in the intake of protein and calcium during the intervention were significantly different between the two groups ($P < 0.001$ and $P = 0.026$, respectively) (Table 1). Haem iron intake was significantly higher during the intervention period in the meat-based group compared to the vegetable-based group (1.0 ± 0.03 vs. 0.2 ± 0.01 mg/d, respectively, $P < 0.001$) (results not shown).

At baseline haemoglobin levels (mean ± SD) were 125 ± 0.6 g/l and serum ferritin levels (median (Q_1–Q_3)) were 16.8 (11.8–25.2) μg/l for the whole group of participants ($n = 57$) with no differences between the women in the two intervention groups. At baseline there were no signs of an acute phase reaction according to serum α_1-anti-chymotrypsin concentration of 0.24 ± 0.03 g/l (mean ± SD). All women had values below the normal cut-off value of 0.6 g/l and there was no change in values at the end of the study, or any significant difference between the two diet groups.

During the intervention period serum ferritin was unchanged, 16.3 (12.7–25.3) μg/l vs. 16.5 (10.3–25.3) μg/l in the meat-based group ($P = 0.071$) while it declined significantly from (median (Q_1–Q_3)) 17.3 (10.9–23.7) μg/l at baseline to 11.2 (8.8–14.6) μg/l ($P < 0.001$) at 20 weeks in the vegetable-based group (Table 2). The mean ± SE values of the log-transformed serum ferritin data were for the meat-based diet prior to the intervention 2.89 ± 0.11 and at the end of the intervention 2.75 ± 0.11 whereas the values for the vegetable-based diet were 2.78 ± 0.10 prior to the intervention and 2.40 ± 0.09 at the end of the intervention, respectively (not shown). At the end of the intervention period haemoglobin was unchanged, 126 ± 0.9 g/l vs. 125 ± 1.1 g/l in the meat-based group ($P = 0.34$) while it had declined significantly from 124 ± 0.9 g/l (mean ± SD) at baseline to

Table 2 Serum ferritin and haemoglobin in women with low iron status prior to and at the end of the 20 weeks dietary intervention study (mean ± SE or median (Q_1–Q_3))

	Meat-based diet $n = 29$		Vegetable-based diet $n = 28$	
	Prior to intervention	End of intervention	Prior to intervention	End of intervention
Serum ferritin (μg/l)*	16.3 (12.7–25.3)	16.5 (10.3–25.3)	17.3 (10.9–23.7)	11.2 (8.8–14.6)
Haemoglobin (g/l)*	126 ± 0.9	125 ± 1.1	124 ± 0.9	121 ± 0.9

* Significant difference between dietary groups at end of intervention: ferritin $P = 0.018$; haemoglobin $P = 0.038$

121 ± 0.9 g/l ($P = 0.003$) in the vegetable-based group. The biological effect of this small difference is insignificant.

Discussion

The results from our study emphasize the importance of dietary composition in the maintenance of iron status in healthy, fertile women with low iron stores. Women on the meat-based diet, i.e. with an average daily consumption of about 150 g meat/fish over a 20-week period maintained their iron status on a stable level. In contrast, iron status decreased in women on the vegetable-based diet, suggesting that this diet is unable to make up for increased iron losses. The two types of diets contained similar amounts of total iron, but presumably differed with respect to iron bioavailability.

In the present study, no associations were found between the changes in serum ferritin and haemoglobin and intake of total iron or calcium, respectively (results not shown). The absence of associations may in part be due to the crude assessment of dietary intake of the various components and of iron stores, which were all evaluated by indirect methods. It is also debatable, whether an inhibitory or enhancing effect on non-haem iron absorption, demonstrated by single meal tests, can be addressed to a test diet consumed over a longer period [24]. This issue has also previously been raised both concerning the enhancing factor ascorbic acid [13] and the possible inhibiting factor calcium [21, 22]. In a study by Cook et al. [5], the difference between enhancing and inhibiting meals, expressed in percentage absorption of non-haem iron, was 5.9-fold when evaluated in single-meal studies and 2.5-fold when evaluated in 2-week diet studies. In a 12-week study of men with adequate iron stores Hunt and Roughead found an adaptation of total iron absorption as the difference in absorption between high- and low-bioavailability diets was reduced from 8-fold to 4-fold when initial absorption was compared with the absorption tested of consumption of the diets of 10 week [15]. In a similar 12-week study of premenopausal women the efficiency of non-haeme iron absorption but not of haeme iron absorption tended to adapt in response to a high or low iron bioavailability diets characterized by total iron intakes of 15.1 mg/day, high versus low meat intake, high versus low ascorbic acid intake and low versus high phytic acid intake whereas biomarkers of iron status were less sensitive [12]. This may indicate either that a 12-week period is too short for detecting changes in iron status or that the adaptive mechanisms in premenopausal women's iron absorption are different from those of men's. Another important finding from that study was that the percentage of total iron absorption was inversely related to body iron stores only in women on a high-iron bioavailability test diet [12].

In the present intervention study, the total iron content (mean ± SE) of both diets was lower (11.0 ± 0.5 and 12.3 ± 0.3 mg/day for the meat-based and the vegetable-based intervention diets, respectively) than the total iron content in the long-term studies of Hunt [12] and lower than the 15 mg/day for women of childbearing age recommended by the Nordic Nutrient Recommendations 2004 [1]. This difference is mainly because iron fortification of commonly consumed foods is not practiced in Denmark. An intake of 15 mg iron/day is difficult to achieve from foods alone unless iron fortified foods are used. This situation makes the bioavailability of the iron consumed in single meals and in the overall diet of uttermost importance. The intervention diet containing approximately 150 g meat and fish/day had an adequate bioavailability to maintain the iron status on a stable level. In contrast, the intervention diet containing approximately 30 g meat and fish/day (but the same iron content as the meat-based diet) had an inadequate bioavailability and elicited a further decline in an already low iron status. Similar findings were reached in studies of African children where the diets were predominantly cereal- and legume based [31].

One of the limitations of the present study is that iron bioavailability was not measured directly. The biomarkers of iron status employed do however represent the most appropriate and most widely used indicators of iron status [1]. Another limitation of our study is that the blood sampling per se could influence the results. The amount of blood sampled was 80 ml in all participants and 50 ml before the last blood samples

were taken. With a mean baseline haemoglobin in the entire series of 125 g/l, 50 ml blood contains 22 mg iron. Assuming that each μ/l of serum ferritin corresponds to approximately 7 mg mobilizable body iron [29] the drawing of 50 ml blood would decrease serum ferritin by 3.0 μg/l, which is almost half of the median decline of 6.1 μg/l observed in the vegetable-diet group (Table 2). Consequently, our study shows that women on a meat-based diet with a presumed high biovailability of iron were able to maintain a stable iron status despite these additional unphysiological blood losses. In contrast, women on the vegetable-based diet experienced a negative iron balance with a significant decrease in serum ferritin because they were unable to increase their iron absorption due to the presumed low bioavailability of the dietary iron. In the present study the intakes of meat and vegetables in the two dietary groups were controlled on a daily rather than on a meal-by-meal basis. The latter is needed together with valid data on specific inhibitors like phytic acid and polyphenols on iron absorption [7]. It was somewhat surprising that the relatively high intake of vitamin C in the vegetable group did not have more of a positive impact on iron balance. In an earlier fully-controlled dietary intervention study with a low content of enhancers and a high content of inhibitors we showed that vitamin C was the most potent enhancer of iron absorption in healthy women with low iron stores [27].

Therefore, the differences in both serum ferritin and haemoglobin in the two groups after 20 weeks intervention reflect a dietary-associated difference in the ability to adapt to an additional stress on iron balance. It should however be noted that the study period of 20 weeks is relatively short compared with the recommended 6–9 months for fortification trials.

The women in our study were selected to have low iron stores. However, we do not know whether the low iron status may be due to an impaired iron absorption per se, possibly in combination with a habitual inadequate dietary iron intake, and/or low iron bioavailability and/or excessive physiological iron losses. Infection with *Helicobacter pylori* may also exert a negative effect on iron status and possibly iron absorption [19]. We assessed the seroprevalence of *Helicobacter pylori* IgG-antibody in the participants (data not shown) and it appeared to be 13%, i.e. similar to the seroprevalence in the Danish background female population [19].

Furthermore, in women of childbearing age, different iron demands due to blood losses from menstruation and pregnancy, as well as methods of contraception, need to be considered. Women using oral contraceptives have shorter duration of menstruation, higher serum ferritin concentration [17], lower absorption of dietary iron and higher faecal ferritin excretion [14] compared to women using other contraceptive methods. This indicates reduced menstrual iron losses and thus reduced iron demands in women on oral contraceptives [17]. In the present study no differences in the use of oral contraception were found between the two intervention groups. This indicates the importance of a balanced diet in relation to the bioavailability of the dietary iron.

In conclusion, biomarkers of iron status in women with initially low iron stores were maintained at a stable level when they consumed a meat-based diet for a period of 20 weeks, despite a relatively low dietary iron intake and blood losses by blood sampling as part of the study. In contrast, iron status declined in women with initially low iron stores when they consumed a vegetable-based diet with the same total iron content as the meat-based diet and similar blood losses. Our results emphasize the importance of the delicate balance between food iron content and ion bioavailability in the recommendations of dietary iron intake.

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