Original Paper

Annals of Nutrition & Metabolism

Ann Nutr Metab 2004;48:103–108
DOI: 10.1159/000077045

Received: May 16, 2003
Accepted: August 13, 2003
Published online: February 25, 2004

Dietary Iron Intake and Iron Status of German Female Vegans: Results of the German Vegan Study

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Key Words
Vegans, female · Dietary iron intake · Iron status · Iron deficiency

Abstract
Background: As shown in previous studies vegetarians and especially vegans are at risk for iron deficiency. Our study evaluated the iron status of German female vegans. ***Methods:*** In this cross-sectional study, the dietary intakes of 75 vegan women were assessed by two 9-day food frequency questionnaires. The iron status was analyzed on the basis of blood parameters. ***Results:*** Mean daily iron intake was higher than recommended by the German Nutrition Society. Still 42% of the female vegans <50 years (young women, YW) had a daily iron intake of <18 mg/day, which is the recommended allowance by the US Food and Nutrition Board. The main dietary sources of iron were vegetables, fruits, cereals and cereal products. Median serum ferritin concentrations were 14 ng/ml for YW and 28 ng/ml for women ≥50 years (old women, OW). In all, 40% (tri-index model (TIM) 20%) of the YW and 12% (TIM 12%) of the OW were considered iron-deficient based on either serum ferritin levels of <12 ng/ml or a TIM. Only 3 women had blood parameters which are defined as iron deficiency anemia. Correlations between serum ferritin levels and dietary factors were not found. ***Conclusion:*** Although the mean iron intake was above the recommended level, 40% (TIM 20%) of the YW were considered iron-deficient. It is suggested that especially YM on a vegan diet should have their iron status monitored and should consider taking iron supplements in case of a marginal status.

The EDEN Foundation, Bad Soden, Germany, and the Stoll VITA Foundation, Waldshut-Tiengen, Germany, sponsored this study.

Introduction

Despite the health benefits that the life style and dietary habits of vegetarians and vegans may provide, vegetarian diets have been associated with deficiencies in cobalamin, riboflavin, calciferol, calcium, zinc, and iron [1–3]. The bioavailability and absorption of iron is dependent on the source of iron, the composition of the diet as well as the iron status in the human body. Heme iron is dominant in animal-derived foods (with exception of eggs

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and milk), while non-heme iron is the only iron compound in plants. The absorption of non-heme iron from plant sources, which is low compared to heme iron, can be improved by dietary constituents such as ascorbic acid and other organic acids and can be decreased by components such as phytic acid and tannins [4–6]. The lack of heme iron as well as a high content of fiber and phytate in vegan diets make an adequate intake of iron questionable [7, 8]. Different definitions and cutoff points are used to classify iron deficiency. A serum ferritin concentration of <12 ng/ml indicates a depletion of iron storage and is therefore often used as an indicator for a marginal iron status. Bindra and Gibson [9] recommend the use of a tri-index model (TIM) to detect iron deficiency. This model is based on 3 independent tests of biochemical iron status representing the 3 different stages of iron deficiency (serum ferritin <12 ng/ml for depletion of iron storage, serum transferrin saturation <16% as an indicator of reduced transport iron supply, and mean cellular hemoglobin concentration, MCHC, <32 g/dl which indicates the final stage of iron deficiency). Currently, iron deficiency and iron deficiency anemia (IDA) are the most prevalent nutritional disorders in the world [10, 11]. Nearly 10% of all premenopausal women in Germany are classified as iron-deficient by health professionals [12, 13]. The purpose of our investigation was to determine the iron status in German vegan women using dietary intake data, information on menstrual cycle, and biochemical parameters (serum iron and ferritin concentrations, MCHC, transferrin saturation and hemoglobin concentrations).

Subjects and Methods

Recruitment and Screening

The German Vegan Study (GVS) was conducted in accordance with the Helsinki Declaration of 1964 as amended in 1983 and 1996. Since there was no intervention, the Ethic Commission of the State of Lower Saxony decided that approval was not required. All volunteers gave written consent prior to participation.

Participants were recruited by advertisements in German magazines. A total of 82 women were eligible for this evaluation because they did not consume iron supplements, fulfilled all study criteria (vegan nutrition at least 1 year prior to the beginning of the study, a minimum age of 18, no pregnancy or child birth during the last 12 months), and completed all parts of the study (pre- and main questionnaire, two 9-day food-frequency questionnaires, and blood sampling). All subjects were screened for general health status by means of a questionnaire.

Definition of 'Vegan'

As previously described, participants were classified as vegan when adhering to either a strict vegan diet (SV, n = 43) or a moderate vegan diet (MV, n = 32). The latter was considered as a vegan diet when it included a maximum of 5% of the ingested energy from eggs, milk and/or dairy products [14]. Mean energy intake (± SD) in MV females from products of animal origin was 1.38 ± 1.30% of the total energy which was provided by the mean daily consumption of 0.47 ± 1.18 g eggs, 5.39 ± 10.1 g milk and dairy products, 2.15 ± 2.54 g butter, and 1.0 ± 2.99 g cheese and curd.

Definition of 'Young Women' and 'Old Women'

Since iron metabolism is affected by iron loss [5], it was necessary to differentiate between premenopausal and postmenopausal women.

Fifty women in the age range of 18–49 years were classified as 'young women' (YW), while 25 women, who were 50 years or older, were classified as 'old women' (OW). This classification was based upon the women's statements regarding their menstruation and their climacteric period. All YW stated that they were not in their climacteric period, while 9 of the women aged 50+ were in their climacteric, and the remaining 16 were postmenopausal.

Dietary Data Collection and Analysis

As described elsewhere [14], each GVS participant was asked to complete two 9-day estimated food frequency questionnaires (FFQ). The FFQ used was a slight modification (complemented for vegan foods, foods of animal origin, except eggs, butter, milk and dairy products, were excluded) of the validated FFQ used in the Giessen Raw Food Study. The food item list was defined on the basis of the dietary intake results of the German National Food Consumption Survey. The single food items were classified into 14 different food groups according to the German National Food Consumption Survey. In order to minimize seasonal differences, one FFQ was sent in autumn, the other in spring. Members of the GVS team developed software (Paradox and Access database) on the basis of the German Nutrient Data Base (BLS II.2, Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin, Germany) to calculate the concentrations of ingested nutrients.

Hematological Measurements

A fasting venous blood sample was taken for the measurement of transferrin, ferritin, serum iron and hemoglobin. Transferrin and ferritin were measured using particle-enhanced immunonephelometry (Behring, Marburg, Germany). Serum iron concentrations were measured by atomic absorption (Perkin Elmer AAS 3100, Ueberlingen, Germany) [15]. The hemoglobin-cyanide method was used to measure hemoglobin [16–18], and MCHC was determined by Coulter® STKS (Beckman Coulter; VCS Technology, Krefeld, Germany).

Transferrin saturation was calculated using the standard formula: serum iron (μmol/l)/total iron-binding capacity (μmol/l), with total iron-binding capacity being defined as transferrin (g/l)·22.5 [19].

Statistical Analyses

A statistical analysis program (SPSS 10.0.7, Chicago, Ill., USA) was used to analyze the data. Results are represented as means ± SD and median plus 5 and 95 percentiles. Normal distribution of data was checked using the Kolmogorov-Smirnov test. In case of skewness, the Mann-Whitney U test was used to reveal statistically significant differences between the 2 subgroups (SV and MV; YW and OW). Given normal distribution, the independent sample t test was used. Dealing with nominal data, the χ^2 test was employed to evaluate statistically significant differences.

Table 1. Characteristics of the study population of female vegans (mean ± SD)

	19–50 years (n = 50)	≥ 50 years (n = 25)	p value
Age, years	35.4 ± 8.11	62.0 ± 8.31	per definition <0.001[a]
Duration of vegan diet, years	5.23 ± 3.60	6.98 ± 4.95	0.129[b]
BMI, kg/m^2	20.4 ± 2.00	21.9 ± 3.06	0.036[a]
Energy intake, MJ/day	7.20 ± 1.91	7.10 ± 1.79	0.831[a]
Energy intake from products of animal origin, % of total ingested energy	0.47 ± 0.96	0.82 ± 1.30	0.493[b]
Protein intake, % of energy	11.9 ± 1.67	11.6 ± 2.72	0.540[a]
Protein intake, g/day and kg body weight	0.90 ± 0.31	0.83 ± 0.22	0.307[a]
Dietary iron intake[1], mg/day	20.0 ± 5.77	19.6 ± 5.14	0.799[a]
Dietary fiber intake, g/day	52.3 ± 15.6	51.0 ± 14.7	0.739[a]
Vitamin C intake, mg/day	299 ± 135	336 ± 219	0.379[a]

[1] Excluding dietary supplements.
[a] t test. [b] Mann-Whitney U test.

Correlation analysis was done to reveal statistically significant associations between hematological factors and dietary components. In case of skewness, the Spearman correlation coefficient was used, and in case of normal distribution, Pearson's correlation coefficient was used.

Statistical significance was set at the 0.05 level.

Results

Subjects' Characteristics

The subjects' characteristics with respect to age-related subgroup allocation are shown in table 1. Statistically significant differences between the age-related subgroups for the shown parameters were only observed for the BMI ($p = 0.036$, t test).

In all, 58.0% of the women aged <50 (YW) and 56.0% of the women aged 50+ (OW) were SVs, the others were classified as MVs. Since YW and OW did not differ in the adherence to the vegan diet (SV or MV; $p = 0.869$, χ^2 test), further analysis will neglect this differentiation. Statistical significances between SV and MV female vegans were only found in the OW sub-cohort: SVs and MVs differed with respect to protein energy intake (SV 12.7 ± 2.59%, MV 10.1 ± 2.50%, $p = 0.017$, t test) as well as protein intake in grams per day and body weight (SV 0.93 ± 0.21 g/day·kg body weight; MV 0.70 ± 0.17 g/day·kg body weight, $p = 0.009$, t test).

Nutritional Intakes and Dietary Sources of Iron

The mean dietary iron intake did not differ statistically significantly between YW and OW (table 1). The German Nutrition Society recommends a daily intake of 15 mg/day for premenopausal and of 10 mg/day for postmenopausal women [20]. 18.0% of the YW and none of the OW had intakes below the German reference levels (table 1). The main sources for iron intake are shown in figure 1. Due to the fact that iron fortification of food was and still is not common in Germany, intake via fortified products can be neglected.

Hematological Status

Hematological status indicators of the YW and OW are shown in table 2. Only the serum ferritin concentrations differed significantly between YW and OW ($p = 0.001$, Mann-Whitney U test). In OW the median ferritin concentration was twice as high as in the YW. Using the TIM [9], 10 of the YW and 3 of the OW were considered iron-deficient. When serum ferritin alone (cutoff point 12 ng/ml) was considered as an indicator for iron deficiency, the prevalence of iron deficiency within the YW sub-cohort doubled (n = 20), while the number of OW classified as iron-deficient stayed the same. Thus, the difference between YW and OW became statistically significant ($p = 0.013$, χ^2 test). When employing the TIM and a low hemoglobin concentration (<120 g/l) to define IDA, 2 women younger than 50 and 1 woman aged more than 50 had IDA. However, correlations between hematological parameters and dietary factors (protein intake while con-

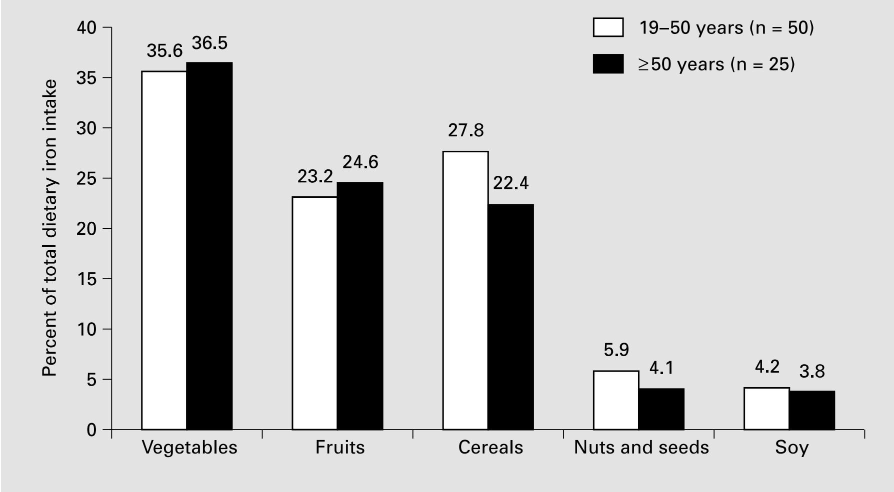

Fig. 1. Main sources of dietary iron intake in the GVS collective.

Table 2. Hematological status parameters of the vegan women (median; 5 and 95 percentiles)

	19–50 years (n = 50)	≥ 50 years (n = 25)	p value
Ferritin, ng/ml	14.0 (5, 84.6)	28.0 (5, 70.5)	0.001[a]
<12 ng/ml, %	40.0	12.0	
<6 ng/ml, %	12.0	8.0	
MCHC[1], g/dl	33.4 (32.2, 34.4)	33.3 (31.5, 34.6)	0.972[b]
<32 g/dl, %	2.0	4.0	
Transferrin saturation, %	20.0 (7.73, 43.6)	17.3 (8.58, 37.9)	0.462[b]
<16%, %	36.0	36.0	
Hemoglobin, g/l	132 (117, 151)	134 (117, 151)	0.390[b]
<120 g/l, %	6.0	8.0	
Iron deficiency anemia, %	4.0	4.0	0.986[c]

MCHC = Mean cellular hemoglobin concentration.
[a] Mann-Whitney U test. [b] t test. [c] χ^2 test.

trolling for dietary subgroup allocation; intake of iron, vitamin C, dietary fiber, alcohol, carbohydrates, fat, soy, tea and coffee) were not found for the total group or for the age-related subgroups.

Discussion

The nutritional adequacy of vegetarian diets has been the focus of several recent studies. Nevertheless, knowledge concerning the adequacy and nutritional effects of vegan diets is still limited mainly because most studies did not differentiate the various types of vegetarianism. The GVS was designed to fill this data gap. This is the first study in Germany that deals with a larger vegan population and was designed, among other things, to determine iron status in vegan women using dietary intake data and several biochemical parameters.

One major problem of dietary questionnaires is to establish a measuring instrument that is valid and at the same time not too inconvenient for the volunteers and thus resulting in high compliance. Weighing records are generally known to give exact results, but they are strongly reactive and the effort for participants is high. Direct, semiquantitative methods like the FFQ used here are easier to handle and result in higher compliance, but they can be less accurate. The validation (standard weighed records) of the FFQ similar to the one used showed that calculated intake of energy and main nutrients was quite accurate, while the intake of minerals and vitamins tended to be overestimated [21, 22].

The calculated iron intake seems to be overestimated through the FFQ in our study, because the mean dietary iron intake (excluding iron intake in the form of supplements) in GVS women was above the German reference level and the recommended daily allowance (18 mg/day for women <50 years and 8 mg/day for women ≥50 years), and also higher than the intakes reported in other vegetarian [20, 23–27] and vegan studies [7, 28]. Only Levin et al. [29] found a higher dietary iron intake in their vegetarian collective (females 29.7 ± 3.0 mg/day). Nevertheless, 18% of the YW did not reach the amount of 15 mg/day recommended by the German Nutrition Society [20]. When considering the US recommendation (18 mg/day) 42% of the YW did not have a sufficient intake [23]. Even though both recommendations envision higher iron intakes for premenopausal than for postmenopausal women, dietary iron intakes were comparable in the 2 age groups. As mentioned earlier further nutritional factors have an influence on iron absorption. Vegetarian and vegan diets provide large amounts of iron absorption inhibitors (i.e. dietary fiber, phytic acid) as well as enhancers (vitamin C, organic acids). However, no correlations were found between the serum ferritin concentration and the intake of absorption enhancers/inhibitors in the GVS cohort.

The nutrient density of iron exceeded the recommendations in 74 of the 75 GVS women. The main dietary sources of iron were vegetables, fruits, as well as cereal products (fig. 1). These items contain non-heme iron as well as iron absorption inhibitors such as dietary fiber and phytic acid. This may be the reason that the volunteers had a low iron status even though the documented intake was sufficient.

Assessing iron stores by plasma analyses of serum ferritin concentrations seems most common [5], but according to Cooper and Zlotkin [30] three or more blood samples are required to determine serum ferritin accurately because of the day-to-day variation. The GVS was designed as a cross-sectional study and blood samples were taken in more than 30 different German cities. For this reason it was not possible to collect three blood samples of each study participant. Yip and Dallman [5] advise the use of a multiple test instead of relying on the serum ferritin concentration alone when assessing iron deficiency. In our study we used a TIM: biochemical iron indices to detect iron deficiency were ferritin (<12 ng/ml), transferrin saturation (<16%) and MCHC (<32 g/dl). It has been shown that the TIM is more likely to detect iron deficiency than using cutoff points for single biomarkers alone [9]. But one should keep in mind that decreased transferrin saturation as well as decreased MCHC marks a more severe stage of iron deficiency than decreased serum ferritin alone [5]. Prevalence of iron deficiency measured only by serum ferritin (<12 ng/ml) is 4 times higher in the GVS women than in the average population: 20 of the 50 YW (40.0%) were considered iron-deficient, whereas only 10% of premenopausal women in Germany are classified as iron-deficient [12, 13]. Using the TIM the prevalence of iron deficiency is 2 times higher than in the average population. IDA is defined by the presence of iron deficiency plus hemoglobin concentrations of <120 g/l. The prevalence of IDA was 4.0% in the GVS women.

One possibility to prevent and treat iron deficiency is to increase the content and bioavailability of iron in the diet, mainly by including meat, fish or poultry and ascorbic acid-rich foods, and by decreasing consumption of tea and milk with meals [5, 31]. The major benefit of modifying the dietary habits in this way is the long-term effect on iron status [31]. But this option is impracticable for a vegan population who, on the one hand, avoid foods of animal origin and, on the other hand, have an already remarkably high mean daily vitamin C intake (YW 299 mg/day, OW 336 mg/day) when compared to the average population. A further possibility to improve iron status is the consumption of iron supplements. Absorption is enhanced when the supplement is taken between meals rather than with meals, and when the supplement is taken with water or juice rather than with tea, coffee or milk [5]. Iron from a multivitamin-mineral supplement is absorbed to a lesser extent than from an equivalent amount of iron given alone [32]. Due to the decreased absorption, it seems advisable to select multivitamin-mineral products that contain 60 mg of iron rather than the recommended dose of 30 mg. The calcium content of those products should be no more than 250 mg [5]. However, there is only little evidence of a long-term benefit (i.e. maintaining an increased serum ferritin concentration) of short-term iron supplementation [33]. Nevertheless, iron supplementation seems to be the best opportunity for a vegan population to treat and prevent iron deficiency. It is suggested that iron status should be monitored before, during and after supplementation because enhanced iron stores may be associated with an elevated risk of cancer [34] and coronary heart disease [35].

Conclusions

Although the mean iron intake of the women was above the recommended daily allowance, 40.0% (TIM 20.0%) of vegan women <50 years and 12.0% (TIM 12.0%) of women ≥ 50 years were considered iron-deficient. Our results indicate that especially young women adhering to a vegan diet should have their iron status monitored on a regular basis and, in case of iron deficiency, should consider taking iron supplements with good bioavailability. Due to the risk of cancer and coronary heart disease by enhanced iron levels, results of the intervention should be checked by assessment of hematological parameters.

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