# Iron status and dietary iron intake in vegetarians

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### **Abstract**

**Background.** Iron is one of the nutrients that require special consideration in a plant-based diet. The wide-spread belief is that meat is the best source of iron and a vegetarian diet increases the risk of its deficiency. This conviction has been the subject of analysis in a growing number of scientific reports.

**Objectives.** The aim of this study was to assess the iron intake and iron metabolism in vegetarians and vegans compared to a control group.

**Material and methods.** A total of 55 vegetarians and 36 healthy volunteers were studied. The following parameters were measured in serum: iron, ferritin, transferrin, transferrin receptor, and hepcidin-25, using the enzyme-linked immunosorbent assay (ELISA) method. The dietary iron intake was assessed using a 24-hour dietary recall.

**Results.** The mean daily intake (DI) of iron was significantly higher in the female vegan group compared to the control group. Iron, hepcidin-25, ferritin and transferrin receptor in serum remained within their normal ranges. The ferritin concentration was significantly decreased and that of transferrin significantly higher in both female groups and in the male vegan group.

**Conclusions.** The obtained results show that the studied parameters, excluding transferrin, remained within normal ranges. However, the ferritin concentration was significantly decreased in the female vegetarian group and also in both vegan groups. This may indicate low iron storage.

Key words: iron, ferritin, transferrin, vegetarianism, hepcidin-25

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# Introduction

Iron is involved in various bodily functions, including one of the most important, which is the transportation of oxygen from the lungs to all cells of the body. Additionally, this element is a component of hemoproteins, where it takes part in the electron transfer and oxygen reduction. Iron is also necessary in erythropoiesis, in plenty of immunological reactions and in the production of leukocytes, nucleic acids, neurotransmitters, collagen, hormones, and neurolemma. Iron is essential for proper cell metabolism and cellular respiration. Cell iron overload results in toxicity related to the increased production of free radicals and lipid peroxidation, which can lead to cell death; therefore, iron homeostasis requires strict regulations. <sup>1</sup>

Food is the only source of iron for the human body, delivering it in both the heme and non-heme form. It is assumed that only 1/3 of the iron in meat is heme, while the remaining 2/3 and all the iron content of plant products is non-heme. Vegetarianism is the practice of eliminating meat and the by-products of animal slaughter from one's diet.<sup>2</sup> Vegetarian diets contain no heme iron.<sup>3</sup> Non-heme iron primarily exists in an oxidized form (Fe<sup>3+</sup>), which is not bioavailable and first requires reduction to the Fe<sup>2+</sup> form to be transported across the intestinal epithelium.<sup>4</sup>

Studies conducted by Hallberg and Rossander-Hulthén showed that the amount of iron that is absorbed from a mixed diet (containing meat) ranges from 14% to 17%. However, the amount of non-heme iron absorbed from a vegetarian diet is between 5% and 12%.5 Another study has revealed similar results: 18% of iron from a mixed diet and 10% from a plant-based diet.6 Moreover, the amount of non-heme iron absorbed from a 1-product meal is 6 times smaller than the amount of heme iron absorbed from a 1-product meal. On the other hand, in mixed meals, non-heme iron bioavailability is just 2 times smaller.<sup>7</sup> This is the reason why the recommendation of iron intake is 1.8-fold greater compared with non-vegetarians. However, this recommendation was based on a test diet that was low in vitamin C and high in factors that reduce iron absorption.<sup>5</sup>

Factors enhancing iron absorption are: iron deficiency (pregnancy, lactation, growth), increased erythropoiesis, the malfunction of protein synthesis, or hemochromatosis. Other components manifesting the aforementioned effect are: amino acids, monosaccharides, animal proteins, ascorbic acid, organic acids, carotenoids, hydrochloric acid, fermented products, products made from fermented soy beans, and internal factor IF12.8

Non-heme iron absorption is inhibited by zinc, copper, manganese, cobalt, nickel, dietary fiber, carbohydrates, phosvitin, egg yolk albumins, oxalic acid, phosphate, phytic acid, salicylic acid, tannin, polyphenols, barbiturate, soporific drugs, and major calcium supplementation. Previously, it was surmised that also soy protein had a negative effect on iron bioavailability, but other studies showed that

iron contained in soy beans is in the ferritin form, which shows high bioavailability.<sup>9</sup>

Previously, researchers focused intently on diet components that enhanced or inhibited non-heme iron bioavailability, which has been proven by more recent studies to be less important than iron status. This is the reason why iron bioavailability is underestimated. 10 It is acknowledged that iron status in the human body is more important than its bioavailability, and that in women, menstrual blood loss (rather than their diet) is a major determining factor in iron metabolism. This means that the amount of iron absorbed depends on iron status of the body – people with low iron levels absorb more and excrete less iron. Additionally, non-heme iron absorption in people with a low iron level is as effective as the absorption of heme iron. Therefore, it is proven that the human body can adapt to new conditions.<sup>7,9,11–13</sup> Iron deficiency related to food intake patterns develops slowly over months or even years. Deficiency symptoms are most often detectable by laboratory diagnostic methods.<sup>14</sup>

The aim of this study was to assess the iron intake based on a 24-hour dietary recall and to estimate iron metabolism (the concentration of iron, ferritin, transferrin, transferrin receptor, and hepcidin-25) in groups of vegetarians and vegans compared to a control group.

## **Material and methods**

This study was performed between February and May 2013 in the Department of Clinical Nutrition at the Medical University of Gdańsk, Poland. Participants aged over 18 years were recruited from Gdańsk, Gdynia and Sopot in Poland via information on websites. This study was part of the nutritional status of vegetarians research (No. MN 01-0098/08). The study received all the necessary approvals, including one from a bioethics review committee of the Medical University of Gdańsk, Poland.

The data was segregated according to gender into:

- a vegetarian female group (n = 21), abbreviated as VEG<sub>1</sub>;
- a vegetarian male group (n = 11), abbreviated as VEG<sub>2</sub>;
- a vegan female group (n = 18), abbreviated as VEG<sub>3</sub>;
- a vegan male group (n = 5), abbreviated as VEG<sub>4</sub>.

Thirty-six omnivores were divided by gender and included in 2 control groups. All participants from both groups were healthy and without any acute or chronic diseases, with a good nutritional status. The subjects were not taking any medication. The inclusion criterion for the study was a minimum of 1 year on a vegetarian diet.

The basic characteristic of the study groups is presented in Table 1.

### **Diet assessment**

A retrospective method of diet assessment – a 24-hour dietary recall (24HR) – was used. This method, in the form of a structured interview, aimed to capture detailed

**Table 1.** The basic characteristic of the study groups

| Group            | Number     | Age [years]                   | BMI [kg/m²]        |
|------------------|------------|-------------------------------|--------------------|
| VEG <sub>1</sub> | 21         | 29.95 ±7.26                   | 22.2               |
| VEG <sub>2</sub> | 11         | 29.64 ±4.06                   | 22.3               |
| VEG <sub>3</sub> | 18         | 32.1 ±10.01                   | 21.5               |
| VEG <sub>4</sub> | 5          | 34.8 ±5.26                    | 23.0               |
| Control<br>F/M   | 36<br>31/5 | F: 27.4 ±9.8<br>M: 32.6 ±12.9 | F: 20.8<br>M: 24.7 |

BMI – body mass index; VEG $_1$  – vegetarian female group; VEG $_2$  – vegetarian male group; VEG $_3$  – vegan female group; VEG $_4$  – vegan male group; F – females; M – males.

information about all foods and beverages consumed by the respondent over the previous 24-hour period. The interview was conducted by a trained nutritionist. The data was analyzed by a computer program called Cronometer® (Cronometer Software Inc., Revelstoke, Canada). This software enables the user to analyze single foods and beverages and estimate their nutrient values. Additionally, the daily iron intake was compared to the Recommended Dietary Allowance (RDA) values established by the National Food and Nutrition Institute in Poland.

### Laboratory parameters

Venous blood was collected after a 12-hour period of fasting, centrifuged and stored at  $-80^{\circ}$ C.

The following biochemical parameters were measured in serum:

- iron, by the in vitro assay method, with Roche Diagnostic (Mannheim, Germany) reagents and a Hitachi 704 device (Roche Diagnostics, Basel, Switzerland);
- ferritin, transferrin receptor and hepcidin-25, by the enzyme-linked immunosorbent assay (ELISA) method and an ELIZAMAT 3000 device (DRG Diagnostics, Marburg, Germany);
- transferrin, by the ELISA method (Assaypro, St. Charles, USA) and an ELIZAMAT 3000 device.

# Statistical analysis

The data is expressed as the mean with standard deviation (SD) and median. The Kolmogorov-Smirnov test was used to verify whether the variable distribution was normal. The differences between the means were evaluated by an independent Student's t test, and the Mann-Whitney U test was used when the distribution of the variables was not normal. Spearman's rank correlation coefficient (R) was used to evaluate the relationships between the variables. Statistical analysis was performed using Microsoft Office Excel 2007 (Microsoft, Redmond, USA) and STATISTICA v. 12.0 (StatSoft Inc., Tulsa, USA). A p-value <0.05 was considered as statistically significant.

### Results

#### Iron intake

The results of the daily iron intake are presented in Table 2 and Fig. 1 for women, and in Table 3 and Fig. 2 for men. The mean daily iron intake was significantly lower in the control group in comparison to the VEG<sub>3</sub> group (p = 0.002). In the vegan female group (VEG<sub>3</sub>), a significantly higher daily intake of iron in comparison to vegetarian female group (VEG<sub>1</sub>) (p = 0.0256) was observed. Among the female participants, no one (0%) from the VEG<sub>1</sub> group and only 2 subjects (18%) from the VEG<sub>3</sub> group implemented the iron intake recommendations for vegetarians. Among the male participants, 67% from the VEG<sub>2</sub> group and 75% from the the VEG<sub>4</sub> group implemented those recommendations.

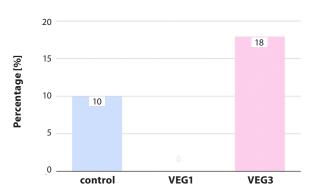


Fig. 1. The percentage of women who met the recommendation for the iron intake

VEG<sub>1</sub> – vegetarian female group; VEG<sub>3</sub> – vegan female group.

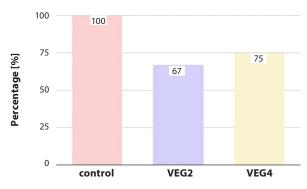


Fig. 2. The percentage of men who met the recommendation for the iron intake

 $VEG_2$  – vegetarian male group;  $VEG_4$  – vegan male group.

### Iron status

The iron concentration was within a normal range in all studied groups (Tables 2,3). There were no significant differences in the iron concentration between any groups.

Table 2. Women: the iron intake and serum concentrations of iron, hepcidin-25, ferritin, transferrin, and transferrin receptor (values are arithmetic mean  $\pm$  standard deviation (SD), median and range)

| Values                                    | VEG₁ group                        | VEG₃ group                         | Control group                      | p-value* | p-value** | p-value*** |  |  |
|---|-----------------------------------|------------------------------------|------------------------------------|----------|-----------|------------|--|--|
| Iron intake [mg] <sup>1</sup>             |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 14.22 ±5.75<br>12.7<br>5.1–27.6   | 19.86 ± 8.87<br>18.1<br>5.1–41.5   | 13.25 ±4.78<br>11.6<br>7.7–25.9    | 0.524    | 0.002     | 0.0256     |  |  |
| lron [μg/dL]²                             |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 110.71 ±43.85<br>106<br>44–177    | 91.28 ±47.14<br>79<br>36–182       | 111.68 ±38.32<br>109<br>41–170     | 0.933    | 0.1056    | 0.191      |  |  |
| Hepcidin-25 [ng/mL] <sup>3</sup>          |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 11.73 ±4.15<br>11.0<br>5.40–21.36 | 12.35 ±5.91<br>11.53<br>1.21–26.10 | 15.82 ±6.16<br>17.12<br>5.04–25.70 | 0.0174   | 0.0899    | 0.699      |  |  |
| Ferritin [ng/mL]⁴                         |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 12.16 ±10.85<br>8.1<br>5.0-45.3   | 11.21 ±13.78<br>6.4<br>5.0-62.3    | 15.55 ±11.75<br>9.8<br>5.0-42.3    | 0.349    | 0.3085    | 0.8098     |  |  |
| Transferrin [g/L] <sup>5</sup>            |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 3.93 ±1.45<br>4.03<br>1.1-6.0     | 3.87 ±1.52<br>3.16<br>1.91–6.00    | 3.19 ±1.15<br>3.1<br>1.1-5.3       | 0.085    | 0.133     | 0.903      |  |  |
| Transferrin receptor [µg/mL] <sup>6</sup> |                                   |                                    |                                    |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 0.70 ±0.43<br>0.55<br>0.35-1.85   | 0.55 ±0.24<br>0.43<br>0.35-1.15    | 0.57 ±0.2<br>0.5<br>0.35-1.05      | 0.211    | 0.831     | 0.188      |  |  |

 $<sup>^1</sup>$  18 mg (x 1.8 for vegetarians and vegans);  $^2$  37–145  $\mu$ g/dL;  $^3$  1.0–39.3 ng/mL;  $^4$  10–120 n/mL;  $^5$  2.0–3.6 g/L;  $^6$  0.378–1.513  $\mu$ g/mL; VEG $_1$  – vegetarian female group; VEG $_3$  – vegan female group; p-value \* – VEG $_1$  group vs control group; p-value \*\* – VEG $_3$  group vs control group; p-value \*\*\* – VEG $_3$  group.

Additionally, a positive correlation between the concentration of iron and ferritin (R = 0.19; p = 0.023) in the VEG<sub>1</sub> group was observed.

### Hepcidin-25

The mean hepcidin-25 level was lower in the VEG $_1$  group in comparison to the control group (p = 0.0174) (Table 2). However, the mean hepcidin-25 concentration was maintained within the references range (1.0–39.3 ng/mL) in all groups. In the VEG $_1$  group, a positive correlation between the hepcidn-25 and ferritin concentration (R = 0.60; p = 0.025) was observed.

#### **Ferritin**

The mean ferritin concentration was maintained within normal limits (for men: 20–250 ng/mL, for women: 10–120 ng/mL) in all groups; the results are presented in Tables 2 and 3.

# Transferrin and transferrin receptor

The mean transferrin concentration was above the reference range in the VEG<sub>1</sub> (3.93  $\pm 1.45$  g/L), VEG<sub>2</sub> (3.87  $\pm 1.52$  g/L) and VEG<sub>4</sub> (4.19  $\pm 0.66$  g/L) groups. In both control groups, the mean transferrin level was maintained

within a normal range (Tables 2, 3). There were no significant differences in the transferrin concentration between any groups.

The mean transferrin receptor concentration was maintained within normal limits. There were no significant differences between any of the vegetarian groups and the control groups.

### Discussion

The mean intake of iron among the vegetarians in our study was 18.53 ±8.23 mg; only 20% of participants met the recommended daily iron intake for vegetarians. However, our results showed that the mean daily iron intake in the female vegan group was significantly higher (19.86 ±8.87 mg) compared to the control group (13.25  $\pm 4.78$  mg). The National Food and Nutrition Institute in Poland recommends a daily intake (DI) of iron of 10 mg for men and 18 mg for women, and a 1.8-fold greater intake for vegetarians and vegans.16 The recommended iron intake for men is 18 mg/day and for women 33 mg/day, at which point meeting the demand exclusively by food consumption becomes difficult and requires considerable knowledge in this area. Nevertheless, 100% of female vegetarians and 82% of female vegans, along with 33% of male vegetarians and 25% of male vegans, did not reach the daily recommendation for the iron intake.

**Table 3.** Men: the iron intake and serum concentrations of iron, hepcidin-25, ferritin, transferrin, and transferrin receptor (values are arithmetic mean  $\pm$  standard deviation (SD), median and range)

| Values                                    | VEG₂ group                         | VEG₄ group                         | Control group                       | p-value* | p-value** | p-value*** |  |  |
|---|------------------------------------|------------------------------------|-------------------------------------|----------|-----------|------------|--|--|
| Iron intake [mg] <sup>1</sup>             |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 22.98 ±7.85<br>21.2<br>14.4–38.6   | 24.4 ±8.72<br>22.5<br>16.0–36.6    | 17.67 ±2.36<br>17.9<br>15.2–19.9    | 0.2879   | 0.2586    | 0.7755     |  |  |
| lron [μg/dL]²                             |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 124.00 ±36.80<br>134<br>66-170     | 121.80 ±40.46<br>127<br>71–166     | 130 ±26.71<br>127<br>95–162         | 0.75     | 0.715     | 0.916      |  |  |
| Hepcidin-25 [ng/mL] <sup>3</sup>          |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 18.12 ±7.96<br>17.87<br>7.22–33.06 | 13.02 ±3.79<br>14.95<br>7.89–16.57 | 20.89 ±7.52<br>19.43<br>12.63–32.15 | 0.523    | 0.07      | 0.2        |  |  |
| Ferritin [ng/mL] <sup>4</sup>             |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 36.29 ±24.75<br>40.5<br>6.0-74.5   | 21.22 ±15.38<br>16.0<br>8.5–46.3   | 55.66 ±39.28<br>78.0<br>12.1–88.7   | 0.246    | 0.105     | 0.234      |  |  |
| Transferrin [g/L] <sup>5</sup>            |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 3.39 ±1.60<br>3.05<br>1.6-6.0      | 4.19 ±0.66<br>4.4<br>3.02-4.60     | 3.03 ±1.11<br>3.16<br>1.4-4.2       | 0.658    | 0.08      | 0.307      |  |  |
| Transferrin receptor [μg/mL] <sup>6</sup> |                                    |                                    |                                     |          |           |            |  |  |
| Mean ±SD<br>Median<br>Range               | 0.52 ±0.18<br>0.45<br>0.35-0.85    | 0.71 ±0.46<br>0.45<br>0.35-1.35    | 0.64 ±0.22<br>0.6<br>0.35-0.95      | 0.249    | 0.767     | 0.2367     |  |  |

 $<sup>^{1}</sup>$  10 mg (x 1.8 for vegetarians and vegans);  $^{2}$  59–158  $\mu$ g/dL;  $^{3}$  1.0–39.3 ng/mL;  $^{4}$  20–250 n/mL;  $^{5}$  2.0–3.6 g/L;  $^{6}$  0.378–1.513  $\mu$ g/mL; VEG $_{2}$  – vegetarian male group; VEG $_{4}$  – vegan male group; p-value\*\* – VEG $_{2}$  group vs control group; p-value\*\* – VEG $_{4}$  group vs control group; p-value\*\*\* – VEG $_{2}$  group vs VEG $_{4}$  group.

Results of other studies also proved that vegetarians did not follow the recommendations established for them. Lee et al. analyzed the 3-day menus of 54 Buddhist vegetarian nuns.<sup>17</sup> The mean iron intake in this group was 14.1 mg/day, while in non-vegetarian Catholic nuns and students, it was 15.2 mg/day and 10.0 mg/day, respectively. It is assessed that among vegetarian females, the iron intake ranges from 11 to 18 mg/day. Additionally, among non-vegetarian girls, a low intake of iron was observed. Broniecka et al. studied 159 non-vegetarian girls at the age of 17–18 years. The median quantity of iron amounted to 6.4 ±2.8 mg/day, which is below the Estimated Average Requirements (EAR), i.e., 8 mg/day.<sup>18</sup> Wolnicka and Taraszewska presented similar results having surveyed 193 non-vegetarian girls aged 11-13 years. The iron content of their diet was 7.4 ±3.1 mg/day.<sup>19</sup> The studies showed that a vegetarian and vegan diet can be associated with an increased risk of nutrient deficiencies and anemia.<sup>20</sup> The elimination of meat and the intake of plant-based products, which contain only non-heme iron, are associated with decreased iron bioavailability. Therefore,  $% \left( x\right) =\left( x\right) +\left( x\right)$ the recommendations for the iron intake are 1.8-fold higher for vegetarians. Iron bioavailability is enhanced by ascorbic acid, resistant starch, oligosaccharides, vitamin A, and β-carotene. 21 Observational studies showed that a vegetarian diet is associated with a higher intake of vitamin C, vitamin E, fibre, magnesium, and  $\beta$ -carotene than a nonvegetarian diet. At the same time, a plant-based diet leaves vegetarians with a lower intake of vitamin  $B_{12}$ , vitamin D and zinc.<sup>2</sup>

It has been proven that iron absorption depends on the composition of the diet and its preparation. However, in subjects whose diet contains only non-heme iron, the absorption mechanisms adapt to new conditions.<sup>3</sup> Roughead and Hunt showed that both heme and non-heme iron absorption was increased in people with low iron levels. However, non-heme iron bioavailability increased even 10-15fold compared to the heme form, for which the increase was only 2–3-fold. The heme iron study involved subjects who ate meals containing beef, which is rich in highly bioavailable iron.21 The British 2003 National Diet and Nutrition Survey has shown that a plant-based diet does not provide less iron compared to a diet that contains meat.<sup>22</sup> Several studies showed that a well-balanced vegetarian diet provides the required iron amounts and sometimes even exceeds the references<sup>9,20,22</sup> Similar results were obtained in an EPIC-Oxford cohort study, published in 2003. A total of 33,883 meat-eaters and 31,546 non-meat-eaters took part in that study. The mean intake of iron was 12.6 mg/day among vegetarian women and 13.9 mg/day among vegetarian men. Moreover, the intake of this mineral was 14.1 mg/ day among vegan women and 15.3 mg/day among vegan men, which makes it the highest intake rate of all groups.<sup>23</sup>

Our study showed no deficiency in iron, hepcidin-25, ferritin, and transferrin receptors in vegetarians and vegans.

The transferrin level was significantly higher in female vegetarian and vegan groups and in the male vegan group, while the ferritin level was lower in the female vegetarian and vegan groups and also in the male vegan group.

In 1989, the US Food and Nutrition Board declared that anemia caused by iron deficiency occurs in vegetarians as often as in non-vegetarians, and this claim has upheld until now.<sup>6</sup> Bhatti et al. showed the iron levels in children and adults among Hindu Brahmins (who observe a strict vegetarian diet) compared to vegetarians from other communities, considered a control group. The mean iron concentration in serum was 95.6 µg/dL among Brahmin boys (control group – 85.7 μg/dL) and 90.7 μg/dL among Brahmin girls (control group – 87.0 μg/dL). The mean iron level was 85 μg/dL among Brahmin men (control group – 83.1 μg/dL) and 72.5 μg/dL among Brahmin women (control group  $-65.5 \mu g/dL$ ). The results show that the mean iron concentration in the Brahmin community was maintained within a normal range and was higher compared to the control group, which may mean that the daily iron intake was adequate.<sup>24</sup> Obeid et al. assessed the iron concentration in vegans, lacto-ovo-vegetarians and occasional meat-eaters (semi-vegetarians). These results show that the mean iron level in all groups was maintained within a normal range and any differences between those groups were insignificant – in the vegan group, the mean iron concentration in blood serum was 80 µg/dL, in vegetarians 91 μg/dL and in semi-vegetarians 93 μg/dL.<sup>25</sup>

In our study, the mean hepcidin-25 concentration in all groups was maintained within the normal range. But in the female vegetarian group, hepcidin-25 was significantly lower than in the control group. Hepcidin-25 synthesis and secretion by the liver is controlled by iron stores within macrophages, inflammation, hypoxia, and erythropoiesis. In response to an increased iron concentration, hepcidin-25 synthesis is upregulated in the liver, which regulates iron absorption, its storage and recycling from macrophages. Hepcidin-25 is a direct inhibitor of ferroportin, a protein that transports iron out of the cells that store it. 26,27 The production of hepcidin-25, depending the content of iron in the body, is carried out by high Fe human hemochromatosis protein (HFE), transferrin receptor 1 and 2 (TfR1 and TfR2), bone morphogenetic protein 6 (BMP6), hemojuvelin, and transferrin. In response to the expanding resources of iron, the liver produces hepcidin-25, which inhibits the intestinal absorption and prevents further excessive accumulation. A decreased level of hepcidin-25 observed in our study among vegetarian women can cause an increased intestinal iron absorption and iron release from macrophages.<sup>28</sup>

In our study, the mean ferritin concentration in all groups was maintained within the normal range. However, its level was significantly lower among female vegetarians and particularly among female and male vegans compared to omnivores. Subjects on a plant-based diet, even if the iron intake was adequate, exhibited a lower ferritin concentration

(but still maintained within normal limits) in comparison to non-vegetarians. However, the mechanism underlying this effect has not been elucidated. 11 Lower ferritin levels mainly concern lacto-ovo-vegetarian women, although only 3 out of 11 have a lower hematocrit and hemoglobin concentration.3 There have been many studies showing a similar association. Kim and Bae examined 107 postmenopausal vegetarian women. The ferritin level in this group was significantly lower than in the control group, but it was maintained within the normal range.<sup>29</sup> In another study, Obeid et al. noticed no differences in the ferritin level between vegans, lacto-ovo-vegetarians and semi-vegetarians.<sup>25</sup> A reduced ferritin level is always related to iron depletion.8 Contrarily, some authors suggested that a high ferritin concentration is associated with an increased risk of metabolic syndrome or chronic degenerative diseases, such as Alzheimer's disease. In 1998, Fernández-Real et al. revealed that there is a strong correlation between a high ferritin level and the development of insulin resistance contributing to an increased risk of type 2 diabetes.<sup>30,31</sup>

In our study, the mean transferrin concentration was shown to be elevated in both the female vegetarian and vegan groups, and also in the male vegan group. An increased transferrin concentration can be associated with iron deficiency and might be due to multiple reasons, such as inadequate food processing, insufficient amount of vitamin C in the diet, low iron content in the diet and other reasons, e.g., malabsorption or blood loss. 32 An increased transferrin concentration, similarly to a decreased ferritin level in this study, may result from the fact that vegetarian food provides only non-heme iron, which manifests lower bioavailability than heme iron. Therefore, the preparation of plant-based meals requires techniques that increase iron bioavailability, including soaking, cooking, sprouting, fermentation, consumption of products rich in vitamin C along with products rich in non-heme iron, and others.8 The results obtained in this study vary from the former research results. In a study by Deriemaeker et al., the mean transferrin level among elderly Dutch vegetarians was higher compared to the control group; however, contrary to our study, it remained within the normal range.<sup>33</sup> Furthermore, Obeid et al. showed that the transferrin concentration in blood serum among lacto-ovo-vegetarians was 2.4 g/L, among vegans 2.3 g/L and among semi-vegetarians 2.3 g/L – there was no real variation in the results between all these groups and all were within the normal range.<sup>25</sup>

In our study, the transferrin receptor was within the normal range in all the groups studied, and there were no significant differences between them. Furthermore, other studies show no differences in the level of transferrin receptor. Walls et al. examined 2 groups of men aged from 59 to 78 years. Those in the  $1^{\rm st}$  group were put on a plant-based diet for 14 weeks, while the men in the  $2^{\rm nd}$  group were on a diet including beef for the same period of time. The transferrin receptor concentration among the meat-eaters

decreased from 4.9  $\pm 2.5~\mu g/mL$  to 4.6  $\pm 2.5~\mu g/mL$  in the 5<sup>th</sup> week of the study, and thereafter to 4.3  $\pm 1.9~\mu g/mL$  in the 12<sup>th</sup> week of the study. In the vegetarian group, the transferrin receptor concentration increased from 4.6  $\pm 1.7~\mu g/mL$  to 4.9  $\pm 2.0~\mu g/mL$  in the 5<sup>th</sup> week of the study and it maintained the same level until the 12<sup>th</sup> week of research. The differences in the transferrin receptor level are associated with the iron bioavailability level. A diet that includes only non-heme iron is associated with an increased transferrin receptor level, as observed in the vegetarian group. However, food rich in heme iron caused a decrease in the transferrin receptor concentration, as observed in the group of meat-eaters.<sup>34</sup>

The limitation of our study is the lack of measurement of hemoglobin and other morphology parameters. Moreover, a single 24-hour dietary recall does not show that habitual intake (because it is less accurate method compared to 72-hour dietary recall); additional methods of evaluating food habits should be used in further studies.

# **Summary**

Some authors have shown that the risk of developing anemia amongst vegetarians who follow a well-balanced diet (rich in vitamin C and iron) is not higher than in the case of non-vegetarians.  $^{35}$ 

Our results suggested a low storage of iron in the vegetarian and vegan groups studied (low ferritin, high transferin levels). However, the decreased level of hepcidin-25 observed in our study among vegetarian women is one of the mechanisms possibly increasing the absorption of iron. We conclude that periodic monitoring of basic biochemical parameters is recommended, especially among vegans. Also, exhaustive information about vegan and vegetarian diet is necessary.

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