Research Article

Natalia Wawrzyniak, Anna Gramza-Michałowska, Joanna Suliburska*

Effect of pumpkin enriched with calcium lactate on iron status in an animal model of postmenopausal osteoporosis

Abstract: The homeostasis of calcium (Ca) and iron (Fe) is disturbed during menopause. The present study aimed to determine the effects of Ca-enriched pumpkin on Fe status in ovariectomized rats. A total of 48 female Wistar rats were divided into six groups. One group was fed a standard diet (C), while the other five groups were ovariectomized and fed a standard diet (OVX), a calcium lactate diet (CaL_OVX), calcium lactate-enriched pumpkin (PCaL_OVX), calcium lactate and alendronate (CaL_OVX_B), and calcium lactate-enriched pumpkin and alendronate (PCaL_OVX_B), respectively. The nutritional intervention lasted 12 weeks and rats were euthanized. Tissue samples were collected, and the iron content in the samples was assessed. A comparison of all groups showed a reduction in iron concentrations in femurs, liver, hair, spleen, and kidneys in the ovariectomized groups than in the control group. The PCaL_OVX_B group had a significantly higher blood hemoglobin concentration than the control group. Moreover, spleen and liver Fe concentrations were the highest in PCaL_OVX and PCaL_OVX_B rats among the treated groups and were comparable with the control group. These results indicate that ovariectomy decreases Fe status in rats. Calcium lactate-enriched pumpkin with and without alendronate can increase Fe concentration in liver and spleen in ovariectomized rats.

Keywords: iron metabolism, ovariectomy, enriched pumpkin, calcium

1 Introduction

Menopause is associated with a reduction in estrogen levels, which leads to a significant decrease in bone mineral density, due to increased bone resorption [1]. Inhibition of endogenous ovarian function also leads to changes in the metabolism of minerals including iron (Fe). Studies that compared the serum of premenopausal and postmenopausal women showed significantly increased Fe concentrations and an increase in Fe stores at the end of menstruation [2,3], as indicated by up to threefold higher ferritin concentration [4]. In men, the accumulation of Fe in the body occurs from the stage of puberty. In the case of women, Fe accumulation takes place in the reproductive period, during which there is a systematic loss of Fe along with the monthly blood, and hence its accumulation begins only after menopause [5]. Fe status is determined from the serum concentration of ferritin, as well as hemoglobin (HGB), which also increases after menopause [6]. Changes in Fe metabolism affect the skeletal system; Fe overload results in the inhibition of bone formation and increased resorption, which is independent of the effect of estrogen [7]. Evidence shows that increased bone resorption activity is associated with an increase in urinary deoxypyridinoline in a rat model of postmenopausal osteoporosis and changes in bone architecture such as a decrease in the number of bone trabeculae and their thinning [8]. In contrast, reducing Fe overload may contribute to the normalization of bone resorption and formation [9], which can be achieved by using agents that control Fe stores, such as hepcidin [10]. If the concentration of Fe is so high that transferrin is unable to bind it, free Fe is deposited in the...
organisms affecting them permanently [11], through various mechanisms, including increased oxidative stress [7].

During menopause, the calcium status is low in women [12]. In menopausal women, insufficient calcium intake results in lower Ca concentration [13] and higher serum parathyroid hormone levels, which increase bone turnover and accelerate resorption [12]. Therefore, a daily calcium dose of at least 1,200 mg is recommended for this group of women [14]. However, most of these women take only half of the recommended daily dose [15]. Thus, to effectively treat osteoporosis, an adequate amount of calcium should be included in the diet and ingredients increasing calcium bioavailability should also be taken [16]. It has been shown that dairy products, legumes, and fortified foods are good sources of calcium and help to maintain its balance [17].

Ca may inhibit the absorption of Fe by affecting the divalent metal transporter (DMT1) or inhibit the transfer of Fe ions to the blood. However, according to studies, this is only a short-term effect, and compensatory actions such as long-term Ca supplementation can prevent the negative effects associated with Fe metabolism [18]. Because both Fe and Ca play a role in bone metabolism and interact at the absorption level, it may be interesting to understand the effects of Ca-enriched food products on Fe status in rats. Pumpkin enriched with calcium is an innovative product, which is also enriched with inulin. Inulin is necessary in the process of osmotic dehydration as an osmotically active substance. Inulin is a fructan polysaccharide that has beneficial effects on the body, changing the composition of the microbiota, stimulating immune functions, reducing constipation. Inulin also affects the bioavailability of minerals [16]. It forms complexes with iron in the intestinal tract, which leads to an increase in its absorption by extending the time of residence in the intestine. It also increases the bioavailability of calcium by stimulating the growth of colon cells, thereby increasing the absorption surface and stimulating the expression of calcium-binding proteins [16,17].

Therefore, this study aimed to determine the effects of Ca-enriched pumpkin on Fe status in ovariectomized rats.

2 Methods

2.1 Materials and reagents

Calcium lactate and inulin were purchased from Agnex (Białystok, Poland). Pumpkin (yellow melon, Cucurbita maxima) was obtained from an organic farm (with the consent of the land owner). Dietary ingredients (minerals, vitamins, and macroingredients) were procured from Sigma-Aldrich (Darmstadt, Germany), Hortimex (Konin, Poland), and Warchem (Warszawa, Poland).

2.2 Osmotic dehydration

Through osmotic dehydration, pumpkin tissue was enriched with calcium lactate. Briefly, calcium lactate was dissolved in an inulin solution (5:1 with distilled water) to make up 5% of the content. Frozen pumpkin flesh cubes were added to this mixture in a 1:5 ratio. The jars containing pumpkin cubes were shaken in a 50°C water bath for 2 h. Then, enriched pumpkin was drained and freeze-dried. The prepared pumpkin powder was added to the diets of rats [19].

2.3 Animals

A total of 48 female Wistar rats aged 12 weeks were obtained from the Center for Advanced Technologies in Greater Poland, University of Adam Mickiewicz in Poznań, Poland. All animal experiments were carried out following the guidelines for the use and care of laboratory animals according to the EU Directive 2010/63/EU. The study was approved by the Local Ethics Committee in Poznań (no. 34/2019).

2.4 Experimental protocols

During the experiment, the rats were fed the standard AIN-93M diet [20]. The animals were divided into six groups of eight each. The average initial body weight of rats was 267.7 ± 16.1 g. Five of these groups (40 rats) were ovariectomized, and a 12-week nutritional intervention was introduced after the recovery period (2 weeks after ovariectomy). One of the ovariectomized (OVX) groups and the control group (C) received the standard AIN-93M diet without modifications, whereas the other four groups received one of the following modified diets: calcium lactate diet (CaL_OVX), calcium lactate-enriched pumpkin (PCaL_OVX), alendronate and calcium lactate (CaL_OVX_B), or calcium lactate-enriched pumpkin and alendronate (PCaL_OVX_B). The dietary components are summarized in the previous study [19]. The design of the experiment is presented in Figure 1. Dietary supplements were added such that the calcium content remained the
same (0.5% Ca in each of modified diet), while the dose of alendronate was adjusted weekly based on the actual body weight of the animals (3 mg/kg of body mass). The rats were provided food and water ad libitum, and the consumption was recorded daily. Body weight was measured weekly and at the end of the experiment using a Bruker LF90II body composition analyzer. After 12 weeks, the rats were euthanized by decapitation. Whole blood was collected for the determination of HGB. Femurs, spleen, pancreas, heart, liver, brain, kidneys, and muscles were isolated for analysis. Hair was collected from the interscapular area of all rats. Tissues were weighed, washed with saline, and frozen at −80°C.

2.5 Fe analysis in diets

To determine the Fe content in the studied diets, 1 g of each diet was burned at 450°C in a muffle furnace until mineralization. Then, the samples were dissolved in 1 mol/l nitric acid (Merck, Kenilworth, NJ, USA). The dissolved samples were diluted with appropriate amounts of deionized water (AAS-3, Carl Zeiss, Jena, Germany), and their mineral content was determined using flame atomic absorption spectrometry (AAS). The method was validated using a certified reference material (BCR191, Sigma-Aldrich, St. Louis, MO, USA) with an accuracy of 92%. All samples were analyzed in triplicate.

2.6 HGB determination in blood

Whole-blood HGB determination was carried out by a commercial laboratory (Alab, Poznań, Poland).

2.7 Fe analysis in tissues

Samples containing pure nitric acid (Merck, Kenilworth, NJ, USA) were mineralized in a Microwave Digestion System (Speedwave Xpert, Berghof, Eningen, Germany) to determine the Fe content in tissues. After digestion, the samples were diluted with deionized water. Flame AAS was used to determine the content of minerals (AAS-3; Carl Zeiss, Jena, Germany) at a wavelength of λ = 248.3 nm. The method was validated using bovine liver as certified reference material (1577C; Sigma-Aldrich, St. Louis, MO, USA) with an accuracy of 91%.

2.8 Statistical analysis

Statistical analyses were performed using the Statistica program (StatSoft, Tulsa, OK, USA). The Shapiro–Wilk test was used to determine the normality of the distribution of the variables. One-way analysis of variance with Tukey’s post hoc test was used to identify the statistical
Body mass gain

Table 1: Daily intake, iron intake, and body mass gain (mean and standard deviation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>OVX</th>
<th>CaL_OVX</th>
<th>CaL_OVX_B</th>
<th>PCaL_OVX</th>
<th>PCaL_OVX_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily intake of diet (g)</td>
<td>25.08 ± 0.63</td>
<td>25.11 ± 1.70</td>
<td>25.90 ± 0.55</td>
<td>25.66 ± 2.29</td>
<td>24.31 ± 1.26</td>
<td>24.84 ± 2.29</td>
</tr>
<tr>
<td>Fe diet content (µg/g dm)</td>
<td>35.77 ± 0.59</td>
<td>35.77 ± 0.59</td>
<td>35.77 ± 0.33</td>
<td>35.77 ± 0.33</td>
<td>35.67 ± 0.91</td>
<td>35.67 ± 0.91</td>
</tr>
<tr>
<td>Fe daily intake (µg)</td>
<td>901.01 ± 23.88</td>
<td>896.77 ± 65.41</td>
<td>938.23 ± 25.99</td>
<td>903.02 ± 66.53</td>
<td>869.48 ± 41.31</td>
<td>869.11 ± 97.35</td>
</tr>
<tr>
<td>Body mass gain (g)</td>
<td>55.45 ± 20.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114.71 ± 32.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>105.75 ± 30.35&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>106.00 ± 27.82&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>71.63 ± 10.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>74.38 ± 20.84&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

C, control group; OVX, ovariectomized group; CaL_OVX, ovariectomized group receiving calcium lactate; CaL_OVX_B, ovariectomized group receiving calcium lactate and alendronate; PCaL_OVX, ovariectomized group receiving calcium lactate-enriched pumpkin; PCaL_OVX_B, ovariectomized group receiving calcium lactate-enriched pumpkin and alendronate; dm, dry mass.

<sup>a,b,c</sup>Significant differences between groups (p < 0.05).

3 Results

The results of the analysis are presented in Tables 1–3. It was observed that Fe content in diets did not differ between the studied groups (Table 1). Similarly, there were no differences in daily Fe consumption between the groups. However, some differences were observed in weight gain. Ovariectomy caused a significant increase in the weight gain of rats, which was minimized by pumpkin enriched with calcium lactate with or without alendronate (PCaL_OVX and PCaL_OVX_B groups).

Table 2: HGB concentration in whole blood and iron content in tissues (mean and standard deviation)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C</th>
<th>OVX</th>
<th>CaL_OVX</th>
<th>CaL_OVX_B</th>
<th>PCaL_OVX</th>
<th>PCaL_OVX_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB (g/dl)</td>
<td>15.29 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.65 ± 0.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.19 ± 0.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.00 ± 0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.00 ± 0.79&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.31 ± 0.56&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Femur (µg/g dm)</td>
<td>87.52 ± 9.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.86 ± 10.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.95 ± 9.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.80 ± 10.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.20 ± 7.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.24 ± 16.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas (µg/g dm)</td>
<td>111.49 ± 11.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.93 ± 11.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.85 ± 12.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.74 ± 10.63&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>105.41 ± 13.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>102.66 ± 13.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hair (µg/g dm)</td>
<td>176.09 ± 16.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>125.24 ± 8.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>153.95 ± 25.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>145.77 ± 24.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.64 ± 19.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140.84 ± 16.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen (mg/g dm)</td>
<td>10.30 ± 2.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.15 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.54 ± 1.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.29 ± 1.13&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.78 ± 1.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.93 ± 1.44&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver (mg/g dm)</td>
<td>22.79 ± 1.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.10 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.20 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66 ± 0.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.84 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart (µg/g dm)</td>
<td>515.93 ± 34.91</td>
<td>535.40 ± 37.33</td>
<td>536.48 ± 34.73</td>
<td>558.14 ± 28.47</td>
<td>524.03 ± 39.35</td>
<td>541.47 ± 32.44</td>
</tr>
<tr>
<td>Brain (µg/g dm)</td>
<td>130.72 ± 16.42</td>
<td>145.25 ± 20.65</td>
<td>134.68 ± 13.12</td>
<td>136.30 ± 20.57</td>
<td>135.06 ± 14.96</td>
<td>138.94 ± 13.54</td>
</tr>
<tr>
<td>Muscle (µg/g dm)</td>
<td>91.27 ± 15.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.71 ± 14.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.15 ± 11.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>81.43 ± 12.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.14 ± 6.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.64 ± 16.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney (µg/g dm)</td>
<td>760.39 ± 124.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>578.32 ± 57.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>690.52 ± 62.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>663.06 ± 43.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>708.21 ± 104.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>664.24 ± 115.31&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

C, control group; OVX, ovariectomized group; CaL_OVX, ovariectomized group receiving calcium lactate; CaL_OVX_B, ovariectomized group receiving calcium lactate and alendronate; PCaL_OVX, ovariectomized group receiving calcium lactate-enriched pumpkin; PCaL_OVX_B, ovariectomized group receiving calcium lactate-enriched pumpkin and alendronate; HGB, hemoglobin; dm, dry mass.

<sup>a,b,c</sup>Significant differences between groups (p < 0.05).
groups). However, ovariectomy did not affect the iron content in the muscles, whereas the muscle’s Fe concentration was decreased in rats fed enriched pumpkin compared to groups C and OVX.

The study also analyzed the effect of a simple factor on Fe content in tissues. Table 3 shows significant changes in Fe content in tissues caused by ovariectomy and feeding with calcium lactate-enriched pumpkin and alendronate. The effect of ovariectomy was investigated by comparing the control group and the ovariectomized group (C:OVX). The effect of Ca-enriched pumpkin on Fe content was investigated by comparing the group receiving calcium lactate and the group receiving calcium lactate-enriched pumpkin (CaL_OVX:PCaL_OVX). The effect of alendronate was determined by comparing the calcium lactate group and the calcium lactate + alendronate group (CaL_OVX: CaL_OVX_B), while the combined effect of enriched pumpkin with alendronate was analyzed by comparing the calcium lactate group and the enriched pumpkin + alendronate group (CaL_OVX:PCaL_OVX_B).

We observed that ovariectomized groups had reduced Fe content in bones, hair, spleen, liver, and kidneys compared to the control group. The enriched pumpkin group (PCaL_OVX) showed higher Fe content in the pancreas and liver, but lower Fe content in muscles compared to the calcium lactate group (CaL_OVX). The group receiving bisphosphonates + calcium lactate (CaL_OVX_B) had reduced Fe content in the liver in comparison to the CaL_OVX group. The group fed bisphosphonates + enriched pumpkin (PCaL_OVX_B) showed higher spleen and liver Fe content compared to the CaL_OVX group.

### Table 3: Significant effect of ovariectomy, Ca-enriched pumpkin, and alendronate on iron content in tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ovariectomy</th>
<th>Enriched pumpkin</th>
<th>Bisphosphonate</th>
<th>Bisphosphonate + enriched pumpkin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Heart</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Compared groups:** ovariectomy, C:OVX; enriched pumpkin, CaL_OVX:PCaL_OVX; bisphosphonate, CaL_OVX:CaL_OVX_B; bisphosphonate + enriched pumpkin, CaL_OVX:PCaL_OVX_B. C, control group; OVX, ovariectomized group; CaL_OVX, ovariectomized group receiving calcium lactate; CaL_OVX_B, ovariectomized group receiving calcium lactate and alendronate; PCaL_OVX, ovariectomized group receiving calcium lactate-enriched pumpkin; PCaL_OVX_B, ovariectomized group receiving calcium lactate-enriched pumpkin and alendronate.

#### 4 Discussion

This study confirmed that ovariectomy losses in the majority of the organs in rats. In contrast, pumpkin enriched with calcium lactate with or without alendronate prevented the loss of Fe in the liver and spleen in ovariectomized rats. Moreover, we found that feeding with Ca-enriched pumpkin with or without alendronate more efficiently reduced Fe losses in organs compared to calcium lactate used alone.

Menopause causes an increase in iron content in women; however, our study showed the opposite effect, as indicated by a decrease in Fe content after ovariectomy. Liu et al. observed a lack of Fe overload in ovariectomized rats in their study, in which ovariectomy did not cause any changes in serum iron concentration in rats [21]. It can be assumed that the decrease in Fe concentrations in the body is related to increased body weight after ovariectomy, and thus increased blood volume and increased Fe losses associated with excessive body weight [22]. It is important to regulate Fe status in people with obesity, mainly due to increased concentration of leptin, which is biologically similar to interleukin 6, and whose increase causes an increase in the secretion of hepcidin from the liver [23]. The excessive adiposity is associated with disturbances of iron homeostasis due to elevated hepcidin expression and increased ferritin level. This dysregulation of iron metabolism in obesity increases the risk of the iron overload [24–26]. It seems that the consumption of enriched pumpkin may be beneficial in people with obesity because: first, it is a source of well-absorbed calcium and, second, it may reduce the risk of iron overload.
Hepcidin, on the other hand, regulates iron status in the body, as it interacts with ferroportin, a protein that controls Fe efflux from cells [27]. High levels of hepcidin block intestinal iron absorption, resulting in erythropoiesis and anemia due to reduced iron content [28]. A decrease in iron content in tissues observed in this study can also be caused by impaired Fe absorption in the duodenum and impaired Fe transport to the blood [29].

In the present study, we observed that calcium-enriched pumpkin increased Fe content in tissues in ovariectomized rats. Pumpkin was enriched not only with calcium but also with inulin – an osmotically active substance necessary for osmotic dehydration. Inulin is a polysaccharide and a prebiotic with beneficial effects on the human body [30]. A study showed that inulin significantly increased Fe absorption in children and adolescents by reducing Fe intake via the regulation of intestinal microbiome and increasing the count of *Bifidobacterium* [31] and *Lactobacillus* and decreasing *Clostridium* bacteria [32]. Furthermore, inulin increases the absorption surface and stimulates the formation of short-chain fatty acids (SCFAs) in the intestines [33], which contribute to strengthening the absorbent surface by the proliferation of epithelial cells [34]. SCFAs also reduce pH, promoting an acidic environment that favors the conversion of iron to its absorbable ferrous state [35]. Studies have shown changes in the composition of intestinal microbiota in the presence of inulin, but there is no evidence of an increase in Fe absorption in women with low iron status [36]. Recently, it was reported that by modulating the intestinal microbiota, inulin influences the metabolism of organs involved in maintaining energy homeostasis, including skeletal muscles [37]. Increased production of SCFAs, which results from the action of inulin, leads to the activation of the AMP protein in the muscles [38]. In contrast, muscle protein kinase activated by AMP takes part in the inhibition of Fe-dependent cell apoptosis (ferropotosis) caused by energy stress [39]. This relationship between AMP and iron may partly explain the lowest Fe concentration observed in muscles in the PCaL_OVX group in this study.

Our study showed that pumpkin enriched with calcium and inulin increases Fe content in soft tissues, while it has no effect on Fe saturation in bones. This finding is in agreement with the study by Jolliiff and Mahan who observed a significant increase in Fe content in the liver after the addition of inulin to the diet [40]. It has been found that inulin increased the formation of the Fe transporter DMT1, ferroportin, and ferritin, by increasing the rate of Fe absorption in the duodenum and liver of chickens [41]. Furthermore, inulin reduced the expression of pro-inflammatory genes and increased the expression of genes encoding Fe storage proteins (CYBRD1, FTL, HEPH, HIF1, LTF, UBE2D1) in the livers of young pigs, with the simultaneous lack of inulin’s effect on the expression of Fe transporters in the intestine (DMT-1 and ferroportin) and the increased expression of Fe regulators, supporting a feedback loop that would prevent Fe overloading [42].

Consumption of inulin positively influences Fe metabolism, improving Fe bioavailability, and also increases the concentration of HGB in the blood, as has been observed in women of reproductive age [43] and in animals [44,45].

In this study, increased iron concentration in the liver was associated with lower weight gain in groups fed with enriched pumpkin. It seems that enriched pumpkin reduced fat content, and this effect might be accompanied by lower inflammation and hepcidin level, resulting in improved iron status [46]. After ovariectomy, iron transfer to bones may have been inhibited by interaction with calcium.

Lowering bone iron content may have clinical implications. Iron is involved in two critical processes related to bone health. The first of them is the production of collagen through the hydroxylation of procollagen, the second – is the metabolism of vitamin D, by regulating the synthesis of this vitamin with cytochrome P450 having heme in its molecule [47]. Iron deficiency, therefore, leads to a decrease in bone mineral density, increasing the rate of bone turnover [48]. In contrast, iron overload in postmenopausal women leads to reduced bone mineral density, which correlates with blood ferritin levels [49]. Excessive deposition of iron in the bones also leads to increased bone turnover [50].

We observed that alendronate decreased Fe level in the liver compared to Ca lactate used alone. However, Ca-enriched pumpkin offset the adverse effect of alendronate in rats. Human and *in vitro* studies have not confirmed the effect of alendronate on Fe content in the liver or on blood morphology [51,52]. The possible explanation for the differences in Fe concentrations is the level of alendronate in the blood [53,54]. Jing et al. showed that a combination of alendronate and lactic acid derivatives may increase the drug’s bioavailability and thus its effect on mineral metabolism [55]. However, another study showed that when consumed with a meal (mainly fruit) the bioavailability of alendronate decreased drastically. Unfortunately, in our study, we did not analyze alendronate concentration in the blood of rats. However, it has been found that bisphosphonates are effective in the treatment of thalassemia-associated osteoporosis [56]. Moreover, some bisphosphonates, such as risedronate, have been shown to decrease the serum ferritin level in postmenopausal women with osteoporosis [57].
The present study showed that calcium-enriched pumpkin increased Fe content in liver and spleen and HGB levels in ovariectomized rats. However, these findings may point to possible side effects of enriched pumpkin in postmenopausal women. After menopause, ferritin and iron levels usually increase in women, so calcium-enriched pumpkin may cause iron accumulation and accelerate bone loss in the body. Further studies are needed to analyze the positive and side effects of this novelty product in postmenopausal women. Nevertheless, studies should explore the potential application of calcium-enriched pumpkin in iron deficiency conditions.

4.1 Strong points and limitations

The strength of the research is the use of a new product, namely pumpkin enriched with calcium lactate. We evaluated for the first time the effect of enriched pumpkin on iron status in ovariectomized rats. In addition, we analyzed the effect of calcium lactate without plant matrix and the effect of alendronate on many parameters of iron metabolism.

This study also has a few limitations, which may have affected the results. The volume of serum collected from rats was not sufficient for the determination of Fe or alendronate. Moreover, only selected parameters of iron metabolism were analyzed; for instance, we did not analyze the levels of ferritin and hepcidin in the blood. In addition, rats’ urine was not collected, so we could not analyze the Fe content in feces and thus calculate the amount of excreted Fe. The sham-operated group was not taken into account, and the results were compared with those of the ovariectomized group fed with the standard diet and the nonoperated control group.

5 Conclusion

Based on the obtained results, we can conclude that: (i) ovariectomy decreases the Fe status in rats and (ii) calcium lactate-enriched pumpkin with or without alendronate increases the Fe content in liver and spleen in ovariectomized rats.

Funding information: This study was funded by the National Science Centre (grant number: 2018/29/B/NZ9/00461).

Author contributions: Conceptualization, N.W. and J.S.; methodology, J.S. and N.W.; software, J.S. and N.W.; validation, N.W. and J.S.; formal analysis, J.S.; investigation, N.W. and J.S.; resources, A.G.M.; data curation, J.S. and N.W.; writing – original draft preparation, N.W. and J.S.; writing – review and editing, all authors; visualization, N.W. and J.S.; supervision, J.S.; project administration, A.G.M.; funding acquisition, A.G.M. All authors have read and agreed to the published version of the article.

Conflict of interest: The authors have declared that they have no conflicts of interest.

Ethics approval: All experimental procedures were performed in accordance with the EU Directive 2010/63/EU for animal experiments. Approval for the study was obtained from the Local Ethics Committee in Poznań (no. 34/2019). The reporting in the manuscript follows the recommendations in the ARRIVE guidelines.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[9] Chen B, Li GF, Shen Y, Huang X, Xu YJ. Reducing iron accumulation: A potential approach for the prevention and...


