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EFFECTS OF OVARIECTOMY AND CALCIUM ENRICHED PUMPKIN ON MAGNESIUM STATUS IN RATS

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ABSTRACT

Background. Calcium (Ca) and magnesium (Mg) are important components of bones, whose homeostasis is disturbed during menopause. Calcium and magnesium metabolism are closely related, so it is important to study the interactions between them. This study aimed to determine the effect of Ca-enriched pumpkin on the Mg content in tissues in an animal model of postmenopausal osteoporosis.
Material and methods. 70 female Wistar rats divided into seven groups. One group was fed a standard diet (C), whereas the other six groups were ovariectomized and fed a standard diet (OVX), a calcium-deficient diet (DEF), a calcium lactate diet (CaL), calcium-lactate-enriched pumpkin (P_CaL), calcium lactate and alendronate (CaL_B), or calcium-lactate-enriched pumpkin and alendronate (P_CaL_B). This nutritional intervention was followed for 12 weeks, and then the rats were euthanized. Tissue samples were collected, and their magnesium content was assessed.
Results. The Mg content in bones was lower in the OVX group (3.15 ±0.19 mg/g) but higher in the DEF group (3.76 ±0.16 mg/g) in comparison with the control group (3.45 ±0.15 mg/g). The Mg content in the muscles and the liver was higher in the P_CaL group (1025.24 ±47.22 µg/g and 2102.09 ±83.35 µg/g) compared with the control group (842.51 ±19.13 µg/g and 1486.15 ±97.12 µg/g). However, the CaL_B and P_CaL_B groups showed a high Mg content in the kidneys (about 156% of the control group).
Conclusion. Ovariectomy and intervention diets revealed various new observations regarding the effect of innovative calcium-rich foods on the Mg content. These results showed that (i) ovariectomy decreases the status of Mg content; (ii) deficiency of Ca in the diet and Ca-enriched pumpkin with alendronate improve the Mg content in bones; and (iii) alendronate promotes the accumulation of Mg in the kidneys. In postmenopausal women, both those treated and untreated with drugs and diet, magnesium status should be monitored.

Keywords: ovariectomy, calcium, magnesium, postmenopausal osteoporosis, ovariectomized rats

ABBREVIATIONS

Ca – calcium
Mg – magnesium
BMD – bone mineral density
AAS – flame atomic absorption spectrometry
TRPM6 – transient receptor potential cation channel subfamily M member 6

INTRODUCTION

Menopause begins in women around the age of 45–50. Clinicians define menopause as the last menstruation in women's lives, and this condition is associated with various changes in metabolism. The primary symptom of menopause is a decline in estrogen levels due to the suppression of endogenous ovarian function (Minkin,

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2019). A low estrogen concentration in the blood has numerous consequences, including a reduction in bone mineral density (BMD), resulting in postmenopausal osteoporosis (Ji and Yu, 2015). Deepening low BMD leads to an increase in bone fragility, and thus an increase in the risk of falls and bone fractures (Miller, 2016). The effects of osteoporosis significantly reduce the quality of life of patients and their families, which is why it is important to prevent and effectively treat this disease (Erhan and Ataker, 2020). Osteoporosis is treated using either pharmacological measures, e.g., the use of alendronate or denosumab, or nonpharmacological methods, which include, among others, an adequate calcium (Ca) intake.

Ca is the primary bone mineral; therefore, its adequate supply to the body is an important preventive factor for osteoporosis. It is present in bones in the form of hydroxyapatite $[(Ca)_{10}(PO_4)_6(OH)_2]$, and about 99% of the total Ca in the body is present in bones (Murshed, 2018). Bone reconstruction, i.e., the replacement of the tissue with a new one, occurs throughout life; however, during menopause, bone resorption dominates over bone formation, which leads to a significant reduction in BMD (Song, 2017). Bone changes occurring during osteoporosis depend on Ca metabolism, and an adequate Ca supply supports the treatment of osteoporosis and increases BMD, thus preventing fractures (Black and Rosen, 2016).

The standard recommendation level of Ca for women during menopause is between 1000 and 1500 mg per day (Black and Rosen, 2016). In many countries, Ca is deficient in the diet, and its daily intake ranges from around 400 mg in Asia to around 700 mg in Africa. Only in the Scandinavian countries is Ca intake around 1200 mg/day (Balk et al., 2017). In Poland, calcium is also not consumed in the right dose (about 700 mg per day) (Skowrońska-Jóźwiak et al., 2016) and according to the Institute of Food and Nutrition, the recommended daily intake for women over 50 is 1200 mg (Jarosz et al., 2020). Ca supplements and Ca-fortified foods can help maintain normal blood Ca levels. Supplements should be used as prescribed by the doctor since frequent excessive dosage leads to side effects such as gastrointestinal complaints and the formation of kidney stones (Chiodini and Bolland, 2018). Ca can be supplemented in the diet using enriched or fortified foods (Harvey and Diug, 2018). Effective saturation of plant tissues with Ca can enrich the diet, thus contributing to an increase in Ca consumption and to the prevention and treatment of osteoporosis (Kulczyński et al., 2021). Osmotic dehydration makes pumpkin tissues saturated with calcium along with the active substance – inulin, which increases the bioavailability of calcium (Krupa-Kozak et al., 2016). The aim of producing pumpkin enriched with calcium was to create a food product, which would be a good source of calcium (Wawrzyniak et al., 2020; Wawrzyniak and Suliburska, 2021). However, excessive calcium levels can interfere with the metabolism of other minerals, including magnesium (Mg), due to numerous interactions (Perales et al., 2006; Pérez-Gallardo et al., 2009).

Ca metabolism and Mg metabolism are related in many ways. Ca absorption is dependent on the level of vitamin D in the body, and Mg is involved in the hydroxylation of vitamin D to its active form, $1{,}25(OH)_2D$, in the kidneys (Rosanoff et al., 2016). In this way, Mg plays an important role in Ca absorption. Even vitamin-D-resistant rickets becomes sensitive to calcitriol again under Mg supplementation (Wesselink et al., 2020). On the other hand, 1,25(OH)2D coordinates the intestinal absorption of Mg, which is related to Ca. Ca deficiency in the diet leads to a high turnover of vitamin D metabolism products and thus a lower vitamin D level (Lips, 2012). Mg deficiency results in an impaired parathyroid hormone (PTH) response (Uwitonze and Razzaque, 2018), and it is well known that PTH is involved in Ca metabolism. In Ca deficiency, PTH is secreted by the parathyroid cells (Goltzman et al., 2018). In addition, Mg influences the active transport of Ca ions through the cell membrane, which is crucial in muscle contraction, conduction of nerve impulses, normal heart rhythm, and vasomotor tension (Gröber et al., 2015).

Since Mg interacts with Ca and supplementation of these two minerals is positively correlated with BMD in postmenopausal women (Mahdavi-Roshan et al., 2015; Mutlu et al., 2007), it is interesting to investigate the effects of the consumption of innovative Ca-rich foods on Mg metabolism. Therefore, this study aimed to determine the effects of Ca-enriched pumpkin on the Mg content tissues in ovariectomized rats while the hypothesis of the study is that pumpkin enriched with Ca affects the status of Mg in ovariectomized rats.

METHODS

Experimental protocols

The rats were fed the standard AIN-93M diet with or without modification during the experiment (Reeves and Suppl, 1997). They were divided into seven groups of 10 rats each. No significant differences in the initial body weight were observed between the rats. Six groups (60 rats) were ovariectomized and subjected to a recovery period. Then, a 12-week nutritional intervention was introduced. The control group (C) and one of the ovariectomized groups (OVX) received the unmodified standard AIN-93M diet, whereas the other five groups received a modified diet: Ca-deficient diet (DEF), calcium lactate diet (CaL), pumpkin enriched with calcium lactate (P_CaL), alendronate and calcium lactate (CaL_B), or calcium-lactate-enriched pumpkin and alendronate (P_CaL_B). The experiment design is presented in Fig. 1, and the dietary components are summarized in the previous study (Wawrzyniak et al., 2022). Calcium lactate and enriched pumpkin were used in such an amount that the diets did not differ in the calcium content. The alendronate dose was set at 3 mg per kg body weight and adjusted weekly.

The rats were provided food and deionized water ad libitum, and intake was recorded daily. Their body weights were measured weekly, and a Bruker LF90II body composition analyzer was used for the analysis at the end of the experiment. The rats were euthanized by guillotine head removal. Femurs, pancreas, spleen, liver, heart, brain, muscles, and kidneys were isolated for analysis. Tissues were frozen at −80°C after washing with saline and weighing. Hair was collected from all rats from the interscapular area.

Materials and reagents

A pumpkin (yellow melon, *Cucurbita maxima*) obtained from an organic farm with the consent of the land owner was used in this study. Inulin and calcium lactate were purchased from Agnex (Białystok, Poland). Minerals and vitamins used for diet preparation were purchased from Sigma-Aldrich (Darmstadt, Germany). Casein, corn starch, dextrin, rapeseed oil, and sucrose were purchased from Hortimex (Konin, Poland).

Osmotic dehydration

The pumpkin tissue was enriched with calcium lactate in the process of osmotic dehydration as follows: the pumpkin flesh was cut into 1-cm^3 cubes and frozen. A solution of inulin and distilled water (50:50) was prepared in jars into which calcium lactate was added until a concentration of 5% was achieved. The frozen pumpkin cubes were added to the solution in a ratio of 1:5, and the mixture was shaken for 2 h in a 50°C

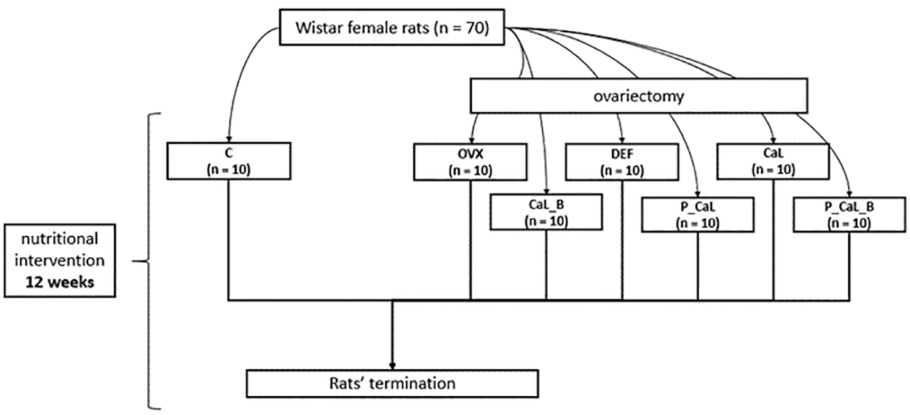

Fig. 1. Scheme of the study; C – control group; OVX – ovariectomized group; DEF – ovariectomized group with calcium-deficit diet; CaL – ovariectomized group with calcium lactate; CaL_B – ovariectomized group with calcium lactate and alendronate; P_CaL – ovariectomized group with calcium-lactate-enriched pumpkin enriched with; P_CaL_B – ovariectomized group with calcium-lactate-enriched pumpkin and alendronate

water bath, freeze-dried, ground, and added as such to the rats' diets (Wawrzyniak et al., 2022).

Animals

A total of 70 female Wistar rats aged 12 weeks were purchased from the Greater Poland Center for Advanced Technologies, University of Adam Mickiewicz in Poznań, Poland. 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.

Mg and Ca analysis in diets

To determine the Mg and Ca content in the diets, 1 g of each diet was burned in a muffle furnace at 450°C until mineralization. Then, the samples were dissolved in 1 mol/l nitric acid (Merck, Kenilworth, NJ, USA). Their mineral content was determined after diluting them with appropriate amounts of $LaCl_3$ (0.5%) and deionized water (AAS-3, Carl Zeiss, Jena, Germany) using flame atomic absorption spectrometry (AAS). A certified reference material was used to validate the method, with a 92% for Ca and 95% for Mg accuracy using brown bread (BCR191, Sigma-Aldrich, St. Louis, MO, USA). All samples were analyzed in triplicate.

Mg analysis in tissues

To determine the Mg content in tissues, the samples with pure nitric acid (Merck, Kenilworth, NJ, USA) were subjected to mineralization in a microwave digestion system (Speedwave Xpert, Berghof, Eningen, Germany). After digestion, they were diluted with deionized water and $LaCl_3$ (0.5%). The flame AAS method was used to determine the content of Mg (AAS-3, Carl Zeiss, Jena, Germany). A certified reference material — bovine liver — was used (1577C, Sigma-Aldrich, St. Louis, MO, USA) to validate the method (with an accuracy of 97%).

Statistical analysis

The Statistica program was used to conduct statistical analyses (StatSoft, Tulsa, OK, USA). To determine the normality of the distribution of the variables, the Shapiro–Wilk test was used. One-way analysis of variance with Tukey's post hoc test was used to identify the statistical differences between the analyzed groups (a *p*-value of <0.05 was considered statistically significant). For comparing two groups, Student's *t*-test was used. The results are presented in tables as mean values ± standard deviation.

RESULTS

The results are presented in Tables 1–3. The Mg content did not differ between the diets (Table 1). Similarly, no differences in the daily consumption of Mg were observed between the groups.

Ovariectomy-induced changes that were or were not compensated for by nutritional intervention were observed (Table 2). Ovariectomy significantly reduced the Mg content in bones, whereas the Ca-deficient diet and calcium lactate or enriched pumpkin (with and without alendronate) significantly increased it, compared with the C and OVX groups. Ovariectomy also led to a reduction in the Mg content in hair, but the dietary intervention did not result in any changes, except for the calcium lactate and alendronate group (CaL_B), whose Mg content was comparable to that of the control group. Although ovariectomy and a Ca-deficient diet did not affect the Mg content in the spleen, liver, heart, brain, and kidneys, significant changes in it were observed after the nutritional intervention. Calcium-lactate-enriched pumpkin significantly reduced the Mg content in the spleen (P_CaL), which was intensified by the addition of alendronate, resulting in a two-fold decrease in the Mg content in the spleen (CaL_B and P_CaL_B), compared with the OVX and control groups. Calcium lactate increased the Mg content in the liver in comparison with the control and ovariectomized group (OVX), as did enriched pumpkin, and alendronate exacerbated this effect. Both calcium lactate and enriched pumpkin decreased the Mg content in the heart, with the addition of alendronate bringing it closer to that of the control group. The modified diets did not significantly affect the Mg content in the brain in comparison with the OVX group, whereas in the kidneys, alendronate significantly increased it in the CaL_B and P_CaL_B groups, compared with the CaL and P_CaL groups. Ovariectomy increased the Mg content in muscles in comparison with the control group, which was not affected by the nutritional intervention.

Table 1. Diet parameters and body weight of rats (mean and standard deviation)

Parameter	Group						
	C	OVX	DEF	CaL	CaL_B	P_CaL	P_CaL_B
Ca content in diet, mg/g	5.63 ±0.37[b]	5.63 ±0.37[b]	0.64 ±0.04[a]	5.68 ±0.24[b]	5.68 ±0.24[b]	5.77 ±0.15[b]	5.77 ±0.15[b]
Mg content in diet, mg/g	0.57 ±0.06	0.57 ±0.06	0.55 ±0.03	0.58 ±0.03	0.58 ±0.03	0.58 ±0.01	0.58 ±0.01
Daily intake of diet, g	25.08 ±0.63	25.11 ±1.70	26.14 ±1.87	25.90 ±0.55	25.66 ±2.29	24.31 ±1.26	24.84 ±2.29
Daily intake of Ca, mg	141.12 ±3.56[b]	141.30 ±9.57[b]	16.77 ±1.20[a]	147.03 ±3.11[b]	139.73 ±12.44[b]	140.31 ±7.26[b]	145.01 ±13.39[b]
Daily intake of Mg, mg	14.30 ±0.36	14.31 ±0.97	14.59 ±1.20	15.23 ±0.51	15.19 ±1.31	14.01 ±0.73	14.50 ±1.56
Body weight g	325.86 ±25.97[a]	421.90 ±55.10[b]	441.00 ±70.97[b]	428.40 ±51.1[b]	433.30 ±51.62[b]	384.11 ±34.02[a,b]	392.30 ±34.88[a,b]

C – control group; OVX – ovariectomized group; DEF – ovariectomized group with calcium-deficit diet; CaL – ovariectomized group with calcium lactate; CaL_B – ovariectomized group with calcium lactate and alendronate; P_CaL – ovariectomized group with calcium-lactate-enriched pumpkin; P_CaL_B – ovariectomized group with calcium-lactate-enriched pumpkin and alendronate; Mg – magnesium.
a, b – significant differences between groups ($p < 0.05$).

Table 2. Magnesium content in the tissues (mean and standard deviation)

Tissue	Group						
	C	OVX	DEF	CaL	CaL_B	P_CaL	P_CaL_B
Femur mg/g dm	3.45 ±0.15[b]	3.15 ±0.19[a]	3.76 ±0.16[c]	3.86 ±0.16[c,d]	3.76 ±0.22[c]	3.88 ±0.19[c,d]	4.05 ±0.14[d]
Pancreas µg/g dm	118.78 ±8.01	109.48 ±8.28	116.51 ±17.38	109.63 ±12.11	112.74 ±9.19	116.38 ±6.18	114.91 ±9.03
Hair µg/g dm	94.79 ±14.38[b]	76.31 ±7.73[a]	78.29 ±6.25[a]	79.04 ±6.99[a]	83.62 ±6.65[a,b]	76.07 ±7.75[a]	82.04 ±4.77[a]
Spleen µg/g dm	1 874.87 ±416.66[c]	1 747.16 ±347.97[c]	1 462.65 ±340.31[b,c]	1 499.35 ±282.13[b,c]	885.92 ±235[a]	1 140.94 ±280.59[a,b]	902.89 ±214.49[a]
Liver µg/g dm	1 486.15 ±97.12[a]	1 402.83 ±112.88[a]	1 401.7 ±142.17[a]	1 870.98 ±142.55[b]	1 948.09 ±140.85[b,c]	2 102.09 ±83.35[c]	2 022.28 ±124.38[b,c]
Heart µg/g dm	1 122.79 ±40.57[c,d]	1 148.86 ±45.68[d]	1 119.4 ±46.74[c,d]	1 031.4 ±41.94[a,b]	1 063.51 ±55.62[b,c]	995.08 ±52.39[a]	1 063.59 ±50.81[a,b,c]
Brain µg/g dm	589.12 ±15.2[b]	568.36 ±26.31[a,b]	558.82 ±12.63[a]	557.18 ±12.06[a]	562.31 ±10.28[a]	569.59 ±13.54[a,b]	568.35 ±13.62[a,b]
Muscle µg/g dm	842.51 ±19.13[a]	994.13 ±44.25[b,c]	970.37 ±36.8[b,c]	952.08 ±40.79[b]	1 020.71 ±54.28[c]	1 025.24 ±47.22[c]	955.68 ±57.28[b]
Kidney µg/g dm	1 041.53 ±24.34[a]	1 036.33 ±64.53[a]	1 048.7 ±66.6[a]	1 052.25 ±61.27[a]	1 623.48 ±107.92[b]	1 054.13 ±83.14[a]	1 631.18 ±116.71[b]

C – control group; OVX – ovariectomized group; DEF – ovariectomized group with calcium-deficit diet; CaL – ovariectomized group with calcium lactate; CaL_B – ovariectomized group with calcium lactate and alendronate; P_CaL– ovariectomized group with calcium-lactate-enriched pumpkin; P_CaL_B – ovariectomized group with calcium-lactate-enriched pumpkin and alendronate; dm – dry mass.
a, b, c, d – significant differences between groups ($p < 0.05$).

Table 3. Significant changes in the magnesium content in tissues

Tissue	Ovariectomy	Ca deficit	Enriched pumpkin	Bisphosphonate	Bisphosphonate + enriched pumpkin
Bone	↓	↑			↑
Pancreas	↓				
Hair	↓				
Spleen			↓	↓	↓
Liver			↑		↑
Heart					
Brain			↑		
Muscle	↑		↑	↑	
Kidney				↑	↑

Compared groups: ovariectomy – C:OVX; Ca deficit – OVX:DEF; enriched pumpkin – CaL:P_CaL; bisphosphonate – CaL:CaL_B; bisphosphonate + enriched pumpkin – CaL:P_CaL_B.
C – control group; OVX – ovariectomized group; DEF – ovariectomized group with a calcium-deficit diet; CaL – ovariectomized group with calcium lactate; CaL_B – ovariectomized group with calcium lactate and alendronate; P_CaL – ovariectomized group with calcium-lactate-enriched pumpkin; P_CaL_B – ovariectomized group with calcium-lactate-enriched pumpkin and alendronate.

Significant changes in the Mg content in tissues are presented in Table 3. The effect of ovariectomy was investigated by comparing the control and ovariectomized groups (C:OVX). Moreover, the effect of Ca deficiency in the diet was evaluated by comparing the ovariectomized and Ca-deficient groups (OVX:DEF). The influence of Ca-enriched pumpkin on the Mg content was investigated by comparing the calcium lactate group and the calcium-lactate-enriched pumpkin group (CL:P_CaL). The effect of alendronate was determined by comparing the calcium lactate group and the calcium lactate and alendronate group (CaL:CaL_B), and the effect of combinations of enriched pumpkin with the drug was analyzed by comparing the enriched pumpkin group and the enriched pumpkin and alendronate group (CaL:P_CaL_B).

Ovariectomy reduced the Mg content in bones, pancreas, and hair but increased the same in muscles, compared with the control group. The Ca-deficient diet increased the Mg content in bones, compared with the ovariectomized group (OVX). The enriched pumpkin group (P_CaL) showed a higher Mg content in the liver, brain, and muscles but a lower Mg content in the spleen in comparison with the calcium lactate group (CaL). Bisphosphonates increased the Mg content in muscles and the kidneys but decreased it in the spleen, compared with the CaL group. The combination of pumpkin and bisphosphonate increased the Mg content in the bones, liver, and heart but decreased it in the spleen in comparison with the CaL group.

DISCUSSION

The results of this study showed that ovariectomy decreased the Mg content in the femur, whereas the Ca-deficit diet and Ca-enriched pumpkin improved the same in bones. Moreover, alendronate enhanced the Mg content in the kidneys.

It is well established that estrogen deficiency during menopause leads to various physiological and molecular changes (Wall et al., 2014). The disturbance in the Mg content in ovariectomy may be attributable to the decline in estrogen levels, which leads to dysregulation of Mg homeostasis by decreasing the intestinal absorption and reabsorption in the kidneys due to reduced activity and transcription of TRPM6 (transient receptor potential cation channel subfamily M member 6) — a channel for Mg^{2+} ions (Cao et al., 2009). Mg deficiency leads to the inhibition of bone growth, an increase in the number of osteoclasts, a decrease in the number of osteoblasts, and thinning of the bone trabeculae (Rude et al., 2003); therefore, a decrease in BMD is possible in the OVX group. The Mg content in hair and serum is closely related to BMD (Song et al., 2007); therefore, in the ovariectomized rats (OVX group), a decrease in the Mg content was observed in both bones and hair. However, a slightly lower Ca content in bones and a lower number of osteoblasts were observed in the previous study on ovariectomized rats, which may show the relationship between Ca and Mg, and bone structure during the development of adverse bone changes as a result of menopause, which is a long-term process (Wawrzyniak et al., 2021). The limitation of this study is that the serum Mg content was not determined, which would have allowed for a broader explanation of the obtained results. As far as clinical trials are concerned, Laires et al. observed a low Mg content in red blood cells in healthy menopausal women, which indicates the dysregulation of factors that control Mg homeostasis during menopause (Laires et al., 2004). It has been reported that the serum Mg content in women with postmenopausal osteoporosis is much lower than the recommended level (Mahdavi-Roshan et al., 2015), and that magnesium deficiency is associated with changes in the structure of apatite crystals in bones, a decrease in PTH, and thus a decrease in vitamin D levels (Mutlu et al., 2007).

In the present study, a reduction in the Mg content of the femurs was also observed in rats consuming a calcium-deficient diet (DEF group). This effect is probably attributable to the fact that Mg competes with calcium; it blocks the calcium channel, which reduces the intracellular calcium content (Houston, 2011). Mg competes with Ca in the formation of hydroxyapatite, forming an insoluble salt by binding to pyrophosphate (Navarro-González et al., 2009), and a high Mg content inhibits osteoblast differentiation, leading to a reduction in mineralization activity (Leidi et al., 2011). Matsuzaki et al. observed a similar relationship and reported that Ca supplementation reduces the Mg content in bones (Matsuzaki et al., 2005). However, some authors did not find this negative correlation between the Ca content in the diet and the Mg content in bones (Hernández-Becerra et al., 2017; 2020; Toba et al., 2000).

Although calcium lactate increased the Mg content in the liver, an innovative food product such as pumpkin enriched with calcium lactate contributed to an even higher accumulation of Mg in the liver. Enriched pumpkin also increased the Mg content in muscles compared with the OVX control group. The accumulation of Mg in the liver and muscles should therefore be attributable to the action of another component of the enriched pumpkin. During osmotic dehydration, inulin was used as an osmotically active substance, which is one of the factors increasing Mg absorption (Coudray et al., 2003; Schuchardt and Hahn, 2017). Inulin and other indigestible oligosaccharides affect both Ca and Mg metabolism by increasing the active and passive transport of these minerals (Scholz-Ahrens and Schrezenmeir, 2002), which may increase the Mg content in tissues.

Alendronate, a drug belonging to the group of bisphosphonates, is an oral therapeutic agent prescribed to women with postmenopausal osteoporosis for the inhibition of bone resorption and increase in bone formation, thereby protecting against bone mineral loss (Wang et al., 2017). Although alendronate does not affect the Mg content in body fluids (Buduneli et al., 2008; Shapses et al., 2011), bisphosphonates can affect Mg metabolism, for example, by impairing renal function, thereby increasing Mg excretion (Gröber, 2019). In general, the use of alendronate is safe and does not interfere with normal kidney function (Jamal et al., 2007; Sadowski et al., 2011); however, its long-term use may contribute to nephrotoxicity as one of the side effects (Benghuzzi et al., 2012; Miura et al., 2009). In the present study, a significant increase in the Mg content was observed in the kidneys after alendronate administration. Through the accumulation of calcium in the kidneys due to alendronate administration (described in the previous study (Wawrzyniak et al., 2022), adequate removal of Mg from the organism is disturbed. The changes in the kidneys can only be speculated, and to determine the changes caused by alendronate, the parameters of kidney functioning need to be analyzed. In this study, a synergistic effect of the ingredients of pumpkin and alendronate was observed on the increase in the Mg content in the femur.

The results of the study may contribute to the next ones, which could consist in enriching another raw material with calcium, e.g., apple or beetroot. Unfortunately, it is not advisable to use calcium-enriched pumpkin in clinical trials due to the very high accumulation of calcium in the kidneys, which was described in a previous article (Wawrzyniak et al., 2022).

STRONG POINTS AND LIMITATIONS

The strength of this study is its use of innovative products with potentially good calcium bioavailability in the protection and treatment of postmenopausal osteoporosis. We used a group with alendronate to compare the activity of the product with an antiosteoporotic drug. The dose of alendronate was adjusted weekly according to the body weight of the animals.

This study also has some limitations, which may have affected the results and limited the discussion. The volume of serum was not sufficient for the determination of Mg. In addition, rats' urine was not collected, and thus Mg excretion was analyzed. The sham-operated group was not taken into account, whereas the results were compared with those of the nonoperated control group and the ovariectomized group with the standard diet.

CONCLUSIONS

Ovariectomy and intervention diets revealed various new observations regarding the effect of innovative calcium-rich foods on the Mg content. These results showed that (i) ovariectomy decreases the status of Mg content; (ii) deficiency of Ca in the diet and Ca-enriched pumpkin with alendronate improve the Mg content in bones; and (iii) alendronate promotes the accumulation of Mg in the kidneys. In postmenopausal women, both those treated and untreated with drugs and diet, magnesium status should be monitored.

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