

Densitometric, tomographic and mechanical parameters of the female Wistar rat skeletal system after lactoferrin and colostrum treatment in the condition of gonadectomy-induced osteopenia

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Summary

Colostrum (COL) is a valuable discharge of the mammary glands of mammal females, which contains many bioactive components that are indispensable for the normal development of the young organism. Lactoferrin (LF) is one of the colostrum components serving a wide range of functions in the organism, e.g. it is involved in iron metabolism and has anti-inflammatory or immune activity. The experiment was conducted on 36 female Wistar rats in conditions of established osteopenia (60 days after gonadectomy). Females from the experimental groups were administered lactoferrin at the doses of 20 and 40 mg/kg b.w. and colostrum at the doses of 0.5 and 1.0 ml/100 g b.w. The results indicate that application of both COL and LF in conditions of established bone tissue atrophy induced by prolonged deficiency of sex steroid hormones has an osteoprotective effect on bone tissue. LF and COL inhibit a decrease in femoral BMC and BMD and improve bone strength parameters at the doses LF = 20 mg/kg b.w. and COL = 5 ml/100 g b.w. However, high doses LF = 40 mg/kg b.w. and COL = 10 ml/100 g b.w. may impede the improvement of bone strength. In femoral cancellous bone tissue, LF reduces the decline in BMC and vBMD, likewise COL, which additionally inhibits atrophy of the cancellous bone tissue. Administration of LF and COL reduced body mass gain and decreased the content of adipose tissue in the OVX rats. The presented results suggest a possibility of therapeutics use of lactoferrin and colostrum for improving the condition of bone tissue.

Keywords: bone, colostrum, lactoferrin, rats, DXA, pQCT

Colostrum is the first discharge of the mammary glands of female mammals and the first food that contains all components ensuring normal growth and development of the newborn organism. It contains over 250 bioactive components (10, 13, 35). These include, for instance, growth factors (IGF-I, IGF-II, TGF- β , EGF, TNF- α , VEGF), immunoglobulins, leukocytes, lactoperoxidases, lysozyme, lactenins, hormones, enzymes, nucleic acids, oligosaccharides, pantothenic and folic acids, vitamins, and mineral compounds (19, 35). The composition of colostrum varies over time after parturition, and hour by hour contains decreasing levels of immunoglobulins, growth factors, hormones,

and other components. In terms of bioactivity, colostrum produced by cows within 12 hours after parturition is most valuable (18, 35).

Lactoferrin (LF) is one of the bioactive components of colostrums: it represents glycoproteins from the family of transferrins with a molecular mass of 80 kDa. One of its functions in the organism is involvement in iron metabolism. Fe²⁺ and Fe³⁺ are the most frequently bound ions, although lactoferrin can also bind Cu²⁺, Zn²⁺, and Mn²⁺ ions. Lactoferrin's concentration is in the range of 4-5 g/L in bovine colostrum, 0.2-1 g/L in bovine milk, and 1-4 mg/mL in human milk (4, 22, 35). It is also present in other body fluids such as

sweat, saliva, tears, secretions of the respiratory and reproductive systems, bile, and pancreatic juice (27, 34). Secondary granules of circulating neutrophils constitute another reservoir of LF. Lactoferrin exhibits antibacterial, antifungal, antiviral, and anticarcinogenic activity and stimulates growth, differentiation, and proliferation of many cell types, including endothelial cells, as well as the immune system (11). Its role in the inflammatory process involves stimulation of the cytotoxic activity of NK cells and monocytes as well as increased numbers and enhanced phagocytic properties of phagocytes. Additionally, it stimulates production of anti-inflammatory cytokines IL-4, IL-10, and IL-11, and inhibits production of proinflammatory cytokines IL-1 β , IL-6, and TNF- α . LF also stimulates NO production (3, 20, 33).

As an antioxidant, lactoferrin is part of antioxidant systems, which protect the cells against damage by scavenging free radicals (7). The increased synthesis of lactoferrin observed in the course of neurodegenerative diseases in the elderly is probably a protective mechanism against the destructive effects of oxidative stress in the organism.

LF excess is removed from circulation mainly by macrophages and monocytes. These cells have high-affinity receptors involved in the conversion of lactoferrin-bound iron into ferritin accompanied by the degradation of lactoferrin. Pharmacokinetic investigations carried out on mice and rats have shown rapid removal of lactoferrin from the blood by the liver hepatocytes and Kupffer cells.

Lactoferrin is currently used as a dietary supplement for iron disorders and immunocompromised condition (21). The ease of oral application of lactoferrin is ensured by its resistance to degradation by gastric juice and digestive enzymes.

Recent research has shown that the bioactive components of colostrum and milk have a beneficial effect on bone metabolism (9). Application thereof in osteopenia and osteoporosis conditions results in reduction of bone loss (32, 35). They suppress induction of osteoclasts and stimulate proliferation of osteoblasts, which contributes to an increase in bone mineral density BMD (23, 31).

In vitro studies have revealed a stimulatory effect of LF on the proliferation and differentiation of osteoblasts and 50-70% inhibition of apoptosis. *In vivo* investigations have proved the osteoprotective effect of LF, oral supplementation of which improves bone mineral density (BMD) and bone fracture strength and prevents reduction of volumetric bone mineral density in osteopenia (1, 5, 26, 27).

The aim of the study was to investigate and compare the effects of application of different doses of colostrum (COL) and lactoferrin (LF) on bone tissue in female Wistar rats with established osteopenia induced by bilateral ovariectomy. Another objective

of this research was to assess the effect of LF and COL supplementation on body composition indicators such as muscle tissue (Lean Mass) as well as adipose tissue (Fat Mass) and its proportion in the total body mass (% Fat).

Material and methods

The experiment was carried out on 36 9-10-week-old female Wistar rats with an initial body weight (BW) of approx. 200 g. After transporting, the animals were left to adapt to the vivarium conditions for 7 days (temperature 22°C \pm 2°C, 12 h light/12 h dark photoperiod, humidity approx. 55%). After the adaptation period, the animals were divided randomly into a sham-operation group (SHO; n = 6) and an ovariectomized group (OVX; n = 30).

The surgery was performed under general anesthesia induced by intramuscular injection of 10.0, 2.0, and 0.05 mg/kg BW of ketamine (Vet Agro Lublin, Poland), atropine (Polfa Warszawa SA, Poland), and Sedazin (Biowet Pulawy, Poland). Next, the rats were placed in individual cages for 3 days to allow the formation of stable post-operative adhesions. After the recovery period, the rats were divided randomly and kept in Eurostandard Type IV (Tecniplast, Italy) cages (3 animals in each) for another 60 days in order to induce and stabilise osteopenic atrophy of bone tissue. After that period, the OVX females were divided into a control group (OVX-PHS) receiving daily physiological saline (0.9% NaCl solution at a dose of 2 mL/kg b.w.) and experimental groups receiving daily 0.5 and 1.0 mL/100 g b.w. of COL (OVX-COL5 and OVX-COL10) and 20 and 40 mg/kg b.w. LF (OVX-LF20 and OVX-LF40). The LF content in colostrum was equivalent to that of LF doses administered separately, i.e. a dose of 0.5 mL/100g b.w. of LF in COL was equivalent to the LF dose of 20 mg/kg b.w., while an LF dose of 1 mL/100g b.w. was equivalent to 40 mg/kg b.w. of LF. The control group (SHO-PHS) was administered physiological saline at a dose of 2 mL/kg b.w. The body weight in control and experimental groups was controlled twice a week. All substances used in the experiment were administered intragastrically through a rat gastric tube (Instech Laboratories, Inc. USA) adjusted to the mass of the rats.

Throughout the experiment, the rats had *ad libitum* access to fresh water and standard rodent feed LSM (Agropol-Motycz, Poland). In order to avoid increased food intake induced by sterilisation, the OVX females received feed aliquots determined based on the daily intake in the SHO group.

After a 40-day period of administration of PHS, COL, and LF, the rats were anesthetized with CO₂, weighed and euthanized by cervical dislocation. Immediately after euthanasia, the animals were scanned using the dual X-ray absorptiometry (DXA) method, to determine the bone mineral density (tBMD), bone mineral content (tBMC) of total skeleton and vertebral column (L2-L4), as well as to ascertain the parameters of body composition (Lean Mass and Fat Mass). Subsequently, their femora were isolated, cleaned of soft tissues and frozen for further analysis.

All animal procedures described followed established guidelines for the care and handling of laboratory animals,

and were approved by the Local Animal Welfare Committee in Lublin, Poland.

DXA method. The bone mineral density, bone mineral content of whole body and isolated femora and the body composition (Lean Mass, Fat Mass) were established with a Norland Excell Plus Densitometer (Fort Atkinson, WI, USA) equipped with Illuminatus Small Subject Scan Software v.4.5.

pQCT method. The scans were performed in the proximal femur metaphysis (DFM) (5 mm from distal end) for analysis of trabecular bone tissue and in the middle diaphysis (MFD) (50% of bone length) for the analysis of cortical bone tissue with the use of a Stratec XCT Research SA Plus peripheral quantitative computed tomography system, with software version 6.2 C (Stratec Medizintechnik GmbH, Pforzheim, Germany).

The scan line was adjusted using a scout view of the pQCT system. Upon completion of scanning, the following parameters were determined: trabecular bone area (Tb.Ar), trabecular bone mineral content (Tb.BMC), trabecular volumetric bone mineral density (Tb.vBMD), cortical bone area (Ct.Ar), cortical bone mineral content (Ct.BMC), cortical volumetric bone mineral density (Ct.vBMD) as well as cortical thickness (Ct.Th), periosteal (Peri.C) and endocortical (Endo.C) circumferences. Analyses of trabecular bone were performed with a threshold of 0.450 cm^{-1} with a contour mode 2 and a peel mode 20, whereas the cortical part was tested with a threshold of 0.900 cm^{-1} and cortical mode 2. The initial scan was performed with the speed of 10 mm/s , and CT-scan of 4 mm/s (12).

Both machines were calibrated using the quality assurance phantoms (QA-Phantom), provided by the manufacturer, and done in agreement with set procedures, before every measurement series.

Strength analysis. The mechanical parameters of isolated femora were examined in a 3-point bending test with the use of a ZwickRoell Z010 (ZwickRoell GmbH&Co. KG, Ulm, Germany) universal testing machine equipment with 1 kN measuring head Xforce HP series. The analysed bones were investigated as a tube model, and external as well as internal diameters were measured using pQCT. The

bones were placed on two holders, and the force was applied downward, perpendicularly to the horizontal axis and at the midpoint of the bone. The load was increased until the bone broke. The data obtained were analysed with testXpert II 3.1 software assistance. The ultimate strength (F_{\max}), work to ultimate strength (W/F_{\max}), and the Young modulus (E_{mod}) were determined.

Statistical analysis. The results were reported as mean values \pm SEM. A one-way analysis of variance (ANOVA) was used to test for significant differences among the groups. To detect significant differences between the individual experimental groups, significant ANOVAs were followed by a post hoc Tukey test for multiple comparisons. Differences were considered significant at $p < 0.05$. Analysis of significant differences was performed with the use of Statistica 8.0 software (StatSoft, Inc. Tulsa, USA).

Results and discussion

The ovariectomy surgeries increased rats' body mass in all the experimental groups in comparison with the control group (SHO-PHS). However, it should be noticed that application of both lactoferrin and colostrum significantly ($p < 0.05$) reduced the body mass gain in comparison with the OVX-PHS group. The highest significant ($p < 0.05$) decrease in body mass was found in the OVX-COL5 group, which exhibited values similar to those in the SHO-PHS group. A significant ($p < 0.05$) body mass decrease was also noted in groups OVX-LF20 and OVX-COL10 (Tab. 1).

The densitometric analysis of the body composition revealed a statistically significant ($p < 0.05$) increase in the parameters of adipose tissue, i.e. fat mass and total fat percentage, in the OVX-PHS group in comparison with the control group. The application of 5 mL of colostrum (OVX-COL5) significantly ($p < 0.05$) decreased the fat tissue gain in comparison with the OVX-PHS group. The results in this group were even slightly lower than the values noted in the control group SHO. Similarly, supplementation with 20 mg of lactoferrin and 10 mL of colostrum significantly

Tab. 1. Effects of colostrum and lactoferrin on body weight, bone mass and length, densitometry and mechanical parameters of bone tissue

Variable	SHO-PHS	OVX-PHS	OVX-LF20	OVX-LF40	OVX-COL5	OVX-COL10
Body weight (g)	413.83 \pm 5.79	525.5 \pm 4.69*	485.67 \pm 7.88*	464.40 \pm 6.19	422.50 \pm 7.07 [#]	434.80 \pm 3.21 [#]
Lean mass (g)	265.45 \pm 2.83	304.45 \pm 2.30	316.27 \pm 3.26*	299.20 \pm 6.62	281.80 \pm 3.03	286.82 \pm 6.67
Fat mass (g)	130.49 \pm 3.82	203.18 \pm 1.76*	148.84 \pm 3.11 [#]	143.66 \pm 1.67 [#]	120.18 \pm 3.58 [#]	127.04 \pm 3.12 [#]
Soft tissue fat%	0.323 \pm 0.06	0.402 \pm 0.02	0.317 \pm 0.01	0.327 \pm 0.01	0.291 \pm 0.02	0.306 \pm 0.02
Total fat%	0.313 \pm 0.02	0.40 \pm 0.002*	0.309 \pm 0.01 [#]	0.319 \pm 0.01	0.28 \pm 0.002 [#]	0.298 \pm 0.004 [#]
Total BMD (g/cm ²)	0.167 \pm 0.003	0.142 \pm 0.001*	0.167 \pm 0.001 [#]	0.170 \pm 0.001 [#]	0.160 \pm 0.002	0.162 \pm 0.001 [#]
Total BMC (mg/mm)	12.08 \pm 0.20	9.89 \pm 0.09*	11.41 \pm 0.08 [#]	11.58 \pm 0.05 [#]	11.06 \pm 0.02	11.57 \pm 0.20 [#]
Total area (mm ²)	66.11 \pm 1.27	82.29 \pm 0.86*	68.49 \pm 1.63 [#]	68.32 \pm 0.891 [#]	69.32 \pm 1.18	71.79 \pm 1.58
Femur BMD (g/cm ²)	0.118 \pm 0.001	0.094 \pm 0.001*	0.114 \pm 0.001 [#]	0.113 \pm 0.002 [#]	0.113 \pm 0.001 [#]	0.114 \pm 0.001 [#]
Femur BMC (mm/mg)	0.449 \pm 0.006	0.389 \pm 0.002*	0.437 \pm 0.005 [#]	0.449 \pm 0.001 [#]	0.442 \pm 0.006 [#]	0.436 \pm 0.004 [#]
Femur area (mm ²)	3.82 \pm 0.07	3.91 \pm 0.04	3.84 \pm 0.02	3.97 \pm 0.03	3.89 \pm 0.02	3.83 \pm 0.02

Explanations: * vs. SHO-PHS, $p < 0.05$, [#] vs. OVX-PHS, $p < 0.05$

($p < 0.05$) reduced the content of adipose tissue in the experimental groups. Administration of lactoferrin and colostrum had an effect on muscle tissue as well. In all the experimental groups, there was an increase in muscle tissue content in comparison with the SHO-PHS group. The highest significant ($p < 0.05$) increase was reported in the OVX-PHS group (Tab. 1).

The application of lactoferrin and colostrum significantly ($p < 0.05$) inhibited the decrease in bone mineral density in the entire skeleton induced by hormone deficiencies in the experimental groups. In the OVX-LF20 group, the values were significantly ($p < 0.05$) higher than in the control SHO-PHS group. An upward trend in the BMD value was found in the other groups. A similar phenomenon was observed in the bone mineral content. In comparison with the OVX-PHS group, a statistically significant ($p < 0.05$) decrease in the BMD and BMC values induced by the application of the different doses of LF and COL was also observed in the femora isolated in the OVX experimental groups (Tab. 1).

The strength analysis of the isolated femora showed a significant ($p < 0.05$) increase in the Young modulus (E_{mod}) in the OVX-LF20 and OVX-COL5 groups in comparison with the OVX-PHS group. The values in these groups were also higher than in the SHO-PHS group. A similar trend was observed for ultimate strength (F_{max}) although the increase in the values was not statistically significant (Tab. 1).

The tomographic examination of rats' distal femora performed after application of the different doses of colostrum and lactoferrin revealed a statistically significant ($p < 0.05$) increase in the Tot.BMC and Tot.vBMD parameters in all the experimental groups, compared with the OVX-PHS group. The highest increase was found in the OVX-LF40 and OVX-COL5 groups. An identical trend towards significant ($p < 0.05$) increases in these values was exhibited by the Tb.BMC and Tb.vBMD parameters. Tomography scanning performed in the midpoint of the femoral bone length demonstrated an increase in the values of Tot.BMC and Tot.vBMD in all the experimental groups in comparison with the OVX-PHS group. These increases, however, were not statistically significant. A similar trend, without statistical significance, was noted for the Ct.vBMD, Ct.Ar, and Ct.Th parameters (Tab. 2).

Besides components with a very high nutritional value, colostrum and mother's milk contain regulatory proteins and peptides that are indispensable for normal development of the organism. These include lactoferrin, proline-rich polypeptide, alpha- and beta-casein, and glycomacropeptide. In terms of biological activity, lactoferrin is an especially interesting protein. One of its properties described recently is the function of a regulator of skeletal morphogenesis processes.

The presented investigations are an attempt at determining the effects of the supplementation of dif-

Tab. 2. Effects of colostrum and lactoferrin on tomographic parameters of bone tissue

Variable	SHO-PHS	OVX-PHS	OVX-LF20	OVX-LF40	OVX-COL5	OVX-COL10
Distal part of femur						
Tot.BMC (mg/mm)	18.10 ± 0.27	12.78 ± 0.28*	14.13 ± 0.24*	14.34 ± 0.45*	14.39 ± 0.42*	13.40 ± 0.19*
Tot.vBMD (mg/mm ³)	794.50 ± 9.78	550.56 ± 6.473*	593.45 ± 6.06*	600.74 ± 7.71*	621.7 ± 4.78*	586.10 ± 6.33*
Tb.BMC (mg/mm)	5.85 ± 0.11	2.24 ± 0.07*	3.09 ± 0.04*	3.37 ± 0.04**	3.39 ± 0.11**	2.96 ± 0.12*
Tb.vBMD (mg/mm ³)	571.18 ± 10.34	220.77 ± 2.83*	288.17 ± 7.41*	303.87 ± 3.73*	324.77 ± 8.44*	285.28 ± 10.35*
Tot.Ar (mm ²)	22.84 ± 0.34	20.95 ± 0.36	23.79 ± 0.25	23.72 ± 0.51	23.13 ± 0.32	22.87 ± 0.26
Tb.Ar (mm ²)	10.26 ± 0.15	9.40 ± 0.16	10.69 ± 0.11	10.67 ± 0.23	10.41 ± 0.15	10.29 ± 0.11
Middle part of femur						
Tot.BMC (mg/mm)	9.56 ± 0.11	9.62 ± 0.11	9.67 ± 0.17	10.02 ± 0.22	9.65 ± 0.10	9.84 ± 0.12
Tot.vBMD (mg/mm ³)	970.28 ± 3.16	890.68 ± 3.68*	935.83 ± 6.71	951.28 ± 6.51	933.85 ± 9.71	943.66 ± 6.09
Tot.Ar (mm ²)	9.86 ± 0.13	10.8 ± 0.11	10.32 ± 0.15	10.57 ± 0.26	10.35 ± 0.09	10.44 ± 0.14
Ct.BMC (mg/mm)	8.80 ± 0.08	8.8 ± 0.10	8.95 ± 0.12	9.36 ± 0.20	8.84 ± 0.09	8.99 ± 0.11
Ct.vBMD (mg/mm ³)	1443.92 ± 1.30	1438.18 ± 1.76	1448.93 ± 1.61	1441.20 ± 1.15	1449.72 ± 1.64	1444.12 ± 2.04
Ct.Ar (mm ²)	6.09 ± 0.06	6.12 ± 0.07	6.18 ± 0.09	6.49 ± 0.14	6.10 ± 0.07	6.23 ± 0.08
Ct.Th (mm)	0.677 ± 0.002	0.634 ± 0.007	0.664 ± 0.006	0.670 ± 0.007	0.653 ± 0.008	0.665 ± 0.006
Peri.C (mm)	11.12 ± 0.07	11.64 ± 0.06	11.38 ± 0.08	11.50 ± 0.14	11.40 ± 0.05	11.45 ± 0.08
Endo.C (mm)	6.87 ± 0.07	7.66 ± 0.06	7.21 ± 0.07	7.13 ± 0.11	7.30 ± 0.07	7.26 ± 0.08
SSI (mm ³)	7.41 ± 1.22	7.90 ± 0.15	7.75 ± 0.13	8.13 ± 0.30	7.69 ± 0.09	7.81 ± 0.12
E mod (Nmm)	4.01 ± 0.10	2.70 ± 0.04*	4.31 ± 0.08 [#]	3.79 ± 0.15	4.06 ± 0.15 [#]	3.21 ± 0.04 [‡]
F max (Nmm)	130.64 ± 2.51	123.55 ± 2.61	144.89 ± 2.78	122.96 ± 4.13	136.67 ± 3.17	147.58 ± 2.23
W/F max	21.58 ± 1.72	45.98 ± 4.27	35.00 ± 1.65	36.76 ± 2.69	42.39 ± 3.92	49.22 ± 1.30

Explanations: * vs. SHO-PHS, $p < 0.05$, [#] vs. OVX-PHS, $p < 0.05$, [‡] vs. OVX-LF20

ferent doses of lactoferrin (LF) and colostrum (COL) on the skeletal system in female rats with established bone tissue atrophy induced by prolonged deficiency of sex steroid hormones (28). Bone atrophy resulting from a decline in oestrogen levels, increased bone turnover, and decreased BMD leads to many changes in bones (10). Both *in vitro* and *in vivo* investigations indicate that the antiresorptive activity of oestrogen is exerted by the RANKL/RANK/OPG system, by means of which osteoblasts regulate the size of osteoclast population (15). Lactoferrin protects bone microarchitecture and enhances VEGF and FGF2 expression in osteoblast precursor cells in a time- and dose-dependent manner, which implies its stimulating effect on angiogenesis depending on the availability of LF in treated animals. Therefore, at adequate availability of LF over a sufficiently long time, development of osteopenic conditions is impaired and bone reconstruction processes are intensified. Moreover, lactoferrin stimulates phosphorylation of p44/p42 MAP kinase in osteoblasts (25, 26, 29). In turn, the compounds contained in colostrum reduce or abolish the decline in the Ca level, thereby protecting against the development of osteoporosis. The use of COL increases the values of BMD and prevents reduction of cancellous bone tissue area after ovariectomy. Colostrum therapy stimulates proliferation of osteoblasts and suppresses osteoclasts in conditions of increased bone turnover (10). Lactoferrin and colostrum prevent bone loss in rats affected by oestrogen deficiency after ovariectomy, which corresponds with the osteopenic condition in osteopenic postmenopausal women (19).

As reported by Cornish et al. (8) and Guo et al. (14), lactoferrin stimulates bone growth in adult mice, and administration thereof to ovariectomized rats contributes to increased bone formation and enlargement of the total bone area, which impedes loss of vBMD and BMD. This may explain why the use of both LF and COL, regardless of the dose, significantly limited the decline in both BMD and BMC in the analysed femoral bones. In turn, Yoshimaki et al. (34) have shown that systematic application of LF contributes to the regeneration of bones with small lesions in developing osteopenia, and LF administration in established osteopenia impedes bone resorption. Oral administration of lactoferrin in ovariectomized rat females improves bone mineral density. This suggests that LF has a beneficial impact on bone metabolism by exerting a local effect on bone tissue with reduced density. *In vitro* investigations of rat osteoblast cultures have demonstrated that bone formation depends on the LF dose used in experiments and on the level of bone mineralisation (16, 27, 34). Similar results were obtained in the *in vivo* study of OVX female rats presented in this paper. Similarly, investigations of rabbit bone cell cultures have revealed antiresorptive activity of lactoferrin (22). Lactoferrin acts differently towards osteoblasts in the subsequent developmental

stages, i.e. it exerts the strongest effect by intensification of osteoblast differentiation in the early stage of development. In turn, the impact is not as strong in the consecutive stages (25, 27, 34). Cornish et al. (8) and Yoshimaki et al. (34) have demonstrated that after application of lactoferrin vBMD is higher than in the ovariectomized control group, which is related to the anabolic effect of LF on osteoblasts and its inhibitory activity towards osteoclasts. No such relationship has been found in the present investigations in cortical bone tissue. This is probably associated with the moderate bone loss observed in the females in this experiment in comparison with other authors' reports, where the process was more advanced and involved more severe, prolonged osteopenia (24). Lactoferrin used in this experiment was introduced earlier than in the investigations carried out by Mountziaris et al. (24), who analysed the impact of LF on bone regeneration after fracture in severe osteopenia, where osteopenic and fracture-induced lesions were more serious. The anti-inflammatory effect of LF applied earlier prevented the development of massive bone loss; hence, the vBMD of cortical tissue of the femoral bones persisted at a level comparable with that in the control groups. As reported by Du et al. (9), application of COL and bovine colostrum acidic proteins (BCAP) significantly increases BMD reduced by lowered oestrogen levels in OVX females. Similar effects of COL and LF application on the femoral BMD have been demonstrated in the present study; moreover, the densitometric analysis of the femoral BMC performed in these experiments did not reveal any significant differences between the SHO group and the treated groups, which is contrary to the results obtained by Du et al. (9). This is probably associated with the fact that BCAPs alone are more potent than BCAPs combined with all other components of COL, which suggests that whole colostrum exerts a different effect on bone tissue than each of its components administered alone.

Similar BMD results upon application of LF in ovariectomized female rats were obtained by Blais et al. (6), who additionally proved that administration of LF improved femur fracture strength. In this study, however, it has not been fully explained why femora of the OVX females supplemented with the higher doses of lactoferrin exhibited lower strength than the bones of OVX females receiving the lower doses, which remained at a similar level as the bones of OVX females supplemented with both COL doses. In their investigations of the effect of various LF doses on the mitogenesis of osteoblast precursors, Palmano et al. (29) demonstrated a similar effect, i.e. the highest LF doses used in the experiment (100 µg/mL) stimulated osteoblast proliferation at a statistically significantly lower level than the values obtained at application of lower LF doses of 1 and 10 µg/mL. Therefore, this suggests that too high lactoferrin doses may have an adverse effect on some bone metabolism processes and

contribute to reduction of bone strength. In contrast, opposite results were presented by Hou et al. (16). Their investigations proved that oral administration of 1000 mg/kg b.w. and 2000 mg/kg b.w. LF in OVX rats not only improved bone mineral density but also contributed to regeneration of bone loss and reconstruction of the microarchitecture of the femur and lumbar vertebrae, which had a direct effect on bone strength parameters (16). Our research has shown that the lower LF doses (20 mg/kg b.w.) had a statistically significant increase in the values of the strength parameters in comparison with the higher LF dose (40 mg/kg b.w.).

Metabolic processes taking place on the extra- and intraosseous surface have a direct impact on changes in total cortical area (Ct.Ar) and cortical thickness (Ct.Th). In this study, no statistically significant differences were found between the groups supplemented with lactoferrin and colostrum; however, in the OVX females receiving the higher LF dose, both Ct.Ar and Ct.Th were characterised by the highest values, which were on average by 8.8% and 5.7% higher than in the OVX-PHS group.

Deprivation of ovarian hormones leads to an increase in body mass and a significant increase in the proportion of fat tissue (28). Many papers have reported that oral administration of lactoferrin in humans and animals leads to reduction of body mass and the level of visceral fat as well as reduction of triglycerides and total blood cholesterol. The action of LF has several aspects and results in e.g. reduction of triglyceride absorption, inhibition of the formation of foam cells by impairment of the uptake of the oxidised form of HDL cholesterol by macrophages, inhibition of deposition of fat cells, and involvement in glucose metabolism by increasing insulin sensitivity (2). In their investigations, Pilvi et al. (30) and Ibrahim (17) have demonstrated that lactoferrin inhibits formation of fat cells by inhibition of the formation of adiponectin, i.e. a precursor of fat tissue growth. It was found in our study that both LF and COL reduced statistically significantly the content of adipose tissue, and the greatest decline was observed after administration of the 5-mL dose of colostrum. Similar effects on body mass were observed in all the experimental groups, in comparison with the OVX-PHS group, and the highest significant decrease in the value of the parameter was noted in the OVX-COL5 group. This suggests that, besides LF, there are other factors in colostrum enhancing its activity. Our study has demonstrated that the use of COL was more effective, as it reduced body mass gain and limited deposition of adipose tissue.

To conclude, it should be emphasised that both COL and LF have an osteoprotective effect on bone tissue in conditions of severe bone loss. COL prevents excessive mass gain induced by inadequate oestrogen levels associated with loss of gonadal function, as in the case of postmenopausal women. Application of LF efficiently inhibits the decline in BMD, as revealed by

the densitometric analysis, and in BMC and vBMD of cortical bone tissue in femora, as shown by tomography. Similarly, a significant protective effect of LF on cortical bone tissue area has been shown. Lactoferrin and colostrum applied at lower doses increased femoral strength, which was not observed in the other OVX groups. As shown by tomography, supplementation with colostrum has no significant impact on the BMC and vBMD of cortical bone tissue in contrast to the significant effect on cancellous bone tissue, i.e. limitation of bone loss and loss of cancellous bone tissue area.

The presented results suggest a possibility of a therapeutic use of lactoferrin for improvement of BMC and BMD, reduction of mass gain and fat deposition, and improvement of bone strength parameters in metabolic diseases of the animal and human skeletal system induced by deficiency of gonadal hormones. Nevertheless, many other unresolved questions in this field can be a starting point for further investigations of LF properties.

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