Praca oryginalna *Original paper*

Alterations in the immunolocalization of non-collagenous proteins in cartilage and bone tissue after gastrectomy, antrectomy, and fundectomy in a rat model

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Summary

Bone, as a tissue, plays a pivotal role in maintaining whole body homeostasis, contributing to, among other things, structural support, blood cell production, and mineral storage. On the other hand, bone homeostasis depends on hormonal factors released by various tissues of the body, including the gastrointestinal tract. Thus, surgical procedures involving the removal of various parts of the stomach wall, such as, antrectomy and fundectomy or partial gastrectomy, may have profound negative effects on bone metabolism and mineralization. These effects may result from changes in the concentration of neuropeptides, such as nesfatin-1, secreted by cells located in the fundus and vestibule of the stomach. Additionally, bone morphogenetic proteins, osteocalcin, osteoprotegerin, and tissue inhibitor of metalloproteinases 2, are regulatory proteins involved in bone metabolism, playing a key role in maintaining the balance between the activity of osteoclasts and osteoblasts, cells responsible for the remodeling of bone tissue. The aim of this study was to investigate the effects of antrectomy, fundectomy, and partial gastrectomy, on bone homeostasis in a rat model. The experiment was carried out on 24 male Wistar rats randomly and equally divided into control (SHO), gastrectomy (GAST), antrectomy (ANT), and fundectomy (FUN) groups. At the beginning of the study, the rats underwent surgical removal of selected parts of the stomach. At the end of the 6-week study, the rats were sacrificed, and femurs were collected. Microscopic images of immunohistochemical reactions (IR) in trabecular and compact bone, as well as isolated articular cartilages and growth plates of femurs were analyzed. The intensity of the immunoreaction (IR) of osteocalcin (OC), osteoprotegerin (OPG), tissue inhibitor of metalloproteinases 2 (TIMP-2), bone morphogenetic protein 2 (BMP-2), and nesfatin-1 was determined, and IR-positive cells were counted in each area of the femur. The quantitative analysis of the intensity of OC IR in growth plate cartilage and trabecular bone showed the highest values after fundectomy in comparison to the other treatments. Moreover, fundectomy increased the intensity of BMP-2 IR in articular cartilage, growth plate cartilage, and trabecular bone. The intensity of OPG IR in articular cartilage and compact bone showed the highest values after antrectomy. The intensity of TIMP-2 IR in the examined parts of the femur was highest in the rats subjected to antrectomy. The intensity of expression of nesfatin-1 IR was highest in articular and growth plate cartilages for rats subjected to gastrectomy. In conclusion, surgical procedures affecting the gastrointestinal tract, such as antrectomy, fundectomy, and partial gastrectomy, can have significant effects on bone metabolism. Our study on a rat model demonstrated varying impacts on bone homeostasis, with different surgeries leading to alterations in the expression of key regulatory proteins involved in bone remodeling.

Keywords: bone turnover, remodeling, nesfatin-1, osteocalcin, osteoprotegrin

Any surgical procedures involving the stomach lead to the loss of digestive capacities and the cessation of enzyme and hormone secretion, resulting in various complications. In both humans and domestic animals, gastrointestinal signs, such as anemia, reflux, diarrhea, steatorrhea, increased bowel movements, intestinal obstruction, and loss of appetite, persist in the post-surgery period. These complications contribute to a decline in overall well-being, malnutrition, nutrient deficiency, and weight loss. The complex etiology of these complications involves alterations in gastrointestinal motility, vagal innervation, hormonal signaling pathways, and the intestinal microbiota (4, 8, 11, 12, 20, 21, 23, 32, 45).

Bone, as a tissue, plays a pivotal role in maintaining homeostasis in the body, contributing to structural support, blood cell production, and mineral storage (6, 9, 37). Procedures such as gastrectomy, antrectomy, or fundectomy, which are commonly performed in gastric cancer treatment, can have profound effects on bone homeostasis (21, 24, 48). Gastrectomy, involving the removal of the stomach; antrectomy, which consists in the removal of the antrum (lower part of the stomach); and fundectomy, the removal of the fundus (upper part of the stomach), disrupt the normal physiological processes of the digestive system. Essential as these procedures are in treating gastric cancer, they can lead to unintended consequences, including alterations in bone metabolism and mineralization (8, 24, 27, 28, 48).

Researches report that patients after total or partial gastrectomy may show changes in bone density and are at an increased risk of fractures due to disturbed bone homeostasis. The mechanisms behind these effects are not fully understood, but they are thought to involve disruptions in nutrient absorption, hormonal imbalances, and changes in the gut microbiota (20, 23, 27, 29, 32, 45). Some of these factors, including nesfatin-1, bone morphogenetic proteins (BMPs), osteocalcin (OC), osteoprotegerin (OPG), and tissue inhibitor of metalloproteinases 2 (TIMP-2), are involved in bone metabolism as regulatory proteins. They play crucial roles in maintaining the balance between the activity of osteoclasts and osteoblasts within the bone structure, thus contributing to bone homeostasis (27, 28, 35, 47, 51).

As previously mentioned, individuals after total or partial gastrectomy may exhibit reduced bone density and are more susceptible to pathological fractures. Furthermore, regulatory proteins implicated in bone metabolism are affected, which contributes to disturbed bone homeostasis (27, 28, 35, 47, 51).

To address gaps in our understanding of how these gastric surgeries impact bone health, this study aimed to investigate the effects of gastrectomy, antrectomy, and fundectomy on bone homeostasis in a rat model. Specifically, the research focused on changes in signals involved in general bone turnover.

Material and methods

Experimental design. All procedures using animals were approved by the Local Ethics Committee for Animal Experiments, University of Life Sciences in Lublin, Poland and were performed according to the Guiding Principles for Research Involving Animals.

The experiment was carried out on 24 male Wistar rats with a body weight of approximately 220-240 g. After a 7-day adaptation to the experimental conditions of the vivarium (temperature 22°C ± 2%, humidity 55% ± 10%, a 12 h day/night cycle), the rats were randomly divided into control (SHO, n = 6), gastrectomy (GAST, n = 6), antrectomy (ANT, n = 6), and fundectomy (FUN, n = 6) groups. The control rats underwent a sham operation, which involved a midline incision of the abdominal wall, a gentle reposition of viscera, and the closing of the incision with stitches. The GAST rats underwent a modified gastrectomy during which all glandular parts of the rat stomach (fundus and antrum) were removed, and a connection between the remaining rumen (nonglandular part of the rat stomach) and the duodenum (end-to-end) was then established without total vagotomy, with care taken to preserve the vagus nerve. In the fundectomy procedure, the fundus of the stomach was removed along the visible borderline between the antrum and the fundus. The remining part of the stomach was connected to the rumen. Antrectomy was achieved by the resection of the antrum. This procedure was completed by connecting the fundus to the duodenum. The rats were fasted for 12 hours prior to surgery. General anesthesia was used for all surgical procedures, with ketamine (15 mg/kg b.w. i.m.) and xylazine hydrochloride (35 mg/kg b.w. i.m.). After surgery, the rats were administered amoxicillin for 3 days (30 mg/rat i.m.) and were housed under the controlled conditions of the vivarium, for a period of 6 weeks. The rats showed normal behavior and no signs (such as bleeding, anastomotic failure, infection, reflux, diarrhea, increased bowel movement, decreased feed intake) that might indicate postoperative complications. The rats were fed ad libitum a standard diet for laboratory rodents, formulated to meet minimal nutritional requirements specified in AIN-93M and had free access to water. The diet contained 160 g protein, 28 g fat, 50 g crude fibre, and 70 g crude ash in 1 kg of feed, with metabolizable energy of 11 MJ/1 kg of dry feed mass. At the end of the 6th week of the experimental period, the rats were fasted overnight (12 hours) and anesthetized in the morning next day. Femora were then collected for further analysis. In the present study, the real control group of rats was excluded in accordance with "the 3Rs" principle and the recommendation of the Ethics Committee to avoid unnecessary use of experimental animals.

Immunohistochemistry (IHC). Immunohistochemical staining of decalcified serial sections of bone was performed according to a previously described protocol (39). Briefly, after deparaffinization and rehydration with distilled water, antigen retrieval was achieved by 10-min enzymatic retrieval with proteinase K (Sigma-Aldrich, St. Louis, MO, USA) at 37°C. Endogenous peroxidase activity was subsequently blocked with a 3% solution of hydrogen peroxide in deionized water for 5 min. After blocking in normal goat

serum, sections were incubated overnight at 4°C with the following primary antibodies: rabbit polyclonal anti-osteoprotegerin (OPG; Abcam, Cambridge, UK, 1 : 100 dilution), rabbit monoclonal anti-inhibitor of metalloproteinases 2 (TIMP-2; Abcam, Cambridge, UK, 1 : 100 dilution), rabbit polyclonal anti-bone morphogenetic protein 2 (BMP-2; Abcam, Cambridge, UK, 1 : 250 dilution), and rabbit polyclonal anti-nesfatin-1 (H-003-22, Phoenix Pharmaceuticals, Burlingame, CA, dilution 1 : 2000) antibodies. The sections were then incubated (30 min) with secondary antibody (peroxidase conjugated goat anti-rabbit, Rockland Immunochemicals, Inc. Limerick, USA, dilution 1 : 500). Negative control sections for each antibody were obtained by identical immunohistochemical staining, excluding the primary antibody. Then the sections were developed in 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO, USA) used as a chromogen for 15 min at room temperature. Counterstaining was performed with haematoxylin (Sigma-Aldrich, St. Louis, MO, USA) (38).

Microscopic images of immunohistochemistry reactions were further analyzed. In trabecular and compact bone, as well as in the articular and growth plate cartilages, the intensity of immunoreaction (IR) of the following proteins was determined: osteocalcin (OC), the most abundant non-collagenous protein in bone, specifically expressed in osteoblasts, a marker of bone turnover, indicating osteoblast activity; osteoprotegerin (OPG), expressed by mature osteocytes and their precursors, a key factor for osteoclast differentiation and activation; tissue inhibitor of metalloproteinases 2 (TIMP-2), a natural inhibitor of matrix metalloproteinases, a group of peptides involved in degradation of the extracellular matrix; bone morphogenetic protein 2 (BMP-2), which stimulates the development of bone and cartilage; nesfatin-1, a factor preventing bone loss. The intensity of IR was measured in six randomly selected areas of the growth plate cartilage and articular cartilage and in twelve randomly selected areas of positive signal in compact and trabecular bone.

In the cytoplasm of the cells, the IR of each protein was described as positive expression (brown color of cytoplasm) or negative expression (blue color of cytoplasm) (3). IR-positive cells were counted in each area analyzed (40). The IHC images were analyzed using the IHC Profiler plugin (44) compatible with Fiji and an optical density (OD) quantitative score for the evaluation of IR intensity (16).

Results and discussion

Osteocalcin. The intensity of OC IR in articular cartilage was stronger in the GAST rats than it was in the other groups, and in the ANT rats compared with the SHO and FUN rats. In growth plate, the strongest IR was observed in the GAST rats. In trabecular bone, IR was stronger in the SHO rats than in the ANT and FUN rats, and in the GAST rats compared to the ANT rats. In compact bone, IR was strongest in the FUN rats. Moreover, OC IR was stronger in the GAST rats than in the SHO rats, and in the ANT rats compared to the SHO and GAST rats. The number of OC-positive cells in articular cartilage was highest in the ANT and GAST groups, whereas the number of OC-positive cells in growth plate was higher in the SHO and FUN rats than it was in the ANT and GAST rats (Fig. 1). No other changes were observed.

About 10% of different non-collagenous proteins are secreted by osteoblasts, osteocytes, and chondrocytes in connective tissue. The cells of this tissue differ in morphology and metabolism, but the type of connective tissue is best characterized by the composition of the extracellular matrix, which determines its division and classification. The extracellular matrix is a dynamic compartment that directly affects cells by limiting the diffusion of substrates. Thus, bone metabolism can be determined by the assessment of certain non-collagenous proteins, such as osteocalcin, a protein responsible for bone mineralization, whose overproduction also indicates intense bone processes related to osteoporosis (19). The findings presented here indicate significant variations in osteocalcin immunoreactivity (OC IR) across different anatomical structures and experimental groups. These variations in OC IR suggest differential regulation of bone metabolism in response to gastric surgery. Specifically, the differences observed in OC IR could indicate alterations in osteoblast and chondrocyte activity, which are crucial for bone formation and homeostasis maintenance. The higher OC IR in certain groups may reflect increased bone turnover, potentially indicative of pathological processes, such as osteoporosis. This is consistent with a study showing an increase in OC mRNA in bone tissue of animals undergoing gastrectomy (43) or with a study that observed an increase in serum OC in people after other bariatric surgery (15). Moreover, it is worth emphasizing that normal articular cartilage is characterized by low OC expression, whereas in progressive degeneration, OC is present in the synovial fluid (41). Moreover, serum OC has been proposed as a possible biomarker for the early detection and monitoring of arthritic degeneration (26, 41). Overall, the current results are consistent with those of other authors who found a high incidence of osteoporosis and changes in bone density and strength after gastric resection (1, 17, 18).

Osteoprotegerin. The intensity of OPG IR in articular cartilage was stronger in the GAST rats than in the SHO and FUN rats, and in the ANT rats compared to the SHO and FUN rats. In growth plate, IR was stronger in the ANT rats than in the SHO and FUN groups, and in the FUN group it was stronger than in the GAST group. In trabecular bone, IR was strongest in the ANT group, and in the GAST rats it was stronger than in the SHO and FUN rats. In compact bone, IR was strongest in the ANT rats, and it was stronger in the FUN rats than it was in the SHO rats. The number of OPG-positive cells in articular cartilage was lowest in the ANT group; in growth plate, it was lower in the

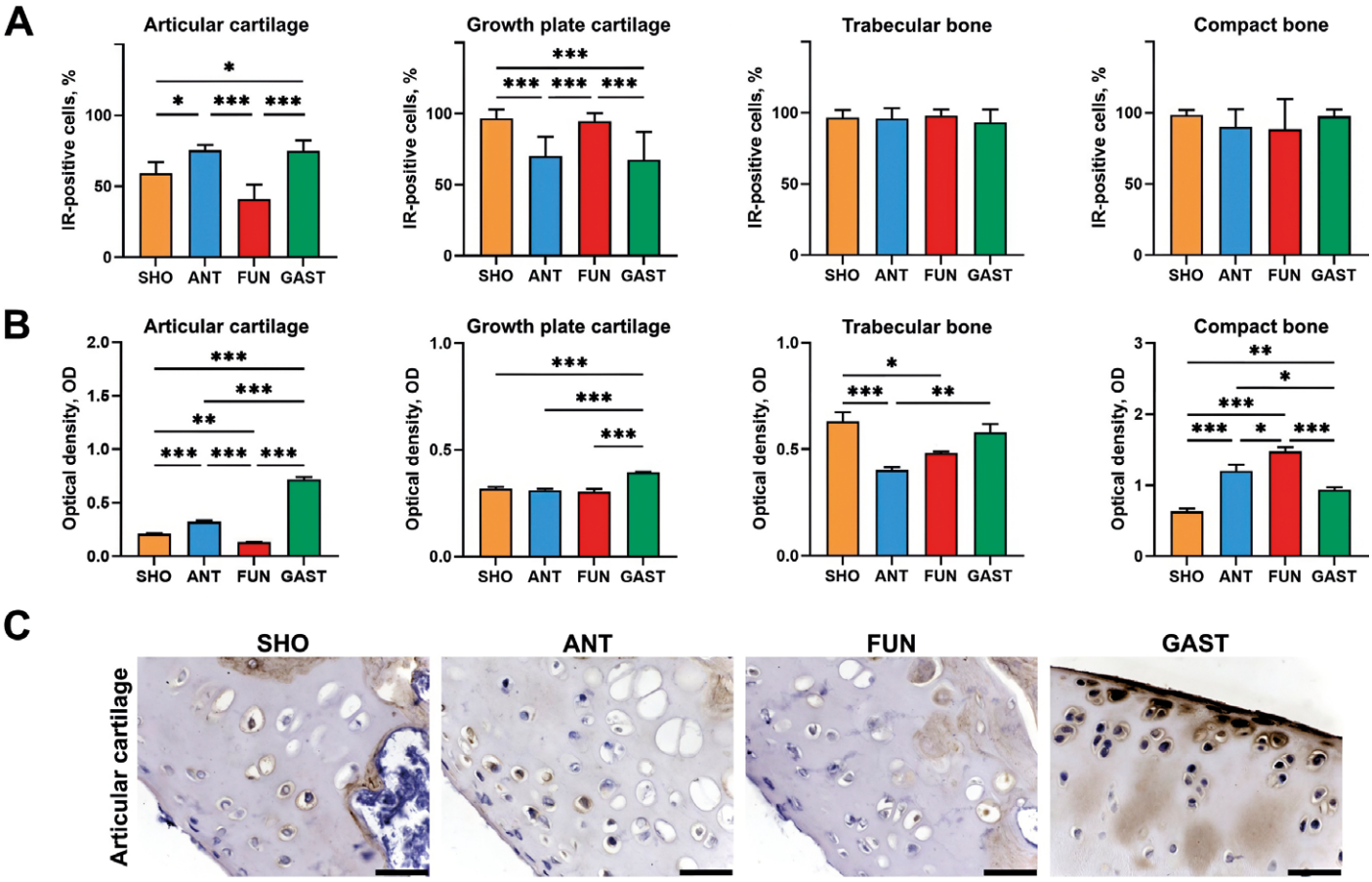

Fig. 1. The immunohistochemical reaction for osteocalcin (OC) in the femora of rats subjected to gastric surgeries: (A) the percentage of immunoreactive IR-positive cells and (B) quantitative analysis of the intensity of OC IR in articular cartilage, growth plate cartilage, trabecular bone, and compact bone measured by comparison of optical density (OD). (C) Representative images of OPG IR in articular cartilage

Explanations: Bar plots show mean values and standard error (whiskers). A p-value range was attributed to the above plots when two groups exhibited significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis test with Dunn's test and ANOVA with Tukey's HSD test). All scale bars represent 200 µm. SHO – sham operated; ANT – antrectomy, FUN – fundectomy, GAST – gastrectomy

GAST rats compared to the SHO and ANT rats; and in trabecular and compact bone, it was lower in the ANT rats than it was in the GAST rats (Fig. 2). No other changes were observed.

Complex physiological mechanisms regulate bone turnover processes. Key bone proteins, including OPG, receptor activator of nuclear factor NF-κB (RANK), and the RANK ligand (RANKL), collectively govern overall bone metabolism. OPG is secreted by various cells, such as osteoblasts, dendritic cells, and lymphocytes, while RANKL is expressed in osteoblasts, osteoclasts, primary mesenchymal cells surrounding cartilage and chondrocytes, as well as endothelial cells and the extracellular space. RANKL plays a critical role in osteoclast differentiation from precursors, enhancing their activity and survival by inhibiting apoptosis and thus promoting bone resorption. This interplay is crucial for bone loss and the development of osteoporosis. Osteoclast development and function are regulated primarily by the interaction between RANKL and the RANK receptor on their surface. These processes are hindered when OPG binds to RANKL, because OPG competes with RANK for binding due to its high affinity for the RANK receptor. These processes are hindered when OPG binds to RANKL, as OPG competes with RANK for binding due to its high affinity for the RANK receptor. An imbalance between RANKL and OPG is typically the underlying cause of various bone disorders. Additionally, proper bone metabolic processes are crucial for maintaining the macro- and microstructure of bones and ensuring adequate mechanical strength. The mechanical resistance of bone depends on mineral density and mechanical properties, such as stiffness and strength, which are influenced by genetic, environmental, geometric, physiological, and pathological factors, as well as bone trabecular microarchitecture, appropriate mineralization, and the presence of microdamage. The present findings suggest that gastrectomy, that is, a total or partial removal of the stomach, can have significant effects on bone metabolism, as indicated by variations in OPG IR and the number of OPG-positive cells in different anatomical structures in the experimental groups. The data show that antrectomy had the strongest impact on OPG IR.

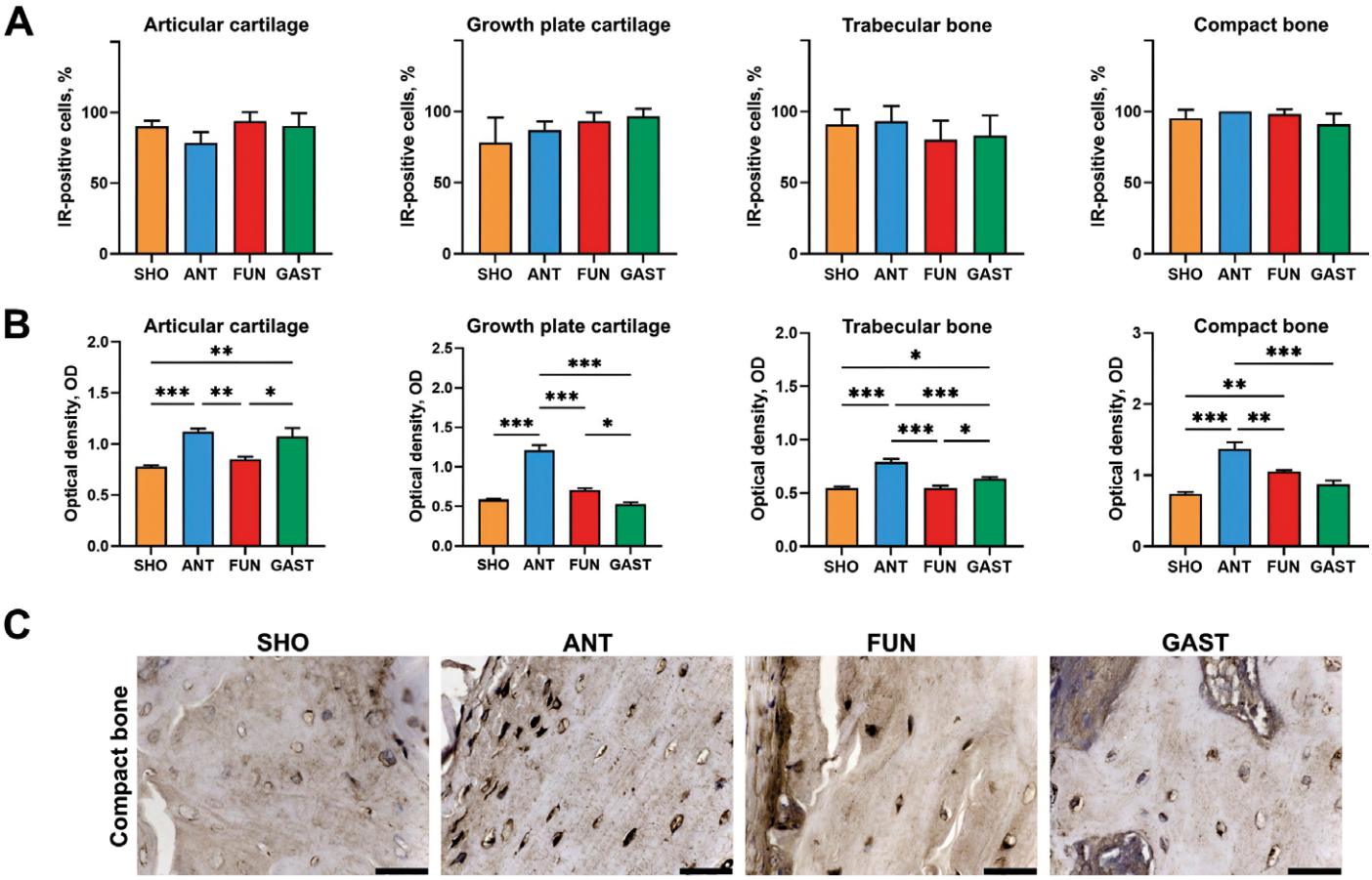

Fig. 2. The immunohistochemical reaction for osteoprotegerin (OPG) in the femora of rats subjected to gastric surgeries: (A) the percentage of immunoreactive IR-positive cells and (B) quantitative analysis of the intensity of OPG IR in articular cartilage, growth plate cartilage, trabecular bone, and compact bone measured by comparison of optical density (OD). (C) Representative images of OPG IR in compact bone

Explanations: Bar plots show mean values and standard error (whiskers). A p-value range was attributed to the above plots when two groups exhibited significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis test with Dunn's test and ANOVA with Tukey's HSD test). All scale bars represent 200 µm. SHO – sham operated; ANT – antrectomy, FUN – fundectomy, GAST – gastrectomy

It indicates that gastrin, ghrelin, and others hormones, which are altered by total or partial gastrectomy, are implicated in bone metabolism and may indirectly influence OPG levels. Previous studies have shown that ghrelin directly regulates bone formation, while pantoprazole-induced bone loss through gastrin secretion suggests a potential link between gastrin and bone health (10, 22, 33). The removal of the antrum and fundus (or the entire stomach), which are involved in gastric acid secretion and hormone production, could lead to changes in nutrient absorption, hormonal balance, or gut microbiota composition that directly affect bone metabolism. The present findings are consistent with those from previous research showing bone loss in rats subjected to total or partial gastrectomy. This suggests that the variations observed in OPG IR and the number of OPG-positive cells may reflect underlying changes in bone metabolism following gastric surgery (18). The current study does not present other analyses except for the IR of selected proteins. However, there is another study presenting opposite results, where total gastrectomy does not affect the OPG (osteoprotegerin) expression in bone tissue (43). On the other hand, another study has shown a decrease in OPG concentration in blood serum 3 months post-gastrectomy (2).

Bone morphogenetic protein 2. The intensity of BMP-2 IR in articular cartilage, growth plate, and trabecular bone was strongest in the FUN rats. Moreover, BMP-2 IR in growth plate was stronger in the GAST rats than it was in the SHO and ANT groups. In compact bone, BMP-2 IR was stronger in the SHO rats than in the ANT and GAST rats, and in the FUN rats it was stronger than in the ANT and GAST rats (Fig. 3). No other changes were observed.

These variations in the signal intensity of BMP-2, a protein related to osteoinduction, suggest differential regulation in response to different types of gastrectomy. This protein regulates mainly the growth of chondrocytes in the cartilage plate. It is well known that BMP-2 is expressed by both osteocytes and osteoblasts (14), and when OPG expression increases, the number of mature osteoclasts decreases, which in turn leads to a decrease in BMP-2 expression. Exactly that relationship between BMP-2 and OPG IR was observed in the

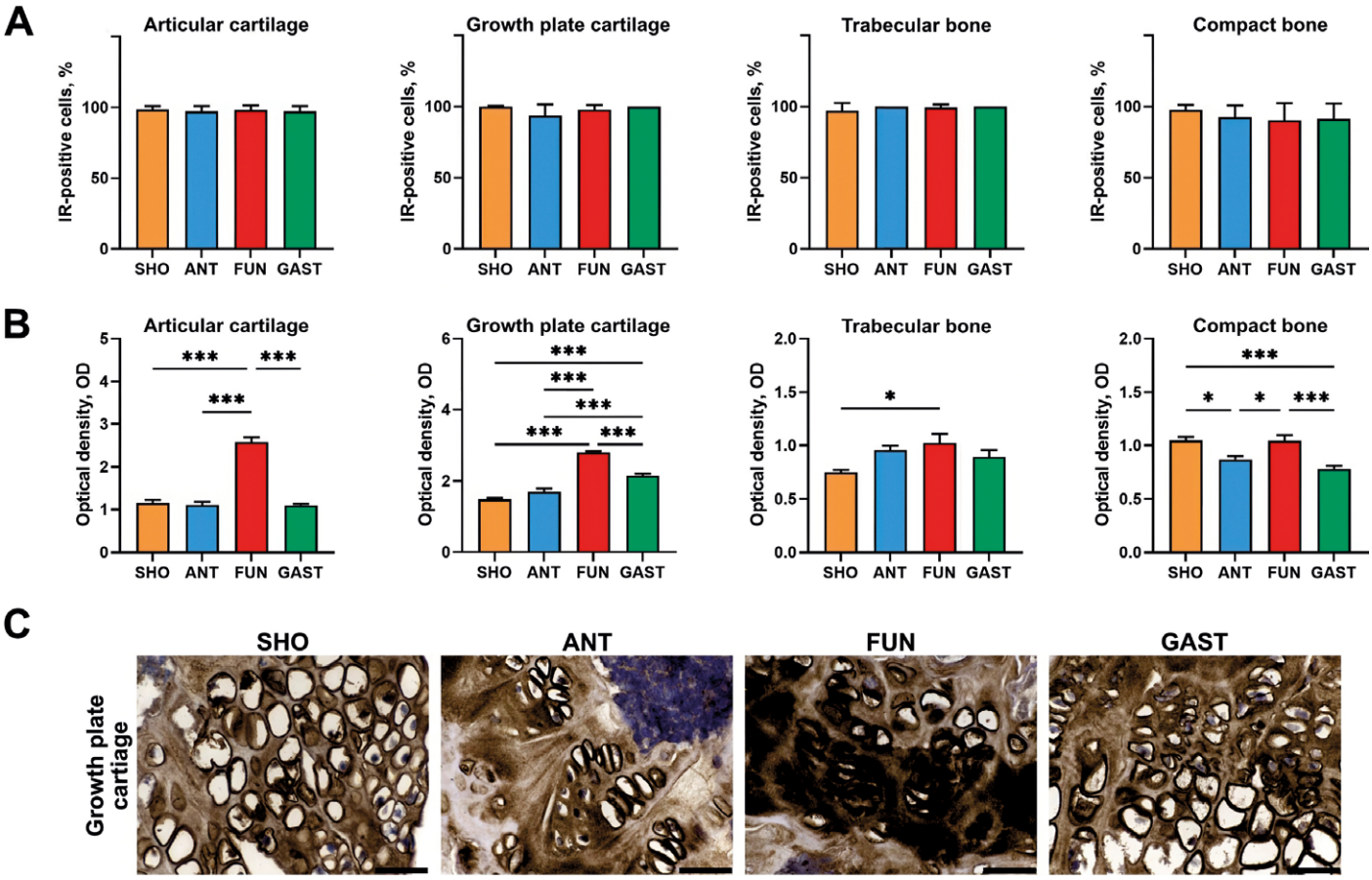

Fig. 3. The immunohistochemical reaction for bone morphogenetic protein 2 (BMP-2) in the femora of rats subjected to gastric surgeries: (A) the percentage of immunoreactive IR-positive cells and (B) quantitative analysis of the intensity of BMP-2 IR in articular cartilage, growth plate cartilage, trabecular bone, and compact bone measured by comparison of optical density (OD). (C) Representative images of OPG IR in growth plate cartilage

Explanations: Bar plots show mean values and standard error (whiskers). A p-value range was attributed to the above plots when two groups exhibited significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis test with Dunn's test and ANOVA with Tukey's HSD test). All scale bars represent 200 µm. SHO – sham operated; ANT – antrectomy, FUN – fundectomy, GAST – gastrectomy

current study, especially in the FUN rats. Moreover, BMP-2 regulates OPG mRNA levels in osteoblasts, increasing the RANKL/OPG ratio and promoting osteoclastogenesis (13). BMP-2 is responsible for the formation of both bone and cartilage tissue, and plays a crucial role during remodeling (34). BMP-2-deficient mice have skeletal malformations due to disturbances in complex events in growth plate cartilage, including proliferation, hypertrophic differentiation, and apoptosis. The hypertrophic zone is especially important, since chondrocytes in this part are invaded by blood vessels, osteoblasts, and osteoclasts in order to initiate the ossification process (25).

Tissue inhibitor of metalloproteinases 2. The intensity of TIMP-2 IR in articular cartilage was highest in the ANT rats, and in the GAST rats it was higher than in the SHO and FUN groups. In growth plate, IR was significantly higher in the GAST rats than it was in the other groups, and in the FUN rats it was higher than in the ANT rats. In trabecular bone, IR was higher in the SHO and FUN rats than in the ANT rats, and in the GAST rats as compared to the ANT rats. In compact bone, TIMP-2 IR was higher in the ANT rats than in the GAST rats (Fig. 4). No other changes were observed.

In articular cartilage, the intensity of TIMP-2 IR was notably higher in the ANT rats than it was in the other groups. Similarly, in the GAST rats, TIMP-2 IR was higher than it was in the SHO and FUN groups. This suggests a potential role of TIMP-2 in regulating cartilage homeostasis after gastrectomy, with differential effects depending on the specific surgical procedure. In the growth plate, TIMP-2 IR was significantly higher in the GAST rats than it was in the other groups, and in the FUN rats it was significantly higher than in the ANT rats. This indicates that gastrectomy, particularly total gastrectomy, may influence TIMP-2 IR in the growth plate, potentially affecting processes related to bone growth and development. In trabecular bone, IR was higher in the SHO and FUN rats than in the ANT rats, and in the GAST rats compared to the ANT rats. This suggests that different types of gastrectomy may affect TIMP-2 levels in trabecular bone, potentially influencing bone remodeling and turnover, as in the case of compact bone.

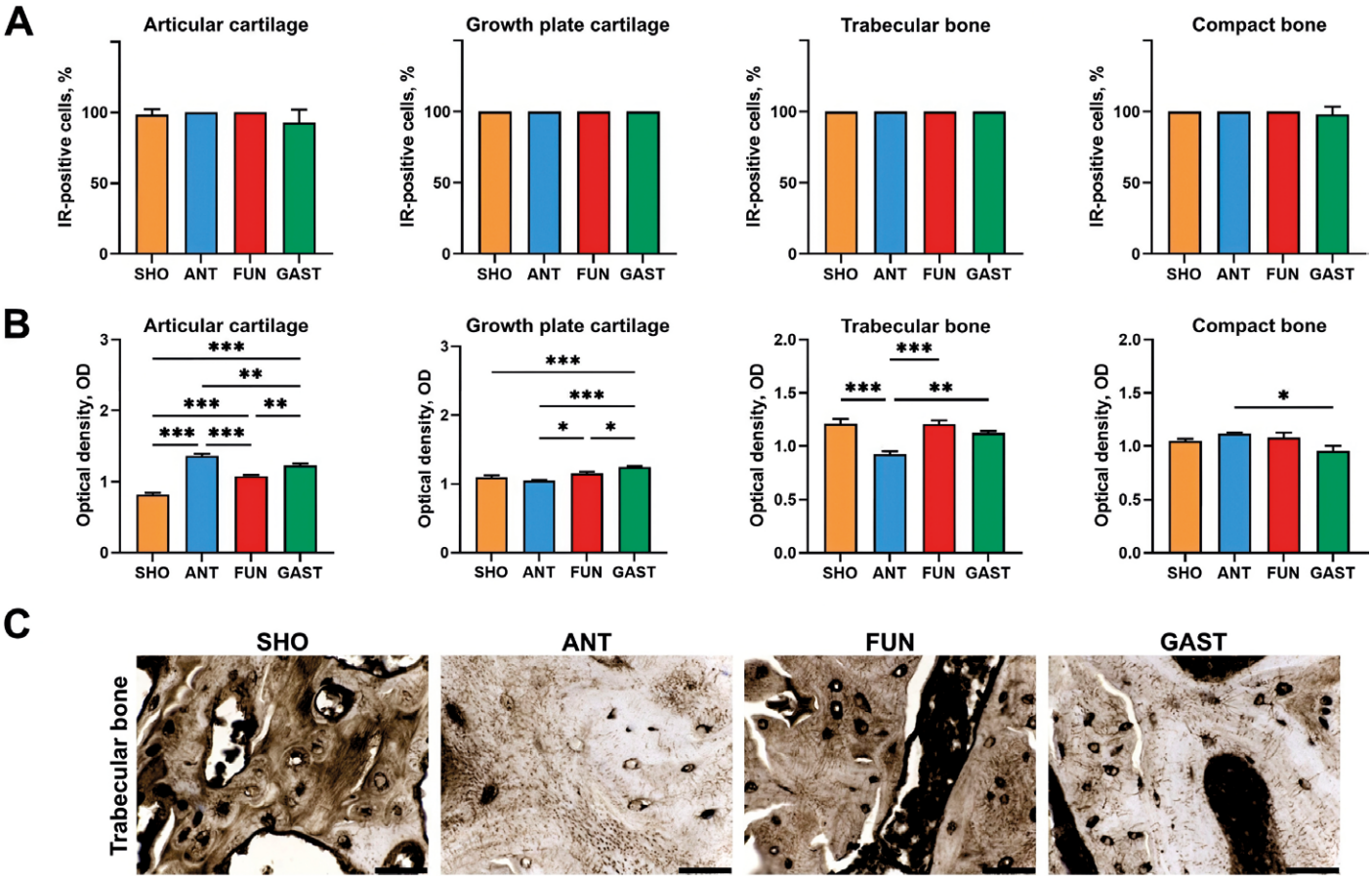

Fig. 4. The immunohistochemical reaction for tissue inhibitor of metalloproteinases 2 (TIMP-2) in the femora of rats subjected to gastric surgeries: (A) the percentage of immunoreactive IR-positive cells and (B) quantitative analysis of the intensity of TIMP-2 IR in articular cartilage, growth plate cartilage, trabecular bone, and compact bone measured by comparison of optical density (OD). (C) Representative images of OPG IR in trabecular bone

Explanations: Bar plots show mean values and standard error (whiskers). A p-value range was attributed to the above plots when two groups exhibited significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis test with Dunn's test and ANOVA with Tukey's HSD test). All scale bars represent 200 µm. SHO – sham operated; ANT – antrectomy, FUN – fundectomy, GAST – gastrectomy

TIMP-2 plays a role in the maintenance of balance between extracellular matrix deposition and degradation in different physiological and pathological processes (5). TIMP-2, as endogenous inhibitor that blocks the extracellular matrix-degrading activity of matrix metalloproteinases, is responsible for the stimulation of osteoclastic bone resorption. Reduced TIMP levels contribute to increased bone resorption through stronger expression of metalloproteinases (49). The different effects of different surgical procedures observed in our study highlight the complexity of the response of bone and cartilage tissue to changes in gastric physiology. These findings underscore the importance of considering the specific type of gastrectomy in understanding its impact on bone loss, as TIMP-2 plays a key role in maintaining the balance between extracellular matrix deposition and degradation, which is essential for bone health and integrity.

Nesfatin-1. The intensity of nesfatin-1 IR in articular cartilage was higher in the ANT and GAST rats than it was in the SHO rats. In growth plate, IR was lowest in the SHO group, whereas in the FUN rats it was higher than in the ANT and GAST rats. In trabecular bone, IR was lower in the SHO and ANT rats than it was in the FUN and GAST rats. In compact bone, IR was highest in the GAST rats, and in the FUN rats it was higher than in the SHO group (Fig. 5). No other changes were observed.

Nesfatin-1 is expressed in the duodenum, pancreas, and colon, and plays a role in enzyme activation, nutrient absorption, and protection of the intestinal walls (29, 42). It reduces food intake and is involved in the regulation of body weight, as well as inhibits gastric motility and emptying, duodenal motility, and the vagally mediated stimulation of gastric acid secretion (46). As shown by previous studies, an increase in the level of nesfatin-1 may be responsible for weight loss after gastric resection (7, 50).

The variations in nesfatin-1 IR across different anatomical structures and experimental groups after gastrectomy provide insights into the potential role of nesfatin-1 in regulating metabolic processes and body weight after gastrointestinal surgery. Since nesfatin-1 is implicated in the regulation of glucose metabolism

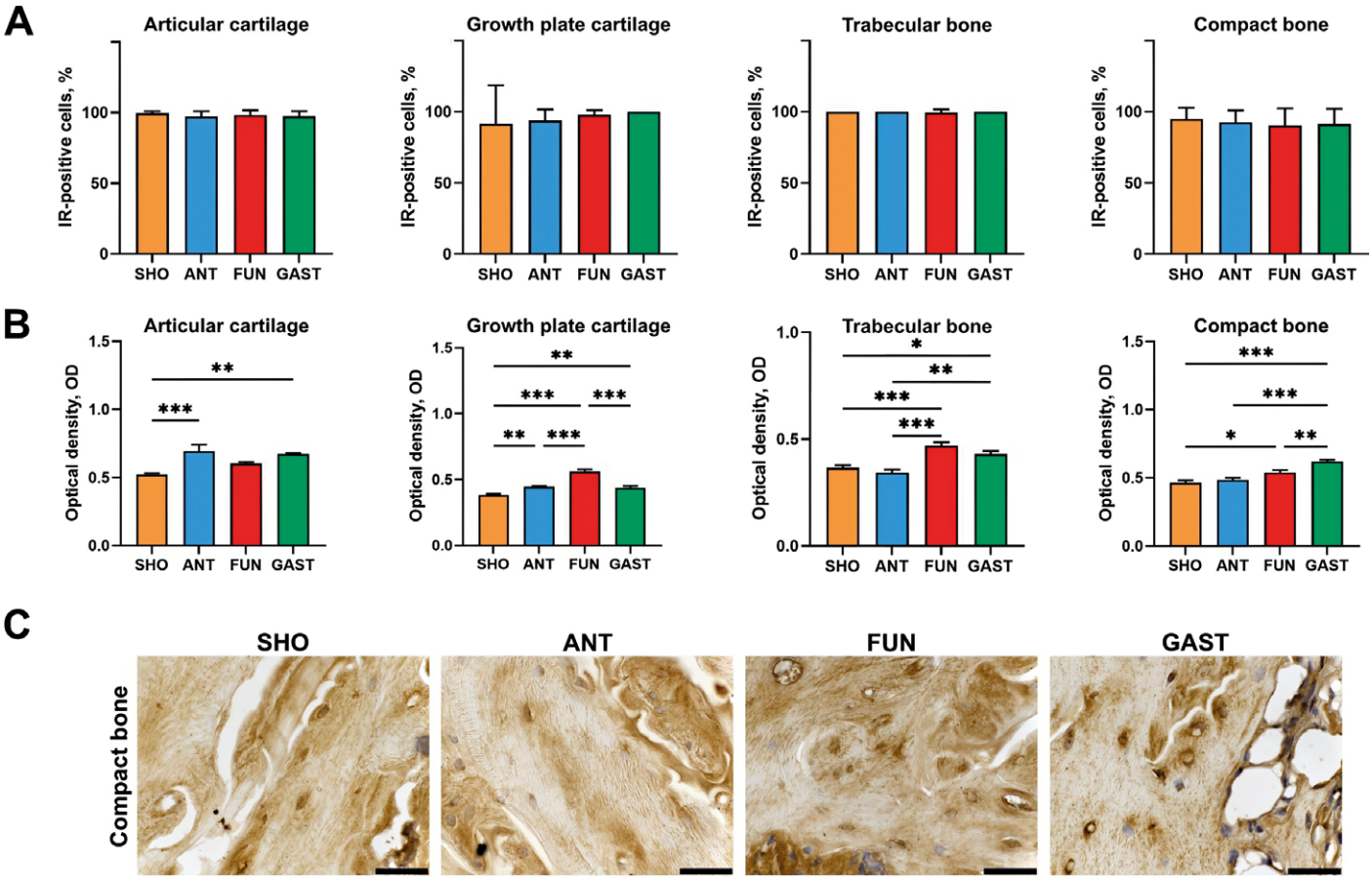

Fig. 5. The immunohistochemical reaction for nesfatin-1 in the femora of rats subjected to gastric surgeries: (A) the percentage of immunoreactive IR-positive cells and (B) quantitative analysis of the intensity of nesfatin-1 IR in articular cartilage, growth plate cartilage, trabecular bone, and compact bone measured by comparison of optical density (OD). (C) Representative images of OPG IR in compact bone

Explanations: Bar plots show mean values and standard error (whiskers). A p-value range was attributed to the above plots when two groups exhibited significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis test with Dunn's test and ANOVA with Tukey's HSD test). All scale bars represent 200 µm. SHO – sham operated; ANT – antrectomy, FUN – fundectomy, GAST – gastrectomy

and body weight, it may play a potential role in mediating the metabolic consequences of gastrectomy. Further, the current study highlights the potential role of nesfatin-1 in modulating the metabolism and mechanical strength of bone after gastrectomy (18). The important role of nesfatin-1 in the metabolism of bone tissue is suggested by other studies, in which exogenous nesfatin-1 limited bone loss, preserved bone architecture, and increased bone strength in established and developing osteopenia (30, 42). A similar study showed an increase in the concentration of nesfatin-1 in the blood serum of rats depending on the type of surgery (18). On the other hand, clinical data showed that nesfatin-1 level decreased in individuals undergoing bariatric surgery (31).

Further research is needed to elucidate the specific mechanisms underlying the effects of nesfatin-1 on bone metabolism and to explore its potential therapeutic implications.

In summary, our research demonstrated a significant impact of bariatric surgery on bone tissue homeostasis through significant changes in the expression of regulatory proteins involved in bone tissue remodeling.

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