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Fundectomy, antrectomy and gastrectomy influence densitometric, tomographic and mechanical bone properties as well as serum ghrelin and nesfatin-1 levels in rats

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Summary

The aim of the present study was to examine the influence of gastrectomy, fundectomy, and antrectomy on bone properties and changes in the levels of ghrelin and nesfatin-1 in rats, as well as to reveal their potential influence on bone metabolism. Twenty-four 2.5-month-old male Wistar rats were divided into four groups: sham-operated animals (SHO) and those subjected to gastrectomy (Gx), fundectomy (Fx), and antrectomy (ANT). After a six-week experiment, the rats were sacrificed, and blood was collected for further nesfatin-1 and ghrelin analysis in serum (RIA methods). The tBMC and tBMD of the whole skeleton, as well as the BMD and BMC of isolated femora, were measured by the DXA method. The femora were also examined by the pQCT method (area, mineral content, volumetric density of the trabecular and cortical parts of diaphysis and distal metaphysis) and by mechanical tests. Gx and ANT induced a decrease in BMD, ultimate force and work to failure of the femur, Tot.vBMD and Ct.Th of the femoral diaphysis, and Tot. BMC, Tot.vBMD, Tot.Ar, and Tb.BMC of femoral metaphysis. Fx lowered Tot.BMC, Tot.Ar, and Tb.BMC of metaphysis. The reduction in Tot.vBMD, Tot.Ar, and Tb.BMC of metaphysis after Gx was greater than after Fx. Moreover, the metaphyseal Tot.Ar and Tb.BMC of the Gx rats were lower than those in the ANT rats. The serum ghrelin concentration was reduced by antrectomy (by 57%), fundectomy (by 71%), and gastrectomy (by 76%). Conversely, the serum level of nesfatin-1 was increased in all the experimental groups (by 28%, 40%, and 65% in Fx, Gx, and ANT, respectively). In conclusion, our data indicate that the removal of different parts of the stomach caused negative changes in bone strength as well as in DXA and pQCT parameters. The Gx-evoked osteopenia and deterioration in bone parameters are more severe than after Fx and ANT. The bone response to gastric resection appeared to differ between cortical and cancellous bones. The changes observed in bone properties are probably a consequence of changes in the endocrine function of the stomach. They suggest that nesfatin-1 plays a yet unknown role in gastrectomy-, fundectomy-, and antrectomy-related bone loss, but further research is required to verify this hypothesis.

Keywords: fundectomy, antrectomy, gastrectomy, ghrelin, nesfatin-1

The maintenance of skeletal integrity depends on various organs, including the gastrointestinal tract, where different dietary components required for normal bone metabolism are derived from diet, e.g. protein, magnesium, zinc, copper, iron, fluoride, vitamins D, A, C, K, and folic acid (18, 48). Consequently, gut disorders characterized by malabsorption of these nutrients lead to the deterioration of bone health. The surgical bypass and/or removal of several regions of the gut can also significantly affect bone metabolism. The surgical removal of the whole stomach (gastrectomy, Gx) or the acid-producing part of the stomach (fundectomy, Fx) has particularly significant negative effects on bone metabolism, initiating osteopenia in humans and animals (1-3, 17, 41, 46-48).

After Gx, the daily dietary intake of calcium, magnesium, phosphorus, iron, zinc, vitamin D, vitamin B_{12}, and folic acid is reported to be lower than the recom-

mendations (33, 37, 38). However, a general nutritional deficiency is unlikely to explain these effects, since the body weight development of Gx rats is very similar to that of a sham-operated group (24, 26, 27, 46-48).

Although gastric acid secretion and gastric acidity have been suggested to facilitate the intestinal absorption of ingested calcium by mobilizing calcium from insoluble complexes in the diet (39), available evidence demonstrates that the negative effect of Gx and Fx on bone is probably not related to the loss of gastric acid (6, 48). In fact, calcium does not seem to be critical, since supplementation with oral and/or systemic calcium has failed to prevent the Gx-evoked osteopenia (24, 28, 39).

Another possibility is that the Gx-evoked osteopenia reflects vitamin D deficiency, though after Gx in humans, pigs, and rats, calcium absorption is impaired, calcium and 25(OH)D levels decrease, and the $1,25(OH)_2D_3$ level increases (11, 32, 50, 53). The malabsorption of calcium and vitamin D with subsequent secondary hyperparathyroidism may play an important role in bone disorders, including osteomalacia and osteoporosis (5, 11, 53).

The gut is an important site of endocrine and neuroendocrine hormone production. There is a considerable crosstalk between the gut and other organs in the body involved in the regulation of metabolism (42). Among the regulatory mechanisms of bone metabolism, hormones produced in the gut may play an essential role. The connection between gut hormones and bone has been defined as an entero-osseous-axis (7). It is, therefore, quite likely that a mechanism for the osteopenic effect of Gx or Fx is through the reduction or loss of endocrine hormones that support normal bone metabolism. The oxyntic mucosa of the stomach is rich in peptide hormone-producing endocrine cells, ECL (enterochromaffin-like cells), and X/A-like cells. Larsson et al. (25) and Zhao et al. (52) showed that gastric fundal extracts (extracts of oxyntic mucosa, EOM) evoked a rise in intracellular calcium in human, mouse, and rat osteoblast cell lines. Moreover, EOM elevated the proliferation of osteoblasts, as well as the expression of collagen type I and osteocalcin *in vitro* (52). These results indicate that osteopenia induced by gastric resection may be due to the lack of special molecules secreted by endocrine cells in the stomach.

The endocrine cells of gastric mucosa are a source of different hormones, e.g. ghrelin, obestatin, apelin, and nesfatin-1/NUCB2. Receptors for these hormones were found in human and rat osteoblasts and osteoblast-like cells (29, 36, 51). However, cells respond to gut peptide stimuli in feeding/fasting states depending on their stage of differentiation and on the duration of exposure to gut hormones (36). Human and murine chondrocytes express nesfatin-1/NUCB2 (20, 44), and osteoblasts express apelin (51) and ghrelin (12, 15).

A positive influence of ghrelin on bone metabolism was demonstrated by studies *in vitro* and *in vivo* (12, 13, 15, 22). However, several investigations indicated no such influence (14, 15). To date, only one paper reports that nesfatin-1 promotes osteogenesis *in vivo* by increasing bone mineral density in ovariectomized rats (29). On the other hand, the effects of obestatin and apelin were observed only *in vitro* (29, 51). It is conceivable that the absence of one or other of the putative peptide hormones of the oxyntic mucosa will lead to negative changes in bone, but the underlying mechanism is still poorly understood. Therefore, the aim of the present study was to examine the influence of gastrectomy, fundectomy, and antrectomy on bone properties and changes in the levels of ghrelin and nesfatin, which would establish grounds for speculation as to their influence on bone metabolism.

Material and methods

Animal procedures. The experimental procedures were approved by the 2nd Local Animal Welfare Committee in Lublin, Poland.

The study was carried out on 24 male Wistar rats aged 2.5 months with an initial body weight of approximately 220--240 g. First, the rats were adapted for 7 days to the experimental conditions of the animal house, and then they were housed under a controlled temperature of 22°C (± 10%) and humidity of 55% (± 10%) with a 12 h day/night cycle. The rats were housed separately in plastic cages with *ad libitum* access to water and LSM Standard Rat and Mouse chow (Agropol-Motycz, Poland). After acclimatization period, the animals were randomly divided into 4 groups, one control and 3 experimental.

Experimental design. Surgical operations were performed after 7-d adaptation, under anesthesia with ketamine, 15 mg/kg b.w. *i.m.* (Biowet-Pulawy, Poland), and Rometar, 35 mg/kg b.w. *i.m.* (Leciva, Czech Republic). Six control rats underwent a sham operation by an abdominal mid-line incision, followed by gentle manipulation of the viscera. Six rats were subjected to gastrectomy (Gx), i.e., resection of the antrum and the glandular part of the stomach followed by joining the duodenum to the nonglandular part of the stomach end-to-end (followed by rumen-duodenostomy end-to-end) (27). In six rats, parts of the acid-producing part of the stomach (fundus, oxyntic gland mucosa) were resected in an operation referred to as fundectomy (Fx). Fx was achieved by dividing the antrum-fundus border along the visible borderline as well as dividing the easily recognizable border between the rumen and the fundus. In six animals, the antrum was removed (ANT), and the fundus of the stomach was jointed to the duodenum.

Densitometric analysis (DXA). After six weeks of the experiment, overnight-fasted rats were weighed and anaesthetized in CO_2, and then blood was collected by cardiac puncture. The rats were killed immediately by cervical dislocation, and total bone mineral content (tBMC) and total bone mineral density (tBMD) of the whole skeleton were measured by the DXA method (Norland Excell Plus X-ray densitometer, Fort Atkinson, WI, USA). Then, femora were isolated, stripped from soft tissues, and frozen at –20°C for further analysis. The quality of bone was evaluated on the basis of its weight, length, bone mineral content (BMC), and areal bone mineral density (BMD). DXA measurements of BMC and BMD were established by Small Subject Scan software (Norland Illuminatus v. 4.4.1) which made it possible to analyze the isolated bones (scout scan speed 100 mm/s, resolution 3.0 × 3.0 mm; measurement scan speed 30 mm/s, resolution 1.0 × 1.0 mm). The machine was calibrated daily using the quality assurance

phantom (QA-Phantom), provided by the manufacturer in agreement with procedures.

Peripheral quantitative computed tomography. The femur was fixed in a test tube filled with 70% ethanol, and then scanned using peripheral quantitative computed tomography (pQCT) (XCT Research SA Plus system with software version 6.2.C; Stratec Medizintechnik GmbH, Pforzheim, Germany). The scans were performed in the middle diaphysis (50% of bone length) for the analysis of cortical bone tissue and in the distal metaphysis (5 mm from distal end) for the analysis of trabecular bone tissue. The following parameters were determined: total bone mineral content (Tot.BMC), total volumetric bone mineral density (Tot.vBMD), total bone area (Tot.Ar), 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), cortical thickness (Ct.Th), as well as periosteal (Peri.C) and endocortical (Endo.C) circumferences. Analyses of the trabecular bone were performed with a threshold of 0.450 cm^{-1}, contour mode 2, and a peel mode 20, whereas the cortical tissue was tested with a threshold of 0.900 cm^{-1} and cortical mode 2. The initial scan was performed at a speed of 10 mm/s, and CT-scan 4 m/s. The pQCT system was calibrated daily with the use of hydroxyapatite containing a quality assurance phantom (pQCT QA-Phantom).

Mechanical testing. Bones were submitted to a 3-point bending test with the use of a Zwick-Roell Testing Machine Z010 (Zwick GmbH&Co. KG, Ulm, Germany) equipped with a moving head with an operation range of 0-10 kN at a constant speed of 10 mm/min. The ultimate strength, work to failure, and the Young modulus were estimated.

Biochemical analysis. The blood samples after clotting were centrifuged and frozen at –80°C for further analysis. The serum concentrations of ghrelin and nesfatin were measured by RIA methods with a Ghrelin Rat, Mouse RIA kit and Nesfatin (1-82) Rat RIA kit (Phoenix Pharmaceutical, Inc., Burlingame, California, USA).

Statistical analysis. All results are expressed as means ± SD. Differences between groups were tested with the one-way analysis of variance (ANOVA) and post hoc Tukey's test as a correction for multiple comparisons. Differences were considered significant at $P \leq 0.05$. All statistical analyses were performed with the use of Statistica software v. 8.0 (StatSoft, Inc., Tulsa, USA, 2008).

Results and discussion

The initial body weight did not differ significantly between the groups. The final body weight was significantly lower (by 15.5%) in the Fx group than in the SHO group. No significant differences were observed in the other experimental groups (data not shown).

The gastric resection did not significantly influence either the femoral weight and length (data not shown) or tBMD. However, in the experimental groups, the values of tBMD were lower than in the SHO group (Tab. 1). A similar tendency was observed for tBMC, whereas significant differences were noted between the SHO and Fx rats (Tab. 1). ANT and Gx induced a significant decrease in femoral BMD (by 10%), but did not significantly influence BMC (Tab. 1). In femoral diaphysis, ANT and Gx diminished ultimate force and work to failure (Tab. 1), but did not cause any significant changes in the Young modulus (data not show). The mean reduction in ultimate force was 17% for both groups, and in work to failure 34% and 43% for the ANT rats and Gx rats, respectively.

The experimental groups did not differ from the SHO rats with respect to Tot.BMC, Ct.BMC, Tot.Ar, Ct.vBMD, Ct.Ar, and Peri.C for femur diaphysis. ANT and Gx decreased significantly diaphyseal Tot.vBMD and Ct.Th, but increased Endo.C. (Tab. 2). ANT reduced Tot.vBMD and Ct.Th by 11% and 13%, respectively, whereas Gx reduced these parameters by 14% and 10%, respectively. Significant differences for Tot.vBMD were also observed between the Fx and Gx rats. Gx lowered Tot.vBMD by 13% compared with Fx. The values of Endo.C. were significantly higher in the ANT and Gx groups: by 11% and 18%, respectively, compared with the SHO group (Tab. 2).

All types of gastric surgery reduced metaphyseal Tot.BMC, Tot.Ar, and Tb.BMC, whereas the values of Tot.vBMD were significantly decreased after ANT and Gx, by about 18% and 33%, respectively (Tab. 2). The greatest reductions in Tot.BMC, Tot.Ar, and Tb.BMC were observed in gastrectomized rats: by 28%, 61%, and 65%, respectively. Significant differences for Tot.vBMD, Tot.Ar, and Tb.BMC were also noted between the Gx group and the Fx group, and for Tot.Ar and Tb.BMC between the Gx and ANT groups. On the other hand, Gx induced an increase in Tb.vBMD and Tb.Ar compared with the levels observed in fundectomized animals (Tab. 2).

The serum ghrelin concentration was reduced by antrectomy (by 57%), fundectomy (by 71%), and gastrectomy (by 76%) (Fig. 1). Conversely, the serum level of nesfatin was increased in all the experimental groups. It increased by 28%, 40%, and 65% after Fx, Gx, and ANT, respectively. Among the experimental groups, the ANT rats

Tab. 1. DXA measurements of bone mineral content (BMC) and bone mineral density (BMD) in the whole skeleton and femur, and femur strength in rats after a sham operation (SHO), fundectomy (Fx), antrectomy (ANT), and gastrectomy (Gx). Data are presented as x ± SD.

Parameters	SHO	Fx	ANT	Gx
tBMC (g/cm)	10.4 ± 2.16	8.76 ± 1.46	10.2 ± 2.68	9.96 ± 1.53
tBMD (g/cm^2)	0.173 ± 0.028	0.164 ± 0.015	0.162 ± 0.011	0.157 ± 0.012
Femur				
BMC (g/cm)	0.475 ± 0.073	0.457 ± 0.063	0.452 ± 0.064	0.449 ± 0.099
BMD (g/cm^2)	0.103 ± 0.011^a	0.099 ± 0.011	0.093 ± 0.007^b	0.92 ± 0.011^b
Ultimate force (N)	227 ± 53.3^a	200 ± 44	188 ± 40.8^b	189 ± 40^b
Work to failure (N·mm)	76 ± 15.3^a	65 ± 41	50.5 ± 26.7^b	43.5 ± 19.8^b

Explanation: a, b – values in rows denoted by different letters differ significantly at $P \leq 0.05$

Tab. 2. Peripheral quantitative computed tomography of the femur of rats after a sham operation (SHO), fundectomy (Fx), antrectomy (ANT), and gastrectomy (Gx). Data are presented as $\bar{x} \pm SD$.

Parameters	SHO	Fx	ANT	Gx
Diaphysis				
Tot.BMC (mg/mm)	10.27 ± 1.10	9.90 ± 1.46	9.48 ± 0.87	10.17 ± 1.34
Tot.vBMD (mg/mm^3)	924.4 ± 50.4^{ac}	912.1 ± 98.7^{c}	824.9 ± 138^{bc}	795.6 ± 46.4^{b}
Tot.Ar (mm^2)	11.17 ± 1.63	10.93 ± 1.72	11.76 ± 2.21	12.82 ± 1.73
Ct.BMC (mg/mm)	9.83 ± 0.89	9.29 ± 1.32	9.10 ± 0.92	9.66 ± 1.17
Ct.vBMD (mg/mm^3)	1441.7 ± 16.5	1443.4 ± 33.0	1444.8 ± 44.5	1418.3 ± 13.9
Ct.Ar (mm^2)	6.82 ± 0.64	6.43 ± 0.89	6.30 ± 0.68	6.82 ± 0.85
Ct.Th (mm)	0.71 ± 0.03^{a}	0.67 ± 0.06	0.62 ± 0.06^{b}	0.64 ± 0.05^{b}
Peri.C (mm)	11.82 ± 0.84	11.69 ± 0.96	12.11 ± 1.14	12.67 ± 0.88
Endo.C (mm)	7.35 ± 0.84^{a}	7.46 ± 0.96	8.18 ± 1.35^{b}	8.66 ± 0.76^{b}
Distal metaphysis				
Tot.BMC (mg/mm)	15.99 ± 2.41^{a}	12.82 ± 1.59^{b}	13.34 ± 2.19^{b}	11.58 ± 2.45^{b}
Tot.vBMD (mg/mm^3)	658.8 ± 36.3^{ac}	590.8 ± 83.7^{c}	540.1 ± 71.1^{bc}	442.3 ± 76.7^{b}
Tot.Ar (mm^2)	4.64 ± 1.08^{a}	2.94 ± 0.93^{b}	3.02 ± 0.96^{b}	1.80 ± 1.17^{c}
Tb.BMC (mg/mm)	423.1 ± 54.4^{a}	302.0 ± 105^{b}	267.3 ± 61.9^{b}	148.5 ± 90.6^{c}
Tb.vBMD (mg/mm^3)	24.23 ± 2.94	21.96 ± 3.33^{a}	25.09 ± 5.07	26.23 ± 3.5^{b}
Tb.Ar (mm^2)	10.88 ± 1.34	9.88 ± 1.49^{a}	11.29 ± 2.27	11.79 ± 1.58^{b}

Explanation: a, b, c – values in rows denoted by different letters differ significantly at $P \leq 0.05$

were characterized by the highest serum concentrations of ghrelin and nesfatin (Fig. 1).

The present study indicated no influence of gastric surgery, except Fx, on body weight. Similar data were obtained by Lehto-Axtelius et al. (27), who observed a somewhat slower weight gain in fundectomized rats. Other authors did not observe any effect of gastric surgery on body weight gain in rats (24, 26, 27, 46-48). In humans, BMD changes after Gx are not associated with weight loss, either (41).

Our study confirmed that the resection of different parts of the stomach induced osteopenia, leading to changes in bone properties. Gx and ANT induced a decrease in BMD, ultimate force and work to failure of the femur, Tot.vBMD and Ct.Th of the femoral diaphysis, and Tot.BMC, Tot.vBMD, Tot.Ar and Tb.BMC of femoral metaphysis. Fx lowered Tot.BMC, Tot.Ar, and Tb.BMC of metaphysis. The reduction in Tot.vBMD, Tot.Ar, and Tb.BMC of metaphysis after Gx was greater than that after Fx. Moreover, the metaphyseal Tot.Ar and Tb.BMC of the Gx rats were lower than those of the ANT rats. The reduction in Tot.vBMD and Ct.Th in the Gx and ANT groups was associated with increased endocortical bone resorption, which was also observed by Iwamoto et al. (19).

The results of the present study indicate that the most severe changes in bone parameters resulted from gastrectomy. The degree of bone changes was also greater after ANT than after Fx. Our findings do not correspond to those of Lehto-Axtelius et al. (27), who found that the osteopenia induced by Fx was quantitatively and qualitatively similar to that induced by the resection of the whole glandular portion of the stomach, whereas the resection of the antrum had an insignificant effect on bone structure.

Furthermore, we observed that the deterioration in bone parameters was greater in femoral distal metaphysis than in femoral diaphysis. Thus, our results revealed a different response of cortical and cancellous bones to gastric surgery. Similar results were reported by Iwamoto et al. (19). In their study, Gx-induced reductions in BMC, BMD, and bone strength were greater at skeletal sites rich in cancellous bone than at sites rich in cortical bone, and the Gx-induced decrease in bone mass was greater in cancellous bone than in cortical bone.

Earlier data indicated that post-gastrectomy osteopenia in rodents was prevented by preserving 10-30% of the oxyntic gland area, the ghrelin and nesfatin-1 producing region of the stomach (28). Several experiments suggest that ghrelin may have an effect on bone homeostasis promoting osteoblast proliferation and differentiation, and calcium accumulation (12, 15, 23, 30). Although ghrelin has a positive effect *in vitro* (12, 23, 30, 45), *in vivo* studies on animals are contradictory, as they show either no association with bone mass in mice (15) or a positive effect in rats (30), whereas i.p. ghrelin infusion significantly increased femoral BMD. Other animal studies show that Gx-induced reduction in ghrelin is associated with osteopenia that is not reversed after the administration thereof (14). Findings from human studies in which BMD was measured are contradictory as well (25, 31, 49). However, after gastric bypass surgery, obese humans were diagnosed with a low serum ghrelin level as well as an increase in bone resorption and bone mass reduction (9, 21). Previously,

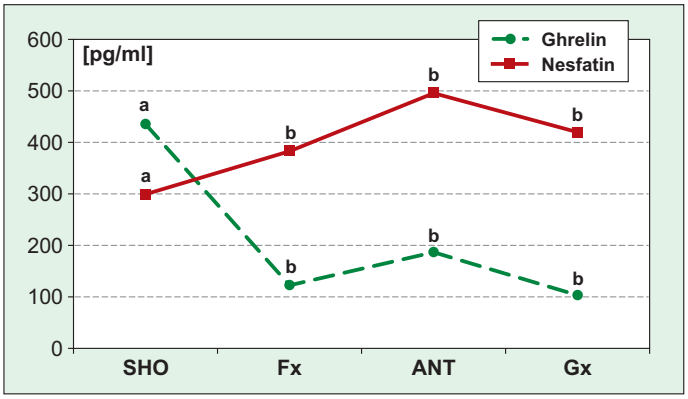

Fig. 1. Serum content of ghrelin and nesfatin in rats after a sham operation (SHO), fundectomy (Fx), antrectomy (ANT), and gastrectomy (Gx).
Explanation: a, b – values denoted by different letters differ significantly at $P \leq 0.05$

Letho-Axtelius et al. (26) indicated that serum ghrelin progressively decreased with the removal of increasing portions of the stomach. In our study, Fx, Gx, and ANT led to a reduction in serum ghrelin, as well as caused negative changes in bone properties. The greatest reduction in serum ghrelin was observed after Gx (by 76%). However, no significant influence of the type of surgery on the serum ghrelin level was noted. The Gx-induced deterioration in bone parameters was more severe than in the other experimental groups, and was accompanied by the greatest reduction in the ghrelin level. However, we did not estimate the correlation between the ghrelin level and bone properties. On the other hand, Napoli et al. (34) indicate a positive correlation between serum ghrelin and trabecular BMD in both elderly male and female subjects. Similar results were presented by Gonnelli et al. (16), who described a significant positive effect of ghrelin on femoral neck pQCT BMD in elderly men. On the other hand, no significant effects of ghrelin on BMD were found in DXA measurement (25, 40, 49). Moreover, long-term treatment of rats with omeprazole, a proton pump inhibitor, was found to cause osteopenia, but the number of X/A-like cells, a source of ghrelin, was not changed after an omeprazole treatment, and the ghrelin levels were not altered (10). These observations suggest that although ghrelin positively influences bone, it does not seem to be a gastric candidate supporting normal bone metabolism.

Fundectomy and gastrectomy reduced the number of X/A-like cells, which are also a source of nesfatin-1/NUCB2. Nesfatin-1 is an 82-amino-acid peptide that was first described in 2006 by Oh et al. (35). They found an anorexigenic protein derived from nucleobindin 2 (NUCB2) and named it NUCB2-encoded satiety- and fat-influencing protein, or nesfatin. Currently, a few available studies describe the effect of nesfatin-1 on cartilage and bone cells. Nesfatin-1/NUCB2 promotes differentiation and mineralization of pre-osteoblastic cells *in vitro* and inhibits osteoclastic differentiation of macrophages, which indicates its important role in the metabolic control of bone (29). Its cytoplasmic expression was detected in mouse and human chondrocytes (20, 44). In addition, nesfatin-1/NUCB2 production was increased during chondrocyte differentiation and after stimulation with pro-inflammatory mediators, such as IL-1 and TNF-α (44). These data suggest that this peptide might play a role in cartilage maturation, as well as in the complex mechanisms of chondrocyte development and differentiation, and might affect endochondral ossification. Furthermore, nesfatin-1 induces pro-inflammatory cytokines alone in primary human osteoarthritis chondrocytes and in combination with IL-1 in murine chondrocytes (44). On the other hand, Jiang et al. (20) reported a higher serum nesfatin-1 concentration in humans with osteoarthritis and a higher nesfatin-1 gene expression in osteoarthritis cartilage. The results of this study suggest a potentially pivotal role of nesfatin-1 in the pathophysiology of osteoarthritis.

In our study, gastric resections caused an increase in the serum nesfatin-1 level. In all the experimental groups, the serum nesfatin-1 level was higher than that in the SHO group, and the highest concentration was observed in the ANT group. These changes are difficult to explain. On the one hand, the increase in the serum nesfatin-1 level may suggest compensatory release of this peptide. On the other hand, an increase in the serum nesfatin-1 level in osteoarthritis observed by Jiang et al. (20) may suggest an effect of pro-inflammatory cytokines on nesfatin-1 release. Cytokines, such as IL-6, IL-1, and TNF-α, are involved in the mechanisms of bone remodelling and in the pathogenesis of osteoporosis (4). These cytokines increase bone resorption, and they are key regulators of osteoclastogenic activity (8, 43) leading to bone loss and negative changes in its structure and properties. It is most likely that the increase in the serum nesfatin-1 level observed in our study was associated with intensification of bone resorption, which was reflected in the negative changes in the bone properties. The deterioration of the bone parameters was accompanied by a high level of nesfatin-1, even in rats after antrectomy. Since our study is the first in which the nesfatin-1 level was determined after gastric resection, further studies are needed to determine the exact role of nesfatin-1 in bone metabolism.

In conclusion, our data indicate that the removal of different parts of the stomach caused negative changes in bone strength, as well as in the DXA and pQCT parameters. The Gx-evoked osteopenia and deterioration in the bone parameters were more severe than after fundectomy and antrectomy. The bone response to gastric resection appeared to differ between cortical and cancellous bones. The observed changes in bone properties are probably a consequence of changes in the endocrine function of the stomach. They suggest that nesfatin-1 plays a yet unknown role in gastrectomy-, fundectomy-, and antrectomy-related bone loss, but further research is required to verify this hypothesis.

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