Fundectomy-Evoked Osteopenia in Pigs Is Mediated by the Gastric-Hypothalamic-Pituitary Axis

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The aim of the study was to determine the effects of gastric impairment in pigs on the axial and peripheral skeletal system properties and to test the hypothesis that fundectomy-evoked osteopenia is related to disturbed gastric-hypothalamic-pituitary axis function. Forty-day-old male piglets were subjected to experimental fundectomy (FX group, *n* = 6) to induce osteopenia, while sham operation was performed in the controls (SHO group, *n* = 6). At the age of 8 months, serum samples were collected, and the animals were sacrificed to obtain lumbar vertebrae (L_1–L_6) and right humerus for analysis. Using quantitative computed tomography (QCT) and dual-energy x-ray absorptiometry (DEXA) methods, bone mineral density and bone mineral content of the vertebrae and humerus were measured. The compression and three-point bending tests were applied to determine mechanical properties of lumbar vertebrae and humerus, respectively. Furthermore, geometric properties of humerus were assessed. Serum concentrations of ghrelin, growth hormone (GH), insulin-like growth factor-1 (IGF-1), and selected macro- and microelements were also determined. Performed fundectomy decreased body weight in pigs by 66% compared with pair-fed sham operated pigs ($P < 0.0001$). Bone weight, bone mineral density, and bone mineral content of the lumbar vertebrae and humerus were significantly decreased in the fundectomized pigs ($P < 0.01$). Mechanical parameters of the lumbar spine and humerus were decreased after the fundectomy, as well. Serum concentrations of ghrelin, GH, and IGF-1 were lowered by 74.4%, 90.6%, and 54.6% in the fundectomized pigs, respectively (all $P < 0.001$). Moreover, the serum concentrations of calcium, magnesium, iron and copper in the fundectomized animals were significantly decreased by 15.5%, 45.3%, 26.7%, and 26.2%, respectively ($P \leq 0.05$). In conclusion, the results obtained showed that both the disturbed gastric-hypothalamic-pituitary axis function and impaired mineral metabolism are associated with development of postfundectomy osteopenia of axial and peripheral skeleton in pigs. Exp Biol Med 232:1449–1457, 2007

Key words: fundectomy; gastric-hypothalamic-pituitary axis; osteopenia; quantitative computed tomography

Introduction

The stomach has a variety of physiological functions that are lost when gastrectomy is performed. Abnormal food transit, disturbed nutrition intake, abnormal digestion and absorption, as well as deficiencies of micronutrients, are the most common problems related to gastrectomy and they have a negative impact on systemic functions in organisms (1). Gastric surgery in humans is a clinically relevant procedure. Among many reasons for performing gastric surgery, the most common are gastric malignancy, advanced peptic ulcer disease, surgical treatment of morbid obesity, and fundal variceal bleeding (2–5). It has been suggested that surgical resection of the different anatomic parts of the stomach may have various consequences for patients with regard to their quality of life and postoperative disorders (1). Considering the anatomic structure of the stomach in nonruminant organisms, three different types of the gastric resection can be performed. The first is total gastrectomy, consisting of surgical extirpation of the glandular part of the stomach followed by anastomosing the duodenum and the esophagus.

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To induce similar effects in rats, the total gastrectomy technique may be modified, and anastomosing the duodenum and the nonglandular part of the stomach end-to-end can be performed (modified gastrectomy). The second type of partial gastrectomy is called fundectomy, and it is carried out by selective resection of the acid-producing part of the stomach—the fundus. As a consequence of the fundectomy, the oxyntic mucosa, rich in peptide hormone-producing cells, is eliminated. The extirpation of the gastrin-producing part of the stomach (antrum) and the following anastomosing of the duodenum and the fundus end-to-end is defined as antrectomy. Total gastrectomy is known to induce osteopenia in humans and experimental animals (6–8). It has been reported that total gastrectomy in rats causes striking effects in several regions of the skeleton, including the trabecular and cortical bone compartments (9, 10). Both gastrectomy and fundectomy are reportedly the most effective in inducing osteoporosis, while the antrectomy has a less negative effect on bones (8). Mechanically, gastrectomy-induced osteopenic changes affect the load-carrying capacity and strength of long bones and all regions of the spine.

Recent findings indicate that the fundus of stomach, notably the acid-producing part (oxyntic mucosa), plays an important role in bone metabolism regulation. The high concentration of H^+ in the gastric juice dissolves dietary calcium, facilitating its absorption in the intestines (11, 12). Although calcium deficiency may contribute to osteoporosis development, it is surprising that gastrectomy-evoked osteopenia is not prevented with calcium administration (8). Moreover, blockade of secretion in the acid-producing part of stomach with the use of the proton pump inhibitor, omeprazole, had no effect on bone tissue properties in rats (13). Gastrectomy is usually accompanied by reduced food intake, which, combined with the effects induced by the peptides released from the peptide hormone–producing cells of the stomach (ECL cells), may be responsible for osteopenia (8).

The lack of knowledge about mechanisms responsible for negative effects of the fundectomy on bone tissue metabolism was considered in undertaking this study. To evaluate effects of long-term fundectomy on skeletal properties, determination of bone morphology, bone mineral density, and geometric and mechanical properties of the lumbar vertebra and humerus was performed. Assessment of ghrelin, growth hormone (GH), insulin-like growth factor-1 (IGF-1), and selected macro- and microelements in the serum of pigs was performed as well.

Materials and Methods

The experimental procedures used throughout this study were approved by The Local Ethics Committee on Animal Experimentation of The Agricultural University of Lublin, Poland.

Experimental Design. The study was performed on 12 pigs of the Large Polish White breed that were randomly assigned to two weight-matched groups at the age of 40 days of life—12 days after their weaning from sows. The animals were kept housed in indoor pens, under standard rearing conditions with constant access to fresh water, and were fed commercial diets (Table 1), supplied in accordance with the stage of production cycle and age of pigs, twice a day (0800 and 1600 hrs). The first group of animals, control group, consisted of 6 males that were subjected to sham operation (SHO group). The second group comprised 6 males in which the fundectomy procedure was executed (FX group). All of the control and experimental animals were sacrificed at the age of 8 months of life, just after blood collection following 24-hr fasting. The lumbar spine and right humeri were isolated for analyses; immediately after isolation and cleaning from remaining soft tissues, each bone was weighed, wrapped in gauze soaked in isotonic saline, and stored at –25°C for further analyses.

Surgical Procedure. All surgical procedures were performed on 40-day-old piglets under general anesthesia. Piglets were fasted for 24 hrs before the surgical procedure; however, they were allowed to drink fresh water until 3 hrs before the operation. In preparation for general anesthesia, piglets were sedated with an im (intramuscular) injection of azaperon (5 mg/kg body wt). Anesthesia was induced and then maintained with an iv (intravenous) injection of ketamine (10 mg/kg body wt) until the end of the procedure. The skin of the abdomen was shaved and then prepared with iodine-based scrub; the surgical site was then accurately draped. Both the fundectomy and sham operation involved a midline abdominal incision. Fundectomy was carried out by resection of the fundus and the glandular part of the cardium of the stomach and followed by anastomosing the remaining tissues, which allowed nondisturbed food passage to the duodenum. As the result of the fundectomy, approximately 50% of the stomach was removed with regard to the distribution of the ghrelin-containing glands (14). After recovery, both the fundectomized and sham-operated piglets were food deprived for next 24 hrs and an iv infusion of the 5% glucose solution (500 ml/piglet/day) was performed. During next 3 days all piglets were fed with one-third of the daily dosage of the diet, which was increased up to 50% until the end of the first week after the fundectomy. Starting on eighth day after the fundectomy, the fundectomized as well as control animals received 100% of the daily dosage of the diet. Throughout the whole period of the study, the pigs from FX and SHO groups were housed indoors in two pens (one pen for each group) equipped with feeders enabling food consumption for all animals within the group at the same time. During each feeding, the animals from FX group were fed *ad libitum,* and the remaining part of the feed was taken out from the feeder and weighed. The amount of feed consumed by FX group of animals was calculated, and after that SHO group received exactly the same amount of the feed for consumption. Thus, identical nutritional conditions were provided during the whole

Table 1. Composition of Diet Administered to Pigs During the Study[a]

	41–70 days of life	71–120 days of life	121–244 days of life
Ingredient			
Wheat middlings (%)	53.00	41.50	15.00
Barley middlings (%)	—	30.00	36.00
Soybean meal (%)	—	—	10.00
Rye middlings (%)	—	—	32.00
Rape middlings (%)	—	—	4.00
Panto Ferkelgold F 250[b] (%)	22.00	—	—
Panto Multivit-Premix M 51[c] (%)	—	3.50	—
Panto Multivit-Premix M 39[d] (%)	—	—	3.00
Corn, grain (%)	25.00	10.00	—
Composition			
Dry matter (%)	87.66	87.28	87.30
Crude protein (%)	16.96	16.66	16.00
ME (MJ/kg)	14.01	13.00	12.63
Crude fibre (%)	2.72	3.56	4.03
Crude fat (%)	1.98	1.98	1.75
Ash (%)	4.60	5.86	5.65
Starch (%)	47.60	45.34	43.60
Ca (%)	0.64	0.82	0.79
P (%)	0.48	0.48	0.48
Available P (%)	0.08	0.14	0.15
Na (%)	0.17	0.25	0.21
Mg (%)	0.20	0.20	0.19
Carbohydrates (%)	4.37	3.49	3.54
Lys (%)	1.17	1.00	0.93
Met (%)	0.55	0.33	0.30
Met + Cys (%)	0.79	0.65	0.63
Trp (%)	0.18	0.19	0.19
Thr (%)	0.74	0.63	0.57
Vitamin A (IE)	15,840	14,000	12,000
Vitamin D (IE)	1,760	1,750	1,200
Vitamin E (mg)	99.00	140	75.00
Vitamin C (mg)	33.00	—	—
Vitamin K (mg)	2.20	1.40	0.90
Vitamin B_1 (mg)	2.20	1.40	0.90
Vitamin B_2 (mg)	6.60	4.24	2.70
Vitamin B_6 (mg)	4.40	4.20	2.70
Vitamin B_{12} (μg)	33.00	35.00	—
Nicotinic acid (mg)	33.00	—	—

[a] Ca, calcium; P, phosphorus; Na, sodium; Mg, magnesium; Lys, lysine; Met, methionine; Trp, tryptophan; Thr, threionine.
[b] The protein-vitamin-mineral concentrate contained per 1 kg of diet: crude protein 38.0%, 14.5% ash, L-lysine 3.8%, crude fibre 3.75%, crude fat 3.5%, Ca 2.7%, P 1.0%, Na 0.7%, vitamin A 72,000 IU, vitamin D_3 8000 IU, vitamin E 450 mg, Cu 600 mg, endo-1,4-β-xylanase 1200 EXU, 3-phytase 2000 FTU, $Ca(H_2PO_4)_2$, lactic acid, formic acid, aromas.
[c] The vitamin and mineral premix contained per 1 kg of diet: Ca 21.0%, P 3.0%, Na 6.0%, L-lysine 7.0%, threonine 1.0%, Mg 2.0%, methionine 2.0%, vitamin A 400,000 IE, vitamin D_3 50,000 IE, vitamin E 4000 mg, Cu 5000 mg, 3-phytase 16,650 FTU, salinomycin-Na 1000 mg.
[d] The vitamin and mineral premix contained per 1 kg of diet: Ca 22.5%, P 3.5%, Na 5.5%, L-lysine 6.5%, Mg 2.0%, 1.0% methionine, vitamin A 400,000 IE, vitamin D_3 50,000 IE, vitamin E 2500 mg, Cu 1000 mg, salinomycin-Na 1000 mg.

period of the study for both the sham-operated and fundectomized pigs.

Determination of Bone Properties. The lumbar vertebrae and humeri were thawed for 4 hrs at room temperature before assessment of bone mineral density and biomechanical testing. Areal bone mineral density (BMD) and bone mineral content (BMC) were determined for each humerus and each lumbar vertebra (L_1–L_6) in the dorso-ventral direction using dual-energy x-ray absorptiometry (DEXA) method and Norland XR-46 apparatus supplied with Research Scan software (Norland, Fort Atkinson, WI). Quantitative computed tomography (QCT) method and Somatom Emotion–Siemens apparatus (Siemens, Erlangen, Germany) supplied with Somaris/5 VB10B software (Version B10/2004A) were used for volumetric bone mineral density (vBMD) determination of the trabecular and cortical bone of each humerus and each lumbar vertebra. The vBMD was measured for the trabecular bone of the vertebral body using 2-mm-thick, cross-sectional QCT scans, placed at 50% of each vertebral body length; the cross-sectional area (A) of the vertebral body at this place was measured automatically. Similar scans were used to measure vBMD of the trabecular and cortical bone compartments of humerus; however, they were placed just below the distal growth plate of humerus and at 50% of this bone length, respectively. Both the trabecular (Td) and cortical bone mineral density (Cd) were determined with sequential measuring scans. Using Osteo CT application package (software Version B10/2004A), the quantitative determination of calcium hydroxyapatite (Ca-HA) for both the trabecular ($Tb_{Ca\text{-}HA}$) and cortical bone ($Cb_{Ca\text{-}HA}$) was performed for each vertebral body. The lumbar vertebrae were scanned together with the water- and bone-equivalent calibration phantom; however, in contrast to Td determination, Ca-HA measuring scans were 10 mm thick. Using Volume Evaluation application package (software Version B10/2004A), the total bone volume (B_{vol}) of each vertebra and humerus was determined. For B_{vol} calculations, the volume-of-interest (VOI) was defined by limiting the minimum and maximum density for the investigated bone at 60 and 3000 Hounsfield units (HU), respectively. Moreover, using this application, the mean volumetric bone mineral density (MvBMD) for each lumbar vertebra and humerus was measured. Geometric properties of humerus were calculated on the basis of measurements of horizontal and vertical diameters of mid-diaphyseal cross-section of this bone. The values of the cross-sectional area (A), the second moment of inertia (Ix), the mean relative wall thickness (MRWT), and the cortical index (CI) were derived as reported previously (15, 16). Furthermore, the relative weight of the investigated bones (bone weight divided by body weight) was calculated.

Mechanical Analysis of Lumbar Vertebrae and Humerus. Before the measurement of the L_1–L_6 vertebrae's bone strength, both the posterior and transversal processes were removed. The biomechanical parameters of each vertebra were measured by compression test at a load rate of 20 mm/min, using the Zwick/Roell Z010 machine (Zwick/Roell, Ulm, Germany). The biomechanical parameters such as ultimate force (F_u), ultimate stress (σ_u), Young's modulus (E), stiffness (S), and work to the ultimate force point (W) were derived. Independent from the sample size, the obtained load-deformation curve allowed determination of parameters such as F_u, W, and S that corresponds to the slope of the linear region of the curve. After adjusting for the sample size of the vertebral body of each vertebra, the load was converted to stress and deformation to strain, in order to obtain a stress-strain curve. The stress-strain curve data were used to determine σ_u and E that correspond to the slope of the curve within the elastic region. Mechanical properties of humerus were determined using three-point bending test, and the values of maximum elastic strength (Wy) and ultimate strength (Wf) were obtained. The distance between bone supports was set at 40% of humerus length, and the measuring head loaded bone samples with a constant speed of 20 mm/min (17, 18).

Analysis of Serum. Blood samples were collected for serum carefully, using standard venipuncture of the subclavian vein to avoid hemolysis. After blood clotting, the serum was immediately separated by centrifugation. GH level in serum was determined with the use of porcine GH enzyme-linked immunosorbent assay (ELISA; Diagnostic System Laboratories, Inc., Webster, TX). The serum concentration of total IGF-1 was determined using the commercial immunoenzymometric assay (IEMA) for the quantitative determination of IGF-1 in serum or plasma (OCTEIA IGF-1, Immunodiagnostic Systems Ltd., Boldon, UK). Using Ghrelin (porcine) Radioimmunoassay Kit (Phoenix Pharmaceuticals, Inc., Belmont, CA), determination of serum level of ghrelin was performed. The concentration of calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and sulfur (S) in the serum was determined using energy-dispersive x-ray fluorescence (ED 2000 Spectrometer, Oxford Instruments, Buckinghamshire, UK). Prior to the measurements, the serum samples were lyophilized and placed on thin Formvar film, which was stretched on polyethylene rings with an i.d. [inside diameter] of 10 mm, and covered with a second film. The serum concentration of macro- and microelements was measured in μg/g of dry matter of the lyophilized sample (19).

Statistical Analysis. All data are presented as mean ± SD. All the values of the investigated parameters obtained from each lumbar vertebra were averaged for each pig. Differences between sham-operated and fundectomized groups were tested for statistical significance with the use of non-paired Student's *t* test. Differences showing a P value ≤ 0.05 were considered statistically significant.

Results

Body Weights. Body weights of the piglets at the beginning of the experiment were not significantly different between the FX group and the control animals (SHO group) and were 10.85 ± 2.54 kg and 10.75 ± 1.86 kg, respectively. Even though animals consumed the same amount of the feed throughout the study in both groups, the mean body weight after 29 weeks from the surgery in the fundectomized animals (FX group) reached 35.34 ± 17.42 kg and was significantly decreased when compared with the value of 105.15 ± 12.94 kg in the sham-operated pigs (SHO group; $P < 0.0001$).

Bone Weight and Length, Bone Mineral Density, and Bone Mineral Content. The weight of the vertebrae and humerus from the pigs subjected to the fundectomy was significantly decreased by 45.0% and 40.9%, respectively, when compared with the sham-operated controls ($P < 0.01$). However, the relative weights of lumbar vertebrae and humerus (expressed as bone weight divided by body weight in grams) were significantly higher in the FX group (0.001187 ± 0.000370 g and 0.004502 ± 0.001024 g, respectively) when compared with the controls (0.000644 ± 0.000064 g and 0.002357 ± 0.000164 g, respectively) ($P = 0.01$). The length of humerus was reduced

Table 2. Effect of the Fundectomy on Morphology, Bone Mineral Density, and Mechanical Properties of Lumbar Vertebrae in Pigs[a]

Investigated parameter	SHO group	FX group
Weight of the vertebra (g)	67.0 ± 1.3	36.9 ± 12*
Total bone volume (cm^3)	41.8 ± 0.92	24.3 ± 7.97*
Mean volumetric bone mineral density (g/cm^3)	1.506 ± 0.007	1.335 ± 0.024**
Trabecular bone mineral density (g/cm^3)	1.456 ± 0.017	1.276 ± 0.033**
Calcium hydroxyapatite density in the trabecular bone (mg/ml)	291 ± 15.5	178 ± 14.2**
Calcium hydroxyapatite density in the cortical bone (mg/ml)	480 ± 10.6	265 ± 37.7**
Bone mineral density (g/cm^2)	0.7734 ± 0.027	0.4495 ± 0.084**
Bone mineral content (g)	15.1 ± 0.29	6.2 ± 2.27**
Cross-sectional area (mm^2)	204 ± 8.14	189 ± 46.6
Ultimate force (N)	8747 ± 371	3278 ± 1093**
Young's modulus (N/mm^2)	923 ± 40	438 ± 74**
Ultimate stress (N/mm^2)	11.83 ± 0.56	5.91 ± 1.35**
Stiffness (N/mm)	1777 ± 130	744 ± 181**
Work to the ultimate force point (N/mm)	44,668 ± 1607	15,356 ± 6658**

[a] Values are mean ± SD of 6 pigs per group. SHO group, pigs subjected to sham operation; FX group, pigs subjected to experimental fundectomy.
* $P \leq 0.01$ and ** $P \leq 0.001$ by non-paired Student's *t* test.

by 16.1% in the fundectomized animals ($P = 0.01$). BMD measurements using DEXA method have showed that the fundectomy significantly decreased BMD in lumbar vertebrae and humerus by 41.9% and 41.4%, respectively ($P < 0.001$). BMC was lowered by 58.9% and 54.4% in the lumbar vertebrae and humerus of the fundectomized pigs, when compared with the values obtained in the sham-operated controls ($P < 0.001$; Tables 2 and 3).

Computed Tomography Evaluation of the Lumbar Spine and Humerus. The results of the lumbar spine and humerus evaluation with the use of computed tomography are shown in Tables 2 and 3. B_{vol} determined in the lumbar spine and humerus showed that fundectomy in pigs decreased this parameter significantly when compared with the sham-operated animals by 41.8% and 42.7%, respectively ($P < 0.01$). Experimental fundectomy induced a 12.4% and 13.3% decrease of the vBMD of the trabecular bone in the lumbar vertebrae and humerus, respectively, when compared with the control pigs ($P < 0.01$). Volumetric BMD of the cortical bone measured in the midshaft of humerus was decreased by 11.8% after experimental fundectomy ($P < 0.05$). MvBMD reached significantly lower values in the FX group for the lumbar vertebra and humerus, when compared with the SHO group and the differences of the MvBMD between the investigated groups were 11.4% and 8.8%, respectively ($P < 0.01$). $Tb_{Ca\text{-}HA}$ of the lumbar vertebrae (quantity of mineralized bone within a unit of volume; mg/ml) was significantly decreased by 38.7% in the FX group when compared with the SHO group ($P < 0.0001$). Similar to the changes

Table 3. Effect of the Fundectomy on Bone Mineral Density, Geometric, and Mechanical Properties of Humerus in Pigs[a]

Investigated parameter	SHO group	FX group
Bone weight (mm)	246.5 ± 12.5	145.7 ± 53.8**
Bone length (mm)	185.2 ± 4.9	155.5 ± 19.9*
Bone volume (cm^3)	148.3 ± 7.8	84.9 ± 33.1**
Trabecular bone mineral density (g/cm^3)	1.432 ± 0.038	1.242 ± 0.094**
Cortical bone mineral density (g/cm^3)	2.680 ± 0.084	2.363 ± 0.275*
Mean volumetric bone mineral density (g/cm^3)	1.500 ± 0.033	1.368 ± 0.075**
Bone mineral density (g/cm^2)	0.991 ± 0.085	0.581 ± 0.152**
Bone mineral content (g)	60.3 ± 5.4	27.5 ± 12.1**
Cross-sectional area (mm^2)	244.5 ± 22.1	124.9 ± 48.8**
Second moment of inertia (mm^4)	14,089 ± 1426	6274 ± 3438**
Mean relative wall thickness	0.784 ± 0.132	0.383 ± 0.131**
Cortical index	43.4 ± 4.0	27.0 ± 7.3**
Maximum elastic strength (N)	4080 ± 1014	1618 ± 771**
Ultimate strength (N)	6150 ± 1170	2167 ± 1306**

[a] Values are mean ± SD of 6 pigs per group. SHO group, pigs subjected to sham operation; FX group, pigs subjected to experimental fundectomy.
* $P < 0.05$ and ** $P < 0.01$ by non-paired Student's *t* test.

Table 4. Effect of the Fundectomy on Serum Concentration of Ghrelin, Growth Hormone, and Insulin-Like Growth Factor-1 in Pigs[a]

Investigated hormone	SHO group	FX group
Ghrelin (pg/ml)	54.6 ± 8.9	14.0 ± 4.3*
Growth hormone (ng/ml)	9.8 ± 6.2	0.93 ± 0.5*
Insulin-like growth factor-1 (ng/ml)	154.4 ± 5.8	70.2 ± 17.9*

[a] Values are mean ± SD of 6 pigs per group. SHO group, pigs subjected to sham operation; FX group, pigs subjected to experimental fundectomy.
* $P < 0.001$ by non-paired Student's *t* test.

Table 5. Effect of the Fundectomy on Serum Concentration of Macro- and Microelements in Pigs[a]

Investigated element	SHO group	FX group
Calcium (mg/g)	1.117 ± 0.089	0.942 ± 0.164*
Phosphorus (mg/g)	2.756 ± 0.107	2.611 ± 0.265
Magnesium (mg/g)	0.340 ± 0.130	0.186 ± 0.080*
Sulfur (mg/g)	10.09 ± 0.55	10.03 ± 0.68
Chloride (mg/g)	35.00 ± 2.19	36.40 ± 3.81
Iron (μg/g)	32.88 ± 5.37	24.11 ± 6.87*
Copper (μg/g)	32.18 ± 6.05	23.75 ± 4.84*
Zinc (μg/g)	15.38 ± 2.81	13.20 ± 5.61

[a] Values are mean ± SD of 6 pigs per group. SHO group, pigs subjected to sham operation; FX group, pigs subjected to experimental fundectomy.
* $P \leq 0.05$ by non-paired Student's *t* test.

observed in the trabecular bone of the lumbar spine, $Cb_{Ca\text{-}HA}$ was decreased in the fundectomized pigs by 44.7% when compared with the sham-operated group ($P < 0.0001$; Table 2).

Mechanical Testing of the Lumbar Spine and Humerus. Mechanical testing of the lumbar spine showed that the fundectomy in pigs decreased the value of the F_u in lumbar vertebrae by 62.5% ($P < 0.0001$). Similar changes of the E, σ_u, S, and W were observed in the group after the fundectomy when compared with the SHO group; however, the values of E, σ_u, S, and W were changed by 52.4%, 49.9%, 58.1%, and 65.6%, respectively ($P < 0.0001$; Table 2). The values of Wy and Wf of humerus were significantly decreased after experimental fundectomy by 60.3% and 64.8%, respectively ($P < 0.001$; Table 3).

Geometric Properties of Humerus. Assessment of the geometric properties such as cross-sectional area, second moment of inertia, mean relative wall thickness, and cortical index of humerus revealed that fundectomy decreased these parameters by 48.9%, 55.5%, 51.2% and 47.7% when compared with the values obtained in the SHO group, respectively ($P \leq 0.001$; Table 3).

Analysis of Hormones and Macro- and Microelements in Serum. Results of hormonal analyses in the pigs from the fundectomized and sham-operated groups are presented in Table 4. Serum concentrations of ghrelin, GH, and IGF-1 were lowered by 74.4%, 90.6%, and 54.6%, respectively, in the fundectomized pigs ($P < 0.001$). Results of the analysis of macro- and microelements in the serum are presented in Table 5. It was shown that the fundectomy performed in pigs significantly decreased serum concentration of the Ca, Mg, Fe, and Cu by 15.5%, 45.3%, 26.7%, and 26.2%, respectively ($P \leq 0.05$).

Discussion

Our findings showed that surgical extirpation of the fundus in pigs induces significant decrease of the body weight gain, which is similar to the body weight loss observed after gastrectomy in humans (1, 7, 20). Due to identical environmental conditions and feeding in both the groups, this dramatic decrease of body weight values of the fundectomized pigs is the result of the elimination of the physiological function of the stomach in growing animals. This drastic effect of fundectomy on the body weight of pigs is really surprising, especially when there were no adverse reactions from the gastrointestinal tract of the pigs such as diarrhea or vomiting during the whole period of the study. However, it should be expected that the effects of long-term fundectomy on final body weight values in growing animals may be more meaningful than in adult animals. This hypothesis seems to be supported by the results of final body weight values obtained in the current study, where these values in the fundectomized and sham-operated animals were almost 3.5 and 10 times higher, respectively, when compared with the initial body weight values obtained at the beginning of the experiment. In other species, the effects of gastrectomy on body weight gain are not clear. While some of the experimental studies on rats have not shown decreased body weight as a consequence of the performed gastrectomy, fundectomy, or antrectomy (8, 10, 11); other studies present gastrectomy as a factor negatively influencing the body weight of these animals (9). This study has also shown that BMD and morphologic characteristics of the lumbar vertebrae and humerus are strongly influenced by the fundectomy. Analysis of bone weight and B_{vol} of lumbar spine and humerus in the pigs showed that preformed fundectomy negatively affects both these parameters. Furthermore, both of these parameters were closely related and this finding has confirmed usefulness of B_{vol} determination with the use of computed tomography technique as a noninvasive tool for bone morphology determination in experimental studies. BMD and BMC values determined by DEXA method were negatively altered by fundectomy, similar to the changes of morphology of the lumbar spine and humerus observed in the fundectomized pigs. The obtained results are in accordance with the investigations performed by Surve and colleagues in rats (10), where nearly 22.9% and 17.4% decrease of BMD was observed for femur and L_5 vertebra, respectively; however, in this study, BMD was determined by bone sample ashing, and modified gastrectomy in experimental animals was applied. In other experiments with rats and modified gastrectomy, 8 weeks after surgery BMC and BMD values were decreased by 18% and 44%, respectively. The fact that postgastrectomy changes of BMD in the trabecular bone of the distal metaphysis of the femur in rats were comparable with those observed in our study is noteworthy (9). Negative effects of the fundectomy on bone tissue were confirmed by assessment of the vBMD and MvBMD values. While vBMD determination was performed for 2-mm-thick slice of the trabecular and cortical bone, the measurement of MvBMD was executed for the whole bone, and the obtained results reflect MvBMD measured for both the trabecular and cortical bone compartments within all anatomic structures of the investigated bone. The changes of vBMD and MvBMD values in lumbar spine as a consequence of the fundectomy reached 12.4% and 11.4%, respectively. The values of vBMD measured for the trabecular and cortical bones of humerus were decreased by 13.3% and 11.8%, respectively, after the fundectomy; while MvBMD was decreased by 8.8%. Furthermore, obtained results have shown that the fundectomy in pigs decreased the Ca-HA density in the trabecular and the cortical bone compartments by 38.7% and 44.7%, respectively, indicating that long-term fundectomy affects both these types of bone tissue. Similar response of the trabecular and the cortical bones were reported in other studies on rats; however, the calvaria served as a model for the cortical bone (8–10).

Bone strength is mostly determined by bone geometry and bone quantity, while the qualitative aspects of bone, which are determined by its mineral content, bone microarchitecture, and microdamages, as well as collagen structure and maturation, contribute to about 30% effect (21). Increased BMD was reported as a factor improving bone mechanical properties, whereas its decreased values induced opposite effects (16, 22, 23). Moreover, BMD assessment was reported as a good predictor for the vertebral fracture risk (24, 25). Considering these data and the results of analysis of geometric parameters such as A, Ix, MRWT, and CI of humerus in pigs subjected to fundectomy and sham-operated pigs, it is concluded that both the BMD and bone geometric properties strongly influenced mechanical strength of humerus and lumbar spine. These effects were expressed by dramatically decreased values of Wy and Wf of humerus as well as by decreased values of the F_u, E, σ_u, S, and W of lumbar vertebrae. The fact that in our studies long-term fundectomy influenced the skeletal system at different range than other tissues is noteworthy. While bone weight was significantly decreased in pigs after the fundectomy, the values of relative bone weight were increased almost two-fold in this group of animals. However, marked reduction of the investigated bone parameters obtained in the FX group is not simply a function of the significantly reduced body size and weight of the animals. This statement is strongly supported by results of variables determined independently of bone size, such as volumetric bone mineral density (Td, Cd, MvBMD), calcium hydroxyapatite density ($Tb_{Ca\text{-}HA}$, $Cd_{Ca\text{-}HA}$), σ_u, and E. Results obtained in this study are in accordance with the findings presented by Puzio and colleagues (11), where mechanical parameters of the femur in rats were decreased by about 20% when investigated 12 weeks after fundectomy. In another study on rats, the ultimate load of the femur shaft was significantly decreased by approximately 30%; however, this study was conducted throughout the 12 months after the experimental gastrectomy (26).

The results obtained in the current study indicate that negative effects of surgical fundectomy on the skeletal system in pigs are mediated by impaired function of the gastric-hypothalamic-pituitary axis. The proposed hypothesis is strongly supported by the results of hormonal analyses, which revealed that fundectomy significantly decreased serum concentrations of ghrelin, GH, and IGF-1 by 74.4%, 90.6%, and 54.6%, respectively, when compared with sham-operated controls. These results are in accordance with other studies performed by Anderson and colleagues (27), who reported that ghrelin, the endogenous ligand for GH secretagogue receptors, is produced in the chromogranin A-immunoreactive X/A-like endocrine cells located in the mucosal layer of the fundus of the stomach. Ghrelin plays an important role in existence of the gastric-hypothalamic-pituitary axis and provides endocrine control of nutritional homeostasis with GH secretion and gastrointestinal motility. Due to physiological stimulation of GH secretion by ghrelin, which is strictly related to enhanced IGF-1 synthesis, the elimination of ghrelin caused by surgical extirpation of the fundic part of stomach in pigs induced negative effects on bone homeostasis maintenance, leading to gastric-hypothalamic-pituitary mediated osteopenia (28). It should be underlined that circulating IGF-1 is mainly produced in the liver *via* regulation by GH and diet. It acts in an endocrine manner, activates remodeling, and exerts anabolic effects on bone tissue (29, 30). IGF-1 is essential for longitudinal bone growth, and it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate. This factor plays an important role in trabecular and cortical bone formation; it stimulates both proliferation and differentiation of osteoblasts, enhances synthesis of collagen type I, and increases activity of alkaline phosphatase and osteocalcin production, exerting anabolic effects on bone mass during growth and adulthood. Due to IGF-1 renal action on tubular reabsorption of phosphate and on the synthesis of calcitriol, through a direct influence on renal cells, IGF-1 can be considered as an important controller of the intestinal absorption and of the extracellular concentration of both Ca and P, the main mineral components of the bone (31). Investigations on humans have shown positive correlation between serum IGF-1 concentration and BMD measured in both the lumbar spine and the peripheral skeleton (32). Considering identical composition and the amount of the feed consumed by animals from both groups, the decreased serum concentration of IGF-1 in the fundectomized pigs is not likely to be simply the result of a different diet. Our hypothesis is in accordance with investigations performed by Puzio and colleagues (11), where serum ghrelin was found to have decreased by approximately 30 times when analyzed 12 weeks after the fundectomy in rats. The other studies on rats have shown a close relationship between the serum ghrelin concentration and the proportion of the fundus resection (33). Studies in weaning pigs have also confirmed our findings and shown that iv ghrelin infusion (3 times daily for 5 days) significantly increased weight gain and GH concentration (34). In humans, ghrelin secretion increases with weight loss to stimulate appetite and increases osteoblastic proliferation and differentiation (35).

The analysis of the serum in pigs has shown decreased concentration of Ca and Mg as the consequence of fundectomy. Contrary to the investigations performed by Maier and colleagues (36), the current study has also shown that concentrations of Fe and Cu were reduced in the fundectomized animals to the concentration that would decrease enzyme activity. While Ca and Mg are mainly stored in bone tissue determining its mineral density and mechanical properties, the conversion of procollagen to collagen is dependent on hydroxylation of peptide-bound proline to hydroxyproline *via* prolyl 4-hydroxylase that is regulated by Fe^{2+}, alpha-ketoglutarate, and ascorbate (12, 37). Moreover, Cu influences enzymatic processes of maturation of collagen that occur in bone tissue by activation of lysyl oxidase, which leads to the formation of immature and mature cross-links that stabilize the collagen fibrils and provide mechanical strength of bones (38). Thus, the fundectomy-evoked macro- and microelement malabsorption may be an additional mechanism responsible for the observed osteopenia. It may be postulated that impaired secretion of gastric acid *via* oxyntic mucosa of the fundus of stomach leads to decreased solubility of Ca, Mg, Fe, and Cu, which limits intestinal absorption of these elements from the diet and induces a negative effect on bone. These data seem to be contrary to the results obtained in studies on rats, where the proton pump inhibitor omeprazole was used to inhibit acid secretion of the stomach, and no adverse effects of this treatment on bone tissue were observed (13).

In conclusion, the current study showed that fundectomy in pigs induced osteopenic changes in the axial and peripheral skeleton similar to those observed in both humans and animals after gastrectomy. The obtained results showed that the development of postfundectomy osteopenia in pigs is mediated by the disturbance of the gastric-hypothalamic-pituitary axis function and impaired mineral metabolism. In addition, this study addresses the question of whether factors regulating gastric-hypothalamic-pituitary axis function may be effective in prevention of fundectomy-induced osteopenia. Considering all these data, which show numerous similarities of the physiology and anatomy between the gastrointestinal tracts and skeletal systems of man and pigs, the fundectomy in pigs can be used as a very attractive model for investigations on physiological, nutritional, and pharmacological factors contributing to maintenance of skeletal homeostasis. Moreover, these observations may serve to determine hormonal and skeletal response in humans undergoing gastric surgery; however, further studies are indicated.

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