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INTERRELATIONSHIPS BETWEEN MORPHOMETRIC, DENSITOMETRIC, AND MECHANICAL PROPERTIES OF MANDIBLE IN 6-MONTH-OLD MALE PIGS

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Abstract

The aim of the study was to evaluate interrelationships between final body weight, and morphometric, densitometric, and mechanical properties of the mandible in 6-month-old Polish Large White pigs exposed to dexamethasone and nanocalcium. The study was performed on 27 males, castrated on the 28th d after weaning, and reared until the age of 6 months, after which the animals were slaughtered, and the mandible was obtained. The pigs were divided into four experimental groups: animals given *per os* nanopartical calcium, animals injected with dexamethasone, animals given both nanopartical calcium *per os* and dexamethasone injections, and animals injected with placebo. After the slaughter, morphological properties of the mandible such as bone weight and length were determined. Using computed tomography technique, volumetric bone mineral density (vBMD) of the cortical bone (Cd), mean volumetric bone mineral density (MvBMD), and total bone volume (Bvol) of whole mandible were measured. Areal bone mineral density (BMD) and bone mineral content were evaluated with the use of dual-energy X-ray absorptiometric method. Using three-point bending test, mechanical parameters such as maximum elastic strength (Wy) and ultimate strength (Wf) of mandible were estimated. Pearson's correlation coefficient (*r)* was determined between all the investigated variables. The obtained results showed a significantly positive correlation between body weight and mandible weight, mandible length, Bvol, Cd, BMD, BMC, Wy, and Wf. However, statistically insignificant correlations of MvBMD and body weight, mandible weight, mandible length, and Bvol were observed. Furthermore, Bvol and Cd were not found to be significantly correlated. In conclusion, this study showed numerous positive correlations between final body weight and densitometric, morphometric, and mechanical properties of the mandible. This bone of pigs may be used as an attractive model for further investigation on metabolic response of the skeleton to physiological, nutritional, toxicological, and pharmacological factors influencing bone tissue metabolism.

Key words: swine, mandible, bone properties, quantitative computed tomography, densitometry.

The aim of the study was to evaluate interrelationships between final body weight and morphometric, densitometric, and mechanical properties of mandible in domestic pigs exposed to administration of dexamethasone and nanocalcium solely and in combination. These preparations were administered to differentiate the process of modelling and re-modelling of bone tissue, considering the negative effect of dexamethasone and the positive effects of calcium on the morphometric, densitometric, and mechanical properties of mandible. Previous studies have documented that dexamethasone and other glucocorticoids have a profound impact on the skeleton, inhibiting bone formation and accelerating bone resorption, especially when administered for prolonged periods (4, 9, 13, 20). In contrast to glucocorticoid treatment, administration of calcium during important periods of prenatal and postnatal life and in the old age improves peak bone mass acquisition in the skeleton and bone mineral density, as well as it leads to reduced osteoporotic fracture risk (1, 17, 18). The choice of the experimental model is the result of the common knowledge on the destructive effects of chronic dexamethasone administration and positive effect of calcium on the properties of bone studied. Determination of bone mineral content (BMC) and bone mineral density (BMD) has become essential in investigation of the calcium metabolism and associated bone diseases. BMD is defined as BMC divided by the area of interest, expressed in g/cm^2. It has been intensely discussed in which part of the skeleton the bone mass should be determined. The vertebral body consists mainly of trabecular bone, which seems to be more affected in several metabolic bone diseases than the cortical bone (7, 19). The long bones of mammal body consist of two types of bone tissue: trabecular and cortical, while the mandible has mainly cortical bone tissue, which makes it different from the other models described. The first observations on the relationship between osteoporosis with loss of bone tissue within the craniofacial bones were made in 1960 (10). The choice of the type of bone for this study was dictated by the fact

that the mandible as part of the skeleton is exposed to bone loss with age and systemic factors (diseases or medications known to interfere with the metabolism of bone tissue), as well as local factors (3, 5, 8, 11, 14, 15). The mandible consists mainly of compact bone representing 79%-89% of the total bone mass within body of the mandible. In humans, after 50 years of age there is a steady loss of the compact compartment that causes the thinning of the lower jaw and an increase in its porosity. This loss depends on gender and age, with the mandible in women smaller in size and of lower bone mass than the mandible in males (5, 8, 24, 26). The amount of trabecular substance in the mandible is small. The volume reflects individual features and does not depend on age and sex, as it does in the case of the volume of compact bone in the jaw.

Material and Methods

The experimental procedures used in this study were approved by the Local Ethics Committee on Animal Experimentation.

Experimental design and sampling procedure. The investigation was conducted on 27 male piglets of Polish Large White breed kept with their mothers until the weaning. Considering higher growth rate and the advantages resulting from lack of sex-differentiated hormonal influences on bone tissue metabolism (androgens or oestrogens), the current study was performed on a model of growing males. The animals were kept in standard breeding conditions that apply for the rearing of pigs. Piglets had permanent access to water and were fed 2 times a day with a mix designed for pigs. The time of experiment included the period from the day of birth to the 180th d of life. Standard castration procedure was performed on 28th d of life, immediately after the weaning. Piglets were reared in a specialised farm and supervised by a veterinarian.

Immediately after birth, the piglets have been divided into four experimental groups. The first group (control) (n=7) was receiving a placebo - physiological saline in intramuscular injection in a volume corresponding to the volume of dexamethasone administered in other groups. The second group (NanoCa group; n=7) was given *per os* nanopartical calcium (AceNano-Calcium Essential, NanoTechWorld, Korea). The third group was receiving 0.2% solution of dexamethasone (Rapidexon, Novartis, The Netherlands) in the intramuscular injection at a dose of 3 mg/pig/48 h. The fourth group received simultaneously *per os* nanopartical calcium and intramuscularly 3 mg/pig/48 h of dexamethasone. Nanopartical calcium was administered in the second and fourth groups at two different dosages – namely 250 mg/pig (since birth up to 4th month of life) and 500 mg/pig (up to 6th month). Euthanasia of the animals was carried out on day 180 of age using the Morbital (Biowet, Poland). After the euthanasia, bone samples were cleaned of remaining bone tissues and morphological properties of the mandible such as bone weight and length were measured. The samples were kept frozen at -25°C until further analyses. They were thawed at room temperature for 2 h before morphologic, densitometric, and biomechanical analyses.

Using quantitative computed tomography (QCT) technique and Somatom Emotion-Simens apparatus (Siemens, Erlangen, Germany), equipped with Somaris/5 VB1OB software (version B10/2004A), volumetric bone mineral density (vBMD) of the cortical bone (Cd) and mean volumetric bone mineral density (MvBMD) and total bone volume of the whole mandible were determined. The measurement of Cd was performed on cross-sectional scan of mandible body positioned just after fourth premolar tooth. Furthermore, total bone volume (Bvol) was determined using volume evaluation software (Siemens, Erlangen Germany). For bone volume and MvBMD determination of whole mandible, the volume-of-interest was limited by minimum and maximum density of the investigated samples at 0 and 3071 Hounsfield units, respectively. The measurement of MvBMD was performed for the whole mandible and the results obtained reflect MvBMD measured for all anatomic structures including trabecular and cortical bones and teeth. Areal bone mineral density (BMD) and bone mineral content (BMC) were evaluated with the use of dual-energy X-ray absorptiometry (DEXA) method and Norland XR-46 apparatus, supplied with Research Scan software (Nortland, Fort Atkinson, USA). The measurement of BMD and BMC was performed for the whole right part of mandible including appeared teeth (Fig. 1). Using an INSTRON 3367 apparatus with Bluehill 2 software (Instron Corp., Canton, USA), that records in a graph the relationship between a force and bone displacement in the three-point bending test, mechanical parameters such as maximum elastic strength (Wy) and ultimate strength (Wf) of right mandible branch were estimated. The distance between bone supports was set at 40% of mandible length, and the measurement of the head loaded bone samples with a constant speed of 50 mm/min at the reference point used for Cd determination was performed.

Statistical analysis. Statistical analysis of the data was performed using Statistica software (version 6.0). Pearson's correlation coefficient (r) was determined for all the investigated variables of the mandible, and $P<0.05$ was considered as statistically significant.

Results

The values of Pearson's correlation coefficient between all the investigated parameters of mandible in male pigs at the age of 6 months are shown in Table 1. Body weight, weight and length of the mandible, and bone volume were found to be positively correlated with all the investigated parameters (all $P<0.05$) except for MvBMD. Mean vBMD of the whole mandible was positively correlated with Cd, BMD, BMC, Wy, and Wf ($P<0.05$). Evaluation of Cd has shown positive correlation of this parameter with all the investigated variables ($P<0.05$), except bone volume. Furthermore, BMD, BMC, Wy, and Wf were found to be positively correlated with all the investigated parameters of the mandible (all $P<0.05$).

Table 1

The values of Pearson's correlation coefficient between all the investigated parameters of mandible in male pigs at the age of 6 months

Investigated parameter	Body weight	Mandible weight	Mandible length	Mean volumetric bone mineral density	Bone volume	Cortical bone density	Bone mineral density	Bone mineral content	Maximum elastic strength	Ultimate strength
Body weight	X	0.72*	0.71*	0.33	0.7*	0.46*	0.57*	0.73*	0.39*	0.63*
Mandible weight	0.72*	X	0.83*	0.37	0.98*	0.46*	0.76*	0.92*	0.62*	0.65*
Mandible length	0.71*	0.83*	X	0.24	0.86*	0.49*	0.58*	0.86*	0.57*	0.50*
Mean volumetric bone mineral density	0.33	0.37	0.24	x	0.22	0.68*	0.76*	0.57*	0.57*	0.66*
Bone volume	0.70*	0.98*	0.86*	0.22	x	0.36	0.71*	0.90*	0.60*	0.58*
Cortical bone density	0.46*	0.46*	0.49*	0.68*	0.36	X	0.56*	0.60*	0.61*	0.68*
Bone mineral density	0.57*	0.76*	0.58*	0.76*	0.71*	0.56*	X	0.91*	0.74*	0.75*
Bone mineral content	0.73*	0.92*	0.86*	0.57*	0.90*	0.60*	0.91*	x	0.76*	0.78*
Maximum elastic strength	0.39*	0.62*	0.57*	0.57*	0.60*	0.61*	0.74*	0.76*	x	0.68*
Ultimate strength	0.63*	0.65*	0.50*	0.66*	0.58*	0.68*	0.75*	0.78*	0.68*	X

* $P<0.05$.

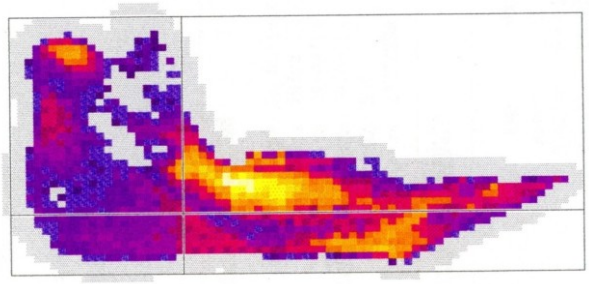


Fig. 1. The measurement of bone mineral density (BMD) and bone mineral content (BMC) in the mandible of pig. The region-of-interest (ROI) was determined on DEXA scan for the whole mandible for each measurement.

Discussion

Little is known about the impact of systemic factors (diseases, drugs) on the mandible bone mass. Bras *et al.* (3) showed that secondary hyperparathyroidism, being a cause of secondary osteoporosis, also causes bone atrophy within the mandible. The prospective work by Kribbs (14) evaluated the effect of two years of administration of 1,000 mg of calcium and 400 IU of vitamin D_3/day for mandible mass in women with postmenopausal osteoporosis. In 83% of the women after the two-year treatment, the mandible bone mass did not change, but even rose. In turn, Jacobs *et al*. (11) evaluated the effect of hormone replacement therapy (HRT) used by 62 women for an average period of 5 years on spine and mandible bone mass. Positive influence was confirmed, with hormone replacement therapy affecting BMD in the studied areas. Local factors also have an effect on the quality of tissue and bone metabolism in the mandible. Among the main adverse conditions periodontal inflammation and alveolar atrophy may be mentioned (12). Von Wowern *et al.* (25) evaluated prospective relationship between periodontitis and the state of skeletal BMD, measured within the mandible, forearm, femoral neck, and lumbar spine, studying the markers of bone turnover. It turned out that in the follow-up of 5-10 years the BMD in the mandible was always significantly lower while BMD in other places of the skeleton and bone markers were not different compared to the control group.

Many authors have asked the question whether BMD of the mandible reflects the state of the skeleton and hence may be a marker of osteopenia or osteoporosis (2). Earlier studies by Von Wowern *et al*. (27) with the use of older methods of SPA (single-photon absorptiometry) and DPA (dual-photon absorptiometry) for the assessment of bone spine, forearm and mandible bones denied the existence of a correlation between them. Kribbs *et al*. (15), who used the same method, noted significant correlations of mandible bone mass with spine and forearm bone mass in 85 women after menopause. However, there is a lack of experimental data on the interrelationships between morphometric, densitometric, and mechanical properties of mandible in domestic pig. The most frequently used and cost-effective experimental model is the rats model, which serves to investigate the bone metabolism. However, there are great differences concerning bone metabolism between humans and rodents, which make the interpretation of the data of biomechanical analyses difficult (16, 21-23). Except for primates, domestic pig as an experimental model is an animal that is the most close to humans. A number of anatomical and physiological similarities in the mastication organ, the digestive system, and the processes of digestion and absorption of nutrients, as well as skeletal structure (anatomical and physiological aspects of bone plate growth) in domestic swine and humans confirm the validity of using this species as a model in the research carried out.

The results of our studies allow noting the positive correlation between body weight, weight of the mandible, mandible length, and bone volume, with all the variables tested with exclusion of MvBMD. Cortical bone density was also positively correlated with all the tested variables excluding bone volume. It is worth to emphasise that BMD, BMC, Wy, and Wf showed statistically significant positive correlation with all studied parameters. In contrast to our research, in the turkey tibia, MvBMD was positively correlated with bone weight, bone length, cortical bone density, bone volume, and maximum elastic strength and ultimate strength. However, in the tibia model, bone weight, bone length, cortical bone density, bone volume, the maximum elastic strength, and ultimate strength were positively correlated (23). In the study concerning vertebral bones in pigs, positive correlation was found between the investigated parameters describing bone morphology, namely bone weight and total bone volume (24). Total bone volume of the lumbar vertebrae was found to be negatively correlated with MvBMD. It is in contrast to our data where a positive, but statistically insignificant correlation was found. The results obtained in our studies, similarly to those from the experiments on pigs' lumbar vertebrae, showed positive correlations between MvBMD and BMD. Like in the study on mandible, mean volumetric bone mineral density was positively correlated with cortical bone density. The analysis of bone mineral content in L1 – L4 vertebrae showed positive correlation of this parameter with bone weight, which was also noted in our investigations (23). The examinations of the lumbar vertebrae and mandible in pigs showed positive and statistically significant correlation between BMC and body weight. The former showed negative, though statistically insignificant, correlation between BMC and MvBMD, while in our investigation on mandible, by contrast, we noted a positive and statistically significant correlation between BMC and MvBMD. In the results of the experiment considering the femur in rams, bone weight was positively correlated with parameters such as bone length, Cd, bone volume. In case of the results of MvBMD in the cited study, as well as in our experiments (mandible in pigs and femur in lambs), the

parameters were insignificantly statistically positively correlated. Wy and Wf in mandible studies were correlated with bone weight, and in lambs the correlation was positive but statistically insignificant. In both series cortical vBMD, Wy, and Wf were positively correlated with all the parameters (25).

In conclusion, this study showed numerous positive correlations between final body weight, densitometric, morphometric, and mechanical properties of mandible. This bone of pigs may be used as an attractive model for further investigations on metabolic responses of the skeleton to physiological, nutritional, toxicological and, pharmacological factors influencing bone tissue metabolism. The mandible of domestic pig may serve as an experimental model reflecting very sensitively changes of skeletal properties and bone mineral metabolism.

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