Relationships between biochemical bone metabolism indices and morphometric, densitometric and mechanical properties of mandible in 6-month-old pigs

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Abstract

Introduction and objective. Mandible is used as a bone model for monitoring bone tissue responses to various factors influencing skeletal homeostasis. Considering the lack of experimental data on interrelationships between bone metabolism indices and morphometric, densitometric and mechanical properties of mandible, the aim of this study was to perform such an evaluation in 6-month-old pigs.

Materials and methods. Quantitative computed tomography was used to determine bone volume, mean volumetric bone mineral density, cortical bone density and cortical bone area. Using dual-energy X-ray absorptiometry, bone mineral density and bone mineral content were measured for ramus, body and whole jaw. In the three-point bending test, maximum elastic strength and ultimate strength of jaw was determined. Assessment of calcium (Ca), phosphorus (P), magnesium (Mg), parathormone (PTH), growth hormone (GH), insulin-like growth factor-1 (IGF-1), alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BAP), osteocalcin (OC) and C-terminal telopeptide of collagen type-I (CTX) in blood was performed.

Results. Statistically significant correlations in relation to the investigated traits of the jaw were found in the case of ALP, OC, CTX, GH and IGF-1. Significant correlations of ALP activity, OC and IGF-1 concentrations with final body weight were stated (p<0.05).

Conclusions. This study shows the highest predictive value of ALP activity determination in relation to assessment of morphological, densitometric and biomechanical properties of mandible. Evaluation of Ca, P, Mg, BAP and PTH has not confirmed its significance for morphological, densitometric and biomechanical properties prediction in the jaw of pigs. ALP activity, OC and IGF-1 concentrations would be prognostic for body weight prediction.

Key words

mandible, bone turnover markers, pig, quantitative computed tomography, dual-energy X-ray absorptiometry, bone biomechanics

INTRODUCTION

Bone is a dynamic tissue possessing the ability to consequent remodelling throughout life. Bone formation and resorption are important processes which are intimately coupled under normal circumstances. Optimal balance between bone formation and resorption is crucial to maintain the biochemical competence of the skeleton, its structural organization, strength and function [1]. Evaluation of biochemical bone turnover markers in plasma or serum provides useful information on bone metabolic processes within the skeleton [2]. C-terminal telopeptide of type I collagen (CTX) is considered as bone resorption marker.

Collagen type I is synthesized in bone and degradated to small peptide fragments (CTX) released into the blood [3]. Osteocalcin (OC) is found exclusively in bone tissue. This is a vitamin K dependent protein produced by osteoblasts, and consists of 3 gamma-carboxyglutamic acid residues involved in calcium ion and hydroxyapatite binding. While *in vivo* function of osteocalcin needs to be investigated, as a tissue matrix constituent it improves the bone formation process. A higher osteocalcin level was observed in postmenopausal osteoporosis due to increased bone turnover. Decreased levels of osteocalcin have been reported in hyperparathyroidism and during long-term glucocorticoid therapy [4]. Insulin-like growth factor 1 (IGF-1) induces biological effects similar to insulin. The peptide is growth hormone (GH) dependent to a high degree, but GH-independent secretion on the tissue level mediates growth-promoting actions in an autocrine and paracrine manner. IGF-1 together with GH induces an

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anabolic effect on bone formation and calcium metabolism. IGF-1 concentration changes with age, nutritional status, body composition and growth hormone secretion [5, 6]. Serum level of bone-specific alkaline phosphatase (BAP) is believed to reflect the metabolic status of osteoblasts and the bone formation process. Measurement of serum level of BAP has proved to be useful in evaluating patients with Paget's disease, osteomalacia, primary hyperparathyroidism, renal osteodystrophy, osteoporosis and metastases to bone tissue [2, 7]. Bone tissue is the main store in the body of macro- and microelements, among which the most important are calcium (Ca), phosphorus (P) and magnesium (Mg), providing high mineral density and mechanical endurance of the skeleton. Calcium and P in bones are deposited as hydroxyapatite crystals and phosphates showing chemical activity enabling ion exchange within body compartments. The important function of Ca, P and Mg in relation to bone tissue is to provide rigidity to the skeleton [8, 9, 10]. If the mechanisms responsible for intestinal absorption of Ca are insufficient to maintain the concentration of ionized calcium, parathyroid hormone (PTH) secretion rises and increases bone resorption. This is the response of the parathyroid glands to Ca deprivation, and finally may lead to osteopenia and osteoporosis [11].

OBJECTIVES

The aim of the study was to investigate the interrelationships between morphological traits, bone mineral density (both areal and volumetric), bone mineral content, biomechanical properties of mandible and blood biochemical bone metabolism indices in 6-month-old pigs. Furthermore, the potential predictive value of the assessment of bone metabolism indices in relation to bone metabolic activity and morphological, densitometric and biomechanical traits of the mandible was studied.

MATERIAL AND METHODS

The experimental procedures used in the presented study were approved by the Local Ethics Committee on Animal Experimentation of the Medical University in Lublin.

Experimental design and sampling procedure. The study was performed on pigs of the Polish Large White (PLW) breed. Twenty-seven orchidectomised males were kept in standard rearing conditions until euthanasia was performed at the age of 6 months. The animals were weaned from their sows on day 28 of life, had free access to drinking water and fed *ad libitum* with a diet prepared in accordance to several stages of the production cycle. Immediately after the birth, the piglets were divided into 4 groups. The first control group (n=7) received saline in intramuscular injection as a placebo. The second group (NanoCa group; n=7) consisted of animals administered orally with nanopartical calcium. The third group (Dex group; n=7) of pigs was treated with dexamethasone (Dexamethasone 0.2% solution) in intramuscular injections at the dose of 3 mg/pig/48 hours. The fourth group (NanoCa/Dex group; n=6) received simultaneously nanopartical calcium and dexamethasone in the same way and dosage as the second and third groups. Nanopartical calcium was administered

per os in the NanoCa and NanoCa/Dex groups at 2 different dosages. While 250 mg/pig of nanopartical calcium was given from birth up to the 4th month of life, during the next 2 months of the experiment the animals received 500 mg/pig. Long-term exclusive or combined administration with dexamethasone and nanopartical calcium was applied in this study to accelerate mineral metabolism in the skeleton of the growing animals. Euthanasia of the animals was carried out on day 180 of their life by intravenous injection of sodium pentobarbital (Morbital, Biowet, Pulawy). Final body weight determination and blood samples collection for plasma and serum were performed after 12-hour fasting before euthanasia was performed. The isolated mandible samples were cleaned of remaining soft tissues, and their morphological properties such as weight and length were determined. The serum and bone samples were stored at the temperature of –25°C until further analyses.

Biochemical analysis of plasma and serum. Concentrations of total calcium, ionized calcium, magnesium and phosphorus were determined in plasma using an automatic Vitalab Flexor analyzer equipped with ion-selective electrodes (AVL List GmbH, Graz, Austria). Alkaline phosphatase (ALP) activity in serum was determined using the colorimetric method. Bone-specific alkaline phosphatase (BAP) concentration in serum of pigs was measured with the use of an immunoenzymometric assay (IEMA (OCTEIA™ Ostease®BAP, Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK). Concentration of osteocalcin (OC) was determined with the use of MicroVue Human Osteocalcin EIA Kit (Enzyme-Linked Immunosorbent Assay; QUIDEL, San Diego, CA, USA). Serum concentration of C-terminal telopeptide of type-I collagen (CTX) was assessed using Serum CrossLaps® ELISA (Immunodiagnostic Systems Nordic a/s, Herlev, Denmark). Growth hormone (GH) concentration in serum was measured with the use of ELISA Kit (USCN Life Science Inc., Wuhan, PRC). Serum level of insulin-like growth factor-1 (IGF-1) was evaluated using immunoenzymometric assay for the quantitative determination of IGF-1 (OCTEIA IGF-1, Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK). Parathormone (PTH) concentration in serum was evaluated using Porcine Intact PTH Elisa Kit (Immunotopics Inc., San Clemente, CA, USA). The results of analyzed hormones, IGF-1 and biochemical markers of bone turnover were obtained using Benchmark Plus microplate spectrophotometer equipped with Microplate Manager Software Version 5.2.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Morphological, mechanical and densitometric evaluation of mandible. Quantitative computed tomography (QCT) technique and Somatom Emotion-Siemens apparatus (Siemens, Erlangen, Germany), equipped with Somaris/5 VBIOB software (version B10/2004A) were used to determine 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. The measurement of Cd was performed on a cross-sectional scan of the mandible body positioned just after the 4th premolar tooth. At the same site, the cortical bone area (CBA) was measured automatically. Total bone volume (Bvol) of the mandible (including teeth) was determined using volume evaluation software (Siemens, Erlangen Germany). For

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bone volume and MvBMD determinations, the volume-of-interest (VOI) was restricted by minimal and maximal density of the investigated samples at 0 and 3,071 Hounsfield units, respectively. The measurement of MvBMD reflects the results obtained within all anatomical structures, including trabecular and cortical bones and teeth. Areal bone mineral density (BMD) and bone mineral content (BMC) were measured with the use of the dual-energy X-ray absorptiometry (DEXA) method and Norland XR-46 apparatus, supplied with Research Scan software (Norland, Fort Atkinson, WI, USA). The measurement of BMD and BMC was performed for 3 different regions of interest (ROIs). The first ROI included measurements of BMD (Mandible bone mineral density – M_{BMD}) and BMC (Mandible bone mineral content – M_{BMC}) performed separately for the whole right and left parts of the mandible. Analogical measurements of BMD and BMC were performed in the second and the third ROI for mandible ramus (MR_{BMD} and MR_{BMC}) and mandible body (MB_{BMD} and MB_{BMC}). In an INSTRON 3367 apparatus supplied with Bluehill 2 software (Instron Corp., Canton, USA), the mechanical parameters, such as maximum elastic strength (Wy) and ultimate strength (Wf) of right and left parts of mandible, were determined. During the 3-point bending test, the distance between bone supports was set at 40% of mandible length, and the measuring head loaded bone samples with a constant speed of 50 mm/min at the reference point used for Cd and CBA determinations.

Statistical analysis. Statistical analysis of the data was performed using Statistica software (version 6.0). Determination of Pearson's correlation coefficients was performed for mean values of the investigated variables (Cd, CBA, BMD, BMC, Wy and Wf) obtained from the right and left parts of the mandible. Pearson's correlation coefficient (r) was determined for all the investigated variables in mandible and serum and p-value <0.05 was considered as statistically significant.

RESULTS

Results presenting Pearson's correlation coefficients of the investigated parameters of serum and mandible are shown in Table 1. Alkaline phosphatase activity was found to be

positively correlated with length, Bvol, M_{BMD}, M_{BMC}, MR_{BMD} and MR_{BMC}, MB_{BMD}, MB_{BMC}, Wy, Wf and final body weight (all p<0.05). Serum osteocalcin concentration in 6-month-old pigs was positively correlated with weight, length, Bvol, M_{BMC}, MR_{BMC}, MB_{BMC}, and final body weight (all p< 0.05). C-terminal telopeptide concentration in serum was found to be significantly negatively correlated with cortical bone area (P < 0.05). Growth hormone concentration was positively correlated with Cd value, while significant negative correlation of this hormone with CBA was stated (both p<0.05). Insulin-like growth factor-1 was positively correlated with the values of CBA and final body weight (p<0.05). Neither the positive nor negative statistically significant correlations with mandible properties were stated evaluating concentrations of ionized and total calcium, magnesium, phosphorus, bone-specific alkaline phosphatase and parathormone (all p>0.05).

DISCUSSION

The mandible in animals and humans was used in previous studies as reference bone for monitoring skeletal system responses to osteopenic and osteoporotic factors, as well as for therapy effectiveness evaluation [12, 13]. Osteoporosis induces comparable negative effects within the stomatognatic system as at different sites of skeleton. Thus, mandibular indices determined using relatively simple radiographic techniques, such as the number of teeth lost, alveolar bone resorption, lamina dura width, cortical bone thickness, bone mineral density and bone mineral content, may serve as useful tools for skeletal bone quantity and quality determination. All these parameters may serve for early effective diagnosis of osteopenia and osteoporosis [14, 15, 16, 17]. In the presented study, the mandible from pigs was selected as an experimental model to investigate relationships of the morphological, densitometric and biomechanical properties with serum biochemical indices of bone turnover processes. It is a noteworthy fact that the bone metabolic processes and functional properties of bones in pigs are similar to those evidenced in humans. The growth plate of bones in pigs reflects the human growth plate in terms of cellular numbers in different zones, cell kinetics, and patterns of closure and

Table 1. Values of Pearson's correlation coefficient estimated for all the investigated variables in serum and mandible of 6-month-old male pigs

Investigated parameter	Mandible weight	Mandible length	MvBMD	Bvol	Cd	CBA	M_{BMD}	M_{BMC}	MR_{BMD}	MR_{BMC}	MB_{BMD}	MB_{BMC}	Wy	Wf	Final body weight
Ca ionized	−0.02	0.10	0.12	−0.02	0.11	0.04	0.01	0.05	0.07	0.24	0.03	0.11	−0.04	0.10	0.01
ALP	0.38	0.53*	0.08	0.45*	0.26	0.18	0.39*	0.49*	0.39*	0.49*	0.42*	0.55*	0.39*	0.39*	0.41*
Total Ca	0.04	0.21	−0.06	0.05	0.03	−0.08	−0.11	0.03	−0.14	0.05	−0.06	0.08	0.02	0.01	0.20
Mg	0.12	0.02	0.22	−0.16	0.26	−0.05	0.08	−0.03	0.27	0.04	0.06	0.02	−0.03	−0.23	−0.01
P	0.04	0.11	0.26	−0.02	0.26	−0.28	0.04	0.06	0.14	0.30	0.11	0.20	0.16	−0.04	−0.05
GH	−0.08	0.13	0.15	−0.09	0.49*	−0.45*	0.07	0.06	0.15	0.11	0.02	0.15	−0.07	0.02	0.11
IGF-1	0.20	0.07	−0.05	0.21	−0.13	0.41*	0.16	0.15	0.11	−0.06	0.19	0.05	−0.11	0.07	0.43*
BAP	0.23	0.25	−0.11	0.28	0.10	0.22	0.15	0.22	0.17	0.34	0.15	0.33	−0.12	0.08	0.36
OC	0.48*	0.39*	0.03	0.53*	0.06	0.14	0.27	0.42*	0.16	0.42*	0.33	0.48*	0.19	0.30	0.45*
PTH	−0.09	−0.25	0.12	−0.17	0.21	−0.09	−0.08	−0.12	−0.02	−0.04	−0.14	−0.22	−0.01	0.01	−0.19
CTX	−0.20	0.03	−0.03	−0.21	0.13	−0.38*	−0.13	−0.14	−0.01	0.11	−0.13	0.02	−0.15	−0.24	−0.21

*Statistically significant correlations at p-value < 0.05.

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mineralization [18]. Moreover, numerous similarities of endocrine features, nutrient digestion and anatomy and physiology of the digestive tract between pigs and humans were found [19]. Considering the physiological functional advantages of the gastro-intestinal tract and skeletal system in the pig model, the acceleration of bone tissue metabolism in this study was induced using experimental treatments with nanostructural calcium and dexamethasone. While oral calcium administration in well balanced dosages is recognized as beneficial for the skeletal system and bone tissue metabolism, and counteracts osteoporosis development, long-term treatment with dexamethasone or glucocorticoids induces negative effects on skeletal morphology, BMD, BMC and mechanical endurance of bones [20, 21, 22, 23]. Therefore, in the experimental animals treated with nanopartical calcium and dexamethasone, separately or simultaneously, altered skeletal bone characteristics, together with changed serum biochemical bone metabolism indices, was anticipated.

Evaluation of the relationships between the investigated traits of mandible and biochemical indices of bone metabolism in 6-month-old pigs have shown that alkaline phosphatase activity was positively correlated with morphological, densitometric and biomechanical properties of the bone. Among morphological traits, significant correlations of ALP activity were found for mandible length and volume. It is worth underlining that ALP activity was positively correlated with BMD and BMC values obtained in all the investigated ROIs of the mandible. Moreover, analogical interrelationships of ALP and both the parameters representing mechanical endurance of the bone were observed.

These results do not seem surprising, especially when one considers that ALP is an indicator of the bone formation process. Even though ALP is considered as a non-specific marker of the anabolic processes in the skeleton, its evaluation in experimental studies have shown positive correlations with specific markers of osteoblastic activity, calcium metabolism and bone formation processes [24, 25, 26]. Our findings are in agreement with the other experimental studies on ovariectomised rats, where serum ALP activity was negatively correlated with BMD of mandible and spine [13]. Furthermore, in a 5-year follow-up on pre- and postmenopausal woman, negative correlations of ALP activity, OC concentration and bone loss were stated, confirming similar dependence between skeletal properties and bone turnover markers to those observed in this study [27].

The other bone formation marker evaluated in our experiment – osteocalcin – has shown to be valuable for determination of metabolic characteristic of the mandible. However, in contrast to ALP activity, positive correlations of OC were found for mandible weight, length and volume, as well as BMC determined at all the investigated ROIs. These results are in accordance with studies on humans in which positive relationships between serum osteocalcin concentration and jaw bone morphology were observed [25]. Assessment of CTX in serum of pigs has also shown its possible usefulness for evaluation of bone morphology since its negative correlation with CBA was observed. Clearly, this bone resorption marker increases in serum in response to catabolic processes and bone mass loss. These findings correspond with the earlier report on humans showing the advantages of plasma CTX determination for assessment of bone metabolism in mandible among osteopenic and osteoporotic patients. Contrary to our study, evaluating BMD, BMC and vBMD for both the whole jaw and its different regions, densitometric analysis in humans was performed only with the use of the DEXA method for mandibular body, and the obtained results were compared with data obtained from the femoral neck [12]. Except for bone turnover markers, serum concentrations of GH and IGF-1 was positively correlated with Cd and CBA, respectively, which confirmed previous findings indicating the importance of somatotrophic axis function in bone growth, development and skeletal homeostasis maintenance. [6, 28]. It is also interesting that ALP activity, OC and IGF-1 concentrations in serum would be valuable for final body weight prediction, especially when one considers the observed positive correlations between these variables. This phenomenon may be explained by experimental data showing circulating IGF-1 as an important factor responsible for improved metabolism and systemic development of the whole body, including the skeletal system [29]. However, its direct effects on bone growth and mineralization in such conditions cannot be excluded [30].

CONCLUSIONS

The presented study shows the highest predictive value of serum ALP activity determination in relation to assessment of morphological, densitometric and biomechanical properties of mandible in 6-month-old pigs. Bone metabolic activity and morphological traits of the mandible may be also monitored with the use of serum osteocalcin and CTX concentrations. Among hormones, serum GH and IGF-1 concentrations were positively associated with vBMD and CBA. Alkaline phosphatase activity, OC and IGF-1 concentrations in serum could also be prognostic for growth rate and final body weight prediction; however, further studies in this field are needed. The evaluation of calcium, magnesium, phosphorus, BAP and PTH, has not confirmed their significance for morphological, densitometric and biomechanical properties prediction in the mandible of pigs.

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