MATERNAL GLUCOCORTICOID TREATMENT AS A MODEL FOR EXAMINING FOETAL GENDER-SPECIFIC EFFECTS ON THE BONE METABOLISM OF NEONATAL PIGLETS

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Abstract

The aim of the study was to investigate gender-specific effects of a maternal treatment with dexamethasone (DEX) at multiple maximum therapeutic doses during the last 25 d of pregnancy on the bone tissue metabolism in neonatal piglets. BMD and BMC were measured. The geometry, histomorphometry, and mechanical properties of bones were determined. The assessment of selected hormones, cytokines, and amino acids was performed in 35-day-old piglets. DEX administered prenatally inhibited neonatal bone development, as well as bone mineral density, and geometrical and mechanical properties of bones. Neonatal GC-induced bone loss was linked with disturbed somatotrophic axis function and decreased serum concentration of GH in male piglets and decreased concentration of IGF-1 in females. Prenatal DEX treatment led to an increase in the concentration of insulin and enhanced activity of bone specific alkaline phosphatase in piglets of both genders.

Key words: piglets, prenatal time, dexamethasone, bone, histomorphometry.

Foetal skeleton growth is determined by genetic factors and can be modified by environmental factors, among which hormonal factors are the most important (4). The most known of these factors are glucocorticoids (GCs), which play a pivotal role in prenatal physiological maturity of many systems. Natural glucocorticoid - cortisol is detectable in early stage of foetal life in pigs (15). On the other hand, the excess of GCs during prenatal period leads to retarded foetal growth or many systemic adult diseases including hypertension, insulin resistance syndrome, bone loss, and osteoporosis (10, 21, 25-27, 30-32). Pigs, horses, dogs, and chickens suffer from different lesions with primary or secondary changes in the bone, associated with the failure of hormone or enzyme systems and influencing the musculoskeletal function (1, 5, 9, 14, 33). The effect of prenatal manipulation of postnatal growth depends on the species studied and the treatment model applied, and on the stage of prenatal period during which foetus is exposed to the harmful factors (19, 29). Although pregnant sows hardly ever are treated with GCs, we propose a model of maternal treatment with GCs to study the experimentally-induced bone loss. Animal models with maternal excess of GCs during pregnancy or selected part of gestation are used to test programming effects that are linked with important, costly and wide-spread bone diseases (21, 23, 30, 31,

34). The excess of dexamethasone (DEX) - a synthetic glucocorticoid - can serve as a model for defining the role of GCs in prenatal programming of physiological processes and behaviour in animals and humans (3, 6, 11, 20, 34, 35). Moreover, movement of maternal GCs across the placenta and an increase in foetal GCs as the result of stress may influence foetal growth (2, 21, 17).

Lack of knowledge about gender-specific effects of a maternal treatment with DEX at multiple maximum therapeutic doses on the bone tissue metabolism of neonatal piglets was considered in undertaking this study. To evaluate the effects of GC maternal treatment on skeletal properties, determination of bone morphology and histomorphometry, bone mineral density, and geometric as well as mechanical properties of long bones were performed. Bone metabolism was assessed by determination of serum activity of bone alkaline phosphatase and concentration of osteocalcin. Moreover, the concentration of various hormones essential for bone formation, selected cytokines, and plasma amino acids were used to monitor the effects of prenatal administration of maximum therapeutic doses of DEX.

Material and Methods

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

Animal, breeding, and experimental design.

Ten multiparous sows (Large Polish White breed) were randomly chosen and mated naturally with the boar of the same breed, then their litters were used in this experiment. All sows showed a similar care for their earlier offspring. Sows were clinically healthy and singly housed in separated cages under standard rearing conditions (controlled constant temperature and humidity, controlled 12:12-h light-dark cycle) with free access to fresh water and fed twice a day (2.3 kg/d) standard commercial diet for pregnant and lactating sows. Twenty five days before delivery, the pregnant sows were randomly divided into two weight- and age-matched groups. The first group of sows (DEX-sows; $n=5$) was treated intramuscularly with DEX (0.2% dexamethasone pro inj., Eurovet Animal Health B.V., The Netherlands) at the maximum dose of 0.08 mg/kg b.w. 48 h until the parturition. A total dose of DEX was about 104 mg per each pregnant DEX-treated sow. The second group of sows (PhS-sows; $n=5$) received physiological saline in the same volume and manner as DEX. The gestation length and mean total number of stillborn and live born piglets in litters did not differ between PhS-sows and DEX-sows.

Piglets were born naturally and had no congenital infections. All of the litters contained eight or more piglets. On the day of the birth (postnatal day 1), litters were weighed and sexed before suckling. Females and males were treated in the same manner and used in the subsequent study. Male piglets were not castrated. Piglets were not separated from mother after farrowing and were held in the same cage. Animals were not transferred from one sow to another. Piglets during suckling did not receive solid feed, iron, or other medication. Twenty-four males and 24 females at the age of 35 d were randomly chosen and assigned to four groups. Piglets from PhS-sows belonged to the control group of males ($n=12$) and females ($n=12$), and piglets from DEX-sows belonged to Dex group of males ($n=12$) and females ($n=12$). Next, piglets were weighed and euthanised by intravenous injections of lethal doses of pentobarbitalum natrium (Morbital, Biowet, Pulawy, Poland).

Bone analysis. Left femora, left humeri, and two ribs (6th-7th) taken from both sides were isolated. After cleaning of remaining soft tissues, the bones were weighed and measured. Each limb bone was wrapped in gauze, soaked in isotonic saline, and stored at -25 °C for further analysis.

Mechanical properties of bones were determined identically for both groups after thawing at room temperature using a three-point bending test in INSTRON 4302 apparatus (Instron, Canton, USA) linked with a computer. Relationship between forces perpendicular to the longitudinal axis of the bone and the displacement were registered. The distance between bone supports was set at 40% of total bone length and

bone samples were loaded with a constant speed of 10 mm/min. The maximum elastic strength (W_y) and the ultimate strength (W_f) of the bones were determined (7).

The geometry of bone was estimated on the basis of measurements of horizontal and vertical diameters of the mid-diaphyseal cross-section of this bone as described previously (7). The cross-section area (A), the mean relative wall thickness (MRWT), and cortical index (CI) were determined (7).

Bone mineral density (BMD) and bone mineral content (BMC) were determined using the dual-energy X-ray absorptiometry (DEXA) method on NORLAND XR 43 apparatus supplied with Research Scan software (Norland, Fort Atkinson, USA). These measurements were performed for the whole bone samples and for proximal part of each limb bone including both the trabecular and cortical bone compartments, and for the neck of ribs (the flattened portion, which extends laterally from the head, about 2.5 cm in length).

Histomorphometry. The fragments of bone from the head of each rib through the neck to a distance of 5 mm pass the tubercle were taken. Samples preparation was conducted as describe previously using the same equipment (32). The following parameters were analysed: the fractal dimension of the trabecular bone (D); the trabecular bone volume (BV/TV %); the trabeculae border line length (mm/mm² trabeculae); the number of osseous lacunae per mm² of trabeculae; osseous lacunae mean area (μm²); the area of osseous lacunae per mm² of trabeculae (mm²); trabecular area per mm² of bone tissue (mm²).

Blood sample collection and analysis. Blood samples were collected from each 35-day-old piglet. The piglets were not fasted before blood collection. One part of the blood samples were allowed to clot, and next they were subsequently centrifuged. The second part was collected in sodium heparinised tubes. Both serum and plasma samples were stored at -80°C for later analyses.

Growth hormone (GH) concentration was determined with the use of Porcine Growth Hormone Enzyme-Linked Immunosorbent Kit (ELISA; Diagnostic System Laboratories, Inc., Webster, USA); bone specific alkaline phosphatase (BAP) activity with the use of BAP Enzyme-Linked Immunosorbent Assay Kit (ELISA; METRA, Quidel Corporation, USA); osteocalcin (OC) concentration using Osteocalcin Enzyme-Linked Immunosorbent Assay Kit (ELISA; Diagnostic System Laboratories, Inc., Webster, USA); leptin concentration with the use of Porcine Leptin Enzyme-Linked Immunosorbent Kit (ELISA; Diagnostic System Laboratories, Inc., Webster, USA); insulin concentration with the use of Porcine Insulin ELISA (Mercodia, SE-754 50 Uppsala, Sweden); the concentration of insulin-like growth factor-1 (IGF-1) using Porcine IGF-1 Enzyme-Linked Immunosorbent Assay Kit (Uscn Life Science Inc. Wuhan 430056, China); the concentration of adiponectin was determined using Human Adiponectin Enzyme-Linked Immunosorbent Assay Kit (ELISA kit, BioVendor Laboratory Medicine, Inc. Czech Republic). The determination of serum hormonal concentrations was performed using commercially available kits and

Benchmark Plus microplate spectrophotometer supplied with Microplate manager Software Version 5.2.1. (Bio-Rad Laboratories, Inc., Hercules, USA).

The plasma samples were analysed using an ion-exchange chromatography on an INGOS AAA 400 automatic analyser (Ingos, Czech Republic), and the concentration of arginine (ARG), isoleucine (ILE), leucine (LEU), lysine (LYS), alanine (ALA), glycine (GLY), glutamine (GLU), glutamate (GLN), ornithine (ORN), and proline (PRO) were determined.

Serum concentrations of calcium, phosphorus, magnesium, iron, and copper were determined using energy-disperse X-ray spectrometry (ED 2000 Spectrometer, Oxford Instruments, UK) and expressed in mg/g dry matter of the lyophilised sample.

Statistical analysis. All results are expressed as means $\pm$ SEM (standard error of the mean). Differences between means were tested with the Two Way ANOVA and *post hoc* Tukey's test as the correction for multiple comparisons. Normal distribution of data was examined using the W. Shapiro-Wilk test and equality of variance was tested by the Levene test. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out by means of STATISTICA 8.0 software (www.statsoft.com).

Results

Body weight. Body weights of female newborn piglets in the control and Dex groups were not significantly different and reached $1,350 \pm 32$ g and $1,197 \pm 22$ g, respectively. Body weights of DEX-treated

male newborn piglets reached the value of $1,389 \pm 42$ g and were significantly lower compared with the value of $1,616 \pm 37$ g obtained in the male control piglets ($P < 0.001$).

The final body weight obtained at the age of 35 d in prenatally DEX-treated females was $4,730 \pm 230$ g and did not differ from the value of $5,850 \pm 539$ g obtained in the female control group. Prenatally DEX-treated males ($7,960 \pm 154$ g) did not weigh significantly less than the control males ($8,441 \pm 290$ g).

Bone weight, length, density, geometry, and mechanics. Results of the morphological and mechanical properties, as well as bone mineral density measurements of limb bones and ribs of piglets are presented in Tables 1 and 2. Prenatal DEX-treatment resulted in a significantly reduced value of ultimate strength of the femur, reduced value of maximum elastic strength and cross-sectional area of ribs, and reduced BMD in male offspring. Moreover, maternal administration of DEX significantly reduced ribs geometry and BMC of the femur and ribs in female piglets at the age of 35 d.

Histomorphometry. Results of the histomorphometric measurements are presented in Table 3. Prenatal DEX treatment significantly reduced the length of trabecular border line, the area of osseous lacunae, the area of osseous lacunae per mm² of trabeculae, and the area of trabecular ribs of male and female piglets when compared with the values of the same parameters obtained in the control group (Fig. 1).

Table 1

The effect of prenatal dexamethasone treatment on mechanical and geometric properties, bone mineral density, and bone mineral content of the femur, humerus, and ribs of male piglets (n=12) at the age of 35 d

Bone Group	Femur		Humerus		Ribs	
	Control	Dex	Control	Dex	Control	Dex
Weight (g)	40.60 $\pm$ 0.86	39.80 $\pm$ 0.49	36.52 $\pm$ 0.46	34.37 $\pm$ 0.65	4.01 $\pm$ 0.10	3.46 $\pm$ 0.08
Length (cm)	9.11 $\pm$ 0.19	9.15 $\pm$ 0.05	8.56 $\pm$ 0.07	8.30 $\pm$ 0.04	11.10 $\pm$ 0.19	11.01 $\pm$ 0.12
Wf (N)	1,542 $\pm$ 134	1,237 $\pm$ 44*	1,196 $\pm$ 41	1,077 $\pm$ 19	108 $\pm$ 3	99 $\pm$ 6
Wy (N)	928 $\pm$ 34	869 $\pm$ 44	830 $\pm$ 35	756 $\pm$ 22	68 $\pm$ 3	51 $\pm$ 2*
A (mm ²)	63 $\pm$ 1.9	62 $\pm$ 1.3	52 $\pm$ 2.3	56 $\pm$ 1	10.3 $\pm$ 1.4	7.1 $\pm$ 0.5*
MRWT	0.60 $\pm$ 0.03	0.51 $\pm$ 0.02	0.57 $\pm$ 0.01	0.52 $\pm$ 0.02	0.45 $\pm$ 0.05	0.36 $\pm$ 0.03
IC	37.1 $\pm$ 1.31	34.7 $\pm$ 0.82	36.5 $\pm$ 0.77	34.4 $\pm$ 0.96	29.5 $\pm$ 2.32	25.0 $\pm$ 1.72
BMD of the whole bone (g/cm ³)	0.379 $\pm$ 0.002	0.377 $\pm$ 0.003	0.387 $\pm$ 0.005	0.354 $\pm$ 0.011*	0.102 $\pm$ 0.003	0.099 $\pm$ 0.002
BMC of distal part (g)	2.833 $\pm$ 0.121	2.869 $\pm$ 0.075	2.952 $\pm$ 0.093	2.789 $\pm$ 0.152*	0.163 $\pm$ 0.002	0.142 $\pm$ 0.008*
BMDp of the whole bone (g/cm ³)	0.399 $\pm$ 0.003	0.349 $\pm$ 0.002*	0.356 $\pm$ 0.005	0.352 $\pm$ 0.009	-	-
BMCp of distal part (g)	2.455 $\pm$ 0.054	1.889 $\pm$ 0.053*	1.972 $\pm$ 0.081	1.912 $\pm$ 0.087	-	-

Presented data are means $\pm$ SEM, * $P < 0.05$ Dex vs. control group. Control group - piglets under prenatal treatment with physiological saline; Dex group - piglets after maternal treatment with DEX.

Wy - the maximum elastic strength; Wf - the ultimate strength; A - the cross-section area; MRWT - the mean relative wall thickness; CI - cortical index; BMD - bone mineral density; BMC - bone mineral content

Table 2

The effect of prenatal dexamethasone treatment on mechanical and geometric properties, bone mineral density and bone mineral content of the femur, humerus, and ribs of female piglets (n=12) at the age of 35 d

Bone	Femur		Humerus		Ribs	
Group	Control	Dex	Control	Dex	Control	Dex
Weight (g)	27.08±0.93	26.30±1.54	24.02±1.27	21.20±0.45	2.71±0.07	2.65±0.16
Length (cm)	8.11±0.06	8.13±0.12	7.21±0.07	7.48±0.09	9.55±0.19	9.62±0.14
Wf (N)	1,122±18	1,018±38	903±29	863±30	76±3	79±4
Wy (N)	704±28	638±34	564±25	542±23	43±4	39±3
A (mm ²)	46±2.2	44±2.4	48±2.5	49±1.8	7.8±0.4	6.7±0.3*
MRWT	0.57±0.03	0.53±0.01	0.55±0.02	0.54±0.01	0.68±0.04	0.44±0.03*
IC	36.3±1.25	37.4±0.54	39.7±1.2	35.2±0.35	38.6±1.59	29.6±1.38*
BMD of the whole bone (g/cm ²)	0.347±0.009	0.318±0.009*	0.356±0.004	0.347±0.009	0.092±0.002	0.093±0.002
BMC of the whole bone (g)	2.619±0.106	2.127±0.099*	2.512±0.161	2.408±0.088	0.116±0.004	0.109±0.002
BMDp of distal part of bone (g/cm ²)	0.347±0.014	0.316±0.009	0.312±0.006	0.304±0.004	-	-
BMCp of distal part (g)	1.892±0.092	1.519±0.028*	1.558±0.122	1.491±0.067	-	-

Symbols are explained in the footnote in Table 1.

Table 3

The effect of prenatal dexamethasone treatment on histomorphometry of ribs of male (n=12) and female (n=12) piglets at the age of 35 d

Group	Male piglets		Female piglets	
	Control	Dex	Control	Dex
Fractal dimension of trabecular bone (D)	1.236±0.011	1.205±0.008	1.207±0.01	1.188±0.011
Number of osseous lacunae per mm ² of trabeculae	215.76±18.69	146.99±7.67*	132.16±15.86	67.57±11.32*
Osseous lacunae mean area (µm ²)	30.59±0.84	28.93±0.65	29.75±0.50	28.55±1.50
Osseous lacunae area per mm ² of trabeculae (µm ²)	6,715.75±693.79	4,318.96±301.72*	3,910.30±474.14	1,975.95±350.75*
Area of trabeculae (mm ² /mm ² bone)	0.223±0.006	0.182±0.011*	0.211±0.008	0.170±0.011*
Area of trabeculae %	20.93±0.58	17.42±1.13*	19.76±0.64	16.19±0.95*
Trabeculae border line length (mm/mm ² trabeculae)	0.019±0.001	0.014±0.001*	0.015±0.001	0.012±0.001*

Presented data are means ±SEM, * P<0.05 Dex vs. control group. Control group - piglets under prenatal treatment with physiological saline; Dex group - piglets after maternal treatment with DEX.

Table 4

The effect of prenatal dexamethasone treatment on the concentration of selected hormones, osteocalcin (OC) and insulin-like growth factor-1 (IGF-1), and the activity of bone alkaline phosphatase (BAP), in serum obtained from male (n=12) and female (n=12) piglets at the age of 35 d

Group	Male piglets		Female piglets	
	Control	Dex	Control	Dex
GH (ng/mL)	6.52±0.36	3.15±0.36*	3.47±0.51	4.59±0.82
IGF-1 (ng/mL)	75.03±5.55	72.33±4.71	75.50±2.72	58.01±4.23*
Insulin (µg/mL)	0.093±0.009	0.116±0.005*	0.088±0.003	0.131±0.013*
BAP (U/L)	95.85±4.42	195.81±5.29*	82.82±2.37	148.65±4.68*
OC (ng/mL)	36.14±0.99	38.80±0.56	25.78±0.58	25.85±4.55
Leptin (ng/mL)	0.877±0.162	0.936±0.055	0.56±0.07	2.42±0.09*
Adiponectin (ng/mL)	0.0904±0.0009	0.0855±0.0026	0.0882±0.0014	0.0900±0.0019

Presented data are means ±SEM, * P<0.05 Dex vs. control group. Control group - piglets under prenatal treatment with physiological saline; Dex group - piglets after maternal treatment with DEX.

GH - growth hormone

Table 5
The effect of prenatal dexamethasone treatment on selected free amino acid concentration (nmol/mL) in plasma of male (n=12) and female (n=12) piglets at the age of 35 d

Group	Male piglets		Female piglets	
	Control	Dex	Control	Dex
Essential				
Arginine	126.6 ± 18.1	130.8 ± 9.4	115.3 ± 9.1	137.0 ± 8.7
Isoleucine	88.3 ± 24.7	112.8 ± 14.8	68.3 ± 9.2	125.1 ± 9.5*
Leucine	147.8 ± 24.0	247.6 ± 30.6*	132.0 ± 10.0	282.0 ± 19.9*
Lysine	176.3 ± 19.3	249.2 ± 25.7*	191.8 ± 7.5	276.3 ± 17.6*
Threonine	79.8 ± 15.3	160.2 ± 28.0*	79.3 ± 15.4	203.8 ± 17.3*
Valine	185.8 ± 38.4	336.8 ± 53.3*	164.2 ± 16.4	396.3 ± 32.9*
Non Essential				
Alanine	562.3 ± 24.2	1,042.0 ± 221.3*	560.8 ± 28.4	1,366.0 ± 157.3*
Glycine	594.5 ± 52.6	913.6 ± 197.7	498.0 ± 16.8	1,236.6 ± 142.5*
Glutamate	167.0 ± 16.6	289.0 ± 27.27*	170.8 ± 21.2	251.3 ± 20.0*
Glutamine	103.1 ± 21.1	94.8 ± 4.2	90.8 ± 14.9	96.6 ± 4.2
Ornithine	75.8 ± 10.4	90.4 ± 6.9	86.6 ± 1.2	97.1 ± 5.0
Proline	771.0 ± 81.6	900.0 ± 87.6	614.5 ± 40.1	1,018.6 ± 38.5*
Serine	93.6 ± 9.2	143.0 ± 17.5*	91.2 ± 6.6	168.0 ± 15.4*

Presented data are means ±SEM, * P<0.05 Dex vs. control group. Control group - piglets under prenatal treatment with physiological saline; Dex group - piglets after maternal treatment with DEX.

Growth hormone, bone specific alkaline phosphatase, osteocalcin, leptin, insulin, and insulin-like growth factor-1 (IGF-1), and adiponectin. Results of biochemical analyses of serum obtained from piglets at the age of 35 d are presented in Table 4. Prenatal DEX-treatment of piglets significantly decreased the concentrations of GH, and increased insulin concentration and the activity of BAP in male piglets when compared with the control group. DEX-treated female piglets had a significantly higher concentration of leptin and insulin as well as the activity of BAP, and a significantly lower concentration of IGF-1 in the serum compared with the control animals.

Plasma concentration of amino acids and serum concentration of macro- and microelements. Plasma concentrations of selected amino acids are presented in Table 5. The concentrations of many non-essential and essential amino acids were significantly affected by DEX in female and male piglets when compared with the control group.

Selected macro- and microelement concentrations in serum from male and female piglets were not influenced by prenatal administration of DEX. Data are not shown.

Discussion

Results of this study indicate that DEX given to pregnant sows inhibits neonatal bone development of piglets. Although, body weight of DEX-treated male piglets (14%) was more reduced than DEX-treated female piglets (11%) compared with the control group, the rate of neonatal development of prenatally DEX-treated piglets was comparable in male and female offspring. In none of the male and female offspring

treated with DEX the growth retardation persisted until weaning, despite the fact that final body weight of females at the age of 35 decreased about 20% comparing with female control group. Furthermore, suckling piglets that underwent the prenatal DEX treatment had enhanced concentration of amino acids, which could constitute a possible additional mechanism involved in attaining the appropriate, optimal body weight. More agents might independently determine the postnatal growth, such as adiponectin that was untouched by treatment with maximum therapeutic doses of DEX. Adiponectin, a hormone of adipose tissue modulates a number of metabolic processes, acts like leptin and can be reduced by the weight decrease (12, 24, 38).

The compensatory growth of male piglets of sows treated with DEX at multiple minimal therapeutic doses during the last 24 d of prenatal time is achieved about 30 d after the birth. The compensation was referred to body weight, and the geometry, as well as weight, and length of the humerus (32).

In spite of the achieved compensation in body and bone weights, female offspring at the age of 35 d had BMD and BMC of the whole femur decreased about 8.3% and 18.7%, respectively. In the same way DEX reduced bone mineralisation of the humerus and the distal part of the femur in males (BMD and BMC by 12.5% and 23%, respectively), while serum concentrations of calcium and phosphorus were unchanged in DEX-treated piglets of both genders (data not shown). However, the amount of bone tissue accumulated in ribs, expressed by the value of BMC, was more decreased in males than females (12% and 6%, respectively) when compared with the controls. Moreover, the results of this investigation showed that DEX administration to pregnant sows during the last 25 d of pregnancy affected the geometry of ribs.

Fig. 1. Trabecular bone of ribs of male and female piglets at the age of 35 d; A – male piglets from the control group; B – female piglets from the control group; C - male piglets from the Dex group - piglets being under the influence of DEX for the last 25 d of prenatal life; D - female piglets from the Dex group - piglets being under the influence of DEX for the last 25 d of prenatal life; 1- trabecular bone, 2 - osseous lacunae, 3 - marrow cavity. H+E staining, seen under UV microscope, 40x.

In the group of females prenatally treated with DEX, in spite of geometrically immature skeleton and reduced mineralisation, no alteration of mechanical parameters was observed. On the basis of the performed analyses of the bone, this study showed that both reduced mineralisation and decreased histomorphometric parameters were responsible for lowered bone mechanical endurance in male piglets. While maternal DEX treatment induced greater geometrical immaturity in females than males, the mechanical parameters were much more reduced and bone loss was much more enhanced in male piglets.

The results of bone analyses obtained in this study are very similar to earlier results, in which smaller doses than the ones presented in this study were investigated (35). Considering previous data related to prenatal manipulation, hormonal mechanisms have been investigated. The major functions of skeleton, *i.e.* support and protection, changed in response to the unfavourable foetal environment, which may be associated with the lack of production of a specific enzyme or hormone and reduced activity of some metabolic function. The regulatory system of foetal development that may influence postnatal bone tissue metabolism and peak bone mass, achieved at the end of the bone maturation, consists from three endocrine axes, *i.e.* the hypothalamic-pituitary-adrenal, endocrine pancreas, and somatotrophic (8, 13, 19, 22, 27, 31, 34, 36). Among many systemic factors - insulin, GH, leptin, and GCs influence bone metabolism. GH is an important modulator of insulin sensitivity, which seems to be the main hormone involved in the short-term control of muscle protein synthesis from nutrients in pigs (18, 28).

Leptin is produced in adipose tissue and directly stimulates bone growth although declines with advancing age in pigs (16, 37).

This study showed that harmful effects of the maternal DEX treatment on neonatal skeletal system development were mediated by changed somatotrophic axis. It is supported by significantly reduced serum concentration of GH in male piglets from the Dex group. Moreover, in spite of decreased concentration of GH, males had unchanged concentration of leptin, OC, and IGF-1, and enhanced level of insulin. While in females, the concentration of IGF-1 decreased, and insulin, leptin, and GH increased. The elevation of insulin concentration following the meal consumption was probably due to the release of leptin. Furthermore, current study showed that DEX given to pregnant sows resulted in enhanced activity of BAP, the biochemical marker of bone formation. On the other hand, the function of growth hormone/IGF-1 axis, affected by maternal DEX treatment, might led to bone loss.

In conclusion, DEX administered prenatally at multiple maximum therapeutic doses inhibited neonatal bone development in pigs, as well as their bone mineral density and the geometrical and mechanical properties of bones. Neonatal GC-induced bone loss was linked with disturbed somatotrophic axis function, decreased serum concentration of GH in male piglets, and lowered concentration of IGF-1 in females. However, further investigations should be performed to study the role of GC in epigenetic modification of the growth and bone development, by suboptimal conditions in prenatal life, and conditions associated with adult bone diseases in animals as well as humans.

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References

1. Céleste C., Ionescu M., Poole A.R., Laverty S.: Repeated intraarticular injections of triamcinolone acetonide alter cartilage matrix metabolism measured by biomarkers in synovial fluid. *J Orthop Res* 2005, **23**, 602-610.
2. Bertram C.E., Hanson M.A.: Prenatal programming of postnatal endocrine responses by glucocorticoids. *Reproduction* 2002, **124**, 459-467.
3. Bloom S.L., Sheffield J.S., McIntire D.D., Leveno K.J.: Antenatal dexamethasone and decreased birth weight. *Obstet Gynecol* 2001, **97**, 485-490.
4. Eastell R., Lambert H.: Diet and healthy bones. *Calcif Tissue Int* 2002, **70**, 400-404.
5. Ekman S., Heinegård D.: Immunohistochemical localization of matrix proteins in the femoral joint cartilage of growing commercial pigs. *Vet Pathol* 1992, **29**, 514-520.
6. Engle M.J., Kemnitz J.W., Rao T.J., Perelman R.H., Farrell P.M.: Effects of maternal dexamethasone therapy on fetal lung development in the rhesus monkey. *Am J Perinatol* 1996, **13**, 399-407.
7. Ferretti J.L., Capozza R.F., Mondelo N., Zanchetta J.R.: Interrelationships between densitometric, geometric and

- mechanical properties of rat femora: inferences concerning mechanical regulation of bone modelling. *J Bone Min Res* 1993, **8**, 395-1399.
8. Fowden A.L., Giussani D.A., Forhead A.J.: Endocrine and metabolic programming during intrauterine development. *Early Human Develop* 2005, **81**, 723-734.
 9. Henson F.M.D., Davies M.E., Jeffcott L.B.: Equine dyschondroplasia (*Osteochondrosis*) – histological findings and type VI collagen localization. *Vet J* 1997, **154**, 53-62.
 10. Holemans K., Aerts L., Van Assche F.A.: Fetal growth restriction and consequences for the offspring in animal models. *J Soc Gynecol Invest* 2003, **10**, 392-399.
 11. Holemans K., Aertis L., Van Assche A.: Fetal growth and long-term consequences in animal models of growth retardation. *Eur J Obstet Gynecol* 1998, **81**, 149-156.
 12. Huang K-C., Lue B-H., Yen R-F., Shen C.G., Ho S-R., Tai T-Y., Yang W-S.: Plasma adiponectin levels and metabolic factors in nondiabetic adolescents. *Obesity Res* 2004, **12**, 119-124.
 13. Jobe A.H., Wada N., Berry L.M., Ikegami M., Ervin M.: Single and repetitive maternal glucocorticoids exposures reduce fetal growth in sheep. *Am J Obstet Gynecol* 1998, **178**, 880-885.
 14. Katta J., Jin Z., Ingham E., Fisher J.: Biotribiology of articular cartilage – a review of the recent advances. *Med Eng Physiol* 2008, **30**, 1349-1363.
 15. Klemcke H.G., McGuire W.J., Christenson R.K.: Cortisol concentration in early porcine embryos. *Life Sci* 1999, **64**, 1307-1312.
 16. Laml T., Preyer O., Szulz-Lobmeyr I., Ruecklinger E., Hartmann B.W., Wagenbichler P.: Umbilical venous leptin concentration and gender in newborns. *J Soc Gynecol Inves* 2001, **8**, 94-97.
 17. Maccari S., Darnaudery M., Morley-Fletcher S., Zuena A.R., Cinque C., Van Reeth O.: Prenatal stress and long-term consequences: implications of glucocorticoids hormones. *Neurosci Biobehav Rev* 2003, **27**, 119-127.
 18. Marshall S., Garvey W.T., Traxinger R.R.: New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. *Exp Biol J* 1991, **5**, 3031-3036.
 19. Miller S.C., Bowman B.M., Jee W.S.: Available animal models of osteopenia - small and large. *Bone* 1995, **17**, 117S-123S.
 20. Nagano M., Ozawa H., Suzuki H.: Prenatal dexamethasone exposure affects anxietylike behaviour and neuroendocrine systems in an age-dependent manner. *Neurosci Res* 2008, **60**, 364-371.
 21. Nathanielsz P.W., Thornburg K.L.: Fetal programming: from gene to functional systems –an overview. *J Physiol* 2003, **547**, 3-4.
 22. Newnham J.P., Moss T.J.: Antenatal glucocorticoids and growth: single versus multiple doses in animal and human studies. *Semin Neonatol* 2001, **6**, 285-292.
 23. O'Regan D., Welberg L.L.A.M., Holmes M.C., Seckl J.R.: Glucocorticoid programming of pituitary-adrenal function: mechanisms and physiological consequences. *Semin Neonatol* 2001, **6**, 319-329.
 24. Silha J.V., Krsek M., Skrha J.V., Sucharda P., Nyomba B.L., Murphy L.J.: Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 2003, **149**, 331-335.
 25. Simmons R.A., Templeton L.J., Gertz S.J.: Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. *Diabetes* 2001, **50**, 2279-2286.
 26. Seckl J.R.: Physiologic programming of the fetus. *Clin Perinatol* 1998, **25**, 939-964.
 27. Seckl J.R.: Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. *Mol Cell Endocrinol* 2001, **185**, 61-71.
 28. Sève B., Ponter A.A.: Nutrient-hormone signals regulating muscle protein turnover in pigs. *Proc Nutr Soc* 1997, **56**, 565-580.
 29. Smink J.J., Buchholz I.M., Hamers N., van Tilburg C.M., Christis C., Sakkers R.J., de Meer K., van Buul-Offers S.C., Koedam J.A.: Short-term glucocorticoid treatment of piglets causes changes in growth plate morphology and angiogenesis. *OsteoArtr Cart* 2003, **11**, 864-871.
 30. Smith N.H., Ozanne S.E.: Intrauterine origins of metabolic disease. *Rev Gynaecol Perinatal Pract* 2006, **6**, 211-217.
 31. Śliwa E., Dobrowolski P.: Perinatal programing of skeletal system. *J Pre-Clin Clin Res* 2007, **1**, 111-117.
 32. Śliwa E., Dobrowolski P., Piersiak T.: Bone development of suckling piglets after prenatal, neonatal or perinatal treatment with dexamethasone. *J Anim Physiol Anim Nutr* 2010, **94**, 293-306.
 33. Śliwa E., Radzki R.P., Puzio I.: Osteochondrosis and tibial dyschondroplasy in chickens, pigs and foals. *Medycyna Wet* 1996, **52**, 156-159.
 34. Śliwa E., Tatar M.R., Nowakowski H., Pierzynowski S.G., Studziński T.: Effect of maternal dexamethsone and α -ketoglutarate administration on skeletal development during the last three weeks of prenatal life in pigs. *J Matern Fetal Neonatal Med* 2006, **19**, 489-493.
 35. Śliwa E., Kowalik S., Tatar M.R., Studziński T.: Effect of maternal administration of dexamethasone during last weeks of pregnancy on foetal skeletal system in pigs. *Bull Vet Inst Pulawy* 2004, **48**, 449-452.
 36. Weiler H., Wang Z., Atkinson S.: Lean body mass and protein and energy utilization for growth are lower in dexamethasone-treated piglets. *Biol Neonate* 1997, **71**, 53-59.
 37. Weiler H.A., Kovacs H., Murdock C., Adolphe J., Fitzpatrick-Wong S.: Leptin predicts bone and fat mass after accounting for the effects of diet and glucocorticoid treatment in piglets. *Exp Biol Med* 2002, **227**, 639-644.
 38. Yokota T., Oritani K., Takahashi I., Ishikawa J., Matsuyama A., Ouchi N., Kihara S., Funahashi T., Tenner A.J., Tomiyama Y., Matsuzawa Y.: Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000, **96**, 1723-1732.