Journal of Pre-Clinical and Clinical Research, 2012, Vol 6, No 1, 14-19
www.jpccr.eu

ORIGINAL ARTICLE

Biochemical bone metabolism markers and morphometric, densitometric and biomechanical properties of femur and tibia in female and gonadectomised male Polish Landrace pigs

Marcin R. Tatara[1], Witold Krupski[2], Barbara Tymczyna[3], Iwona Łuszczewska-Sierakowska[4]

[1] *Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences, Lublin, Poland*
[2] *II Department of Radiology, Medical University, Lublin, Poland*
[3] *Department of Conservative Dentistry, Medical University, Lublin, Poland*
[4] *Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Life Sciences, Lublin, Poland*

Tatara MR, Krupski W, Tymczyna B, Łuszczewska-Sierakowska I. Biochemical bone metabolism markers and morphometric, densitometric and biomechanical properties of femur and tibia in female and gonadectomised male Polish Landrace pigs. J Pre-Clin Clin Res. 2012; 6(1): 14-19.

Abstract

Estrogens and androgens are critical regulators of bone metabolism and maintain bone mass throughout live in humans and animals. The aim of the presented study was to compare biochemical bone turnover markers and hormones influencing bone tissue metabolism and skeletal properties in female and orchidectomised male pigs at slaughter age. To achieve this aim, femur and tibia from 6-month-old pigs were investigated in terms of morphometric, densitometric and biomechanical properties. Serum evaluation of osteocalcin (OC), bone-specific alkaline phosphatase (BAP), growth hormone (GH), insulin-like growth factor-1 (IGF-1) and cotrisol in newborn and 90-day-old pigs was performed. This study shows a significantly higher growth rate and final body weight gain in orchidectomised males when compared to females ($p<0.05$). The obtained results have not shown statistically significant differences in weight, length, volumetric bone mineral density of the trabecular and cortical bone, vertical and horizontal diameters of the mid-shaft, cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength of femur and tibia in females and orchidectomised males ($p>0.05$). Bone formation markers such as BAP and OC assessed in serum were not significantly different between both the investigated groups ($p>0.05$). Furthermore, serum concentrations of GH, IGF-1 and cortisol were not gender-differentiated ($p>0.05$). In conclusion, the differences in body weight gain were not influenced by gender-differentiated serum concentrations of GH, IGF-1 and cortisol. Lack of significant differences of morphological, densitometric and biomechanical properties of femur and tibia between the groups was related to very similar levels of OC, BAP, GH, IGF-1 and cortisol. The data obtained provide novel physiological information on bone metabolism markers and regulators, as well as properties of femur and tibia in female and gonadectomised male pigs. The results obtained in this study may therefore be useful in experimental approaches for studying the effects of physiological, nutritional, pharmacological, toxicological and environmental factors on bone metabolism and skeletal system properties in pigs.

Key words

volumetric bone mineral density, quantitative computed tomography, femur, tibia, pig

INTRODUCTION

Estrogens and androgens are critical regulators of bone metabolism and maintain bone mass during life. Estrogen deficiency induced by menopause leads to accelerated bone resorption and significant bone loss in the skeleton. Ovariectomy in animals is commonly used in experimental models to induce osteopenia and osteoporosis. In males, orchidectomy results in marked bone loss, induced by increased bone resorption since testosterone and estrogen are responsible for bone mass accretion and maintenance [1, 2]. Hypogonadism causing osteoporosis in men may be treated with androgens which reverse bone tissue loss [3, 4]. It has been shown that testosterone exerts its effects in males by suppressing bone turnover, and that the effects may be mediated by the estrogen pathway [5]. Androgens may be converted to estrogens by aromatase in various organs, tissues and cells, such as the testes, brain, liver. prostate, placenta, skin, adipocytes and osteoblasts [6, 7, 8]. In aromatase-deficient humans, delayed maturation of skeletal tissue and osteoporosis has been observed; however, bone loss was normalized by estrogen replacement therapy, indicating the essential role of estrogen in maintaining bone mass in males [2, 9, 10].

Alpha-ketoglutarate (AKG) and β-hydroxy-β-methyl-butyrate (HMB) were recognized as natural bioactive

Address for correspondence: Marcin R. Tatara, Department of Animal Physiology, University of Life Sciences, Akademicka 12, 20-950 Lublin, Poland.
E-mail: matatar99@gazeta.pl; matatar@o2.pl

Received: 17 January 2012; accepted: 29 May 2012

This copy is for personal use only - distribution prohibited.

This copy is for personal use only - distribution prohibited.

metabolites of amino acids (glutamine and leucine) influencing bone tissue metabolism and skeletal properties in vertebrates. Alpha-ketoglutarate administration in animals has induced positive effects on the maintenance of skeletal development and bone tissue homeostasis [11, 12, 13]. Administration of AKG during neonatal life in sheep and pigs has induced beneficial effects on the programming of skeletal system development in terms of bone mineral density, morphological and mechanical properties [14, 15, 16, 17]. Studies with rats and humans have shown that HMB solely or in combination with arginine and glutamine results in increased collagen deposition [18]. Moreover, prenatal and neonatal exposure of pigs and sheep to HMB treatment has induced long-term beneficial effects on bone mineral density, morphological and mechanical properties of the skeletal system investigated at slaughter age [16, 19].

Considering the lack of literature data, the aim of the presented study was comparison of biochemical bone turnover markers and hormones influencing bone tissue metabolism and skeletal properties in female and orchidectomised male pigs. To achieve this aim, femur and tibia from 6-month-old pigs were investigated in terms of morphometric, densitometric and mechanical properties. Serum evaluation of osteocalcin (OC), bone-specific alkaline phosphatase (BAP), growth hormone (GH), insulin-like growth factor-1 (IGF-1) and cotrisol in newborn and 90-day-old pigs was also performed.

MATERIALS AND METHODS

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

Experimental design and sampling procedure. The study was performed on 290 pigs obtained from 24 sows of Polish Landrace (PL) breed. Pregnant sows were divided into 4 groups and kept under identical breeding and environmental conditions until the partum.

First group consisted of control sows (n = 6) treated with placebo ($CaCO_3$ dissolved in saline at the daily dosage of 0.05 g/kg of body weight), while the second group of sows (n = 6) received alpha-ketoglutaric acid (AKG group) as a neutral water solution. The third group of sows (n = 6; HMB group) was treated with calcium salt of β-hydroxy-β-methylbutyric acid (CaHMB dissolved in saline) at the daily dosage of 0.05 g/kg of body weight. To the fourth group belonged sows (n = 6; AH group) that underwent combined administration of alpha-ketoglutarate and β-hydroxy-β-methylbutyrate at the same dosages as in the AKG and HMB groups.

The pregnant sows were administered orally with *placebo*, AKG and HMB each day throughout the last 2 weeks of gestation. During the whole period of pregnancy and lactation, all sows were fed a well-balanced diet. The piglets obtained from the sows were assigned to the control group (n = 71), AKG group (n = 73), HMB group (n = 72) and AH group (n = 74). The body weights of the newborns were determined and blood samples taken from the subclavian vein of unsuckled piglets for analysis. Mortality of the piglets was similar in each group and in total reached 10.7%. At the age of three days of life the male piglets were castrated, and at the age of four weeks of life they were weaned. At the age of 90 days of life, blood samples were collected from 12-hour fasted pigs. Blood samples of newborns and 90-day old pigs were collected from one male and one female from each litter, with the closest body weight values to mean body weight of the whole litter. The obtained samples of serum were stored at –25 °C until biochemical analyses. During the whole period of the study, the piglets had free access to fresh water and feed *ad libitum*, prepared according to the stages of the production cycle. At the age of 6 months, the pigs (127 females and 133 males) were slaughtered and the left femur was isolated for further analysis.

Biochemical analysis of serum from piglets. Determination of osteocalcin in the serum of newborn and 90-day-old pigs was performed by the Enzyme-Linked Immunosorbant Assay (ELISA) method (Human Osteocalcin ELISA, Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Bone-specific alkaline phosphatase activity in the serum of newborn piglets was assessed with the use of an enzyme immunoassay (METRA BAP EIA kit, Quidel Corp., San Diego, CA, USA). In 90-day-old pigs, the measurement of BAP concentration in serum was performed using OCTEIA Ostase® BAP immunoenzymometric assay (Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK). Growth hormone concentration in serum was measured with the use of Porcine Growth Hormone Enzyme-Linked Immunosorbant Assay (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Serum concentration of IGF-1 was determined using immunoenzymometric assay (IEMA, OCTEIA IGF-1, Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK). Cortisol ELISA assay was used to determine cortisol concentration in serum of piglets (DiaMetra, Inc., Foligno, Italy). The results of biochemical analyses were obtained with the use of Benchmark Plus microplate spectrophotometer supplied with Microplate Manager Software Version 5.2.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Determination of bone morphometric, densitometric and mechanical properties. The left femur was isolated from pigs 24 hours after the slaughter and cleaned of remaining soft tissues. Bone length and bone weight were determined and the bone samples stored at –25 °C until further analysis. The volumetric bone mineral density (vBMD) of femur and tibia was determined using the quantitative computed tomography (QCT) technique in SOMATOM EMOTION SIEMENS apparatus (Siemens, Erlangen, Germany) equipped with Somaris/5 VB10B software. The volumetric BMD in femur and tibia was determined for both the trabecular and cortical bone. Using 2-mm thick, cross-sectional metaphyseal QCT scans, the trabecular bone mineral density (Td) was assessed. In femur, Td was measured for the distal metaphysis, while in tibia both the proximal and distal metaphyses were evaluated. The cortical bone mineral density (Cd) was determined on the measuring scans placed at the mid-shaft of femur and tibia. Geometrical properties of the femur and tibia were determined on the basis of measurements of horizontal and vertical diameters of the mid-diaphyseal cross-sections of the bones, and the values of cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were obtained [20, 21, 22].

This copy is for personal use only - distribution prohibited.

The mechanical properties of femur and tibia were determined using the 3-point bending test in an INSTRON 4302 apparatus (Instron, Canton, MA, USA) linked to a computer, registering the relationship between forces perpendicular to the longitudinal axis of the bone and the resulting displacement. The values of maximum elastic strength (Wy) and ultimate strength (Wf) were determined. The distance between bone supports was set at 40% of total bone length. The measuring head loaded bone samples with a constant speed of 20 mm/min.

Statistical analysis. All data are presented as means ± SEM. The data were found to be normally distributed in accordance with Kolomogorov-Smirnov test. Statistical analysis was performed using non-paired Student's *t* test and Tukey's test. The level of statistical significance was set at p-value < 0.05.

RESULTS

The body weight of 6-month-old females reached 103 ± 1 kg and were significantly different when compared to the value of 106 ± 1 kg obtained in gonadectomised males (p=0.03). Daily body weight gain was found to reach a significantly higher value in orchidectomised males (583 ± 7 g) when compared to this parameter in females (550 ± 6 g; p<0.001) (Tab.1). Neither osteocalcin concentration nor BAP activity in serum was found to be significantly different between newborn females and males (p>0.05). Growth hormone, IGF-1 and cortisol concentrations in the serum of newborn females and males were not significantly different (p>0.05). Results of osteocalcin, BAP, GH, IGF-1 and cortisol concentrations in serum of 90-day-old piglets are presented in Table 2. The obtained results did not reveal statistically significant differences in the evaluated parameters between the groups (p>0.05). Results of morphometric, densitometric and biomechanical evaluation of femur and tibia are shown in Table 3 and 4. Weight and length of femur and tibia were not significantly different between the groups of females and gonadectomised males (p>0.05). Determination of vBMD in the trabecular and cortical bone compartments of femur and tibia did not show significant gender-related differences (p>0.05). Assessment of vertical and horizontal diameters (both internal and external) of femur and tibia at mid-shaft showed very similar values of these variables in both the investigated groups of animals (p>0.05). Similary, the evaluation of geometrical properties of the mid-shaft of femur and tibia did not reveal statistically significant differences in

Table 1. Serum concentration of biochemical markers of bone metabolism and hormones in newborn female and male piglets of Polish Landrace breed

Investigated parameter	Males (n = 24)	Females (n = 24)
Osteocalcin (ng/mL)	23.8[a] ±3.2	22.7[a] ±2.2
Bone alkaline phosphatase activity (U/L)	26.1[a]±1.2	25.9[a] ±1.3
Growth hormone (ng/mL)	37.1[a] ±2.8	39.2[a] ±2.8
Insulin-like growth factor-1 (µg/L)	84.6[a] ±5.4	82.4[a] ±7.4
Cortisol (ng/mL)	328.0[a] ±25	338.0[a] ±20

[a]Identical superscript letters indicates lack of statistically significant differences between mean values in a row. The level of statistical significance was set at p-value < 0.05

Table 2. Serum concentration of biochemical markers of bone metabolism and hormones in 90-day- old female and gonadectomised male pigs of Polish Landrace breed

Investigated parameter	Males (n = 24)	Females (n = 24)
Osteocalcin (ng/mL)	4.86[a]±0.29	4.66[a]±0.18
Bone alkaline phosphatase concentration (µg/L)	279.0[a]±10.9	289.0[a]±14.4
Growth hormone (ng/mL)	3.04[a]±0.47	3.97[a]±0.52
Insulin-like growth factor-1 (µg/L)	48.8[a]±5.6	49.9[a]±7.9
Cortisol (ng/mL)	143.0[a]±13	161.0[a]±18

[a]Identical superscript letters indicates lack of statistically significant differences between mean values in a row. The level of statistical significance was set at p-value < 0.05

Table 3. Morphometric, densitometric and biomechanical properties of femur in female and gonadectomised male Polish Landrace pigs at the age of 6 months

Investigated parameter	Males (n = 133)	Females (n = 127)
Bone weight (g)	323.8[a]±2.8	317.5[a]±3.0
Bone length (mm)	198.0[a]±0.7	198.4[a]±0.8
Trabecular bone mineral density (g/cm^3)	1.411[a]±0.005	1.407[a]±0.005
Cortical bone mineral density (g/cm^3)	2.523[a]±0.012	2.534[a]±0.010
Vertical internal diameter (mm)	16.1[a]±0.1	16.4[a]±0.2
Vertical external diameter (mm)	26.7[a]±0.2	26.8[a]±0.2
Horizontal internal diameter (mm)	14.4[a]±0.1	14.6[a]±0.1
Horizontal external diameter (mm)	23.4[a]±0.1	23.7[a]±0.1
Cross-sectional area (mm^2)	309.7[a]±3.8	312.1[a]±3.8
Second moment of inertia (mm^4)	19 288[a]±425	19 754[a]±481
Mean relative wall thickness	0.656[a]±0.010	0.641[a]±0.009
Cortical index	39.16[a]±0.4	38.68[a]±0.4
Maximum elastic strength (N)	4765[a]±115	4627[a]±113
Ultimate strength (N)	6125[a]±116	5823 [a]±111

[a]Identical superscript letters indicates lack of statistically significant differences between mean values in a row. The level of statistical significance was set at p-value < 0.05

Table 4. Morphometric, densitometric and biomechanical properties of tibia in female and gonadectomised male Polish Landrace pigs at the age of 6 months

Investigated parameter	Males (n = 133)	Females (n = 127)
Bone weight (g)	208.2[a]±2.8	201.9[a]±2.3
Bone length (mm)	180.4[a]±0.7	179.2[a]±0.6
Trabecular bone mineral density of the proximal metaphysis (g/cm^3)	1.398[a]±0.006	1.389[a]±0.005
Trabecular bone mineral density of the distal metaphysis (g/cm^3)	1.570[a]±0.007	1.553[a]±0.008
Cortical bone mineral density (g/cm^3)	2.553[a]±0.007	2.534[a]±0.007
Vertical internal diameter (mm)	10.0[a]±0.2	9.8[a]±0.2
Vertical external diameter (mm)	17.9[a]±0.2	17.5[a]±0.2
Horizontal internal diameter (mm)	15.5[a]±0.2	15.1[a]±0.1
Horizontal external diameter (mm)	25.1[a]±0.1	24.8[a]±0.2
Cross-sectional area (mm^2)	230.3[a]±2.7	224.3[a]±3.8
Second moment of inertia (mm^4)	6626[a]±304	5963[a]±218
Mean relative wall thickness	0.745[a]±0.026	0.734[a]±0.015
Cortical index	41.4[a]±0.4	41.4[a]±0.7
Maximum elastic strength (N)	4693[a]±83	4787[a]±83
Ultimate strength (N)	6520[a]±96	6755[a]±103

[a]Identical superscript letters indicates lack of statistically significant differences between mean values in a row. The level of statistical significance was set at p-value < 0.05

This copy is for personal use only - distribution prohibited.

cross-sectional area, second moment of inertia, mean relative wall thickness and cortical index between the groups of females and orchidectomised males ($p>0.05$). Biomechanical properties of femur and tibia were not significantly differentiated between females and orchidectomised males since the values of maximum elastic strength and ultimate strength were comparable in both groups ($p>0.05$).

DISCUSSION

Growth hormone and IGF-1 are important factors of the somatrotropic axis stimulating systemic growth and development in animals and humans. Cortisol is the final agent of the hypothalamic-pituitary-adrenal axis, playing an important role in both the programming and regulation of growth and metabolic processes [23, 24]. Increased production of GH stimulates IGF-1 secretion that is an essential factor for longitudinal bone growth, stimulating the proliferation and differentiation of chondrocytes in the epiphyseal plate. Anabolic effects of IGF-1 on the skeleton is also related to trabecular and cortical bone formation resulting from proliferation and differentiation of osteoblasts, increased type I collagen synthesis, as well as BAP and OC production [25, 26]. Until attainment of whole body and skeletal maturity and acquisition of peak bone mass (PBM), the bone formation processes dominate over resorption and the somatotropic axis shows increased activity. Thus, in the presented study on pigs, the evaluation of GH, IGF-1, cortisol, OC and BAP was carried out. Obtained results, however, did not show significant differences of GH, IGF-1, cortisol, OC and BAP between the groups of females and orchidectomised males.

This observation is in accordance with the data obtained in humans where serum concentrations of GH and IGF-1 were evaluated in representative groups of girls and boys at mean age between 9-14 years of life. Serum concentrations of GH and IGF-1 obtained at 4 different Tanner stages in normal weight and obese children also did not show statistically significant differences between girls and boys [27]. In other studies performed on boys ($n = 579$) and girls ($n = 540$), serum concentration of IGF-1 was significantly different between the groups at 1st, 2nd and 4th Tanner stages. Investigation of both these cohorts at 3rd and 5th Tanner stages did not show statistically significant difference of IGF-1 concentrations between girls and boys. Furthermore, the evaluation of serum BAP concentrations showed significant differences between girls and boys only at 4th and 5th Tanner stages [28]. The results of a multicentre study on serum concentration of IGF-1 in 3,961 healthy humans aged between 1-80 years showed significant gender-related differences. Significantly higher values of IGF-1 concentration were stated in males when compared to age-matched females. The differences at the ages of 1-3 years of life reached nearly 20 μg/L, and were the highest between 11-13 years of life (ca. 70 μg/L). In subjects over 20 year of age, the gender-related differences reached the range of 6-26 μg/L, and were the smallest between the ages of 35-50 [29]. Studies on healthy children aged from 2 months to 18 years of life and evaluation of serum concentration of bone formation markers showed a significantly higher concentration of BAP in boys than in girls aged over 13. The measurement of OC concentration in the latter study did not show significant differences between girls and boys, which is in agreement with the presented study [30].

Studies on young (25-35 years) and older (65-75 years) women and men have shown gender-related differences in bone formation markers. Plasma BAP activity and OC concentrations reached significantly higher values in older women than in older men. Furthermore, the group of young women had a significantly lower OC level when compared to age-matched men; however, plasma activity of BAP was similar in both these groups [31]. Similar to our studies, reports on the measurement of serum GH concentration in an obese line of pigs have not shown significant gender-related differences from birth to 2 weeks of life. A significantly higher concentration of GH in serum was found in males at 3, 4 and 5 weeks of life when compared to females; the gender-related difference disappeared one week later. Furthermore, in that study, significant differences in serum IGF-1 between male and female piglets were not reported [32]. On the other hand, studies on Duroc pigs have shown that serum concentration of IGF-1 measured in pigs weighing 105 kg reached the greatest values in boars (159.3-256.4 ng/mL) than in gilts (101.1-146.4 ng/mL) and barrows (86.2-116.2 ng/mL), and the differences between all the groups were statistically significant [33]. Hormonal evaluation in castrates and gilts has not shown the effects of gender on plasma cortisol level at the age of 9 and 12 weeks of life. This is in agreement with the presented study. At slaughter age (6 months, 100 kg of body weight), the plasma concentration of cortisol was found to be differentiated between castrates and gilts only in NN Hal genotype, whereas lack of gender-related differences of this hormone was observed in Nn and nn animals [34]. Another report on intact males and gilts, where serum concentrations of cortisol, GH and IGF-1 were monitored between birth and 42 days of life, gender-related differences were stated only in overall GH concentrations. Significantly higher values were stated in males than in gilts (8.7 *versus* 6.8 ng/mL) [35].

The obtained results of bone properties evaluation in the presented study did not show significant differences between females and orchidectomised males. Bone weight, length, vBMD of the trabecular and cortical bones, vertical and horizontal diameters, cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength of femur and tibia reached very close values in castrates and gilts at slaughter age. These data are in contrast to results obtained on Wistar rats, where geometrical and biomechanical parameters of femur reached significantly higher values in adult intact males than in females. Bone mineral density in that study was significantly higher in females when compared to males [20]. Similar to our results in pigs, the final body weight attained significantly higher values in male rats than in females. Investigations of skeletal properties in 8-12-year-old girls ($n = 190$) and boys ($n = 186$) have revealed gender-related differences. Significantly higher geometrical traits of tibia, such as cross-sectional area, cortical bone area and diameter of medullary cavity, determined a higher mechanical endurance to acting forces in males than in females. However, vBMD of the cortical bone was significantly higher in females than in males, even though body weight, height and pelvic limb length were not differentiated between the groups [36]. Other reports on humans have not proved gender-related differences of vBMD from birth to puberty, which is in agreement with the data in the presented study [37, 38, 39, 40]. Extensive study of

the bones of pigs, such as femur, humerus, third and fourth metacarpal, third and fourth metatarsal, rib and thoracic vertebrae, has shown that the bending moment of bones from boars was comparable to that of bones from gilts or barrows. When expressed as force per unit area, the ultimate stress of bones from boars was less than that of bones from gilts or barrows. However, the ash content in bones was not gender-differentiated [41].

In conclusion, the presented study has shown a significantly higher growth rate and final body weight gain in orchidectomised males when compared to females. These differences were not associated with gender-differentiated serum concentrations of GH, IGF-1 and cortisol determined at slaughter age of 6 months, since the values of these indicators were comparable in both the investigated groups. Lack of significant differences of morphological, densitometric and biomechanical properties of femur and tibia between the groups was related to very similar levels of bone formation markers (OC and BAP), and the hormones determining growth, development and homeostasis maintenance of bones in the skeleton, such as GH, IGF-1 and cortisol. The data obtained provide novel physiological information on bone metabolism markers and regulators, as well as the properties of femur and tibia in female and gonadectomised male pigs. Thus, the results obtained in this study may be useful in experimental approaches for studying the effects of physiological, nutritional, pharmacological, toxicological and environmental factors on bone metabolism and the properties of the skeletal system in pigs.

Acknowledgments

This study was supported by Grant No. 2 P06Z 012 29 from Ministry of Education and Science in Warsaw, Poland.

REFERENCES

1. Ishimi Y, Yoshida M, Wakimoto S, Wu J, Chiba H, Wang X, Takeda K, Miyaura C. Genistein, a soybean isoflavone, affects bone marrow lymphopoiesis and prevents bone loss in castrated male mice. Bone. 2002; 31: 180-185.
2. Matsumoto C, Inada M, Toda K, Miyaura C. Estrogen and androgen play distinct roles in bone turnover in male mice before and after reaching sexual maturity. Bone. 2006; 38: 220-226.
3. Ross RW, Small EJ. Osteoporosis in men treated with androgen deprivation therapy for prostate cancer. J Urol. 2002; 167: 1952-1956.
4. Vanderschueren D, Vandenput L. Androgens and osteoporosis. Andrologia 2000; 32: 125-130.
5. Anderson FH, Francis RM, Peaston RT, Wastell HJ. Androgen supplementation in eugonadal men with osteoporosis: effects of six months' treatment on markers of bone formation and resorption. J Bone Miner Res. 1997; 12: 472-478.
6. Harada N, Utsumi T, Takagi Y. Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. Proc Natl Acad Sci. USA 1993; 90: 11312-1136.
7. Simpson ER, Maahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev. 1994; 15: 342-355.
8. Terashima M, Toda K, Kawamoto T, Kuribayashi I, Ogawa Y, Maeda T, Shizuta Y. Isolation of a full-length cDNA encoding mouse aromatase P450. Arch Biochem Biophys. 1991; 285: 231-287.
9. Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med. 1998; 339: 599-603.
10. Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med. 1997; 337: 91-95.
11. Kowalik S, Śliwa E, Tatara MR, Krupski W, Majcher P, Studziński T. Influence of alpha-ketoglutarate on mineral density and geometrical and mechanical parameters of femora during postnatal life in piglets. Bull Vet Inst Pulawy 2005; 49: 107-111.
12. Tatara MR, Brodzki A, Krupski W, Śliwa E, Silmanowicz P, Majcher P, Pierzynowski SG, Studziński T. Effects of α-ketoglutarate on bone homeostasis and plasma amino acids in turkeys. Poult Sci. 2005; 84: 1604-1609.
13. Tatara MR, Silmanowicz P, Majcher P, Krupski W, Studziński T. Influence of alpha-ketoglutarate on cortical bone atrophy after denervation of the humerus in turkey. Bull Vet Inst Pulawy 2005; 49: 113-116.
14. Andersen NK, Tatara MR, Krupski W, Majcher P, Harrison AP. The long-term effect of α-ketoglutarate, given early in postnatal life, on both growth and various bone parameters in pigs. J Anim Physiol Anim Nutr. 2008; 92: 519-528.
15. Harrison AP, Tygesen MP, Sawa-Wojtanowicz B, Husted S, Tatara MR. α-Ketoglutarate treatment early in postnatal life improves bone density in lambs at slaughter. Bone. 2004; 35: 204-209.
16. Tatara MR, Śliwa E, Krupski W. Prenatal programming of skeletal development in the offspring: effects of maternal treatment with ***β***-hydroxy-***β***-methylbutyrate (HMB) on femur properties in pigs at slaughter age. Bone. 2007; 40: 1615-1622.
17. Tatara MR, Tygesen MP, Sawa-Wojtanowicz B, Krupski W, Majcher P, Harrison AP. Bone development: the effect of short term alpha-ketoglutarate administration on long term mechanical properties of ribs in ram lambs. Small Rum Res. 2007; 67: 179-183.
18. Williams J, Abumrad N, Barbul A. Effect of a specialized amino acid mixture on human collagen deposition. Ann Surg. 2002; 236: 369-375.
19. Tatara MR. Neonatal programming of skeletal development in sheep is mediated by somatotrophic axis function. Exp Physiol. 2008; 93: 763-772.
20. Ferretti JL, Capozza RF, Mondelo N, Montuori E, Zanchetta JR. Determination of femur structural properties by geometric and material variables as a function of body weight in rats. Evidence of sexual dimorphism. Bone.1993; 14: 265-270.
21. Ferretti JL, Capozza RF, Mondelo N, Zanchetta JR. Interrelationships between densitometric, geometric and mechanical properties of rat femora: inferences concerning mechanical regulation of bone modelling. J Bone Miner Res. 1993; 8: 1389-1395.
22. Tatara MR, Krupski W, Majcher P, Studziński T. Long-term denervation of the humerus in turkeys as an experimental model for osteopenia. Poult Sci. 2005; 84: 718-722.
23. Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C. Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. J Clin Endocrinol Metab. 1998; 83: 135-139.
24. Fowden AL, Giussani DA, Forhead AJ. Endocrine and metabolic programming during intrauterine development. Early Hum Dev. 2005; 81: 723-734.
25. Rizzoli R, Bonjour J-P, Ferrari SL. Osteoporosis, genetics and hormones. J Mol Endocrinol. 2001; 26: 79-94.
26. Ueland T. Bone metabolism in relation to alterations in systemic growth hormone. Growth Horm IGF Res. 2004; 14: 404-417.
27. Falorni A, Bini V, Cabiati G, Papi F, Arzano S, Celi F, Sanasi M. Serum levels of type I procollagen C-terminal propeptide, insulin-like growth factor-I (IGF-I), and IGF binding protein-3 in obese children and adolescents: relationship to gender, pubertal development, growth, insulin, and nutritional status. Metabolism 1997; 46: 862-871.
28. Léger J, Mercat I, Alberti C, Chevenne D, Armoogum P, Tichet J, Czernichow P. The relationship between the GH/IGF-I axis and serum markers of bone turnover metabolism in healthy children. Eur J Endocrinol. 2007; 157: 685-692.
29. Brabant G, von zur Mühlen A, Wüster C, Ranke MB, Kratzsch J, Kiess W, Ketelslegers JM, Wilhelmsen L, Hulthén L, Saller B, Mattsson A, Wilde J, Schemer R, Kann P; German KIMS Board. Serum insulin-like growth factor I reference values for an automated chemiluminescence immunoassay system: results from a multicenter study. Horm Res. 2003; 60: 53-60.
30. Rauchenzauner M, Schmid A, Heinz-Erian P, Kapelari K, Falkensammer G, Griesmacher A, Finkenstedt G, Högler W. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. J Clin Endocrinol Metab. 2007; 92: 443-449.
31. Yan L, Prentice A, Zhou B, Zhang H, Wang X, Stirling DM, Laidlaw A, Han Y, Laskey A. Age- and gender-related differences in bone mineral status and biochemical markers of bone metabolism in Northern Chinese men and women. Bone. 2002; 30: 412-415.

This copy is for personal use only - distribution prohibited.

32. Buonomo FC, Klindt J. Ontogeny of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II) and IGF binding protein-2 (IGFBP-2) in genetically lean and obese swine. Domest Anim Endocrinol. 1993; 10: 257-265.
33. Suzuki K, Nakagawa M, Katoh K, Kadowaki H, Shibata T, Uchida H, Obara Y, Nishida A. Genetic correlation between serum insulin-like growth factor-1 concentration and performance and meat quality traits in Duroc pigs. J Anim Sci. 2004; 82: 994-999.
34. Nyberg L, Lundström K, Edfors-Lilja I, Rundgren M. Effects of transport stress on concentrations of cortisol, corticosteroid-binding globulin and glucocorticoid receptors in pigs with different halothane genotypes. J Anim Sci. 1988; 66: 1201-1211.
35. Carroll JA, Veum TL, Matteri RL. Endocrine responses to weaning and changes in post-weaning diet in the young pig. Domest Anim Endocrinol. 1998; 15: 183-194.
36. Ahamed Y. Sex-specific changes in bone structure and strength during growth: pQCT analysis of the mid-tibia. MS Thesis. British Columbia University, 2007.
37. Gilsanz V. Phenotype and genotype of osteoporosis. Trends Endocrinol Metab. 1998; 9: 184-190.
38. Gilsanz V, Gibbens DT, Roe TF, Carlson M, Senac MO, Boechat MI, Huang HK, Schulz EE, Libanati CR, Cann CC. Vertebral body density in children: effect of puberty. Radiology 1988; 166: 847-850.
39. Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med. 1991; 325: 1597-600.
40. Trotter M, Dixon BB. Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. Anat Rec. 1974; 179: 1-18.
41. Crenshaw TD, Peo ER, Lewis AJ Jr, Moser BD, Olson D. Influence of age, sex and calcium and phosphorus levels on the mechanical properties of various bones in swine. J Anim Sci. 1981; 52: 1319-1329.

This copy is for personal use only - distribution prohibited.