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Original article

The effect of obesity on the bone morphometry and histomorphometry in male and female Wistar rats

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Abstract

The study was undertaken to determine the effect of continuation or changes of the diet on the morphometry and histomorphometry of bone in male and female Wistar rats with experimentally induced obesity by high energetic diet. Sixty-four 90-day-old Wistar rats obtained from obese parents (16 male, 16 female) and control parents (16 male, 16 female) were used in this study. After 21 days of the baby period, rats were divided into four groups: obese rats fed with high energy feed (F/F), control rats fed with a standard diet (C/C), obese rats with changed diet from high energy diet to control diet (F/C) and control rats with changed diet from control diet to high energy diet (C/F). After 90 days of experimental feeding, the rats were sacrificed. Thereafter, body weight and the isolated humerus were measured and next, the histological stainings and counts were done. Our results revealed that change in the parent's diet from F to C in the female leads to increased bone growth length and reduction of body weight in female and male. Reverse diet changes (from C to F) lead to decreased bone length only in the female. Moreover, the continuation by offspring of both sexes with a high-energy diet contributes to a reduction in osteocytes, reduction in bone marrow cavity and cortical expansion, but a change in nutrition from parents' standard diet to high-energy diet leads to increase in osteocytes dimensions. The continuation of feeding with F diet promotes the accumulation of adipocytes in the bone marrow in female and male, and correction of nutrition from F to standard diet leads to a reduction in their number in the bone marrow compared to groups continuing feeding with high-energy diet.

Key words: bone tissue, histomorphometry, humerus, obesity, osteocytes, adipocytes, rats

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Introduction

Obesity nowadays is one of the most dangerous and the fastest growing civilization diseases in the world (James et al. 2004). According to World Health Organization (WHO), in 2014 more than 600 million adults were obese, it stood for about 13% of the world's adult population; still, 39% of adults were overweight. The situation is also disturbing in children and adolescents, as more than 41 million of them were obese or overweighted in 2016 (WHO 2018). Obesity is defined as abnormal fat accumulation in an organism that may cause health impairment (WHO 2000). Obesity may be conducive to increase a risk of the occurrence of type 2 diabetes, some types of cancer, cardiovascular diseases as well as a stroke, endocrinal disorders, osteoarthritis, and premature death worldwide (Goodpaster et al. 2005). Adipose tissue is not only a source of energy in the organism but it is also an endocrine organ (Fantuzzi 2005). However, depending on localization fat tissue plays a different role in the secretion of various levels of some substances (Kershaw and Flier 2004). For example, subcutaneous fat secretes more leptin and adiponectin (Fontana et al. 2007) than visceral fat, but visceral fat is the main source of some interleukins, like interleukin 6 (IL-6) (Lenchik et al. 2003). In addition, adipocytes produce other biologically active substances that play an important role in bone metabolism, like tumor necrosis factor α (TNF-α), tissue factor (TF), resistin, visfatin, as well as some enzymes connected with steroid hormones etc. (Skowrońska et al. 2009, Bieńko et al. 2016).

The relationship between fat and bone should be considered in different aspects. The first of them is the relationship between body weight and bone density. The second is connected with the relationship between bone marrow adipose tissue (MAT) and the age-associated bone loss (Deque 2008, Suchacki et al. 2016). Some studies have demonstrated that high body mass index (BMI) is protective against the development of osteoporosis and osteoporotic fractures in men and women. In slim people with a BMI lower than normal, weight loss is associated with low bone mineral density (BMD) (De leat et al. 2005, Villareal et al. 2006). On the other hand, obesity in childhood may lead to fragility fractures and may lead to early development of osteoporosis in adulthood (Abramowicz et al. 2013). Also, MAT is significant in bone turnover and bone marrow adipose tissue quantification could be useful in the diagnosis of osteoporosis (Justesen et al. 2001). Despite the fact that bone marrow (both red marrow and yellow marrow) plays an important role in the bone remodeling processes and formation of blood cells, its other metabolic roles are largely unknown (Gesta et al. 2007). Adipocytes located in a bone marrow are a source of energy as well as numerous substances. Likely, the size and quantification of the adipocyte in a bone marrow contribute to the regulation of bone metabolism. Significantly, the balance between bone and adipose tissue is determined by stromal cells differentiation into osteoblasts or adipocytes. Aging-related bone loss or osteoporosis are associated with an increase of adipogenesis in bone marrow (Parhami et al. 1999). Additionally, bone thickness, bone weight and also bone mineral content (BMC), BMD and the width of the marrow cavity – they are strongly connected with bone metabolism and the content of fat in the body (Cao 2011, Shapses and Sukumar 2012).

The aim of the study was to determine the effect of obesity programming on the morphology of the male and female Wistar rats. For this purpose, we chose a humerus as a reference bone, because we intended to examine if there were any morphological and histological changes in the humerus (belonging to the support type limb) in the offspring, under the influence of the continuation or change in the eating habits of the parents. Numerous studies have shown morphology and/or histology of tibiae or femur as references bones from pelvic limb, but there is no specific data concerning the limb bone of the thorax, which is the humerus.

Materials and Methods

Animals

The experiment on animals was carried out according to the guidelines of animal welfare of the World Organization for Animal Health and approved by the Second Local Animal Ethics Committee of Life Sciences in Lublin, Poland. Sixty-four 90-day-old Wistar rats obtained from obese parents (16 male, 16 female) and control parents (16 male, 16 female) were used in this study. After 21 days of baby period, rats were divided into four groups: obese rats fed with high energy feed (F/F), control rats fed a normal diet (C/C), obese rats with diet changed from high energy diet to control diet (F/C) and control rats with diet changed from control diet to high energy diet (C/F) and allowed water *ad libitum*. The high-energetic diet (F) contains 17.60 MJ/kg but control diet (C) contains 11.50 MJ/kg of energy. Composition of control and high-energetic diet is presented in Table 1.

Animals (8 males and 8 females in each group) were maintained at a constant temperature (22 ± 2°C), on a 12 hours light/dark cycle. After 90 days of the experiment, all the rats were sacrificed by an overdose of carbon dioxide and dislocation of the cervical vertebrae with spinal cord disruption. Body mass was

Table 1. Composition of control and high-energetic diet.

Ingredients	Control diet	High-energetic diet
Energy MJ/kg	11.50	17.60
	% Energy	
Carbohydrate	75	65
Protein	19	19
Fat	6	16

recorded and then the right and left humerus of each animal were dissected. The right humerus mass and length was measured with the use laboratory scale (AS.R. RADWAG 310.R2) and electronic caliper (HITEC 101-45, 0.01 mm Resolution) and then histological analysis were performed.

Histological Methods

Tissue sections were made following conventional methods and stained with Hematoxylin and Eosin (H&E). The specimens obtained from each group were fixed in 4% neutral buffered (pH = 7.4) formaldehyde solution for 24 h at 4°C and decalcified with ethylenediaminetetraacetic acid (EDTA) for histologic examination (Suvarna et. all 2012). After decalcification, specimens were rinsed in water and transferred to ammonia solution to neutralize acids. Next, the specimens were washed in tap water, dehydrated in serially graded ethanol solutions, defatted in methanol, and embedded in paraffin blocks by using standard procedures. After being embedded in paraffin, 10μm-thick sections were prepared and stained with H&E. Samples were rehydrated in a graded series of ethanol in each case related to the ethanol concentration of the first staining solution. Finally, sections were washed in 96% ethanol, xylene and mounted in DPX (Sigma-Aldrich, Germany) for morphological evaluation.

Morphological and morphometric analysis

Histological analysis consisted of two parts: qualitative (morphology) and quantitative (morphometric). The analysis of the histological sections was performed by a single examiner under blinded conditions with the aid of a light microscope Olympus CX41 in the transmitted mode at final magnifications of x4, x10, x20, x40 and x60. One section in each staining per subject was analyzed. Morphologic analysis of the parts was performed by photomicrography. Magnification depended on the need to visualize the desired structures and the following characteristics of each group were observed in bone tissue. Total cell counts were counted at an original magnification of x40 in 10 subsequent adjacent visual fields, representing the whole width of the non-union. All specimens were blinded for cell counts. Fat cells identified as empty oval spaces were enumerated under magnification x20 in ten consecutive microscopic fields of humerus metaphysis and an average number of cells per high power field (x20 magnification) of each group was calculated. With the help of the following criteria the different cell types were identified: osteoblasts, which are mononuclear cells, were counted if they lined up the external surface of bone trabeculae and the surface of Haversian canals. Osteocytes were counted if they were embedded in the mineralized bone matrix. Cells with two to fifteen nuclei in small resorptive excavations (Howship's lacunae) on the bone surface were counted as multinuclear osteoclasts. All parameters were measured in 10 fields/slide from 10 slides for each rat with a calibrated image analysis system that consisted of a computer equipped with morphometric software Cell D Soft Imaging System (SIS) with an attached digital camera Colorview IIIu (Soft Imaging System, Olympus). The analyses consisted of a descriptive analysis of the rat humerus. The following parameters were examined and assessed:

- cortical bone thickness: cortical thickness (measured from the periosteum to the endosteum) and the width of the marrow cavity in μm at three different points in each section was measured (Surve et. al 2001) (Fig. 1)
- the quantification of osteocytes: the number of osteocytes per mm^2 lacunae containing nuclei was counted in each section. The measurement of osteocytes concerned were: length (μm; the long axis of the soma), width (μm; the short axis of the soma), area of soma (μm^2) and cell diameter (μm) (Vashishth et. al 2000) (Fig.1)
- the number of adipocytes in the marrow cavity were counted in each section of rat humerus metaphysis.

Statistical analysis

The chi-square and One-way analysis of variance (ANOVA) tests were used to evaluate statistically the

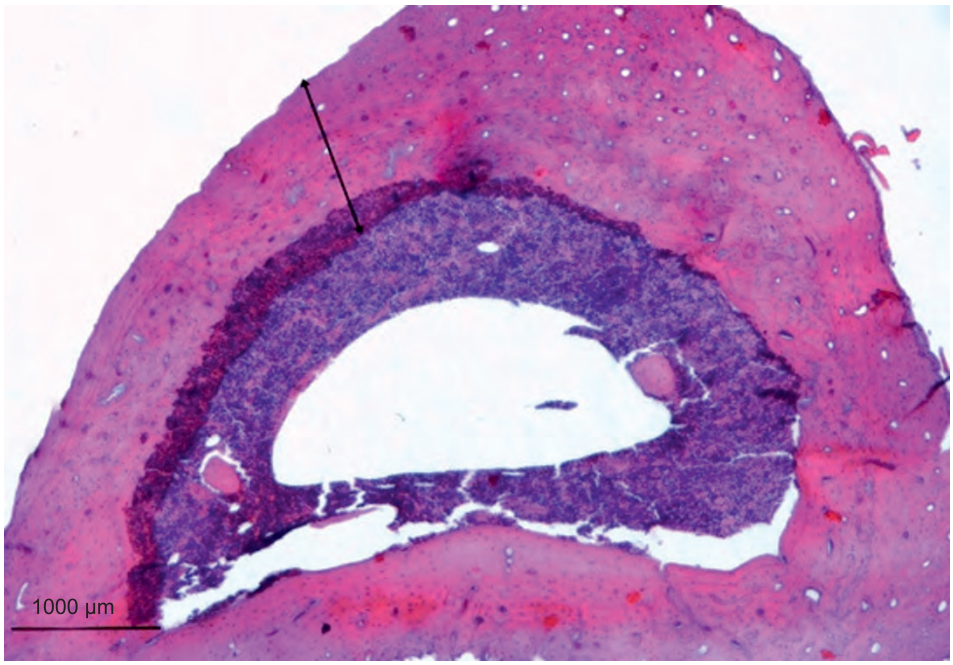

Fig. 1. Photomicrograph of the rat at the 90-day study humerus sections stained by H&E. The normal cortical bone thickness and numerous osteocytes, osteocyte lacunae and Volkman's canals. The arrow shows the width between the periosteum to the endosteum. Scale Bar=1000 μm

Table 2. Body mass and morphometric parameters of isolated humerus bones in males.

Male group	Body mass (g)	Bone length (mm)	Bone mass (g)
C/C	515 ± 16 a	29.92 ± 0.92 a	0.52 ± 0.01
F/F	573 ± 20 * a	28.84 ± 2.07 a	0.60 ± 0.03† a
F/C	461 ± 16 *# a	29.69 ±1.40 a	0.56 ±0.02
C/F	517 ± 15 a	28.12 ± 0.79 a	0.49 ± 0.01#

* vs. C/C ($p<0.05$). # vs. F/F ($p<0.05$). $ vs. F/C ($p<0.05$). † vs. C/F ($p<0.05$). a vs. female ($p<0.05$)

Table 3. Body mass and morphometric parameters of isolated humerus bones in females.

Female group	Body mass (g)	Bone length (mm)	Bone mass (g)
C/C	369 ±11 b	27.26 ± 0.67 b	0.52 ± 0.02
F/F	414 ± 16 * b	27.24 ± 2.03$ † b	0.48 ± 0.09 b
F/C	332 ±12 *# b	28.16 ±1.04*#† b	0.49 ± 0.03
C/F	365 ± 9 b	26.15 ± 1.79*#$ b	0.44 ± 0.01

* vs. C/C ($p<0.05$). # vs. F/F ($p<0.05$). $ vs. F/C ($p<0.05$). † vs. C/F ($p<0.05$). a vs. male ($p<0.05$)

results obtained from all groups. To detect significant differences between the individual experimental groups, significant ANOVAs were followed by post hoc Tukey test for multiple comparisons. Differences between groups were considered significant at p value <0.05. Analysis of significant differences was performed with the use of Statistica 13 software (StatSoft, Inc. Tulsa, USA)

Morphometric analysis

The following parameters were measured by computerized image analysis using a morphometric software Cell D Olympus Soft Imaging System (SIS).

Results

Analysis of the body mass as well as morphometric analysis of the humerus in males and females

The measurement of the isolated humerus of the rat males and females of both control and experimental groups allowed to determine average lengths of bones (mm) and average masses of bones (g). The above data together with the body mass are presented in Tables 2 and 3.

The statistically significant highest body mass was found in both male and female rats which carried on nourishment with high-energetic diet started in parents (10.1% and 10.9% in males and females, respectively) compared to the control group C/C (the group when nourishment changed from high energetic to standard one (F/C) ($p<0.05$). The lowest body mass was found in males (461 ± 16g) and females (332 ± 12g) coming from the parents fed with high-energetic diet for which the diet was changed to the control one (F/C) compared to the groups carrying on the standard parents' nourishment (C/C) as well as high-energetic diet (F/F), which was statistically proved ($p<0.05$). Moreover, remarkably lower values of the final body mass in females were found compared to those of corresponding experimental groups in females ($p<0.05$) (Tables 2 and 3).

The smallest (28.12 ± 0.79 mm) average length of the humerus bones was found in males whose parents were fed with the control diet and which obtained the high-energetic diet C/F after separation. The biggest average length of the humerus (29.92 ± 1.02 mm) was found in the male group C/C with no statistically significant differences between the groups. The largest statistically significant average mass of the humerus (0.49 ± 0.09 g) was found in males of the group F/F (vs. C/F; $p<0.05$) which was by 13.3% larger compared to the group C/C. The smallest average bone mass (0.44 ±0.01) was observed in males of the group C/F. It was smaller by 18.3% compared to the group F/F ($p<0.05$). The average mass of the humerus in males in the group F/C was (0.56 ± 0.02g) without statistically significant differences compared to the group CC and the other experimental ones (Table 2).

Table 3 presents the average body masses (g), average bone lengths (mm) and bone masses (g) in females. The greatest (28.16 ± 1.04 mm) average lengths of the humerus bones were found in the group F/C which was statistically the longest (vs. C/C, F/F, and C/F; $p<0.05$) and the longest by 3.2% compared to those in females from the group C/C. The smallest (26.15 ± 1.79 mm) statistically significant values of bone lengths were obtained in females from the group C/F (vs. C/C, F/F, and F/C; $p<0.05$). The smallest (0.44 ± 0.01g) average mass of humerus bones in females was observed in the group C/F which was lower by 13.3% and statistically significant compared to the group C/C ($p<0.05$). In the other female groups no statistically significant differences were found in the examined bones mass and their values were similar (Table 3).

Body mass and bone length in all-female groups were significantly smaller compared to those in males in the respective experimental groups. Only the bone mass in the female F/F group was much smaller compared to the respective male group ($p<0.05$).

Morphometric parameters of the humerus in males

The morphometric analysis (Figs. 2 and 3) showed that osteocytes in the group F/F possessed statistically lowest average length values (6.95 ± 0.24 μm) compared to the group C/C and F/C, and C/F ($p<0.05$). The statistically the lowest average values of osteocytes width (6.51 ± 0.11 μm) were found in rats in the experimental group F/C (vs. C/C, F/F, C/F; $p<0.05$) but the highest ones (9.32 ± 0.15 μm) in the male humerus in the group C/F (vs. C/C, F/F, F/C; $p<0.05$). The osteocytes surface area of the studied bones was statistically the lowest (31.24 ± 1.10 μm^2 and 30.03 ± 0.53 μm^2) being similar in the respective F/F and F/C groups compared to that of osteocytes in males from the group C/C ($p<0.05$). The highest average values of this parameter (44.05 ± 0.86 μm^2) were found in the humerus of the group C/F vs. C/C, F/F, F/C ($p<0.05$). The lowest statistically significant value of osteocytes diameter (8.38 ± 0.22 μm) was obtained in the group F/F but the highest one (10.47 ± 0.13 μm) in the group C/F ($p<0.05$) (Table 4).

The highest values of the marrow cavity (1509.40 ± 91.29 μm) were obtained in the group F/F but the lowest ones (1315.69 ± 8.83 μm) in the group F/C. No statistically significant differences were found between the experimental groups and the group C/C as well as in relations to each other (Table 4).

In males from the group F/F, the lowest average values of cortical bone thickness (370.5 ± 10.31 μm) were found, which were statistically significant compared to the group C/C ($p<0.05$). The other experimental groups (F/C and C/F) did not show statistically significant differences compared to the group C/C and in relations to each other. The average values of this parameter were insignificantly higher than in the group C/C (Table 4).

The largest statistically significant number of adipocytes (55.4 ± 1.10) was obtained in males from the group F/F. It was as much as 24.2% higher compared to that from the group C/C ($p<0.05$). A larger number

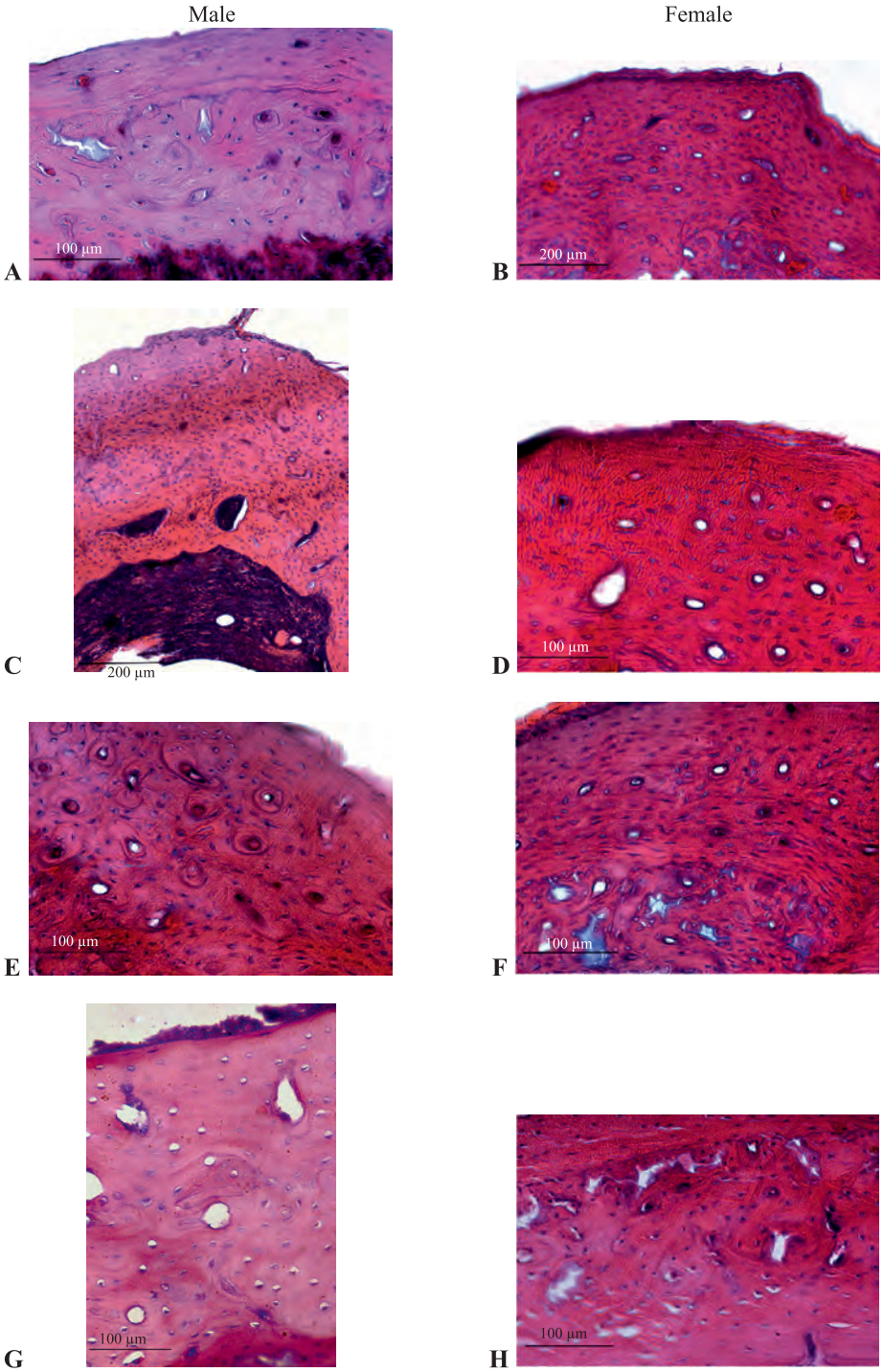

Fig. 2. Photomicrograph of a section of rat at the 90-day study humerus diaphysis compact bone stained by H&E showing blood vessels and osteocytes inside their lacunae. The endosteal surface of the cortical bone is smooth. A-B- Control/Control (A-Scale Bar=100 μm; B- Scale Bar=200 μm) C-D- Control/Fat (C-Scale Bar=200 μm; D- Scale Bar=100 μm); E-F- Fat/Control Scale Bar=100 μm; G-H- Fat/Fat Scale Bar=100 μm

(but statistically insignificant) of fat cells was found in the groups F/C and C/F compared to that in the red bone marrow in the C/C group despite the lack of statistically significant differences compared to the group C/C (Table 4).

Analysis of morphometric parameters of the humerus in females

The results of analysis of the effects of parents' continuation of nourishment habits (C/C and F/F) as well as their changes (C/F and F/C) by female offspring are presented in Table 5. The statistically significant largest

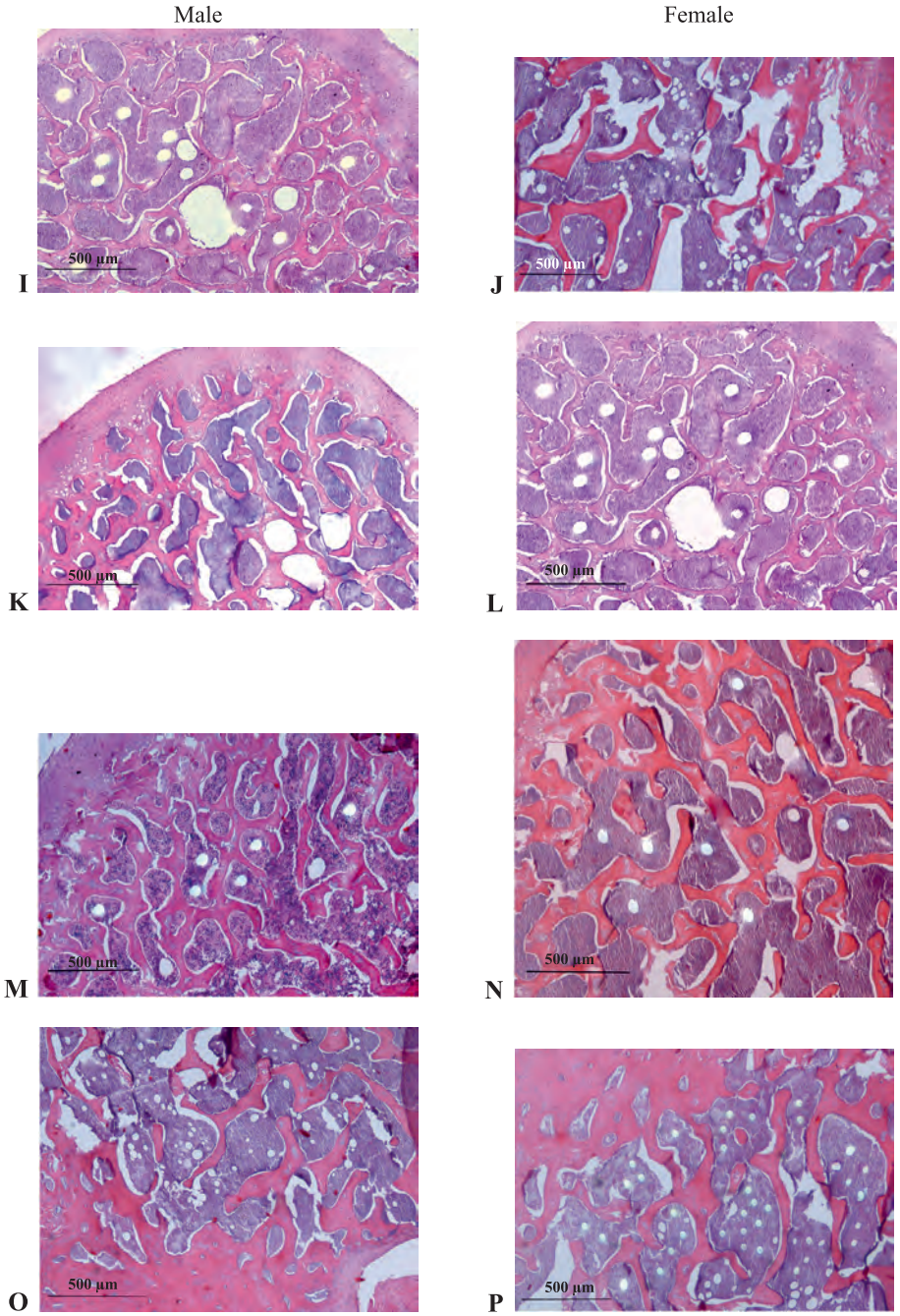

Fig.3. Photomicrograph of a longitudinal section of the rat at the 90-day study distal humerus metaphysis stained by H&E showing cancellous bone consisting of a network of bony trabeculae separated by interconnecting spaces containing islands and clusters of hematopoietic cells admixed with adipocytes. Osteocytes within their lacunae in between bone lamellae. I-J- Control/Control; K-L- Control/Fat; M-N- Fat/Control; O-P- Fat/Fat. Scale Bar=500 μm

length of osteocytes (8.79 ± 0.18 μm) was observed in females in the group C/F (vs. C/C, F/F, F/C; $p<0.05$) but the smallest one (6.77 ± 0.12 μm) in the group F/F (vs. F/C, C/F; $p<0.05$). The statistically significant smallest average values of osteocytes width (7.34 ± 0.12 μm) were found in the experimental group F/C (vs. C/F; $p<0.05$) but the largest ones (7.89 ± 0.15 μm) came from osteocytes of the bone in the females of the group C/F (vs. F/C; $p<0.05$). The osteocytes surface area was statistically the smallest (33.75 ± 1.99 μm^2) in the group F/F compared to the all other groups ($p<0.05$). The largest value of the osteocytes surface area (49 ± 3.06 μm^2) was obtained in the group C/F (vs. C/C, F/F, C/F; $p<0.05$). The largest statistically significant diameter of osteocytes (10.79 ± 0.62 μm) was found in females from the group C/F (vs. C/C, F/F, F/C; $p<0.05$) but the smallest (9.06 ± 1.00 μm) in females from the group F/F (vs. C/C, F/C, C/F; $p<0.05$).

The largest values of the diameter of marrow cavity

Table 4. Morphometric parameters of osteocytes, marrow cavity, the thickness of cortical bone as well as the number of adipocytes from the red bone marrow in males from the control and experimental groups.

Group	Osteocytes				Marrow cavity & cortical bone		Adipocytes
	Length (μm)	Width (μm)	Area (μm^2)	Diameter (μm)	Diameter of the marrow cavity (μm)	Width of the cortical bone (μm)	Number of cells
C/C	8.42±0.22	8.28±0.15	42.33±0.85	9.15±0.12	1328.62± 45.23	312.4± 21.32	42.00±1.25
F/F	6.95±0.24*†	7.16±014 *$†	31.24±1.10 *†	8.38±0.22†	1509.40± 91.29	370.5 ±10.31*	55.40±1.10*
F/C	8.22±0.17	6.51±0.11*#†	30.03±0.53 *†	9.26±0.17	1315.69±28.83	334.9± 14.01	50.20±1.20 #
C/F	8.49±0.13	9.32±0.15*#$	44.05±0.86 *#$	10.47±0.13 #	1461.75±39.62	349.0±11.54	49.40±1.00 #

* vs. C/C ($p<0.05$). # vs. F/F ($p<0.05$). $ vs. F/C ($p<0.05$). † vs. C/F ($p<0.05$).

Table 5. Morphometric parameters of osteocytes, marrow cavity, the thickness of humerus cortical bone and number of adipocytes in the red bone marrow in females from control and experimental group.

Group	osteocytes				Marrow cavity & cortical bone		adipocytes
	Length (μm)	Width (μm)	Area (μm^2)	Diameter (μm)	Diameter of the marrow cavity (μm)	Width of the cortical bone (μm)	Number of cells
C/C	7.36±0.24	7.55±0.22	39.27± 2.23	9.56 ± 0.97	1273.53±49.07	325.69±6.21	48.70± 3.50
F/F	6.77±0.12 $ †	7.51±0.16	33.75± 1.99 * $ †	9.06± 1.00 * $ †	776.24± 19.30 * $ †	390.63±7.03 * †	80.30±5.25* $ †
F/C	8.07±0.44 * # †	7.34±0.12†	39.07 ±2.36 # †	9.54± 0.89 # †	1086.32± 17.56#	408.78±9.58 * †	70.29±2.10* #
C/F	8.79±0.18 * # $	7.89±0.13$	49.00 ± 3.06 * # $	10.79±0.62 * # $	1253.37± 31.50#	343.38±9.21 # $	67.71±4.50* #

* vs. C/C ($p<0.05$). # vs. F/F ($p<0.05$). $ vs. F/C ($p<0.05$). † vs. C/F ($p<0.05$).

(1273.53 ± 49.07 μm) were obtained in females of the group C/C. The lowest statistically significant values of the marrow cavity diameter (776.24 ± 19.30 μm) were found in the group F/F (vs. C/C, F/C, C/F; $p<0.05$) but the highest (1253.37 ± 31.50 μm) in females of the group C/F (vs. F/F; $p<0.05$). The highest statistically significant average values of thickness (408.78 ± 9.58 μm) of the cortical bone were observed in females of the group F/C (vs. C/C, C/F; $p<0.05$) and the lowest (343.38 ± 9.21 μm) in the group C/F (vs. F/F, F/C; $p<0.05$) (Table 5).

In females of the group F/F, the highest statistically significant number (80.30 ± 5.25) of adipocytes (vs. C/C, F/C, C/F; $p<0.05$) was found. It was by 39.4% higher compared to the group C/C. The lowest statistically significant number of adipocytes (67.71 ± 4.50) was observed in the red bone marrow in females of the group C/F (vs. C/C, F/C; $p<0.05$) (Table 5).

Discussion

In the studies an attempt was made to evaluate the effects of standard (C/C) and high-energetic (F/F) nourishment continuation as well as the changes of parents' feeding habits (C/F or F/C) on morphometric parameters of the humerus in rats. The evaluation of changes in the organism after continuation or change of feeding started in rat parents was made after 10 weeks from the separation time. The final body mass in rats of both genders was statistically higher in the groups carrying on the high-energetic diet started by parents which is consistent with the data about the animals fed with the high-energetic diet reported in the literature (Cao et al. 2009, Fehrendt et al. 2014, Doucette et al. 2015). The change of F diet into C causes remarkable inhibition of fat tissue accumulation in the organism responsible for body mass increase in both gender rats. The total bone mass increases with the increasing body mass during organism growth as well as with the increasing body mass in adults. The studies on

mice by Cao et al. (2009) showed that after the 14-week high-fat diet no significant growth of tibia mass was observed. However, Tucker et al. (2002) proved that the high fat and carbohydrate contents diet favours reduction of bone mass in middle-aged people. The own studies showed a different character of changes due to which the males fed with the high-energetic diet had a larger bone mass compared to those whose diet was changed into the standard one. Such relation was not found in females which is in agreement with the results obtained by Cao et al. (2009). According to the studies by Bielohuby et al. (2010), the high-fat diet in young rats results in deterioration of bone growth in length, reduction in bone BMD and abatement of their mechanical properties. Only in rat females for which parents' feeding habits were changed from the high-energetic diet to the standard diet significant inhibition of bone growth in length was observed. In the females originating from the parents with induced obesity for which feeding was changed into the standard one, the length of the humerus increased indicating a favorable effect of balanced nourishment even in the offspring whose parents were characterized by nourishment-induced obesity.

The own studies did not reveal statistically significant changes in bone lengths affected by high-energetic feeding or changes of parents' feeding habits in male offspring. Similar results in mouse males offspring were obtained by Cao et al. (2009). Slight fluctuations of bone mass changes can be due to adipogenesis connected with the increased discharge of leptin from the fatty tissue and lower absorption of calcium from the food. This may result from consuming too much fat in the food dose (Sakata et al. 2003).

Continuation of high-energetic diet started in parents by offspring results in a reduction of morphometric parameters of osteocytes connected with their length, surface area width, and diameter. Statistically significant reduction of the marrow cavity diameter and increase in the width of cortical bone were found in the humerus in females and males compared to the control nourishment groups (C/C). Similar dependencies were obtained in mice by Fehrendt et al. (2014). The application of high-energetic diet increased the number and size of osteocytes compared to those of the control group. Moreover, it was proven that with the animal age, a larger fat tissue does not protect against degeneration changes in the bone tissue (Fehrendt et al. 2014). The greatest changes of morphometric parameters concerning the increase in the length, width, and diameter of osteocytes were found in females whose parents were fed in the control way and the diet was changed into the high-energetic one. Similar research results were obtained by Sakata et al. (2003). They proved that with the body mass increase due to a larger content of fat tissue, the increase of proliferation, as well as differentiation of osteoblasts and osteocytes, take place. There is also enlarged the surface area of osteocytes with the accompanied intensified mineralization of the bone tissue (Sakata et al. 2003).

As follows from the own studies continuation of high-energetic diet caused the increase of adipocytes number in the red bone marrow in males compared to those continuing the standard feed of their parents. Similar results were observed in mice when the high-fat diet was applied and the content of fat tissue increased in the bone marrow adipose tissue (MAT) (Scheller et al. 2016). Studying mice, Doucette et al. (2015) found that the high-fat diet resulted in MAT increase in the red bone marrow with the simultaneous lack of effect on the bone mass. Moreover, in females, each change of parents' feeding habits resulted in the accumulation of a larger number of adipocytes in the red bone marrow compared to the control group C/C. This may be connected with the hormonal metabolism and various concentrations of estrogens and androgens dependent on the gender which directly affects the metabolic processes in the bone marrow and bone tissue. The analysis of morphometric parameters in the red bone marrow proved that each change of feeding habits in offspring increased of adipocytes number compared to the offspring which carried on feeding on a high-energetic diet (F/F).

Conclusion

Comes from the studies, continuation of parents feeding habits by offspring using the high-energetic diet for weeks after separation causes an increase in the humerus mass only in males (by 13.3%). Moreover, it does not cause intensification or inhibition in the increase of bone length with the enlarged body mass compared to the control group. The change of parents' diet from F to C in females results in the larger growth of bone length but causes a reduction in the final body mass in both genders. The contrary change of parents' feeding habits (C/F) in females contributes to inhibition of humerus bone length increase with the comparable final body mass in the control group. In both females and males continuation of the diet (F) leads to a reduction of morphometric parameters regarding osteocytes as well as marrow cavity and broadening of the cortical bone. On the other hand, the change of parents' standard diet into the high-energetic one results in the increase of osteocytes morphometric parameters. In addition, in rat males and females continuation of high-energetic diet favours the accumulation of adipocytes number in the adipose tissue and

feeding correction from the high-energetic to the standard one results in the reduction of their number in the red bone marrow compared to the groups feeding on the F diet.

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