CHANGES OF BONE TISSUE IN PATIENTS WITH CHRONIC ARTERIAL INSUFFICIENCY OF LOWER LIMBS (DIABETES MELLITUS TYPE II) AFTER INSULIN AND NONINSULIN THERAPY

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Abstract. There was radiology study of condition of vascular bed and bone tissue in patients with chronic arterial insufficiency who had combined therapy (by insulin and metformin) and noninsulin therapy (by metformin or glimepiride or their combination). Direct connection was established between continued administration of insulin and cortex bone regeneration. Authors haven’t found an influence of metformin or glimepiride on bone tissue. Also the changes of bone mineral density in toes and calcaneus were directly proportional to the aggravation of ischemic changes of diabetic foot.

Keywords: bone mineral density, chronic arterial insufficiency, diabetes mellitus type II, insulin therapy, metformin.

Introduction
The problem of chronic arterial insufficiency (CAI) associated diabetes mellitus type II (DM2) is diagnosed in more than 75% of the population over the age of 50 years with this pathology [1]. The diabetic foot syndrome and atherosclerosis of the lower extremities take the leading place among peripheral obliteratorarteriopathy [2,3]. In the last few years in literature more and more works were devoted to an issue of complex changes in the tissue of the lower extremities with CAI associated with DM2 [4]. According to the scientific research, the problems caused by changes in bone will be important for the state, society and health care system [5,6].

Materials and methods
The study included 60 patients (all men) length of 175±10 cm, weight 76±13 kg with a diagnosis of CIA, caused by etiological factor of DM2. According to the level of transcutaneous oxygen pressure patients were divided into two groups of thirty people: 1) patients with with TcPO2 =30–50 mmHg; 2) patients with with TcPO2 ≤ 30 mmHg. These two groups were subdivided due to method of therapy also in two subgroups: 1) patients, who had combined therapy which included insulin (average daily dose – 36±8 units per day) and metformin (average daily dose – 1500±500 mg per day); 2) patients who had noninsulin therapy treatment by metformin (average daily dose – 1500±500 mg per day) or glimepiride (average daily dose – 4,5±1,5 mg per day) or their combination. It should be noted a metformin was temporary canceled before contrasting radiological examination. The surveillance was carried out by patients taking mentioned types of treatment for at least 5 years. Pursuant to the history of the disease patients were excluded who had bone fractures of the lower limbs and diseases that could lead to changes in bone mineral density. Groups of patients at the time of the study were reliably comparative on clinical data. For control, data on bone mineral density of the lower extremities in 15 healthy people were used. General clinical and laboratory examination was conducted to all patients after traumatic injuries. State of the vascular bed were studied during angiography and duplex sonography. The aver-
The age of examined patients was 55 ± 12 years, the control group (C) 36 ± 4.5 years.

Determination of bone mineral density of the femur neck was performed on the dual energy X-ray densitometer (technology – DEXA) (Fig. 1). Processing of the results of mineral density was performed according to the guidelines of the International Society for Clinical Densitometry (ISCD – 2003, 2005, 2007) [8]. The determined T-score is the amount of standard deviations above or below the average for peak of bone mass. The value of this criterion complied with the percent reduction of mineral density. T-score fluctuations within the range 1.0 SD refers to norm. A decrease of BMD between -1 and -2.5 SD is diagnosed like osteopenia (pre-osteoporosis condition). If the decrease of BMD exceeds -2.5 SD of peak indices (T-score ≥ -2.5 SD), results correspond to osteoporosis.

Also mineral density was determined by using multidetector computed tomography (MDCT) (“AquillonPrime” Toshiba). The examination included:

1) MDCT hip joint, slice thickness of 0.5 mm by Protocol HIP;
2) MDCT ankle joints, same thickness under the protocol Ankle / Foot.

In the study of bone studied the state of cortical and spongy substances. In determining bone mineral density (BMD) have used Hounsfield units (HU). The size of the aperture was the same in similar measurements, but various sizes for different areas. BMD was measured separately for cortical and spongy substances that were identified manually: 1) both in the neck of the femur (in axial projection); 2) plantar and dorum of both heel bones (in the axial and sagittal sections); 3) in the heads and shifts of all metatarsal bones (in axial and sagittal sections) by the method D. Robertson and others (2000). Also measured density subordinatess of tissues and for determined a Mönckeberg’s disease presence.

Analysis of the results of research conducted on workstations program VITREA.

In determining the bone mineral density (BMD) with SCT there were used relative values, so-called Hounsfield units (HU), and they were additionally substituted in the following equation to estimate apparent physical density of the bone (Est-vol.BMD, g·cm⁻³).

\[\text{Est-vol.BMD, g·cm}^{-3} = 0.114 + 0.916 \times 10^{-3} \text{ (HU)}\]

The non invasive assessment of vascular bed of lower limbs was made using duplex and doppler sonography (“Acuson Antares Premium Edition” Siemens). There were studied such quantitative parameters as: peak systolic velocity (PSV), volume flow (VF) and semiquantitative (relative) parameter – pulsatility index (PI), resistivity index (RI). Also the anatomy structure and the degree of artery’s local stenosis were assessed.

Additionally there were conducted multi-detector computed tomography angiography (MDCTA). The survey was carried out on the machine “Aquillon Prime” Toshiba (Japan) in modes: slice thickness of 0.5 mm X 32 mm X 64 0.5); pitch- standard (Pitch Factor 0813 /Helical Pitch 65,0), rotating tubes – 0.5 sec. For receiving qualitative MDCT scans using dual-head injector 30 ml 0.9% aqueous solution of sodium chloride were introduce intravenously to test the antecubital vein passability. Further nonionic monomer injected contrast medium Iohexolum 300 mg of iodine per milliliter (bolusof 1 mg per kilogram of body weight administered at a rate of 4.5 mL/s for 22 seconds. After entering the contrast vein washed with 30 ml of 0.9% aqueous solution of sodium chloride. Imaging of the aortic bifurcation began in achieving triggering density of 180 HU. According to the report “CT Vascular Femoral Run-off”, after a delay of 2 seconds at the valve, there was the first series of scans starting from the top of the diaphragmatic cupola and ending - below the knees. The second series of scans took place in 11.3 seconds after contrast administration from the beginning below the knee and ending below the ankle joint (including toes). There were assessed following vascular indicators: a total area of the arteries, a ratio “wall / lumen” of artery, an occlusion level, a presence of collaterals.
Statistical analyses were conducted by using data of application package «Statistica 6.0». The reliability of the results was provided by using standard diagnostic methods and definition the Student’s t-test.

**Results and conclusions**

For patients in the control group (C) BMD of the calcaneus ranged from 743.8±16.87 HU (M + m) for trabecular bone (TB) and 1308±112 HU (M + m) for cortical bone (CB). The following parameters for femoral neck, metatarsal bones and proximal phalanx of 1-st toe were established: femoral neck – TB: 650±56 HU (M + m); CB: 1195±44 HU(M + m); 1-st metatarsal bone: head – TB: 689±36,33 HU (M + m); CB: 1256±100 HU(M + m); diaphysis – CB: 1898±178,33 HU (M + m); 2-st metatarsal bone: head – TB: 655±65 HU (M + m); CB: 1290±56 HU (M + m); proximal phalanx of 1-st toe – TB: 540±45 HU (M + m); CB: 1307±67 HU (M + m).

Hemodynamic parameters during ultrasonographic examination were: in anterior tibial artery PSV was 21.0±5.6 cm/sec, PI= 0.8±0.4; RI=0.52±0.6; VF corresponded to 47.4±1.5 ml/min. In posterior tibial artery PSV=22.6±7.8 cm/sec; PI was 0.7±0.1; RI= 0.5±0.2 VF corresponded to 42.34±6.1 ml/min. The spectrum form of blood flow remained systolic peak. 4 patients (26.6%) had thickening and increase in echogenicity distal artery walls due to calcification, diffuse thickening of the complex “intima-media” to complete loss of differentiation in the layers.

During MDCTA-scans additionally was established 9 patients had stenosis in iliac-femoral segment (6- unilateral; 3- bilateral); 6 in femoral-popliteal segment (1- unilateral; 2- bilateral); 3 – in peripheral segment (1- unilateral; 2- bilateral).

The second group had non-insulin therapy, including treatment by metformin or glimepiride or their combination and the level TePO2 = 41±5 mmHg. The index T-score is -0.5, the average value mineral density is 1.067±0.098 g/cm², M±SD. BMD of the femoral neck ranged from 655.8±56 HU (M + m) for TB and 882±22 HU (M + m) for CB; for calcaneus – TB: 767±57 HU (M + m); CB: 977±57 HU (M + m). The following parameters for metatarsal bones were established: 1-st metatarsal bone: head – TB: 578±65 HU (M + m); CB: 1245±46 HU (M + m); diaphysis – CB: 1834±53 HU (M + m); 2-st metatarsal bone: head – TB: 556±65 HU (M + m); CB: 1345±55 HU (M + m); diaphysis – CB: 1723±45 HU (M + m); 3-st metatarsal bone: head – TB: 444±34 HU (M + m); CB: 1310±145 HU (M + m); diaphysis – CB: 1650±44 HU (M + m); 4-st metatarsal bone: head – TB: 446±78 HU (M + m); CB: 1181±31 HU (M + m); diaphysis – CB: 1388±56 HU (M + m); 5-st metatarsal bone: head – TB: 488±12 HU (M + m); CB: 1110±88 HU (M + m); diaphysis – CB: 1290±56 HU (M + m); proximal phalanx of 1-st toe – TB: 540±45 HU (M + m); CB: 1307±67 HU (M + m).
HU (M + m); diaphysis – CB: 1256±87 HU (M + m); 5-st metatarsal bone: head – TB: 377±43 HU (M + m); CB: 812±34 HU (M + m); diaphysis – CB: 901±78 HU (M + m); proximal phalanx of 1-st toe – TB: 512±12 HU (M + m); CB: 811±12 HU (M + m).

Hemodynamic parameters during ultrasonographic examination were: in anterior tibial artery PSV was 23.0±4.5 cm/sec, PI = 0.7±0.3; RI = 0.51±0.3; VF corresponded to 43.3±1.2 ml/min. In posterior tibial artery PSV = 20.3±4.5 cm/sec; PI was 0.8±0.3; RI = 0.4±0.3 VF corresponded to 44.2±4.5 ml/min. The spectrum form of blood flow remained systolic peak. 3 patients (20%) had thickening and increase in echogenicity distal artery walls due to Mönckeberg medial calcific sclerosis, diffuse thickening of the complex “intima-media” and a losing of differentiation in the layers.

During MDCTA-scans additionally was established 7 patients had stenosis in iliac-femoral segment (5 – unilateral; 2 – bilateral); 4 – in femoral-popliteal segment (2 – unilateral; 2 – bilateral); 4 – in peripheral segment (1 – unilateral; 3 – bilateral).

The third group had combined insulin-metformin therapy and the level TcPO2 = 23±5 mmHg. Some patients in this group were prepared for further amputations toe in the clinic. The index T-score is -0.5, the average value mineral density is 1.003±0.043 g/cm², M ± SD. BMD of the femoral neck ranged from 754.8±55 HU (M + m) for TB and 891±42 HU (M + m) for CB; for calcaneus – TB: 588±78 HU (M + m), CB: 891±91 HU (M + m). The following parameters for metatarsal bones were established: 1-st metatarsal bone: head – TB: 301±21 HU (M + m); CB: 723±33 HU (M + m); diaphysis – CB: 1200±44 HU (M + m); 2-st metatarsal bone: head – TB: 514±35 HU (M + m); CB: 1226±87 HU (M + m); diaphysis – CB: 1592±56 HU (M + m); 3-st metatarsal bone: head – TB: 478±87 HU (M + m); CB: 1312±43 HU (M + m); diaphysis – CB: 1314±98 HU (M + m); 4-st metatarsal bone: head – TB: 404±32 HU (M + m); CB: 922±32 HU (M + m); diaphysis – CB: 1321±11 HU (M + m); 5-st metatarsal bone: head – TB: 211±12 HU (M + m); CB: 711±43 HU (M + m); diaphysis – CB: 856±12 HU (M + m); proximal phalanx of 1-st toe – TB: 312±32 HU (M + m); CB: 567±32 HU (M + m). Proximal phalanx of 1-st toe – TB: 312±32 HU (M + m); CB: 567±32 HU (M + m).

Hemodynamic parameters during ultrasonographic examination were: in anterior tibial artery PSV was 5.7±2.5 cm/sec, PI = 0.37±0.05; RI = 0.24±0.07; VF corresponded to 10.3±3.2 ml/min. In posterior tibial artery PSV = 4.8±4.7 cm/sec; PI was 0.33±0.001; RI = 0.19±0.05 VF corresponded to 9.54±3.4 ml/min. Blood flow spectrum shape was characterized by the absence of acute systolic peaks, had smooth character with a low systolic and high diastolic components. 4 patients (26.6%) had changes of artery walls due to Mönckeberg medial calcific sclerosis, diffuse thickening of the complex “intima-media” and an absence of differentiation in the layers.

During MDCTA-scans additionally was established 7 patients had stenosis in iliac-femoral segment (3 – unilateral; 2 – bilateral); 4 – in femoral-popliteal segment (3 – unilateral; 1 – bilateral); 6 – in peripheral segment (1 – unilateral; 5 – bilateral).

The fourth group had non-insulin therapy, including treatment by metformin or glimepiride or their combination and the level TcPO2 = 17±6 mmHg. Some patients in this group were prepared for further amputations toe in the clinic. T-score is at -1.3-1.5, the average value mineral density 0.906±0.033 g/cm², M±SD, corresponds to osteopenia. BMD of the femoral neck ranged from 655.8±51 HU (M + m) for TB and 805±44 HU (M + m) for CB; for calcaneus – TB: 534±12 HU (M + m), CB: 701±34 HU (M + m). The following parameters for metatarsal bones were established: 1-st metatarsal bone: head – TB: 233±41 HU (M + m); CB: 545±55 HU (M + m); diaphysis – CB: 877±45 HU (M + m); 2-st metatarsal bone: head – TB: 315±44 HU (M + m); CB: 1001±30 HU (M + m); diaphysis – CB: 988±34 HU (M + m); 3-st metatarsal bone: head – TB: 478±87 HU (M + m); CB: 1312±43 HU (M + m); diaphysis – CB: 1314±98 HU (M + m); 4-st metatarsal bone: head – TB: 404±32 HU (M + m); CB: 922±32 HU (M + m); diaphysis – CB: 1321±11 HU (M + m); 5-st metatarsal bone: head – TB: 211±12 HU (M + m); CB: 711±43 HU (M + m); diaphysis – CB: 856±12 HU (M + m); proximal phalanx of 1-st toe – TB: 312±32 HU (M + m); CB: 567±32 HU (M + m).
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Hemodynamic parameters during ultrasonographic examination were: in anterior tibial artery PSV was 4.7±1.6 cm/sec, PI = 0.21±0.04; RI = 0.19±0.04; VF corresponded to 8.3±4.2 ml/min. In posterior tibial artery PSV = 3.9±5.8 cm/sec; PI was 0.22±0.02; RI = 0.14±0.04 VF corresponded to 8.56±3.7 ml/min. Blood flow spectrum shape was characterized by the absence of acute systolic peaks, had smooth character with a low systolic and high diastolic components. 3 patients (20%) had changes of artery like third group.

During MDCTA-scans additionally was established 4 patients had stenosis in iliac-femoral segment (2 – unilateral; 2 – bilateral); 4 – in femoral-popliteal segment (3 – unilateral; 1 – bilateral); 11 – in peripheral segment (5 – unilateral; 6 – bilateral).

The estimated data have shown the decreasing in bone mineral density in patients with non-insulin therapy are gradually according to the circulatory disorders’ appearance and decreasing of TcPO2 level (Fig.2). A decline in BMD is more expressed for bones located more distally, namely the foot bones compared to the femoral bone that can be explained by aggravation of the CAI in the distal parts of the leg (the diabetic foot syndrome).

It should be noted the difference of received data in patients with insulin therapy and non-insulin treatment. In first ones there were higher parameters of mineral density of cortical part of bones. At the same time, the level of trabecular component’s density was low. The mineral density of cortical and trabecular bones were decreased equally in patients with noninsulin therapy. Our results correlate with received data of laboratory study of model of diabetic rats of A.K. Picke (Germany, 2016), where it was revealed that insulin treatment increases cortex bone mass, but absolutely hasn’t effect on the state of spongy material. Dynamic histomorphometry has confirmed low levels of bone formation in trabecular tissue and periosteal layer of the cortex, that in part was later increased in the treatment by insulin [8]. Also it should take into account a duration of insulin therapy to reliably assert its influence on bone regeneration.

Additionally such factor as obliterative atherosclerosis which is always present in patients with diabetic angiopathy causes changes of bone mineral density (Fig.3). That’s why assessing of bone tissue condition should be complemented by study of vascular bed.

The dual-energy densitometry of femoral necks has shown low sensitivity and specificity in patients with level TcPO2 = 30–50 mmHg (61% and 59% respectively). Higher parameters of sensitivity and specificity were in patients with noninsulin therapy and level TcPO2< 30 mmHg (85% and 78% respectively).

This quantitative analysis improves the quality and objectification of diagnostic peripheral artery diseases. There is a direct correlation between the data of CAI and MDCT of foot bones (sensitivity – 99,2%, specificity – 92,6%).

Conclusions

The proposed technique of quantitative analysis of BMD allows to improve prognosis of the postoperative and rehabilitation period for patients in whom amputation cannot be turned away and, most importantly, it allows to evaluate fully the bone tissue. Respectively it will more accurately determine the connection between insulin therapy and bone regeneration.

References


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**Fig. 1.** Dual-energy X-ray densitometry of femoral neck.
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