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Bone mineral density and vitamin D status in war veterans after lower limb amputation

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The study aimed to assess bone mineral density (BMD) and vitamin D status in war veterans after unilateral lower limb amputation (ULLA). Methods. In the case-control study, 64 men aged 20–54 years were examined and divided into two groups: healthy subjects without any diseases or conditions affecting bone metabolism (control group) and men who received ULLA (study group). The analysis was performed depending on the presence and level of amputation. BMD was measured by two energy X-ray absorptiometry, and vitamin D status was assessed by serum dihydroxyvitamin D (25(OH)D) levels. Results. Significantly lower BMD values were found in the femoral neck and the hip of the amputated ($p = 0.00002$ and $p = 0.0002$, respectively), but not in the contralateral side or lumbar spine in amputees compared with controls. Significantly worse BMD indices were found in the femoral neck and the hip in men with transfemoral amputation compared with those with transtibial amputation at the level of the amputation, but not the contralateral side. BMD of the femoral neck of the amputated side significantly correlated with the duration of the post-amputation period ($r = -0.47$; $p = 0.01$). Significantly lower serum level of 25(OH)D was found in amputees compared to controls (25.9 ± 4.8) and (32.0 ± 9.8) ng/ml; $p = 0.002$). 50 % of the control group and 81.25 % of the study group had low side of 25(OH)D. Conclusions. The results demonstrated BMD changes in subjects after ULLA, a significant relationship between BMD and the duration of the post-amputation period, and a high proportion of vitamin D deficiency and insufficiency, which should be taken into account when planning rehabilitation measures in this category of patients.

Мета. Оцінити мінеральну щільність кісткової тканини (МЩКТ) та статус вітаміну D у військовослужбовців після односторонньої ампутації нижньої кінцівки (НК). Методи. Обстежено 64 чоловіків віком 20–54 років, поділених на 2 групи: здорові особи без будь-яких захворювань і станів із визначеним впливом на метаболізм кісткової тканини (контрольна група) та потерпілі після ампутації однієї НК (досліджувана група). Аналіз проводили залежно від наявності й рівня ампутації. Вимірювання МЩКТ здійснювали за допомогою двофотонної рентгенівської абсорбціометрії, статус вітаміну D оцінювали за сироватковим рівнем ди-гідроксिवітаміну D (25(OH)D). Результати. Виявлено достовірно нижчі показники МЩКТ шийки стегнової кістки (ШСК) та проксимального відділу стегнової кістки (ПВСК) ампутованої кінцівки ($p = 0,00002$ та $p = 0,0002$, відповідно), проте не контрлатеральної НК чи хребта в ампутованих осіб порівняно з показниками контролю. Зафіксовано достовірно гірші показники МЩКТ ШСК та ПВСК у чоловіків із трансфеморальною ампутацією порівняно з особами з транстібіальною на рівні ампутованої, проте не контрлатеральної НК. МЩКТ ШСК ампутованої НК достовірно корелювала з тривалістю постампутаційного періоду ($r = -0,47$; $p = 0,01$). Виявлено достовірно нижчі показники сироваткового рівня 25(OH)D в осіб із ампутацією порівняно з контролем ($25,9 \pm 4,8$) та ($32,0 \pm 9,8$) нг/мл; $p = 0,002$). Низькі рівні 25(OH)D мали 50 % осіб контрольної та 81,25 % досліджуваної групи. Висновки. Результати дослідження продемонстрували погіршення стану кісткової тканини на тлі односторонньої ампутації НК, достовірний зв'язок між МЩКТ і тривалістю постампутаційного періоду та високу частку дефіциту й недостатності вітаміну D, що слід ураховувати під час планування реабілітаційних заходів у цієї категорії хворих. Ключові слова. Ампутація нижньої кінцівки; мінеральна щільність кісткової тканини; 25(OH)D; ДРА; остеопороз.

Keywords. Lower-limb amputation; bone mineral density; 25(OH)D; DXA; osteoporosis

Introduction

The full-scale Russian invasion of Ukraine over the past three years has led to an unprecedented increase in the number of combat gunshot and mine-explosive injuries among both military personnel and civilians. Among these injuries, limb amputations (LAs) occupy a special place, which not only disrupt the integrity of the musculoskeletal system, but are also characterized by profound physiological, psychoemotional, and social consequences for the injured individuals and society as a whole. Important complications of amputations are post-traumatic stress disorder, phantom pain, infectious lesions, and post-immobilization osteoporosis, which impedes adequate rehabilitation of patients and causes the development of low-traumatic fractures in the long term [1–2].

Unfortunately, there is limited recent literature on the development of post-immobilization osteoporosis and its complications in amputees [3–6], and the available information on the impact of amputation on the rate of disease progression is fragmentary and does not fully reflect its real consequences for the health of injured people. Most of the existing studies or systematic reviews on post-immobilization osteoporosis focus on the features of bone loss in astronauts [7, 8] and people with chronic diseases that lead to prolonged immobilization [9, 10], and there are very few high-quality scientific studies on the comprehensive assessment of bone tissue in people after LA. Therefore, further observations of the state of bone tissue in this category of injured people can help to identify the features of its loss, develop personalized approaches to the prevention and treatment of post-immobilization osteoporosis, and also reduce the number of complications due to prolonged rehabilitation and disability. Studying this issue will contribute to the formation of new protocols for physical therapy and drug correction taking into account anatomical and functional changes after amputation. The results will have not only clinical, but also humanitarian and strategic value for the post-war recovery of the country, the reintegration of veterans into society and the formation of an effective military medicine system in the future. Purpose: to assess bone mineral density (BMD) and vitamin D status in servicemen after unilateral amputation of the lower limb.

Material and methods

To achieve this goal, we conducted a study on the basis of the Superhumans Center, Lviv Territorial Medical Association Clinical Hospital for Planned

Treatment, Rehabilitation and Palliative Care, and the State Institution “D. F. Chebotaryov Institute of Gerontology of the National Academy of Medical Sciences of Ukraine” (Kyiv) in June–August 2024, a case-control study was conducted with the participation of 64 men aged 20–54 years (mean age 35.0 ± 8.3).

Two groups were selected for analysis: healthy men without any diseases and conditions with a certain impact on bone metabolism (control group, $n = 32$, mean age (34.9 ± 9.0) years) and individuals who had one lower limb amputation as a result of military operations and were in the “Superhumans” center for the purpose of primary or secondary prosthetics (study group, $n = 32$, mean age (35.1 ± 7.8) years). In the future, for analysis, men in the second group were divided into two subgroups: A — individuals with transfemoral amputation and B — with transtibial amputation.

The study was conducted in compliance with the requirements and provisions of the Helsinki Declaration on Human Rights (2000), the Council of Europe Convention on Human Rights and Biomedicine (1997), the Fundamentals of Ukrainian Legislation on Health Care (1992), and the current national ethical standards for conducting clinical research. The study was approved by the local ethics committee of the State Institution “D. F. Chebotaryov Institute of Gerontology of the National Academy of Medical Sciences of Ukraine” (Protocol No. 4 dated 20.06.2024). All participants provided written informed consent to participate in it. The study employed general clinical, instrumental and laboratory research methods.

Measurement of the main anthropometric indicators (height and body weight) with calculation of body mass index (BMI) was carried out using a calibrated stationary height meter “Seca202”, RP-200 and medical scales “Zdorovya” in the morning, on an empty stomach, in a calm state after urination, in light clothing, without shoes and hats. The results of height measurement were recorded with an accuracy of 1 cm, body weight — up to 0.1 kg.

In the individuals of the study group, the adjusted body weight (weight (corrected)) was also calculated, which reflects the approximate value of the “full” weight in a patient without an amputation using the formula:

$$\text{weight (corrected)} = \text{weight (actual)} - P,$$

where weight (actual) is the patient's body weight (kg) without taking into account the prosthesis; P is the fraction of total body weight lost due to amputation

(in decimal format, e. g. $0.059 = 5.9\%$). For amputation of one limb below the knee joint it is $\sim 5.9\%$ ($p = 0.059$), above 16% , full foot $\sim 1.5\%$ [11].

BMI was calculated using the standard formula ($\text{BMI} = \text{body weight (kg)} / \text{height (m)}^2$) for the control group and a similar but adapted formula with the adjusted body weight index (adjusted body weight) depending on the level of LA (adjusted BMI = adjusted body weight (kg) / height (m)²) for the men in the study group.

Bone mineral density was measured using the two-photon X-ray absorptiometry (DXA) method using two DISCOVERY densitometers with automatic calculation of the Z-score using densitometer software. The indicators were measured at the level of the proximal femur (PF) and its neck in the amputated and contralateral limbs, as well as at the level of the lumbar spine (LS) L_I–L_{IV}. The study was performed in the morning by two qualified specialists. Preparation and positioning of the subjects, autocalibration and quality control of densitometers using a QA phantom were performed in accordance with the manufacturer's requirements.

Laboratory studies included measurement of the level of dihydroxyvitamin D (25(OH)D) in blood serum by electrochemiluminescent immunoassay (ECLIA) using an automated analyzer Cobas e411 (Roche Diagnostics) using original Roche reagents. Venous blood sampling of the subjects was performed in the morning on an empty stomach, followed by centrifugation (at 2,000 rpm for 10 min to obtain serum) and analysis.

Statistical analysis was performed using the "Statistika 10.0" Copyright© StatSoft, Inc. 1984–2001 software, Serial number 31415926535897. The nature of the distribution of the results was determined by the Shapiro-Wilk criterion. Under normal distribution conditions, data were presented as mean and standard deviation ($M \pm SD$), under non-normal distribution conditions, as median (Me) and interquartile range [LQ–UQ]. Relationships between indicators were assessed using Pearson or Spearman correlation analysis depending on the nature of their distribution. Differences between quantitative indicators of the studied groups were assessed using Student's t-test for unrelated samples and Mann-Whitney U Test, differences between percentage indicators were assessed using χ^2 and were considered significant at $p < 0.05$.

Results

The analysis of the results showed that the subjects did not differ in age ($t = 0.11$, $p = 0.91$), body weight

(81.4 ± 13.9) in the control and (77.1 ± 13.8) kg in the study groups, respectively, $t = 1.2$; $p = 0.22$), adjusted body weight ((81.4 ± 13.9) and (88.7 ± 16.8) kg, respectively, $t = 1.9$; $p = 0.07$) and BMI ((25.2 ± 3.6) and (25.0 ± 4.1) kg/m²; $t = 1.1$; $p = 0.82$), although the individuals of the study group had significantly lower height indicators ((175.6 ± 5.2) and (179.6 ± 6.3) cm, respectively; $t = 2.7$; $p = 0.008$) and higher values of adjusted body weight ((28.7 ± 5.0) and (25.2 ± 3.6) kg/m², respectively, $t = 3.2$; $p = 0.002$).

Traumatic amputation in patients of the study group was confirmed at the level of the lower leg ($n = 21$) or thigh ($n = 11$). The duration of the post-amputation period in the examined patients of this group was from 1.5 to 27 months and was, on average, 5.0 (4.0–7.0) months. It did not differ depending on the level of amputation (trans-tibial (5.0 (3.5–6.0) months or transfemoral (6.0 (4.0–7.0) months; in $Z = 1.28$; $p = 0.20$). Also, patients in the study group did not differ among themselves in terms of age ($t = 0.62$; $p = 0.54$), height ($t = 0.34$; $p = 0.73$), body weight ($t = 0.68$; $p = 0.50$) and BMI ($t = 0.85$; $p = 0.40$), although men with transfemoral amputation had significantly higher adjusted body weight ($t = 2.11$; $p = 0.04$) and BMI ($t = 2.20$; $p = 0.04$).

The study of risk factors for low BMD found that among 65.6% of the subjects in the study group and 31.3% of the control group smoked ($\chi^2 = 7.57$; $p = 0.006$). Previous high-traumatic fractures were reported by 18.8 % of the subjects and 15.6 % of the control group. Concomitant chronic respiratory, cardiovascular, and gastrointestinal diseases were reported by 31.3 % of the subjects and 18.8 % of the control group ($\chi^2 = 1.22$; $p = 0.24$).

Analysis of DXA indicators (Table 1) in the subjects, depending on the presence of traumatic amputation, revealed significantly lower BMD data in the subjects in the study group compared to control values at the level of the femoral neck (FN) and the PF of the amputated limb ($t = 4.7$; $p = 0.00002$ and $t = 4.0$; $p = 0.0002$, respectively). In contrast to the above, we did not find any significant differences in BMD at the level of the FN and PF of the limb contralateral to the amputation ($t = 0.2$; $p = 0.84$ and $t = 1.0$; $p = 0.33$, respectively). BMD at the level of the LS tended to differ but did not reach significant values ($t = 1.8$; $p = 0.07$, Table 1).

Low BMD of the FN and PF ($Z\text{-index} \leq -2\text{ SD}$) at the level of the amputated limb was found in 42 and 39 % of cases in the study group and in no patient at the level contralateral to the LA, while in the control

group, low BMD ($Z\text{-index} \leq -2$ SD) was not recorded in any man at either the FN or PF levels.

Evaluation of the results of DXA in the study group depending on the level of amputation (Table 2) revealed significantly worse indicators in men with transfemoral amputation compared to those with transtibial amputation at the level of the amputated, but not contralateral to the amputation limb. We did not obtain any significant differences in BMD of the LS depending on the level of amputation.

BMD of the FN (Fig. 1) of the amputated limb significantly correlated with the duration of the post-amputation period ($r = -0.47$; $p = 0.01$). Similar relationships at the level of the PF of this limb were less pronounced ($r = -0.35$; $p = 0.06$) and absent at the level of the LS ($r = -0.09$; $p = 0.64$) and the proximal part of the contralateral. Assessment of the rela-

tionship between BMD indices of the FN of the amputated limb and the duration of the post-amputation period in patients in the study group depending on the level of amputation (transfemoral or transtibial) revealed a statistically significant relationship in individuals with amputation at the thigh level ($R = -0.78$; $p = 0.008$), but not at the lower leg level ($R = -0.20$; $p = 0.40$). Analysis of serum 25(OH)D levels in the subjects depending on the presence of lower limb amputation revealed significantly lower values in the study group compared to the control group ((25.9 ± 4.8) and (32.0 ± 9.8) ng/ml; $t = 3.2$; $p = 0.002$). Low serum 25(OH)D levels were found in 50 % of the control group and 81.25 % of the subjects in the study group. Vitamin D deficiency was observed in 6.25 % of men in the study and control groups, 75 and 43.75 % had vitamin D deficiency, and

Table 1

Dual-energy X-ray densitometry measurements in patients based on lower limb amputation status

Indicator / Group	Control group	Study group	t	p
BMD of the LS, g/cm ²	1.06 ± 0.11	1.00 ± 0.11	1.8	0.07
Z-score of the LS, SD	-0.31 ± 1.05	-0.71 ± 1.03	1.5	0.13
BMD of the FN of the contralateral limb, g/cm ²	0.87 ± 0.14	0.87 ± 0.12	0.2	0.84
Z-score of the FN of the contralateral limb, SD	-0.06 ± 0.98	-0.12 ± 0.83	0.3	0.78
BMD of the PF of the contralateral limb, g/cm ²	1.00 ± 0.20	1.04 ± 0.13	1.0	0.33
Z-score of the PF of the contralateral limb, SD	0.07 ± 0.81	0.18 ± 0.83	0.6	0.58
BMD of the FN of the amputated limb, g/cm ²	0.87 ± 0.14	0.66 ± 0.20	4.7	0.000020
Z-score of the FN of the amputated limb, SD	-0.06 ± 0.98	-1.61 ± 1.48	4.8	0.000010
BMD of the PF of the amputated limb, g/cm ²	1.00 ± 0.20	0.78 ± 0.22	4.0	0.000200
Z-score of the PF of the amputated limb, SD	0.07 ± 0.81	-1.51 ± 1.46	5.2	0.000003

Note. SD — sigma deviation.

Table 2

Dual-energy X-ray densitometry indicators in the examined patients depending on the level of lower limb amputation

Indicator / Group	Subgroup A	Subgroup B	t	p
BMD of the LS, g/cm ²	0.97 ± 0.11	1.02 ± 0.12	1.0	0.31
Z-score of the LS, SD	-1.02 ± 0.98	-0.56 ± 1.05	1.2	0.25
BMD of the FN of the contralateral limb, g/cm ²	0.85 ± 0.11	0.87 ± 0.13	0.5	0.65
Z-score of the FN of the contralateral limb, SD	-0.31 ± 0.80	-0.02 ± 0.84	0.9	0.38
BMD of the PF of the contralateral limb, g/cm ²	1.03 ± 0.07	1.04 ± 0.15	0.3	0.75
Z-score of the PF of the contralateral limb, SD	0.08 ± 0.46	0.23 ± 0.96	0.5	0.64
BMD of the FN of the amputated limb, g/cm ²	0.47 ± 0.12	0.76 ± 0.16	5.0	0.000030
Z-score of the FN of the amputated limb, SD	-3.11 ± 0.87	-0.86 ± 1.10	5.6	0.000005
BMD of the PF of the amputated limb, g/cm ²	0.59 ± 0.18	0.88 ± 0.17	4.3	0.000200
Z-score of the PF of the amputated limb, SD	-2.81 ± 1.21	-0.86 ± 1.11	4.4	0.000100

Notes: A — amputation of the lower limb at the thigh level, B — at the shin level.

18.75 and 50 % had normal serum 25(OH)D levels. However, we did not find any significant differences ($t = 0.42$; $p = 0.68$) in the level of 25(OH)D depending on the level of amputation. In the case of transfemoral amputation, it was (25.4 ± 3.84) ng/ml, and in the case of transtibial amputation, it was (26.2 ± 5.21) ng/ml.

Discussion

Due to the Russian invasion of Ukraine, the number of injuries and amputations of limbs has increased dramatically in recent years, both among the civilian population and among military personnel. LA is a complex surgical procedure that has not only important pathophysiological and anatomical consequences, but also significantly affects psychological health and quality of life of patients. One of the critical aspects that often remains underestimated is the impact of amputation on bone health. The loss of a limb leads to dramatic changes in mechanical stress, bone metabolism and overall functioning of the body. However, to date, studies on the impact of amputations on bone health are fragmentary and do not fully reflect the real consequences of war for the health of military personnel. This makes it impossible to plan the necessary medical measures, early diagnosis of post-immobilization osteoporosis and effective rehabilitation of patients.

Loss of sufficient mechanical load is a major factor in the development of post-immobilization osteoporosis following amputation. According to Wolff's Law, formulated by the German anatomist Julius Wolff in the 19th century, bone tissue constantly adapts to the loads acting on it, ensuring an adequate rate of bone remodeling [12]. The presence of increased mechanical load leads to an increase in bone

strength, its absence, on the contrary, causes progressive bone loss and the development of post-immobilization osteoporosis. A decrease in mechanical stress on the bone, as occurs after amputation or during prolonged bed rest, leads to an imbalance of remodeling processes with a predominance of bone resorption.

Post-immobilization osteoporosis, which develops as a result of inactivity (immobilization, prolonged bed rest, paralysis, and amputation), is one of the most powerful factors leading to rapid bone loss [13–15], which exceeds that even in women in the first years after menopause [16]. Post-immobilization osteoporosis involves various pathophysiological components, including changes in bone tissue cells such as reduced osteoblast activity, increased osteoclast activity, and disrupted osteocyte function. These factors result in a higher rate of bone resorption and alterations to bone microarchitecture, such as thinning of trabeculae and fewer connections between them, which contribute to decreased bone strength and a greater risk of fractures. Additionally, local blood circulation and neural regulation may be impaired, further promoting bone demineralization. To date, the effect of amputation on BMD indicators has not been studied sufficiently. Studies and systematic reviews have shown reduced BMD in individuals after amputation [4–6, 17–19]. Most observations are confirmed in the residual limb (stump), and some of them in the preserved limb, which indicates a bilateral effect of amputation. According to scientists, changes in BMD in the contralateral limb are the result of modifications in walking patterns and body weight distribution of the patient. Although the healthy limb is mostly subjected to increased load to compensate for the lost function, it may be insufficient to maintain optimal BMD, or a decrease in overall physical activity leads to systemic effects.

A study by D. A. Bembien et al. [4] demonstrated a rapid and marked loss of bone strength in individuals with traumatic amputations within the first 6 months after surgery, which was not recovered within 12 months after resuming walking. The reduction in regional femoral BMD at the level of the amputated limb was 11–15 %. The study confirmed not only the loss of trabecular but also cortical bone. Similarly, J. H. Flint et al. [5] showed a significant loss of BMD after combat amputation of the lower limb, emphasizing that the traumatic nature of the surgery may additionally influence these processes. In a retrospective case-control study [5] of 156 lower limb amputation patients (121 unilateral, 35 bilateral), 42 % had a lower Z-score (-1.2 ± 1.0 SD) in bilateral amputation patients compared with unilateral ampu-

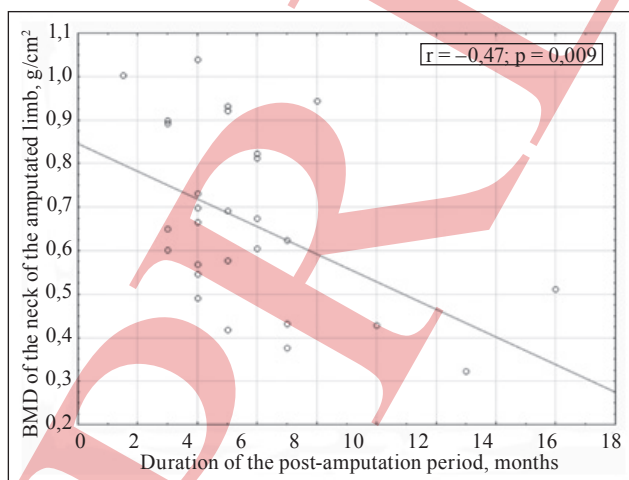


Figure. Relationship between BMD of the femur on the side of amputation and the duration of the post-amputation period in patients in the study group

tation patients (-0.6 ± 1.1 SD, $p = 0.005$). In addition, a significant difference in BMD between the intact and amputated limbs was found in unilateral amputation patients (odds ratio (OR) -1.0 ; 95 % CI -1.1 to -0.8 ; $p < 0.001$). Important factors for low BMD were the long period before resumption of walking [OR = 1.39; 95 % CI: 1.003–1.93; $p = 0.048$] and a higher level of amputation (OR = 7.27; 95% CI: 3.21–16.49; $p < 0.001$).

The important significance of the level of amputation (transfemoral or transtibial) on BMD obtained in our study was also confirmed by V. D. Sherk et al. [17], which the authors attribute to the features of the changed load redistribution.

The results of our investigation also coincide with the data of another recently published cohort study ADVANCE involving 153 male military personnel from the United Kingdom with lower limb amputees compared with the control group [6]. The authors found a significant decrease in BMD of the femoral neck compared to controls (t-score -0.08 SD vs. -0.42 SD; $p = 0.0001$). This decrease was significant only at the level of the femoral neck of the amputated limb ($p = 0.0001$) and was greater for individuals with transfemoral versus transtibial amputations ($p < 0.001$), with no differences in BMD of the lumbar spine. An important conclusion of the authors of the article is that BMD loss in amputees is mechanical, not systemic, and is caused by altered physical load on the limbs. Therefore, local strategies to stimulate bone turnover, in particular through adequate physical rehabilitation, may be effective in the recovery of individuals with amputees.

However, the idea of local bone loss at the level of the amputated limb is not shared by all researchers. W. Ngo et al. [18] in their review note that amputation can cause systemic adaptations in the entire musculoskeletal system, including changes in muscle strength, balance and walking patterns. This may indirectly affect the BMD of other parts of the skeleton, in particular due to a general decrease in physical activity or a change in load distribution. It is obvious that in order to compensate for the lost function of the amputated limb, the healthy one is often subjected to increased but abnormal load, which can lead to changes in BMD in it or, conversely, to a local increase in density depending on individual movement patterns and the use of the prosthesis.

In view of the above, the results of a retrospective cohort study of 44 British male veterans of World War II with major unilateral lower limb amputations [19] (mean age 73.0 years, and at the time of amputation — 26 years (17–57 years)) are interesting.

Transfemoral amputations were in 34% of patients, transtibial — in 66 %. BMD on the amputated side of the femoral neck, trochanter and Ward's triangle was significantly lower than on the contralateral limb ($p < 0.0001$). The t-score was, respectively, -2.26 SD on the amputated and -1.10 SD on the healthy limb ($p < 0.00001$). Given the long post-amputation period in the patients of this observation, it becomes obvious that there is no recovery of BMD in the amputated limb even long after amputation. It is obvious that low BMD in the amputated limb can be a significant factor in low-trauma fractures in this category of individuals. Another important factor that affects bone remodeling and the risk of fractures is vitamin D deficiency and insufficiency [20]. According to the results of a recent study in Ukraine, the average level of total vitamin D in serum in men aged 20–40 years is 30.1 ng/ml [21]. Studies of the status of this vitamin in amputees demonstrate a high proportion of its deficiency or insufficiency. Thus, according to É. Smith et al. [22] 68.8 % of men with lower limb amputees had vitamin D deficiency, 10.4 % had its insufficiency. Lower percentages of vitamin deficiency and insufficiency were demonstrated in the study by D. Bemben et al. [4] in subjects in the early post-amputation period in the period before the prosthesis was installed (respectively, 12 % of cases had its deficiency and 25 % had its insufficiency). The results of our observation showed a lower serum level of vitamin D in amputees compared to control values, but there were no significant differences in groups depending on the level of amputation. Low serum 25(OH)D values were found in 81.25 % of men after amputation (vitamin D deficiency was 6.25 %, insufficiency was 75 %), which may have a negative impact on bone tissue remodeling and the rate of its loss. Thus, the results of the study indicate that lower limb amputation is a significant risk factor for the development of post-immobilization osteoporosis. It is obvious that the loss of mechanical load on the stump bones leads to rapid demineralization of bone tissue, while changes in movement patterns and the general level of activity may cause a less pronounced decrease in BMD in the contralateral limbs, increasing the risk of osteoporosis and fractures. Understanding these mechanisms is critical for developing effective prevention and rehabilitation strategies. A comprehensive approach that includes early mobilization, individualized exercise programs [23], adequate nutritional support, and, when necessary, pharmacological treatment is key to minimizing the negative consequences of amputation on bone health and improving functional outcomes for patients.

Our observation has a number of limitations. These include its design (a single-center case-control study), the small number of subjects and the inclusion of only male patients with unilateral lower limb amputation, and the large range of time from amputation to examination (1.5–27 months).

Conclusions

The results of the study demonstrated a significant decrease in BMD of the femoral neck in men after unilateral amputation at the level of the amputated limb compared with the indicators of healthy men and the absence of significant differences on the contralateral side to the amputation. BMD indicators correlated with the duration of the post-amputation period and were lower in subjects with transfemoral amputation compared with indicators after transtibial.

After unilateral amputation of the lower limb, 81.25 % of cases had low levels of 25(OH)D in the blood serum (vitamin D deficiency — 6.25 %, insufficiency — 75), which may have a negative impact on the development of osteoporosis and low-traumatic fractures in the long term after amputation.

Conflict of interest. The authors declare no conflict of interest.

Prospects for further research. It is obvious that due to the growing consequences of the Russian invasion of Ukraine and the increase in the number of people with injuries and amputations, high-quality multicenter prospective studies using modern instrumental and laboratory methods are needed to study in detail the state of bone tissue and the characteristics of its loss in this category of patients.

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References

- Kaur, Y., Cimino, S. R., Albarico, M., Mayo, A. L., Guilcher, S. J., Robinson, L. R., Hanada, E., & Hitzig, S. L. (2020). Physical function outcomes in patients with lower-limb amputations due to trauma: A systematic review. *JPO journal of prosthetics and orthotics*, 33(2), 88–95. <https://doi.org/10.1097/jpo.0000000000000313>
- Esfandiari, E., Yavari, A., Karimi, A., Masoumi, M., Soroush, M., & Saeedi, H. (2018). Long-term symptoms and function after war-related lower limb amputation: A national cross-sectional study. *Acta orthopaedica et traumatologica turcica*, 52(5), 348–351. <https://doi.org/10.1016/j.aott.2017.04.004>
- Jin, S., An, C. H., Jeong, H. Y., Choi, W., Hong, S., Song, H. K., Kim, H. S., Lee, Y. K., Kang, H. J., Ahn, D., & Yang, H. (2024). Importance of bilateral hip assessments in unilateral lower-limb amputees: A retrospective review involving older veterans. *Journal of clinical medicine*, 13(14), 4033. <https://doi.org/10.3390/jcm13144033>
- Bemben, D. A., Sherk, V. D., Ertl, W. J., & Bemben, M. G. (2017). Acute bone changes after lower limb amputation resulting from traumatic injury. *Osteoporosis international*, 28(7), 2177–2186. <https://doi.org/10.1007/s00198-017-4018-z>
- Flint, J. H., Wade, A. M., Stocker, D. J., Pasquina, P. F., Howard, R. S., & Potter, B. K. (2014). Bone mineral density loss after combat-related lower extremity amputation. *Journal of orthopaedic trauma*, 28(4), 238–244. <https://doi.org/10.1097/bot.0b013e3182a66a8a>
- McMenemy, L., Behan, F. P., ... & Kaufmann, J. (2023). Association between combat-related traumatic injury and skeletal health: bone mineral density loss is localized and correlates with altered loading in amputees: the Armed Services Trauma Rehabilitation Outcome (ADVANCE) Study. *Journal of bone and mineral research*, 38(9), 1227–1233. <https://doi.org/10.1002/jbmr.4794>
- Coulombe, J. C., Senwar, B., & Ferguson, V. L. (2020). Space-flight-induced bone tissue changes that affect bone quality and increase fracture risk. *Current osteoporosis reports*, 18(1), 1–12. <https://doi.org/10.1007/s11914-019-00540-y>
- Harris, L. R., Jenkin, M., & Herpers, R. (2022). Long-duration head down bed rest as an analog of microgravity: Effects on the static perception of upright. *Journal of vestibular research*, 32(4), 325–340. <https://doi.org/10.3233/ves-210016>
- Saleh, I., Akbar, A., Hasan, H. S., Yulisa, N. D., & Aprilya, D. (2025). Clinical characteristics and bone mineral density score in post-stroke neuromuscular deficit. *Journal of clinical medicine research*, 17(2), 119–124. <https://doi.org/10.14740/jocmr6070>
- Zheng, X., Qi, Y., Zhou, H., Kang, H., Tong, Y., & Bi, L. (2021). Bone mineral density at the distal femur and proximal tibia and related factors during the first year of spinal cord injury. *International journal of general medicine*, 14, 1121–1129. <https://doi.org/10.2147/ijgm.s297660>
- Osterkamp, L. K. (1995). Current perspective on assessment of human body proportions of relevance to amputees. *Journal of the american dietetic association*, 95(2), 215–218. [https://doi.org/10.1016/s0002-8223\(95\)00050-x](https://doi.org/10.1016/s0002-8223(95)00050-x)
- Chen, J., Liu, C., You, L., & Simmons, C. A. (2010). Boning up on Wolff's law: Mechanical regulation of the cells that make and maintain bone. *Journal of biomechanics*, 43(1), 108–118. <https://doi.org/10.1016/j.jbiomech.2009.09.016>
- Rolvien, T., & Amling, M. (2021). Disuse osteoporosis: Clinical and mechanistic insights. *Calcified tissue international*, 110(5), 592–604. <https://doi.org/10.1007/s00223-021-00836-1>
- Grygorieva, N., Dedukh, N., Parubets, M., & Bystrytska, M. (2022). Disuse (post-mobilization) osteoporosis: Literature review and clinical case series. *Pain, joints, spine*, 12(3), 94–107. <https://doi.org/10.22141/pjs.12.3.2022.335>
- Alexandre, C., & Vico, L. (2011). Pathophysiology of bone loss in disuse osteoporosis. *Joint bone spine*, 78(6), 572–576. <https://doi.org/10.1016/j.jbspin.2011.04.007>
- Buettmann, E. G., Goldscheitter, G. M., Hoppock, G. A., Friedman, M. A., Suva, L. J., & Donahue, H. J. (2022). Similarities between disuse and age-induced bone loss. *Journal of bone and mineral research*, 37(8), 1417–1434. <https://doi.org/10.1002/jbmr.4643>
- Sherk, V. D., Bemben, M. G., & Bemben, D. A. (2008). BMD and bone geometry in Transtibial and Transfemoral amputees. *Journal of bone and mineral research*, 23(9), 1449–1457. <https://doi.org/10.1359/jbmr.080402>
- Ngo, W., Finnerty, C., Finco, M., Holley, B., & Menegaz, R. A. (2022). Additive effects of diabetes and lower-limb amputation on osteoarthritis with comparison to diabetic and healthy controls. *The FASEB journal*, 36(S1). <https://doi.org/10.1096/fasebj.2022.36.s1.r3505>
- Kulkarni, J., Adams, J., Thomas, E., & Silman, A. (1998). Association between amputation, arthritis and osteopenia in British male war veterans with major lower limb amputations. *Clinical rehabilitation*, 12(4), 348–353. <https://doi.org/10.1191/026921598672393611>
- Lips, P., & Van Schoor, N. M. (2011). The effect of vitamin D on bone and osteoporosis. *Best practice & research clinical endocrinology & metabolism*, 25(4), 585–591. <https://doi.org/10.1016/j.beem.2011.05.002>

21. Grygorieva, N., Solonenko, T., & Musiienko, A. (2023). Vitamin D deficiency during the COVID-19 pandemic and war in Ukraine. *Pain, joints, spine*, 13(1), 7–14. <https://doi.org/10.22141/pjs.13.1.2023.352>
22. Smith, É., Comiskey, C., Carroll, Á., & Ryall, N. (2011). A study of bone mineral density in lower limb amputees at a national prosthetics center. *JPO Journal of prosthetics and orthotics*; 23(1): 14–20. <https://doi.org/10.1097/jpo.0b013e318206dd72>
23. Behan, F. P., Bull, A. M., Beck, B. R., Brooke-Wavell, K., Müller, R., Vico, L., Isaksson, H., Harvey, N. C., Buis, A., Sherman, K., Jefferson, G., Cleather, D. J., McGregor, A., & Bennett, A. N. (2024). Developing an exercise intervention to minimise hip bone mineral density loss following traumatic lower limb amputation: *A Delphi study. British journal of sports medicine*, 58(21), 1251–1257. <https://doi.org/10.1136/bjsports-2024-108721>

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BONE MINERAL DENSITY AND VITAMIN D STATUS IN WAR VETERANS AFTER LOWER LIMB AMPUTATION

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