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Variations in the Amino Acid Composition of Human Bone Tissue

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Abstract

With the use of HPLC technique, studies on the amino acid composition of human bone tissue within the age range of 30–79 years were performed. Gender-features concerning the qualitative and quantitative amino acid content have been revealed. It is demonstrated that the clinical course of coxarthrosis does not lead to significant changes in amino acid composition of human bone tissue.

Key words: HPLC, bone tissue, chemical composition, coxarthrosis, cluster analysis

INTRODUCTION

Bones constitute a solid basis of a mammalian organism, including human beings. Human skeletal system takes an active part in the work of the musculoskeletal system and metabolic processes those provide the homeostasis of a living organism [1, 2]. Such a function of the bone tissue is provided by the morphological structure and the complicated multicomponent composition comprising organic matter (protein collagen, non-collagenous proteins, lipids, *etc.*) and inorganic matter (calcium phosphate). The phosphates of calcium and collagen impart to bones the mechanical strength, hardness, rigidity, elasticity and a high resistance with respect to compressive forces. The structural chemical units of the substances of protein nature are presented by amino acids those are often involved in the metabolic processes of organisms. Meanwhile, the literature mainly describes the structural organization of collagen, whereas the amino acid composition of human bone tissue is poorly considered [3–5].

It is known that the bone vital activity is based on the two interrelated processes such as bone formation that consists in the mineralization of the collagen matrix, and the destruction of the bone material formed [6]. These processes occur continuously throughout entire human life being called bone remodelling [7, 8]. The violation thereof leads to developing different kinds of osteoarticular disorders.

Currently, the diagnosis and correction of such states represents one of socially important and incompletely solved problems. So, there is still no consensus concerning the changes in the organic composition occurring with aging and different bone diseases.

The purpose of this work consisted in determining the qualitative and quantitative amino acid content in human bone under coxarthrosis, as well as in revealing the features of the amino acid composition thereof within people age range from 30 to 79 years.

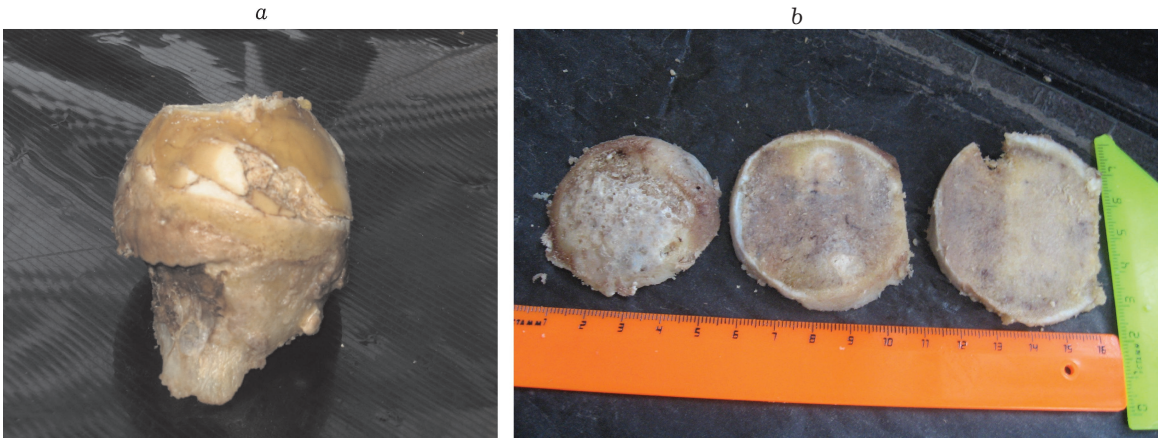


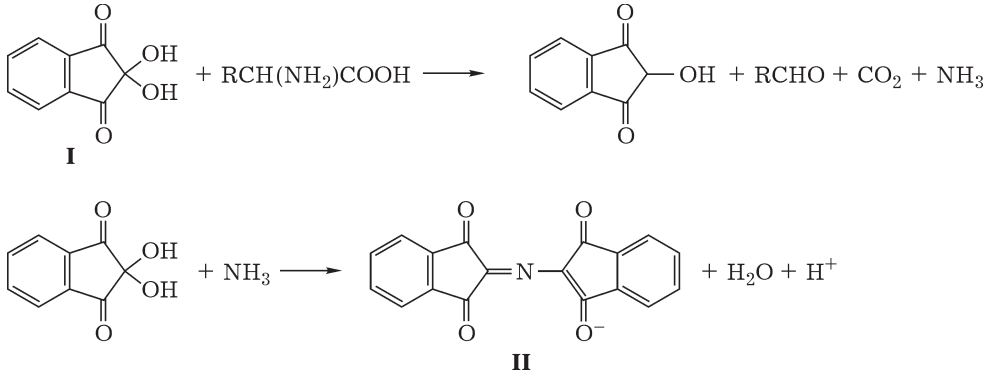
Fig. 1. Human femoral bone head in the case of coxarthrosis (*a*) and the horizontal upper, middle and lower slices thereof (*b*).

MATERIALS AND METHODS

The material of our investigations was presented by our collection of femoral bone heads in the quantity of 100 pcs (Fig. 1, *a*) removed from patients with coxarthrosis (men and women), the residents of the Omsk Region within the age from 30 to 80 years old. To reveal age-and-gender changes in bone tissue, all the material was distributed in four age groups: the first group 30–49 years old, the second group 50–59 years old, the third group 60–69 years old, and the fourth group 70–79 years old. For assessing the dynamics of changes in the course of coxarthrosis we obtained three horizontal slices of individual femoral bone heads with the thickness of 0.2–0.5 cm: the upper, middle and lower (the order of succession is given in the cartilage – femoral bone direction, see Fig. 1, *b*), with subsequent grinding thereof. Then the average dried powder samples were analyzed according to State Standard GOST 17681–82.

The investigation of the amino acid composition of bone tissue was performed by means of ion-exchange high performance liquid chromatography (HPLC) on an AAA 39M amino acid analyzer. As the ion exchange resin, we used sulphonated polystyrene.

The sample preparation technique was based on obtaining protein hydrolysates for bone samples by means of acidic demineralization of the peptide bonds and their subsequent separation into different amino acid fractions. Acidic hydrolyzing the medium sample (1.5 g) by 6 M HCl solution was performed at 105 °C for 24 h [9]. For the sequential isolation of amino acids from the analyzer ion-exchange column, we used buffers with three values of pH, such as 3.5, 4.25, 9.45. For the detection and quantification of amino acids, the eluate obtained was mixed



Scheme 1.

with a solution of ninhydrin (1,2,3-indantrione hydrate $C_9H_6O_4$). Coloured product **II** was determined by means of spectrophotometry ($\lambda_{eff} = 570$ nm, for proline $\lambda_{eff} = 440$ nm) (Scheme 1) using a SF-46 spectrophotometer.

The final result was determined from the peak area and the retention time of corresponding amino acid. The amino acid content was determined using a calibration curve technique (solutions with a known concentration of corresponding amino acids being used as standards). As the final result of the optical density (D) (absorbance) we took the arithmetic mean of triplicate measurements. Allowable discrepancies between the results of two parallel determinations of D did not exceed 10 %, whereas those between the results obtained under different conditions (confidence probability $P = 0.95$) were lower than 25 %. The detection limit of the amino acid composition was equal to 10^{-4} mass %.

The statistical data processing was carried out with the use of a StatSoft Statistica 6.0 software package.

RESULTS AND DISCUSSION

In the analyzed samples of powdered bone tissue there were 13 amino acids identified (Table 1). It was established that in the case of

TABLE 1

Amino acid composition of human bone tissue in the case of coxarthrosis ($n = 3$, $P = 0.95$), mass %

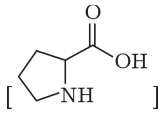
Amino acids [Formula]	Abbreviated name	Bone slices		
		upper	middle	lower
Glycine $[H_2NCH_2COOH]$	Glu	2.33±0.34	2.38±0.36	2.42±0.45
Alanine $[CH_3CH(NH_2)\ COOH]$	Ala	0.8±0.13	0.72±0.3	0.84±0.18
Valine $[(CH_3)_2CHCH\ (NH_2)\ COOH]$	Val	0.29±0.05	0.3±0.05	0.3±0.07
Leucine $[(CH_3)_2CHCH_2\ CH(NH_2)COOH]$	Leu	1.11±0.19	1.03±0.26	1.07±0.02
Isoleucine $[CH_3CH_2CH\ (CH_3)CH(NH_2)COOH]$	Ile	1.41±0.24	1.34±0.33	1.36±0.38
Threonine $[CH_3CH(OH)CH(NH_2)\ COOH]$	Thr	0.29±0.05	0.31±0.06	0.31±0.07
Serine $[HOCH_2CH(NH_2)COOH]$	Ser	0.29±0.15	0.23±0.04	0.23±0.05
Methionine $[CH_3SCH_2CH_2CH(NH_2)COOH]$	Met	0.13±0.02	0.13±0.03	0.13±0.03
Glutamic acid $[HOOCCH_2CH_2CHNH_2COOH]$	Gly	1.84±0.30	1.88±0.32	1.92±0.42
Lysine $[H_2N(CH_2)_4CH\ (NH_2)COOH]$	Lys	0.72±0.13	0.74±0.13	0.76±0.18
Arginine $[HN=C(NH_2)NH(CH_2)_3CH(NH_2)COOH]$	Arg	0.49±0.1	0.49±0.11	0.5±0.13
Phenylalanine $[PhCH_2CH(NH_2)\ COOH]$	Phe	0.24±0.04	0.25±0.05	0.25±0.06
Proline 	Pro	0.7±0.17	0.73±0.18	0.74±0.22

TABLE 2

Concentration series of amino acids series in organomineral aggregates (OMA) from a human organism

OMA	Series of amino acids
Bone slices (morbid affection)	
Lower	Glu > Gly > Ile > Leu > Ala > Lys > Pro > Arg > Thr > Val > Phe > Ser > Met
Middle	Glu > Gly > Ile > Leu > Lys > Pro > Ala > Arg > Thr > Val > Phe > Ser > Met
Upper	Glu > Gly > Ile > Leu > Ala > Lys > Pro > Arg > Thr > Ser > Val > Phe > Met
Pathogenic	
Odontoliths	Gly > Ser > Ala > Glu > Asp > Lys > Phe > Val > Leu > Thr > Ile > His > Arg > Tyr > Met [11]
Salivoliths	Gly > Ser > Phe > Lys > Arg > Asp > Tyr > Leu > Glu > Val > Ala > His > Ile > Thr > Met [11]
Kidney stones (phosphate type)	Gly > Lys > Ala > Pro > Thr > Val > Glu > Ser > Phe > Arg > Met > Leu > Ile [12]

damaged samples the qualitative and quantitative composition of amino acids in all bone slices (upper, middle, lower) was about the same value, however, only a decrease in the total content (4 mass %) was observed the upper slice as compared to the middle and lower slices. This might be connected with the fact that the collagen fibres of the upper slice, as the part most affected by coxarthrosis, are sclerosed and the lowest solubility is inherent therein [10].

We have performed the ranking of amino acids according to the content thereof in the bone tissue. The resulting concentration series were compared with the amino acid set of pathogenic organomin-

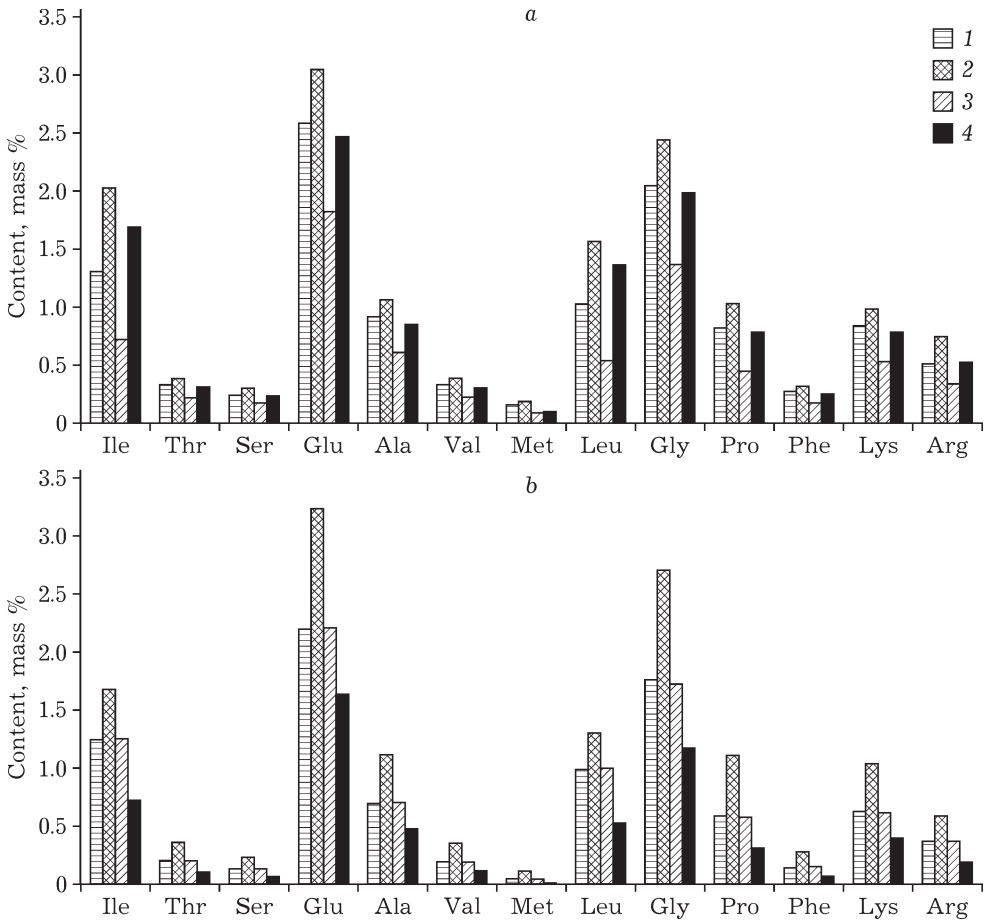


Fig. 2. Amino acid content in bone tissue for men (*a*) and women (*b*) in different age groups, years: 30–49 (*1*), 50–59 (*2*), 60–69 (*3*), 70–79 (*4*).

eral aggregates (OMA) of a human organism (odontoliths, salivoliths, nephrolithss [11, 12]).

It can be seen (Table 2) that the composition of all of the new biominerals exhibits glutamic acid to prevail, which indicates a special role thereof in the formation of biominerals. It is known that this amino acid in an organism at physiological pH values (*e. g.*, blood pH 7.3–7.5) is in anionic form, whereby it interacts with the positively charged areas of the surface of the bone apatite [12, 13].

The structure-forming role in the formation of physiogenic (bones and teeth) and pathogenic biominerals is played by glycine [11, 12, 14]. However, the data of Table 2 demonstrate that the abovementioned amino acid, in contrast to the pathogenic OMA, dominates in bone tissue, since it represents the main component of the ordered crystalline polypeptide chain of collagen (–Glu–X–Y–), whose fibre parts serve as crystallization nuclei for bone apatite [4, 5, 7, 8 15].

Physiogenic biominerals also contain nonpolar amino acids (leucine and isoleucine) in great quantities, whereas pathogenic biominerals contain polar amino acids (serine, proline, and lysine) and non-polar ones (alanine, phenylalanine).

In order to establish the age and gender characteristics of the amino acid composition inherent in human bone tissue we performed statistical analysis of the data concerning the content of amino acids in the lower slices of bone using the Student's *t*-test and the cluster analysis.

These calculations have demonstrated that before 60 years, both the group of men, and the group of women, exhibit an identical trend in the amino acid composition of bone samples. After 60 years, there were significant differences observed in the content of amino acids with respect to gender and age characteristics. Thus, the bone tissue of men and women of the third and fourth age groups differ significantly between each other in the content of amino acids therein ($P = 0.95$, $t_{calc} = 2.14 > t_{tab} = 2.06$ и $t_{calc} = 2.50 > t_{tab} = 2.06$, respectively). No such differences in the samples of bone tissue were revealed for the first and second age groups.

Meanwhile, for the bone samples belonging men one can observe a significant decrease in the total amino acid content as passing from the first to the second and third age groups (Fig. 2, *a*). So, a significant difference in the Student's *t*-test can be noted between the first and the third categories of age ($t_{calc} = 2.38 > t_{tab} = 2.06$, $P = 0.95$), the second and third ones ($t_{calc} = 2.85 > t_{tab} = 2.06$, $P = 0.95$). From the data presented in Fig. 2 one could see that after 60 years old the bone tissue of men generally exhibit a decrease of the amount of amino acids, whose biological role was discussed earlier: glutamic acid and leucine, leucine and isoleucine. The bone samples taken from women, a significant decrease in the concentrations of amino acids is observed within the age range of 70–79 years (see Fig. 2, *b*).

The cluster analysis also confirms the results obtained. In the dendrogram (Fig. 3, *a*), one could two clusters those differ from each other in amino acid composition: the first cluster includes the first age group (sample No. 1) and the second (sample No. 3) age group, whereas the second cluster includes the third age

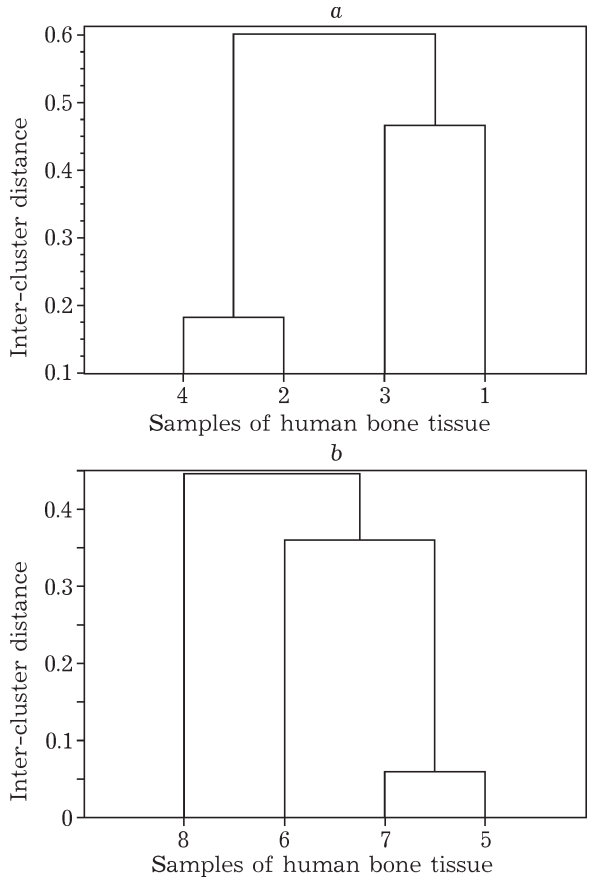


Fig. 3. Dendrograms of bone tissue for men (*a*) and women (*b*) of different age groups (cluster analysis), years: 30–49 (1), 50–59 (2), 60–69 (3), 70–79 (4).

group (sample No. 2) and fourth age group (sample No. 4).

With the use of Student's *t*-test we demonstrated that the fourth age group differs in amino acid composition from the other age groups (the difference between the first and fourth, third and fourth groups is $t_{calc} = 2.19 > t_{tab} = 2.06$, that between the second and the fourth groups is $t_{calc} = 2.93 > t_{tab} = 2.06$; $P = 0.95$). In the dendrogram of the bone tissue of women (see Fig. 3, *b*) one could see two clusters. The first cluster (sample No. 8) belongs to the fourth group of age and is characterized by the smallest total amino acid content (6.83 mass %), whereas for the other age groups it is equal to 10.07–14.91 mass %. The second cluster (samples Nos. 5–7) includes the other categories of age, most similar between each other in the amino acid composition.

The decrease in the average concentrations of amino acids in the bone tissue for men of the third age group and for women of the fourth age group, could be, to all appearance, caused by a decrease in collagen content resulting from the general process of organism aging and the presence of comorbidities (*e. g.*, osteoporosis), and so on [16].

CONCLUSION

Thus, it has been found that the distribution of amino acids in the head of the femoral bone (from hyaline cartilage to the femoral bone) in patients with coxarthrosis is not changed in the course of the disease. As far as the bone tissue of men and women within the age range of 30–79 years is concerned, the amino acid composition before 60 years old does not depend on age and gender characteristic of the samples. After 60 years old there was a variation of the amino acid content observed with respect to gender and age characteristics: within the age range of 60–69 years the content of amino acids in men's bone tissue exhibit a decrease, whereas in women's bone tissue it remains unchanged within the age range of 70–79 years, it is not changed in men but exhibits a decrease in women's bone tissue.

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