

Effect of L-tryptophan on the bone biophysical properties and oxygen consumption in rats with diet-induced obesity

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The purpose of the study was to evaluate the effect of L-tryptophan on the biophysical properties of bone tissue and oxygen consumption in rats with diet-induced obesity. The study was conducted on 40 male 3-months-aged Wistar rats. The photometric determination of phosphorus and calcium concentration in the femoral bones was conducted. The rate of oxygen consumption was measured according to chronoamperograms. The biophysical properties of femurs were measured by a methodic three-point bending test. It was shown that the content of calcium (by 15%) and phosphorus (by 20%) was significantly higher in the femurs of rats that received L-tryptophan (at a dose of 80 mg/kg per os) for 28 days compared to the control parameters. The rate of oxygen consumption, density and biophysical properties of the femurs did not change. As a result of the consumption for three months of a high-calorie diet (580 kcal/100 g) in experimental rats clear features of obesity evolved. Thus, they had a greater visceral fat mass (by 145%), a visceral fat mass to body weight ratio (by 122%), and an obesity index (by 145%). In rats with alimentary obesity, the calcium and phosphorus content in the femurs was significantly reduced by 28 and 24%, respectively, and the rate of oxygen consumption was 45% lower than in control animals. Femur bearing capacity, strength limit, and stiffness were significantly lower, namely on 23, 11, and 37%, respectively. Administration of L-tryptophan to rats, against the background of consumption of a high-calorie diet, inhibited the development of obesity. Visceral fat mass and its ratio to body weight in this group of rats were 38 and 23% lower, respectively, compared to the obese group. The concentration of calcium (by 32%) and phosphorus (by 25%) and oxygen consumption rates (by 31%) were significantly greater compared to rats fed only the high-calorie diet. Our research shows that the administration of L-tryptophan to obese animals can prevent the development of negative changes in bone tissue.

Key words: obesity; visceral fat; femurs; oxygen consumption; biophysical properties; calcium; phosphorus.

INTRODUCTION

Alimentary obesity and related disorders of energy metabolism are one of the actual problems of modern medicine. In 1997 obesity was recognized by the WHO as an epidemic of the XXI century. According to the WHO, in 2016 more than 1.9 billion adults were overweight, of whom more than 650 million were obese. It is estimated that by 2025, up to 40% of men and 50% of women in the world will be obese [1].

It is established that the pathogenesis of obesity is based on the imbalance between energy intake with food and its expenditure. There is a lot of clinical and experimental

evidence that almost all organs and systems of the body are involved in the pathological process caused by excess body weight. Obesity has been shown to lead to a number of serious diseases, such as hypertension, type 2 diabetes, and coronary heart disease [2].

Adipose tissue can affect bone tissue both directly and through the production of adipokines. Of the known adipokines synthesized by adipose tissue, leptin and adiponectin have the greatest effect on bone metabolism [3]. Many studies have shown the negative effects of excess body weight on bone mineral density (BMD) in women, men and adolescents [4].

Studies of obese women have shown significant positive relationships between leptin levels and alkaline phosphatase levels, body mass index (BMI) and body fat. There was a negative relationship between leptin levels and Ca^{2+} levels in the blood, as well as between adiponectin levels, BMI and adipose tissue content. There was a significant positive correlation between leptin levels and BMD of the spine and proximal femur. These facts suggest that fat mass affects BMD indirectly through changes in adipokines production [5]. Although many studies have shown a direct relationship between serum leptin levels and human BMD [6-8], some researchers, by contrast, have found an inverse relationship [9, 10]. The complex effect of leptin on bones can be explained by its ability to act positively directly in peripheral tissues or negatively through central regulatory mechanisms, which leads to the activation of the sympathetic nervous system. Therefore, the assumption of «double control» of leptin over bone formation has been suggested [11]. Some researchers have suggested that subcutaneous fat has a protective effect on bone tissue and visceral fat has a negative effect [12].

Serotonin is one of the most important transmitters involved in the regulation of energy homeostasis, which is the stimulation of some and suppression of other neurons in the hypothalamus by peripheral hormones [13, 14]. Serotonin, synthesized in the intestine, inhibits the formation of bone tissue. Serotonin, synthesized in the brain, in contrast, increases bone mass, enhances bone formation, and inhibits bone resorption [15].

The essential amino acid tryptophan easily enters the bloodstream into the brain and effectively increases the production of serotonin by the central nervous system (CNS) [16]. It has also been shown that tryptophan is responsible for the synthesis of vitamin B3 (nicotinic acid) in the liver, which is a major component of NAD^+ and NADP - essential coenzymes that regulate hundreds of metabolic processes in the body from digestion to the immune system.

Tryptophan also promotes the release of growth hormones and helps suppress appetite. Studies in rats after ovariectomy and tryptophan derivatives administration have shown an increase in serum N-terminal procollagen type 1 propeptide (PINP), a marker of bone formation, and an improvement in bone microarchitecture without estrogenic side effects. The authors suggest that these derivatives may be new components of anabolic drugs for the treatment of bone diseases [17, 18]. Thus, although the physiological role of tryptophan in the body is not in doubt, the mechanisms of its influence on the development of alimentary obesity and osteoporosis remain undisclosed and need further study.

The purpose of the work was to investigate the effect of L-tryptophan on the biophysical properties of bones and oxygen consumption in young rats with diet-induced obesity.

METHODS

Research design. An experimental single-center, prospective, single-sample controlled, randomized study without blinding was conducted. Randomization was carried out by the block method according to the age and weight of the animals.

The study was conducted on 40 male Wistar rats taken in the experiment at the age of 3 months. Rats were divided into 4 groups: group I – control; group II – animals that received tryptophan orally daily for 28 days at a dose of 80 mg/kg of body weight and were on a standard vivarium diet; group III – rats that received a high caloric diet for three months (45% fat and 31% carbohydrates); group IV – rats that were on a high caloric diet for three months and additionally received L-tryptophan at a dose of 80 mg/kg body weight. Weekly body weight was recorded. Rats were decapitated under etheric anesthesia under the terms of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Research (Strasbourg, 1986), as well as the requirements of the Biomedical

Ethics Committee of the Bogomoletz Institute of Physiology NAS of Ukraine.

Visceral fat was isolated using the dissection method. Visceral fat mass and the mass of the femurs were determined.

The obesity index (IO) was calculated by the formula:

$IO = M/100$, where M is the mass of visceral fat.

The volume of the bones was measured by the volumetric method, and their density and ash content were calculated. The content of mineral elements in the femur was determined by the method of ashing. To do this, the bones were burned in a muffle at a temperature of 700°C for at least 5 h. The ash was weighed and dissolved in hydrochloric acid. The content of calcium and phosphorus in the resulting solution was determined by a photometric method using standard test kits from Philisit Diagnostics (Ukraine).

Biophysical properties of the femurs were measured by a three-point bending test on the device “Osteotest” (Ukraine). According to the obtained curve of the dependence of bone elongation on load, the bearing capacity, stiffness, and energy of elastic deformation of the femurs were calculated [19].

At the point of bone fracture, the diameter and thickness of its wall were also measured, and the strength limit was calculated according to the formula:

$E = M/W$, where $M = P \cdot l/4$, where: P is the bearing capacity; l is the distance between the supports.

$W = (3,14 \cdot b_1 \cdot a_1^3/64 - 3,14 \cdot b_2 \cdot a_2^3/64) \cdot (a_1/2)$, where: a_1, b_1 are the outer diameters of the bone; a_2 and b_2 are the inner diameters of the bone.

Oxygen consumption by the diaphyseal part of the femur was measured using a platinum electrode according to the method of Schirmacher [20]. The spongy part of the bone fragments was washed in saline at a temperature of 28°C, pH 7.4. The mass of bone fragments ranged from 70 to 100 mg, thickness from 130-500 µm. Femoral fragments were placed in a

2 cm³ thermostated cell filled with saline at 37°C, pH 7.4. Chronoamperogram, reflecting the change in oxygen tension over time, was recorded for 30 min. Oxygen consumption per minute was calculated from the curve of oxygen decline over time per 100 g of tissue. The oxygen solubility coefficient was taken to be 0.024 ml/ml/mm Hg at a temperature of 37°C.

The obtained data were processed by the methods of variational statistics using the software Statistica 6.0 for Windows (“StatSoft”, USA) and Excel 2010 (“Microsoft”, USA). The normality of the distribution of digital arrays was checked using the Shapiro-Wilk W-test. In the case of normality of the distribution, the Student’s t-test was used to estimate the reliability of the difference between the control and experimental groups. Differences were considered significant at $P < 0.05$. One-way analysis of variance (ANOVA) was also used. Multiple pairwise comparisons of groups were performed using Tukey’s HSD test with a significance level of 0.05.

RESULTS AND DISCUSSIONS

Obesity markers (visceral fat mass, fat mass to body weight ratio and obesity index) in rats that received L-tryptophan (II group) remained close to control indicators. In rats that were on a high-calorie diet (group III, HC), all the studied parameters were significantly higher than the control values. Namely, the visceral fat mass was 145% higher and the visceral fat mass to body weight ratio was 122% higher compared to the control (Table 1). Such changes in morphometric parameters indicated the development of obesity in rats of the III group. In experimental rats (IV group) these morphometric parameters increased less clearly, compared with controls, and were significantly lower than those recorded in rats of the III group. In experimental rats, which received the HC diet and L-tryptophan together (group IV), these indicators, compared to the control, increased less clearly and were significantly lower than the indicators recorded

in rats of group III. Thus, the mass of visceral fat and its ratio to body weight in rats of group IV were 38 and 23% less than in rats of group III. However, these indicators were significantly higher than in control animals (Table 1).

Our data showed that tryptophan did not significantly affect the risk of obesity in animals that were on a normal calorie diet (330 kcal/100 g). However, rats that received L-tryptophan at the same time as high-calorie food (580 kcal/100 g) developed less obesity, than rats that received only the HC diet and showed all signs of alimentary obesity. That is, L-tryptophan inhibited the increase in visceral fat mass and the development of obesity in experimental rats that received the HC diet.

The results of the “ANOVA” analysis on such indicators of obesity as visceral fat mass, visceral fat mass to body weight ratio and obesity index showed that the parameters of at least one of the groups of rats are probable different from the others at the significance level of 0.05. By pairwise comparison using the Tukey test, it was shown that the most probable difference in obesity index indicators was between groups III and I, as well as between groups II and III, and the minimum difference in these indicators was between groups II and I. No difference was observed when comparing groups IV with I and II with IV. When comparing the groups according to the visceral fat mass to body weight ratio, the most probable difference was observed between groups III and I, as well as between groups II and III, and the smallest difference was between groups II and I. No difference was observed when comparing groups

IV and I. When comparing groups by visceral fat mass, it was shown that the most probable difference was between groups III and I, as well as between groups II and III, and the smallest difference was between groups IV and III.

The results of our studies coincide with the data of other researchers, who observed normalization of body weight, and weight loss of visceral fat under the influence of tryptophan in rats with alimentary obesity [21, 22]. This effect of tryptophan can be explained by the fact that it increases the level of serotonin in the brain. As a result, appetite and carbohydrate intake are reduced and body weight is normalized. It is believed that one of the mechanisms of the serotonin influence on the development of obesity may be its interaction with leptin. Leptin levels increase with obesity, they increase stress-induced food intake, affecting the hypothalamic nuclei.

Bone tissue differs from other types of connective tissue in the body in that it contains a lot of mineral elements. The main mineral elements of bone tissue are calcium, magnesium and phosphorus. Calcium and phosphorus salts make up 40-45% of the mass of cortical bone and determine its mechanical strength and stiffness [23]. In experimental rats that were on standard chow and received tryptophan, the calcium content in the femurs was 15% higher than in control animals. In rats that consumed the HC diet, the calcium content in the femurs was significantly 28% less, compared to the control. In the femurs of rats of the IV group, the calcium content was not significantly different from the control level (Fig. 1).

Table 1. Indicators of obesity in rats (M ± m)

Indicators	Group I	Group II	Group III	Group IV
Visceral fat mass, g	19.0 ± 1.4	19.6 ± 1.1	51.1 ± 2.4*	28.7 ± 1.4*^
The ratio of the mass of visceral fat to body weight	0.046 ± 0.004	0.051 ± 0.005	0.120 ± 0.007*	0.078 ± 0.006*^
Obesity index	0.19 ± 0.02	0.19 ± 0.02	0.51 ± 0.07*	0.29 ± 0.03*^

Note: here and in a Table. 2, *P < 0.05 compared with the control group; ^P < 0.05 compared to group III. The I group is the control; group II – rats that received L-tryptophan; group III – rats that were on a high-calorie diet; group IV – animals that received a high-calorie diet and tryptophan together

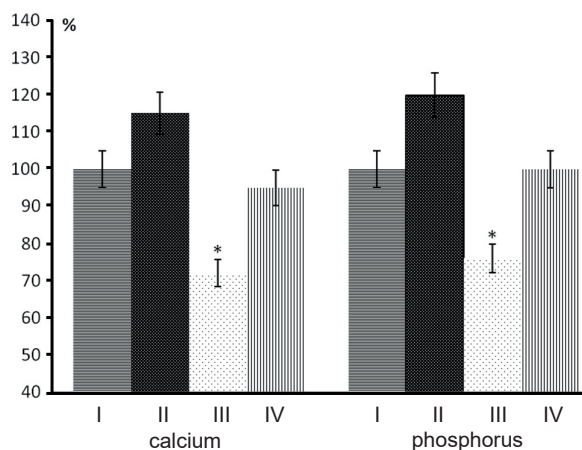


Fig. 1. Content of calcium and phosphorus in the femurs of experimental rats (% of control). The I group is the control; group II – rats that received L-tryptophan; III – rats that were on a high-calorie diet; IV – animals that received a high-calorie diet and tryptophan together. * $P < 0.05$ compared with the control group

The phosphorus content in the femurs of experimental rats varied similarly to changes in calcium. The phosphorus concentration in the femurs of the II group of rats was 20% higher, and in the III group of rats was 24% less compared to the control. But in the IV group of rats, it remained at the same level as in the control group. Attention is drawn to the fact that the calcium/phosphorus ratio in the femurs of obese rats had a clearly defined tendency to decrease by 13%, and in rats of the II group, on the contrary, it had a tendency to increase by 12%. In the femurs of the IV group of rats, the Ca/P ratio did not differ from the indicators recorded in the III group.

The rates of oxygen consumption of the bone tissue in the II group of rats did not differ from the control values. The oxygen consumption of the bone tissue in animals that received the HC diet was 45% less compared to control values (Fig. 2). The data suggest a close relationship between the development of obesity and the intensity of energy metabolism in the femur of rats. The administration of L-tryptophan on the background of the HC diet led to less pronounced changes in oxygen consumption compared with the III group. In rats of group IV,

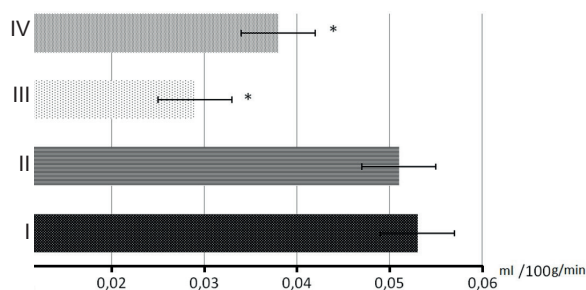


Fig. 2. Oxygen consumption by the diaphyseal part of the femur. The I group is the control; group II – rats that received L-tryptophan; III – rats that were on a high-calorie diet; IV – animals that received a high-calorie diet and tryptophan together. * $P < 0.05$ compared with the control group

the rate of oxygen consumption was 31% higher than in group III. But it remained lower than in the control group of animals by 28%.

The results of our research give grounds to talk about the activation of metabolic remodeling processes in bone tissue under the influence of L-tryptophan in obese animals. Measurements of bone morphometric parameters revealed that the density of the femurs in rats of the III group was 8% less than in control animals. In experimental rats of the II group, which received L-tryptophan, the density of the femurs remained at the control level. In IV group rats this figure tended to decrease. The ash content, which reflects the content of mineral elements, in groups III and IV, tended to decrease, but in rats of group II, remained close to the baseline.

The biophysical properties of the femurs did not change in rats fed a normal diet and L-tryptophan. In rats that consumed the HC diet, the bearing capacity of the femurs was 23%, the tensile strength 20% and the energy of elastic deformation was 46% lower than control values. Femoral stiffness, by contrast, was significantly 45% higher. Some indicators of the biophysical properties of femurs in the IV group of rats were also significantly lower than in control animals. Thus, the bearing capacity was 20%, and the energy of elastic deformation was 40% lower than in control animals. At the same time, the strength and stiffness of bones approached the

level of control indicators (Table 2). A decrease in the biophysical properties of bones is probably associated with a decrease in calcium content and bone density in rats due to obesity.

Other researchers have also shown a positive effect of tryptophan on bone tissue. A study of men and women of different ages found higher levels of hip joint mineral density and stiffness index in people who consumed more tryptophan [17]. A number of studies have correlated the tryptophan content in the blood with the mineral density of the femoral neck. An increase in the level of the N-terminal propeptide of procollagen type I (a marker of bone formation) was detected in the serum of rats after the administration of tryptophan derivatives. Stimulation of bone marrow cell proliferation under the influence of L-tryptophan has been shown in the elderly with osteoporosis [18]. Different tryptophan metabolites have been shown to have opposite effects on bone tissue. A number of tryptophan metabolites (e.g. 3-hydroxykynurenine, kynurenic acid and anthranilic acid) have a detrimental effect on bones and reduce bone mineral density, which increases the risk of fractures. Other metabolites (e.g. 3-hydroxyxanthurenic acid, picolinic acid, quinoline acid) increase bone density and reduce the risk of fractures [24, 25].

Our data indicate that alimentary obesity leads to a decrease in the rate of oxygen consumption by bone tissue, a decrease in the content of mineral elements and the density of the femurs of experimental rats. Such changes naturally caused a decrease in the biophysical properties of femurs. A decrease in the intensity of oxygen consumption by bone tissue under the influence of obesity indicates inhibition

of oxygen-dependent metabolism and bone remodeling processes. Rats fed a balanced diet did not show signs of alimentary obesity, and L-tryptophan did not affect the biophysical properties and oxygen exchange of bone tissue. In rats that were on the HC diet and had obvious signs of alimentary obesity, administration of L-tryptophan improved most of the investigated parameters of bone tissue, such as the rate of oxygen consumption, calcium and phosphorus balance, tensile strength and stiffness. These indicators approached the control values. Our research shows that the administration of L-tryptophan to obese animals can prevent the development of negative changes in bone tissue.

CONCLUSION

The results of our research indicate that obesity worsens strength indicators of the femurs of young animals. Such changes in the biophysical properties of femurs reduce the bone's ability to withstand loads and increase the risk of fractures. A decrease in the biophysical properties of femur bones is associated with a decrease in the content of calcium and phosphorus in bone tissue, a decrease in bone density, as well as a possible violation of bone microarchitecture. The introduction of L-tryptophan against the background of both a standard vivarium diet and the HC diet contributed to an increase in the content of calcium and phosphorus in bone tissue and the normalization of its biophysical properties. The obtained results may be important for experimental and practical medicine when solving issues of complex treatment and prevention of bone tissue disorders in obesity.

Table 2. Biophysical indicators of the femurs (M ± m)

Indicators	Group I	Group II	Group III	Group IV
Bearing capacity, kgf	21.8 ± 2.6	22.0 ± 1.5	19.1 ± 1.6	17.5 ± 1.4
Stiffness, kgf/mm	24.9 ± 1.7	24.5 ± 1.5	36.1 ± 1.2*	24.4 ± 2.3
Energy of elastic deformation, kgf·mm	9.9 ± 0.3	10.1 ± 0.4	5.4 ± 0.8*	6.0 ± 0.7*
Tensile strength, kgf/mm ²	14.0 ± 0.5	15.4 ± 1.3	11.2 ± 0.9	12.7 ± 0.1

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and the interrelations of coauthors of the article.

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ВПЛИВ L-ТРИПТОФАНУ НА БІОФІЗИЧНІ ВЛАСТИВОСТІ КІСТОК І СПОЖИВАННЯ КИСНЮ У ЩУРІВ З ДІЄТИНДУКОВАНИМ ОЖИРІННЯМ

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Мета дослідження – вивчити вплив L-триптофану на біофізичні властивості кісток і споживання кисню у молодих щурів-самців лінії Вістар (3 міс) з дієтиндукованим ожирінням. У стегнових кістках визначали: концентрацію кальцію та фосфору фотометричним методом, швидкість споживання кисню – за даними хроноамперограми, біофізичні властивості – методом триточкового навантаження на вигин. Показано, що у стегнових кістках щурів, яким протягом 28 днів вводили L-триптофан (80 мг/кг, per os), вміст кальцію та фосфору був вірогідно більшим (на 15 і 20% відповідно) порівняно з контрольними значеннями. Швидкість споживання кисню, щільність та біофізичні властивості стегнових кісток не змінювалися. Після 3-місячного споживання висококалорійного раціону (580 ккал/100 г) у дослідних щурів розвивалися чіткі ознаки ожиріння. Так, у них спостерігали більшу масу вісцерального жиру (на 145%), відношення маси вісцерального жиру до маси тіла (на 122%) та індекс ожиріння (на 145%). У щурів з аліментарним ожирінням вміст кальцію та фосфору у стегнових кістках був вірогідно меншим на 28 та 24% відповідно, а швидкість споживання кисню на 45% нижчою, ніж у контрольних тварин. Несуча спроможність стегнових кісток була вірогідно менша на 23%, межа міцності – на 11% та жорсткість – на 37%. Введення L-триптофану щурам, на фоні споживання висококалорійного раціону, гальмувало розвиток ожиріння. Маса вісцерального жиру та відношення її до маси тіла в цій групі щурів були меншими на 38 та 23% відповідно порівняно з групою щурів з ожирінням. Вміст кальцію (на 32%) і фосфору (на 25%) та швидкість споживання кисню (на 31%) стегновою кісткою були вірогідно більшими порівняно зі значеннями у щурів, які отримували лише висококалорійний раціон. Проведені нами дослідження свідчать, що введення L-триптофану може попереджувати розвиток негативних змін у кістковій тканині при ожирінні.

Ключові слова: ожиріння; вісцеральний жир; стегнові кістки; споживання кисню; біофізичні властивості; кальцій; фосфор.

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