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AGE FEATURES OF INFLUENCE METABOLIC SYNDROME ON FETAL EMBRYONIC DEVELOPMENT OF THE OFFSPRING OF MALE RATS

Comparative study of embryo-fetal death in females fertilized by males with metabolic syndrome, induced in adult or juvenile age has shown that the offspring of adult rats did not have significant abnormalities in embrio- and fetogenesis. At the same time it has been revealed 4% post-implantation death of offspring in male rats with metabolic syndrome induced in the juvenile age. The pre-implantation loss in this group was 6 folds higher than in control. Accordingly, the total mortality of the offspring rose 2.4 times in comparison with control.

Key words: metabolic syndrome, rats, embrio- and fetogenesis.

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AMINO ACIDSLEVELS IN RATS UNDER PROGESTERONE LONG-TERM ADMINISTRATION

Amino acids levels in blood serum of rats under progesterone long-term administration have been determined. The studies show that levels of most amino acids content decrease in rats under progesterone long-term administrationcompared with control group of rats. Obtained data give evidence that progesterone long-term administration significantly influence on amino acids metabolism.

Key words:amino acids, progesterone, obesity.

Introduction. The rising prevalence of overweight and obesity is a major global health challenge and concern. Obesity has become a global epidemic and threat to public health [1]. Analysis of the Global Burden of Disease study 2013 revealed that during the last three decades, the prevalence of overweight and obesity rose 27.5 % in adults and 47.1 % in children [2]. The prevalence of obesity is highest in the developed countries; however, almost two thirds of the obese population live in developing countries [3].

The development of obesity requires a persistent state of positive energy balance [4,5]. Antiobesity strategies need to overcome the strong homeostatic mechanisms driving reduced energy expenditure. Accordingly, much effort has been devoted in this front during the last two decades, with the recognition of the major metabolic roles of numerous neuropeptides and transmitters, as well as multiple peripheral hormones [6-8], not only in the regulation of feeding, but also in the modulation of energy expenditure.

The use of drugs based on steroid hormones, particularly progesterone, can be one of many factors contributing to obesity. It is known that progesterone, which enters the female body artificially leads to fat accumulation effect. Ovarian steroids, such as progesterone, control a vastness of physiological processes, such as puberty, reproduction, growth, development and metabolic rate. In addition, some reports suggest the use of progesterone-containing preparations as contraceptive or for the hormone replacement therapy to cause sufficient weight gain by causing hyperphagia and increased fat deposition in the body [9]. A lack of the accurate and scientifically recognized explanation of the mechanism of the body fat increase causes a difficult situation being currently present in the obesity treatment.

Amino acids, especiallyBranched-chain (BCAAs) amino acids, are critical nutrient signals that affect metabolism, either directly or indirectly. It has been shown that a positive association exists between a BCAA-rich diet and metabolic health, including the regulation of body weight, muscle protein synthesis, and glucose homeostasis [10,11]. In spite of the positive effects of BCAAs on metabolic health, an elevation in the level of BCAAs correlates with an increasing risk of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) in humans and in rodent models [12]. Despite the positive effects of BCAAs on metabolism, the strong association of BCAA levels with insulin resistance and metabolic syndrome suggests that increased levels of BCAAs may cause insulin resistance and T2DM, although this remains a speculation for now. The mechanism underlying that correlation is not yet fully understood.

In this study, we search changes of amino acids levels of rats under progesterone administration.

Materials and methods.Research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe's ‘Europe Convention for the Protection of Vertebrate Animals' used for experimental and other scientific purposes' (1997). The general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001) and other international agreements and national legislation in this field. Animals were kept in a vivarium that was accredited in accordance with the ‘standard rules on ordering, equipment and maintenance of experimental biological clinics (vivarium)'. Instruments to be used for research are subject to metrological control.

Thepresentstudyusedwhite nonlinear femalerats weighing210±20gatthebeginingofexperiment.. The animals of each experimental group were housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of 22±3C, a 12 h light/12 h dark cycle and a relative humidity of 60±5%.Studies were conducted on 20 rats that were divided into two groups of 10 animals each: 1 – control group; 2 – progesterone-induced obese group.

Progesterone oil solution (Biopharma, Kyiv, Ukraine) was administered in the dorsal neck region of rats (10 mg/kg body weight, treated daily for 28 days) to modulate obesity. Rats belonged to the control group were injected with the oil used for the progesterone administration. To confirm the development of obesity, we have determined the body mass index and Li index.Blood serum was taken after the 28 days of progesterone injectionto determine amino acids levels.

Amino acids blood serum levels were determined by using of ion exchange chromatographyanalyzer(Spekman, Stein, Moore).

Statistical analysis of data was carried out by the software package ‘Statistica7.0'. For the analysis of data distribution type, Shapiro-Wilks criterion was used. As the data were normally distributed, we used Student's t test for independent samples. Mean values (M) and standard deviations (SD) were calculated. Significant difference was considered at $p \leq 0.05$.

Results and discussion. We determined amino acids levels of the rats under progesterone long-term administration.BCAA is about 40% of the essential amino acids and 35% of muscle amino acids. Part of BCAA in hormonal regulation processes is very important. This amino acids increase secretion of insulin by pancreatic β-cells and activate mTOR signal transduction pathway. Accordingly, measure of serum BCAA levels was the first stage of our investigations.It has been established that valine, leucine levels in the experimental group were in 1.58, 1.5times lower than that of the control group.Isoleucine level was not significantly changed (Fig 1.).

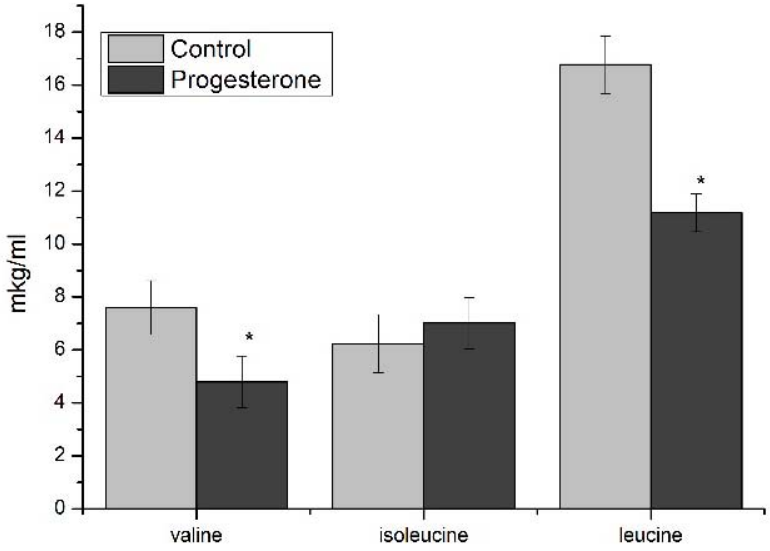

Fig. 1. Valine, isoleucine, leucine levels in serum of rats under progesterone long-term administration compared to the control group

Data are shown as the mean ± SD of 10 animals; asterisk (*), p <0.05 in comparison with control group

Despite the negative effects of BCAAs on metabolism, the strong association of BCAA levels with insulin resistance and metabolic syndrome elevating the level of BCAA slead stop ositive effects that improve metabolic parameters such as body composition, glycaemia levels, and satiety. Hypothalamic leucine is a potential nutrient signal that may reduce food intake by activating mammalian target of rapamycin (mTOR) [13]. BCAAs control hormone release in both the gastrointestinal tract and in fat deposits. Treatment with leucine for six weeks increased adiponectin and decreased cholesterol in the plasma of previously obese mice, without changing body weight or fat mass [14]. Considering that BCAAs are essential amino acids that cannot be synthesized de novo in organisms. The level of circulating BCAAs could be contributed to by dietary intake and by degradation of protein in tissue.

Thenextstageofourworkwastoanalyzecontain of lysine and other essential amino acids. These changesin comprising with control group were shown on the Fig.2.

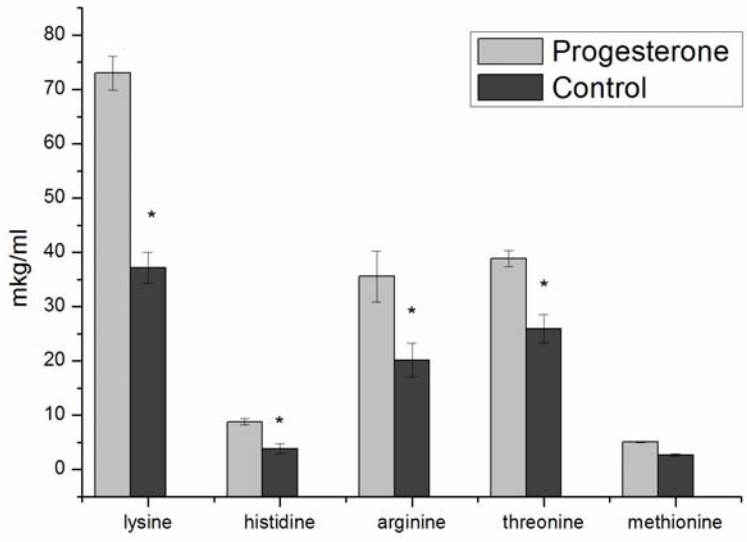

Fig. 2. Lysine, histidine, arginine, threonine, methionine levelslevels in serum of rats under progesterone long-term administration compared to the control group

Data are shown as the mean ± SD of 10 animals; asterisk (*), p < 0.05 in comparison with control group

Investigations were shown decrease concentration of lysine in blood serum of rats that were administrated by progesterone in 1,58 time compared with control group of animals. Also, long-term progesterone administration have led to decreasing of histidine and arginine contains in 1,5 and 1.96 times respectively.

Investigations of threonine blood serum concentration were shown decreasing of this index in group of animal with progesterone induced obesity in 2,27 time compared with control group (Fig.2). Also, decrease of methionine concentrationhas been shown.

Decrease of essential amino acids levels in blood serum of animals under long-term administration of progesterone correlated with previous studies [15].

Consistent with these results, other studies were shown that total body fat was decreased significantly by deprivation of Phe, Thr, Trp or Met in comparison with the control mice. Deficiency of any of these five EAAs for 7 days resulted in significant reduction in body weight, to the smallest extent by Lys deprivation. Similar changes were observed for abdominal fat mass [15]. There was report about in crease in the concentration of l-homo arginine (hArg) in the maternal plasma during human pregnancy. This observation, along with a well-known function of h-Arg, the methylene homologue of l-arginine (Arg), as a substrate fornitric oxide (NO) synthase, wastheigniti on for the start of intense research on the physiology and pathology of h-Arg. The circulating concentration of h-Arg was found to below patients suffering from various diseases, And h-Arg emerged with in only very few years as a novel cardiovascular risk factor [16]. Consentration of progesterone also increases during the pregnancy. This natural correlation can explain effects of progesterone long-term administration on hArg and Arg levels.

Aromatic amino acids contain is important index in diagnostics of numbers of diseases. Thus, the next stage of our work was investigating of aromatic amino acids concentrations in blood serum of rats under artificial progesterone-induced obesity (Fig.3).

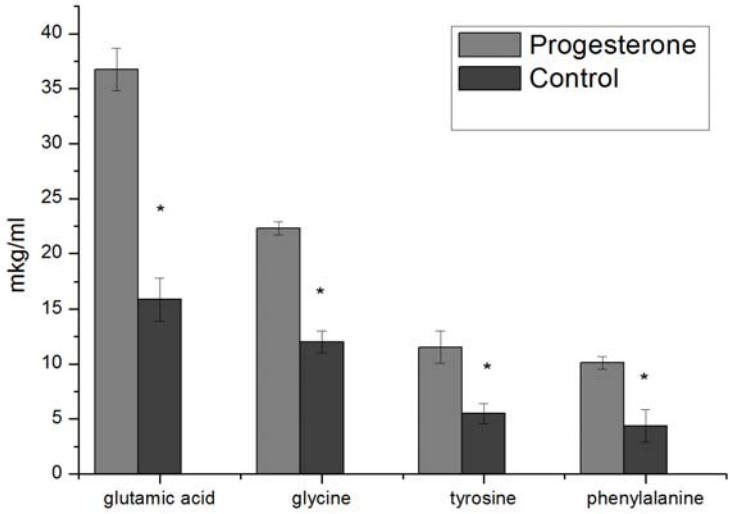

Fig. 3. Glutamic acid, glycine, tyrosine, phenylalanine levels in serum of rats under progesterone long-term administration compared to the control group

Data are shown as the mean ± SD of 10 animals; asterisk (*), $p < 0.05$ in comparison with control group

28-days progesterone administration have led to deacerase of phenylalanine contain at 1,5 compared with control group. Investigation of histidine blood serum contain have shown decrease of this index in 1,9 time. Decrease of tyrosine blood serum contain at 2,3 time have been shown.

Aromatic amino acids are the substrates for synthesis of neurotransmitters. Changes of content of aromatic amino acids can bere as on of disfunction of synthesis of this neurotransmitters. Significant decrease of some amino acids, in particular BCAAs and aromatic, canshow disorders of transport processes and metabolism of amino acids. This, directly or mediated, leads to disorders in a row of neurotransmitters and hormonal systems. Cutteve of in crease of content of row of amino acid, in particular BcAA aromatic, can specify on credible violation of mechanisms of transport and metabolism of amino acid, that straight and mediated can resultin a disbalance in process row of neyromediatornikh and hormonal systems.

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Recent study was shown that dietary methion in erestriction produces a highly beneficial metabolic phenol type by increasing energy expenditure, limiting fat deposition, And enhancing insulin sensitivity [17]. That work makes a compelling case that liver and adipose tissue are key targets of the diet, where the transcriptional responses effectively remodel the integration of lipid metabolism between the two tissues. For example, the MR diet diminishes the capacity of the liver to synthesize and export lipid, while simultaneously increasing these functions in white adipose tissue.

This results shown significant influence of long-term progesterone administration on blood serum levels of most amino acids.

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ВМІСТ АМІНОКИСЛОТ У ЩУРІВ ЗА УМОВ ДОВГОТРИВАЛОГО ВВЕДЕННЯ ПРОГЕСТЕРОНУ

Було визначено рівень амінокислот у сироватці крові щурів за умов розвитку ожиріння, індукованого введенням прогестерону. Дослідження показали, що має місце зниженнярівнів більшості амінокислотв сироватці крові щурів хворих ожирінням, яке було індуковане введенням прогестерону, у порівнянні зі здоровою контрольною групою щурів. Отримані дані свідчать про те, що довготривале введення прогесетрону значно впливає на метаболізм амінокислот.

Ключові слова: амінокислоти, прогестерон, ожиріння.

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СОДЕРЖАНИЕ АМИНОКИСЛОТ У КРЫС В УСЛОВИЯХ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ ПРОГЕСТЕРОНА

Было определено уровеньаминоислот в сыроватке крови крыс при условиях развития ожирения, индуцированного введением прогестерона. Исследования показали, что имеет место понижение уровней большинства аминокислот в сыворотке крови крыс больных ожирением, которое индуцировалось введением прогестерона, в сравнении со здоровой контрольной группой крыс. Полученные данные свидетельствуют о том, что длительноевведениепрогестероназначительновлияет на метаболизмаминокислот.

Ключевые слова:аминокислоти, прогестерон, ожирение.