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Rat Serum Amino Acid Content under Conditions of Obesity Caused By Separate and Combined Consumption of High-Calorie Diet And 10% Fructose Solution.

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ABSTRACT

Urgency of obesity problem lies in the fact that the number of people who are overweight is increasing progressively. The high prevalence, disability and early mortality in obesity and low efficiency of overweight correction and treatment modern methods are the main forcing factor to seek new therapeutic approaches. The majority of weight normalization methods eliminate the consequence but not the cause of the disease. The aim of this work was to investigate the content of amino acids in the serum of rats under conditions of obesity-induced consumption of high-calorie diet, consumption of 10% solution of fructose and consistent intake of high-calorie diet and 10% fructose solution. During the research, we have shown changes in tryptophan content. Our results have shown an increase of tryptophan content in the blood and a decrease in the brain of rats in experimental groups. So the next step was to investigate the blood content of amino acids that compete with tryptophan for ways of passing the blood-brain barrier. Our studies indicate the involvement of amino acids in reducing the amount of serotonin in the brain of rats, which probably influences the development of obesity.

Keywords: Obesity, high-calorie diet, 10% fructose solution, amino acids, tryptophan, serum rats

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INTRODUCTION

Obesity is among the most prominent global public health concerns of our time. The epidemic dimension of obesity prevalence and related disorders represents an increasing problem in both developed and developing countries [1].

The underlying mechanisms, which cause the development of obesity and increase obesity-associated insulin resistance remain unknown. Today it is believed that understanding the problem is focused on the control of blood glucose. Indeed, the essential mechanisms of glucose uptake studies in cells, mechanisms of glucose-stimulated insulin secretion, and characterize the natural history and epidemiology of type 2 diabetes [2]. Although the risk of diabetes is higher in people with overweight and obesity [3], these patients have perfectly normal blood glucose. In this connection it is necessary to go beyond studies of glucose metabolism only when considering the etiology and consequences of insulin resistance. Insulin resistance is condition of a broad perturbation of metabolic physiology involving considerable changes in fat and amino acid metabolism in addition to glucose.

Reducing body weight requires manipulation of the energy balance equation to produce energy deficits. This can be accomplished through diet and exercise, pharmacological interventions, or surgical means. However, each of these methods comes with disadvantages. For instance, many diet and exercise lifestyle interventions suffer from a lack of long-term (≥1 yr) adherence [4]. Furthermore, pharmacological and/or surgical means to reduce body weight are typically expensive and are sometimes accompanied by potentially unpleasant and/or dangerous side effects [5,6]. As such, consideration of alternative weight loss methods is warranted.

Diet is certainly important in the incidence of obesity and its negative consequences, such as cancer [7], aging [8], cardiovascular disease [9,10] and a number of other pathological conditions [11,12], but the mechanisms responsible for these pathological changes are not yet clearly elucidated. Therefore, finding safe and effective methods for reducing body weight in obese individuals is essential.

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.

Animals and housing conditions

Stadies conducted on 60 Wistar rats and divided to four groups of 15 animals each. The animals of each experimental group were individually 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%.

Animals and diet

Rats of group 1 (Control) were given water ad libitum and were fed by a standard chow during 70 days of the experimental period. Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week.

The (Fr10) group was fed by standard Purina chow and received 10% fructose in drinking water ad libitum during 70 days of the experimental period [13]. Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week. Rats of group 3 (HCD) were fed by a high-carbohydrate diet, which contained: standard chow (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), vegetable oil (1%) and water ad libitum [14]. Food consumption was measured daily at the same time (09:00 to 10:00 h). The body weights were determined once a week.

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The (HCD_Fr10) group was fed by, a high-carbohydrate diet, which contained: standard chow (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), vegetable oil (1%) and received ad libitum 10% sucrose in its drinking water during 70 days of the experimental period. Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week.

Biochemical, anthropometrical and nutritional determinations

Body length of all animals was measured; body mass index (BMI) (the ratio of body weight (g) of rats to the square of the body length (cm2)) and Lee obesity index (the ratio of 1/4 of cube root of body weight (g) to body nose-to-anus length (cm)) were calculated [15].

After 70 days of the the experimental period the animals were sacrificed. The rats' blood was collected in tubes, brain was removed and weighed. Blood samples were kept at a temperature of 38°C for at least 40 min and centrifuged for 15 min at 1,000×g, followed by collecting of serum. Brain was homogenized in the TRIS-acetic buffer at the rate of 5 ml buffer per half of the brain. Homogenate was centrifuged for 15 min, and then the supernatant was harvested for further research.

The tryptophan content was determined in serum and brain using ion-exchange chromatography and fluorescence methods which were described previously [16,17]. Amino acid composition was determined by an automatic amino acid analyzer T-339 ("Microtekno", Czech Republic).

Statistics

Statistical analysis of data was carried out by the software package 'Statistica 7.0'. 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

Serotonin neurons in the brain participate in the control of appetite. In general, serotonin neurons function in neuronal circuits that diminish food intake [18-21]. In previous studies conducted on animals with type 2 diabetes, we have shown the involvement of serotonin biosynthetic pathway in general and in particular in the development of this disease [22]. As obesity increases the risk of diabetes, it was appropriate to transfer data from a that study on models of obesity.

Treatments that enhance serotonin function reduce food intake, whereas those that diminish serotonin function stimulate food intake [23,24]. The synthesis of serotonin in the brain is controlled in part by the availability of its amino acid precursor, L-tryptophan. Protein molar rate of tryptophan is 1.1%, making it the rarest amino acid found in proteins. So in the course of research, we have determined the content of tryptophan in serum and brain of experimental and control rats. Studies have shown an increase in the content of tryptophan in the serum of all experimental groups compared with the control group (fig. 1).

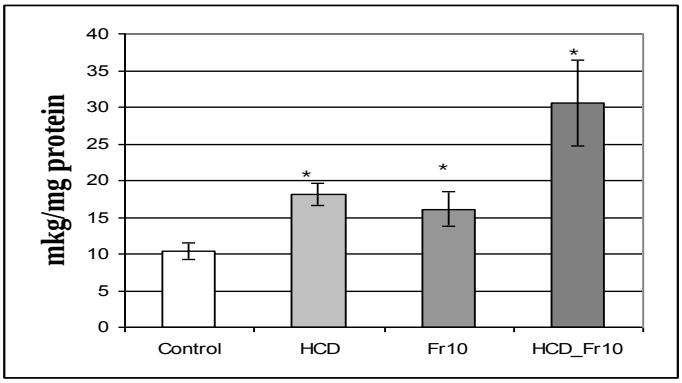

Figure 1: The content of tryptophan in serum of obesity rats
*p<0.05 compared to the control.

These results indicate an increase in the content of tryptophan in serum in all investigated groups of animals. In the group of animals that were on high calorie diet the studied parameter increased by 1.7 times compared to the control value. The tryptophan content in the group of animals that consumed 10% solution of fructose increased by 1.6 times. Joint high calorie diet and 10% fructose solution consumption have led to increase the tryptophan content in 2.9 times compared with the values of the control group.

The same trend was observed in the study of tryptophan content in blood serum of rats with type 2 diabetes. Obese patients who received tryptophan 750 mg per os twice a day had significant weight loss, compared with a placebo group [24]. The study have shown why obese people often have uncontrollable appetites – they have an insufficient amount of tryptophan in relation to other amino acids in their blood. However, we have shown hte increase of this indicator content.

Tryptophan in blood is found in two forms: free and bound to albumin. Tryptophan-albumin complex cannot pass the blood-brain barrier, the remaining free tryptophan can easily cross it and enter the brain. The determining factor of the free tryptophan and tryptophan-albumin complex ratio is the plasma concentration of free fatty acids, which are also bind to albumin. According to the literature, an increase in the concentration of free fatty acids in obesity, which form a complex with albumin, can displace tryptophan from its binding, resulting in increased content of free tryptophan in plasma. Another reason for the increase in the content of tryptophan in the blood serum of rats with obesity can be caused by the competition with other large neutral amino acids on the path of the blood-brain barrier.

Tryptophan is transported across the blood-brain barrier by a specific carrier for which tryptophan and all other large neutral amino acids also compete. Some studies have shown that the level of brain tryptophan depends on the ratio between plasma concentrations of free tryptophan and other LNAAs principally tyrosine, phenylalanine, and the branched-chain amino acids such as: leucine, isoleucine, and valine [26]. While others [27] have shown that the ratio of total tryptophan and other LNAAs in serum is a better predicting parameter for brain tryptophan levels. Therefore, on the next stage of work, we have determined the tryptophan content in the brain of rats under conditions of obesity.

As seen in Figure 2, obesity causes a decrease in the tryptophan content in the brain of rats compared to control. A decrease in the tryptophan content was by 1.5 and 2.6 times in the group of animals that were on high-calorie diet and that consumed 10% solution of fructose, respectively. Also it was shown a decrease of this index by 3.1 times in terms of the combined consumption of 10% solution of fructose and high-calorie diet compared to the control value.

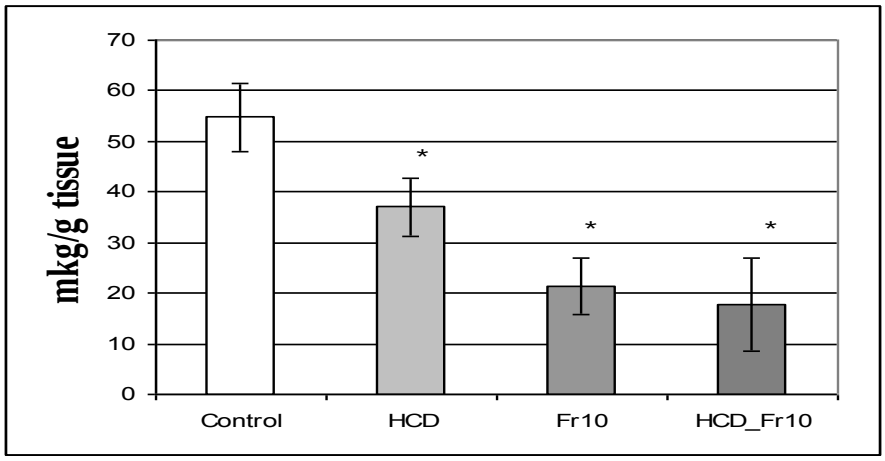

Figure 2: The content of tryptophan in brain of obesity rats
*p<0.05 compared to the control.

Tryptophan pools in the brain, in turn, are influenced by the uptake of tryptophan from the circulation. Uptake process depends on a competitive, saturable transport carrier shared by tryptophan and several other large neutral amino acids (tyrosine, phenylalanine, and the branched-chain amino acids). In humans, there are 9 essential amino acids, 3 of which are valine, isoleucine and leucine, which compose

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BCAAs. They form nearly 40% of the preformed amino acids required by mammals. Also valine, isoleucine and leucine form approximately 35% of the essential amino acids in muscle proteins. BCCAs are important nutrient signals increasing insulin secretion in islet beta cells and mammalian mTOR signaling in most tissues. So the next our step was to investigate the content of branched-chain amino acids in the blood of rats (fig. 3). We have shown an increase of valine, isoleucine and leucine contents by 1.5, 1.7 and 1.3 times, respectively under conditions of high calorie diet consumption by rats compared with the control group. It was shown an increase of valine, leucine and isoleucine content in 1.9, 1.8 and 1.6 times, respectively in the group of rats with obesity induced by the consumption of 10% solution of fructose. Under conditions of high calorie diet consumption combined with 10% fructose solution no significant changes the contents of the studied amino acids were observed.

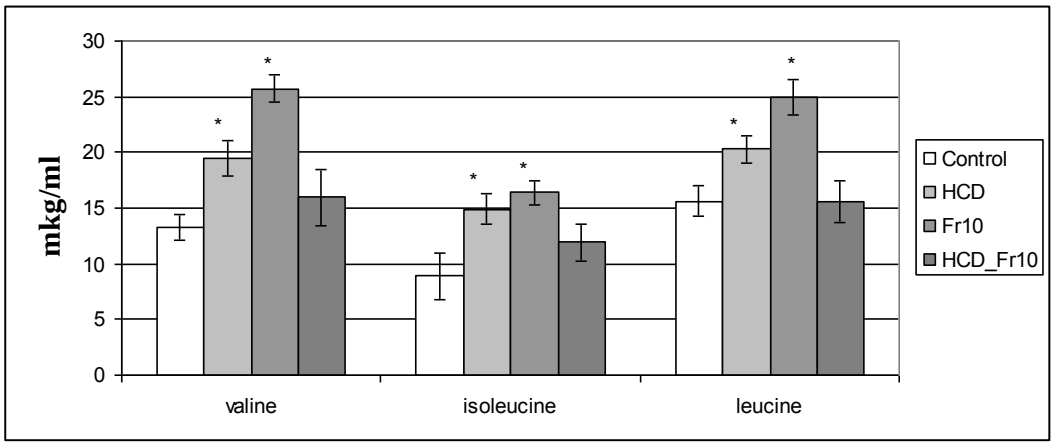

Figure 3: The content of BCAA in serum of obesity rats

*p<0.05 compared to the control.

Our studies have shown an increased content of amino acids that compete with tryptophan for passage of blood-brain barrier. Assuming that chronic elevations in BCAA impair transport of aromatic amino acids into cells and tissues, this could contribute to reduced production of neurotransmitters such as serotonin (derived from tryptophan) and catecholamines (derived from phenylalanine and tyrosine) in the central nervous system. Indeed, imbalances in the serum molar ratio of tryptophan (Trp) to its large neutral amino acid competitors (sum of Phe + Leu + Ile + Val + Tyr), the so-called “tryptophan ratio” have been related to depression [28,29] and obesity[30,31].

A high rate of flux through BCAA catabolic pathways and accumulation of glutamate may increase transamination of pyruvate to alanine. Increases in alanine, a highly gluconeogenic amino acid, could contribute to development of glucose intolerance in obesity. Our study has shown a higher content of alanine in the serum of rats under conditions of obesity (Table 1).

Table 1: The content of amino acids (mkg/ml) in serum of obesity rats

	Control	HCD	Fr10	HCD_Fr10
ornithine	8,25±0,88	6,79±1,19	10,38±0,94*	3,39±0,84*
aspartic acid	7,12±0,25	7,92±0,97	10,29±0,57*	5,15±0,84*
serine	16,01±1,77	32,92±4,86*	37,37±3,01*	28,47±2,75*
proline	11,51±0,59	14,37±2,46	14,36±1,99	21,56±1,05*
alanine	27,05±2,06	30,54±4,05	66,31±2,96*	36,65±4,98*
cysteine	1,43±0,15	2,86±0,28*	2,86±0,37*	2,85±0,30*
glutamine	47,97±2,11	93,86±3,41*	108,46±6,01*	45,89±6,09

*p<0.05 compared to the control.

In obesity, nutrient overload, especially fat consumption, has often been considered the major cause, but increased protein intake can also contribute through elevated levels of circulating amino acids [32]. These excesses are detected by the nutrient sensitive kinase, mammalian target of rapamycin complex 1 (mTORC1), which is a master regulator of protein synthesis, lipid synthesis, gene transcription and autophagy pathways. Activation of mTORC1 is dependent upon the availability of sufficient concentrations of amino acids, especially

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the branched chain amino acids[32,33]. The activity of mTORC1 is modulated by three major pathways. One pathway involves the branched chain amino acids, especially leucine. BCAAs have been associated with the stimulation of skeletal muscle protein synthesis via mTORC1 [34]. Leucine promotes the translocation of inactive mTORC1 to the lysosomal compartments which contain activated Rheb. Leucine has also been shown to interact with the AMPK pathway. In a study using rat skeletal muscle, elevated concentrations of both leucine and glucose were found to decrease AMPK kinase activity, increase protein synthesis and mTORC1 activity and cause insulin resistance [35]. This suggests that a common mechanism exists wherein glucose and leucine modulate mTORC1 activity, protein synthesis and insulin resistance.

So the next step was to analyze the content of lysine and other essential amino acids. We wave shown an increase in the content of all investigated essential amino acids compared to control, as seen in Figure 4.

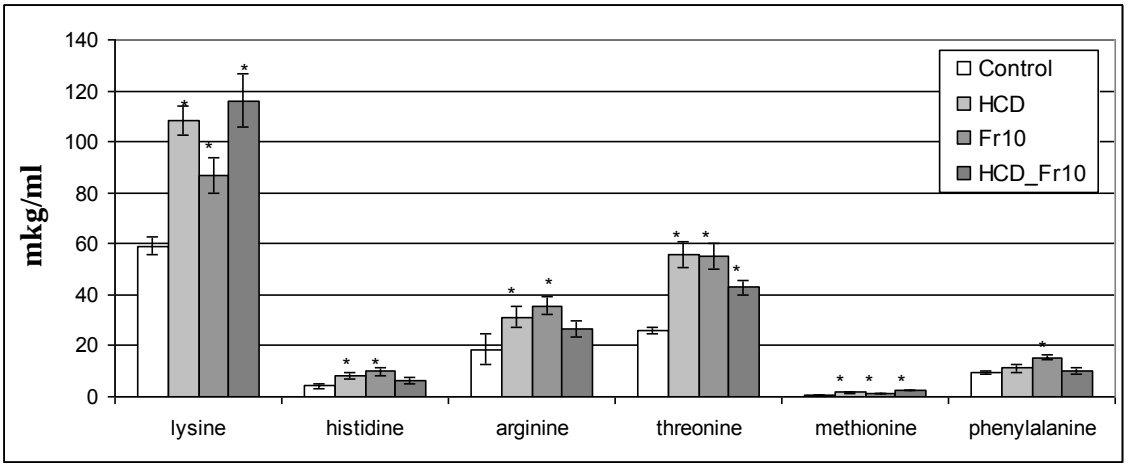

Figure 4: The content of essential amino acids in serum of obesity rats
*p<0.05 compared to the control.

Because of competitive transport, brain tryptophan uptake and, ultimately, serotonin synthesis are influenced by plasma concentrations not only of tryptophan but also of the other LNAAs. The plasma ratio of the concentration of tryptophan to the sum of the concentrations of the other LNAAs (tyrosine + phenylalanine + leucine + isoleucine + valine) (the plasma tryptophan ratio), which summarizes this competitive relation, has been proven to be a useful and reliable predictor of brain tryptophan uptake and central serotonin synthesis [36,37].

In further studies we have found an increase in the content of glutamic acid and glycine under conditions of obesity-induced consumption of high-calorie diet and a separate solution of fructose. However, the increase in the content of tyrosine is observed only in the group of rats that consumed 10% solution of fructose (fig. 5).

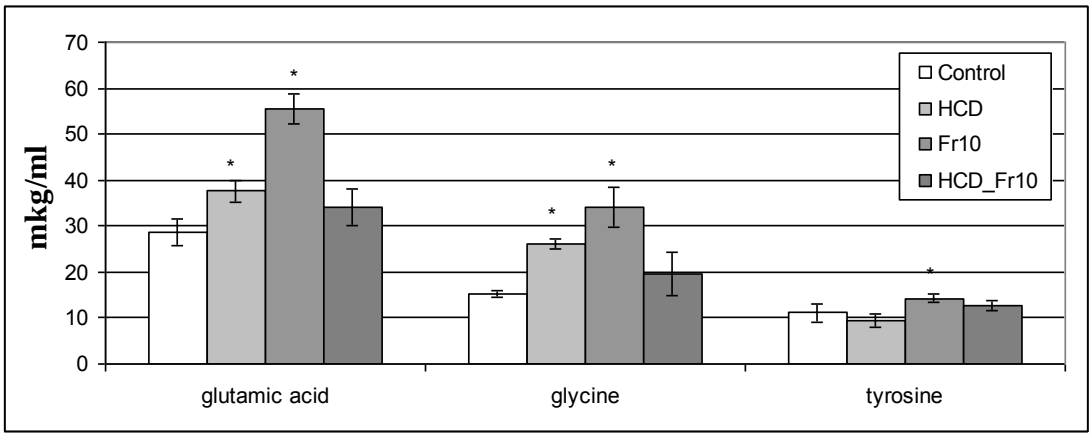

Figure 5. The content of amino acids in serum of obesity rats
*p<0.05 compared to the control.

The aromatic amino acids, phenylalanine and tyrosine are elevated in obese rats compared to lean subjects. This may be explained by the fact that the “large neutral amino acids”, which include both BCAA and aromatic amino acids, compete for transport into mammalian cells by the large neutral amino acid transporter (LAT1)

We have found no significant changes in the ornithine, aspartic acid, proline, alanine content in the blood of rats that consumed high-calorie diet. However, in terms of consumption of 10% solution of fructose and joint consumption of 10% solution of fructose and high-calorie diet we have observed changes in the content of all the named amino acids.

CONCLUSIONS

Regarding LNAAs, particularly the BCAAs, elevations in their plasma concentrations, where present, could be due to insulin insensitivity. Insulin secretion lowers plasma concentrations of BCAAs [38], probably by promoting their uptake into peripheral tissues [39, 40]. The development of insulin insensitivity for amino acids in obesity could raise plasma BCAA concentrations. Our results are consistent with the results of Wang, who has shown in his studies an increased risk of diabetes and insulin resistance under conditions of high content of BCAAs.

Obesity come with widespread metabolic perturbations that affect the entire body and the branched chain amino acids appear to be among the most distinctly perturbed metabolites. Monitoring their levels may have important clinical implications. Since, they play an important and critical role in a wide range of physiological processes. A healthy and disease state has many competing energy and proliferative demands, and complicates the use of BCAAs as biomarkers. The main requirement for the use of BCAAs in the diagnosis, monitoring and forecasting of disease is the presence of additional information. This information may include carbohydrate analysis (glucose and insulin levels), nutritional and dietary factors, along with weight status.

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