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Serum Serotonin And Other Biochemical Parameters In Conditions Of High-Calorie Diet In Rats.

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ABSTRACT

Serotonin, also known as 5-hydroxytryptamine (5-HT), is involved in formation of satiety and maintenance of a normal energy homeostasis. The study was conducted on white nonlinear rats with initial weight 200-215 g. Experimental obesity was simulated by a long-term (15 weeks) feeding with a high calorific food. In order to study the mechanism of obesity development in the groups of experimental animals, 5 rats were being randomly selected every 3 weeks from baseline, to obtain biological material. Our analysis confirmed the implication of serotoninergic system in conditions of obesity alterations and found the augment level of tryptophan and serotonin in serum of obese rats. It was established the more intense synthesis of GLUT-4 in adipocytes. There were also observed multiple changes of insulin receptors content: decrease in adipocytes membrane fractions of obese rats and increase in adipocytes cytosol that may be a result of cell walls damage. It is known that insulin is a key regulator of intracellular translocation process of the GLUT-4 to plasma membrane, but also serotonin possesses such effect. Serotonin can influence the glucose distribution and deposition as fat in adipocytes in conditions of high-calorie diet.

Keywords: serotonin, GLUT-4, high-fat diet, insulin receptor, obesity.

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INTRODUCTION

Over the past decades, the problem of overweight and obesity has become of a global medical and social significance [1]. In economically developed countries, nearly 50% of the population suffers from overweight, while 30% of them suffers from obesity. The wide spread occurrence, threat of disability among the working age patients and extremely high mortality, resulted from obesity, require additional efforts aimed at establishing the pathophysiological mechanisms of this condition in order to timely identify and implement large-scale preventive and curative measures.

Obesity is a complex, multifactorial disease, characterized by excessive accumulation of fat (triglycerides) in different parts of the body, followed by the development of complications. The external (nutrition, physical activity level), psychological inheritance, and medical factors, these all affect the body weight and distribution of adipose tissue in the body to a different extent [2]. It is proved that weight is the subject of a complex neurophysiological regulation, where neurotransmitters play a special role: serotonin, norepinephrine and dopamine – biogenic amines, involved in the regulation of feeding behavior [3, 4].

Serotonin, also known as 5-hydroxytryptamine (5-HT), is involved in formation of satiety and maintenance of a normal energy homeostasis [5]. In these latter days, much attention has been focused on establishing causal links between obesity and changes in functioning of the neurotransmitter. Nowadays, it is a proved fact that obesity causes inefficiency of serotonergic systems in the brain: reduced synthesis of serotonin, its increased binding to receptors and increased efficiency of its reuptake. As a result, concentration of serotonin in the synaptic cleft decreases, leading to changes in eating behavior – the patient's hunger is based rather on need to stimulate the serotonergic central nervous system than on the basal metabolism [6]. Nowadays, serotonin's involvement in the brain pathogenetic mechanisms of obesity is more studied and understood, while the role of circulating serotonin in regulation of metabolic processes, including support for carbohydrate and fat homeostasis, is not fully understood. To that end, researches in this area are absolutely relevant.

This study was aimed to determine relationship between the level of peripheral serotonin and the development of insulin resistance in rats with experimental model of obesity. To do this, they investigated the content of serotonin in blood and activity of enzymes involved in its biosynthesis and degradation on the one hand, and on the other hand, they identified a number of indicators, reflecting the development of insulin resistance in experimental rats.

METHODS

The study was conducted on white nonlinear rats with initial weight 200-215 g. Experimental obesity was simulated by a long-term (15 weeks) feeding of experimental animals with a high calorific food, which consisted of a standard feed (47%), sweet concentrated milk (44%), corn oil (8%) and plant starch (1%) (diet #C 11024, Research Dietes, New Brunswick, NJ). In order to study the mechanism of obesity development in the groups of experimental animals, 5 rats were being randomly selected every 3 weeks from the experiment start day, to obtain biological material. Serum was obtained from whole blood. The blood sample was left at 37º C for 4 hours and then centrifuged at 2000 g for 15 min. to remove fibrinogen and related proteins. Membrane fraction and cytosol of cells of adipose tissue of rats were obtained from the total homogenate by differential centrifuging method [7]. Additionally, they studied the performance of control rats (before the high-calorie diet - HCD), who were on a standard vivarium diet.

In the group of rats, involved in the experiment, they monitored fasting blood glucose concentration. Glucose content was determined using a glucometer "Hlyukofot II", manufactured by company "Norma" (Ukraine). The study was conducted according to the instructions of the device.

Biochemical analysis of blood serum was carried out under the enzymatic method, using a biochemical analyzer Statfax 1904 plus and test kits of Bio Meriex company (France).
Insulin level was determined in serum of fasting experimental animals under the ELISA method, using polyclonal antibodies against insulin. The anti-insulin antibodies fraction was obtained independently from serum of immunized rabbit, gradually purified using the affinity chromatography on columns of protein A-Sepharose (Amersham, USA) and insulin-Sepharose, which was prepared under the standard method of

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immobilization of ligands on the matrix using bromine cyan [8]. All procedures for obtaining, cleaning and testing of the antibodies specificity, were performed according to recommendations of standard protocols [9, 10].

Insulin resistance state in animals with experimental obesity was judged based on results of the insulin-tolerance test, performed on 15th week of the experiment [11]. To do this, we determined fasting glucose concentration in blood, and then the rats were administered intravenous injections of insulin solution ("Monodar", Ukraine) at the rate of 0.75 U per 1 kg of the animals mass. In 15, 30, and 60 min after administration of insulin, we sampled 100 ml of blood from the rats' tail vein, using an intravenous catheter, and measured the concentration of glucose. Based on the test results, we generated glycaemic curves, reflecting declining rate of the blood glucose level of experimental animals in response to exogenous insulin.

The serotonin and tryptophan contents were determined using ion-exchange chromatography and spectrofluorometric methods [12-14]. The content of insulin receptor (IR) and glucose transporter (GLUT-4) were determined under ELISA method, using commercial monoclonal antibodies against β-subunit of IR rats and polyclonal anti-GLUT-4 antibodies, respectively (Millipore, USA). Here, protein of cytosolic and membrane fractions of the rats' adipose tissue, in concentration of 10 mg/ml, preliminary solubilized in 1% non-ionic detergent Triton X-100, was used as antigen. The IR and GLUT-4 contents in the test samples were judged by adsorption values of corresponding specific antibodies.

Statistical analysis was performed using the Microsoft Excel statistical analysis software. The Student's parametric criterion was used for assessment of the inter-group differences. The difference between readings were considered as statistically significant at $p < 0.05$.

RESULTS AND THEIR DISCUSSIONS

Based on the tests, it was determined that an average weight of visceral fat in rats, exposed to a 15 weeks HCD, was 2-fold higher than in control animals (Fig. 1). These results suggest that by the end of the experiment, the tested animals suffered obesity, which was further confirmed by change of basic biochemical parameters of blood serum (Table 1).

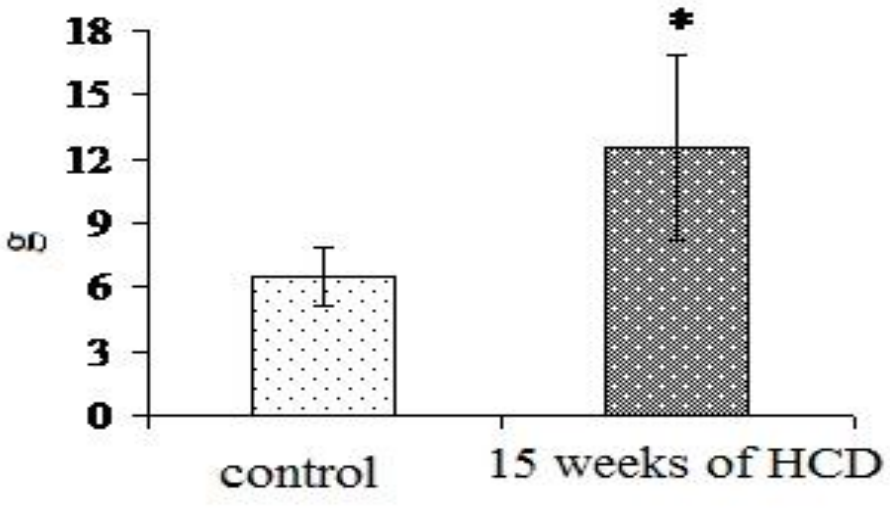

Figure 1: Visceral fat mass in control rats and animals, exposed to a high-calorie diet over 15 weeks; * – p < 0.05 as compared with control animals.

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Table 1: Basic biochemical parameters in blood serum of rats under conditions of long-term exposure to HCD

Value	Control	15th week
Glycosylated hemoglobin, micromoles of fructose/ g of hemoglobin	0.09 ± 0.02	0.326 ± 0.07*
Triglycerides, g/l	0.80 ± 0.12	1.13 ± 0.14*
Cholesterol, mmol/l	2.44 ± 0.59	1.56 ± 0.32
High-density lipoprotein, mmol/l	0.74 ± 0.12	0.40 ± 0.04*
Low-density lipoprotein, mmol/l	0.17 ± 0.03	0.18 ± 0.03
Aspartate transaminase, u/l	148.0 ± 13.5	160.3 ± 17.7
Alanine transaminase, u/l	27.1 ± 5.1	46.4 ± 7.9*
Bilirubin total, mmol/l	1.2 ± 0.3	2.0 ± 0.2*
Indirect bilirubin, mmol/l	0.30 ± 0.15	0.65 ± 0.05*
Total protein, g/l	84.4 ± 9.1	77.7 ± 5.6

* – p < 0.05 as compared with control animals.

Based on our results, the development of obesity in experimental group was accompanied by a decrease in sensitivity of peripheral tissues to the insulin hypoglycemic effect. This conclusion was made based on comparison of the insulin-tolerance test data, conducted in a control group of animals and rats, exposed to a 15 weeks HCD.

Judging from the above glycaemic curves, generated under the results of tests in rats with experimental obesity, the fasting glucose was significantly higher as compared with control animals (fig. 2). Besides, animals of the experimental group were reported to have a decreased rate of glucose uptake by peripheral tissues in response to exogenous insulin (fig. 2). These results can be explained by insulin resistance development.

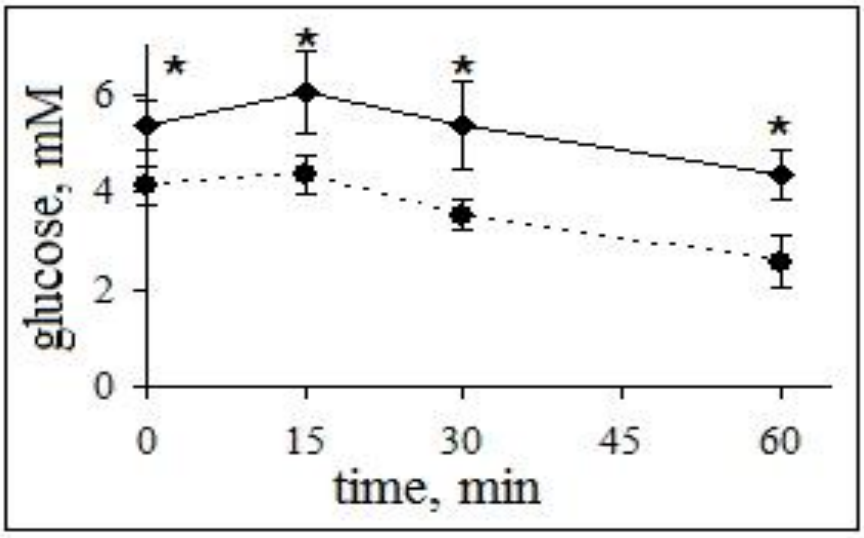

Figure 2: Glycaemic curves, obtained during insulin-tolerance test in a control group of animals (●- - -●) and rats, exposed to a 15 weeks high-calorie diet (◆——◆);
*** – p < 0.05 as compared with control animals.**

In our opinion, testing the values in experiment's dynamics will provide more information about the obesity development mechanism and will help to establish whether there is a relationship between formation of the insuline-resistance status and functioning of serotoninergic system under a long-term exposure of laboratory animals to HCD.

Study of glucose concentration in blood of experimental rats, confirmed that glucose level in blood during the dynamics, varied and not always was higher compared with control animals. Thus, concentration of glucose in blood of fasting rats, after 6 week of exposure to HCD, increased by 75% as comparing to the control group of animals, while in 9th and 12th weeks of experiment the value decreased to control values, and again it significantly increased only on 15th week of the experiment (fig. 3, A).

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The study of insulin content in blood of experimental rats confirmed that a long-term exposure of animals to HCD was accompanied by a progressive hyperinsulinemia (fig. 3, B). At the same time, content of insulin in the blood serum of rats with an obesity model (on 15th week of experiment) was 2 times higher than the present value of control animals (fig. 3, B).

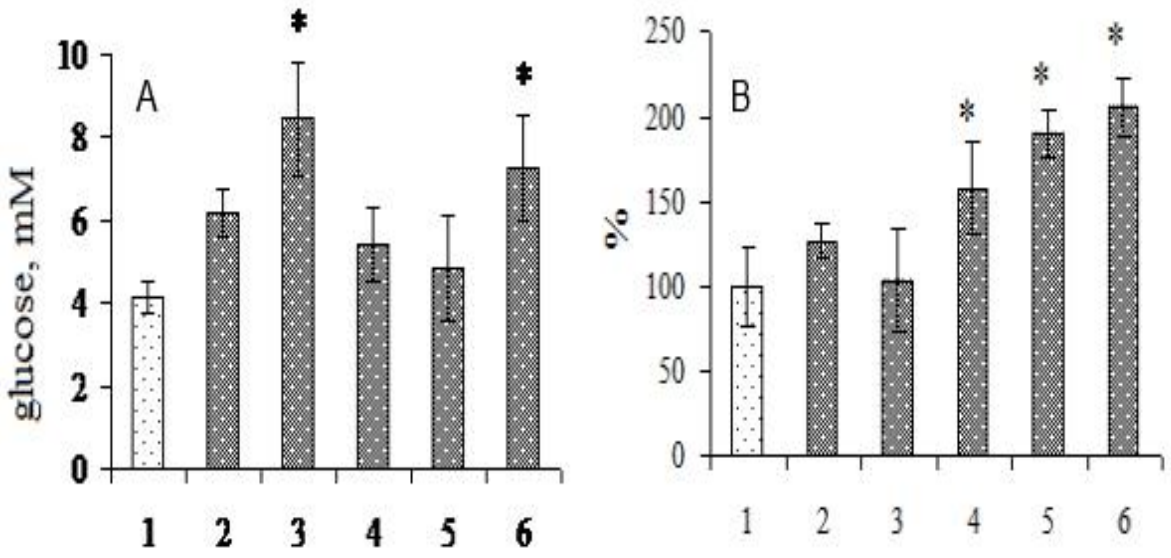

Figure 3: The concentration of glucose (A) and relative insulin content (B) in blood of experimental fasting rats: 1 – control, 2 – 3 weeks HCD, 3 – 6 weeks HCD, 4 – 9 weeks HCD, 5 – 12 weeks HCD, 6 – 15 weeks HCD; * – $p < 0.05$ as compared with control animals.

After data analysis of changed values of glucose and insulin content in the blood serum of experimental animals, it was concluded that the consumption of high-calorie foods can lead to hyperglycemia in response to which β-cells of the pancreas start secreting more insulin (fig. 3, B). Due to the hypoglycemic effects of the latter, the blood glucose level comes to normal for a while, which is confirmed by decrease of glucose level in blood on weeks 9 and 12 of the experiment (fig. 3, A). Increased concentration of glucose at later stages is probably a result of reduced sensitivity of peripheral tissues to biological effects of hormone, which, in general, is consistent with our results for development of insulin resistance in a group of experimental animals on week 15 of experiment (fig. 2).

Insulin is a hormone that is characterized by numerous biological effects. In addition to regulation of carbohydrate and lipid metabolism, insulin also affects the metabolism of proteins [15, 16]. Hence, according to reports, it increases the rate of receipt of various amino acids into the cells, which is associated with its anabolic effect [17, 18]. An interesting observation was described regarding influence of insulin on the tryptophan level in blood serum – the content of the amino acid was increasing in response to administration of exogenous insulin [19]. When attempting to identify a source, responsible for increase in tryptophan, it was assumed that insulin promoted release of the amino acid from liver and increased binding of tryptophan to albumin, which prevented its free flow to peripheral tissues [19]. It is also known that increased content of insulin in blood is accompanied by increased admission of tryptophan into brain, resulting from reduced effect of other circulating amino acids [20].

As far as we noted that development of obesity in experimental animals was accompanied by hyperinsulinemia, we wondered if the content of circulating tryptophan was changing under conditions of our experiment. Our tests confirmed increase of tryptophan content in blood serum of experimental animals, exposed to HCD. Thus, on week 3 of the experiment, the present value was 3 times increasing the value of control animals (fig. 4, A). Despite the gradual decrease of tryptophan in the blood serum of experimental animals, further, its level failed to reach control values and remained significantly high until the end of the

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experiment (fig. 4, A). Therefore, we can say that obesity is accompanied by increased content of tryptophan in blood serum, which may be a result of high levels of circulating insulin in the group of experimental animals.

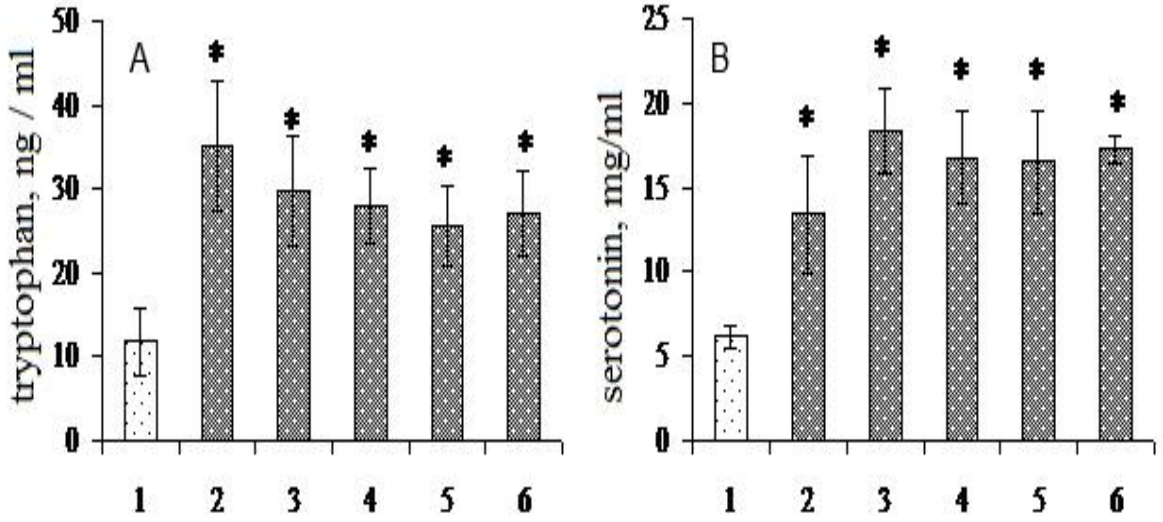

Figure 4: Concentration of tryptophan (A) and serotonin (B) in serum of experimental fasting rats: 1 – control, 2 – 3 weeks HCD, 3 – 6 weeks HCD, 4 – 9 weeks HCD, 5 – 12 weeks HCD, 6 – 15 weeks HCD;
*** – $p < 0.05$ as compared with control animals.**

Tryptophan is an essential amino acid that is a precursor in the synthesis of serotonin [21]. Therefore, our further logical research established serotonin content in blood serum of experimental animals. It was proved that exposure to HCD results in increasing of circulating serotonin content. Thus, on week 3 of HCD, its content was 2 times higher than in control animals, and on week 6 the value increased for another 40% as compared with the previous value, and remained at that level until the end of the experiment (fig. 4, B).

Thus, development of obesity in experimental animals is accompanied by increased serotonin content in blood serum, which is likely associated with high content of its precursor – tryptophan. It is interesting to note that serotonin was also regulating a number of metabolic processes, namely, it could affect the concentration of glucose in blood [22]. There are study confirming that serotonin could contribute to development of hyperglycemia, which, most likely, is the result of increased level of adrenal catecholamine [23]. However, the current prevailing opinion suggests that neurotransmitter is a hypoglycemic agent, and its antagonists, on the contrary, promote the increased concentrations of circulating glucose [24, 25]. The mechanism where serotonin promotes hypoglycemia, is not sufficiently understood at present, however, the proved data confirm that it is not associated with a change in content of insulin in blood, as far as level of circulating hormone never increases after intraperitoneal administration of tryptophan, 5-HT precursor [24, 25]. As an alternative hypotheses for the nature of hypoglycemic effect of 5-HT – the neurotransmitter is capable of influencing the glucose utilization process by cells of peripheral tissues, such as muscle and fat. Recent studies have shown that activation of 5-NT_{2A} receptors of myoblastoma were leading to increased expression of the glucose transporter gene GLUT-3 [21], which can be the immediate cause of increased glucose transport into cells. Other studies confirmed that serotonin precursors (e.g. 5- hydroxytryptophan) may lower glucose level in animals' blood within 1 hour after administration [25]. According to research findings of the post-receptor path of signal transduction from muscle 5-NT_{2A} receptors, stimulation of the receptors by both serotonin and specific 5-HT_{2A}-agonist, causes rapid activation of glucose transport by increasing its carriers as a part of the plasma membrane. However, as mentioned before, the key signaling proteins of insulin cascade (i.e. IRS1, PI3K, or PKBA) are not involved in the intracellular translocation process of glucose transporters, whereas signaling molecules of serotonin receptors remain unknown [26]. Thus, biochemical mechanisms of serotonin action as a hypoglycemic agent are still unknown.

Since there is a recorded evidence that serotonin activates a translocation process of intracellular glucose transporters to plasma membrane, where they act as channels for glucose to enter the cells, the purpose of our investigation was to find out whether the GLUT-4 content changes in subcellular fractions of

adipose tissue during the experiment. The adipose tissue has become the object of our study due to a direct link between the obesity pathogenesis and accumulation of masses of the tissue.

The research resulted in observation that no significant fluctuations in the GLUT-4 content in cytosol of cells of adipose tissue were noted during the experiment. The maximum deviation of the value from control level (down for 14%), was reported on week 9 of HCD. It is ought to note that instead, the GLUT-4 content in the plasma membranes of the adipose tissue cells increased by 26% on week 3 of HCD, as compared with the control group of animals. However, over the next weeks of experiment, they reported a gradual decrease of the value and complete return to its control level (fig. 5).

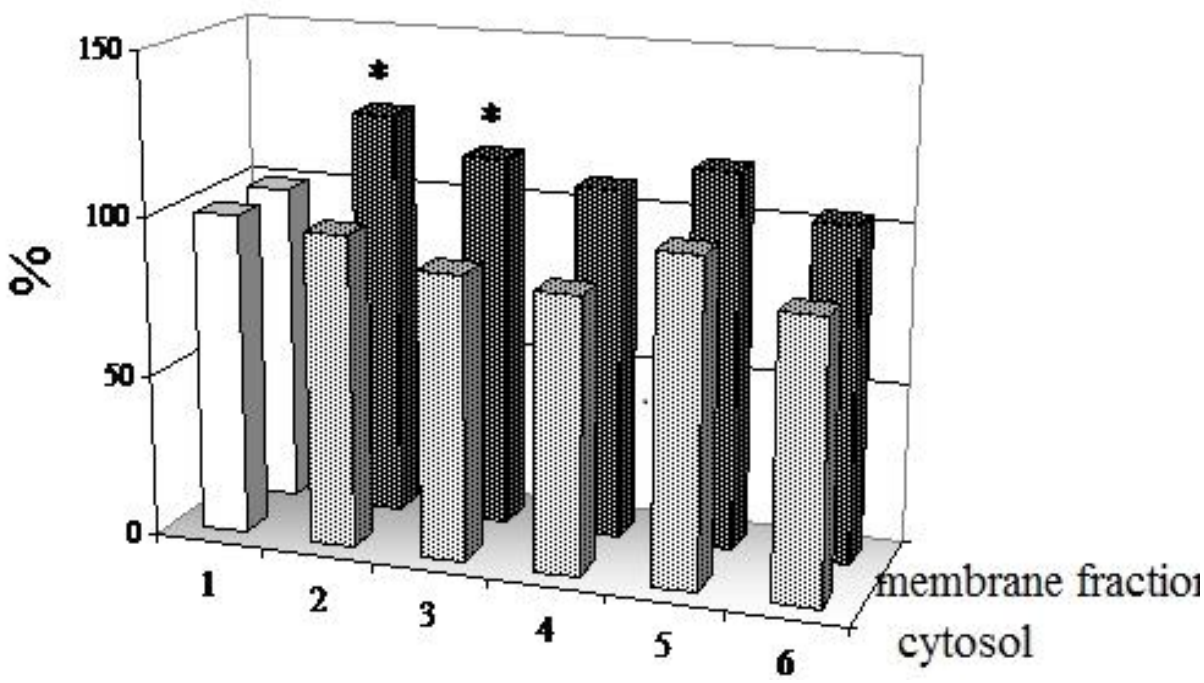

Figure 5: Relative content of GLUT-4 in membrane fraction and cytosol of cells of adipose tissue of rats at HCD: 1 – control, 2 – 3 weeks HCD, 3 – 6 weeks HCD, 4 – 9 weeks HCD, 5 – 12 weeks HCD, 6 – 15 weeks HCD; * – $p < 0.05$ as compared with control animals.

Of course, one cannot make a final conclusion on correlation between the circulating serotonin level and increased GLUT-4 content in plasma membranes of cells within adipose tissue of experimental animals. It is known that insulin is a key regulator of intracellular translocation process of the GLUT-4-containing vesicles, which regulates this process through the activation of the insulin receptor. To that end, high content of the glucose protein-transporter, being a part of plasma membranes, may result from increased level of circulating hormone in a group of experimental animals, which fact was reported during our experiment. Due to the fact that the key role in the insulin metabolic effects belongs to its receptor, we have investigated the content of insulin receptor (IR) being a part of plasma membrane and the cytosol of cells of adipose tissue.

Based on the performed tests, the following trend was noted. Thus, on week 6 of HCD, they reported reduction of IR in plasma membrane of cells within adipose tissue by 26% as compared with control animals. The lowest value (decrease for 35% as compared to control animals) was reported on week 9 of the experiment. Starting from week 12 of the high-calorie diet, they reported a gradual increase in IR content in membranes of the tested tissue, and on week 15, they reported only 10% difference between the values of control and experimental animals, however, it remained significantly lower as compared with control animals. During the first twelve weeks of experiment, they have reported no changes in IR content in the cytosol of cells of adipose tissue of rats, exposed to HCD. At the later stages of experiment (15^{th} week), they reported a significant increase in the IR content, by 25% as compared with control animals (fig. 6).

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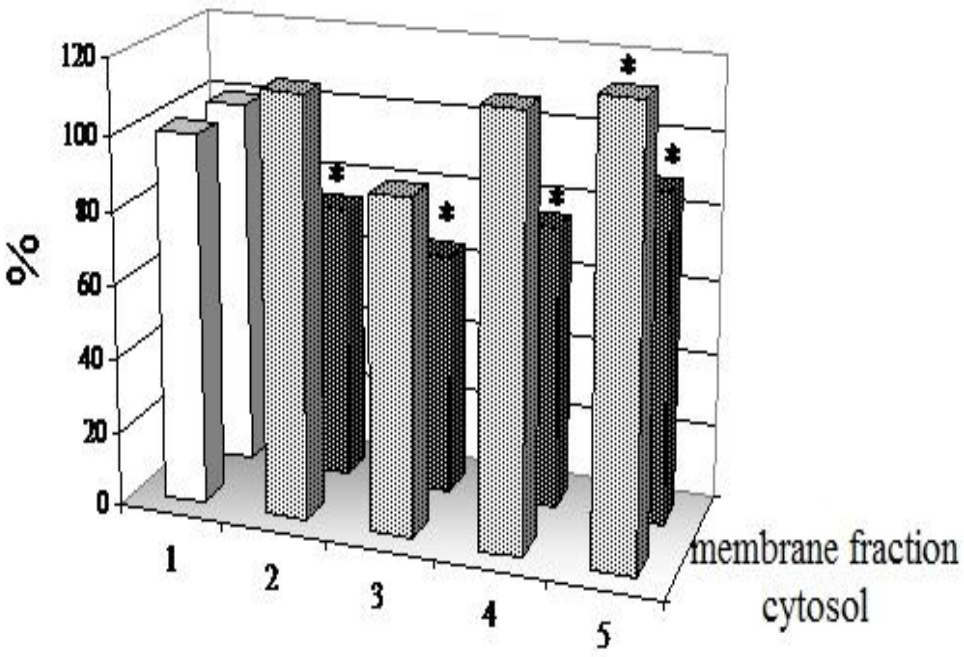

Figure 6: The relative content of insulin receptor in membrane fraction and cytosol of cells of adipose tissue of rats at HCD: 1 – control, 2 – 6 weeks HCD, 3 – 9 weeks HCD, 4 – 12 weeks HCD, 5 – 15 weeks HCD, 6 – 18 weeks HCD (no data for week 3);
*** – $p < 0.05$ as compared with control animals**

Thus, we have established multiple changes to IR content in membrane fractions and cytosol of cells of adipose tissue of rats, exposed to long-term HCD. Reduction of IR content in membranes of adipose tissue, on the one hand, can be caused by violation of integrity of the adipocyte lipid bilayer, resulting from activation of the peroxidation processes, which has been discussed in a number of papers, and on the other hand, it can result from a compensatory reaction in response to increase of insulin in the blood serum of experimental animals. Increase of IR content in the cytosol of cells of the tissue may indicate activation of protein synthesis, however, due to damage to the plasma membrane, there probably occur disturbance of intracellular translocation processes of IR, thereby the newly- synthesized receptor molecules cannot enter membrane and accumulate in cytosol.

CONCLUSION

Thus, we have found that a long-term consumption of high-calorie food can be a powerful factor, contributing to the complex changes of metabolic processes of a body and become a prerequisite for the formation of obesity. Our deliverables suggest that development of insulin resistance in experimental rats is an integral part of pathogenesis of the disease. A mechanism for occurrence of the insulin resistance state under obesity conditions is still not well studied. According to our data, apart from the classical pathogenic factors, including hyperglycemia and hyperinsulinemia, the increased content of circulating serotonin can be a prerequisite for reducing of the body tissues sensitivity to the insulin metabolic effect under the conditions of the tested pathology. This neurotransmitter, indirectly, through increased secretion of adrenaline, may cause the increased production of glucose by liver and decreased glucose utilization by tissues of the body, which can maintain a high blood glucose levels and promote a long-term hyperglycemia in experimental animals. Besides, serotonin, acting through 5-NT_{2A} receptors on the surface of adipocytes, can activate MAP kinase path, involved in the regulation of gene expression. This action may result in reduced expression of adiponectin, which is considered by some authors as an independent factor of insulin resistivity development. It should be noted that further research in this area is absolutely important, as far as understanding of serotonin involvement in development of obesity may be potentially useful in developing new strategies aimed at correcting carbohydrate metabolism under this pathological condition.

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