ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Food Behavior of Rats Under Development of Obesity.

Karpovets TP*, Konopelnyuk VV, Savchuk OM, and Ostapchenko LI.

ESC "Institute of Biology", Taras Shevchenko National University of Kyiv, Ukraine.

ABSTRACT

The effects of long-term consumption of 10% fructose solution, high calorie diet, and their combination have been studied. It is shown that a 10-week high calorie food and 10% fructose solution consumption by rats has led to the development of obesity as a result of changes in food behavior and basic biochemical parameters. It was concluded that these models are adequate and are close by biochemical parameters to human obesity and can be used in studies of the pathogenesis of obesity in animals.

Keywords: food behavior, obesity, fructose, high-calorie diet, rat

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Regulation of feeding behavior has always attracted the attention of physiologists, biochemists and physicians, but recently this line of research has gained special importance because of the growing problem of obesity. Obesity reduces life expectancy by an average of 3 - 5 years in patients with little excess weight and up to 15 years with marked symptoms of morbidity [1]. In fact, two out of three human death cases occur from diseases associated with disruption of lipid metabolism and obesity [2]. If humanity can solve the problem of obesity, the average life expectancy will increase by 4 years. Urgency of obesity problem lies in the fact that the number of people who are overweight is increasing progressively. This growth is 10% of the previous amount for every 10 years. It is estimated that if this trend continues, by the middle of next century the entire population of the developed countries would be obese [3, 4].

Despite such a pronounced problem, the current state of obesity treatment remains unsatisfactory. We know that most of those who need treatment cannot proceed to it because of the fear of the necessity to abide by long half-starved monotonous diets. Most fail to achieve normal body weight, and often results achieved are much lower than expected. Also, in most patients, even after successful treatment of relapse is observed that restores the original or even higher body weight. It is known that 90% of patients reduce their initial pre-treatment body weight at 6 months after it [5]. The prevention of obesity is also complicated. Although risk factors for this disease are almost certain, their use the prophylaxis is still quite limited.

Thus, the high prevalence, disability and early mortality in obesity and low efficiency of modern methods of correction and treatment of overweight are the main forcing factor to seek new therapeutic approaches. The majority of weight normalization methods eliminate the consequence, not the cause of the disease. Therefore, the study of various experimental models of obesity, which can be adequate on pathogenic levels to this disease in humans, is a perspective and promising trend in modern science.

The aim of this study was to investigate changes in eating behavior in rats under development of obesity, caused by different experimental models.

MATERIALS AND METHODS

Experiments were carried out on white nonlinear female rats with initial weighing of 135-140 g in compliance with the standards of the Convention of Bioethics of the Council of Europe in 1997, European Convention for the protection of vertebrate animals that used for experimental and other scientific purposes, the general ethical principles of animal experiments approved by first National Congress of Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this field.

During the first week, all rats received standard food «Purina rodent chow» and water ad libitum. On the 8th day the animals were randomly divided into 4 groups. Animals of the first group (“NC”) have been fed with a standard food and water for 10 weeks. Animals of the second group (“FR10”) have been fed with a standard food and 10% fructose solution instead of water ad libitum [6]. Animals of the third group ("HCD") were on a high-calorie diet, which consisted of a standard meal (60%), pork fat (10%), eggs (10%), sugar (9%), peanuts (5%), dry milk (5%) and sunflower oil (1%) and water ad libitum [7]. Animals of the fourth group (“HCD-FR10”) were on a high-calorie diet combined with 10% fructose solution instead of water ad libitum.

Body weights were recorded once a week. Feed and fluid (water or fructose solution) intake were recorded daily in all animal groups. Biochemical analysis of serum (content of total and direct bilirubin, creatinine, urea, uric acid, albumin and total protein, the activity of alanine aminotransferase (ALT), aspartat aminotransferase (AST), α-amylase and alkaline phosphatase) were carried out using semi-automatic biochemical analyzer Microlab 300 (Vital Scientific, The Netherlands).

Statistical analysis was performed using statistical analysis applications of Microsoft® Excel. To assess inter-group differences the parametric Student test was used. The difference between the parameters was considered statistically significant at $p<0,05$.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Studies indicate the development of obesity in rats in the experimental group compared with the control group of rats.

Figure 1 shows the dynamics of body weight increase in rats throughout the study period. The initial weight of the animals in the control group was 142±2,081 g, after 10 weeks of an experiment a gradual increase of this indicator was shown. On the last week of studies the weight of the animals in the control group was 229±1,154 g, which was 87 g more than the initial. In the group of rats that consumed 10% solution of fructose an increase of body weight by 105 g was recorded compared with the initial weight, which was 147±10,406 g. The initial weight of rats that were on a high-calorie diet was 145±17,481 g. Throughout the period of the development of obesity it was shown a gradual increase in body mass of rats and after 10 weeks of experiment the weight of animals was 263±13,631 g. Thus, the body weight of HCD group rats had been increased by 118 g compared with initial weight. The largest increase in body weight was observed in the group of rats that were on the combined consumption of 10% solution of fructose and high-calorie diet. The initial weight in this group was 145±9,377 g. The increase in body weight after 10 weeks of experiment was 126 g compared with the initial weight of the animals in the group.

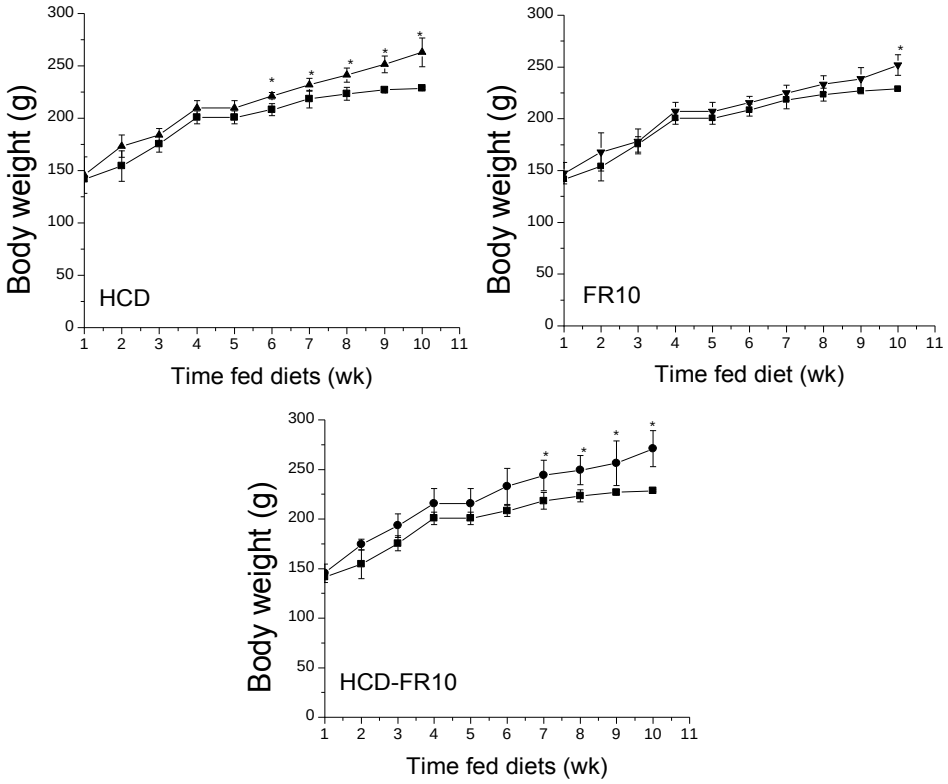

Figure 1: Body weight of rats in the "FR10", "HCD" and "HCD-FR10" groups compared with a control group of animals

1 – control group
2 – experimental group

Note: * - p <0.05 differences credible with respect to the control

Changes in body weight of all studied groups after 10 weeks of obesity were analyzed. In the course of these studies it was found the increase in body weight of rats in FR10 group on 24 g, in HCD group on 35 g, and in HCD-FR10 group on 43 g compared with the control group (Table 1).

ISSN: 0975-8585

Table 1. Basic characteristics of animals

	NC	FR10	HCD	HCD-FR10
Food intake (g)	15,7±1,283	13,2±1,249	18,9±2,795	15,6±2,875
Fluid intake (ml)	15,4±1,590	45,5±6,732	18,7±2,872	24,7±5,173
The initial weight (g)	142±2,081	147±10,406	145±17,481	145±9,377
The final weight (g)	229±1,154	252±10,066	263±13,631	271±18,126
BMI (r/см^2)	0,49	0,55	0,57	0,59

Thus, analyzing the changes in body weight in all study groups compared with the control group after 10 weeks of experiment, we can conclude that the most weight was gained by the group of animals, which was on the joint consumption of 10% solution of fructose and high-calorie diet.

The next stage of our work was to study the food intake by rats of all experimental groups. Feelings of appetite is being controlled by so-called "satiety factors" and "hunger substances" - leptin, arenterin, cholecystokinin, insulin, endorphins, norepinephrine and serotonin [8, 9]. When overeating, saturation center adapts to higher levels of glucose, insulin and leptin [10]. Its sensitivity to the stimulant effects is being reduced and the hunger center is not being inhibited causing the consumption of large quantities of food.

As shown in Figure 2, the control group of rats consumed an average of 16±2,875 g of standard feed per day. Group of rats that consumed 10% solution of fructose ate an average of 13±1,249 g of standard feed, which is 1.2 times less than the control group of animals. The rats that were on high calorie diet ate an average of 19 ± 2,795 g of high-calorie food, which is 1.2 times higher than control rats. However, the group of animals that consumed 10% solution of fructose combined with a high-calorie diet ate an average of 15,6 ± 1,283 g of feed, which actually does not differ from the values of the control group animals.

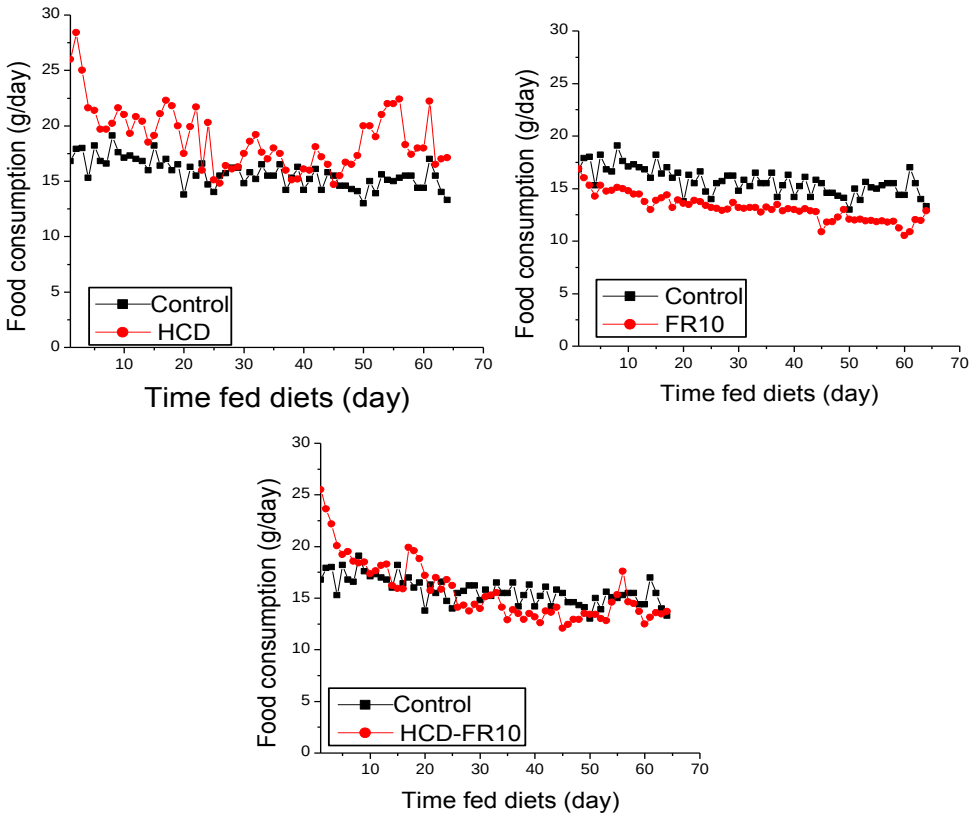

Figure 2: Food consumption by rats in the "FR10", "HCD" and "HCD-FR10" groups compared with a control group of animals

Humoral regulation of eating behavior is understudied. In the past two decades, there were studies showing the role of various neuropeptides and neurotransmitters of the nervous system in the regulation of food intake. It was found that the feeding behavior is being reduced under the increase activity of the catecholamines. This principle is used in catecholamine-agonist drugs [11- 13]. The role of other neurotransmitter – serotonin has been investigated either. In preliminary results, we have investigated the biosynthetic pathway of serotonin in the brain of rats that were on a high-calorie diet and have shown reduction of serotonin [14]. The secretion of serotonin is crucial in the formation of a feeling of satiety: it was shown that an increase of its level in hypothalamic structures causes a feeling of satiety and the reduction of eating behavior, and a decrease - on the contrary [15]. The anorectic effect of some serotonin agonists (fenfluramine, fluoxetine, sibutramine etc.) is based on the influence of serotonin on feeding behavior. These agonists increase the reuptake of the neurotransmitter into the synaptic cleft and reduce its procession by monoamine oxidase. Initially, these drugs were developed and offered as antidepressants and fluoxetine is widely used nowadays as antidepressant. Indeed, the reduction of cerebral serotonin causes depression. In previous studies we have shown changes in the content of serotonin in rats with type 2 diabetes under the administration of meta-Chlorophenylpiperazine [16]. Meta-Chlorophenylpiperazine (mCPP) is a chemical compound, a derivative of trazodone and nefazodone, which affects the regulation of the central nervous system associated with serotonin [17]. MCPP as an agonist of 5-HT1, 5-HT1A, 5-HT2 and 5-HT2C, and an antagonist of 5-HT2B, 5-HT3 serotonin receptors is widely used in studies of nervous disorders associated with impaired serotoninergic system [18, 19, 20, 21]. It also increases the serotonin transporter-dependent release of serotonin [22, 23, 24, 25]. Our results have showed that the mCPP enhances the release of serotonin via its transporter, inhibits the reuptake of serotonin, and enhances serotonergic transmission in the hypothalamus. This leads to a change in eating behavior and forcing animals to consume more food, not from the needs of the basal metabolism but because of the need to stimulate the central nervous system serotonergic system. It is known that sometimes depression can be accompanied by a strengthening of eating behavior and hyperphagia. Food leads to increased levels of brain serotonin and mood improvement. It is believed that some nutritional disorders like as hyperfagic stress response, and premenstrual hyperphagia are linked to the reduction of brain serotonin levels. Hyperphagia usually develops in smokers during nicotine withdrawal.

This is confirmed by the fact that the use of serotonin agonists effectively eliminates these types of hyperphagia.

Changes in fluid intake of experimental groups are shown in Figure 3. Animals that were on a high-calorie diet consumed water. Studies have found that HCD rats consumed an average of 18,7±2,872 ml of water per animal, which is 1.2 times higher than the control group of animals, which consumed an average of 15,4±1,591 ml. Group of animals that were on high-calorie diet combined with the 10% solution of fructose consumed 1.6 times more fluid (fructose) than the control group. Animals of the FR10 group consumed an average of 45,5±6,732 ml of fluid (fructose), which is 3 times higher than the value of the control group.

The next stage of our study was to determine the main parameters of biochemical analysis of serum, which can estimate the functional state of the organism and characterize the metabolic changes that occur on a background of experimental obesity.

Uric acid plays a significant role in the development of obesity. The increase in its content leads to a decrease in the bioavailability of nitric oxide, the development of endothelial dysfunction and, as a consequence the insulin resistance. It was shown the increase of uric acid in the serum of HCD and HCD-FR10 rats in 1.4, and 1.5 times respectively compared with a control group. No significant changes in the content of the studied parameter in the F10 group were observed. Important role in diagnostics plays the determination of urea in the blood. It was found the reduction of this parameter in 1.6, 1.2 and 2 times in the serum of rats of all experimental models of obesity. A 1.2-1.4 times increase compared with control in creatinine and albumin levels in serum has been found in all obesity groups.

ISSN: 0975-8585

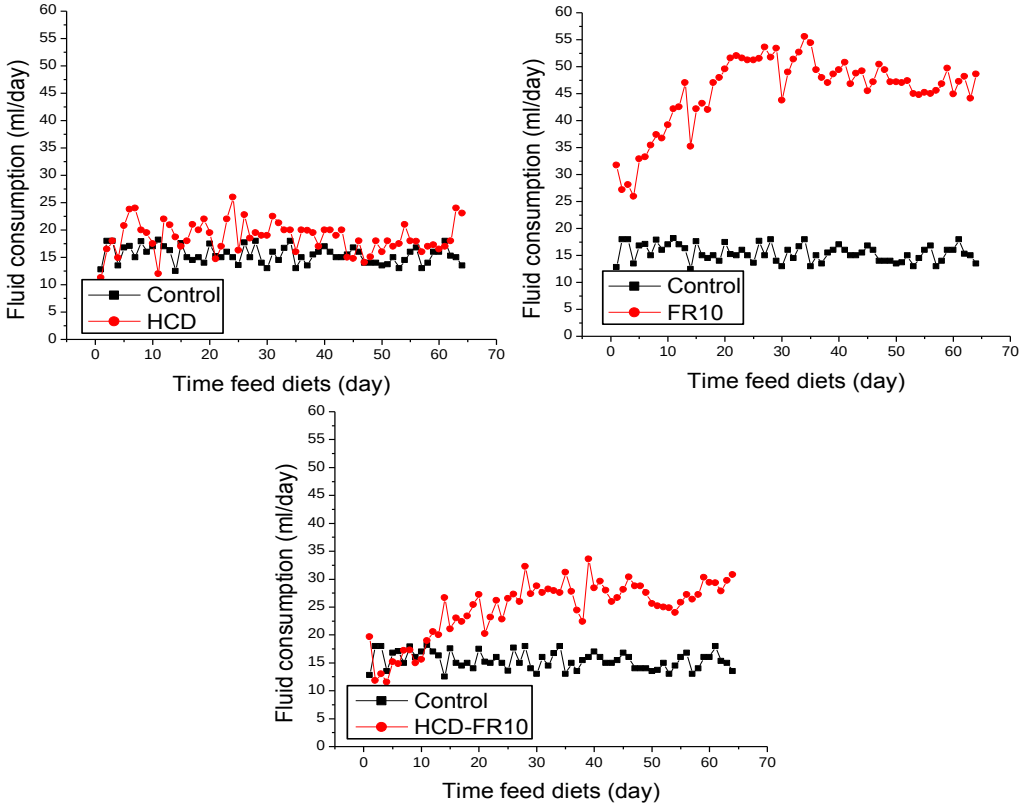

Figure 3: Fluid consumption by rats in the "FR10", "HCD" and "HCD-FR10" groups compared with a control group of animals

Table 2: Biochemical parameters of blood serum of rats of obesity models compared to control

	NC	FR10	HCD	HCD-FR10
Creatinine, µmol/l	45,85±0,84	65,54±4,91*	54,78±4,03*	60,1±7,12*
Urea, mmol/l	10,38±1,94	6,51±1,02*	8,82±1,67	5,14±1,70*
Total bilirubin, µmol/l	3,9±0,41	1,06±0,36*	2,9±0,5*	1,44±0,61*
α-amylase, IU/l	592,4±15,3	375,8±78,1*	523±83	416,12±95,22*
ALT, IU/l	85,64±16,7	31,83±10,4*	46,94±10,6*	40,92±12,68*
AST, IU/l	195,225±18,3	177,73±37,3	143,5±19,2*	220,81±65,98
ALP, IU/l	497,3±108	253±57,7*	270,8±61*	341,71±141,48
Albumine, g/l	34,6±1,27	39,32±2,43*	38,91±1,74*	37,18±0,26*
Uric acid, µmol/l	125,10±12,7	111,51±41,6	169,40±6,11*	185,86±23,93*
Total protein, g/l	64,35±0,62	74,17±3,68*	72,52±3,65*	76,91±25,07*

Note: * - p <0.05 differences credible with respect to the control

Thus, it was found that in rats of obesty groups may develop a kidney disease, as evidenced by elevated creatinine, hyperuricemia, and elevated albumin content. Today the determination of marker enzymes activity is widespread in clinical practice, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - a group of enzymes that are involved in transamination reactions of amino acids in the processes of protein and carbohydrate metabolism. The activity of these enzymes is most commonly used in the diagnosis of liver disease, myocardial infarction, renal insufficiency and deficiency of B vitamins. We have shown the reduce in the activity of ALT in 2.7, 1.8 and 2.1 times in the serum of rats that consumed 10% solution of fructose, high-calorie diet combined with 10% solution of fructose and HCD compared with the control group of animals (Table 1). The study of AST activity have shown the reduction of this parameter in the

Fr10 and HCD groups in 1.1 and 1.4 times compared to the control. These data indicate the possible development of vitamin B6 deficiency, which is widespread in patients with obesity [26]. The determination of alkaline phosphatase (ALP) activity is wide spread clinical diagnosis of hepatobiliar and skeletal systems, and avitaminosis. We have shown the decreased activity of alkaline phosphatase in 2 times in serum of rats with obesity induced by the consumption of 10% fructose solution. In further studies we have found the decreased activity of alkaline phosphatase in 1.8 and 1.5 times in the serum of HCD-FR10 an HCD rats compared with the control group (Table 1). The reduction of ALP activity may be linked to deficiency of vitamins B6 and B12, which develops with the progression of obesity [26, 27].

Also, we have shown the reduction of total bilirubin in serum of rats of experimental models of obesity (Table 1). According to the literature [28] the reduction of total bilirubin levels in serum occurs in the development of coronary heart disease that is a complication of obesity. Also, the studies have shown the reduced activity of pancreatic marker enzyme - α-amylase in serum of rats of experimental obesity groups (Table 1). According to the literature [29], decreased activity of α-amylase in the blood of patients with obesity observed during the development of nonalcoholic fatty pancreatic disease.

CONCLUSIONS

Thus, we have shown that the 10-week consumption of 10% fructose solution, high calorie diet, and their combination leads to changes in food behavior and the development of obesity in rats, as evidenced by changes in key physiological and biochemical parameters. These results suggest that these models are adequate and similar in physiological and biochemical parameters of obesity in humans and can be used in animal studies to more in-depth study of the biochemical and molecular mechanisms of development and progression of this disease.

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