METABOLIC CHANGES IN THE BODY AS THE RESULT OF LONG-TERM USE OF ARTIFICIAL SWEETENER-SODIUM CYCLAMATE

[1]Khabibullaev Sanjarbek Murodilla ugli,
[1]Yuldashev Nasirdjan Mukhamedjanovich,
[2]Mamazulunov Nurmukhammad Khusanboy ugli
[1]Tashkent pediatric medical institute, Tashkent, Republic of Uzbekiston
[2]Andijan State University, Andijan, Republic of Uzbekiston
https://doi.org/10.5281/zenodo.10003469

Abstract. *The study aims to evaluate the effect of sodium cyclamate, a sweet flavoring substance, on carbohydrate metabolism in rats after long-term administration. Sodium cyclamate was administered orally at a dose of 10 mg/kg daily to rats for 60 days. To study biochemical indicators in the blood of animals, blood was taken from animals before cyclamate injection (control group), on the 30th and 60th days of the experiment. On the 60th day, the animals were slaughtered and the amount of glucose and glycogen in their liver was studied. It was found that long-term consumption of sodium cyclamate leads to significant disturbances of metabolic processes in the body. Significant hyperglycemia and hyperinsulinemia developed from the 30th day. Long-term administration of cyclamate developed insulin resistance in animals. Administration of cyclamate also led to strong changes in blood biochemical indicators. The obtained results make it possible to limit the use of sodium cyclamate in the food industry or to propose replacing it with another natural sugar substitute.*

Keywords: *sodium cyclamate, insulin resistance, hyperglycemia, glycated hemoglobin, blood biochemical indicators.*

Introduction. Sugar substitutes are chemical compounds or products that are perceived as sweet by the taste receptors of the human tongue and are used as substitutes for sugar and similar sweet products (honey, molasses). they are used to sweeten (sweetener) various foods. Sugar substitutes include substances with a sweet taste (molasses, honey) obtained by processing natural raw materials, as well as pure chemical compounds. Usually, sugar substitutes have a higher level of sweetness than sugar, and the caloric level, on the contrary, is lower. According to the International Association of Sweeteners and Low-Calorie Manufacturers classification, sugar substitutes include fructose, xylitol, and sorbitols, and sweeteners include cyclamate, sucralose, thaumatin, stevioside, and lactulose [1].

We can add fructose, stevia, saccharin, and sodium cyclamate to the sugar substitutes widely used in the food industry. Non-caloric or very low-calorie sugar substitutes are increasingly being consumed by people with obesity and type 1 diabetes. However, recent studies have shown that long-term use of sugar substitutes can lead to weight gain over time and lead to the development of glucose intolerance with changes in the function and composition of the gut microbiota [2]. Based on the above data, it is of great interest to study the role of sugar substitutes in the development of type 2 diabetes [3, 4, 5, 6]. Although cyclamate was discovered in 1937, it was used as a low-calorie sugar substitute in the 1950s and 1960s. It is a salt of cyclohexanesulfamic acid and is 30 times sweeter than sucrose. It has a bitter taste but has a good

sweetness synergy with saccharin. It is soluble in water and its solubility can be increased by making sodium or calcium salt. Cyclamate has very low toxicity, but it is metabolized by intestinal bacteria into cyclohexylamine, which is more toxic [7]. In rats, 18.9% of daily cyclamate intake is degraded to cyclohexylamine (SCF 2000) [8]. The concentration of cyclohexylamine in plasma depends on the degree of degradation of cyclamate by intestinal flora and excretion of cyclohexylamine from the blood. The recommended daily dose of Cyclamate is 10 mg per 1 kg of body weight.

The purpose of the study. Evaluation of the effect of sodium cyclamate, which is a sweet flavoring substance, on carbohydrate metabolism in rats after long-term administration

Research material and methods. To study the effect of sodium cyclamate on carbohydrate metabolism, 25 white male experimental rats weighing 180-200 g were selected. They were kept in quarantine for 14 days from the time they were brought to the vivarium. Experimental animals were kept at a room temperature of 22±3°C, relative humidity of 30-70%, 12 hours of light, and 12 hours of darkness. For 2 months, sodium cyclamate dissolved in water at a daily dose of 10 mg/kg approved by the American Food and Drug Safety Committee was orally administered to the experimental animals. Glucose tolerance test, insulin, albumin, total protein, glucose, ALT, AST, uric acid, creatinine, cholesterol, triglyceride, HDL, and LDL were determined in the experimental animals as a control (intact) before sodium cyclamate administration, 30 and 60 days after administration.

Blood was taken into gel test tubes for simultaneous immunoassay and biochemical analysis, the gel completely separated blood from elements and blood serum. Blood was collected from animals using a G-24 gauge needle from the tail vein [9]. On the 60th day of the experiment, experimental animals were killed by decapitation under light ether anesthesia. The obtained blood was centrifuged in an EBA 200 centrifuge (Hettich company) at a speed of 3000 rpm for 15 minutes. Biochemical parameters in blood plasma were determined using Humastar 100 automatic analyzer using Human (Germany) reagents, and immunoassay diagnosis using Vektor best (Russian Federation) reagents using Mindray MR 96A analyzer. The homeostatic model (HOMA-IR) for assessing insulin resistance based on the obtained numbers D.R. Matthews et al. (1985) according to [10] and the insulin resistance index (ISI) according to M.H. Duncan et al. (1995) was calculated according to [11]. To determine the amount of glucose and glycogen in the liver tissue of the experimental animals, before the start of the experiment and at the end of the experiment, the animals were lightly sedated with diethyl ether and decapitated. Before decapitation, the body weight of the animals and the weight of the extracted liver were determined. 100 mg of liver tissue was extracted and the amount of glucose and glycogen was determined using enthrone reagent. 0.2% enthrone reagent prepared with 95% sulfuric acid, glucose standard solution, and 30% potassium hydroxide solution were used for the experiment [12]. The obtained results were statistically processed using Student's t-test.

Research results and their analysis. To evaluate the glucose tolerance test in experimental animals, 2 g/kg of glucose was dissolved in water and administered to each rat through a gavage. Blood glucose levels were determined before administration, 30, 60, 90, and 120 minutes after administration of glucose. According to the results, it was found that before the administration of sodium cyclamate, the amount of glucose in all rats was normal, and the level of glucose absorption was in good condition.

We can see that sodium cyclamate consumption for 30 days caused an average increase of 66% and 53% at 120 minutes compared to the control group until the 90th minute of absorption. On the 60th day of the experiment, sodium cyclamate caused an average increase of 73% in all phases of glucose absorption compared to the control group (Figure 1).

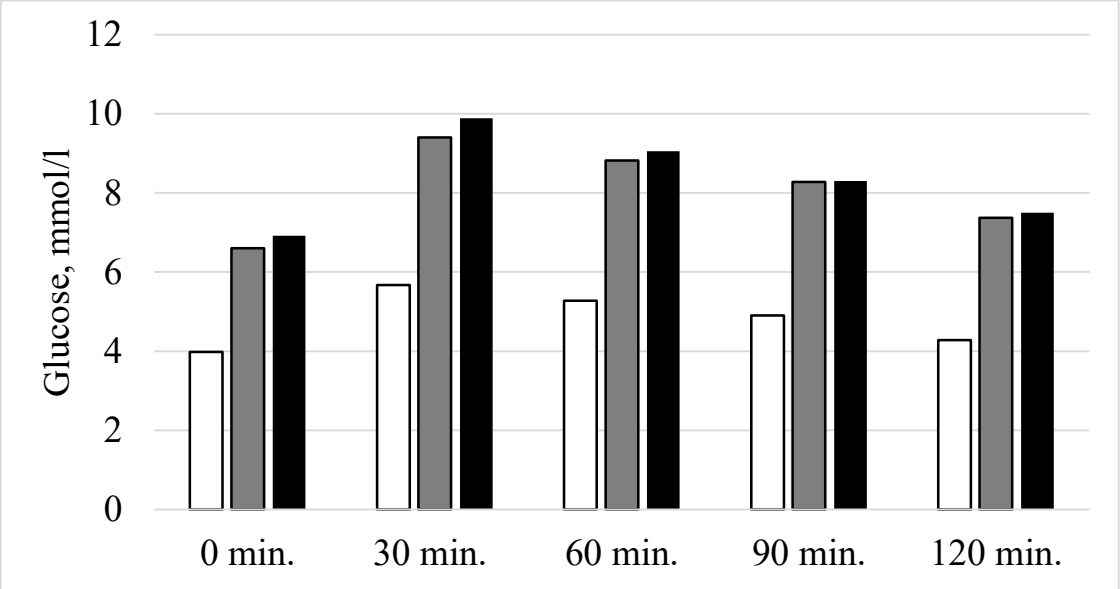

Figure 1. Level of glucose tolerance in experimental animals
White bars - intact animals, gray bars - sodium cyclamate-injected animals on day 30, and black bars - sodium cyclamate-injected animals on day 60.

The obtained results showed that before the start of the experiment, the amount of glucose in the blood of experimental animals was equal to 4.4±0.8 mmol/l (Table 1). It was determined that the amount of insulin in the blood of these animals was equal to 9.5±0.91 mU/l. Calculations showed that the control HOMA-IR was 1.86 and the ISI was 1.67. We can see that when sodium cyclamate was administered to experimental animals for 30 days, the amount of glucose in the blood increased by 61.2 % compared to the control group, and when it was administered for 60 days, it increased by 68.4 %.

These increases were statistically reliable ($p< 0,001$). The amount of insulin was 95.6 % ($p < 0,001$) higher than the control value on the 30th day of the experiment, and 66.4 % ($p < 0,001$) on the 60th day. On the 30th day and at the end of the experiment, HOMA-IR and ISI were 3.1 and 2.8 times higher than the control, respectively. It was found that the amount of glycated hemoglobin in the blood increased by 70.3 % compared to the control on the 30th day of the experiment and by 75.9 % at the end of the experiment ($p < 0,001$)

According to the results, sodium cyclamate which substitutes for artificial sugar, when taken for a long time, affects carbohydrate metabolism in the body and causes an increase in the amount of glucose in the blood.

In addition, the results show that hyperinsulinemia has developed in the body under the influence of sodium cyclamate. A sharp increase in HOMA-IR and ISI indicates that insulin resistance has occurred in experimental animals. Chronic consumption of sodium cyclamate had a significant effect on blood biochemical indicators (Table 2).

Analyzing protein and nitrogen-fixing compounds metabolism, sodium cyclamate administration for 30 days increased total protein by 14.5 % ($p<0,05$). On the 60th day of the experiment, the total protein content was only 5.1 % higher than the intact value.

Table 1.

Effect of sodium cyclamate on blood carbohydrate metabolism indicators (M±m)

	Weight, g	Glucose, mmol/l	HbA1c, %	Insulin, mU/l	HOMA -IR	ISI
Control (n=20)	175,8 ± 4,0	4,40 ± 0,09	3,37 ± 0,08	9,50 ± 0,21	1,8	1,6
Experiment 30 days (n=20)	171,4 ± 4,0	7,14 ± 0,19**	5,74 ± 0,10^	18,67 ± 0,10^	5,9^	5,3^
Experiment 60-days (n=13)	164,3 ± 5,7	7,46 ± 0,13**	5,93 ± 0,07^	15,88 ± 1,06	5,3*	4,7*

Note: * - $p < 0, 05$, ** - $p < 0,01$, ^ - $p < 0,001$.

Administration of sodium cyclamate to experimental animals also led to an increase in albumin: on the 30th day of the experiment, it was 18.7 % more than the control, and on the 60th day - 16.8 % more. Before the start of the experiment, the amount of urea in the blood of animals was 66.20 ± 0.89 mmol/l, after 30 days it increased by 21.5 %, and after 60 days by 44.1 %. ($p<0,05$).

Table 2.

Effect of sodium cyclamate on biochemical indicators (M±m)

Indexes	Intact group (n=20)	Sodium siklamat, 10 mg/kg	
		30-day (n=20)	60-day (n=13)
Indicators of metabolism of protein and nitrogen storage compounds			
Total protein, g/l	66,20 ± 0,89	75,8 ± 0,57*	69,6 ± 0,71*
Albumin, g/l	37,32 ± 0,68	44,3 ± 0,90^	43,6 ± 0,78^
Urea, mmol/l	4,99 ± 0,13	6,06 ± 0,21^	7,19 ± 0,18**
Creatinine, mkmol/l	36,66 ± 0,82	68,58 ± 1,10^	78,54 ± 2,18^
Enzymes			
ALAT, U/l	55,45 ± 2,06	85,56 ± 1,47*	96,74 ± 2,36*
ASAT, U/l	113,4 ± 1,9	122,5 ± 1,7**	121,0 ± 6,4
Indicators of metabolism of lipids			
Cholesterol, mmol/l	1,04 ± 0,02	1,51 ± 0,09*	1,33 ± 0,07^
Triglycerides, mmol/l	0,67 ± 0,03	0,76 ± 0,04	0,93 ± 0,06**
LDL, mmol/l	0,22 ± 0,05	0,24 ± 0,01	0,34 ± 0,03*
HDL, mmol/l	0,53 ± 0,02	0,56 ± 0,02	0,99 ± 0,07^
Indicators of mineral metabolism			
Na^+, mmol/l	143,7 ± 1,0	151,3 ± 1,36^	133,9 ± 1,9^
K^+, mmol/l	5,08 ± 0,13	2,93 ± 0,21*	2,81 ± 0,15*
Ca^{2+}, mmol/l	2,09 ± 0,01	0,86 ± 0,02^	0,79 ± 0,02^

The amount of creatinine in the blood is 36.66±0.82 in the intact group, and we can see that this indicator increased by 77.7 % on the 30th day of the experiment, and by 103.5 % on the

60th day (p<0,001). According to the literature, chronic administration of sodium cyclamate may cause bladder cancer in experimental animals [13].

The effect of cyclamate on liver tissue was significant, it was found that the ALAT enzyme increased by 59.7 % on the 30th day of the experiment, and by 74.5 % on the 60th day. These increases were statistically reliable. 8.0 % excess of AST enzyme on the 30th day of the experiment is statistically reliable (p < 0,01), and the 6.7 % increase on the 60th day was unreliable (p > 0,05). According to the results, the amount of cholesterol and triglyceride during the experiment was equal to 1.04 ± 0.02 and 0.67 ± 0.03 before the beginning of the experiment, and on the 30th day of the experiment, it was 45.1 and 13.9 %, respectively. And in 60 days we can see that it increased by 27.8 and 38.2 %. From these results, we know that sodium cyclamate has a negative effect on lipid metabolism. The administration of sodium cyclamate caused an increase in the amount of LDL and HDL by 54.5 and 86.8 %, respectively, on the 60th day of the experiment, without affecting the amount of LDL and HDL in the blood of experimental animals on the 30th day of the experiment. In addition, chronic consumption of sodium cyclamate affects the metabolism of trace elements, increasing the amount of Na^+ by 5.3 % on the 30th day of the experiment, and decreasing the amount of K^+ and Ca^{2+} by 43.4 and 59 %, respectively. It was found to cause a decrease in the amount of K^+ and Ca^{2+} by 6.8, 45.7, and 63.4 %, respectively.

Table 3.

Changes in the amount of glucose and glycogen in liver tissue under the influence of sodium cyclamate (M±m)

	Intact group, (n = 5)	Experimental group (60 days, n = 8)
Weight of rats, g	175,60 ± 2,08	222,58 ± 6,1**
Weight of liver tissue, g	6,18 ± 0,33	8,63 ± 0,3
Glucose, mkg/g	2,50 ± 0,03	1,37 ± 0,02^
Glycogen, mkg/g	2,25 ± 0,03	1,23 ± 0,02^

Note: *-p< 0, 05, **-p < 0,01, ^-p < 0,001.

As a result of long-term intake of sodium cyclamate, a substance that gives a sweet taste, changes in glycogen synthesis in the liver were found. According to the results of the experiment, we can see that the amount of glucose and glycogen in the liver tissue decreased by 41.99 and 41.83 %, respectively, as a result of the consumption of sodium cyclamate for 60 days (Table 3). The amount of glycogen accumulated in the liver tissue of healthy experimental animals was almost 2 times higher than that of those who consumed a sugar substitute. It was found that synthetic sodium cyclamate solution administered chronically affects carbohydrate metabolism in the body and negatively affects glycogen production.

Conclusion. The results obtained at the end of the experiment indicate that even the permitted daily dose of 10 mg/kg sodium cyclamate, a sweet flavoring substance, can affect carbohydrate metabolism in the body and cause hyperglycemia and insulin resistance in long-term, chronic intake. Cyclamate does not undergo metabolic changes in the body and causes a violation of carbohydrate storage. The results of scientific research show that it is necessary to reduce the

use of sugar substitutes in the food industry and in the production of soft drinks in order to prevent the development of insulin resistance, obesity, and type 2 diabetes. The results of the experiment indicate that sodium cyclamate can also affect kidney function (a sharp increase in the amount of urine and creatinine). Here, our results partially support the findings of Bopp et al. that chronic administration of sodium cyclamate induces bladder cancer in experimental animals [13]. According to the researchers, bladder cancer that occurs in experimental animals as a result of continuous administration of cyclamate is primarily the result of damage to the bladder wall by cyclamate metabolites. However, additional research is needed to determine whether sugar substitutes have a direct effect on the development of insulin resistance.

Conflict of Interest: The author declares that there is no conflict of interest regarding the study.

REFERENCES

1. Генделека Г.Ф., Генделека А.Н. Использование сахарозаменителей и подсластителей в диетотерапии сахарного диабета и ожирения // Международный эндокринологический журнал (Міжнародний ендокринологічний журнал (укр.)) – 2013. – № 2 (50). – С. 34-38.
2. Влияние сахарозаменителей и подсластителей на безвредность кондитерских изделий. Жаббарова С. // Universum: Технические науки: электрон. научн. журн. 2019. № 2(59).
3. Effect of artificial sweeteners on insulin resistance among type-2 diabetes mellitus patients. Kushagra Mathur, Rajat Kumar Agrawal. // J Family Med Prim Care. 2020 Jan; 9(1): 69–71.
4. State of insulin receptors in damage to the pancreas in an experiment. Gurina A.E., Mikaelian N.P., Terentev A.A. // The journal of scientific articles “Health & education millennium”, 2014, volume 16, no. 3. P. 22-23.
5. Insulin signaling in type 2 diabetes-experimental and modeling analyses reveal mechanisms of insulin resistance in human adipocytes. Cecilia Brännmark, Elin Nyman, Siri Fagerholm // J Biol Chem. 2013 Apr 5; 288(14): 9867–9880.
6. Sweetened beverage intake and risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes. - Löfvenborg J.E., Andersson T., Carlsson P.O., Dorkhan M., Groop L., Martinell M., et al // Eur J Endocrinol. 2016; 175: 605–14.
7. Toxicological aspects of cyclamate and cyclohexylamine. Crit Rev Toxicol. Bopp B.A., Sonders R.C., Kesterson J.W. // Crit Rev Toxicol 16: 213–306.
8. Revised opinion of the Scientific Committee on Food on cyclamic acid and its sodium and calcium salts – (SCF) Scientific Committee on Food (2000)___// http://ec.europa.eu/food/fs/sc/scf/out53_en.pdf. Accessed 5 May 2000.
9. Васильева С.В., Карпенко Л.Ю., Душенина О.А. Поиск оптимальных способов забора крови у лабораторных крыс в условиях хронического опыта. Генетика и разведение животных. 2022; (4): 56-60. https://doi.org/10.31043/2410-2733-2022-4-56-60
10. Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F., Turner R.C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9. doi: 10.1007/BF00280883. PMID: 3899825.
11. Duncan M.H., Singh B.M., Wise P.H., Carter G., Alaghband-Zadeh J. A simple measure of insulin resistance. Lancet. 1995 Jul 8;346 (8967):120-1. doi: 10.1016/s0140-6736(95)92143-5. PMID: 7603193.

12. Практикум по биохимии: Учеб. пособие/Под ред. С.Е. Северина, Г.А. Соловьевой. Изд. МГУ, 1989. – 23-24 с.
13. Toxicological aspects of cyclamate and cyclohexylamine. Crit Rev Toxicol. Bopp B.A., Sonders R.C., Kesterson J.W. // Crit Rev Toxicol 16: 213–306.