RESEARCH PAPER

Long-Term Saccharin Consumption: Effects on Insulin Resistance and Metabolic Parameters in Rats

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ABSTRACT

Background. Saccharin is widely used as a non-caloric sweetener, yet its long-term metabolic safety at permissible daily doses remains controversial.

Objective. To evaluate the effects of 60-day oral saccharin administration at 5 mg/kg/day on carbohydrate, protein, lipid and mineral metabolism, liver enzymes, body weight and insulin resistance indices in rats using baseline values as internal controls.

Methods. Adult rats received saccharin (5 mg/kg/day, orally) for 60 days. Metabolic, hepatic, lipid and mineral parameters, together with insulin-based (HOMA-IR, FIRI, QUICKI, Caro) and non–insulin-based (TG/HDL-C, TGI, MI) insulin resistance indices and body weight, were assessed at baseline (pre-treatment), day 30 and day 60. Each animal's baseline served as its own control, and changes over time were analyzed statistically ($p<0.05$).

Results. Saccharin induced progressive hyperglycemia (glucose +53.4% at day 30 and +80.5% at day 60 vs baseline) with parallel increases in glycosylated hemoglobin (+64.7% and +82.4%, respectively) and insulin (+40.8% at day 30, remaining +23.2% at day 60). Albumin and total protein decreased by 33.2% and 19.3%, while ALT activity rose by about 70% over baseline. Total cholesterol and HDL declined (−15.2% and −25.8%, respectively), accompanied by a 50.4% rise in atherogenicity coefficient. Serum sodium, potassium and calcium fell by 15.8%, 42.9% and 31.2%, respectively. Body weight increased by 52.8%, and insulin resistance indices markedly worsened (HOMA-IR and FIRI ≈+120%, MI up to +252.2%), whereas QUICKI decreased by about 9%.

Conclusion. Even at the acceptable daily intake level, chronic saccharin administration produced significant baseline-relative disturbances in glucose homeostasis, protein and lipid metabolism, liver enzyme activity, mineral balance and insulin sensitivity in rats, indicating a substantial metabolic risk profile.

Keywords: Saccharin, hyperglycemia, insulin resistance, liver enzymes, lipid metabolism, rats.

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INTRODUCTION

Saccharin represents the first widely used sugar substitute, produced synthetically via two primary methods. In the first approach, saccharin is obtained through the electrochemical oxidation of σ-toluenesulfonamide using potassium permanganate and chromic acid, proceeding through the formation of corresponding carboxylic acids. In this process, the ortho isomer undergoes dehydration to yield the sweet-tasting form. In the second method, methyl anthranilate is diazotized using sulfur dioxide and chloride to isolate sulfonyl chloride. Upon subsequent ammonification of this compound, saccharin is formed. Numerous studies have investigated the positive and negative effects of saccharin. Experiments conducted in the studies revealed that saccharin exposure leads to reduced caloric demand, paradoxically resulting in increased fat accumulation in the body, heightened appetite, and the development of obesity [1]. Experiments conducted by Suez et al. colleagues have demonstrated that saccharin exposure disrupts the gut microbiota, thereby contributing to the development of insulin resistance [2]. Another study established a direct association between saccharin consumption and metabolic syndrome; wherein impaired glucose control contributes to the development of obesity and diabetes **Error! Reference source not found.**[3]. Experiments in rats, dogs, and humans have shown that saccharin consumption can induce cancer. Due to its potential to cause bladder cancer in rats, saccharin was banned for consumption by the FDA (USA) in 1977. However, following extensive subsequent research, it was reapproved for use.

Non-caloric sweeteners exert effects through taste receptors, disrupting appetite regulation and potentially leading to the development of obesity [4, 5]. The sweet taste signals to the body that a large number of calories is incoming. In response, the organism produces digestive enzymes accordingly. When saccharin induces enzyme secretion but no matching calories are delivered, the demand for calories increases. This disrupts the balance between sweet taste perception and caloric intake, resulting in elevated glucose levels and reduced satiety [1]. This condition heightens the

demand for energy and contributes to body weight gain. Conversely, saccharin exposure may promote water retention and suppress energy requirements, thereby also leading to increased body weight [6, 7].

MATERIAL AND METHODS

To investigate the effects of saccharin on carbohydrate metabolism, 25 male Wistar albino rats weighing 140–160 g were selected. The experiments were carried out at the Toxicology Laboratory vivarium of the Committee for Sanitary and Epidemiological Well-fare and Public Health under the Ministry of Health of the Republic of Uzbekistan (based on the contract between the Committee dated March 10, 2025).

Prior to the start of the experiments, the purchased rats were kept under quarantine for 14 days and subsequently acclimatized for an additional week. Experimental animals were maintained under controlled laboratory conditions at a room temperature of 22 ± 3 °C, relative humidity of 30–70%, with a 12-hour light/dark cycle. Housing and feeding of the rats were conducted in accordance with the requirements of ICSMC 33215-2014.

The study protocol was approved by the Ethics Committee under the Ministry of Health of the Republic of Uzbekistan (No. 4/19-1667, dated 20.05.2024). All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 2011).

For the experimental group, saccharin was administered orally at a dose of 5 mg/kg body weight once daily for 2 months, dissolved in distilled water. Prior to the start of the experiment, blood samples were collected from the animals under light ether anesthesia for the determination of baseline glucose, insulin, glycated hemoglobin (HbA1c), and biochemical parameters, which served as control values. After 30 and 60 days of treatment, the same parameters were re-assessed, and differences between time points were evaluated.

For the study, saccharin manufactured by LLC “NovaProdukt AG” (Moscow, Russian Federation) was purchased from the “Korzinka” retail chain belonging to Anglesey Food LLC, located in Tashkent, Uzbekistan. For biochemical and immunoassay analyses, blood samples were collected from the tail vein of the experimental animals. For this purpose, the rats were placed in specially designed metal restrainers adjusted to their body length, leaving the tail exposed. To induce hyperemia, the tail was immersed in warm water at 40–50 °C for several minutes. After drying, the tail vein was punctured using a G-24 injection needle (Vasilyeva et al., 2023), and the collected blood was transferred into gel-containing yellow-top tubes to obtain serum of sufficient quality.

Blood sampling was performed at baseline (prior to the start of the experiment), and on days 30 and 60 of the study. The samples were centrifuged using a TDZ4-WS centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., China) at 3000 rpm for 5 minutes to separate plasma from cellular components. Hemolyzed blood samples were excluded from further analysis.

Biochemical Laboratory Examination Methods

For the determination of biochemical parameters including glucose, glycated hemoglobin, albumin, total protein, urea, creatinine, cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), sodium, calcium, and potassium, reagents manufactured by Human (Germany) were used, purchased from *Osiamedika LLC*. The measurements were performed using the Humastar 100 automated biochemical analyzer (Human, Germany) at the *Scientific Laboratory*, of the Tashkent State Medical University.

Serum insulin levels were quantified using the Rat Insulin ELISA Kit (ELK Biotechnology Co., Ltd., USA), with assays performed on the Mindray MR-96A semi-automated immunoassay analyzer (manufactured in the People’s Republic of China).

Assessment of Insulin Resistance.

In the study, insulin resistance was assessed using several insulin-based indices, including HOMA-IR, FIRI, Caro, and QUICKI.

The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to D.R. Matthews et al. (1985) as follows: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL)] / 22.5 [8].

The fasting insulin resistance index (FIRI) was determined based on measured fasting insulin and glucose levels in experimental animals, using M.N. Duncan's formula [9]: FIRI = [fasting glucose (mmol/L) × fasting insulin (μU/mL)] / 25.

The Caro index was computed according to F. Caro[10] as the ratio of fasting glucose (mmol/L) to fasting insulin (μU/mL).

Insulin resistance according to QUICKI was calculated using the following formula[11]: QUICKI = 1 / [log (fasting insulin) + log (fasting glucose)].

The following non-insulin-based indices were also employed for insulin resistance evaluation:

The TG/HDL-C index[12] was determined by calculating the ratio of triglycerides (mmol/L) to high-density lipoprotein cholesterol (mmol/L).

The triglyceride-glucose (TyG) index[13] was computed using the formula: TyG = ln [triglycerides (mg/dL)×glucose (mg/dL)/2].

The metabolic index (MI) was determined according to Roytberg using the formula[14]: MI = [triglycerides (mmol/L)×glucose (mmol/L)] / [HDL-C (mmol/L)] ².

Statistical Analysis

Statistical processing of the experimental data was performed using the JMP statistical software package. The significance of differences was evaluated using one-way

ANOVA and nonparametric pairwise comparisons via the Wilcoxon method.

RESULTS AND DISCUSSION

When saccharin was administered orally to experimental rats at a dose of 5 mg/kg/day, dissolved in distilled water for 60 consecutive days, significant changes were observed in carbohydrate metabolism parameters. Prior to the initiation of the experiment, the baseline glucose level in the experimental animals was 4.40 ± 0.09 mmol/L (Table 1).

By day 30 of the experiment, this parameter reached 6.75 ± 0.16 mmol/L, representing a 53.4% increase compared to the baseline. By day 60, the glucose level was 7.94 ± 0.12 mmol/L, corresponding to an 80.5% elevation relative to the initial value. The observed changes were statistically significant ($p<0.05$).

The progressive increase in glucose levels under saccharin exposure indicates the development of hyperglycemia.

Table 1. Effects of Saccharin on Blood Carbohydrate Metabolism Parameters, *M±m*

	Glyukoza, mmol/l	HbA1C,%	Insulin, mU/l
Baseline (*n = 20*)	4,40±0,09	3,4±0,11	12,5±0,65
Day 30 of the Experiment (*n = 20*)	6,75±0,16	5,6± 0,08	17,6±0,73
p	0,001	0,001	0,001
Change Relative to Baseline, %	+ 53,4 %	+ 64,7 %	+ 40,8 %
Day 60 of the Experiment Change Relative to Baseline (*n = 18*)	7,94± 0,12	6,2±0,02	15,4±0,58
p	0,001	0,001	0,01
Change Relative to Baseline, %	+ 80,5 %	+ 82,4 %	+23,2 %

Paralleling the rise in glucose, the glycosylated hemoglobin content also progressively increased throughout the experimental period. Prior to the experiment, the baseline glycosylated hemoglobin level was 3.4 ± 0.11%. By day 30 of the experiment, it had increased by 64.7%, and by day 60, it had risen by 82.4%. These elevations were also statistically significant ($p < 0.05$).

When insulin levels were measured in the experimental animals, the baseline value prior to the experiment was 12.5 ± 0.65 mU/L. By day 30 of the experiment, it had risen to 17.6 ± 0.73 mU/L, representing a 40.8% increase compared to the initial level. This parameter remained elevated through the end of the experiment at 15.4 ± 0.58 mU/L, showing a 23.2% increase relative to baseline.

In a study conducted by Azeez and colleagues, blood glucose levels were found to increase by 75% after 2 months and by 67% after 4 months of saccharin exposure [7]. In another experiment, saccharin consumption over 1.5 months led to an increase in blood glucose levels in rats [15]. When saccharin was administered to rats for 7 weeks, blood glucose levels were found to increase by 17.1% compared to the control group [16].

To assess the impact of saccharin on overall metabolism, parameters of protein and lipid metabolism, liver enzymes, and mineral content were evaluated.

Table 2. Effect of Saccharin on General Metabolism

Indicators	Baseline	Saccharin, 5 mg•kg/day	
		Day 30	Day 60
Indicators of Protein and Nitrogen-Retaining Compound Metabolism			
Total protein, g/L	72,24±1,28	66,21±1,05^	58,31±0,92^
Albumin, g/L	42,57±1,12	30,21±0,75^	28,43±1,24^
Urea, mmol/L	5,45±0,13	8,39±0,20^	7,19±0,18^
Creatinine µmol/L	47,25±1,16	84,11±1,79^	87,25±2,10^
Liver enzymes			
ALT, U/L	71,40±2,27	124,1±4,27^	120,72±4,03^
AST, U/L	119,58±6,08	160,15±6,18^	170,14±5,97^
Indicators of Lipid Metabolism			
TC, mmol/L	1,65±0,09	1,33±0,07*	1,40±0,07*
TG, mmol/L	0,93±0,07	0,78±0,05	0,86±0,05
HDL-C, mmol/L	0,19 ± 0,01	0,16± 0,01*	0,17 ± 0,01
LDL-C, mmol/L	0,64±0,04	0,57±0,03*	0,47±0,03*
VLDL-C, mmol/L	0,68±0,02	0,53±0,02*	0,47±0,02*
AC	1,41 ± 0,09	1,62 ± 0,19	2,12 ±0,22*
Indicators of Mineral Metabolism			
Na^+, mmol/l	140,26±1,57	132,12±1,43*	118,10±5,60*

K^+, mmol/l	5,34±0,80	3,86±0,15*	3,05±0,10*
Ca^{2+}, mmol/l	2,12±0,04	1,82±0,07*	1,46±0,08*

*p>0.05, ^p<0.001, statistically significant difference compared to the initial value.

The primary indicator of protein metabolism, albumin, was determined to be 42.57 ± 1.12 g/L at the start of the experiment (Table 2).

By the midpoint of the experiment, this parameter had decreased by 29% to 30.21 ± 0.75 g/L. By the end of the experiment, it further declined to 28.43 ± 1.24 g/L, representing a 33.2% reduction compared to the baseline value. These decreases in albumin levels were statistically significant (p<0.005). When total protein levels were measured, the baseline value was 72.24 ± 1.28 g/L. Similar to albumin, this parameter progressively declined throughout the experiment. By the midpoint, it reached 66.21 ± 1.05 g/L, and by the end, it had decreased by 19.3% compared to baseline to 58.31 ± 0.92 g/L. This reduction at the conclusion of the experiment was statistically significant ($p < 0.05$).

When saccharin was administered chronically at the permissible daily dose of 5 mg/kg for 60 days, the activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) progressively increased through the end of the experiment. The baseline ALT activity was 71.40 ± 2.27 U/L. By day 30, this parameter had risen by 74% to 124.1 ± 4.27 U/L compared to baseline. Although ALT activity showed no substantial further change from day 30 to day 60, it remained 69.1% elevated relative to initial levels. Both measurements were statistically significant compared to baseline ALT ($p < 0.05$).

To evaluate saccharin's effects on lipid metabolism, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and the atherogenicity coefficient were measured at baseline, mid-experiment, and endpoint. Results indicated that the permissible daily dose of saccharin led to reductions in blood lipid component levels. Prior to the experiment, the baseline total cholesterol level was 1.65 ± 0.09 mmol/L. By the midpoint of the experiment, this parameter had decreased to 1.33 ± 0.07 mmol/L, and by the endpoint, it was 1.40 ± 0.07 mmol/L. These reductions were statistically significant compared to baseline, representing decreases of 19.4% and 15.2%, respectively. Prior to saccharin administration, baseline triglyceride levels were 0.93 ± 0.07 mmol/L. By the experiment's midpoint, they had decreased by 16.1% to 0.78 ± 0.05 mmol/L. At the endpoint, triglyceride levels were 0.86 ± 0.05 mmol/L, representing a 7.9% reduction from baseline. These triglyceride reductions were not statistically significant. When HDL levels were evaluated, the baseline value was 0.64 ± 0.04 mmol/L, decreasing to 0.57 ± 0.03 mmol/L at midpoint and 0.47 ± 0.03 mmol/L at endpoint. These results indicate HDL reductions of 10% at midpoint and 25.8% at endpoint compared to baseline. The endpoint reduction was statistically significant ($p < 0.05$). VLDL levels, which were 0.68 ± 0.02 mmol/L at baseline, decreased by an average of 22% compared to initial values at both midpoint and endpoint. This substantial decline in VLDL levels resulted in a 50.4% increase in the atherogenicity coefficient (AC) by day 60 of the experiment.

When evaluating saccharin's impact on mineral metabolism, progressive reductions in serum sodium, potassium, and calcium levels were observed throughout the experiment. Baseline sodium concentration prior to saccharin administration was 140.26 ± 1.57 mmol/L, which decreased by 15.8% to 118.10 ± 5.60 mmol/L by experiment endpoint.

Potassium levels were 5.34 ± 0.80 mmol/L at baseline, declining by 27.7% to 3.86 ± 0.15 mmol/L at the midpoint and further to 3.05 ± 0.10 mmol/L at endpoint—a 42.9% reduction from initial values that was statistically significant ($p < 0.05$).

Calcium concentration was 2.12 ± 0.04 mmol/L before treatment, falling to 1.82 ± 0.07 mmol/L by day 30 and 1.46 ± 0.08 mmol/L by day 60, representing reductions of 14% and 31.2% relative to baseline, respectively. These decreases in calcium were also statistically significant ($p < 0.05$).

In a study conducted by Azeez and colleagues, among the blood biochemical parameters, albumin levels decreased by up to 39% after 60 days, while total protein levels declined by up to 7%. In contrast, serum creatinine concentration increased by 114% and urea levels rose by 27% over the 60-day period [7]. In another experiment investigating saccharin effects, its 35-day consumption reduced albumin levels by 40.4% and total protein by 20.1% in rat blood, while simultaneously increasing urea and creatinine concentrations by 13.6% and 42.3%, respectively [16]. Other authors administered saccharin at 10 mg/kg/day via gavage to experimental rats for 1 month and, upon examining biochemical parameters, found that total protein and albumin levels in blood were partially reduced compared to the control group, while urea and creatinine concentrations increased by 150% and 48%, respectively [17].

When examining saccharin's effects on liver enzymes, administration of the permissible dose of 5 mg/kg/day to rats for 2 months resulted in increases of 63.3% in ALT activity and 3.5% in AST activity compared to pre-treatment baseline values [7]. In another experiment, saccharin consumption over 35 days was found to increase ALT and AST activities by 33.4% and 29.7%, respectively, compared to the control group [16]. In an experiment conducted by subsequent authors, the effects of saccharin at 10 mg/kg/day were investigated over 1 month, revealing

increases in ALT and AST activities by 51.7% and 33.7%, respectively, compared to the control group [17]. When saccharin was administered to male and female rats for 6 weeks, ALT and AST activities in the blood of male rats were found to be higher compared to both females and the control group [15]. In experiments conducted by Abed and colleagues, saccharin exposure resulted in significantly elevated ALT and AST enzyme activities in rats compared to the control group. The marked increase in AST activity occurs due to hepatocyte cell damage. Hepatocyte injury increases cell membrane permeability, thereby facilitating the release of enzymes from cells into the bloodstream.

Furthermore, increased catalase activity along with elevated levels of uric acid, isoprostanes, and 8-hydroxy-2'-deoxyguanosine indicate enhanced oxidative stress in the liver under saccharin exposure [7].

Consumption of saccharin at a dose of 5 mg/kg/day for 2 months affected lipid metabolism, causing reductions in total cholesterol and triglyceride levels by 23.2% and 20.4%, respectively, as well as decreases in HDL and VLDL levels by 28.3% and 13.8% [7]. In another experiment, saccharin exposure was found to reduce total cholesterol levels by 44.9% and triglyceride levels by 22.7% in rat blood plasma. When saccharin was administered to rats at a daily dose of 10 mg/kg for 1 month, reductions were observed in total cholesterol, triglycerides, HDL, and VLDL levels in blood plasma [17].

Indexes	Saccharin		
	Baseline (n=20)	Experimental days	
		Day 30 (n=20)	Day 60 (n=18)
Body weight, g	115,7±3,0 (97,9÷173,8)	146,2±4,5*	176,8±5,1*
Daily body weight, g		+ 1,02	+ 1,02
HOMA-IR	2,48±0,14 (1,62÷3,76)	5,30±0,26*	5,46±0,23*
FIRI	2,23±0,12 (1,46÷3,48)	4,77±0,23*	4,91±0,08*
Caro	0,37±0,02 (0,19÷0,48)	0,39±0,02	0,53±0,02*
QUICKI	0,33±0,003 (0,31÷0,35)	0,30±0,002*	0,30±0,002*
TG/HDL-C	1,40±0,10 (0,63÷2,64)	1,52±0,10	1,89±0,15*
TGI	8,06±0,06 (7,66÷8,79)	8,31±0,05*	8,58±0,04*
MI	9,61±0,87 (3,23÷16,96)	20,48±1,48*	33,85±3,45*

The effects of saccharin on blood lipid levels have been documented in numerous studies [7, 18]. The reductions observed in the lipid spectrum are attributable to saccharin's indirect effects on fat metabolism and lipid peroxidation. Cyclic imides present in saccharin may influence enzymes involved in lipid metabolism regulation, potentially binding to lipoprotein receptors in rat and human cells and inducing their dehydration [7]. The reductions in cholesterol and triglyceride levels may be associated with decreased total cholesterol synthesis. This is because in vivo studies have demonstrated that saccharin reduces the activity of liver enzymes-acetyl-CoA synthetase and citrate lyase [7, 18].

Under saccharin exposure, mitochondrial citrate-as well as cytoplasmic acetyl-CoA, which serves as a substrate for cholesterol and fatty acid synthesis-is reduced. Additionally, decreased activities of hepatic acetyl-CoA carboxylase, glycerol-3-phosphate acyltransferase, and phosphatidate phosphohydrolase have been observed [7]. The reduced enzyme activities inhibit triglyceride synthesis. The mechanism underlying decreased blood lipid levels is also associated with ApoA. As the primary protein of VLDL, ApoA undergoes changes under saccharin influence. This exerts negative effects on antioxidant systems and diminishes VLDL's ability to bind phospholipids [19].

Effect of Saccharin on Insulin Resistance Indices

In animals administered saccharin, body weight increased by 26.4% and 52.8% on days 30 and 60 of the experiment, respectively, compared to baseline values. The average daily weight gain of the animals was 1 g. In long-term experiments conducted in rats, saccharin administration at a dose of 5 mg/kg/day was found to increase body weight by 59% on day 60 and by 67% on day 120 [7]. Other authors have reported that saccharin administration to rats for six weeks leads to an increase in body weight [15]. In another experiment conducted in rats, saccharin consumption at a dose of 25 mg/kg/day for 8 weeks was found to increase body weight by 71.8% compared to pre-administration levels [20].

Saccharin consumption resulted in HOMA-IR values increasing by 113.7% and 120.2% on days 30 and 60, respectively, compared to baseline. Similarly, FIRI values rose by 113.9% and 120.2% on days 30 and 60 relative to initial levels. The Caro index showed no change until day 60, when it increased by 43.2% from baseline. QUICKI values were 9.1% lower than baseline on both days 30 and 60 of the experiment (Table 3).

Table 3. Effect of Saccharin on Body Weight and Insulin Resistance Indices

*p>0.05, statistically significant difference compared to the initial value.

Analysis of non-insulin-based insulin resistance indices revealed that saccharin administration led to changes in the TG/HDL-C ratio that were not statistically significant compared to baseline.

The TGI parameter, although not markedly elevated, showed a progressive increase: by day 30 of the experiment, it rose by nearly 8%, and by day 60 by approximately 11%. The MI parameter was 71.8% higher than the baseline value on day 30; however, by day 60 it did not differ substantially from this level.

Administration of saccharin to the experimental animals resulted in an increase in the TG/HDL-C parameter only by day 60 of the experiment, reaching 35.0% above the baseline value. Although the increase in TGI was statistically significant, it did not exceed the baseline level substantially.

In contrast, the changes in MI were considerably more pronounced. This parameter exceeded the baseline values by 113.1% and 252.2% on days 30 and 60 of the experiment, respectively.

Saccharin may influence host metabolic signaling through intestinal nutrient-sensing pathways. Activation of sweet taste receptors (TAS1R2/TAS1R3) in the gut has been shown to regulate glucose transporters such as GLUT2, thereby enhancing glucose absorption [21]. Furthermore, artificial sweeteners have been implicated in altered incretin responses, including GLP-1 secretion, which may contribute to dysregulated insulin signaling [22].

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