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Metabolic Biomarker Alterations in a Rat Model of Artificial Sweetener Consumption: Implications for Human Health

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Abstract

Due to some variables, especially the rising incidence of being overweight and hyperglycemia, the consumption of NNS has risen significantly globally in recent years. The use of NNS has produced conflicting findings in studies; some data suggest that it leads to weight gain and arises in hunger, while others claim that it has a major impact on weight loss and diabetes management. Using an in vivo rat model, the goal of this investigation is to look at the effects of several sweeteners that are not nutritious, affecting physical size, fasting blood sugar, Overall cholesterol (OC), triglycerides (TC), Extremely Hard lipoprotein (EHL), and Minimal Density lipoprotein (MDL), as well as body weight. According to the findings, stevia caused the rat's weight to drop by 50 grams after eight weeks, whereas, in the other groups, it stayed essentially the same, except for sucrose (G1) and acesulfame-K (G6), which caused a noticeable weight gain. In every group, there was a general rise in LDL and (OC)and a fall in HDL. In terms of shedding pounds and regulating blood sugar levels while fasting, stevia (G2), Sucralose (G3), aspartame (G5), and saccharine (G4) were discovered to be the most advantageous. In addition, conflicting findings from several studies underscore the ambiguity around the impacts of NSS.

Keywords: Minimal Density lipoprotein (MDL), Artificial sweeteners, Fasting blood sugar, Metabolism.

Introduction

Non-nutritive sweeteners (NNS) have become increasingly necessary due to the prevalence of obesity and diabetes (1). The usage of NNS must be recommended, as advised by the American Diabetes Association, in order to support a healthy diet. Diabetes and being overweight are indicators of risk for NNS. Growing overweight people frequently use it to reduce carbohydrates from processed sugar, and those with diabetes to manage their sugar levels (2). These conditions include hyperlipidemia, cardiovascular disease, and metabolic syndrome. Chemically, sweeteners that are not nutritious are entirely different from carbs and are essentially inert substances. Many synthetic products, such as beverages, confections, baked goods, energy drinks, etc., use sugar substitutes. G3, G5, cyclamate, saccharin, and G6 are some of the most frequently employed NNS (3).

Studies on the use of NNS have produced conflicting outcomes; a few instances suggests that it causes one to put on weight and feel more hungry; some assert that it significantly affects the control of blood sugar levels and reduces weight. Regarding the conflicting opinions and data around these items, NNS use is also linked to negative side effects, such as increased body weight, insulin and glucose digestion alterations, renal damage, neurological diseases, and even malignancies (4). According to reports, using sugar substitutes for a long time has

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interfered with the body's ability to regulate glucose levels. That has been shown to reduce the central nervous system's reward response to elevated sugar intake, alter insulin secretion, and increase issues (5). Various sweetness with various chemical compositions has been created. While G2 also contains sweetness and powerful antioxidant effects, G3 has a structure comparable to that of carbohydrates. It is useful because it can endure at various temperatures and pH levels. Saccharin is also a very polar substance. Urination quickly eliminates it after somewhat sluggish digestion (6). The impact of several sugars that are not nutritious on body weight, observing a fast blood sugar (WC), (TC), Extremely Hard lipoprotein (EHL), and Minimal Density lipoprotein (MDL), will be investigated using a rat in vivo model (8). In the study's findings, it was discovered that chromium caused the rat's burden to drop by fifty grams after eight weeks, while the weights of the other groups stayed essentially constant, except G1 and acesulfame-K, which caused a noticeable increase in weight. Contradictory findings from many studies illustrate the unpredictability of the effects of NSS.

This study (8) assessed the influence of sugar-based sweeteners on the liver function test and documented the change in ALP, AST, and ALT levels. G4 and G5 significantly raised the level of ALT from 40 U/L to 80 U/L when measured by Alanine Aminotransferase (ALT). These two NNS have the highest levels of alanine aminotransferase. This illustrates the hepatic stress brought on by NNS use and implies that human use should be restricted. In the lab, in vivo, and observational clinical research, this investigation (9) intends to investigate the impact of Acesulfame Potassium (Ace K) and its probable mechanism to elicit uterine contraction. Analyzed the influence on contractions of the uterus and the relevant signaling system using ex vivo and in vitro investigations. Ace K's preference for producing proteins associated with contractions was examined using a prolonged, high-dose treatment. These results imply that women who experience uncomfortable uterine hypercontraction should be more aware of zero- or low-calorie soft beverages or food items containing Ace K.

Article (10) suggested a mathematical framework developed to forecast long-term effects on the kinetics of conversion to these sweeteners under the age of administration. Our results shed light on the organic metamorphosis encouraged by artificial sweeteners and emphasize the necessity to assess these environmental pollutants for any potential antibiotic-like adverse effects. The article (11) explains these effects. It explores that ingesting sweet-tasting but low- or non-calorie food and drinks alter trained responses that ordinarily support glucose and energy homeostasis. Regular use of high-intensity sweeteners may interfere with metabolism, which would seem to be the opposite of what is intended. In mice fed a high-fat, high-fructose (HF-HF) diet, the study (12) examined the effects of sesamolin on kidney damage, gastrointestinal (GI) barrier dysfunction, and intestinal microbiota imbalance, as well as the underlying relationships between them. According to the findings, sesamolin inhibited metabolic diseases and improved kidney function measures. According to a histology study, sesamolin decreased renal cell epithelium aging and brush boundary destruction. According to the survey, sesamolin lessens kidney damage by restoring the

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intestinal barrier and regulating gut microbiota, which may be associated with its effects on lowering endotoxemia and related metabolic disorders.

In work (13), synthetic and biogenic APs made healthy mice intolerant to glucose. As a result, ongoing exposure to these APs, either separately or in conjunction, may be of concern, particularly for the population with a status associated with glucose metabolic diseases. By comparing an individual's contact level of food additives in the community, APs were introduced to mice's usual diet at a level that was multiple of the acceptable daily intake (ADI) level in order to assess the effects of APs on the gut microbiota.

The study (14) sought to determine if the benefits of glucose on blood sugar, calorie ingesting, and diet-induced overweight have a bearing on dropping weight by beverages with sugar (caloric or non-caloric). For eight weeks, rats were fed a high-fat diet and given sweetened water to make them obese and intolerant to glucose. For the following six weeks, mice were randomly assigned to consume glucophage dissolved in water, HFCS, or saccharin, an artificial sweetener. Every group demonstrated better glucose tolerance than before metformin treatment after 6 weeks. In conclusion, it is advised to limit non-nutritive sweetener intake while using metformin to prevent the medication's therapeutic effects on body weight and glucose homeostasis from being harmed.

The objective of the article (15) was to establish a rat replica to investigate the consequences of SSB intake and exercise on melancholy and anxiety-like behaviors in rats and to corroborate further the relationship between fizzy beverage intake and behavioral abnormalities in humans. Our findings indicate that long-term use of SSB has no harmful effects on sadness and anxiety and can postpone weight increase in growing mice. Neither the behavioral advantages of exercise nor the related genetic indicators were substantial in this research environment. Overall, research is still needed to fully understand how SSB use affects mood and behavior.

In the present study, we want to observe different artificial sweeteners' impacts on weight gain, fasting blood sugar levels, OC, triglycerides, EHL, and MDL. G1, G2, G3, G4, G5, and G6 are some of the sweeteners in this list.

Materials and Methods

Samples

A total of 56 albino rats, aged 21 to 28 days, were utilized in the investigation. They were divided into 7 groups using a physical randomization procedure, with 7 rats per group. Each rat weighed between 260 and 360 grams and was housed in a controlled environment with a temperature of 24 +/- 3 °C and a cycle of 13 hrs of light and 11 hrs of darkness. The rats received food and water as required. All animals followed the animal welfare committee's guidelines in their care.

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Biochemical Analysis

We utilized the colorimetric analysis implements from Biolabo (France) to evaluate the amount of glucose, Minimal Density lipoprotein (MDL), Extremely Hard lipoprotein (EHL), Whole (OC), overall proteins (OP), triglycerides (TC), and fasting blood sugar (FBS). The following substances were bought locally: G6, G4, G2, G3, and G4. The group separation is depicted in table 1.

Table (1): Tabulation for G1 to G6.

Group (G)	Treatment	Dosage
Control Group (CG)	Distilled Water	-
G1	12% Sucrose Solution	-
G 2	Stevia	210mg/perkg/ per day
G 3	Sucralose	4g/perkg/per day
G 4	Saccharine	28mg/per kg/ per day
G 5	Aspartame	260mg/per kg/ per day
G 6	Acesulfame-K	260mg/per kg/ per day

The outermost vascular plexus was used to gather 12 blood samples, each weighing three milliliters, from every regulated and randomized group. Each piece was collected in an uncoated, simple tube and left to clot for half an hour to one hour at room temperature. This process was used to divide plasma at 3200 rpm for approximately fifteen minutes to determine the biochemical characteristics.

The quantity of red quinone imine released when blood sugar is burned measured to determine the fasting blood sugar in the serum. Enzymatic techniques assessed total protein molecules, total cholesterol, and triglycerides. The Fried Ewald formula was used to determine the low-density lipoprotein concentration.

$$LDL - C\left(\frac{mg}{dl}\right) = TC - (HDL - C + VLDL - C) \quad (1)$$

Analytical statistics

Bar graphs were used to display all the data concerning each group separately. (ANOVA), the Fisher's test, subsequent to Kruskal-Wallis, was used to figure out the different categories, and an assortment of t-tests to assess the modifications between groups were used to analyze the variances. With a $P \leq 0.05$, statistics showed that each number was considerable.

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Results and discussion

According to a thorough review of weight changes in all eight groups after eight weeks, the control group's mean weight (in grams) grew marginally from 218 to 258, while G1 raised the average weight by 130 grams in the same amount of time.

G6, which only slightly made the weight go up. Only G2 showed a weight loss impact; the rat lost 27 grams of weight compared to the other groups, where the weight was nearly unchanged (Figure 1a). G3 and G6, which mutually contributed to the slight, insignificant rise in sugar levels, were the next two substances to significantly raise the blood sugar level at rest from 95 mg/dl to 125 mg/dl, as was predicted. We discovered positive G2, G5, and saccharin outcomes in managing diabetes (Figure (1)). The information was represented as mean SD, *<0.05 (before and after), and $<0.05 (after and before).

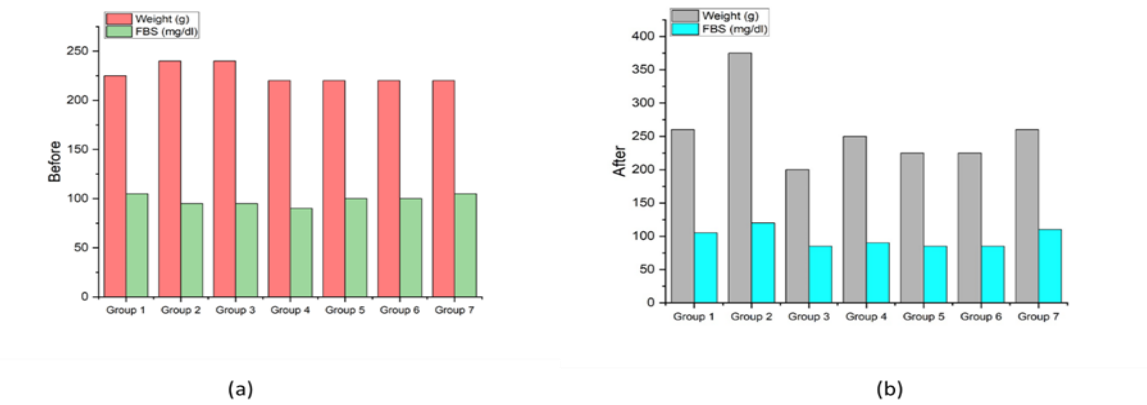

Figure (1): Comparing the weight and FBS of the laboratory rats tested on NNS to the controls

After an eight-week examination of OC, every group showed an increase in cholesterol; however, G2 and G3 showed the least rise, while G6 showed the most growth, going from 118 mg/dl to 128 mg/dl. While LDL levels have raised in all groups, G2 and G3 only slightly increased LDL levels, and HDL levels were reduced in nearly all groups, with G1 and G6 showing the most substantial reductions (Figure (2)). The information was represented as mean SD, *<0.05 (before and after), and $<0.05 (after and before).

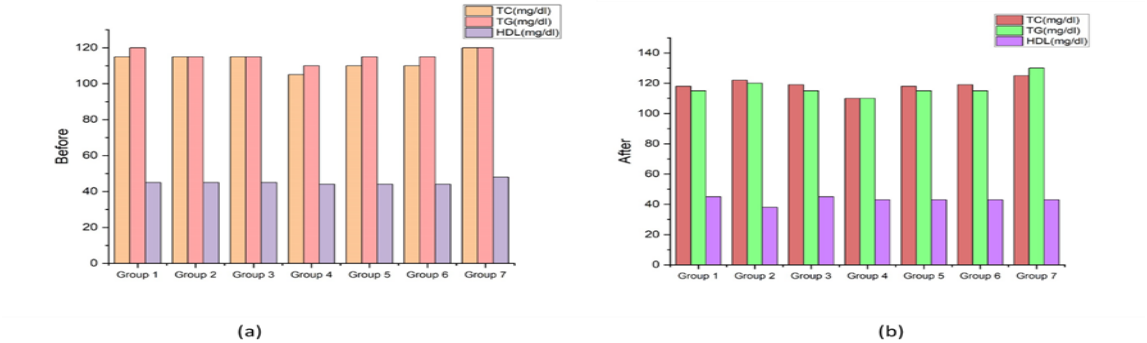

Figure (2): Comparing the lipid profile (mg/dl) of the plasma from laboratory rats tested on NNS to both the positive and negative responses

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G1-G6 showed a clear rise in triglycerides, although the climb was negligible in the other categories. Only the managed group's TG level was decreased (Figure (2)). Accordingly, VLDL in the G6 group alone was equally impacted (Figure (3)). Data presented as mean SD, * <0.05 (before and after), and p< 0.05 (after and before).

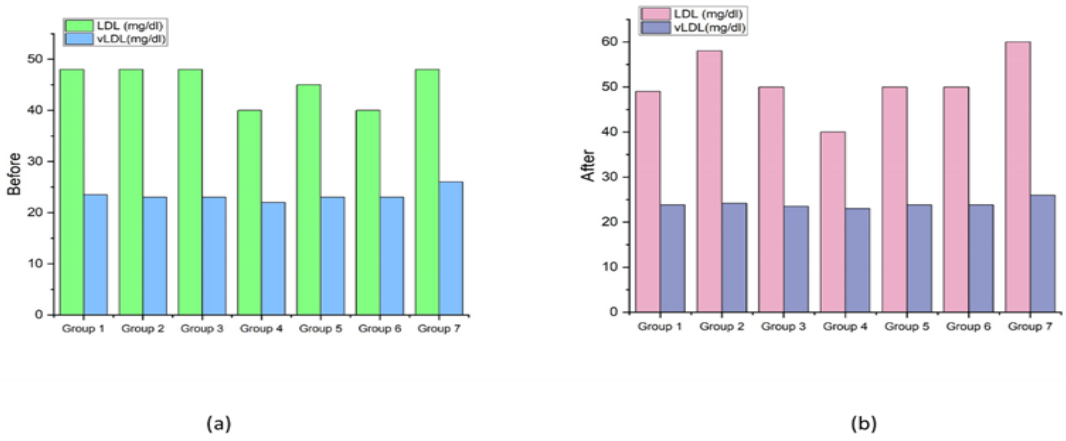

Figure (3): Comparing the calculated lipid characteristics (mg/dl) of the laboratory rats tested on NNS to both positive and negative aspects

Discussion

The consumption of non-nutritive sweeteners for regulating or lowering body weight has risen substantially, and numerous research is being done to evaluate the effects. (16). Through our study, we discovered that, in contrast with the rise in load seen in the G1 group and CG, all other groups showed load maintenance, while only G2 caused the weight to decrease. Similar to this, research conducted to emphasize the effects of various sugars' impact on body composition showed that, except for saccharin, no rise in body weight was observed in rats (17). These data show the variance in outcomes brought about by saccharin ingestion, but the improvement is still less than that of the G1 group.

In the present study, we discovered that, in contrast to the rise in weight in the G1 and the controlled groups, all the other groups showed the maintenance of weight, whereas only G2 caused the weight to decrease. Numerous research has been conducted to assess these impacts since using artificial sweeteners to regulate or lower weight has expanded dramatically. (16). Similar to this, a study conducted to highpoint the effects of different sweeteners on obesity revealed /, that saccharin caused increased body weight in rats (17). However, the rise is still reduced when compared to the G1 group. These data show a variance in outcomes from the use of saccharin.

Compared to G1 consumption, NNS has been beneficial in lowering blood sugar levels; however, the degree of control differs depending on the sweetener. One tablet of G3 in one cup of tea was assigned to the study's predicted dose of 2% G2 extracts. G2 extracts were shown to be a possible replacement for G3 with no spike in glucose levels 120 minutes after

ingestion and a constant HbA1c based on the careful study of fasting blood sugar level, HbA1, and lipid profile after eight weeks (18). While G3 exhibited a small increase from 92 mg/dl to 96 mg/dl, G2 in FBS demonstrated more promising outcomes, going from 98 mg/dl to 88 mg/dl. When compared to using regular sugar, both of them have proved advantageous. Sweeteners' impact on HDL, LDL, and (OC) is currently unknown. Our findings show an overall rise in OC, LDL, and decreased or regulated HDL.

Another study, however, found that while both low and high quantities of G4 significantly increase liver enzymes and damage the kidneys and the liver, G4 use can help lower triglycerides (TC), EHL, and MDL (19).

Various methods of treatment, such as natural remedies (20), minerals (21); vitamins (22); and dietary products (23), such as artificial sweeteners, have been used to control hyperglycaemic and lipid parameters. In reality, diabetic individuals are the ones who utilize NNS the most frequently. Because diabetes causes abnormalities in the glucose and lipid profiles, we did this study to prevent the inappropriate use of NNS in both those with diabetes and the broader public.

Conclusion

Regarding losing weight and regulating fasting blood sugar levels, G2, G5, and saccharin are the most advantageous. While (OC) was controlled, triglycerides rose in all groups. The outcomes fluctuate based on the sweetener used, and the findings from numerous research have contradictory inferences, which shows the degree of ambiguity in the impact of NSS. It has been discovered that using NNS is significantly more advantageous than using G1.

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