RESEARCH ARTICLE

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Does Saccharin Have Effects on Appetite, Energy Intake, And Serum Ghrelin? A Randomized, Controlled, Cross-Over Study in Healthy Males

ABSTRACT

Objective: Instead of sugar, artificial sweeteners that do not contain energy are widely used. However, contrary to popular belief, artificial sweeteners are thought to affect metabolism. Thus, purpose of this present study was to evaluate effects of saccharin on serum ghrelin, appetite, and food consumption.

Methods: Nine healthy males aged 20-29 participated in the randomized, controlled, and cross-over study. Each participant received 300 ml water, and 300 ml water containing 75 grams sucrose and 240 milligrams saccharin. At baseline, 30th, 60th, 90th, 120th, and 180th min, Visual Analog Scale was applied to evaluate appetite, and blood samples were taken to analyze ghrelin. After 180th min, participants consumed ad libitum diet, and kept 24-hours dietary food intake records until the end of this day.

Results: At 60th and 120th min, mean ghrelin level was higher in drinks containing only water and saccharin compared to drink containing sucrose ($p=0.001$, $p=0.003$ respectively). In addition, in 90th min following drink consumption, mean ghrelin level was higher in drink containing saccharin than sucrose test drink ($p=0.001$). Mean prospective food consumption and desire to eat score at 120th min after drink consumption was higher in saccharin test drink than sucrose test drink ($p<0.05$). Difference between energy and macronutrient intake was statistically insignificant ($p>0.05$).

Conclusions: In this study, which examined the effect of acute intake of saccharin an artificial sweetener, it is remarkable that high ghrelin levels and high scores related to appetite in some intervals after drink consumption containing saccharin. However, studies on the longer-term consumption of saccharin are needed to clarify these effects on appetite metabolism.

Keywords: Saccharin, Energy Intake, Appetite, Ghrelin Level, Artificial Sweetener.

Sakarinin İştah, Enerji Alımı ve Serum Ghrelin Üzerinde Etkisi Var mı? Sağlıklı Erkeklerde Randomize, Kontrollü, Çapraz Bir Çalışma

ÖZET

Amaç: Günümüzde şeker yerine enerji içermeyen yapay tatlandırıcılar yaygın olarak kullanılmaktadır. Ancak bilinenin aksine yapay tatlandırıcıların metabolizmayı çeşitli yönlerden etkilediği düşünülmektedir. Bu çalışmanın amacı sakarinin serum ghrelin düzeyi, iştah ve besin tüketimi üzerindeki etkilerini değerlendirmektir.

Gereç ve Yöntem: Randomize, kontrollü ve çapraz olarak yapılan çalışmaya 20-29 yaşları arasında dokuz sağlıklı erkek katılmıştır. Her katılımcıya 300 ml su, 75 gram sükroz içeren 300 ml su ve 240 miligram sakarin içeren 300 ml su verilmiştir. Başlangıç, 30., 60., 90., 120. ve 180. dakikalarda iştahı değerlendirmek için Görsel Analog Skala uygulanmış ve ghrelin analizi için kan örnekleri alınmıştır. Yüzsekseninci dakikadan sonra katılımcılar ad libitum beslenmişler ve her uygulama gününün sonuna kadar 24 saatlik besin tüketim kaydı tutmuşlardır.

Bulgular: Altmışıncı ve 120. dakikalarda sadece su ve sakarinli içeceklerde ortalama ghrelin düzeyi sükroz içeren içeceğe kıyasla daha yüksektir (sırasıyla $p=0.001$, $p=0.003$). Ayrıca içecek tüketimini takip eden 90. dakikada sakarin içeren içecekte ortalama ghrelin düzeyi sükroz içerene göre daha yüksektir ($p=0.001$). İçecek tüketiminden sonraki 120. dakikada ortalama besin tüketme potansiyeli ve yemek yeme isteği skorları, sakarin test içeceğinden sonra sükroz test içeceğine kıyasla daha yüksektir ($p<0.05$). Enerji ve makro besin ögesi alımlarında uygulamalar arasında farklılık bulunmamıştır ($p>0.05$).

Sonuç: Bir yapay tatlandırıcı olan sakarinin akut tüketim sonuçlarının incelendiği bu çalışmada, sakarin uygulamasında bazı ölçüm zamanlarındaki ghrelin düzeyinin ve iştah ile ilgili skorların yüksek olması dikkat çekicidir. Ancak iştah metabolizması üzerindeki bu etkilerin netlik kazanması için, sakarinin daha uzun süreli tüketimini ele alan çalışmalara gereksinim vardır.

Anahtar Kelimeler: Sakarin, Enerji Alımı, İştah, Ghrelin Düzeyi, Yapay Tatlandırıcılar

INTRODUCTION

Artificial sweeteners are defined as food additives frequently used in different foods and drinks that give an intensely sweet taste and reduce the energy density of the foods and drinks (1). In the communique, which covers sweeteners used to sweeten food and drinks prepared by the Turkish Food Codex Regulation and sweeteners offered directly to the consumer (2). Although the safety of artificial sweeteners such as aspartame, acesulfame potassium, advantame, neotame, saccharin, stevia, and sucralose have been approved by the Food and Drug Administration of America (FDA) (3), the results of the studies on the health effects of sweeteners cause controversy (4-6). Because the studies show that sweeteners are localized in the small intestine, and their mechanisms are not only related to taste in the tongue (4).

In this study, saccharin, used as an artificial sweetener, is 300 times sweeter than sucrose and was approved by the FDA in 1970 (7, 8). It is resistant to heat and acidity, and the acceptable daily intake (ADI) level determined by the FDA is 5 mg/kg/day (9). Eighty milligrams (mg) saccharin can provide 25 g sucrose's sweetness in quantity (10). Saccharin, first discovered in 1878, has the oldest historical background compared to all of the artificial sweeteners used to present (11). Saccharin, the only artificial sweetener used in the United States for a while, was proposed to be banned by the FDA in 1977 in line with the results of animal studies. However, this caused reactions, and it was decided that more studies should be conducted, and a warning label should be mandatory on all products containing saccharin. Based on the results of subsequent studies, although the effects of saccharin on human metabolism could not be clearly explained, it was removed from the carcinogen list in 2000, and the requirement to have warning labels that showing saccharin ingredient in products was removed (12). Today, artificial sweeteners are used in many products such as soft drinks, diet drinks, diet desserts, chewing gums, candies, biscuits, and crackers (1).

Weight gain and other adverse health outcomes due to frequent consumption of sugar-sweetened foods and drinks increased the trend towards the consumption of artificial or non-nutritional sweetener-containing products. However, sweeteners are not physiologically inert compounds. Potential biological mechanisms of sweetener consumption that may affect energy balance, metabolic function, effects on hormone release, cognitive processes, intestinal microbiota, and taste receptors should be investigated (13).

People might easily consume artificial sweeteners and foods and beverages containing these sweeteners to limit their daily energy intake, thinking that they will not harm their health and that they will not exceed the acceptable daily intake. However, until recently, these sweeteners were thought to be metabolically ineffective in the human body, some recent studies have led to some doubts that it increases ghrelin secretion and appetite (6, 14-16). Although the effects of various these sweeteners have been investigated in the mentioned studies, preferring saccharine as an artificial sweetener, and giving the standard breakfast to the participants in this study expresses the novelties of the study. In addition, the most important is the first study conducted with healthy people in Türkiye focused on the food consumption effects of saccharin that frequently is used in packaged products, beverages and is sold in boxes alone. It was hypothesized that saccharin can affect the blood ghrelin level, increase energy intake by changing appetite so that saccharin can act like sugar in the body. Therefore, this study was conducted to evaluate the effects of sucrose and the non-energy sweetener saccharin on the ghrelin hormone, appetite, and energy intake in healthy adult males.

MATERIAL AND METHODS

Participants: Informative posters were placed on the boards of the Faculty of Health Sciences, Ankara University, and interested people were asked to come to the study room. It was decided that volunteers were accepted for the study after being evaluated to meet the inclusion criteria of the study. In the study, volunteer male was found that between the ages of 20-29, having normal body weight (body mass index 18.5-24.9 kg/m^2), not exercising regularly, not having weight change more than 3 kilograms (kg) in the last six months, not having any disease diagnosed by the physician, who do not have gastrointestinal problems and food allergy/intolerance, have not undergone any surgery in the last one year, not taking prescription medication, not having disease diagnosed by a physician, not using non-prescription drugs, supplementation or pre-probiotic, and did not consume foods containing sweeteners and/or sweeteners in the last one week were included.

Ethics: The study was conducted in accordance with the Declaration of Helsinki. The study protocol received institutional review board approval and that all participants provided informed consent in the format required by the relevant authorities and/or boards. All procedures were approved by the Ethics Committee of the University Clinical Research Ethics Committee of Ankara University (Decision No: 17-1171-19, dated 2019). The participants' principle of volunteering was taken as a basis, and each participant signed the informed volunteer consent form.

Research Design: '3*3 Latin Square Trial Design' was used in the research to calculate the number of participants. According to this study design, all trials were applied to each individual to eliminate individual differences. At the beginning of the experiment, it is necessary to apply all tests

to all individuals and do randomize for determination of which application to start with. Since there are three different applications in the study, it was found adequate to be done with nine volunteers according to the mentioned Latin square trial design (17). Also, some similar studies evaluating the effects of aspartame and sucralose on glucose homeostasis and appetite were included in the study were considered when calculating this number. There were ten and eight subjects respectively in these studies of Tey et al., and Brown et al. (5, 18). Since it is known that women's energy intake varies before, during and after the menstrual cycle, only men were included in the study (19). The inclusion of only males in the study ensured that interindividual differences were minimized and increased the reliability of the study data.

Before the participants were included in the study, basic anthropometric measurements (body weight, height) were taken to evaluate their body mass index (BMI). All anthropometric measurements were measured in accordance with the technique and method (20). Participants with body mass index <18.5->24.9 kg/m^2 were not included in the study. Body fat ratios, muscle mass, and visceral fat levels of those with normal body weight were determined with a personal body analyzer (TANITA BC601) while having an empty stomach, wearing thin clothing, and without shoes. It was taken into consideration that the participants had similar characteristics in terms of body composition. Then, the general and health information of the participants was questioned.

Participants who did not have regular exercise habits were asked to record 24-hour physical activity on any day of the week since their different physical activity levels may affect the study results, especially their appetite. The total energy expenditure was obtained by multiplying minutes of activity types with activity factors related to the type of activity being calculated. Then this total energy was divided by 1440 to find the physical activity level. Participants with a physical activity level of 1.40-1.69 were considered sedentary or slightly active, participants with 1.70-1.99 as active or moderately regarded as active, participants with 2.00-2.40 considered severely active. Only sedentary or slightly active participants were included in the study. It was ensured that the participants sit and wait during the study after fasting for at least 10 hours (h) and did not consume anything other than the experimental design samples. It was stated to the participants that they should not consume foods and beverages containing sweeteners at least one week before starting the study, avoid heavy physical activity before each application day, do not consume anything unusual at dinner, maintaining the usual diet, and come with at least 10 h of hunger on the day of the application. The consumption status of the participants before the interventions was confirmed by evaluating the Continuous Glucose Monitoring System (GCMS-Medtronic iPro) reports placed the day before. After making sure that they did not consume anything, their data were included in the study. The inclusion criteria and the flow diagram of the study are shown in Figure 1.

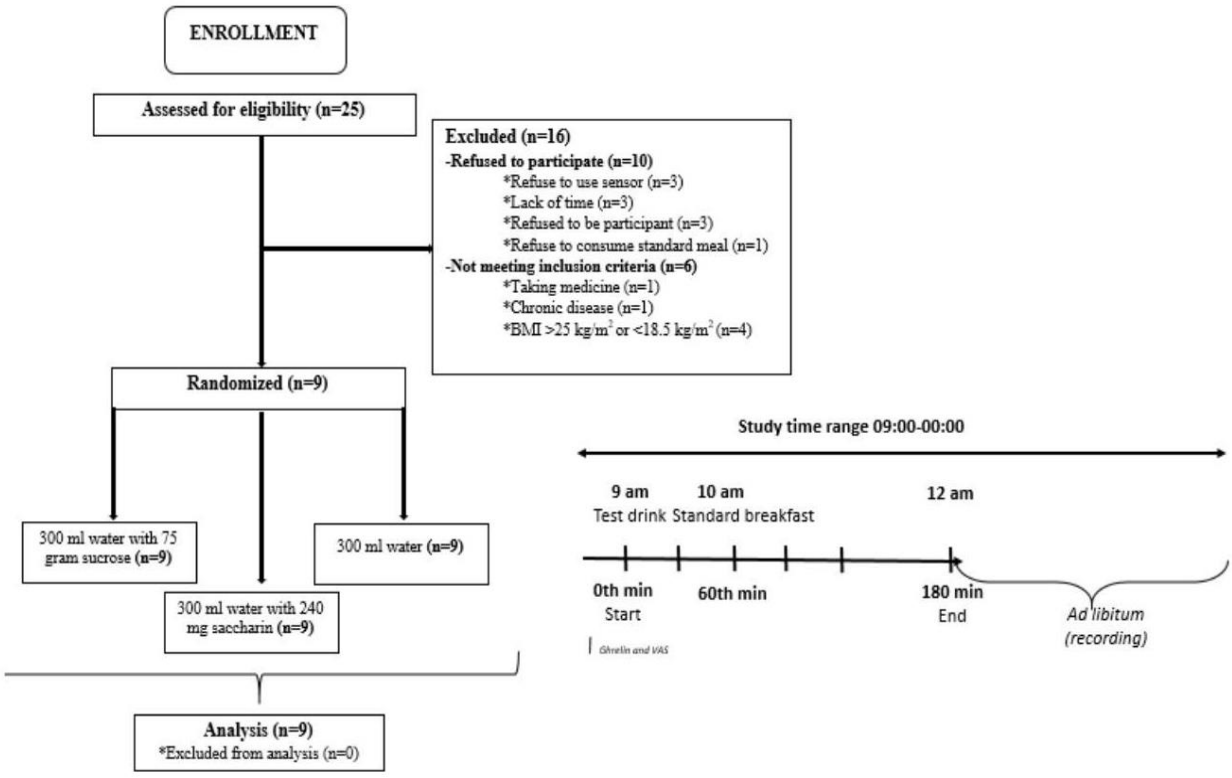

Figure 1. Flow diagram of study

Test Drinks and Breakfast Meal: This study was planned as a randomized and cross-over design. There was a washout time of 4-7 days between trials for each participant. The test drinks to be applied in the study were planned as 300 ml of water containing 75 grams (g) of sucrose (tea sugar), 300 ml of water containing 240 mg of saccharin with the same sweetness, and 300 ml of plain water without any sweetener added. Participants received 3.7±0.51 (2.8-4.3) mg saccharin per kg. It has been ensured that there were at least five days between the consumption of each test drink. Participants were asked to finish their test drinks within 5 minutes. Saccharin was chosen because it is one of the most used artificial sweeteners, especially in Türkiye.

A breakfast meal consisting of a standard 100 g white bread, 60 g white cheese, 150 g apple, and 200 ml unsweetened tea was planned 60 min after consuming the test drink. Breakfast contains 488 kilocalories (kcal) of energy, 80 g of carbohydrate, 20 g of protein, and 10 g of fat. By serving breakfast meals, complications related to prolonged hunger were prevented, and the effect of hunger on the data to be obtained was prevented. Participants were asked to finish breakfast within 20 min.

The individuals were asked to come to the Nutrition Principles Laboratory of the faculty at 08:50 on the application days, they were given a test drink at 09:00, and they were made to consume breakfast at 10:00. Blood samples were taken at 0., 30., 60., 90., 120. and 180. min to determine the serum total ghrelin level and to evaluate the effect of test drinks on the ghrelin level.

After obtaining the data, the participants were asked to return to their routine lives and to record in detail the food and drinks they consume as ad libitum until the end of the day (00:00). The daily energy and nutrient amounts of the participants from the food consumption record were calculated with the BEBİS 7.2 (Nutrition Information System) program.

Appetite Assessment: In order to evaluate the appetite of the participants, Visual Analogue Scale (VAS) was applied immediately after blood samples were taken at 0., 30., 60., 90., 120. and 180. min. With the Visual Analogue Scale (VAS), they were asked to evaluate their appetite between "not hungry at all" (0 mm) and "very hungry" (100 mm). The questions about appetite on the scale are hunger, satiety (fullness), prospective (forward-looking) eating power, estimated amount of food, and the estimated amount of sugary food consumption. The answers given by the participants to the VAS questions were quantitatively valued with the help of a 100 mm VAS scale.

Blood Analysis: Intravenous blood samples were taken for blood ghrelin analysis. An intravenous (intravascular) cannula was placed in the participants by the research doctor. A pink cannula was preferred for blood collection to ensure that the participants were not exposed to injection in every application and provided comfort and convenience. Veins located in the antecubital fossa (vena basilica, vena cephalica) were preferred because less pain remains in the arm, and they have large vessel diameters. The blood collection process was studied in accordance with asepsis and antisepsis prevention, three cc of blood was taken from the cannula in each application and transferred to purple capped EDTA (Ethylenediaminetetra-acetate) tubes. These tubes were kept in a cool place, and after the last blood was taken, they were taken to the laboratory where the analysis would be done. Blood samples collected in purple-capped tubes were centrifuged at 5000 RPM for 10 min in the laboratory and portioned after separating the plasma and stored at -80 C. After the data of all participants were completed, the plasma ghrelin level was analyzed by the ELISA method with the Affymetrix eBioscience brand kit.

Statistical Analysis: The analysis of the data was done in SPSS (IBM SPSS Statistics 23.0. Armonk, NY, USA Corp; 2013) for Windows 15 package program. In this study, it was hypothesized that there is an association between acute saccharin consumption and both ghrelin secretion and appetite.

The area under the curve of the blood ghrelin and VAS scores of the individuals was calculated using Microsoft Office Excel 2013 package program. The mean ($\bar{X}$) and standard deviation (SD), and min-max values were shown for dependent variables with a normal distribution. Nominal variables were given as number and percentage (%).

The regularity of the distribution for each parameter was evaluated using the Shapiro–Wilk test, and it was determined that the data showed normal distribution. The comparison among trials was performed using the Repeated Measures ANOVA test for the specified variables. Bonferroni correction was applied to find the difference between binary groups. Interim analysis was not done; statistical analysis was made in the period following the collection of data. The results were considered statistically significant at the $p<0.05$ level.

RESULTS

A total of 9 male volunteers with a mean age of 23.6±3.17 years participated in the study. The participants' mean body mass index value was 21.4±1.73 kg/m^2, and the body fat ratio was varied between 5.5-18.7% (Table 1).

Table 1. Mean and standard deviation values of age, body mass index, body analysis and physical activity levels of the participants (n=9)

Characteristics (n:9)	**X̄±SD**	**Min-Max**
Age (years)	23.6±3.17	20-29
BMI (kg/m^2)	21.4±1.73	19.1-24.1
Body fat ratio (%)	12.2±4.68	5.5-18.7
Muscle mass (kg)	54.3±3.88	49.1-60.7
Visceral fat level	1.7±1.06	1-3.5
Physical activity level	1.5±0.12	1.37-1.69

There was no statistically significant difference in serum ghrelin levels at the beginning and 30th min after consuming test drinks (p>0.05). After the consumption of test drink containing water and saccharin, the mean ghrelin level was higher at 60th and 120th than sucrose test drink consumption (p<0.05). In addition, the mean ghrelin level at 90 min following the consumption of the test drink containing saccharin was higher than the consumption of the sucrose test drink (p<0.05). The serum ghrelin level was significantly lower in the 180th min following consumption of the sucrose-containing test drink compared to water consumption (p<0.05) (Table 2).

Table 2. Mean and standard deviation values of serum ghrelin of participants at baseline and after test drinks consumption (n=9)

Ghrelin (pg/ml) / Intervals (min)	**Test Drinks**			**p**
	Saccharin	**Water**	**Sucrose**	
	X̄±SD	**X̄±SD**	**X̄±SD**	
Baseline	2249.4±1318.40	2205.0±1140.88	2230.0±1138.08	0.87
30	2289.5±1287.31	2273.8±1198.56	1851.7±1087.48	0.05
60	2424.8±1317.18^{a}	2356.8±1258.02^{a}	1493.2±891.89^{b}	0.001*
90	2291.9±1246.59^{a}	2136.7±1261.10^{ab}	1405.1±938.12^{b}	0.001*
120	1779.2±1013.06^{a}	1756.5±1156.13^{a}	1105.6±670.07^{b}	0.003*
180	1561.5±942.81^{ac}	1539.8±954.62^{ab}	1115.5±685.01^{c}	0.01*
p	0.003*	0.001*	0.0002*	

*Repeated Measures ANOVA Test *p<0.05*

^{abc} *Statistically significant difference between interventions*

For mean serum ghrelin, at the 60th and 120th minutes, sucrose was different from the others, at the 90th-minute saccharin and sucrose were different, and at the 180th minute, water and sucrose were different.

For saccharin trial, mean serum ghrelin was different at 90th minute from at 120th and 180th minutes. For water trial, mean serum ghrelin was different at 180th minute from at 30th and 60th minutes. For sucrose trial, mean serum ghrelin was different at baseline from at 60th, 120th, and 180th minutes. mean serum ghrelin was different at 30th minute from at 120th minute.

When the serum ghrelin responses of individuals were examined after consumption of test drink, ghrelin release after consumption of saccharin and water was higher than ghrelin release after sucrose consumption during the study (p<0.05). No statistically significant difference was found between the effects of saccharin and water consumption on the ghrelin response (p>0.05) (Table 3).

Table 3. Serum ghrelin responses of participants at baseline and after test drinks consumption (n=9)

Area under the curve (AUC) for ghrelin (pg/dlxmin)	**Test Drinks**			**p**
	Saccharin	**Water**	**Sucrose**	
	X̄±SD	**X̄±SD**	**X̄±SD**	
0-120 min	270615.5±148274.29^{ab}	262530.2±14334.50^{b}	192532.0±111356.99^{c}	0.002*
120-180 min	50110.8±28763.34^{ab}	49443.3±31560.30^{b}	33316.5±20175.86^{c}	0.014*
0-180 min	320726.3±175719.31^{ab}	311973.5±173046.54^{b}	225848.5±131022.54^{c}	0.002*
p	0.001*			

*Repeated Measures ANOVA Test *p<0.05*

^{abc} *Statistically significant difference between interventions*

For mean serum ghrelin responses, sucrose was different from the others.

For all trials, there were differences between AUCs.

As a result of the appetite scale applied after consuming the test drinks, the mean desire to eat and the prospective food consumption of the participants at the 120th min were found to be statistically significantly higher in saccharin application than sucrose application (p<0.05). It was determined that the mean scores obtained from other parameters of the appetite scale did not differ (p>0.05) (Figure 2).

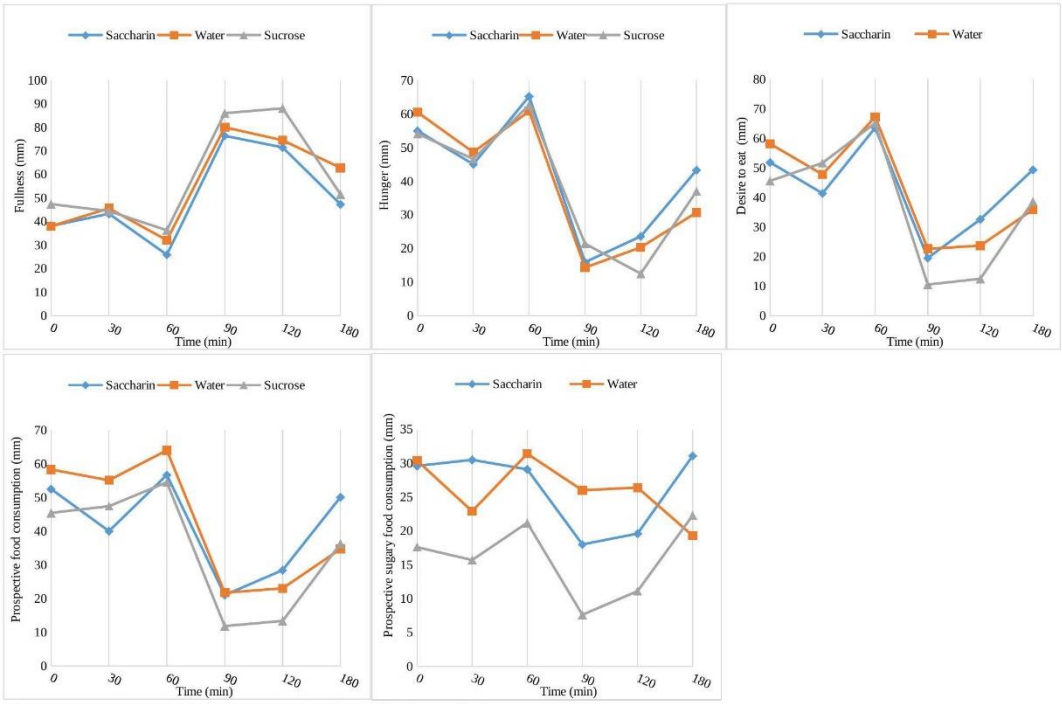

Figure 2. Appetite status of participants at baseline and after test drinks consumption

After the application ended, when the food consumption record data recorded by the participants until the end of the day were evaluated, no significant difference was found between the groups in terms of mean energy, macronutrients, and fiber intake (p>0.05) (Table 4).

Table 4. Energy and nutrient intakes of the participants on the test day

Energy and Nutrients	Test Drinks			p
	Saccharin	Water	Sucrose	
	X̄±SD	X̄±SD	X̄±SD	
Energy (kcal)	1602.7±477.96	1676.7±647.88	1334.5±403.21	0.12
Carbohydrate (g)	203.0±73.10	219.1±106.53	142.1±32.94	0.06
Protein (g)	51.9±17.34	61.7±28.22	48.9±12.53	0.31
Fat (g)	62.8±21.58	59.4±23.49	62.5±27.83	0.92
Dietary fiber (g)	18.0±11.59	21.5±16.74	12.9±6.44	0.14

*Repeated Measures ANOVA Test *p<0.05*
[abc] Statistically significant difference between interventions
There was no difference between values.
The breakfast meal consumed between 09:00 and 12:00 in the morning, which was the application period, was not included.

DISCUSSION

Appetite, which has a very complex mechanism, is controlled by neural and hormonal systems, and many factors affect it. The main hormone known to be associated with appetite is ghrelin (21). While the preprandial level of ghrelin depends on the activation of the autonomic nervous system (22), the decrease in the postprandial level depends on the macronutrient content of the meal (23). Because there is evidence that the subunits of taste receptors are located on ghrelin cells, but the mechanism is not fully known (24). In addition, simple sugars, it is known that artificial sweeteners also activate sweet taste receptor (25). The fact that artificial sweeteners used to restrict energy intake and provide sweet taste has this effect, suggesting that if artificial sweeteners are consumed in the meal, it may affect the next preprandial ghrelin levels and, therefore the appetite level. While this effect was found in some animal studies (26, 27), no such effect was found in others (28, 29). In this study, in which the effect of saccharin on appetite was examined, the strength of desire to eat and the prospective food consumption score after the 120th min after consumption of saccharin were found to be higher compared to sucrose consumption (p<0.05) (Figure 2). In the Visual Analogue Scale (VAS) score application, the scores of the expressions with the potential to increase energy intake such as hunger, prospective food consumption, and prospective sugary food consumption were higher in saccharin application. However, when the energy, macronutrients, and fiber intakes were compared after the applications, no significant difference was found (p>0.05) (Table

4). This result suggests that saccharin may have an acute effect on appetite. However, it should be considered that this effect on appetite may continue in case of continuous saccharin consumption. In a study conducted with 30 healthy male individuals comparing the effects of test drinks containing aspartame, monk fruit, stevia, and sucrose, there was no difference between the daily total energy intake of individuals consuming a drink containing sweetener and the daily total energy intake of individuals consuming a drink containing sucrose. However, it was determined that individuals who consume beverages containing sweeteners have higher energy intake at the next meal than the other group (6). In a study in which the effect of consumption of a diet drink sweetened with aspartame and a standard beverage was compared with healthy adult males and females, the total energy intake was similar (30). In another study, the effects of aspartame, monk fruit, stevia, and sucrose, which have the same sweetness ratio, on energy intake in 10 healthy male individuals were examined, and it was reported that the energy intake of those consuming non-energy sweeteners was higher than natural sweeteners and sucrose (5). It was first suggested in the aspartame study published in 1986 that the consumption of non-energy sweeteners increased appetite, and it was reported that aspartame beverage causes more hunger than water or glucose-containing drink (31). In a study by Rogers et al. (1988) on the appetite and nutritional intake of saccharin, aspartame, acesulfame-K and glucose and water with an equally sweet taste, participants were given a test meal one hour after the consumption of the test drink. As a result, it was stated that sweetener consumption stimulated hunger, and especially aspartame significantly increased the desire to eat (16). These data support the view that artificial sweeteners can increase energy intake and cause weight gain by changing appetite (5, 13, 14). Besides, in a study in which 1453 adults were followed for 28 years between 1984 and 2012, it was found that there was a relationship between long-term use of low-energy sweeteners and the prevalence of obesity and type 2 diabetes (14). Although different types and doses of artificial sweeteners used in studies cause contradictory results, artificial sweeteners such as saccharin, aspartame, acesulfame-K, and sucralose are thought to increase appetite.

This effect of artificial sweeteners on appetite may be due to their ability to increase orexigenic hormone levels. Therefore, the effect of saccharin on the ghrelin level was also examined, and it was found that the mean ghrelin level was higher at the 60th, 90th, and 120th min following the consumption of the test drink containing saccharin compared to the consumption of the sucrose test drink ($p<0.05$) (Table 2). In other words, ghrelin releases after consumption of saccharin and water during the study were higher compared to ghrelin release after sucrose consumption ($p<0.05$) (Table 3). This result shows that saccharin can affect appetite by increasing the blood ghrelin level. There is no study investigating the effect of saccharin on the ghrelin level in the literature, but the results of studies conducted with other sweeteners support the results of this study (14-16). In the study of Brown et al. (2011) comparing the effect of sucrose and sucralose, it was stated that sucrose provided a moderate decrease in acylated ghrelin level and sucralose did not have this effect. Based on this result, the researchers stated that nondecreasing the ghrelin level may increase energy intake (18).

From another point of view, the increase in ghrelin level after saccharin consumption may not only increase the appetite but also increase the desire to consume saccharin or sugar-containing foods and beverages. This view is supported by the study showing that intraperitoneally injected ghrelin in mice increases the consumption of food containing saccharin and that the increase in ghrelin level causes an increase in consumption of sweet-tasting food regardless of energy content (15). Excessive food consumption caused by sweet and tasty flavors may be due to decreased activation of orexigenic neuropeptides and the opioid system (32).

It is also suggested that artificial sweeteners affect not only ghrelin but also other hormone levels that affect appetite (4, 15, 29). It is important and necessary to evaluate orexigenic and anorexigenic hormones together to explain the effect of saccharin on appetite more clearly.

Among the strengths of the study was that the study results suggested that artificial sweeteners like saccharine, known to be completely ineffective, may be particularly effective on appetite. Also, standard breakfast was given to prevent long-time hunger and only males were included to avoid various influences on data of the study. One of the limitations of the study was that it was to determine the sample size at a minimal level because the study included invasive interventions. In addition, ad libitum meals could have been given after trials so that a comparison of daily energy and nutrient intakes could have been made. Thus, it could have been tested whether there was a difference between the energy and nutrients taken in the acute period. These are among the limitations of this research.

CONCLUSION

In this study, it was determined that saccharin increased ghrelin release from the 60th min, and also increased the desire to eat at the 120th min and the food consumption potential in healthy and normal-weight adult males. It is important to evaluate this effect of saccharin in individuals with different health problems such as obesity, metabolic syndrome, or diabetes, and in individuals of

different ages and genders. Besides, the acute effect of saccharin was investigated in this study. Longitudinal studies including other parameters affecting the appetite are needed to reveal the effects of saccharin on appetite metabolism more clearly.

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