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Short communications

Weight reducing effect and safety evaluation of rare sugar syrup by a randomized double-blind, parallel-group study in human

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ABSTRACT

Rare sugar syrup is a sweetener obtained from high-fructose corn syrup under slightly alkaline conditions, which promotes the formation of rare sugars. Here, the physiological impact and safety of rare sugar syrup in humans was investigated by a randomized double-blind parallel experiment. Thirty-four subjects with an average body mass index of 25.6 kg/m^2 were divided into two groups. Subjects consumed either a test drink containing rare sugar syrup or an isocaloric control drink containing high-fructose corn syrup on a daily basis for 12 weeks. Results showed significant decreases in body weight, body fat percentage and waist circumference in the rare sugar syrup group compared to the control. No adverse events with regard to hepatic and renal function or blood parameters were observed. Our study conclusively suggests, for the first time, that rare sugar syrup is a safe sweetener, and that continuous consumption of this syrup could help weight management.

1. Introduction

High-fructose corn syrup (HFCS) and high intensity sweeteners are used in a wide range of food and beverage products. The rise in these consumption, however, is reported to be associated with the growing risk of diabetes and obesity in the United States (Gross, Li, Ford, & Liu, 2004; Swithers, 2013). Because these issues relate to complex factors, such as appetite, excessively consumed amount, kinds of carbohydrate, and genetic or social background of consumer, it is needed to cautiously discuss and propose a solution to the social demand that is the development of better sweeteners smoothing these problems.

Recently, some interesting biological properties have been reported for rare sugars. 'The International Society of Rare Sugars' defined rare sugars as monosaccharides and their derivatives that are present in limited quantities in nature. For

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example, allulose (psicose) is a rare sugar that has been well-studied in terms of its efficacy, safety and production using microorganisms. Allulose has nutritional benefits such as prevention of diabetes (Chung, Oh, & Lee, 2012; Matsuo & Izumori, 2009) and improvement of lipid metabolism (Matsuo et al., 2001). In addition, the inclusion of around 4% allulose in HFCS suppresses abdominal fat accumulation caused by the long-term ingestion of HFCS (Yamada et al., 2010).

As a natural and economically-feasible method for obtaining syrup containing around 5% allulose, the quantity of which is sufficient to suppress fat accumulation, we reisomerized HFCS under mildly-basic conditions, similar to those used in food processing applications such as sugar refining. This method produces other monosaccharides, such as allose, sorbose and mannose, which have been reported to improve lipid and carbohydrate metabolism or immune function (Kimura et al., 2005; Toyota, Fukushi, Katoh, Orikasa, & Suzuki, 1989; Yamada et al., 2014).

The obtained syrup containing rare sugars, called rare sugar syrup (RSS), has 90% of the sweetness of sucrose, and with a richer and cleaner taste than HFCS. Accordingly, we reasoned that RSS might provide an alternative to HFCS, which may reduce the likelihood of obesity caused by the consumption of HFCS.

We previously tested the safety and efficacy of RSS for daily use as a sweetener in a series of feeding experiments using rats. Our study showed ingestion of RSS caused a reduction in body weight and abdominal fat with no detectable adverse effects (Iida et al., 2013; Matsuo et al., 2011). However, detailed information concerning the safety and efficacy of RSS for humans is being reported now for the first time. Based on our previous animal study, we conducted this experiment in humans to know more about an effective safe dose of rare sugar syrup, and to evaluate biomarkers during long-term consumption.

2. Materials and methods

2.1. *Subjects*

This study was approved by the ethics committee of Isogo Central Hospital (Kanagawa, Japan) in accordance with the spirit of the Declaration of Helsinki, and conducted under the supervision of the principle investigator. Informed consent was obtained from 34 volunteers (17 males and 17 females) with an average BMI score of 25.6 kg/m^2 (22–63 years old). A BMI score of more than 25 kg/m^2 is considered as obese as defined by the Japan Society for the Study of Obesity. Exclusion criteria included people with hepatic or renal dysfunction. Subjects participating in this study were randomly divided into two groups by the minimization method based on inspected medical centers, age, gender, BMI, body weight, body fat ratio and waist circumference. Other background details for each group are given in Table 1.

Table 1 – Background of the subjects.

	Control	RSS
Number of subjects	17	17
Male–female ratio	Male: 9, female: 8	Male: 8, female: 9
Age (years)	42.4 ± 2.6	41.7 ± 2.8
Weight (kg)	70.4 ± 2.5	70.2 ± 3.0
Height (cm)	165.8 ± 2.6	165.7 ± 2.5
BMI (kg/m^2)	25.6 ± 0.6	25.4 ± 0.6
Body fat ratio (%)	28.4 ± 1.7	28.2 ± 2.1
Waist circumference (cm)	83.9 ± 2.6	84.7 ± 2.7

Data are expressed as mean ± SEM.

2.2. *Experimental diets*

The compositions of RSS and HFCS were analyzed by high performance liquid chromatography with a MCI GEL CK 08EC column (8 i.d. × 300 mm; Mitsubishi Chemical Corporation) and refractive index detector. RSS was composed of 44.3% glucose, 31.9% fructose, 6.0% allulose and 17.8% other saccharides, in which 7.5% mannose and sorbose were detected as non-dividable peaks and 4.6% oligosaccharides were also detected. The oligosaccharides analyzed were primarily disaccharides (3.7%) including 0.7% maltose, 1.2% isomaltose, and 0.9% maltulose derived from oligosaccharides in HFCS (Osaki & Yoshino, 1982; Shiraishi, Kawakami, & Kusunoki, 1985). As a reference, we confirmed around 1% allose in RSS using other method. HFCS was composed of 41.8% glucose, 52.3% fructose and 5.9% oligosaccharides.

The energy exchange ratios of glucose and fructose are 4 kcal/g, and that of allulose is 0 kcal/g. Other saccharides for which the energy exchange ratios were not determined were calculated as 4 kcal/g based on the maximum calorie obtained for the carbohydrate. The test drink containing 30 g of RSS (114 kcal) and the control drink containing 28 g of HFCS (114 kcal) as dry solid base were then used as the experimental diets. Each experimental diet had the same calorific value and was prepared in the form of an identical 200 g jelly drink, with an indistinguishable flavor and color.

HFCS used as the sweetener for the control drink was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). RSS used for the test drink was made by Matsutani Chemical Industry, Co., Ltd. (Hyogo, Japan).

2.3. *Study design*

The experiment was based on a randomized double-blind, two-group parallel, placebo-controlled comparison study. The 20-week study period comprised 4 weeks for pre-treatment observation, 12 weeks for treatment, and 4 weeks for post-treatment observation. During the treatment phase the subjects consumed either a test drink or a control drink 30 minutes before breakfast on a daily basis. Subjects were instructed to make seven scheduled visits to the medical center for testing as follows: firstly at the beginning of the pre-treatment observation period and then during weeks 0, 2, 4, 8 and 12 of the treatment period, and finally at the end of the post-treatment observation period.

During each visit body weight, body fat ratio, waist circumference, hip circumference, systolic blood pressure and diastolic blood pressure were measured and blood and urine samples were collected. Each subject was interviewed by a doctor to investigate any subjective symptoms in body condition (e.g.,

headache, dizziness, gastric problems) and/or any changes in daily habits.

All measurements of blood and urine collection were taken by SRL, Inc. Co., Ltd. (Tokyo, Japan). The laboratory parameters tested were as follows: white blood cells, red blood cells, hemoglobin, hematocrit, platelets, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactase dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP), cholinesterase, creatine phosphokinase (CPK), total protein (TP), serum amylase, albumin (Alb), albumin globulin to ratio (A/G), total bilirubin, direct bilirubin, indirect bilirubin, urea nitrogen (BUN), uric acid, creatinine, sodium, potassium, chloride, calcium, magnesium, iron, inorganic phosphate, total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol, free fatty acid (FFA), phospholipid (PL), glucose, hemoglobin A1c (HbA1c), insulin, acetoacetic acid, 3-hydroxybutyric acid, and total ketone body. In addition, leptin, adiponectine, retinol-binding protein (RBP), des-acylghrelin, and high sensitivity TNF-α, oxidized low-density lipoprotein, lipid hydroperoxide, and serum pentosidine were tested at 0 and 12 weeks. All subjects were instructed to visit the medical center after at least 10 h of fasting over the previous night to minimize any extraneous dietary influences on the test results.

Urine test strips were used to detect protein, glucose, urobilinogen and occult blood. These results of each subject were expressed as false-positive (±) or positive (1+, 2+, 3+).

The subjects were asked to record all foods and beverages consumed for 3 days before each visit. Nutritional analysis was conducted by dieticians to calculate daily intake of energy, protein, fat, carbohydrate, saturated fatty acid, unsaturated fatty acid, cholesterol and dietary fibre. The amount of exercise was calculated by the numbers of walking steps recorded with a pedometer for 3 days before each visit except for week 2.

2.4. *Statistical analyses*

All the values measured are shown as the mean ± standard error of the mean (SEM). The physical examinations, hematological examinations, urine specific gravity, nutrient intake and number of walking steps were compared between the groups by the unpaired Student's t-test. Bonferroni multiple comparison test was used to compare baseline values and values during the treatment and observation periods in each group. For physical examination, repeated two-way ANOVA was used to assess time-by-treatment interaction. Urine glucose, protein, urobilinogen and occult blood were compared between the groups by using the Mann–Whitney U-test. Wilcoxon t-test was used to compare baseline values and values during the treatment and observation periods in each group. A risk factor of $p < 0.05$ was considered statistically significant.

3. Results and discussion

None of the subjects in this study were excluded based on the exclusion criteria, a criteria to exclude the test subjects based on abnormal blood, physical parameters, irregular lifestyles, or being judged as unfit by the principle investigator.

3.1. *Physical examination*

Data on physical examination are shown in Fig. 1 and Table 2. Hip circumference decreased significantly in the RSS group at weeks 4, 8 and 12 compared to week 0 and body weight, BMI, body fat ratio and waist circumference decreased significantly at weeks 8 and 12 compared to week 0. Diastolic blood pressure decreased significantly in the RSS group at week 12 compared to week 0 and by comparison with the control group.

Although a tendency to "return to an original condition" in terms of body weight was seen during the observation period in the RSS group, we believe our findings demonstrate that continuous consumption of RSS can control weight gain. Moreover, discontinuing the consumption of RSS appears to return the subject to his/her original condition without any measurable damage to organs or tissues.

The anti-obesity activity of RSS is thought to be caused by rare sugars present in the preparation. One of the bioactive ingredients contained in RSS is allulose. In the current study, RSS made from HFCS contained 6% allulose, which was sufficient to limit the accumulation of visceral fat during the intake of caloric sweetener.

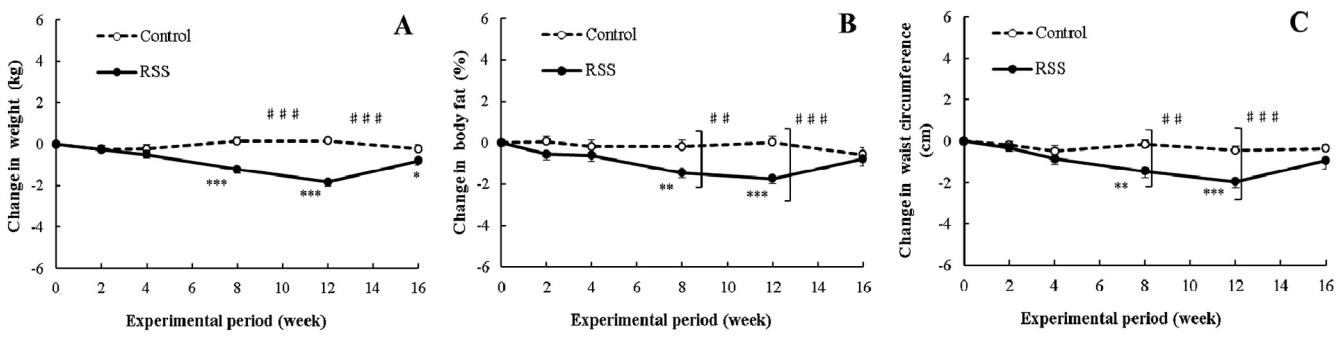

Fig. 1 – Amount of changes in body weight (A), body fat ratio (B) and waist circumference (C) during a 12-week treatment period and 4-week observation period in slightly obese human subjects. Values are expressed as mean ± SEM. Significant differences were shown: $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ between two groups by unpaired t-test and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, at 0 week vs. 2, 4, 8, 12, 16 week by Bonferroni's multiple comparison test.

Table 2 – Alteration in physical parameters during treatment of either the RSS or control drinks for 12 weeks and after the observation periods.

Item	Week 0	Week 2	Week 4	Week 8	Week 12	Observation	p-value time × treatment
Weight (kg)							$p < 0.001$
Control	70.36 ± 2.47	70.12 ± 2.43	70.13 ± 2.44	70.51 ± 2.46	70.52 ± 2.43	70.12 ± 2.43	
RSS	70.24 ± 3.03	69.98 ± 3.05	69.74 ± 3.04	69.02 ± 3.00***	68.39 ± 3.04***	69.43 ± 3.02*	
BMI							$p < 0.001$
Control	25.57 ± 0.59	25.48 ± 0.58	25.49 ± 0.59	25.63 ± 0.61	25.63 ± 0.58	25.49 ± 0.58	
RSS	25.42 ± 0.59	25.32 ± 0.59	25.24 ± 0.60	24.98 ± 0.59***	24.74 ± 0.59***	25.13 ± 0.60*	
Body fat ratio (%)							$p < 0.001$
Control	28.39 ± 1.72	28.45 ± 1.75	28.22 ± 1.73	28.21 ± 1.78	28.41 ± 1.73	27.82 ± 1.72	
RSS	28.16 ± 2.12	27.61 ± 2.07	27.56 ± 2.07	26.73 ± 2.04**	26.44 ± 2.04***	27.41 ± 2.11	
Waist circumference (cm)							$p < 0.001$
Control	83.87 ± 2.59	83.69 ± 2.59	83.38 ± 2.55	83.72 ± 2.56	83.42 ± 2.54	83.51 ± 2.49	
RSS	84.72 ± 2.73	84.39 ± 2.77	83.87 ± 2.67	83.26 ± 2.68**	82.75 ± 2.67***	83.79 ± 2.68	
Hip circumference (cm)							$p < 0.01$
Control	95.92 ± 1.48	95.78 ± 1.46	95.78 ± 1.50	95.74 ± 1.52	95.45 ± 1.49	95.68 ± 1.53	
RSS	98.90 ± 2.15	98.66 ± 2.16	98.35 ± 2.14*	97.75 ± 2.16*	97.31 ± 2.14***	98.40 ± 2.11	
Systolic blood pressure (mmHg)							
Control	122.80 ± 2.50	122.70 ± 2.70	121.20 ± 2.60	121.90 ± 2.40	121.90 ± 2.40	122.50 ± 2.60	
RSS	117.70 ± 3.30	118.50 ± 3.10	117.00 ± 3.20	117.60 ± 3.40	117.10 ± 2.90	120.60 ± 2.50	
Diastolic blood pressure (mmHg)							
Control	76.90 ± 2.30	76.70 ± 2.10	76.20 ± 2.20	74.40 ± 2.50	74.50 ± 2.40 #	75.10 ± 2.40	
RSS	74.30 ± 2.00	74.10 ± 2.10	72.60 ± 2.00	70.60 ± 1.80	68.20 ± 1.60*	71.80 ± 1.70	

Values are expressed as mean ± SEM.
p-value for time-by-treatment interaction was assessed by repeated two-way ANOVA.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$: Significantly different from 0 week (Bonferroni's multiple comparison test).
# $p < 0.05$: Significantly different between two groups (unpaired t-test).

There are several possible mechanisms for the observed anti-obesity activity elicited by allulose. One of the mechanisms involves allulose induced activation of glucokinase translocation in the liver that facilitates glycogen biosynthesis (Toyoda et al., 2010). The effect of glycogen synthesis from glucose in the liver is to restrict glucose usage, which promotes the metabolism of fat as an energy source. Ochiai, Onishi, Yamada, Iida and Matsuo (2014) reported that the inclusion of allulose in the diet of rats increased their energy expenditure, which might be induced by enhanced fat metabolism initiated by glucokinase translocation.

Other saccharides present in RSS, such as allose and sorbose, also have the potential to suppress an increase in abdominal fat. There were many reports suggesting that allose reduces oxidative stress (Kimura et al., 2005); one mechanism being decreased expression of NADPH oxidase. These effects may contribute to an amelioration of insulin resistance. Sorbose was reported to suppress the activity of α-glucosidase (sucrase and maltase) and to decrease serum insulin levels (Yamada et al., 2014). Co-administration of both glucose and fructose is known to lead to more energy expenditure than a single administration of glucose or fructose (Blaak & Saris, 1996).Though adequate intake amount of RSS is thought to be an important factor, an appropriate balance of these sugars in RSS may have contributed to the observed reduction in body weight and body fat in this study.

3.2. *Hematological examination*

As shown in Table 3, though slight variances were observed in some parameters, there were no abnormalities in hepatic function, renal function or blood parameters. These significant variations in hematological parameters were within the standard range. In particular, intake of test carbohydrates did not increase HbA1c value and oxidation of serum lipid in either of the groups.

In the control group, cholinesterase, hemoglobin, potassium, magnesium and chloride were significantly different at intake or observation periods compared to week 0. The RSS group displayed a significant increase in white blood cells at week 4 (RSS; 6876.5 ± 577.7/μl, control; 5505.9 ± 296.2/μl, $p < 0.05$) and decrease in sodium at the observation period (RSS; 140.4 ± 0.4 mEq/l, control; 141.5 ± 0.4 mEq/l, $p < 0.05$) compared to the control group.

The variations of obesity markers (leptin, adiponectin, RBP, des-acylghrelin and TNF-α) are shown in Fig. 2. RBP decreased significantly in the RSS group at week 12 compared to week 0. Leptin in the RSS group increased significantly over the same period. Des-acylghrelin increased significantly in both the control group and the RSS group. Leptin is known to be an appetite-suppressing hormone that increases energy metabolism. RBP is reported to be an insulin-resistant cytokine that is highly expressed in visceral fat (Klöting et al., 2007). The

Table 3 – Alteration in hematological parameters during treatment of either the RSS or control drinks for 12 weeks and after the observation periods.

Item	Standard range	Week 0	Week 2	Week 4	Week 8	Week 12	Observation
ALP (U/l)							
Control	115–359	205.2 ± 10.9	202.2 ± 9.8	199.4 ± 9.6	201.3 ± 8.1	199.5 ± 11.1	195.1 ± 10.6
RSS		203.4 ± 14.2	190.9 ± 12.4	195.5 ± 13.4	201.2 ± 13.3	191.6 ± 11.4	192.9 ± 12.1
AST (U/l)							
Control	10–40	18.6 ± 0.9	18.1 ± 0.9	18.2 ± 0.9	17.7 ± 1.0	17.2 ± 0.9	17.3 ± 0.9
RSS		19.4 ± 1.2	19.3 ± 1.1	18.1 ± 1.0	18.8 ± 0.9	18.9 ± 1.1	18.8 ± 0.9
ALT (U/l)							
Control	5–40	18.9 ± 1.6	18.6 ± 1.5	18.2 ± 1.5	17.9 ± 1.4	17.8 ± 1.4	14.9 ± 1.0
RSS		19.5 ± 1.7	17.7 ± 1.8	17.0 ± 1.6	17.9 ± 1.6	17.2 ± 1.5	16.8 ± 1.4
LDH (U/l)							
Control	115–245	168.6 ± 4.9	153.8 ± 4.0***	161.0 ± 4.3	156.6 ± 5.3*	163.4 ± 4.2	157.8 ± 5.4
RSS		174.8 ± 8.0	169.1 ± 8.1	154.4 ± 7.4*	161.5 ± 7.0	167.6 ± 8.8	162.3 ± 7.5
γ-GTP (U/l)	M: ≦70						
Control	F: ≦30	31.2 ± 4.3	30.4 ± 5.0	29.4 ± 4.6	35.0 ± 6.6	31.7 ± 5.3	29.4 ± 4.5
RSS		24.7 ± 3.4	22.0 ± 2.6	23.0 ± 3.1	27.1 ± 4.0	25.4 ± 4.4	25.9 ± 4.1
CPK (U/l)	M: 62–287						
Control	F: 45–163	95.2 ± 9.1 #	85.1 ± 6.4 ##	106.8 ± 13.0	92.9 ± 7.0	92.1 ± 8.2 #	106.4 ± 22.4
RSS		137.5 ± 14.9	151.8 ± 21.2	132.1 ± 16.9	122.2 ± 13.7	156.9 ± 29.0	142.8 ± 20.1
TP (g/dl)	6.7–8.3						
Control		7.41 ± 0.08	7.25 ± 0.09	7.28 ± 0.08	7.31 ± 0.09	7.27 ± 0.13	7.30 ± 0.11
RSS		7.41 ± 0.11	7.26 ± 0.08	7.29 ± 0.09	7.28 ± 0.08	7.29 ± 0.10	7.35 ± 0.10
Alb (g/dl)	4.0–5.0						
Control		4.72 ± 0.06	4.61 ± 0.05	4.62 ± 0.05	4.63 ± 0.05	4.64 ± 0.07	4.61 ± 0.06
RSS		4.59 ± 0.10	4.48 ± 0.08	4.52 ± 0.08	4.51 ± 0.09	4.54 ± 0.09	4.56 ± 0.08
A/G							
Control		1.77 ± 0.07	1.79 ± 0.07	1.78 ± 0.07	1.78 ± 0.08	1.81 ± 0.08	1.75 ± 0.06
RSS		1.65 ± 0.06	1.63 ± 0.06	1.66 ± 0.06	1.66 ± 0.05	1.66 ± 0.05	1.68 ± 0.06
Total bilirubin (mg/dl)	0.3–1.2						
Control		0.73 ± 0.09	0.69 ± 0.05	0.72 ± 0.07	0.78 ± 0.06	0.78 ± 0.05	0.71 ± 0.06
RSS		0.61 ± 0.07	0.69 ± 0.08	0.70 ± 0.08	0.79 ± 0.10	0.72 ± 0.10	0.71 ± 0.07
Direct bilirubin (mg/dl)	≦0.4						
Control		0.21 ± 0.02	0.18 ± 0.02	0.19 ± 0.02	0.21 ± 0.01	0.23 ± 0.02	0.20 ± 0.02
RSS		0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.03	0.21 ± 0.03	0.23 ± 0.03	0.21 ± 0.02
Indirect bilirubin (mg/dl)	≦0.8						
Control		0.52 ± 0.06	0.51 ± 0.04	0.53 ± 0.05	0.57 ± 0.05	0.55 ± 0.04	0.51 ± 0.04
RSS		0.44 ± 0.05	0.51 ± 0.06	0.51 ± 0.06	0.58 ± 0.08	0.49 ± 0.07	0.50 ± 0.05
Uric acid (mg/dl)	M: 3.7–7.0						
Control	F: 2.5–7.0	5.15 ± 0.29	4.99 ± 0.27	4.88 ± 0.27	5.05 ± 0.30	5.10 ± 0.28	4.90 ± 0.26
RSS		5.29 ± 0.32	4.92 ± 0.31	5.02 ± 0.33	5.06 ± 0.27	5.04 ± 0.30	5.05 ± 0.29
BUN (mg/dl)	8.0–22.0						
Control		13.65 ± 0.81	12.05 ± 0.35	13.45 ± 0.57 #	13.42 ± 0.66	13.39 ± 0.72	13.34 ± 0.74
RSS		12.18 ± 0.61	12.09 ± 0.59	11.82 ± 0.48	12.15 ± 0.60	11.86 ± 0.58	12.27 ± 0.79
Creatinine (mg/dl)	M: 0.61–1.04						
Control	F: 0.47–0.79	0.665 ± 0.024	0.666 ± 0.025	0.651 ± 0.028	0.672 ± 0.023	0.684 ± 0.029	0.671 ± 0.026
RSS		0.718 ± 0.026	0.706 ± 0.023	0.718 ± 0.022	0.728 ± 0.024	0.712 ± 0.024	0.717 ± 0.020

(continued on next page)

Table 3 – (*continued*)

Item	Standard range	Week 0	Week 2	Week 4	Week 8	Week 12	Observation
Triglyceride (mg/dl)	50–149						
Control		90.2 ± 14.8	96.9 ± 13.7	87.4 ± 9.5	86.6 ± 11.2	103.8 ± 15.9	88.4 ± 15.7
RSS		99.3 ± 11.9	94.4 ± 11.4	86.2 ± 12.6	92.2 ± 12.0	90.0 ± 12.4	97.9 ± 13.8
T-Cho (mg/dl)	150–219						
Control		209.1 ± 9.3	205.2 ± 7.0 #	207.9 ± 6.5 #	212.9 ± 7.0 ##	203.9 ± 7.1 #	197.1 ± 7.3
RSS		190.1 ± 7.3	182.1 ± 6.2	185.6 ± 6.7	184.9 ± 5.4	182.1 ± 6.7	181.5 ± 5.9
LDL-C (mg/dl)	70–139						
Control		121.4 ± 8.8	115.9 ± 6.0	119.9 ± 5.8	122.6 ± 5.8	116.4 ± 6.1	110.7 ± 6.0
RSS		108.2 ± 6.8	106.4 ± 5.1	108.2 ± 5.2	106.5 ± 5.6	106.1 ± 6.3	101.9 ± 5.4
HDL-C (mg/dl)	M: 40–86						
Control	F: 40–96	65.6 ± 3.8	66.2 ± 4.1	66.9 ± 4.7	69.5 ± 5.8	68.1 ± 5.4	63.7 ± 4.6
RSS		62.7 ± 3.4	57.4 ± 2.6	60.8 ± 3.5	60.0 ± 3.0	61.2 ± 3.3	60.4 ± 3.7
FFA (μEq/dl)	140–850						
Control		590.5 ± 59.7	419.0 ± 45.3	462.1 ± 66.3	442.4 ± 59.6	484.8 ± 47.0	446.6 ± 59.3
RSS		489.0 ± 40.4	470.2 ± 56.6	453.9 ± 50.7	455.8 ± 51.1	517.3 ± 66.2	514.4 ± 55.5
PL (mg/dl)	160–260						
Control		217.4 ± 8.3	212.6 ± 6.6	215.2 ± 6.4	220.4 ± 7.2 #	217.8 ± 7.4	211.2 ± 8.4
RSS		206.2 ± 6.0	196.5 ± 6.2	202.0 ± 6.3	202.3 ± 5.1	199.6 ± 6.6	203.3 ± 6.4
Glucose (mg/dl)	70–109						
Control		86.5 ± 2.5	87.5 ± 2.0	87.4 ± 1.2	86.8 ± 2.1	86.9 ± 2.5	85.4 ± 1.7
RSS		88.0 ± 2.1	86.2 ± 2.0	86.4 ± 1.9	88.8 ± 1.7	87.2 ± 1.6	86.5 ± 1.2
HbA1c (%)	4.3–5.8						
Control		4.80 ± 0.05	4.84 ± 0.06	4.75 ± 0.05	4.68 ± 0.06	4.68 ± 0.05	4.76 ± 0.06
RSS		4.86 ± 0.07	4.85 ± 0.07	4.78 ± 0.07	4.75 ± 0.07	4.74 ± 0.08	4.73 ± 0.07
Insulin (μIU/ml)	1.84–12.2						
Control		5.980 ± 0.760	6.101 ± 0.566	5.706 ± 0.609	5.239 ± 0.694	5.912 ± 0.716	5.413 ± 0.721
RSS		6.072 ± 0.741	5.698 ± 0.702	6.114 ± 0.851	6.455 ± 0.579	6.809 ± 0.953	5.552 ± 0.717
Acetoacetic acid (μmol/l)	≦55						
Control		42.1 ± 10.4	16.5 ± 3.6	19.9 ± 5.1	21.5 ± 5.1	20.2 ± 4.5	22.6 ± 5.4
RSS		23.6 ± 5.4	24.5 ± 5.1	20.0 ± 7.6	23.7 ± 4.8	31.8 ± 9.5	23.7 ± 3.9
3-Hydroxybutyric acid (μmol/l)	≦85						
Control		138.1 ± 43.9	44.9 ± 10.1	58.5 ± 16.4	58.8 ± 17.4	51.9 ± 13.9	69.2 ± 20.3
RSS		55.4 ± 15.6	62.6 ± 14.9	50.0 ± 19.3	49.6 ± 11.2	79.2 ± 29.8	69.6 ± 15.6
Total ketone body (μmol/l)	≦130						
Control		180.2 ± 53.3	61.4 ± 13.7	78.4 ± 21.5	80.4 ± 22.4	72.1 ± 18.1	91.8 ± 25.5
RSS		79.0 ± 20.9	87.1 ± 19.9	70.0 ± 26.8	73.4 ± 15.8	111.1 ± 39.0	93.3 ± 19.4

Values are expressed as mean ± SEM.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$: Significantly different from 0 week (Bonferroni's multiple comparison test).

$^{\#}p < 0.05$, $^{\#\#}p < 0.01$: Significantly different between two groups (unpaired *t*-test).

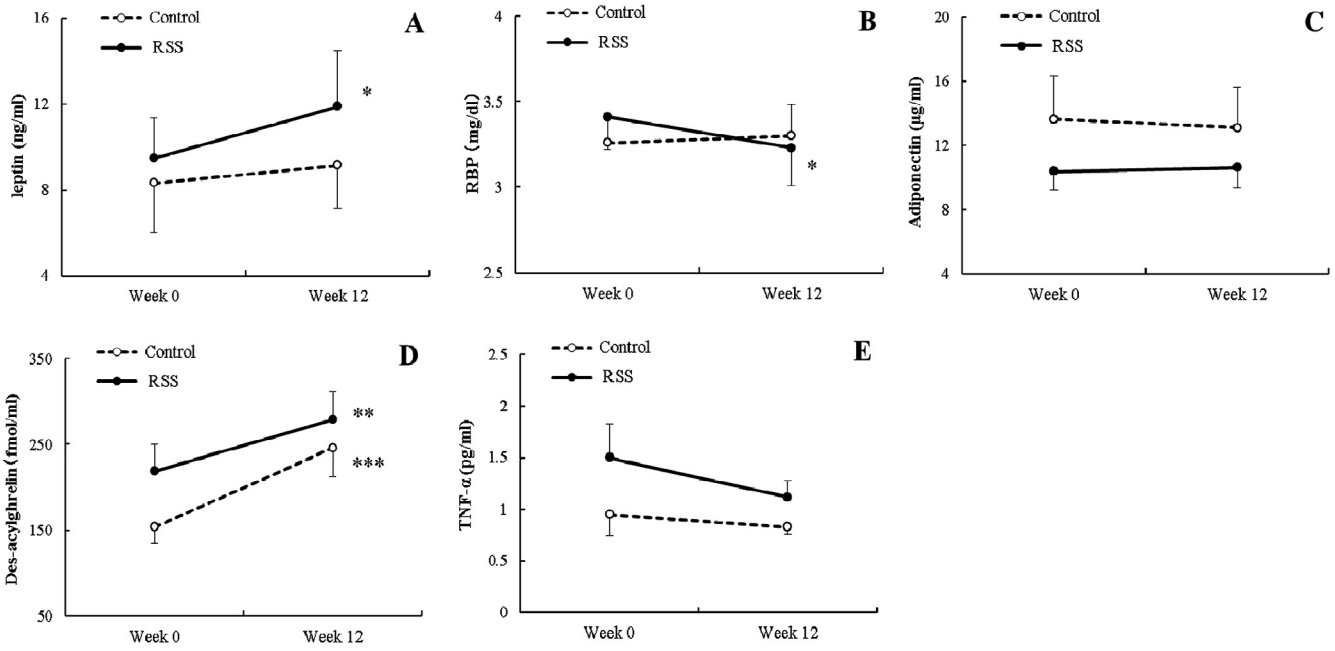

Fig. 2 – Changes in leptin (A), RBP (B), adiponectin (C), des-acyl ghrelin (D) and TNF-α (E) during treatment of either the RSS or control drinks for 12 weeks. Values are expressed as mean ± SEM. Significant differences of 12 week from 0 week are shown: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by paired t-test.

improved changes in physical parameters associated with these obesity-related factors demonstrate the potential of RSS as a sweetener having the advantage of reducing visceral fat.

3.3. *Urinalysis*

Protein was detected in the urine of each subject in the control group i.e., (±) at week 0 and the observation period. Each subject in the RSS group gave a (±) result for protein in the urine at week 0, (1+) at week 8 and (±) at week 12. For the occult blood test, positive or false-positive markers in the control group were detected as (±, ±) at week 0, (±, 2+) at week 2, (2+) at week 4, (±, ±, 2+) at week 8 and (±, ±, ±) at the observation period. These markers in the RSS group were detected as (1+) at week 0, (±) at week 4, (±, ±) at week 8 and (±) at the observation period. Glucose and urobilinogen were not detected in the urine samples from any of the subjects. No significant changes were observed in any of the urine parameters.

3.4. *Consultations*

The daily nutrient intake (energy, fat, protein, carbohydrate) and number of walking steps for each group are indicated in Table 4 and no significant changes were observed in these parameters. Also, no significant differences were shown in the other nutrient intake (saturated fatty acid, unsaturated fatty

Table 4 – Alteration in daily intake of energy and nutrient factors, and exercise amount during treatment of either the RSS or control drinks for 12 weeks and after the observation periods.

Item	Week 0	Week 2	Week 4	Week 8	Week 12	Observation
Intake energy (kcal)						
Control	1715.6 ± 60.4	1676.5 ± 55.9	1741.6 ± 60.3	1752.4 ± 66.0	1721.6 ± 68.8	1721.2 ± 55.4
RSS	1677.8 ± 52.6	1645.9 ± 64.0	1672.2 ± 74.1	1679.5 ± 51.5	1711.1 ± 47.2	1682.6 ± 52.8
Fat (g)						
Control	63.0 ± 3.1	60.9 ± 2.6	62.7 ± 3.3	63.4 ± 2.3	63.4 ± 3.1	60.1 ± 2.5
RSS	65.0 ± 2.5	64.6 ± 2.8	62.7 ± 2.6	63.8 ± 2.5	64.3 ± 2.4	63.4 ± 2.8
Protein (g)						
Control	53.8 ± 3.1	54.7 ± 2.6	56.4 ± 3.0	54.6 ± 3.2	52.7 ± 2.9	54.7 ± 2.8
RSS	52.9 ± 2.1	49.6 ± 2.7	53.4 ± 2.8	51.7 ± 2.1	54.0 ± 2.2	53.2 ± 2.5
Carbohydrate (g)						
Control	230.9 ± 7.7	221.7 ± 7.0	231.4 ± 8.7	237.5 ± 9.5	233.6 ± 9.6	232.0 ± 7.9
RSS	223.2 ± 7.4	223.7 ± 8.7	221.9 ± 9.6	227.0 ± 8.2	229.0 ± 6.8	224.4 ± 8.3
Number of walking steps						
Control	9623.9 ± 813.9		9632.1 ± 838.0	9616.9 ± 815.8	9616.4 ± 828.3	9622.4 ± 823.3
RSS	8900.7 ± 685.5		8908.2 ± 758.8	8865.5 ± 717.5	8922.1 ± 729.0	8834.1 ± 738.9

Values are expressed as mean ± SEM, and expressed as average 3 days before each visit.

acid, cholesterol and dietary fiber). Moreover, no adverse events directly related to RSS intake were reported during the period of the study.

Appetite throughout the day is more important than temporary calorie restriction resulting in a larger appetite. The appetite of both glucose and fructose are affected by the rate of ATP/AMP turnover (Lane & Cha, 2009). In terms of allulose, the amount of the food intake is closely related to body weight in some animal experiments involving the feeding of this sugar (Matsuo & Izumori, 2006; Ochiai et al., 2014). In the current clinical trial, there was no difference in the daily nutrient intake between the RSS and control groups as reflected by the value of des-acylghrelin (Fig. 2). Because subjects took only 3 day records before the experimental day, differences between the groups might not have been detected.

4. Conclusions

In the current human trial, the efficacy and safety of ingesting new sweetener "RSS" over a 12-week period were investigated by a randomized double-blind parallel experiment for the first time. Significant reductions of body weight, body fat percentage and waist circumference in the rare sugar syrup group were observed compared to the high fructose corn syrup group. We also confirmed that no adverse effects or abnormal blood parameters were observed with regard to lipid and carbohydrate metabolism, hepatic and renal functions. RSS also gives natural sweetness, which resembles that of sucrose, even if combined with high intensity sweetener, such as stevia, sucralose, acesulfame-K or aspartame, because of its superior masking ability. A calorie sweetener blended with RSS and high intensity sweeteners might be a highly effective means of countering obesity.

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