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Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus

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ABSTRACT

This trial evaluated the effects of 16 weeks of consumption of 1000 mg rebaudioside A ($n = 60$) a steviol glycoside with potential use as a sweetener, compared to placebo ($n = 62$) in men and women (33–75 years of age) with type 2 diabetes mellitus. Mean ± standard error changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%) and placebo (0.09 ± 0.05%; $p = 0.355$) groups. Changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 μU/mL and 3.3 ± 1.5 μU/mL), and C-peptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) did not differ significantly ($p > 0.05$ for all). Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess vs. placebo. These results suggest that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

1. Introduction

Steviol glycosides, primarily stevioside and rebaudioside A, are natural constituents of the plant *Stevia rebaudiana* Bertoni (JECFA, 2005). Both stevioside and rebaudioside A are ~250 times sweeter than sucrose, and have the potential to serve as non-caloric sweeteners (JECFA, 2005). Stevioside is already in use as a food sweetener in a number of South American and Asian countries. Several studies have reported hypoglycemic and hypotensive effects of stevioside and *Stevia* extracts, particularly among individuals with type 2 diabetes and hypertension (Chan et al., 1998; Chan et al., 2000; Jeppesen et al., 2000; Hsieh et al., 2003; Jeppesen et al., 2003; Gregersen et al., 2004; Chen et al., 2005). Stevioside and the aglycone steviol were shown to stimulate glucose-dependent insulin secretion from islets in an *in vitro* mouse model (Jeppesen et al., 2000). In these studies, steviol was more potent than stevioside, and glucose concentrations of at least 119 mg/dL were required for insulin release. In a clinical investigation of subjects with type 2 diabetes, Gregersen et al. (2004) observed a modest blunting of the postprandial glycemic response following a meal containing 1000 mg stevioside. However, more recent examinations have not provided clear evidence to support the previous results (Ferri et al., 2006; Jeppesen et al., 2006). In a three-month study of subjects with type 2 diabetes, Jeppesen et al. (2006) reported that fasting blood glucose and glycosylated hemoglobin (HbA_{1c}) were not significantly lowered by intake of 1500 mg/d of stevioside compared with placebo. Additionally, the incremental area under the glucose concentration curve following test meal administration at the end of the treatment period was also unaltered relative to placebo.

Abbreviations: ALT, alanine transaminase; d, day; dL, deciliter; GGT, gamma glutamyl transferase; HbA_{1c}, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; JECFA, Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; kg, kilogram; LDL-C, low-density lipoprotein cholesterol; m^2, meters squared; μU, microunits; mg, milligram; mL, milliliter; mmHg, millimeters mercury; *n*, number; ng, nanogram; non-HDL-C, non-high-density lipoprotein cholesterol; total-C, total cholesterol; U/L, units per liter; WHO, World Health Organization.

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Structurally, stevioside and rebaudioside A differ only by the presence of one additional glucose moiety on rebaudioside A. Following oral consumption, both are metabolized to steviol in the gastrointestinal tract (JECFA, 2005). Because of the similarities in the metabolism of rebaudioside A and stevioside, and the fact that both glycosides are contained in *Stevia* extracts, it has been hypothesized that rebaudioside A may have hypoglycemic and antihypertensive effects similar to those reported with stevioside consumption. Although fewer studies have been conducted using rebaudioside A, the available pre-clinical, animal, and clinical data do not support these hypotheses (Dyskrog et al., 2005; Carakostas et al., 2008; Maki et al., 2008). The present study was designed to provide data on the effects, if any, of steviol glycosides on glucose homeostasis in individuals with type 2 diabetes. The investigation did not include subjects with type 1 diabetes since the purported mechanism of action for steviol glycosides involves enhanced secretion of insulin from the pancreas when there is impaired response to glucose stimulation.

The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) specifically requested additional studies involving repeated exposure to dietary and therapeutic doses of steviol glycosides in people with diabetes to help define an acceptable intake of steviol glycosides (JECFA, 2005). The present study, conducted as part of a clinical program designed to address the questions raised by the JECFA, examined the safety of 16 weeks of rebaudioside A consumption in men and women with type 2 diabetes mellitus, with particular attention to any potential glycemic and hemodynamic effects. The dosage provided in the present study was 1000 mg/d, which corresponds to more than seven times the mean projected daily intake for adults with diabetes (1.4 mg/kg body weight/d, calculated based on mean body weight for subjects in the present study) and more than two times the mean expected daily intake for high-intake adult consumers with diabetes (4.5 mg/kg body weight/d) (Renwick, 2008).

2. Methods

2.1. Study design

This was a randomized, double-blind, placebo-controlled clinical trial conducted at six research sites in the United States. Good Clinical Practice Guidelines, the Declaration of Helsinki (2000), and the US 21 Code of Federal Regulations (Part 50 – Protection of Human Subjects) were followed in the conduct of the study. An institutional review board (Schulman Associates Institutional Review Board, Inc., Cincinnati, OH) approved the protocol before study initiation. Signed written informed consent was obtained from all subjects and they were advised of their right to withdraw from the study at any time.

2.2. Subjects

To be eligible, men and women (18–74 years of age) were required to have type 2 diabetes mellitus that was diagnosed at least one year prior to screening; $HbA_{1C} \leqslant 9.0\%$ at screening; and to have been treated for at least 12 weeks with stable dose(s) of one to three oral hypoglycemic agents, basal insulin (intermediate or long-acting injections that provide a steady, low level of insulin throughout the day and night), or a combination of basal insulin plus one to three oral hypoglycemic agents. Qualified subjects were required to have a body mass index of 25–45 kg/m^2, be willing to maintain their habitual diets and physical activity patterns, and have no plans to change their smoking habits during the study period.

Individuals with significant renal, pulmonary, hepatic, or biliary disease; a recent history of a cardiovascular event or revascularization procedure; or any gastrointestinal condition that could potentially interfere with the absorption of the study product were not enrolled. Individuals with poorly controlled hypertension (resting seated systolic blood pressure $\geqslant$160 mmHg or diastolic blood pressure $\geqslant$100 mmHg) were excluded. However, persons with controlled hypertension on a stable dose of medication for at least six weeks were allowed to enter the study. Women of childbearing potential who were unwilling to commit to using a medically approved form of contraception, or who were pregnant, lactating, or planning to be pregnant during the study were not enrolled.

The use of certain medications during the study was prohibited, including inhaled insulin, insulin pumps, or non-basal insulin; incretin mimetics and dipeptidyl peptidase IV inhibitors; systemic corticosteroids; or unstable doses (change in the six weeks prior to screening) of medications for the management of blood lipids and lipoproteins.

2.3. Study product

The study included a two-week single-blind placebo lead-in and a 16-week double-blind treatment period. To be eligible for randomization, subjects were required to be at least 80% compliant with taking placebo capsules (microcrystalline cellulose) during the lead-in period. Subjects were randomly assigned to receive placebo or rebaudioside A (97% purity; rebiana, the common name for rebaudioside A) in 250 mg capsules provided by Cargill, Incorporated, Wayzata, MN. Randomization was stratified according to insulin use. Subjects took four capsules each day: two 250 mg capsules (rebaudioside A or placebo) with the first meal of the day and two 250 mg capsules (rebaudioside A or placebo) with the evening meal to achieve a daily dosage of 1000 mg. Compliance was assessed by capsule count and subject interview. Percent compliance was calculated as 100× the number of doses consumed/the expected number of doses. Subjects visited the clinic four times at four-week intervals during the 16-week treatment period for laboratory assessments. Study coordinators contacted the subjects between the clinic visits at four-week intervals beginning two weeks after randomization, to reinforce study instructions and answer questions.

2.4. Diet

Subjects were instructed to maintain a stable diet during the study. Three-day diet records completed at the beginning and end of the study were used to confirm dietary stability. Diet records were analyzed using the Food Processor® Nutrition Analysis & Fitness Software (version 8.6.0, ESHA Research, Salem, OR).

2.5. Laboratory and blood pressure measurements

Subjects fasted for 10–14 h prior to each clinic visit. Medpace Laboratories (Cincinnati, OH) performed all laboratory tests. Standard clinical laboratory evaluations included serum chemistry, hematology, and urinalysis. Glycosylated hemoglobin was measured by high-performance liquid chromatography (Gruber and Koets, 1979; Cefalu et al., 1994). Fasting glucose was measured by photometry following a reaction with a hexokinase reagent (Kadish and Hall, 1965). Fasting insulin (Allauzen et al., 1995) and C-peptide (Service et al., 1992) were analyzed using electrochemiluminescence immunoassays. Total cholesterol (total-C) and triglycerides were measured by photometry following enzymatic reactions. High-density lipoprotein cholesterol (HDL-C) was isolated by a 2-step precipitation method, and measured by photometry following an enzymatic reaction (Warnick et al., 1982). Low-density lipoprotein cholesterol (LDL-C) concentration in mg/dL was calculated according to the Friedewald equation (Friedewald et al., 1972) as follows: LDL-C (mg/dL) = total-C − HDL-C − Triglycerides/5. No LDL-C value was calculated when the triglyceride concentration was above 400 mg/dL, because the equation is not valid under those circumstances. Non-HDL-C was calculated as the difference between total-C and HDL-C.

Blood pressure was measured at each clinic visit using an automated vital signs monitor (Welch Allyn® Model 53000, Beaverton, OR). Blood pressure measurements were taken at 2 min intervals for 10 min while the subject was resting quietly in a seated position. The first two measurements were discarded and the remaining four were averaged. Subjects requiring antihypertensive medications either took them in the evening before the scheduled visit or at the end of the visit.

2.6. Adverse events and hypoglycemia diary

Subjects reported any adverse events that occurred during the study. In addition, subjects completed a hypoglycemia diary daily during the treatment period in which they documented the frequency and severity of hypoglycemic episodes. Based on answers to the diary questions, the subject assessments of hypoglycemic episodes were classified according to the following categories: severe (required assistance from another person to actively administer carbohydrate, glucagon, or other resuscitative actions); documented symptomatic (an event during which typical symptoms were accompanied by a measured glucose concentration $\leqslant$70 mg/dL); asymptomatic (an event during which typical symptoms were not present but a measured glucose concentration $\leqslant$70 mg/dL was documented); probable symptomatic hypoglycemia (an event during which typical symptoms of hypoglycemia were not accompanied by a glucose determination); or relative hypoglycemia (an event during which the person with diabetes reported any of the typical symptoms of hypoglycemia, but had a measured glucose concentration >70 mg/dL) (American Diabetes Association Working Group on Hypoglycemia, 2005).

2.7. Statistical analyses

Statistical analyses were generated using SAS version 9.1.3, service pack 4 (SAS Institute, Cary, NC). All tests of statistical significance were completed at the 5% level, two-tailed. Baseline and safety data were analyzed from all subjects who received at least one dose of study product. Differences between treatment group responses were evaluated in a modified intent-to-treat population including all subjects who received at least one dose of study product and provided at least one post-randomization HbA_{1c} value. Incomplete data were imputed by taking the average of two surrounding data points. If no subsequent data points were available, the value of the previous non-baseline visit was carried forward to the subsequent visit.

The primary outcome variable was the change from baseline to end of treatment in HbA_{1c}. The study was designed to provide 90% power ($\alpha = 0.05$, two-sided) to detect a 0.5% difference in HbA_{1c} response between treatment groups, assuming a standard deviation of 0.8%. Changes in fasting glucose, insulin, and C-peptide; body weight; resting systolic and diastolic blood pressures; and fasting total-C, LDL-C, HDL-C, non-HDL-C and triglycerides were also evaluated. Differences between treatment groups in all outcome variables were assessed by analysis of covariance. The initial model for each variable included terms for baseline value, treatment, site, and treatment by site interaction. The model was reduced in a stepwise manner, eliminating terms with p-values $\geqslant 0.10$, and the final model contained only terms with $p < 0.10$ or treatment group and baseline value. If it was determined that the distribution of residuals could not be approximated by a normal curve (Shapiro–Wilk test $p < 0.05$), then values were ranked in ascending order (tied values were given a mean rank) prior to running statistical models.

An index for changes in the dosages of diabetes medications or new medications for treating diabetes was calculated for each subject. The following values were assigned to diabetes medications and summed: 3 = an increase in dose or the addition of a new medication; 2 = no change; and 1 = a reduction in dose or removal of a medication. Variations in the total number of medications used by each subject were accounted for by dividing the sum by the number of diabetes medications used at baseline. The Kruskal–Wallis statistic was utilized to test for any differences between treatment groups.

Differences between treatment groups in the frequencies of adverse events were assessed by Fisher's exact (two-tailed) test, and the differences in clinical laboratory test values were evaluated by analysis of covariance as described above.

3. Results

3.1. Subject characteristics

Of the 175 individuals screened, 122 persons with previously diagnosed type 2 diabetes mellitus were randomly assigned to receive either rebaudioside A 1000 mg/d ($n = 60$) or placebo ($n = 62$) for 16 weeks. Six subjects (two in the rebaudioside A group and four in the placebo group) did not complete the study. The reasons for discontinuation included: withdrawal of consent (placebo, $n = 1$), protocol violation [placebo, $n = 1$ (subject started a weight-loss diet)], a work conflict (placebo, $n = 1$), and adverse events [rebaudioside A, $n = 2$ (gastrointestinal hemorrhage and hyperglycemia) and placebo, $n = 1$ (bronchitis)]. A full description of the adverse events is included in the Adverse Events section below.

Baseline and demographic characteristics were not significantly different between treatment groups (Table 1). Participants were predominantly of non-Hispanic white race/ethnicity and ranged in age from 33 to 75 years. Body mass index was approximately 34 kg/m^2 in both groups. In addition to the diabetes medication use, which was required for study entry, a majority of subjects in both groups were taking antihypertension and lipid management drugs. Thiazolidinedione use was higher in the placebo group compared with rebaudioside A, a difference which approached statistical significance ($p = 0.054$). Mean study product compliance in the rebaudioside A and placebo groups was 96.3% and 100.0%, respectively ($p = 0.207$).

3.2. Glycosylated hemoglobin and diabetes medications

Glycosylated hemoglobin concentrations at baseline and throughout treatment are shown in Fig. 1. Glycosylated hemoglobin levels were not significantly different between treatment groups at baseline or in the changes from baseline to weeks 4, 8, 12, and 16 (Table 2). Glycosylated hemoglobin data were imputed by last-observation carried forward for four subjects (two in the

Table 1
Baseline characteristics of all randomized subjects

Characteristic	Rebaudioside A ($n = 60$)	Placebo ($n = 62$)	p-value
n (%)			
Male	32 (53.3)	30 (48.4)	0.585
Female	28 (46.7)	32 (51.6)	
Race/ethnicity			
Non-Hispanic White	41 (68.3)	45 (72.6)	0.959[a]
African American	13 (21.7)	12 (19.4)	
Hispanic	5 (8.3)	4 (6.5)	
Other	1 (1.7)	1 (1.6)	
Diabetes medication			
Insulin	7 (11.7)	6 (9.7)	0.722
Sulfonylurea	20 (33.3)	26 (41.9)	0.327
Metformin	44 (73.3)	44 (71.0)	0.771
Thiazolidinedione	17 (28.3)	28 (45.2)	0.054
Antihypertension medication	34 (56.7)	44 (71.0)	0.100
Dyslipidemia medication	40 (66.7)	39 (62.9)	0.664
Mean ± SEM			
Age (y)	59.1 ± 1.2	61.5 ± 1.1	0.155
Body mass index (kg/m^2)	33.7 ± 0.6	33.6 ± 0.6	0.912
Weight (kg)	97.6 ± 2.1	98.3 ± 2.3	0.813
Height (cm)	169.9 ± 1.2	170.8 ± 1.3	0.629

Abbreviation: SEM = standard error of the mean.
[a] $P = 0.607$ for an additional comparison of Non-Hispanic White vs. all other race/ethnicity categories.

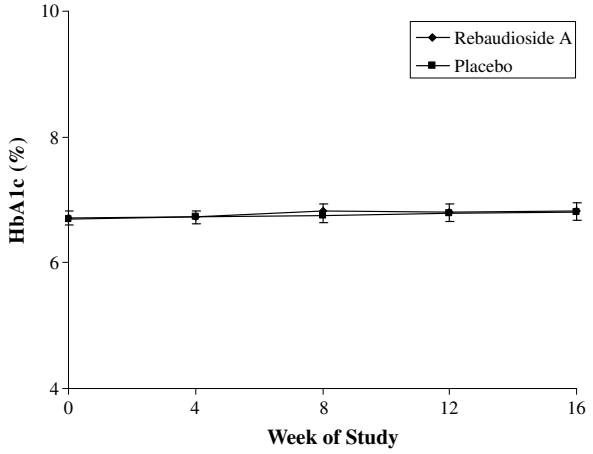

Fig. 1. Mean ± standard error of the mean HbA_{1c} (mg/dL) at baseline (average of values at weeks −2 and 0) and 4, 8, 12, and 16 weeks of treatment for subjects receiving rebaudioside A ($n = 60$) or placebo ($n = 62$). Error bars are shown above the data points for rebaudioside A and below the data points for placebo.

Table 2
Glycosylated hemoglobin at baseline and changes from baseline to weeks 4, 8, 12, and 16[a]

Glycosylated hemoglobin (%)	Rebaudioside A ($n = 60$)	Placebo ($n = 62$)	p-value[b]
Mean ± SEM			
Baseline	6.71 ± 0.11	6.70 ± 0.10	0.964
Week 4 △	0.02 ± 0.03	0.02 ± 0.03	0.982
Week 8 △	0.11 ± 0.05	0.04 ± 0.04	0.574
Week 12 △	0.11 ± 0.05	0.07 ± 0.05	0.248
Week 16 △	0.11 ± 0.06	0.09 ± 0.05	0.355

Abbreviation: SEM = standard error of the mean.
[a] Baseline = the average of values at weeks −2 and 0. Change from baseline is signified by △.
[b] p-values for the change from baseline are for the analysis of covariance with baseline value as the covariate. All values were ranked prior to analysis.

rebaudioside A group and two in the placebo group). The results did not differ materially when the data were analyzed with and without the imputed data points (data not shown).

The diabetes medication index showed no significant differences between the rebaudioside A and placebo groups regarding the changes in number and dosages of diabetes medications during the treatment period (rebaudioside A, 1.6 ± 0.1 vs. placebo, 1.4 ± 0.1; $p = 0.122$).

3.3. Fasting glucose, insulin, and C-peptide

Fasting levels of glucose, insulin, and C-peptide at baseline (average of values at weeks −2 and 0) and the changes from baseline to treatment (average of weeks 12 and 16) are shown in Table 3. Fasting glucose concentrations at baseline and throughout treatment are shown in Fig. 2. There were no significant differences between groups in the values at baseline for fasting glucose and C-peptide. Changes from baseline to treatment for glucose (rebaudioside A, 7.5 ± 3.7 mg/dL vs. placebo, 11.2 ± 4.5 mg/dL; $p = 0.966$) and fasting C-peptide (rebaudioside A, 0.13 ± 0.09 ng/mL vs. placebo, 0.42 ± 0.14 ng/mL; $p = 0.313$) also were not different between groups. Insulin levels at baseline were significantly higher in the rebaudioside A group (17.3 ± 1.5 μU/mL) compared with placebo (14.6 ± 1.4 μU/mL, $p = 0.046$). However, the changes from baseline to treatment were not different between groups (rebaudioside A, 1.0 ± 0.6 μU/mL vs. placebo, 3.3 ± 1.5 μU/mL; $p = 0.979$).

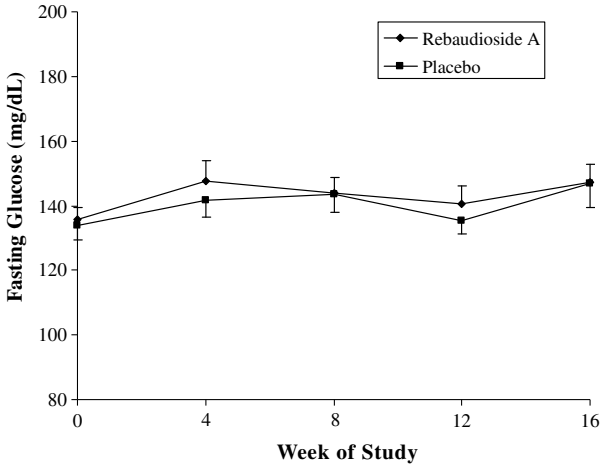

Fig. 2. Mean ± standard error of the mean fasting glucose concentrations (mg/dL) at baseline (average of values at weeks −2 and 0) and 4, 8, 12, and 16 weeks of treatment for subjects receiving rebaudioside A ($n = 60$) or placebo ($n = 62$). Error bars are shown above the data points for rebaudioside A and below the data points for placebo.

Table 3
Fasting glucose, insulin, C-peptide, body weight, blood pressure, and lipids at baseline and following treatment[a]

Parameter	Rebaudioside A ($n = 60$)	Placebo ($n = 62$)	p-value[b]
	Mean ± SEM or median (min, max)[c]		
Baseline glucose (mg/dL)	135.8 ± 3.6	134.3 ± 4.1	0.433[d]
△ (mg/dL)	7.5 ± 3.7	11.2 ± 4.5	0.966[d]
Baseline insulin (μU/mL)	17.3 ± 1.5	14.6 ± 1.4	0.046[d]
△ (μU/mL)	1.0 ± 0.6	3.3 ± 1.5	0.979[d]
Baseline C-peptide (ng/mL)	3.9 ± 0.2	3.5 ± 0.2	0.102[d]
△ (ng/mL)	0.1 ± 0.1	0.4 ± 0.1	0.313[d]
Baseline body weight (kg)	97.8 ± 2.1	98.4 ± 2.3	0.841
△ (kg)	0.0 ± 0.3	0.2 ± 0.3	0.832[d]
Baseline systolic blood pressure (mmHg)	121.6 ± 1.4	126.0 ± 1.6	0.051[d]
△ (mm Hg)	−0.2 ± 1.0	−0.9 ± 1.1	0.775
Baseline diastolic blood pressure (mmHg)	72.5 ± 1.0	71.3 ± 1.1	0.429
△ (mm Hg)	0.2 ± 0.8	−1.1 ± 0.7	0.132
Baseline total-C (mg/dL)	170.7 ± 4.2	168.7 ± 4.1	0.737
△ (mg/dL)	−1.5 ± 3.4	−3.0 ± 2.0	0.833
Baseline LDL-C (mg/dL)	92.9 ± 3.5	92.3 ± 3.4	0.874[d]
△ (mg/dL)	−2.1 ± 3.0	−5.8 ± 1.8	0.132[d]
Baseline HDL-C (mg/dL)	46.5 ± 1.5	46.7 ± 2.2	0.543[d]
△ (mg/dL)	0.3 ± 0.7	0.7 ± 0.7	0.676[d]
Baseline non-HDL-C (mg/dL)	124.2 ± 4.4	122.0 ± 4.1	0.662[d]
△ (mg/dL)	−1.8 ± 3.3	−3.7 ± 2.0	0.240[d]
Baseline triglycerides (mg/dL)	128.8 (62.0, 904.0)	133.5 (39.0, 478.5)	0.992[d]
△ (mg/dL)	8.3 (−158.0, 352.5)	12.0 (−83.5, 1050.0)	0.387[d]

Abbreviations: HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, max = maximum, min = minimum, non-HDL-C = non-high-density lipoprotein cholesterol, SEM = standard error of the mean, total-C = total cholesterol.

[a] Baseline = the average of values at weeks −2 and 0 and change (signified by △) is the change from baseline to the average of values at weeks 12 and 16.

[b] p-values for the change from baseline are for the analysis of covariance with baseline value as the covariate.

[c] Triglycerides were not normally distributed, therefore median (minimum, maximum) values are shown as a measure of central tendency.

[d] Values were ranked prior to analysis.

3.4. Body weight, blood pressure, and lipids

Body weight, blood pressure, and fasting lipid measurements at baseline (average of values at weeks −2 and 0) and the changes from baseline to treatment (average of values at weeks 12 and 16) are shown in Table 3. There was a near-significant between group difference in mean baseline systolic blood pressure (rebaudioside A, 121.6 ± 1.4 mmHg vs. placebo, 126.0 ± 1.6 mmHg; $p = 0.051$). However, the changes from baseline to treatment were not significantly different between groups. Baseline and changes from baseline in body weight, diastolic blood pressure, and fasting lipids were not significantly different between rebaudioside A and placebo groups.

3.5. Diet

Intakes of total energy; percentages of energy from carbohydrate, protein, total fat, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids; the ratio of polyunsaturated to saturated fatty acids; and intakes of total dietary fiber, soluble dietary fiber, sodium, potassium, calcium, and magnesium were not significantly different between groups at baseline or in the changes from week 0 to week 16 (data not shown). At baseline, subjects in the placebo group had a slightly higher mean percentage of energy from alcohol compared with the rebaudioside A group (1.7 ± 0.5% vs. 0.3 ± 0.1%, $p = 0.043$), however mean changes from baseline to week 16 were not significantly different between groups (rebaudioside A, 0.17 ± 0.12% vs. placebo, −0.17 ± 0.33%; $p = 0.224$).

3.6. Adverse events and hypoglycemia diary

A total of 50 subjects reported at least one adverse event during the study [rebaudioside A, $n = 27$ (45.0%) vs. placebo, $n = 23$ (37.1%); $p = 0.462$]. There were no differences between groups in the occurrence of any adverse event. Two adverse events occurred in at least 5% of subjects in either group: gastroenteritis (rebaudioside A, 5.0% and placebo, 4.8%), and upper respiratory tract infection (rebaudioside A, 10.0% and placebo, 6.5%).

In the rebaudioside A group, four of the adverse events were classified as severe (influenza-like symptoms, gastroenteritis, gastrointestinal hemorrhage [a serious adverse event], and a cyst)

compared with three (gastroenteritis, fracture, bronchitis) in the placebo group ($p = 0.715$). The subjects with a gastrointestinal hemorrhage (rebaudioside A) and with bronchitis (placebo) discontinued from the study. Both of these adverse events were judged by the investigator to be unrelated to treatment, and both subjects recovered completely. An additional subject in the rebaudioside A group discontinued due to hyperglycemia which was judged by the investigator as unlikely to be related to treatment. This subject discontinued at week 4 after a fasting glucose level of 407 mg/dL was obtained.

Information regarding hypoglycemic events collected in the daily diary showed no differences between rebaudioside A and placebo groups in the occurrence and severity of hypoglycemic episodes at any point during the study ($p > 0.05$ for all). The percentages of rebaudioside A and placebo subjects, respectively, reporting hypoglycemic episodes were 11.7% and 19.4% at week 0; 11.7% and 14.5% at week 4; 11.7% and 6.5% at week 8; 8.3% and 8.1% at week 12; and 8.3% and 6.5% at week 16.

3.7. Clinical laboratory tests

Selected serum chemistry and hematology parameters at screening and the changes from screening to week 16 are shown in Table 4. The mean level of alanine transaminase (ALT) was significantly increased from baseline in the rebaudioside A group vs. placebo (1.7 ± 1.2 U/L *vs.* −1.5 ± 0.8 U/L, $p = 0.005$). The mean concentration of gamma-glutamyl transferase (GGT) was also significantly increased from baseline for rebaudioside A (2.0 ± 1.8 U/L) compared with placebo (−0.7 ± 1.1 U/L, $p = 0.020$). Following treatment, mean ALT was 25.3 ± 1.4 U/L for rebaudioside A vs. 22.9 ± 1.3 U/L for placebo, and mean GGT was 29.2 ± 2.9 U/L for rebaudioside A vs. 28.2 ± 3.2 U/L for placebo. Normal ranges for these parameters are 6–41 U/L for ALT and 11–52 U/L [male] and 7-38 U/L [female] for GGT (Medpace Reference Laboratories 2007). A shift analysis indicated that 3/60 rebaudioside A subjects and 3/62 placebo subjects with low or normal ALT levels at screening shifted to high levels following treatment ($p = 0.967$). For GGT, 3 of 60 rebaudioside A subjects and 0 of 62 placebo subjects shifted from low or normal to high levels ($p = 0.075$). Only one subject had a liver enzyme value that was three times the upper limit of normal (GGT of 161 U/L in a male in the placebo group). There were no other statistically significant or clinically relevant changes during treatment in the serum chemistry or urinalysis parameters measured. The percentage of basophils increased in the rebaudioside A group compared with placebo (0.09 ± 0.06% vs. −0.04 ± 0.05%; $p = 0.029$). None of the other hematology parameters showed significantly different changes between groups.

Table 4
Serum chemistry and hematology parameters at screening (week −2) and the changes from screening to week 16[a]

Parameter	Rebaudioside A ($n = 59$)	Placebo ($n = 62$)	p-value[b]
Mean ± SEM			
Screening alanine transaminase (U/L)	24.0 ± 1.3	24.4 ± 1.5	0.906[c]
△ (U/L)	1.7 ± 1.2	−1.5 ± 0.8	0.005[c]
Screening aspartate transaminase (U/L)	21.6 ± 0.9	22.6 ± 1.0	0.508[c]
△ (U/L)	0.6 ± 0.9	−0.9 ± 0.7	0.105[c]
Screening alkaline phosphatase (U/L)	73.0 ± 2.3	76.0 ± 2.8	0.759[c]
△ (U/L)	0.3 ± 1.4	−1.7 ± 1.6	0.234[c]
Screening blood urea nitrogen (mg/dL)	16.6 ± 0.6	16.1 ± 0.7	0.313[c]
△ (mg/dL)	0.1 ± 0.5	0.6 ± 0.5	0.447
Screening creatinine (mg/dL)	0.9 ± 0.0	0.9 ± 0.0	0.334[c]
△ (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.971[c]
Screening gamma glutamyl transferase (U/L)	27.7 ± 2.4	29.0 ± 3.0	0.910[c]
△ (U/L)	2.0 ± 1.8	−0.7 ± 1.1	0.020[c]
Screening red blood cell count ($10^6/\mu L$)	4.7 ± 0.1	4.5 ± 0.1	0.075
△ ($10^6/\mu L$)	−0.1 ± 0.0	−0.1 ± 0.0	0.768
Screening white blood cell count ($10^3/\mu L$)	6.6 ± 0.2	6.5 ± 0.2	0.731
△ ($10^3/\mu L$)	0.0 ± 0.2	−0.1 ± 0.2	0.934[c]
Screening basophil[c] (%)	0.36 ± 0.04	0.42 ± 0.04	0.241
△ (%)	0.09 ± 0.06	−0.04 ± 0.05	0.029
Screening hemoglobin (g/dL)	13.7 ± 0.2	13.5 ± 0.2	0.440
△ (g/dL)	−0.2 ± 0.1	−0.2 ± 0.1	0.746
Screening hematocrit (%)	41.4 ± 0.5	40.9 ± 0.5	0.456
△ (%)	−0.3 ± 0.3	−0.4 ± 0.3	0.772[d]

Abbreviations: SEM = standard error of the mean.
[a] Change from baseline to week 16 is indicated by △.
[b] p-values for the change from baseline are for the analysis of covariance with baseline value as the covariate.
[c] Basophil (%) is presented because it was the only subset of white blood cells that showed a significant difference in treatment responses.
[d] Values were ranked prior to analysis.

4. Discussion

The results of the present trial show that 1000 mg/d consumption of rebaudioside A for 16 weeks did not affect glucose homeostasis or resting blood pressure in men and women with type 2 diabetes mellitus. These findings corroborate the results reported elsewhere in this supplement showing that consumption of 1000 mg/d for four weeks did not affect blood pressure in healthy adults (Maki et al., 2008). The results of the present study differ from those in some previous reports which suggested that stevioside and *Stevia* extracts may alter blood pressure and glucose homeostasis, particularly in subjects with hypertension or diabetes (Chan et al., 1998; Chan et al., 2000; Jeppesen et al., 2000; Hsieh et al., 2003; Jeppesen et al., 2003; Gregersen et al., 2004; Chen et al., 2005). This was the first examination of the effects of chronic consumption of rebaudioside A in individuals with type 2 diabetes mellitus.

The standard accepted measure of chronic glycemic control is HbA_{1c} (Miegs et al., 2002; Rohlfing et al., 2002; Saudek et al., 2006; American Diabetes Association, 2007). During the 120-day life cycle of a red blood cell, glucose gradually binds to the A_{1c} form of hemoglobin, reflecting the average concentration of blood glucose while the cell is in circulation. Therefore, HbA_{1c} represents an individual's average glycemia over the previous two to three months (Sacks et al., 2002). The normal range of HbA_{1c} found in healthy persons is 4% to 6% (American Diabetes Association, 2007). The American Diabetes Association recommends an HbA_{1c} concentration <7% for subjects with diabetes mellitus (American Diabetes Association, 2007). In the current study, the mean baseline HbA_{1c} was 6.7% in both groups, suggesting that these individuals started the study with good average glucose control. If rebaudioside A exerted a hypoglycemic effect, as has been proposed by some for stevioside, it would be expected that HbA_{1c} levels would have declined over the 16 weeks of rebaudioside A consumption compared with placebo. Glycosylated hemoglobin levels rose slightly in both groups during the treatment period (rebaudioside A, 0.11% and placebo, 0.09%), and there was no difference in HbA_{1c} changes for rebaudioside A vs. placebo.

The lack of an effect of rebaudioside A on HbA_{1c} in the current study was supported by the measurements of other indicators of glycemic control, including fasting levels of glucose, insulin, and C-peptide; the index of changes in number and dosages of diabetes

medications; and hypoglycemic episodes reported in a daily diary. With the administration of a new hypoglycemic drug or product, sharp increases in hypoglycemia may occur early in the treatment phase and eventually decrease as glycemic control stabilizes (American Diabetes Association Working Group on Hypoglycemia, 2005). That does not appear to have been the case in the present study. Because the hypoglycemia diary was maintained throughout the study and analyzed every four weeks, the authors are confident that there was no early hypoglycemia associated with rebaudioside A consumption.

Systolic and diastolic blood pressure responses showed no differences between groups. Although this study was not designed to specifically address the effects of rebaudioside A in individuals with hypertension, 57% and 71% of the subjects in the rebaudioside A and placebo groups, respectively, were taking antihypertension medications.

Rebaudioside A was well-tolerated and generally had no effects on laboratory measurements of safety. Differences in the mean changes during the study for ALT and GGT between the placebo and rebaudioside A groups were statistically significant, but were very small and considered to have no clinical significance. The increase in ALT in the rebaudioside A group (1.7 U/L) was approximately equal to the decrease in the placebo group (−1.5 U/L), suggesting the difference was likely due to random variation. Mean levels of both of these parameters were still well within normal ranges. None of the subjects had an ALT concentration higher than three times the upper limit of normal, and one individual (in the placebo group) had a GGT greater than three times the upper limit of normal. A small, but statistically significant increase was noted for the basophil count in the rebaudioside A group compared with placebo. However, the total white cell count showed no treatment-related difference. There was no evidence of an increase in the incidence of anemia in rebaudioside A treated subjects, and no other indicators of disease associated with basophilia. Therefore it is likely that this finding was incidental. A large number of laboratory tests were performed, and we would expect ~5% to be statistically significant by chance.

The results of this trial add to the body of literature (Dyskrog et al., 2005; Maki et al., 2008) suggesting that rebaudioside A is well-tolerated and lacks the pharmacological effects on blood pressure and glucose homeostasis that have been reported in some studies of stevioside and *Stevia* extracts. In a rat model of type 2 diabetes, feeding 0.025 g/kg body weight/d rebaudioside A for eight weeks produced no changes in blood pressure or glycemic control (Dyskrog et al., 2005). A clinical investigation of men and women with normal blood pressure consuming 1000 mg/d rebaudioside A for four weeks failed to show any evidence of effects on blood pressure (Maki et al., 2008). The rebaudioside A dose of 1000 mg/d corresponds to more than seven times the mean estimated daily intake for adults with diabetes (1.4 mg/kg body weight/d, calculated based on the mean body weight for subjects in the present study) and more than two times the mean expected daily intake for high-intake adults with diabetes (4.5 mg/kg body weight/d) (Renwick, 2008).

The current trial was the first long-term study of rebaudioside A in persons with type 2 diabetes. It specifically addressed the JECFA's request for additional studies involving repeated exposure of steviol glycosides in people with diabetes. These results suggest that chronic intake of 1000 mg/d of rebaudioside A was well-tolerated and did not produce hypoglycemia or alter blood pressure in men and women with type 2 diabetes.

Conflict of interest statement

Author Tarka received financial support from Cargill for consulting services.

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