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The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure

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ABSTRACT

Rebaudioside A and stevioside are steviol glycosides extracted from the plant *Stevia rebaudiana* Bertoni and are used in several countries as food and beverage sweeteners. This randomized, double-blind trial evaluated the hemodynamic effects of 4 weeks consumption of 1000 mg/day rebaudioside A vs. placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of ~41 (range 18–73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mmHg and 110.7/71.2 mmHg for the rebaudioside A and placebo groups, respectively. Compared with placebo, rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-h ambulatory blood pressures responses. These results indicate that consumption of as much as 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

1. Introduction

Rebaudioside A and stevioside are naturally occurring steviol glycosides from the leaves of the plant *Stevia rebaudiana* Bertoni (JECFA, 2005). *Stevia* extracts have been used for many years as high-intensity sweeteners in several countries, primarily in South America and Asia. Stevioside and rebaudioside A differ by one additional glucose moiety on rebaudioside A. Both are metabolized to steviol in the gastrointestinal tract (JECFA, 2005). Because of the similarities in their metabolism, rebaudioside A and stevioside are expected to produce similar physiological effects.

Steviol glycosides have been reported to have antihypertensive properties in animals and humans (Melis and Sainati, 1991a,b; Melis, 1992a,b; Chan et al., 1998; Hsieh et al., 2003; Jeppesen et al., 2003; Liu et al., 2003). Results from long-term clinical trials (1–2 years) in China studying men and women with mild to moderate essential hypertension have suggested antihypertensive effects of stevioside at intakes of 750 and 1500 mg/day (Chan et al., 2000; Hsieh et al., 2003). However, other studies have not shown measurable effects of steviol glycosides on blood pressure in humans. Geuns et al. (2007) reported that administration of stevioside (750 mg/day for 3 days) failed to significantly alter blood pressure in nine subjects with normal blood pressure. Ferri et al. (2006) also reported a lack of any antihypertensive effect of stevioside in patients with untreated, mild hypertension following 6 weeks of stevioside administration at dosages up to 15.0 mg kg^{-1} day^{-1}.

Most studies assessing antihypertensive properties have evaluated stevioside, steviol, or mixtures of steviol glycosides (i.e., *Stevia*

Abbreviations: ABPM, ambulatory blood pressure monitor; BMI, body mass index; bpm, beats per minute; d, day; DBP, diastolic blood pressure; dL, deciliter; FAO, Food and Agriculture Organization of the United Nations; HR, heart rate; JECFA, Joint FAO/WHO Expert Committee on Food Additives; kg, kilogram; m^2, meters squared; MAP, mean arterial pressure; mg, milligram; mmHg, millimeters mercury; min, minute; *n*, number; NHANES, National Health and Nutrition Examination Survey; oz, ounce; SBP, systolic blood pressure; SEM, standard error of the mean; *t*, time; WHO, World Health Organization.

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extracts). To date, little information has been generated regarding consumption of rebaudioside A alone (also referred to by the common name of rebiana). Maki et al. (2008) showed that consumption of 1000 mg/day of rebaudioside A for 16 weeks did not alter blood pressure or glucose homeostasis in subjects with type 2 diabetes mellitus.

The present investigation was designed to examine the potential effects of 4 weeks of daily consumption of 1000 mg of rebaudioside A on blood pressure and heart rate (HR) in healthy men and women with normal baseline blood pressure (<120 mmHg systolic blood pressure (SBP) and <80 mmHg diastolic blood pressure (DBP) as defined in the Seventh Report on Prevention, Detection, Evaluation and Treatment of High Blood Pressure; Chobanian et al., 2003). The study was conducted as part of a clinical program designed to address questions raised by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) pertaining to the potential for pharmacological effects of steviol glycosides (JECFA, 2005).

2. Methods

2.1. Study conduct

This was a randomized, double-blind, placebo-controlled trial conducted at six clinical research sites in the United States. It was performed under Good Clinical Practice Guidelines, the Declaration of Helsinki (2000), and US 21 Code of Federal Regulations (Part 50 – Protection of Human Subjects). An institutional review board (Schulman Associates Institutional Review Board, Inc., Cincinnati, OH) approved the protocol before the initiation of the study. All subjects provided informed consent and authorization for release of protected health information to the investigators before protocol-specific procedures were carried out.

2.2. Subjects

Participants included healthy men and women, 18–74 years of age, with normal blood pressure (defined as <120 mmHg SBP and <80 mmHg DBP; Chobanian et al., 2003) who were not taking antihypertensive medications. Subjects were instructed to maintain their habitual diet and physical activity patterns throughout the study. A two-week single-blind placebo lead-in period was utilized to evaluate subject compliance. To be eligible for randomization, subjects were required to be at least 80% compliant with placebo capsules during the lead-in.

Individuals with body mass index (BMI) $\geqslant$35.0 kg/m^2 were excluded from participation as were those with a history or clinical evidence of significant cardiovascular, gastrointestinal, renal, pulmonary, hepatic, or biliary disease. Additionally, volunteers with fasting blood glucose $\geqslant$126 mg/dL or diagnosed diabetes mellitus (type 1 or 2) were excluded as were women who were pregnant, lactating, planning to be pregnant during the study, or those of childbearing potential who were not using an approved method of contraception. Subjects who were smokers could not have been planning to change smoking habits during the study period.

2.3. Treatments

Following the placebo lead-in, subjects were randomly assigned to receive four 250 mg capsules/day of either rebaudioside A (97% purity; rebiana, the common name for rebaudioside A) or placebo (microcrystalline cellulose) for the four-week double-blind treatment period. Two capsules were taken with the first meal of the day and two with the evening meal. Compliance was assessed by capsule count and subject interview. Percent compliance was calculated as 100 $\times$ the number of doses consumed/the expected number of doses. Following an overnight fast of 10–14 h, subjects came to the clinic at weekly intervals during the double-blind period for hemodynamic testing. They were instructed to abstain from vigorous physical activity for at least 12 h prior to each clinic visit, and no tobacco products were allowed for a minimum of 1 h prior to the visits.

2.4. Hemodynamic measurements

2.4.1. Resting

Resting, seated blood pressures were assessed at every clinic visit (weeks -2, -1, 0, 1, 2, 3, and 4) using an automated blood pressure device (Welch Allyn® Model 3500, Skaneateles Falls, NY). Measurements were taken at 0, 2, 4, 6, 8, and 10 min. Blood pressure declines with seated rest and the rate of decline lessens over time (Sala et al., 2006). To reduce the variability in the point estimate for seated, resting blood pressure, the first two measurements were discarded and the remaining four were averaged. Each subject was assigned to a specific cuff and monitor for all measurements. Differences between treatment groups in the changes from baseline (average of weeks -1 and 0) to treatment (average of weeks 1, 2, 3, and 4) were calculated for SBP, DBP, and mean arterial pressure (MAP). Mean arterial pressure was calculated as DBP $+\frac{1}{3}$(SBP $-$ DBP).

2.4.2. Meal tests

At weeks 0 and 4, supine (after lying for 5 min) and standing (after standing for 2 min) blood pressure and heart rate were measured before and for 2 h after consumption of a standard breakfast meal with two 250 mg capsules of rebaudioside A or placebo. At week 0, all subjects received placebo, and at week 4, subjects received their randomly assigned treatment. The standard breakfast meal was an 8 fluid oz chocolate-flavored shake (250 kcal; Ensure®, Abbott Nutrition, Columbus, OH) which was consumed over a 10 min period. Pre-meal blood pressure and HR measurements were taken at $t = -5$ min, where $t = 0$ was the start of the test meal. Post-meal blood pressure and HR measurements were obtained at t = 30, 60, 90 and 120 min. These four values were averaged to determine the post-meal response. The changes from pre-meal to post-meal at weeks 0 and 4 for supine and standing SBP, DBP, MAP, and HR were calculated.

2.4.3. 24-h Ambulatory monitoring

At the completion of the meal tests at weeks 0 and 4, each subject was fitted with an ambulatory blood pressure monitor (ABPM; SE-25S 24-h Ambulatory Blood Pressure Monitoring System, Tiba Medical, Portland, OR), which automatically collected data every 30 min for 24 h. Subjects were asked to complete a diary during this time to record their daytime activities, as well as the times for retiring and arising in the morning. Blood pressure monitoring data were considered inadequate if $\geqslant$30% of the required data points were missing, there were gaps in data greater than 2 h, or if the subject spent <6 h or >12 h from the time of retiring to the time of arising (Hermida et al., 2005). Changes from week 0 to week 4 in morning, daytime, nighttime, and 24-h SBP and DBP were calculated. Nighttime blood pressure was defined as the average blood pressure recorded from the time the subject went to bed until the time he/she got out of bed; morning blood pressure was defined as the average during the first 2 h after arising; and daytime blood pressure was defined as the average recorded during the remainder of the day.

2.5. Laboratory, diet, and physical activity measurements

Basic laboratory testing, including serum chemistry, hematology, and urinalysis, was performed by Medpace Laboratories (Cincinnati, OH) at screening and following treatment. Diet records were collected and analyzed at baseline and the end of the treatment period using the Food Processor® Nutrition Analysis and Fitness Software (version 8.6.0, Salem, OR). Physical activity was assessed with the Stanford 7-day Physical Activity Questionnaire administered at weeks 0 and 4 (Sallis et al., 1985).

2.6. Statistical analyses

The trial was designed to have at least 80% power to detect a 4.5 mmHg difference in resting, seated SBP response for rebaudioside A vs. placebo following 4 weeks of treatment, assuming a pooled standard deviation of 7.5 mmHg. Statistical analyses were generated using SAS version 9.1.3, service pack 4 (documentation is available at http://support.sas.com/documentation/onlinedoc/91pdf/index_913.html; SAS Institute, Cary, NC). All tests of statistical significance were completed at an α level of 0.05, two-tailed. Assumptions of normality of residuals were investigated for each response variable using the Shapiro–Wilk test. If it was determined that the distribution could not be approximated by a normal curve (p for the Shapiro–Wilk test $\leqslant$0.05), then values were ranked in ascending order (tied values were given a mean rank) prior to running statistical models. Values throughout are presented as mean ± standard error of the mean (SEM) unless otherwise specified.

Baseline and safety data were evaluated for all subjects enrolled in the study who received at least one dose of study product. Blood pressure and HR data were analyzed for all subjects who received at least one dose of double-blind study product and provided at least one post-randomization blood pressure data point. In cases where an intermediate data point(s) was missing, the average of the two surrounding values was used in its place. The value at the previous non-baseline visit was carried forward to the subsequent visit, if no subsequent data point(s) were available.

Changes from baseline in blood pressures and HR were analyzed by analysis of covariance using SAS PROC GLM. The initial model for each variable included terms for baseline (pre-treatment) value, treatment, site, and treatment by site interaction. The model was reduced in a stepwise manner, eliminating terms with p-values $\geqslant$0.10 until only terms with $p < 0.10$ or treatment remained in the model. A similar procedure was followed for the blood pressure and heart rate changes from the pre-meal value during each meal test except that the pre-meal value for that day was used as the covariate rather than the pre-treatment value. Data for resting, seated and 24-h blood pressure responses were also analyzed for pre-specified subgroups split at the sex-specific median values for SBP in order to assess the effects of rebaudioside A in subjects with low-normal blood pressure.

3. Results

3.1. Subjects characteristics

A total of 100 healthy subjects with normal blood pressure were randomly assigned to receive either rebaudioside A 1000 mg/day ($n = 50$) or placebo ($n = 50$). Two subjects (both in the placebo group) did not complete the study. One was lost to follow-up in week 3, and the other discontinued the study during the final week of the treatment period due to a serious adverse event (gastroenteritis). The investigator judged this event to be unrelated to the study product.

Baseline and demographic characteristics were not significantly different between treatment groups (Table 1). Participants ranged in age from 18 to 73 years of age and were predominantly females of non-Hispanic white race/ethnicity. Body mass index was somewhat higher in the rebaudioside A group than in the placebo group (25.7 vs. 24.3 kg/m^2, $p = 0.058$). Mean study product compliance was 99% in both groups, and all subjects were at least 80% compliant.

3.2. Hemodynamic measurements

3.2.1. Resting, seated blood pressures and heart rate

Values for resting, seated SBP, the primary outcome variable, indicated that there were no significant differences between the rebaudioside A and placebo groups at baseline or during the treatment period (Table 2). Values for resting, seated DBP and MAP were also not significantly different between rebaudioside A and placebo groups at baseline or in the changes from baseline to treatment. In addition, there were no significant differences between the rebaudioside A and placebo groups for SBP, DBP or MAP when the changes from baseline to each visit (weeks 1, 2, 3, and 4) were considered individually (data not shown).

3.2.2. Ambulatory blood pressures

Twenty-four hour ambulatory blood pressure monitoring of SBP and DBP revealed no significant differences between rebaudioside A and placebo treatment groups at week 0 or in the changes from week 0 to week 4 for morning, daytime, nighttime, and overall continuous 24-h readings (Table 3).

3.2.3. Supine and standing hemodynamic responses during meal tests

Supine and standing values pre-meal and the changes from pre- to post-meal for SBP, DBP, MAP, and HR during the meal tests at weeks 0 and 4 are shown in Table 4. Supine and standing SBP and DBP values are shown in Figs. 1 and 2.

At week 0, there were no significant differences between groups in pre-meal values or the changes from pre-meal to post-meal values for supine SBP, DBP, MAP and HR. This was also true for the pre-meal measurements of these parameters at week 4.

The placebo group showed slight reductions in both supine SBP and DBP (−0.8 and −1.9 mmHg, respectively) compared to the pre-meal value at week 4. In contrast, the rebaudioside A group showed slight increases for the corresponding pre- to post-meal changes (1.5 and 0.6 mmHg, respectively). No statistically significant difference was present for SBP, while the differences in response reached statistical significance for DBP ($p = 0.045$) and

Table 1
Baseline characteristics of all randomized subjects

Characteristic	Rebaudioside A ($n = 50$)	Placebo ($n = 50$)	p-Value
	n (%)		
Male	12 (24.0)	9 (18.0)	0.461
Female	38 (76.0)	41 (82.0)	
Race/ethnicity			
Non-Hispanic white	40 (80.0)	38 (76.0)	0.641[a]
African American	1 (2.0)	4 (8.0)	
Hispanic	7 (14.0)	5 (10.0)	
Asian	1 (2.0)	1 (2.0)	
Other	1 (2.0)	2 (4.0)	
	Mean ± SEM		
Age (year)	42.1 ± 1.9	41.0 ± 2.3	0.717
Body mass index (kg/m^2)	25.7 ± 0.5	24.3 ± 0.5	0.058
Weight (kg)	71.1 ± 1.8	67.7 ± 1.7	0.168
Height (cm)	166.2 ± 1.4	166.6 ± 1.2	0.830

Abbreviation: SEM = standard error of the mean.
[a] $p = 0.629$ for an additional comparison of Non-Hispanic white vs. all other race/ethnicity categories.

Table 2
Changes from baseline to treatment for resting, seated blood pressure measurements[a]

Resting, seated measurements	Rebaudioside A ($n = 50$)	Placebo ($n = 50$)	p-Value[b]
	Mean ± SEM		
Baseline SBP (mmHg)	110.0 ± 1.2	110.7 ± 1.3	0.683
SBP Δ (mmHg)	−1.3 ± 0.7	−0.4 ± 0.8	0.237
Baseline DBP (mmHg)	70.3 ± 0.9	71.2 ± 0.9	0.529
DBP Δ (mmHg)	−1.3 ± 0.5	−0.7 ± 0.5	0.154[c]
Baseline MAP (mmHg)	83.6 ± 0.9	84.3 ± 1.0	0.554
MAP Δ (mmHg)	−1.3 ± 0.6	−0.6 ± 0.6	0.192

Abbreviations: DBP = diastolic blood pressure, MAP = mean arterial blood pressure, SBP = systolic blood pressure, SEM = standard error of the mean.
[a] Change (signified by Δ) represents the change from baseline (average of weeks −1 and 0) to treatment (average of weeks 1, 2, 3, and 4).
[b] p-Values for the response variables are for the analysis of covariance with baseline value as the covariate.
[c] Values were ranked prior to analysis. Median values were similar to the means shown.

Table 3
24-h Ambulatory blood pressure monitor readings at baseline (week 0) and the change from baseline to week 4[a,b]

Parameter	Rebaudioside A	Placebo	p-Value[c]
	Mean ± SEM		
Morning			
Baseline SBP (mmHg)	111.7 ± 1.4	114.1 ± 2.0	0.336[d]
Baseline DBP (mmHg)	68.3 ± 1.5	69.7 ± 1.5	0.732[d]
SBP Δ (mmHg)	2.0 ± 2.0	2.8 ± 2.0	0.426[d]
DBP Δ (mmHg)	1.0 ± 2.1	1.6 ± 1.8	0.392[d]
Daytime			
Baseline SBP (mmHg)	115.3 ± 1.6	117.1 ± 1.5	0.582[d]
Baseline DBP (mmHg)	70.8 ± 1.2	70.9 ± 1.3	0.915[d]
SBP Δ (mmHg)	1.2 ± 1.6	0.0 ± 1.7	0.833[d]
DBP Δ (mmHg)	1.0 ± 1.8	2.4 ± 2.4	0.523[d]
Nighttime			
Baseline SBP (mmHg)	98.1 ± 1.0	99.7 ± 1.2	0.329
Baseline DBP (mmHg)	57.4 ± 0.9	57.6 ± 0.8	0.418
SBP Δ (mmHg)	1.7 ± 1.2	1.1 ± 1.2	0.638[d]
DBP Δ (mmHg)	−0.2 ± 0.9	−0.1 ± 0.8	0.742
24-h			
Baseline SBP (mmHg)	111.0 ± 1.1	110.7 ± 1.3	0.833
Baseline DBP (mmHg)	66.8 ± 1.0	65.8 ± 0.8	0.701
SBP Δ (mmHg)	0.1 ± 1.0	2.2 ± 1.0	0.277[d]
DBP Δ (mmHg)	−0.7 ± 0.9	1.6 ± 0.7	0.072

Abbreviations: DBP = diastolic blood pressure, SBP = systolic blood pressure, SEM = standard error of the mean.
[a] Morning blood pressure = average blood pressure during the first 2 h after awakening; daytime blood pressure = average recorded during the rest of the day; nighttime blood pressure = average recorded from the time the subject went to bed until the time he/she got out of bed.
[b] Change (signified by Δ) is the change from baseline (week 0) to week 4.
[c] p-Values for the response variables are for the analysis of covariance with baseline value as the covariate.
[d] Indicates values were ranked prior to analysis. Median values were similar to the means shown.

Table 4
Supine and standing blood pressure and heart rate measurements during meal tests at weeks 0 and 4

Parameters	Week 0			Week 4		
	Rebaudioside A ($n = 50$)	Placebo ($n = 50$)	p-Value	Rebaudioside A ($n = 50$)	Placebo ($n = 48$)	p-Value[a]
	Mean ± SEM			Mean ± SEM		
Supine pre-meal						
SBP (mmHg)	111.5 ± 1.5	109.7 ± 1.3	0.367	109.5 ± 1.2	111.5 ± 1.3	0.260
DBP (mmHg)	69.6 ± 1.1	68.9 ± 1.1	0.647	67.8 ± 1.0	69.9 ± 1.0	0.154
MAP (mmHg)	83.6 ± 1.1	82.5 ± 1.1	0.486	81.7 ± 1.0	83.7 ± 0.9	0.140
HR (bpm)	62.9 ± 1.5	66.1 ± 1.2	0.098	63.3 ± 1.5	67.0 ± 1.3	0.071
Δ *Post-meal*[b,c]						
SBP (mmHg)	0.0 ± 0.9	0.9 ± 0.7	0.674	1.5 ± 1.0	−0.8 ± 1.0	0.180
DBP (mmHg)	−1.3 ± 0.6	−1.2 ± 0.7	0.811	0.6 ± 0.6	−1.9 ± 0.8	0.045
MAP (mmHg)	−0.9 ± 0.6	−0.5 ± 0.6	0.955	0.9 ± 0.7	−1.5 ± 0.8	0.043
HR (bpm)	2.9 ± 1.0	4.5 ± 0.6	0.172[d]	4.4 ± 0.7	2.8 ± 0.8	0.404
Standing pre-meal						
SBP (mmHg)	115.1 ± 1.5	114.1 ± 1.3	0.595	112.8 ± 1.5	114.2 ± 1.1	0.163[d]
DBP (mmHg)	75.0 ± 1.1	74.9 ± 1.2	0.931	72.4 ± 1.2	74.7 ± 1.5	0.016[d]
MAP (mmHg)	88.4 ± 1.1	88.0 ± 1.1	0.773	85.9 ± 1.1	87.9 ± 1.1	0.036[d]
HR (bpm)	74.7 ± 1.5	78.9 ± 1.4	0.045	74.6 ± 1.7	79.9 ± 1.5	0.020
Δ *Post-meal*[b,c]						
SBP (mmHg)	0.8 ± 1.1	1.7 ± 0.9	0.645	1.1 ± 1.0	0.4 ± 0.9	0.745
DBP (mmHg)	−0.5 ± 0.7	−0.5 ± 0.9	0.907[d]	1.4 ± 0.8	−1.3 ± 1.2	<0.001[d]
MAP (mmHg)	−0.1 ± 0.7	0.2 ± 0.8	0.867	1.3 ± 0.7	−0.7 ± 0.8	0.020[d]
HR (bpm)	4.6 ± 0.8	3.7 ± 1.0	0.852	5.3 ± 1.1	1.9 ± 1.1	0.138

Abbreviations: DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, SEM = standard error of the mean.
[a] p-Values for the response variables are for the analysis of covariance with baseline value as the covariate.
[b] Post-meal is the average of values collected at 30, 60, 90, and 120 min post-meal.
[c] Change (signified by Δ) is the change from pre-meal to post-meal.
[d] Values were ranked prior to analysis. Median values were similar to the means shown.

MAP ($p = 0.043$). The supine HR response at week 4 did not differ significantly between treatment groups.

At week 0, there were no significant differences between rebaudioside A and placebo groups in pre-meal or the changes from pre-meal to post-meal values for standing SBP, DBP and MAP. Pre-meal standing HR was significantly higher at week 0 in the placebo group compared with the rebaudioside A group (78.9 vs. 74.7 bpm; $p = 0.045$), but the pre-meal to post-meal changes were not significantly different between groups.

Pre-meal levels for standing DBP (74.7 vs. 72.4 mmHg; $p = 0.016$), MAP (87.9 vs. 85.9 mmHg; $p = 0.036$) and HR (79.9 vs. 74.6 bpm; $p = 0.020$) were significantly higher in the placebo group at week 4. Similar to the pattern observed for supine pressures, the rebaudioside A group showed relative increases from pre-meal in DBP (1.4 mmHg) and MAP (1.3 mmHg) that were significantly different from small declines in the placebo group (−1.3 mmHg, p <0.001 and −0.7 mmHg, $p = 0.020$, respectively). No significant differences were observed for standing SBP or HR responses at week 4. Standing SBP (Fig. 2, Panel A) and DBP (Fig. 2, Panel B) at week 4 were also similar for rebaudioside A and placebo in the first 2 h following dosing.

3.3. Subgroup and sensitivity analyses

Pre-specified analyses conducted on data for resting, seated and 24-h blood pressures for subgroups with baseline SBP split at the sex-specific median (< and ⩾108 mmHg for females and < and ⩾117 mmHg for males) are shown in Table 5 (below the median) and Table 6 (above the median). In the lower baseline SBP-subgroup, there was a small relative reduction from baseline to treatment for rebaudioside A vs. placebo observed for resting, seated MAP (−0.3 vs. 1.5 mmHg, $p = 0.036$). Otherwise, 24-h blood pressure responses in the two SBP-subgroups did not differ significantly between rebaudioside A and placebo treatment groups.

3.4. Body weight, diet, and physical activity

Baseline or changes from baseline in body weight did not differ significantly between the rebaudioside A and placebo groups (data not shown). Baseline values and changes from week 0 to week 4 in intakes of total energy; percentages of energy from carbohydrate, protein, fat, saturated fatty acids, poly- and monounsaturated fatty acids, the ratio of polyunsaturated to saturated fatty acids, and alcohol; dietary fiber; soluble dietary fiber; sodium; potassium; calcium; and magnesium were not significantly different between the rebaudioside A and placebo groups (data not shown). Baseline values and changes from week 0 to week 4 in physical activity were not significantly different between rebaudioside A and placebo treatments (data not shown).

3.5. Tolerance

Rebaudioside A was well tolerated. Sixteen subjects (32%) reported adverse events in the rebaudioside A group compared to 18 subjects (36%) in the placebo group ($p = 0.833$). One serious adverse event (gastroenteritis) was reported in the placebo group. None of the adverse events were believed by the investigators to be related to the study products. No signs or symptoms of hypotension (e.g., lightheadedness or dizziness) were reported in association with rebaudioside A use. One subject in the rebaudioside A group had a vagal response to a blood draw. Laboratory test results (chemistry, hematology, and urinalysis) indicated no clinically meaningful or statistically significant differences between the rebaudioside A and placebo groups (Table 7).

4. Discussion

Several studies in the published literature have examined the effects of steviol glycosides, primarily stevioside, on blood pressure in animals and humans. A number of these have reported antihy-

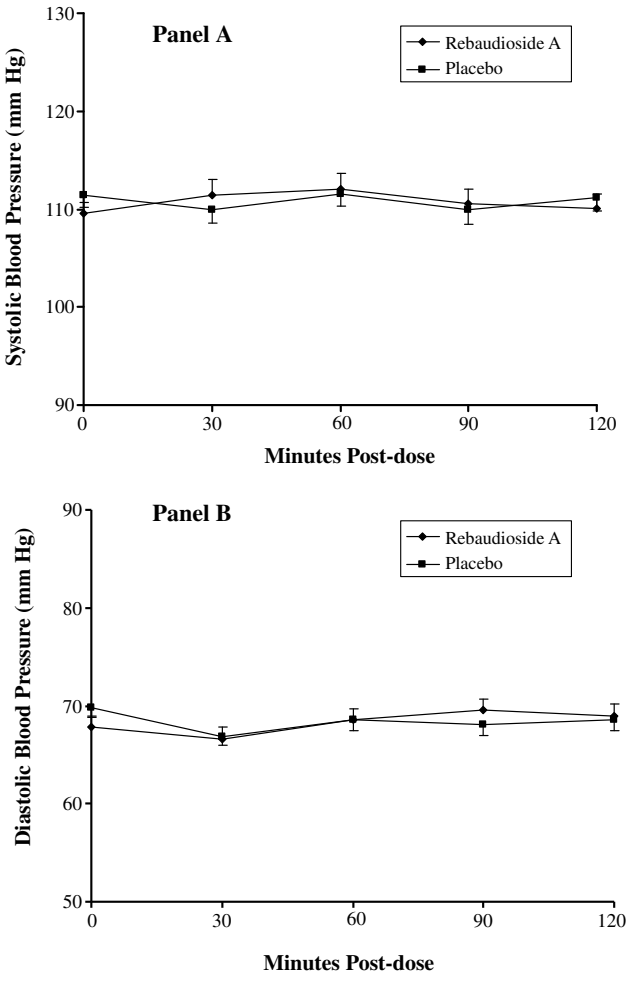

Fig. 1. Supine systolic blood pressure (Panel A – top) and supine diastolic blood pressure (Panel B – bottom) at pre-dose (0 min) and 30, 60, 90, and 120 min after dosing with rebaudioside A or placebo at week 4.

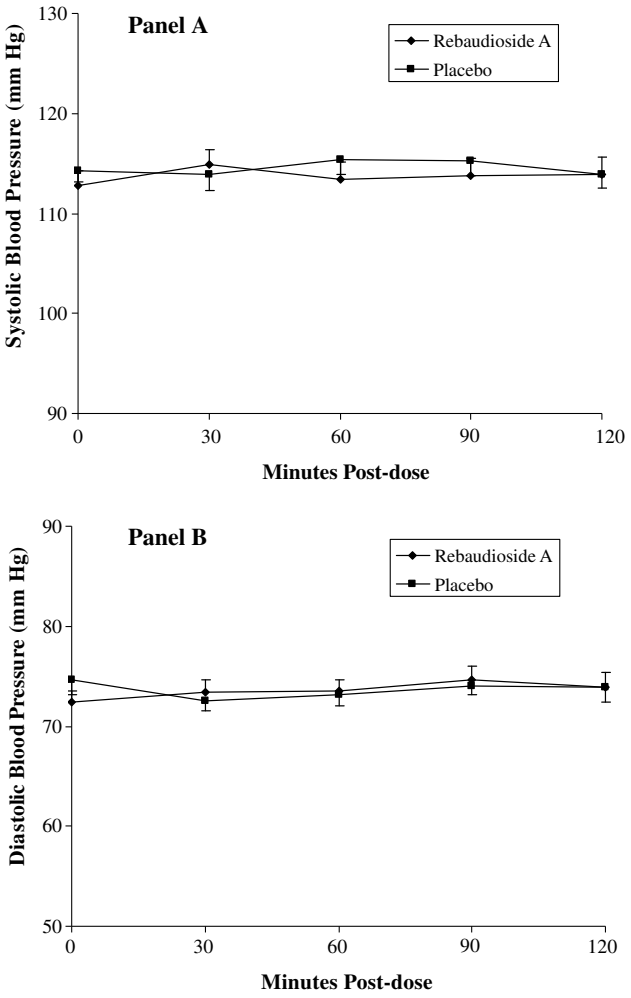

Fig. 2. Standing systolic blood pressure (Panel A – top) and standing diastolic blood pressure (Panel B – bottom) at pre-dose (0 min) and 30, 60, 90, and 120 min after dosing with rebaudioside A or placebo at week 4.

pertensive properties (Chan et al., 1998, 2000; Hsieh et al., 2003; Jeppesen et al., 2003; Liu et al., 2003; Dyrskog et al., 2005; Ferri et al., 2006). Although results from some of the studies support a blood pressure-lowering effect of stevioside (Chan et al., 1998, 2000; Hsieh et al., 2003; Jeppesen et al., 2003; Liu et al., 2003), particularly in individuals with elevated blood pressure, other, more recently conducted studies in normotensive and hypertensive individuals have failed to support these findings (Ferri et al., 2006; Geuns et al., 2007; Maki et al., 2008).

Investigations to address the effects of rebaudioside A on blood pressure lowering are limited (Carakostas et al., 2008). In Goto-Kakizaki rats, a model of type 2 diabetes, 8 weeks of daily ingestion of rebaudioside A had no effect on blood pressure (Dyrskog et al., 2005). In a study designed to investigate the effects of chronic consumption of rebaudioside A on glucose homeostasis in subjects with type 2 diabetes mellitus, Maki et al. (2008) showed that consumption of 1000 mg/day of rebaudioside A for 16 weeks did not significantly alter blood pressure. This finding corroborates the results of the current trial in a separate sample of men and women with diabetes mellitus, more than half of whom were taking antihypertensive medications.

The purpose of the current trial was to assess the potential hemodynamic effects of rebaudioside A in individuals with normal and low-normal blood pressures, in order to address specific questions raised by JECFA at their 63rd meeting about potential adverse effects of steviol glycosides in this population. Subjects were enrolled in the study who met the criteria for normal blood pressure (<120 mmHg SBP and <80 mmHg DBP). Furthermore, specific statistical evaluation of the subgroup of subjects with baseline SBP below the sex-specific median was pre-specified to assess effects in subjects with low-normal blood pressures (baseline SBP <108 mmHg for females and <117 mmHg for males). This analysis was included in order to address the JECFA question regarding potential effects in asymptomatic hypotensive individuals. Asymptomatic hypotensive individuals are relatively rare and thus extremely difficult to identify for the purposes of a clinical study. According to data from the National Health and Nutrition Examination Survey (NHANES), mean values for Americans aged 40–59 are 124 mmHg and 75 mmHg for SBP and DBP, respectively (Ong et al., 2007). Therefore, the lower SBP-subgroup included individuals whose values were well below the US population average.

The present study was designed to provide 80% power to detect a 4.5 mmHg difference in SBP response for rebaudioside A vs. placebo with $\alpha = 0.05$, two-tailed. In fact, the study had >90% power due to lower than anticipated subject variability, which increased the sensitivity to detect minor changes in blood pressure. The subjects were also considered to be fully compliant with study product consumption, based upon 100% of individuals enrolled consuming >80% of the expected doses of study product during the treatment period.

Table 5
Changes from baseline to treatment for blood pressure measurements in the subgroup of subjects with baseline systolic blood pressure below the sex-specific median[a,b]

Parameters	Rebaudioside A (n = 28)	Placebo (n = 20)	p-Value[c]
	Mean ± SEM		
Resting, seated measurements			
Baseline SBP (mmHg)	104.4 ± 0.8	103.1 ± 1.7	0.444
SBP Δ (mmHg)	0.3 ± 0.8	2.4 ± 0.8	0.114
Baseline DBP (mmHg)	67.2 ± 0.9	67.2 ± 1.6	0.970
DBP Δ (mmHg)	−0.5 ± 0.5	1.1 ± 0.7	0.091[d]
Baseline MAP (mmHg)	79.6 ± 0.7	79.1 ± 1.5	0.752
MAP Δ (mmHg)	−0.3 ± 0.6	1.5 ± 0.6	0.036
24-h measurements			
Baseline SBP (mmHg)	108.5 ± 1.2	107.1 ± 1.9	0.504
SBP Δ (mmHg)	−0.5 ± 1.5	1.6 ± 1.6	0.798[d]
Baseline DBP (mmHg)	66.2 ± 1.1	65.3 ± 1.3	0.618
DBP Δ (mmHg)	−1.5 ± 0.9	0.0 ± 1.0	0.347

Abbreviations: DBP = diastolic blood pressure, MAP = mean arterial pressure, SBP = systolic blood pressure, SEM = standard error of the mean.
[a] Median baseline SBP <108 mmHg for women and <117 mmHg for male.
[b] Change (signified by Δ) in resting, seated measurements represents the change from baseline (average of weeks −1 and 0) to treatment (average of weeks 1, 2, 3, and 4), and Δ in 24-h measurements represents the change from baseline (week 0) to week 4.
[c] p-Values for the response variables are for the analysis of covariance with baseline value as the covariate.
[d] Values were ranked prior to analysis. Median values were similar to the means shown.

Table 6
Changes from baseline to treatment for blood pressure measurements in the subgroup of subjects with baseline systolic blood pressure above the sex-specific median[a,b]

Parameters	Rebaudioside A (n = 22)	Placebo (n = 30)	p-Value[c]
	Mean ± SEM		
Resting, seated measurements			
Baseline SBP (mmHg)	117.0 ± 1.4	115.7 ± 1.0	0.870[d]
SBP Δ (mmHg)	−3.2 ± 1.2	−2.2 ± 1.1	0.716
Baseline DBP (mmHg)	74.3 ± 1.5	73.9 ± 0.9	0.437[d]
DBP Δ (mmHg)	−2.3 ± 1.0	−1.8 ± 0.7	0.395[d]
Baseline MAP (mmHg)	88.6 ± 1.3	87.8 ± 0.7	0.476[d]
MAP Δ (mmHg)	−2.6 ± 1.0	−2.0 ± 0.7	0.699
24-h measurements			
Baseline SBP (mmHg)	114.2 ± 1.8	113.1 ± 1.6	0.661
SBP Δ (mmHg)	1.0 ± 1.4	2.6 ± 1.2	0.368[d]
Baseline DBP (mmHg)	67.7 ± 1.7	66.1 ± 1.0	0.362
DBP Δ (mmHg)	0.4 ± 1.8	2.8 ± 1.0	0.291

Abbreviations: DBP = diastolic blood pressure, MAP = mean arterial pressure, SBP = systolic blood pressure, SEM = standard error of the mean.
[a] Median baseline SBP ⩾108 mmHg for women and ⩾117 mmHg for male.
[b] Change (signified by Δ) in resting, seated measurements represents the change from baseline (average of weeks −1 and 0) to treatment (average of weeks 1, 2, 3, and 4); and Δ in 24-h measurements represents the change from baseline (week 0) to week 4.
[c] p-Values for the response variables are for the analysis of covariance with baseline value as the covariate.
[d] Values were ranked prior to analysis. Median values were similar to the means shown.

An intake of 1000 mg/day represents more than 10 times the mean predicted intake of rebaudioside A for consumers of the sweetener, and approximately 4 times the mean predicted daily intake for high-intake consumers (Renwick, 2008). No statistically significant difference was observed between the rebaudioside A and placebo groups for resting, seated SBP, the primary outcome variable. Likewise, no statistically significant differences were noted for SBP in any of the secondary outcome variables.

Table 7
Serum chemistry and hematology parameters at screening (week −1) and the changes from screening to week 4[a]

Parameter	Rebaudioside A (n = 50)	Placebo (n = 50)	p-Value
	Mean ± SEM		
Screening alanine transaminase (U/L)	22.0 ± 1.5	19.9 ± 1.1	0.264
Alanine transaminase Δ (U/L)	−1.0 ± 0.9	0.6 ± 1.4	0.331
Screening aspartate transaminase (U/L)	22.6 ± 1.0	20.9 ± 0.8	0.180
Aspartate transaminase Δ (U/L)	−0.9 ± 0.7	0.1 ± 0.7	0.288
Screening alkaline phosphatase (U/L)	65.2 ± 2.7	65.9 ± 2.3	0.830
Alkaline phosphatase Δ (U/L)	0.8 ± 0.8	0.0 ± 0.9	0.476
Screening blood urea nitrogen (mg/dL)	13.7 ± 0.6	14.5 ± 0.6	0.306
Blood urea nitrogen Δ (mg/dL)	0.1 ± 0.4	−0.8 ± 0.4	0.141
Screening creatinine (mg/dL)	0.8 ± 0.0	0.8 ± 0.0	1.000
Creatinine Δ (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.155
Screening gamma glutamyl transferase (U/L)	20.1 ± 1.7	17.7 ± 1.5	0.278
Gamma glutamyl transferase Δ (U/L)	−0.7 ± 0.7	0.6 ± 0.7	0.162
Screening red blood cell count (10^6/μL)	4.4 ± 0.1	4.4 ± 0.1	0.669
Red blood cell count Δ (10^6/μL)	0.0 ± 0.0	0.0 ± 0.0	0.201
Screening white blood cell count (10^3/μL)	6.0 ± 0.2	6.3 ± 0.2	0.356
White blood cell count Δ (10^3/μL)	0.3 ± 0.2	0.2 ± 0.2	0.884
Screening basophil (%)[b]	0.51 ± 0.06	0.47 ± 0.07	0.655
Basophil Δ (%)	−0.09 ± 0.07	−0.05 ± 0.10	0.741
Screening hemoglobin (g/dL)	13.3 ± 0.2	13.6 ± 0.2	0.292
Hemoglobin Δ (g/dL)	0.0 ± 0.1	−0.1 ± 0.1	0.396
Screening hematocrit (%)	39.8 ± 0.5	40.8 ± 0.4	0.124
Hematocrit Δ (%)	0.3 ± 0.3	−0.2 ± 0.3	0.192

SEM = standard error of the mean.
[a] Change from baseline to week 4 is indicated by Δ.
[b] Basophil (%) is presented because it was the only subset of white blood cells that showed a significant difference between rebaudioside A and placebo in another study (Maki et al., 2008).

There were no statistically significant differences in pre-meal supine blood pressure measurements at weeks 0 or 4, while pre-meal standing DBP and MAP, but not SBP, at week 4 were lower in the rebaudioside A group. However, because there were a large number of statistical comparisons made for the secondary and subgroup analyses, some statistically significant differences would be expected to occur by chance. Nonetheless, the mean differences between the rebaudioside A and placebo groups in DBP from the secondary analyses, despite being statistically significant, were small (<3 mmHg) and raised no safety concerns. In addition, subjects reported no signs or symptoms suggesting intolerance.

The meal challenge was conducted to assess acute effects (up to 120 min post-dose) of rebaudioside A on blood pressure. However, results from a subsequent pharmacokinetic study conducted in humans (Wheeler et al., 2008) indicate that if there were any effects of rebaudioside A on blood pressure, these would unlikely be apparent until several hours after consumption. Steviol glucuronide, the principal metabolite for steviol glycosides, did not appear in the blood until approximately 4–5 h post-dose and reached maximum concentrations at 12 h. Therefore, based upon peak plasma concentrations of the principal metabolite it would be expected that subjects in the rebaudioside A treatment group would have had circulatory levels of steviol glucuronide at the time of the meal tolerance test at week 4 from the previous day's consumption, but these levels would not have been expected to increase post-dose during the meal challenge period.

To the authors' knowledge, steviol glycosides have not been reported to cause elevations in blood pressure. Therefore, it is notable that the supine and standing post-meal DBPs in the rebaudioside A group increased relative to placebo at week 4, although all differences were small (<3 mmHg). Examination of

the values during the post-meal period showed that the DBPs in the placebo and rebaudioside A groups were nearly identical. The rises in the post-meal supine and standing DBPs in the rebaudioside A group were attributable to slightly lower pre-meal values, thus were not indicative of a hypertensive effect of acute rebaudioside A consumption.

In 2004, following a review of the available safety data for steviol glycosides, JECFA set a temporary acceptable daily intake limit of 0–2 mg/kg body weight (JECFA, 2005). The Committee assigned a temporary acceptable daily intake on the basis that there were some indications that stevioside, a steviol glycoside, may have blood pressure-lowering effects in subjects with hypertension. As a result, JECFA requested additional data/information regarding the potential effects of steviol glycosides in individuals with low blood pressure.

The present trial had sufficient statistical power to detect modest effects on blood pressure (>90% power to detect a 4.5 mmHg difference in SBP) and showed that 1000 mg/day of rebaudioside A administered over a 4-week period was well tolerated and had no significant effect on most blood pressure values measured, including resting, seated SBP, the primary outcome variable. Small (<3 mmHg), but statistically significant changes in DBP and MAP were noted in some secondary analyses, but were not considered clinically meaningful from a safety perspective. Therefore, the results of the current study indicate that approximately 4 times the estimated mean daily intake of rebaudioside A by high-intake consumers (Renwick, 2008) was well tolerated and had no clinically important impact on blood pressure in men and women with normal and low-normal resting blood pressures.

Conflict of interest statement

Authors Tarka and Bisognano received financial support from Cargill for consulting services.

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