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Stevia Leaf to Stevia Sweetener: Exploring its Science, Benefits and Future Potential[1,2]

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Footnote: Supplemental Table 1 on the relative sweetness of steviol glycosides versus sucrose and, Supplemental Table 2 on the effect of steviol glycosides on fasting blood glucose, insulin and HbA_{1c} are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents.

Key words: stevia, stevia leaf extract, steviol glycosides, health effects, ADI, EDI, diabetes, obesity, dietary intake, taste, consumer, metabolism, safety

Abbreviations used: ADA, American Diabetes Association; ADI, acceptable daily intake; AHA, American Heart Association; AND, The Academy of Nutrition and Dietetics; AUC, area under the curve; BW, body weight; EDI, estimated daily intake; EFSA, European Food Safety Authority; ESL, fist water extract; FSANZ, Food Standards Australia New Zealand; GK, Goto-Kakizaki; GLUT, high affinity glucose transporter; GRAS, generally recognized as safe; HbA_{1c}, glycated hemoglobin; HFCS, high fructose corn syrup; HPLC, high performance liquid chromatography; iAUC, incremental AUC; IVGTT, intravenous glucose tolerance test; JECFA, Food and Agriculture Organization/World Health Organization's Joint Expert Committee on Food Additives; LC-MS, liquid chromatography-mass spectrometry; LNCS, low and no-calorie sweeteners; NOAEL, no observed adverse effect level; non-GMO, non-genetically modified organism; Reb, rebaudioside; SACN, UK Scientific Advisory Commission on Nutrition; SCF, Scientific Committee on Food; SE, steviol equivalents; SL, dried stevia leaves; SLE95, stevia leaf extract with > 95% purity; STZ, streptozotocin; UK, United Kingdom; US, United States; USP, United States Pharmacopoeia; WHO, World Health Organization.

Abstract

Steviol glycoside sweeteners are extracted and purified from the *Stevia rebaudiana* Bertoni plant, a member of the *Asteraceae (Compositae*) family that is native to South America, where it has been used for its sweet properties for hundreds of years. With continued rising rates of obesity, diabetes and other related co-morbidities, in conjunction with global public policies calling for reductions in sugar intake as a means to help curb these issues, low and no-calorie sweeteners (LNCS) also known as high-potency sweeteners such as stevia are gaining interest among consumers and food manufacturers. This appeal is related to stevia being plant-based, zero calorie and a sweet taste that is 50 – 350 times sweeter than sugar, making it an excellent choice for use in sugar- and calorie-reduced food and beverage products. Despite the fact that the safety of stevia has been affirmed by several food regulatory and safety authorities around the world, insufficient education about stevia's safety and benefits, including continuing concern regarding the safety of LNCS in general, deters health professionals and consumers from recommending and or using stevia. Therefore, the aim of this review and the stevia symposium that preceded this review at the American Society for Nutrition's annual conference in 2017 was to examine in a comprehensive manner, the state of the science for stevia, its safety, potential health benefits and future research and application. Topics covered include metabolism, safety and acceptable intake, dietary exposure, impact on blood glucose and insulin levels, energy intake and weight management, blood pressure, dental caries, naturality and processing, taste and sensory properties, regulatory status, consumer insights and market trends. Data for stevia is limited in the case of energy intake and weight management as well as the gut microbiome, therefore the broader literature on LNCS were reviewed at the symposium and therefore are also included in this review.

Introduction

Stevia rebaudiana Bertoni is a small perennial shrub of the *Asteraceae (Compositae)* family that is native to Paraguay, Brazil and Argentina. The leaves of this plant have been used by indigenous people for centuries in medicines and to sweeten drinks such as maté, a green herbal tea (1–3). The plant was first brought to the attention of the rest of the world by the botanist Moises Santiago Bertoni in 1887, who learnt of its properties from the Paraguayan Indians (1, 3). The chemical characterization of the natural constituents of the plant known as steviol glycosides, responsible for its distinct sweet taste was not identified until 1931 when two French chemists, Bridel and Lavielle isolated stevioside, a primary steviol glycoside from stevia leaves (1). Japan was the first country to commercialize and use crude unpurified extracts of *Stevia rebaudiana* in the 1970s on a large-scale (2). Its use eventually spread to several countries in Asia and Latin America (4). In the 1990s stevia extract was available in the United States (US) as a dietary supplement in health food stores, however, early formulations were known to have a licorice flavor with a sweet or bitter after-taste which limited their wide-spread commercial development (2, 5). The presence of essential oils, tannins and flavonoids in the crude extracts were partly responsible for some of the off tastes, hence efforts were made to purify extracts and chemically characterize steviol glycosides (5).

Following the isolation of stevioside, several other steviol glycosides such as rebaudiosides (Reb) A, B, C, D, E and dulcoside A were identified and isolated from stevia leaves (6). Generally, the most abundant steviol glycosides in stevia leaves are stevioside (4-13% w/w), Reb A (2-4%) and Reb C (1-2% w/w) (7, 8). In recent years, more than 40 steviol glycosides have been identified, e.g., Reb F, G, H, I, J, K, L, M, N, O, Q, stevioside A, D, E etc.

(9–12). Most of the steviol glycosides derived from the plant are four-ring diterpenes that have a backbone of 13-hydroxy-ent-kaur-16-en-19-oic acid, known as steviol (1, 12). The various glycosides differ only in the number and type of monosaccharides attached at the R1 (OH) and R2 (H) position of the aglycone, steviol. Glucose, fructose, rhamnose, xylose and deoxy glucose are examples of sugars that are attached to the steviol backbone (12). The two primary steviol glycosides, stevioside and Reb A differ only by one glucose moiety at R1; stevioside has two glucose molecules, while Reb A has three.

The stevia plant is now commercially cultivated in Argentina, Brazil, Columbia, Paraguay, China, Japan, Malaysia, South Korea, Vietnam, Israel, Australia, Kenya, and the United States. High-purity steviol glycosides are approved as sweeteners by all major regulatory authorities across the globe and more than 150 countries have approved and/or adopted its use in foods and beverages. Reb A was the first commercial steviol glycoside launched in the marketplace (13).

Metabolism of Steviol Glycosides

The absorption, metabolism and excretion of steviol glycosides have been extensively reviewed by multiple scientific authorities and experts including the European Food Safety Authority (EFSA) (14) and recently by Magnuson et al. (15). Steviol glycosides are undigested in the upper gastrointestinal tract. They are hydrolyzed or degraded only when they come into contact with microbiota in the colon that cleave the glycosidic linkages, removing the sugar moieties, leaving behind the steviol backbone that is absorbed systemically, glucuronidated in the liver and excreted via urine in humans, and via feces in rats (15).

In vitro studies demonstrate that human saliva, salivary α-amylase, pepsin, pancreatin, pancreatic α-amylase as well as jejunal brush border enzymes of mice, rats, and hamsters are not able to hydrolyze the glycosidic bonds present in stevioside (16). However, the gut microbiota of humans, rodents and hamsters are able to degrade stevioside to steviol (16). Incubation of stevioside and Reb A with human fecal microbiota demonstrated that both were completely hydrolyzed to steviol in 10 and 24 hours, respectively (4, 17). The released sugar moieties are not absorbed and are most likely quickly utilized by the gut microbes as an energy source, thus making it a zero calorie sweetener (2). An *in vitro* model of the intestinal barrier has shown that the transport of stevioside and Reb A through the monolayers is very low, whereas the absorptive transport of steviol is high, suggesting that steviol is not metabolized by gut microbiota and is absorbed from the intestine (18). Bacteroides species are primarily responsible for the hydrolysis of steviol glycosides in the gut via their beta-glucosidase activity (17).

Evidence from *in vitro* investigations are consistent with human metabolism studies that revealed no detectable presence of the glycosides in plasma, suggesting no uptake from the gut and little or no stevioside or Reb A in urine or feces (19–22). These studies also demonstrate that steviol is absorbed quickly and transported to the liver where it is conjugated with glucuronic acid to form steviol glucuronide which in humans is excreted in urine (19–22). **Figure 1** summarizes the absorption, metabolism and excretion pathway of steviol glycosides in humans.

Wheeler et al. (21) compared the pharmacokinetics and metabolism of stevioside and Reb A in healthy adults over a 72-hour period. Peak plasma levels occurred at 8 hours and 12 hours for stevioside and Reb A, respectively and a half-life ($t_{1/2}$) of 14-16 hours was observed for both. Intake of Reb A resulted in significantly lower steviol glucuronide concentrations (59%) than after stevioside (62%) consumption. The differences in steviol glucuronide levels are attributed

to the simpler structure and faster bacterial degradation of stevioside compared to Reb A. Fecal recovery of steviol accounted for approximately 5% of the original dose for both compounds. The pharmacokinetic analyses revealed that stevioside and Reb A undergo similar metabolic and elimination processes in humans.

Most of the earlier studies on steviol glycoside metabolism were on Reb A or stevioside (a.k.a. primary or major glycosides). However, the similarities in the microbial metabolism of several steviol glycosides were confirmed in *in vitro* studies of pooled human fecal homogenates of healthy male and female Asian and Caucasian subjects (12, 23). Reb A, B, C, D, E, F, M, dulcoside A (a.k.a. minor glycosides) and steviolbioside (an intermediate metabolite), which contain different sugar moieties (glucose, rhamnose, xylose, fructose and deoxyglucose) and different linkage types (αβ (1-2), β-1, β (1-2), β (1-3), and β (1-6)), were all degraded to steviol within 24 to 48 hours. No differences between male and female subjects or between ethnicities were observed. These data suggest that the different steviol glycosides have similar hydrolysis rates to that of Reb A and therefore would be expected to have similar steviol absorption rates, metabolism and pharmacokinetics as Reb A. This was also confirmed in an animal model comparing the metabolism of Reb A and Reb D (24). These data demonstrate that both major and minor steviol glycosides appear to share a common metabolic fate.

Safety and Acceptable Daily Intake of Steviol Glycosides

The safety of steviol glycosides from numerous toxicological, biological, and clinical studies has been reviewed in several publications (2, 7, 14, 25, 26). As described in the regulatory section of this review, all major global scientific and regulatory bodies have determined high-purity steviol glycosides to be safe for consumption by the general population. The majority of the regulatory

approvals pertain to high-purity (≥ 95%) steviol glycosides. Unpurified crude extracts of stevia have been reported to cause adverse effects on fertility in animals (27, 28), which have not been observed with well-characterized high-purity steviol glycosides approved for food and beverage use. Therefore studies conducted with crude extracts have been determined to be not relevant to the safety assessment of high-purity steviol glycosides by knowledgeable scientific experts and regulatory authorities.

Potential effects of high purity steviol glycosides on acute and long-term toxicity, reproductive and developmental toxicity, and carcinogenicity have been conducted primarily in rodents but also in other animal models (29–34). Steviol glycosides are excreted primarily as steviol glucuronide in the urine in humans, whereas in rats, free steviol and steviol glucuronide are excreted primarily in the feces via the bile, with less than 3% appearing in the urine (2, 35). This inter-species difference is due to the lower molecular weight threshold for biliary excretion in rats compared to humans (2). Although the elimination routes of steviol glycosides differ between humans and rats, this is of no toxicological significance as the metabolism and pharmacokinetics are similar in the two species (2). In other words, the majority of the tissues and cells of the body are exposed to similar concentrations of the same metabolites for a similar amount of time following consumption of steviol glycosides in both species, so the potential for development of a toxicological effect is similar even though the final route of excretion is different. Therefore, the rat is an appropriate test animal for safety of consumption of steviol glycosides and toxicological data generated from rat studies are applicable to humans (2).

The acceptable daily intake (ADI) is the amount of a substance that an individual can consume daily over a lifetime without any appreciable health risk. It is established by regulatory agencies based on the results of toxicology testing. The No Observed Adverse Effect Level

(NOAEL), which is the highest dose fed to animals in long-term studies with no adverse toxicological effect is considered the basis of the ADI. The NOAEL is divided by safety factors (typically 100) to account for intra- and inter-species differences to ensure the ADI is safe for all potential consumers, including subgroups such as children. The current ADI for steviol glycosides is based on a toxicity and carcinogenicity study that tested stevioside (95.6% purity) at concentrations of 0, 2.5 and 5% of the diet of rats for 2 years, resulting in consumption levels of 0, 970 and 2387 mg . kg^{-1} . d^{-1} (36). This study evaluated potential effects on physiology (body weight, food consumption, final organ weight), behavior, ophthalmology, biochemistry (blood chemistry, hematology, urine analysis, liver enzymes), and histological changes in tissues. At all the doses tested, stevioside had no effect on cancer development. No adverse effects were observed in rats consuming stevioside at 2.5% of diet or lower. At the highest dose (5% of diet), changes were observed for kidney and body weight and survival rates. Therefore, the NOAEL for this study was 2.5% of the diet, or 970 mg . kg^{-1} . d^{-1}, and when converted to steviol equivalents, 383 mg steviol equivalents (SE) . kg^{-1} . d^{-1}.

Applying a 100-fold safety factor to 383 mg SE results in an ADI of 0 to 4 mg SE . kg^{-1} . d^{-1}. The ADI is expressed in steviol equivalents because all steviol glycosides are metabolized to steviol, allowing the ADI to apply to all steviol glycosides. Steviol glycosides differ in structure and molecular weight, and therefore contribute relatively different amounts of steviol per gram of steviol glycoside. Therefore, using the conversion factor of 0.33 for Reb A versus 0.40 for stevioside, which factors in molecular weight, the number of glucose units and steviol per gram, the ADI for Reb A equates to 12 mg . kg^{-1} . d^{-1} and for stevioside it is 10 mg . kg^{-1} . d^{-1}.

An important study that established the safety of steviol glycosides for consumption by pregnant women and children was a reproductive and developmental study of Reb A (> 97%

purity) (31). Rats were fed up to 2273 mg . kg^{-1} . d^{-1} of Reb A for two generations while body weight, food intake, growth and development, survival, reproductive performance and sexual maturation were monitored. No adverse reproductive or developmental effects were observed in any of the generations at the highest dose. Similar results were reported in reproductive toxicology studies with purified stevioside (29, 37). Early studies in rats with crude extracts of *Stevia rebaudiana* had observed reduced fertility (27) or lower seminal vesicle weights compared to controls (28), but studies with high-purity steviol glycoside extracts (31, 36, 37) have not observed any negative effects on sexual organs, levels of sexual hormones, mating behavior, fertility, gestation length, offspring survival and sexual maturation. The lack of adverse effects following exposures to high doses of high-purity steviol glycoside prior to and during critical periods of fertility and pregnancy, during lactation, and throughout growth and development of the offspring to adulthood for two generations demonstrates the safety of steviol glycosides for consumption by pregnant women and children at or below the established ADI.

Despite the extensive review and conclusions of safety experts that steviol glycosides are not mutagenic, two publications have questioned whether adequate testing of the genotoxic potential of steviol glycosides have been performed (38, 39). In response to their concern, Urban et al. (40) conducted a comprehensive and extensive review of all published *in vitro* and *in vivo* studies. Much of the concern were from a few older *in vitro* studies where steviol was reported to be mutagenic using a highly specific bacterial strain, Salmonella typhimurium TM677 which requires growth conditions that are not applicable to humans. Urban et al.'s (40) review found consistently negative results for Reb A and steviol, and all negative results for stevioside except for one study. The *in vivo* study by Nunes et al. (41) that was positive has been criticized for its methodology and data interpretation by several reviewers (20, 40, 42, 43). Hence Urban et al.

(40) concluded that the database of *in vitro* and *in vivo* studies for steviol glycosides is robust with no evidence that steviol glycosides are genotoxic.

In addition to *in vitro* and animal studies, human safety studies have also been conducted. Reb A doses of up to 1000 mg/day for 1-4 months and stevioside doses of 750 mg/day for 3 months were well tolerated and had no adverse effects on blood pressure or fasting blood glucose in healthy, hypertensive and type 1 and type 2 diabetic subjects (44–46). Nor were there any significant clinical changes in serum chemistry, hematology and urine analysis. Most of the safety studies have been conducted on Reb A and stevioside because they are the most abundant steviol glycosides in the *Stevia rebaudiana* Bertoni plant. However, all major and minor steviol glycosides are degraded to steviol by human microbiota and therefore share the same metabolic fate. A series of *in vitro* tests with human fecal homogenates confirmed this for several of the minor steviol glycosides Reb B, C, D, E, F, M, dulcoside A, and steviolbioside (12, 23), thus making the studies on Reb A and stevioside applicable to the minor steviol glycosides as well.

Another concern raised by some is the allergenic potential of steviol glycosides due to the common taxonomy of the stevia plant with plants that can induce hypersensitivity in some individuals (e.g., ragweed, goldenrod, chrysanthemum, echinacea, chamomile, lettuce, sunflower and chicory). A comprehensive literature search found no evidence of allergenic potential of purified steviol glycosides (47). According to Urban et al. (47) the few cases of allergic reactions that have been reported in the literature occurred before the introduction of high-purity steviol glycosides into the marketplace. Similarly, human studies with high-purity steviol glycosides have reported no negative gastrointestinal side effects such as bloating, gas, diarrhea, nausea or borborygmus (44–46) that are sometimes associated with certain caloric and nonnutritive sweeteners that include, fructose, sugar alcohols and allulose, a.k.a. psicose (48–51).

Overall, the safety data for high-purity steviol glycosides has been thoroughly evaluated and their use as a plant based zero-calorie sweetener has been approved across the globe. It has been conclusively determined that foods and beverages containing approved levels of high-purity stevia leaf extract sweeteners (i.e., steviol glycosides) are safe for all individuals, including children, pregnant and nursing women, and individuals with diabetes.

Dietary Exposure

To ensure safety of consumption, the estimated daily intake (EDI) of a food additive should not exceed the ADI. Hence prior to approval of use, potential intakes are estimated using proposed food usage levels in various food categories, together with information from food consumption surveys. The EDI for steviol glycosides has been estimated for various populations (**Table 1**). In most instances, the EDI for steviol glycosides is less than the ADI and due to the conservative nature by which they are assessed, estimated intakes are generally recognized as over estimations of what might be actual or average consumer intakes.

Surveys have been utilized in various global jurisdictions to determine daily consumption estimates of high-purity steviol glycosides. The Food and Agriculture Organization/World Health Organization's Joint Expert Committee on Food Additives (JECFA) assessed international dietary exposure estimates using a model that assumed steviol glycosides would replace all sweeteners used in or as food, based on the relative sweetness of steviol glycosides to sucrose (52). The Committee estimated maximum intakes of 1.3 - 5 mg SE . kg^{-1} . d^{-1} worldwide. However, the Committee acknowledged that these estimates were highly conservative and indicated that actual intakes were more likely to be 20–30% of these values (52). Renwick et al. (53) estimated Reb A intakes for adults, children and diabetic children using equivalent intake

calculations based on existing LNCS consumption surveys for North America, Australia and Europe. For the general population, mean intake ranged from 0.4 – 0.7 mg SE . kg^{-1} . d^{-1} and for adults and children, high intakes (90^{th} percentile and above) were 1.1 – 1.7 mg SE . kg^{-1} . d^{-1}.

In 2011, Food Standards Australia and New Zealand (FSANZ) during their review to expand the approval of steviol glycosides considered 3 dietary exposure assessment models; a 30% market share scenario, and two 'brand loyal' scenarios (54). Although the 90^{th} percentile dietary exposures of one of the brand loyal scenarios were 110% of the ADI for Australian children aged 2–6 years, and 100% of the ADI for New Zealand children aged 5–14 years, the FSANZ concluded that all 3 models were likely an overestimation. Health Canada (55) used two approaches in their exposure assessment in 2012. Method 1 substituted all table-top sweeteners and method 2 assumed maximum authorized use in all food categories. Both approaches resulted in mean intakes that were well below the ADI. Although the maximum use levels (95^{th} percentile) marginally exceeded the ADI for children 1-3 and 4-8 years, Health Canada considered these estimates insignificant from a health perspective.

In 2014, following a request from the European Commission, EFSA carried out a revised exposure assessment of steviol glycosides *(E 960)* to those previously done in 2010 and 2011 (56). The EFSA panel concluded that overall, the mean exposure estimates remained below the ADI of 4 mg SE . kg^{-1} . d^{-1} across all population groups, except for toddlers in one country (Netherlands). However, the panel did not consider this to be significant enough to change the outcome of the safety assessment. In a re-evaluation, as part of a US GRAS submission (GRN 619) in 2016, estimated intakes of steviol glycosides for the general population were below the ADI (57). The highest intake was in non-diabetic children, with an intake of 3.28 mg SE . kg^{-1} . d^{-1} at the 95^{th} percentile. Dewinter et al. (58) estimated intakes in type 1 diabetic children who

are often at the highest risk of exceeding the ADIs for sweeteners due to their potentially high consumption of sugar substitutes, in their effort to manage a reduced carbohydrate/sugar diet. At the 95th percentile, all age groups had intakes below the ADI, except for 4-6 year olds, who exceeded it at 4.75 mg SE . kg^{-1} . d^{-1}. Due to the conservative nature of the analyses, the authors concluded that there is little chance that type 1 diabetic children will exceed the ADIs. To date, based on estimated dietary exposure assessments from different countries and regions of the world, at typical patterns of consumption of foods and beverages containing steviol glycosides, it is unlikely that either adults or children, including diabetic adults and children will exceed the ADI for steviol glycosides. Although there is no safety concern, it would be valuable to have future research efforts investigate actual dietary intake in adults, children and subsets of the population that are expected to be high consumers of steviol glycosides and to understand trends over time.

Effect of Steviol Glycosides on Health and Related Biomarkers

Background

The new WHO sugars guideline recommends that adults and children reduce their intake of added sugars to less than 10% of total energy intake, and recommend a further reduction to below 5% for additional health benefits (59). This guideline is part of WHOs efforts to halt the rise in diabetes, obesity and premature deaths by 25% by 2025 (59). The UK Scientific Advisory Commission on Nutrition (SACN) also recommends a reduction of free sugar to ≤ 5% (60). For an adult, the 10% and 5% guidelines are equivalent to about 50 g and 25 g of sugar per day, respectively. According to WHO estimates, intake of added sugars among adults ranges from 7-8% of total energy in Hungary and Norway to 16-17% in Spain and the United Kingdom (59).

The range for children is higher, varying from 12% in Denmark, Slovenia and Sweden to nearly 25% in Portugal (59). In the US, added sugar intake has been declining but remains high, with adults and 2-18 year olds consuming 14% and 17% of total energy intake, respectively in 2011-2012 (61). These levels are above the recommended maximum of 10% of total energy in the US (62), as is the case for several other countries.

Postprandial Blood Glucose and Insulin Effects

It is well established that the intake of sucrose or glucose creates a postprandial spike in blood glucose and insulin (63). Hence it is of interest to determine if high-purity steviol glycosides influence postprandial blood glucose and insulin levels. A few human studies have examined this effect in single-meal evaluations comparing a reduced-sugar/calorie meal with steviol glycosides versus a full-sugar/calorie meal, while other studies have examined the effect of steviol glycosides in capsules, as supplements, with no dietary manipulation (**Table 2**). Three randomized controlled trials observed a significant reduction in postprandial blood glucose with purified steviol glycosides utilized in reduced-sugar/calorie meals (64, 65) or supplement form (66) in healthy subjects and diabetics. Anton et al. (64) observed a significant reduction in postprandial blood glucose ($p < 0.01$) and insulin ($p < 0.05$) levels when stevia was consumed in a mid-morning meal compared to sucrose in lean and obese subjects. Similarly, Jeppesen et al. (65) noted a significant decrease in postprandial blood glucose ($p < 0.05$), including a 156% lower area under the curve (AUC) for blood glucose ($p < 0.01$) in subjects with type 2 diabetes. Gregersen et al. (66) investigated the postprandial effect of 1000 mg of steviol glycosides (91% stevioside) compared to a 1000 mg maize starch placebo given in capsule form along with an isocaloric meal in 12 type 2 diabetics who had stopped taking hypoglycemic medication prior to

the test. Despite no sugar, carbohydrate or calorie difference between the test groups, stevioside significantly reduced postprandial blood glucose by 18% ($p < 0.004$) in addition to the AUC for glucose ($p < 0.02$) versus placebo. There was a trend towards an increased insulin response (AUC) and a 40% increase in the insulinogenic index (ratio AUC insulin to AUC glucose) ($p < 0.001$) when stevioside was consumed versus placebo.

Three other studies (20, 67, 68) observed no significant impact on postprandial blood glucose in healthy or diabetic subjects when steviol glycosides were consumed as supplements. However, Jeppesen et al. (67) observed a 45% reduced insulin response in the placebo group ($p <0.05$), and an insulin level that was maintained in the stevioside group, suggesting that steviol glycosides may have a positive effect on beta cell function in type 2 diabetic subjects. In the IVGTT, the insulin response increased after injection of glucose by 21% in the stevioside group compared to placebo ($p<0.05$). The patients included in this study may already have been in a late stage of diabetes and therefore, may have had limited beta cell function, which may explain the different results compared to other human and animal studies.

Overall, when the comparison between steviol glycosides and the control involves a sugar/carbohydrate or calorie differential, postprandial blood glucose reductions have been observed, and this effect is largely due to a sugar and calorie substitution, as observed in the studies by Jeppesen et al. (65), and Anton et al. (64). On the other hand, the postprandial blood glucose decrease observed in the Gregersen et al. (66) study, which had no calorie differential between treatment and control, suggests that at certain doses, stevioside may have a potential blood glucose lowering effect in diabetics. These results may not be evident in diabetic subjects who continue taking their hypoglycemic medication as in the study by Maki et al. (68). Similarly, Maki et al. (68) did not see any change in postprandial insulin levels, whereas in

studies where diabetics stopped their hypoglycemic medication, there was evidence of a potential increase in insulin levels (66, 67). Additional research is needed to more clearly determine if steviol glycosides have an independent effect on insulin and postprandial blood glucose levels in individuals with diabetes, if it is specific to any one steviol glycoside, as well as the mechanism and doses at which these effects may be observed.

Fasting Blood Glucose and Insulin Effects

Long-term studies indicate high-purity steviol glycosides in supplement form within interventions that have no dietary carbohydrate or calorie manipulation do not significantly reduce fasting blood glucose, insulin, or glycated hemoglobin (HbA_{1c}) levels (**Supplemental Table 1)**. Studies were conducted in healthy subjects, type 1 and type 2 diabetic subjects, hyperlipidemic and hypertensive subjects with a wide range of doses (20, 45, 46, 67, 69–71). These studies had differing protocols involving diabetic subjects, with some continuing their hypoglycemic medications and others stopping just prior to the beginning of the study. Although none of the fasting blood glucose measures were significantly changed by the steviol glycoside treatment, it is noteworthy that in one study 750 mg/d of stevioside maintained fasting blood glucose levels over a 3-month period, whereas in the placebo group there was a significant increase compared to baseline among type 1 diabetic subjects who continued their hypoglycemic medication (46). A similar result was observed in a study by Jeppesen et al. (67), where 1500 mg/d stevioside was consumed for 3 months by type 2 diabetic subjects who had stopped their hypoglycemic medications. A significant difference between treatment and placebo groups for fasting glucose ($p < 0.007$) and HbA_1 ($p < 0.01$) was observed. These findings suggest that stevioside at levels above the ADI may help maintain a static diabetic state, which could be

beneficial to individuals with diabetes in minimizing or slowing down the progression of diabetes. Further, a meta-analysis of several of these studies by Onakpoya and Heneghan, (72) revealed a small but significant reduction in fasting blood glucose (-0.63 mmol/L, $p < 0.00001$). However, the clinical relevance of a reduction of 0.63 mmol/L observed in the meta-analysis may be limited.

Jeppesen et al. (73) also examined the effect of supplementing 500 mg of steviol glycosides, together with post-exercise oral carbohydrate versus isocaloric carbohydrate supplementation on muscle glycogen re-synthesis in 15 male cyclists. The glycogen re-synthesis rate was increased by 35% ($p < 0.02$) and glycogen levels were significantly higher ($p < 0.009$) with steviol glycosides vs placebo. More research is needed to understand how steviol glycosides may confer these effects.

Potential Mechanisms Related to Blood Glucose

It is clear that one indirect way in which steviol glycosides and other LNCS lower postprandial blood glucose levels is through the displacement of sucrose or other carbohydrates (74). However, for steviol glycosides, a few *in vitro* and animal studies suggest a potential independent and more direct mechanism involving insulin secretion, signaling and release, up-regulation of key genes, and enhanced glucose absorption in primarily diabetic models. Jeppesen et al. (75) was the first to demonstrate that both stevioside and steviol (1 nmol/L to 1 mmol/L) dose-dependently enhance insulin secretion from incubated mouse islets in the presence of glucose ($p < 0.05$). The insulinotropic effects of stevioside and steviol were critically dependent on the glucose concentration and occurred at or above 8.3 mmol/L glucose ($p < 0.05$). To

367 determine if stevioside and steviol act directly on pancreatic beta-cells, the beta-cell line INS-1
368 was used. Both stevioside and steviol potentiated insulin secretion from INS-1 cells ($p < 0.05$).
369 Animal studies of steviol glycosides suggest an effect on insulin secretion and sensitivity
370 and gluconeogenesis. Jeppesen et al. (76) performed an IV glucose tolerance test with and
371 without 0.2 g . kg^{-1} . d^{-1} stevioside in type 2 diabetic Goto-Kakizaki (GK) and normal Wistar
372 rats. In diabetic rats, stevioside significantly suppressed the blood glucose response (iAUC, $p <$
373 0.05) while concurrently increasing the insulin response (iAUC, $p < 0.05$). Chen et al. (77)
374 reported that 0.5 mg . kg^{-1} . d^{-1} stevioside provided by gastro gavage lowered blood glucose
375 levels in normal rats, as well as in two models of diabetic rats in a dose-dependent manner, not
376 only by enhancing insulin secretion but also by slowing down gluconeogenesis in the liver by
377 decreasing levels of phosphoenol pyruvate carboxykinase (PEPCK), an enzyme involved in the
378 metabolic pathway of gluconeogenesis. Nordentoft et al. (78) in a 9-week intervention study in
379 diabetic KKAy mice treated with 20 mg . kg^{-1} . d^{-1} observed that the stevioside derivate,
380 isosteviol, had a high bioavailability from the colon, improved glucose and insulin sensitivity by
381 upregulating the gene expression of key insulin regulating genes and insulin transcription factors.
382 Chang et al. (79) observed that a single oral administration of 0.5 mg . kg^{-1} . d^{-1} stevioside for 90
383 minutes decreased plasma glucose concentrations and reversed the glucose-insulin index, a
384 measure of insulin action on glucose disposal in rats fed fructose-rich chow for 4 weeks.
385 Repeated administration of stevioside delayed the development of insulin resistance in these rats
386 and increased the response to exogenous insulin in STZ-diabetic rats. Philippaert et al. (80)
387 demonstrated that 0.5 mg. kg^{-1} . d^{-1} stevioside given orally two hours before a glucose tolerance
388 test significantly lowered blood glucose levels in normal wild type mice but not in *TRPM5* mice.
389 *TRPM5* is a Ca^{2+}-dependent cation channel found in type II taste receptor cells on the tongue and

in insulin producing β-cells in the pancreas. *TRPM5* knockout mice have decreased glucose tolerance due to impaired glucose-induced insulin release.

A study of Reb A on metabolic syndrome outcomes, suggests similar outcomes to stevioside. Jeppesen et al. (81) fed rats a high fructose diet for 16 weeks followed by the intake of 8.4 mg/d Reb A, 16.8 mg/d aspartame or high fructose corn syrup (HFCS) at 13% of total caloric intake for 8 weeks. Incremental AUC glucose was significantly lower for the Reb A group compared to the HFCS group ($p < 0.05$) following a glucose tolerance test. Insulin resistance measured by HOMA-IR ($p < 0.005$) as well as hepatic triglyceride content ($p < 0.05$) were significantly reduced in the Reb A and aspartame groups. In addition, expression of fatty acid metabolism genes Srebf1 in liver and Fas in liver and muscle were significantly lower in the Reb A group compared to the HFCS group ($p < 0.001$).

Overall the research supports a beneficial effect and no adverse effects of steviol glycosides for blood glucose management when steviol glycosides are used to reduce or substitute sugar and calories in a food, meal or diet. The longer-term safety studies that range from 3 months to a year, in normal individuals and those with diabetes indicate that steviol glycosides are safe and have a neutral effect on fasting blood glucose, insulin and HbA_{1c} at doses of up to 1500 mg/d. One meta-analysis suggests a modest reduction in fasting blood glucose. The doses studied in several long-term studies were well above the ADI. Some preclinical and clinical studies suggest a potential independent effect of steviol glycosides in lowering postprandial blood glucose levels, enhancing insulin secretion and improving insulin sensitivity in diabetic subjects with some mechanistic evidence for these effects. Additional clinical studies are needed to clarify and confirm these findings.

Energy Intake and Weight Control

Full replacement of caloric sweeteners with LNCS in foods and beverages can provide a desirable sweet taste with little or no sugar and calories. In light of several recent policy recommendations to reduce sugar in the diet (59, 62, 82), LCNS including steviol glycosides offer a simple and effective way to reduce both sugar and calories in the diet and thereby also offer a helpful way to manage both energy intake and body weight.

Steviol glycosides. To date two studies (64, 83) have evaluated the effect of steviol glycosides on satiety and energy intake (**Table 3**). Anton et al. (64) observed no increase in subjective satiety but found energy intake was significantly decreased over the day when two reduced energy/sucrose preload meals with steviol glycosides were consumed 20 minutes prior to an *ad libitum* lunch and dinner. Thirty-one subjects consumed 309 kcal less during the steviol glycoside versus sucrose treatment ($p < 0.001$). There were no differences in energy intake at lunch or dinner, therefore the daily energy difference was primarily due to the energy difference in the two preloads. Energy compensation was 24% during the steviol glycoside period. A second study evaluated the effects of steviol glycosides consumed in water versus a sucrose control one hour before an *ad libitum* lunch in 30 males and observed no difference in satiety ratings but noted a total daily energy intake reduction of 70 kcal (83). The energy compensation during the steviol glycoside period was 73%. The higher energy compensation in this study compared to the first could possibly be attributed to several factors including the number and use of different preloads, the time interval between the preload and the *ad libitum* meal, and the fact that the Tey et al. study (83) was not statistically powered to assess energy intake differences, but was powered to detect a 30% difference of the blood glucose treatment. Across the two studies

436 the average energy compensation was about 50%, similar to the average energy compensation
437 observed for other LNCS (84).
438
439 *Low and no-calorie sweeteners.* Due to the absence of clinical trials on the effect of steviol
440 glycosides on body weight, the symposium included a brief review of the impact of LNCS on
441 energy intake and body weight, as it would be anticipated that the effect would be similar for
442 steviol glycosides if a study were carried out. Research demonstrates that there is no precise
443 physiological balancing of energy intake against energy expenditure. Consumption of energy
444 either in excess or deficit of immediate energy requirements is not fully compensated for by
445 adjustments in intake at the next meal or at subsequent meals (85). Hence, reduced energy intake
446 by LNCS use should be helpful to those attempting to maintain or lose weight. Consistent with
447 this, a recent meta-analyses of 69 acute and long-term randomized controlled studies in human
448 participants between 1970 and 2015 found clear evidence that consumption of LNCS in place of
449 (some) sugar in the diet reduces energy intake and body weight (84). Despite these findings,
450 claims persist that LNCS hinder rather than help appetite and weight control.
451 Based on a rodent model, one claim has suggested that by "decoupling" sweetness from
452 caloric content, LNCS disrupt the animal's learned ability to regulate energy intake (86, 87). In
453 these studies, rats that consumed saccharin-sweetened yogurt increased their intake of food that
454 led to increased weight gain, body fat accumulation and decreased caloric compensation
455 compared to rats that consumed glucose-sweetened yogurt (86, 87). A basic premise underlying
456 these studies is that sweet taste is a valid predictor of increased energy intake. However, this can
457 be challenged, since sweetness does not reliably predict the energy content of foods (88).
458 Furthermore, there is also the question whether rats, or humans, rely only on simple taste-

nutrient relationships to control energy intake. It is more likely that signals triggered by nutrients detected in the gut post-absorptively dominate in influencing satiety (85). Recent research has failed to replicate the earlier "decoupling" findings. In two experiments Boakes et al. (89, 90) observed that rats intermittently fed glucose gained more weight and/or fat mass than rats intermittently fed saccharin. This is opposite to the results reported by Swithers et al. (86). The discrepancy between these two sets of results appears to be explained by the fact that Swithers et al.'s (86) excluded rats that showed low acceptance of the saccharin-sweetened yogurt. Boakes et al. (90) show that this biases the sample towards faster-growing rats, as saccharin acceptance is associated with later weight gain on chow. In other words, the result reported by Swithers et al. (86) and quoted widely to support the LNCS 'confuse your body' claim, is a procedural artefact. Boakes et al.'s (89) results on the other hand are plausibly explained by a lack of full compensation for the higher energy content of the glucose-sweetened yogurt. This was confirmed in a systematic review where 59 out of 68 animal studies of continuous exposure to LNCS showed no significant weight change or decreased body weight (84).

Another claim suggests that repeated exposure to sweetness encourages a "sweet tooth" and therefore the increased intake of sweet, energy-containing foods and drinks (91, 92). This assertion was tested in two recent studies. In a sample of 39 participants, the desire to consume apple juice, apple, and apple pie was significantly reduced ($p < 0.05$) when a LNCS drink was consumed prior to the meal than when water was consumed (93). A second study tested the effect of consuming sweet drinks on sweet and savory food intake. On 3 separate occasions, 50 participants were presented with a savory snack (Doritos®) and a sweet snack (chocolate chip cookies) following consumption of water, LNCS soda or a regular sweetened soda (93). The consumption of the sweet snack was significantly reduced following the intake the LNCS soda (p

482 < 0.05) and the regular soda ($p < 0.01$) compared to water. In contrast, the intake of the savory
483 snack was not significantly impacted by the ingestion of the sweetened beverages. These results
484 are consistent with the phenomenon of "sensory-specific satiety", which is the reduction in liking
485 or reward value of a recently eaten versus recently uneaten food or taste (94, 95). It is also
486 consistent with the findings from a 6-month intervention study where participants who
487 substituted caloric beverages with LNCS beverages significantly reduced their intake of desserts
488 compared to participants who substituted caloric beverages with water (96). In another study,
489 participants who reduced their intake of sweet foods and drinks for 3 months showed an increase
490 in perceived sweet-taste intensity (at low concentrations of sucrose), but no change in perceived
491 pleasantness of sweet test products (97). Finally, randomized-controlled trials have generally
492 found no effect on body weight between a diet moderately high in sugars versus a diet where free
493 sugars were replaced by the isoenergetic exchange of lower sugar carbohydrates (98), again
494 showing that sweetness per se does not encourage increased energy intake.

495 For LNCS to successfully contribute to reduced energy intake, it is necessary that
496 compensatory energy intake not occur. To address this issue a systematic review and meta-
497 analysis examined both short term ($\leq$ 1 day) and sustained (> 1 day) randomized controlled
498 studies (84). The short-term analysis evaluated 218 comparisons from 56 papers that examined
499 the effect of a LNCS preload versus sugar, unsweetened product, water, nothing or placebo
500 capsules on subsequent energy intake. Most of the comparisons (83%) were LNCS versus sugar,
501 where it was observed that LNCS when substituted for sugar consistently reduced short-term
502 energy intake. LNCS intake versus sugar resulted in 70% energy compensation in children and
503 43% compensation in adults, leading to an average compensation across all studies of 50%.
504 Energy intake also did not differ for LNCS comparisons with water, unsweetened product, or

505 nothing. The sustained energy intake analysis included 10 comparisons from 9 studies that
506 ranged from 10 days to one year in overweight, obese, and normal weight participants, and in all
507 instances, the use of LNCS led to a reduction in energy intake. Results of another study
508 completed after this review were consistent with the findings of Rogers et al. (84) where it was
509 noted that LNCS beverage consumption with meals did not increase total energy intake,
510 macronutrient intake or sweet foods selected, either in those who were habitual or non-habitual
511 consumers (99), contrary to the concern that LNCS might increase energy intake by decoupling
512 sweetness with energy content, or by enhancing preference for sweets, or other potential
513 mechanisms reviewed by Mattes and Popkin (100).

514 The relationship between LNCS intake and body weight have been examined by several
515 observational (i.e. prospective cohort) studies and randomized controlled trials. Randomized
516 control studies provide the highest quality of evidence. **Table 4** summarizes the findings of
517 recent systematic reviews and meta-analyses (74, 84, 101–106). Results from 7 systematic
518 reviews of prospective cohort studies were mixed, with the majority showing no clear trend. One
519 meta-analysis observed a very slight decrease in BMI (-0.002 kg/m^2) (84), whereas another
520 observed a slight increase in BMI (0.03 kg/m^2) and no significant association with body weight
521 or fat mass (102). In observational studies, it is not possible to control for all potential
522 confounding factors and therefore the possibility of residual confounding remains, as well as the
523 possibility of reverse causality (106). Of the 6 systematic reviews and 2 meta-analyses of
524 randomized controlled trials, most demonstrate a decrease in body weight and or BMI with
525 LNCS use. Both meta-analyses reported that LNCS use was found to reduce BMI and or body
526 weight (84, 102). Miller and Perez (102) found LNCS use was significantly associated with
527 reduced body weight (-0.80 kg), BMI (-0.24 kg/m^2), waist circumference (-0.83 cm), and fat

mass (-1.10 kg). Similarly, Rogers et al. (84) reported a significant reduction in body weight when LNCS was substituted for sugar (-1.35 kg) or water (-1.24 kg).

Collectively the research to date demonstrate that the consumption of LNCS, including steviol glycosides consistently help reduce energy intake, contrary to the suggestion that LNCS might increase energy intake. In addition, studies show that exposure to sweetness does not train taste preference and encourage a "sweet tooth." There is in fact, no human clinical study that would suggest that a sustained exposure to "sweetness" with LNCS would lead to an increase in energy intake. With regards to steviol glycosides, despite differences in study design, the two available studies (64, 83) demonstrate an energy reduction benefit with an average energy compensation of 50%. Overall, the current evidence is consistent with a recent expert consensus paper (107), which concluded that LNCS help to reduce energy when used in place of higher energy ingredients. Claims that LNCS increase appetite and body weight are clearly contradicted by evidence showing that consumption of LNCS can be expected to contribute to healthy weight management. It is also safe to assume that steviol glycosides would likely result in similar weight reduction benefits observed in randomized controlled studies of other LNCS.

Blood Pressure

Six randomized clinical trials with 8 clinical study arms have investigated the effect of steviol glycosides on blood pressure from 4 weeks to 2 years. Two clinical arms conducted in healthy adults with normal blood pressure observed no significant differences between consumption of steviol glycosides and the placebo control (44, 46). Four clinical arms found no significant impact of steviol glycosides on blood pressure in individuals with type 1 and type 2 diabetes, but in all four instances, the subjects continued taking their blood pressure medications if they were

hypertensive (45, 46, 67). Subjects with mild to moderate hypertension who were not on blood pressure medication were investigated in two studies and both demonstrated a modest blood pressure lowering effect with 750 – 1500 mg of stevioside/day (70, 71). The steviol glycoside interventions were provided in supplement form with no dietary manipulation, with the purpose of examining their safety and independent effect on blood pressure.

A meta-analysis of 7 randomized controlled trials that assessed steviol glycosides in both acute single-meal and long-term settings showed a non-significant difference in systolic blood pressure, but a significant decrease for diastolic blood pressure (-2.24 mm Hg, p=0.03) (72). However, significant heterogeneity was observed, likely due to differences in the composition of the steviol glycosides, doses utilized, continued use of blood pressure and antidiabetic medications by subjects, and the inclusion of subjects with normal blood pressure. Most of these studies were designed to investigate the safety of steviol glycosides within these contexts, with several studies using doses that were 3-4 times the ADI with no negative impact, further supporting the safety of steviol glycosides.

Gut Microbiota

The human gut microbiota is a large and complex population of microorganisms. Over 1000 species have been identified in total, with around 160 being present in the gut of any one individual (108). Over 90% of the species fall into two main phyla, Firmicutes and Bacteroidetes; other common phyla include Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria (109). There is also evidence that the microbiota may also be involved in obesity and type 2 diabetes (110). It has however proven more difficult to identify the microorganisms involved in these conditions.

The relative proportions of the phyla and their component genera and species, as well as gut microbial metabolism, can vary markedly between individuals and can be influenced by a variety of factors including early colonization in the immediate post-natal period, host genetics, exposure to drugs and environmental chemicals (111). Mounting evidence, however, indicates that diet, both habitual, and long-term and shorter-term dietary changes, appear to be the most significant factors influencing the overall composition of the gut microbiota and its functionality.

Because of their extensive use in foods, the interactions of LNCS and gut microbiota have been the subject of numerous studies in laboratory animals and human subjects, although LNCS are unlikely to have a clinically meaningful impact because they are consumed at such low levels. Nevertheless, some studies on saccharin, aspartame and sucralose have shown effects on microbiota composition or metabolism, but only at very high doses above normal human consumption, or in studies with design issues or lacking appropriate controls (112–116). LNCS are a structurally diverse group of compounds that have very different metabolic fates following consumption as reviewed by Magnuson et al. (15). Most (e.g., acesulfame K, saccharin, aspartame and sucralose) are not metabolized by gut bacteria. The only two exceptions are steviol glycosides and cyclamate. The latter is converted by microbiota to cyclohexylamine, which is subsequently absorbed and excreted in urine (117).

Studies on the impact of steviol glycosides on the gut microbiota are few. Gardana et al. (17) incubated human fecal suspensions with stevioside or Reb A for 24 hours. Decreases were seen in numbers of total anaerobes, bacteroides and lactobacilli with stevioside, and in total aerobes, bifidobacteria and enterococci in incubations with Reb A. In all cases the changes in number were small (less than 1 log). Similarly, Kunová et al. (118) noted in another *in vitro* study that the growth of lactobacilli and bifidobacteria strains were poor in the presence of

steviol glycosides compared to a glucose control. Denina et al. (119) also observed the lack of growth of Lactobacillus *reuteri* strains following the incubation of stevioside and Reb A for 24 hours. A study in BALB/c mice given Reb A orally for 4 weeks at 5.5 mg or 139 mg . kg^{-1} . d^{-1} (1.8 mg SE . kg^{-1} . d^{-1} or 46 mg SE . kg^{-1} . d^{-1}) versus water reported no changes in viable counts of the major groups in faeces, or in diversity indices of total bacteria (120). The only difference was an increased diversity of lactobacilli at the higher dose, which was over 10 times the ADI of 4 mg SE . kg^{-1} . d^{-1}. Thus, the current evidence indicates that steviol glycosides have minimal impact on gut microbiota.

Although there is no effect of steviol glycosides on gut microbiota, data do indicate that steviol glycosides are metabolized by gut bacteria. The microbiota provides an important role in the breakdown of dietary ingredients by providing enzymes that are not present in humans (121). Although glycosylases are common among members of the microbiota, Gardana et al. (17) found the ability to deglycosylate steviol glycosides appears to reside only within the *Bacteroides* genus. Cultures of clostridia, bifidobacteria, coliforms, lactobacilli, enterococci tested were unable to metabolize stevioside or Reb A. Human variability in hydrolysis of steviol glycosides is expected to be minimal because *Bacteroides* is by far one of the most abundant bacterial groups found in the large intestine (122).

Dental Caries

The relationship between the consumption of sugar and the incidence of dental caries has been well established. Two short-term clinical studies have been conducted with stevia. Brambilla et al. (123) showed that the plaque pH of sucrose ($p < 0.01$) was significantly lower after a single rinse versus stevioside or Reb A at identical concentrations at 5, 10, 15 and 30 minutes after

rinsing in 20 adults. The reduced growth of *S. mutans* in a biofilm model was also observed with stevioside and Reb A. Zanela et al. (124) reported that the accumulation of plaque in 200 children was not reduced in daily mouth rinses containing 0.5% stevioside with 0.05% sodium fluoride versus 0.12% chlorhexidine with 0.05% sodium fluoride. Counts of *S. mutans* did not differ between the groups, but the results may have been confounded as 20% of the children in all groups had low levels of *S. mutans* at baseline. Furthermore, a comparison of stevioside with sucrose may have been a more appropriate comparison rather than chlorhexidine. A study in rat pups infected with *Streptococcus sobrins* observed that after 5 weeks of treatment, stevioside and Reb A were non-cariogenic, in contrast to sucrose where deep fissure and surface caries and the highest number of *S. sobrin* counts were noted (125). Two additional *in vitro* studies report on the effects of stevia versus typical pharmacological interventions. In one study the inhibitory effect of chlorhexidine was greater against *S. mutans* growth than stevia extract in aqueous and alcoholic solutions (126), and another study demonstrated positive but lower antimicrobial properties of stevia extracts versus two positive controls, Vancomycin and Azithromycin (127). Overall, the data suggests that steviol glycosides are not cariogenic and may have beneficial effects in preventing dental caries versus nutritive sweeteners (e.g., sucrose, high fructose corn syrup, etc.). However, additional long-term human studies using stevia in place of cariogenic nutritive sweeteners are warranted.

Naturality and Processing of Steviol Glycosides

High-purity stevia is extracted and purified from stevia leaves in a manner that is similar to that of sucrose from sugar cane. Specific parameters involved in the extraction and purification of steviol glycosides can vary among stevia producers, but in all instances, it starts with the leaves of the

643 *Stevia rebaudiana* Bertoni plant which are harvested, dried and crushed (128, 129). They are
644 then steeped in warm water similar to a tea infusion (130). Steviol glycosides are soluble in
645 water due to their monosaccharide moieties and can be extracted in large-scale commercial
646 processes with a yield of up to 100%. This water extract is dark brown because of other
647 constituents in the leaves such as protein, fiber, dyes, polyphenols, minerals and salts which are
648 also extracted. Purification steps remove the non-sugar constituents, and the remaining steviol
649 glycosides are spray-dried to an off-white intermediate that contains 80-95% steviol glycosides
650 (131). This end-product is further purified by crystallization using water and or ethanol mixtures
651 to a white end-product with a purity of at least 95%. These purification steps are physical
652 processes used to remove unwanted constituents of the leaves that enable steviol glycosides to be
653 concentrated (13). The process of extraction and purification does not affect the chemical
654 identity of the steviol glycosides, allowing them to remain as they were when located intact in
655 the leaves. Some have called into question this conclusion and therefore the naturality or natural
656 authenticity of high-purity stevia leaf extract. To address this question, a recent study determined
657 if steviol glycoside molecules are altered and or if their pattern is changed during the process of
658 extraction and purification from the leaves of the stevia plant to the high-purity end-product
659 (131).

660 Three separate batches of a large-scale commercial extraction and purification process
661 which included the dried leaves (SL), the first water extract (ESL) and the final product, a stevia
662 leaf extract with a purity of more than 95% (SLE95) were examined (131). All 9 steviol
663 glycosides (*rebaudioside A, -B, -C, -D, -F,* r*ubusoside, steviolbioside, dulcoside A, stevioside)* listed in
664 JECFA's 2010 specification (129) were detected and were well separated using high
665 performance liquid chromatography (HPLC) and mass spectrometric detection. The samples

from all 3 processing steps showed comparable chromatograms with the same pattern and retention times per the USP reference standard, with the exception of Reb D, which eluted quite early and could only be detected in the end-product. A mass spectrometric detector was applied, with HPLC conditions that were comparable to those applied in the first round of testing and the identities of all 9 steviol glycosides including Reb D were confirmed unambiguously in the leaves, the first water extract and the high-purity end product (131).

The relative distribution of the sweeteners for every batch was also calculated. It was found that the relative amounts of Reb A, C and F, dulcoside A and stevioside were comparable across samples of SLE95, ESL and SL. A slight tendency of depletion was seen for rubusoside, Reb B and steviolbioside in the SLE95 samples in comparison to the ESL and SL samples in each series. However, the most salient point is that the 9 steviol glycosides detected in the leaves were found in the water infusion (ESL samples) and the high-purity end product powder (SLE95 samples) in a similar pattern. These results confirm that steviol glycosides tested in this study are not chemically modified or degraded during the traditional large-scale commercial extraction and purification processes used to produce high-purity steviol glycoside sweeteners, thus providing support for the natural authenticity of steviol glycosides.

Alternate Technologies for Steviol Glycoside Production

Recent innovations in the production of “steviol glycosides” by glycosylation, bioconversion (also known as biotransformation) and from genetically modified yeast have focused on reducing cost and improving taste by minimizing the lingering bitter aftertaste or off-flavors that have been found with some steviol glycosides.

Glycosylation is based on the premise that taste is improved when one or more sugar moieties (usually glucose units) are added to the steviol glycoside molecules extracted from the stevia plant (132, 133). The process starts with purified stevia leaf extract that is produced using traditional extraction and purification methods. The extract is then treated with the enzyme cyclodextrin glycosyl transferase that enables the transfer of glucose from a sugar source such as corn starch to steviol glycosides, thus modifying their chemical structure. The end product of glycosylation is a structurally modified form of stevia that consists of several new glycosylated steviol glycosides that are not found in the stevia plant, and with less of the unaltered steviol glycosides.

The recent discovery of the genes that encode the biosynthesis of steviol glycosides like Reb A, D and M has led to the development of Reb A, D and Reb M production in genetically modified yeast strains of S*accharomyces cerevisiae* (134, 135) and *Yarrowia lipolytica* (136). These strains of yeast are genetically engineered to express the steviol glycoside metabolic pathway of the stevia plant, allowing them to produce the enzymes, the intermediates and steviol glycosides such as, Reb A, D and M in a fermenter with corn dextrose or glucose as a sugar source. Steviol glycosides produced from genetically modified yeast are not derived from the stevia plant and do not use any part of the stevia plant in the process.

Another recent technology known as biotransformation or bioconversion starts with traditionally extracted steviol glycosides such as stevioside or Reb A, that are then transformed using multiple genetically modified yeast namely, *Pichia pastoris* strains A and B as noted in a recent US GRAS notification (137). These genetically modified yeast are engineered to contain specific enzymes of the biosynthesis pathway of steviol glycosides that selectively transfer glucose units from a glucose source such as corn dextrose to the starting material, typically

711 stevioside, converting it to Reb E and then to Reb M or other desired steviol glycosides. The
712 end-products, while identical to those found in the stevia plant are not from the plant, but are
713 made using this bioconversion process.

714 Traditional extraction and purification of steviol glycosides from the stevia leaves
715 remains a good way to produce high-purity steviol glycosides that are non-GMO and do not
716 affect the natural authenticity of the product. Recent proprietary traditional non-GMO breeding
717 methods have resulted in new stevia varieties such as a variety known as Starleaf™ by
718 PureCircle Ltd. that has been developed to contain the desirable steviol glycosides, Reb M and
719 D, at levels that are twenty times higher than historically known in stevia plant varieties (138).
720 These breeding methods are making available better tasting steviol glycoside sweeteners that are
721 plant-based, enabling greater reductions in the sugar content of foods and beverages.

722

723 **Taste and Sensory Aspects**

724 The intensity of sweetness and flavor profiles differ widely among the different steviol
725 glycosides (**Supplemental Table 2**). In general, the sweetness potency of LNCS including
726 steviol glycosides is dependent on sucrose reference concentrations. For example, the relative
727 sweetness of Reb A and stevioside are 180 - 350 times than that of sucrose in a 2.5% to 10%
728 aqueous solution. Recent advances in stevia research have found that some of the minor steviol
729 glycosides like Reb M and D have a higher sweetness intensity, are more sugar-like in taste and
730 have minimal aftertaste compared to steviol glycosides like Reb A and stevioside (139–142,
731 PureCircle, unpublished data). The relative sweetness of all of the minor steviol glycosides to
732 that of sucrose is not fully known, as the focus has been on combinations of steviol glycosides.
733 However, from research on proprietary combinations it is known that the minor steviol

glycosides contribute to both sweetness and flavor modification which can influence how a combination works in a given food or beverage matrix versus another (PureCircle, proprietary data).

Replacing sugar in food and beverage products is not simple because sugar provides texture, viscosity and mouthfeel and has no lingering aftertaste that not all LNCS can mimic perfectly. For example, in baking, sugar not only provides sweetness, it also contributes to crispness, cell structure, browning, tenderization and shelf stability, all of which influence mouthfeel, sweetness, flavor perception and control of water activity. Therefore, when sugar is reduced in a baked food, bulking agents such as maltodextrin, sugar alcohols or fibers, and hydrocolloids or proteins are used with stevia, to mimic the characteristics of sugar, provide moisture and texture that full-sugar versions provide. In recent studies, for 20 - 50% reduced-sugar muffins with stevia, cocoa fiber and inulin were used to provide the optimal level for texture, sweet taste and flavour (143, 144). Stevia is generally heat stable and may even enhance flavors in baked goods such as salt, spice and brown aromatics (PureCircle, proprietary data).

Commercially sold high-purity stevia leaf extracts may contain either a single steviol glycoside (e.g., Reb A) or various combinations of steviol glycosides. Unlike other sweeteners, stevia's sweetness is naturally derived from over 40 steviol glycosides, which makes stevia more complex to work with, versus single compound sweeteners. In addition, some of the challenges of LNCS including stevia are that they can have "off" tastes such as bitter and metallic, slow-onset and sweet tastes that linger (145). Reb D and Reb M have a relatively clean sweet taste, while stevioside and Reb A although sweet, can also impart bitter, metallic and or licorice-like tastes to varying degrees depending on the level used (5). Aside from the range of sweetening potency, each of the steviol glycosides have different solubilities and exhibit unique sensory and

functional attributes that also allow them to modify and or enhance flavors such as lemon, fruity, floral, brown and spicy notes.

Most consumers do not want to compromise on taste and prefer the taste of sucrose. Therefore, the goal when working with high-potency LNCS is to as closely as possible replicate the taste and functionality of sucrose. Taste perception is influenced by product matrix and in the case of stevia, sweet taste can be significantly improved through the use of unique high-purity steviol glycoside combinations, optimally designed for a given food or beverage matrix. These innovations point to taste advantages that are far superior versus the use of any single steviol glycoside such as Reb A or Reb M alone (146), thus helping to achieve maximum sugar reduction while imparting a more sugar-like taste without adding calories or bitter off notes. **Figure 2** illustrates results from a sensory study with 30 panelists that compared a sucrose control versus two high-purity stevia leaf extract products in acidified water, namely, Reb A (97%) and a proprietary ingredient that contained a combination of steviol glycosides (PSB-1198) sold by PureCircle Ltd. Acidified water is used as it is representative of characteristics of select market beverages that use stevia. Panelists reported a lingering off taste and less upfront sweetness for the Reb A versus the PSB-1198, demonstrating the advantage of this steviol glycoside combination. The results indicate the taste profile of PSB-1198 was closer to the taste profile of sucrose (PureCircle, proprietary data).

Research in the area of taste science can offer additional clues to enhancing stevia's overall palatability. Humans perceive 5 basic tastes: sweet, umami, bitter, salty and sour. Of these, sweet and bitter tastes are of most relevance to stevia (147). Taste perception can change when multiple taste stimuli are presented together in a food or beverage versus one stimuli, known as a binary taste interaction (148). The sweet and bitter tastes found in steviol glycosides

interact and the overall bitterness threshold of steviol glycosides may be affected (149). Sweet and bitter tastes are detected by different taste receptor cells (147, 150). According to Backmanov (147), human taste perception, especially bitter tastes, can vary greatly among individuals, due to genetic variation. A sensory study of 10 trained panelists combined with *in vitro* cell-based receptor assays determined how steviol glycosides are sensed by the tongue (149). Results indicated that two receptors, TAS2R4 and TAS2R14 mediate the bitter taste in steviol glycosides. The researchers also noted that there are 3 key structural features that appear to modulate the sweet and bitter taste in steviol glycosides, namely glycone chain length, pyranose substitution, and the C16 double bond. Steviol glycosides that had more glucose molecules attached to them were sweeter and less bitter.

Research on sweet taste receptor cells may also be utilized to optimize the taste of steviol glycosides. The area of a taste receptor cell that tastants bind to is referred to as a docking site (151). Findings from a docking study on 8 steviol glycosides showed significant variation in the docking positions of all steviol glycosides tested. Docking scores predicted the sweetness potency of steviol glycosides. The researchers noted that the interaction of the C-13 and C-19 glucose molecules with a specific set of active docking sites was responsible for its characteristic taste (152). These results suggest that modifying steviol structures and enabling their binding towards a specific point in the sweet taste receptor cells may be a useful means of enhancing the taste quality and sweetness index of steviol glycosides.

Regulatory Status

The safety and use of steviol glycosides has been reviewed and considered by multiple scientific bodies and regulatory agencies around the world. High-purity stevia leaf extracts have been

803 approved and or adopted for use in foods and beverages in more than 150 countries and or regions
804 including, the US, European Union, Middle East, Australia, New Zealand, Canada, China, Japan,
805 Korea, Malaysia, India, Mexico, Brazil, Chile, Paraguay, Argentina, Egypt, Ghana, South Africa,
806 Kenya, and many other countries in Asia, Europe, Latin America and Africa.

807 In the US, extracts from stevia have been used as dietary supplements since the
808 1990s (18) and the use of high-purity steviol glycosides in foods and beverages have been
809 determined to be "generally recognized as safe" (GRAS) based on the evidence from published
810 toxicology studies and the review of product specific data by qualified experts who evaluate
811 safety of use (153). High-purity Reb A received GRAS status (GRN 252) with a no-objection
812 letter from the US FDA in 2008 (130). To date, according to the US FDA's GRAS Notice
813 Inventory the agency has issued more than 40 "no objection" letters on GRAS notices for steviol
814 glycosides. A high-purity stevia specification, with 9 steviol glycosides (*rebaudioside A, -B, -C, -*
815 *D, -F, rubusoside, steviolbioside, dulcoside A, stevioside)* at a minimum 95% purity was
816 established by the Codex Alimentarius Committee in 2010 (129). In 2011, Codex adopted steviol
817 glycosides as a food additive with the establishment of food use standards across a variety of food
818 and beverage categories. The French Food Safety authority was the first in Europe to assess the
819 safety of Reb A and approve its use in 2009. A favorable scientific opinion by EFSA (14) led to the
820 approval of ten steviol glycosides by the European Commission (EC) in 2011, which included the
821 9 approved by JECFA and Reb E. After an initial approval in 2008, FSANZ made revisions in
822 2010 and 2011 to include higher levels of use and select food categories. Hong Kong and Swiss
823 approvals happened in 2010, and between 2011 and 2012, Health Canada and several countries in
824 Asia, Latin America and the Russian Federation approved the use of steviol glycosides for foods
825 and beverages. Between 2014 and 2016, high-purity steviol glycosides were approved in India,

several Southeast Asian countries and the Gulf Cooperation Council countries of the Middle East.

Investigations with lower purity products such as RebA-80 (80% steviol glycoside purity) and RebA-50 (50% steviol glycoside purity) versus pure Reb A led to the realization that mixtures of steviol glycosides may offer superior taste to that of pure Reb A. This led to the development of several stevia sweetener products composed of different combinations and purity levels. Also, the study of minor steviol glycosides led to an improved understanding of their taste and functionality. As a result, between 2013 and 2016, there have been 3 US GRAS notices that include Reb M and or Reb D (134, 154, 155). GRN 473 and 512 are for Reb M extracted from the leaves of the stevia plant (154, 155). While, GRN 626 is for Reb M and D produced by a genetically engineered strain of yeast, *Saccharomyces cerevisiae* (134). Reb M has also been approved by EFSA, FSANZ, and Health Canada. A recent GRAS notice (GRN 619) with a no-objection letter from the US FDA in 2016 expands the use of stevia to include the safe use of 40 plus steviol glycosides (57). Additionally, JECFA's most recent 2017 safety review and proposal supersedes previous specifications, by proposing the use of all natural-origin steviol glycosides (50 plus) containing a steviol backbone conjugated to any number, or combination of the principal sugar moieties, in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni including, glucose, rhamnose, xylose, fructose, and deoxyglucose (156). This new proposed specification is expected to be adopted by Codex in the year 2018.

Of the two known genetically modified yeast *Yarrowia lipolytica* (136) and *Saccharomyces cerevisiae* (135) engineered to produce steviol glycosides, to date JECFA has approved the use of Reb A produced "from multiple gene donors expressed in *Yarrowia lipolytica"* at a minimum of 95% purity (157). Additional ingredients using alternate technologies have been approved or have GRAS status. Between 2011 and 2016, several US GRAS notices with no objection letters from

the US FDA (e.g., GRN 452, 656, 448, 375, 337, 607) for glucosylated steviol glycosides allowed their commercialization (132, 158–162). China, the US, Japan, Malaysia and Korea also allow the use of glucosylated stevia ingredients. In addition, two steviol glycoside ingredients (GRN 667 and 715) produced via bio-conversion have US GRAS status (137, 163).

Food categories and the authorized levels of use for steviol glycosides by regulatory authorities vary from one region to another. They generally include flavored and carbonated beverages, dairy products including fermented milk products, edible ices, table top sweeteners, fruit and vegetable preparations, jams and jellies, cocoa and chocolate products, confectionary and chewing gum, a variety of sauces, breakfast cereals, some bakery products, processed fish products, foods for special dietary purposes, alcohol, several regional sweet and savory snack-based products, desserts, and food supplements (164, 165).

Stevia's primary advantage is that it is a plant-based sweetener of natural-origin. There is no global definition or agreed upon claim for the term "natural." However, stevia leaf extract or steviol glycosides from the *Stevia rebaudiana* Bertoni plant are clearly defined as a natural sweetener in the food regulations of Korea, Malaysia and Japan, and reported as the "natural constituents" of the stevia plant in JECFA's 69th meeting report (26). The WHO in its recent publication on reducing sugar in manufactured foods also recognized stevia as a natural sweetener in its categorization of non-caloric sweeteners (i.e., natural versus artificial) (166). It is generally acknowledged as a natural-origin sweetener in the US and imagery and "natural" phraseology is used in many parts of the globe to convey to consumers the use of natural-origin plant-based stevia sweeteners. The labeling of steviol glycosides in the ingredient list of a food or beverage product can vary from one country to another. Examples include: stevia leaf extract, steviol glycosides, Reb A, rebiana, stevia, and in Europe, steviol glycosides (E960), etc.

872

873 **Consumer Insights and Market Trends**

874 Across the globe, increased consumer awareness about the potential health benefits of reducing
875 calories and sugar has resulted in a shift in consumer preferences for reduced-calorie/sugar foods
876 and beverages, increasing the potential role of sugar substitutes in helping to address these
877 preferences. In addition, an increasing interest in clean label, organic and natural LNCS that do
878 not compromise taste and function has helped to increase awareness about the benefits of stevia
879 and the increased demand for stevia-based products.

880 The global growth of stevia is estimated to cross USD one billion by 2021 based on
881 current market trends (167). The approval of high-purity stevia leaf extracts around the world has
882 spawned hundreds of food and beverage launches. According to data accessed from Mintel's
883 global products database, the number of products with stevia has grown considerably in the past
884 5 years (168). Since 2011 alone, a total of 14,000 plus products were launched with stevia
885 globally (**Figure 3**) and in 2016, 45% of the stevia-based products were in foods and 55% in
886 beverages.

887 There is limited peer-reviewed research on consumer and healthcare professional
888 perception and attitudes regarding LNCS. To determine aided awareness, belief and sentiment
889 about LNCS including stevia, nationally representative population samples of approximately
890 1000 adults, aged 18-64 from the US, UK, Germany, China, India, Brazil, and Mexico were
891 surveyed between 2011-2017 (PureCircle, proprietary data). Fifty percent of the respondents
892 were male and 50% were female. The surveys contained approximately 30 sweetener-related
893 questions. The results indicated that across markets at initial launch, stevia awareness ranged
894 from 8-35% which has grown as high as 77%, in Mexico (**Figures 4 A-E**). The increase in

consumer awareness of stevia over time appears to correspond with the increases in product launches in a given country. In the same studies participants were asked about their impression of stevia and their belief of stevia as a natural-origin, plant-based ingredient based on a 5-point Likert scale that ranged from very positive to very negative (**Figure 5**). Positive responses (very positive + moderately positive) to the question on the overall impression of stevia ranged from 57-87% across several countries. Belief that stevia is natural ranged from 48-86% across countries (Figure 5). There appeared to be a relationship between overall impression of stevia and the belief that stevia is natural and vice-versa.

An online beverage survey of 3361 US adults 18 years and older reported that less than 40% of participants identified added sugars as a primary concern when choosing beverages, despite dietary guidance to reduce added sugar in the diet (169). This study also reported a considerable level of consumer misunderstanding or confusion about the types of sugars in beverages. Another online study in the UK found that 65% of the participants reported no knowledge of the WHO sugar intake guidelines (170). Subjects (77% female respondents) were asked to identify and classify 13 caloric sugars (added sugars) or LNCS (aspartame and saccharin) on the food label, and only 4% correctly classified 10 or more from the ingredient lists. The authors noted that even well-educated consumers struggled to understand added sugars on food labels.

A study on the perception of LNCS by dietitians from 5 European countries (France, Germany, Hungary, Portugal and the United Kingdom) indicates that dietitians are uncertain, ambivalent or have fears about adverse health effects of LNCS (171). Their knowledge and opinion of LNCS translated to varied approaches; some dietitians were undecided, some had the opinion that LNCS should not be used, others felt LNCS should only be used as a transitional

product, while another group recommended or at least allowed the use of LNCS. Despite the lack of strong scientific evidence, some dietitians believed that sweet taste stimulates appetite. Uncertainty about possible adverse health effects and or the safety of LNCS, and distrust of the industry were reasons why dietitians avoid recommending LNCS. The authors of this study identified a clear need for authoritative positions and recommendations from appropriate and trusted sources as key to alleviating the ambiguity, uncertainty and fear.

According to Euromonitor's July 2017 report on sugar and sweeteners, global consumers purchased 73 g of total sugars/day in 2015, of which 22% was from table sugar, 19% from fruits (intrinsic sugar), and 16% from soft drinks (172). Sweet snacks such as biscuits, snack bars and confectionary jointly provided over 20 g of sugar per capita/day in some of the high sugar consuming markets. Consumer perception is a critical factor, and according to Euromonitor, there appears to be a shift towards natural sweeteners, particularly natural full caloric sweeteners such as honey, coconut sugar, and brown rice sugar. According to Euromonitor, future development is expected to focus on natural sweeteners (172).

Authoritative Positions on the Use of Nonnutritive Sweeteners

Nutrition and health-related organizations such as The Academy of Nutrition and Dietetics (AND), The American Heart Association (AHA) and the American Diabetes Association (ADA) currently have positions and or scientific statements that support the use of LNCS, including stevia (74, 173). The AND position paper graded the stevia data that they included in their evaluation as "fair" and, the overall conclusion for LNCS states that "consumers can safely enjoy a range of nutritive and nonnutritive sweeteners when consumed within an eating plan that is guided by current federal nutrition recommendations, such as the Dietary Guidelines for

Americans and the Dietary Reference Intakes, as well as individual health goals and personal preference" (173). A 2012 joint scientific statement of the AHA and ADA on the use and health perspective of LNCS, which included the review of evidence on stevia available at that time, concluded that when used judiciously, LNCS could facilitate reductions in added sugar intake, thereby resulting in decreased energy intake and weight loss/control, with beneficial effects on related metabolic parameters, as long as the substitution does not lead to consuming additional calories as compensation (74). In addition, the Council on School Health of the American Academy of Pediatrics in their position on *snacks, sweetened beverages, added sugar for schools* also acknowledged the potential use of LNCS for energy reduction in school-aged children (174). Further, a recent expert panel in the UK concluded that natural origin sweeteners such as stevia, in blends with sugars, offer consumers a way to help meet the UK recommendation of no more than 5% of energy from free sugars (175).

Although all major regulatory authorities around the world have approved and support the use of high-purity steviol glycosides in foods and beverages, policy positions and or scientific statements on LNCS use similar to the ones by the AND and the AHA/ADA are lacking in many other parts of the globe. This is a critical gap, as these statements offer actionable direction for practitioners and healthcare professionals who serve as an important and respected source of information and advice the public often needs. More research and education is needed to understand and help both consumers and healthcare professionals make informed choices based on credible scientific evidence.

Summary and Conclusion

Several global and country-level authoritative dietary guidelines recommend a reduction in added sugar intake due to the growing prevalence of overweight, obesity and diabetes around the world. These guidelines include recommendations to keep added sugar intake less than 10% of total calorie intake, and as low as 5% for additional health benefits according to the WHO (59) and SACN (60). Replacement of caloric sweeteners in foods and beverages with high-purity stevia leaf extract sweeteners i.e., steviol glycosides is a useful and cost-effective tool in reducing added sugar intake.

Natural-origin steviol glycosides are the natural sweet constituents of the leaves of the *Stevia rebaudiana Bertoni* plant that remain unaltered during extraction and purification. The safety of consumption of high-purity steviol glycosides at or below the ADI is well established. Although there are opportunities for additional research as outlined in sections of this proceedings, evidence to date demonstrates that steviol glycosides are safe, non-cariogenic, non-hypertensive and have minimal impact on the gut microbiota. Human studies have reported no negative gastrointestinal side effects. When used to displace carbohydrate and sugar in the diet, studies with high-purity steviol glycosides in healthy individuals and those with diabetes support a reduction in postprandial blood glucose as well as reduced sugar and energy intake. There is no evidence that shows an increase in appetite for sugar or sweet products when LNCS or stevia containing foods are consumed. Therefore, stevia leaf extract sweeteners are a beneficial and critical tool in sugar and calorie reduction, diabetes, weight management and healthy lifestyles. Recent innovations have resulted in better tasting natural-origin high-purity stevia leaf extracts that help both product developers and consumers make the switch from full-calorie/sugar products to reduced or zero-calorie/sugar-added products to assist in meeting dietary guidelines consistent with current health and nutrition policy recommendations.

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Figure Titles and Legends:

FIGURE 1 Steviol glycoside metabolism in humans

FIGURE 2 Sweetness temporal profile intensity over time. Arrows indicate where the addition of steviol glycosides provide upfront sweetness and reduce linger with PSB-1198, a combination of steviol glycosides versus Reb A97 alone, making PSB-1198 taste more like sucrose.

FIGURE 3 Number of stevia food and beverage products launched globally: 2011- August 2017. Source: Mintel GNPD, data accessed August 2017 (168).

FIGURE 4A-E Consumer awareness of stevia around the globe. A: United States, B: United Kingdom, C: Germany, D: China, E: Mexico. Consumer research time points (year) vary across countries as they are influenced by the timing of regulatory approvals of high-purity steviol glycosides, market interest, etc.

FIGURE 5 Positive consumer sentiment and percent that believe stevia is natural. General consumer sentiment and belief that stevia is a natural-origin plant based sweetener was assessed by asking participants the following questions, respectively: What is your overall impression of each of the following sweeteners? How much would you agree or disagree that x sweetener is natural? Each was ranked from very positive to very negative (5-point scale). (Stevia was one of the sweeteners evaluated and only data for stevia is shown).