

# ***Stevia rebaudiana* Bertoni and its effects on human health: current concepts and future directions**

Edward Rojas, MD<sup>1,2</sup>

Valmore Bermúdez, MD, MPH, MgS, PhD<sup>3,2</sup>

Yasaman Motlaghzadeh, MD, MPH<sup>1</sup>

Justin Mathew, MD<sup>1</sup>

Enzamaría Fidilio, MD<sup>4</sup>

Judith Faria, MD<sup>5</sup>

Joselyn Rojas, MD<sup>6</sup>

Mayela Cabrera de Bravo, MD, MgS, MPH, PhD<sup>7</sup>

Julio Contreras, MgS, PhD<sup>3</sup>

Linda Pamela Mantilla, MgS<sup>3</sup>

Lissé Angarita, MgS, PhD<sup>8</sup>

Paola Amar Sepúlveda, MgS, PhD<sup>9</sup>

Isaac Kuzmar, MD, MBT, MsCN, MBA, PhD<sup>10</sup>

**1:** Rutgers University. Department of Medicine. Newark, New Jersey.

**2:** Endocrine and Metabolic Diseases Research Center, School of Medicine. University of Zulia. Maracaibo – Venezuela.

**3:** Grupo de Investigación Altos Estudios de Frontera (ALEF). Universidad Simón Bolívar, Colombia.

**4:** Endocrinology and Nutrition Department, Vall d'Hebron University Hospital, Barcelona, Spain.

**5:** St. Michael's Medical Center. Department of Medicine. Newark, New Jersey.

**6:** Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA, 02115.

**7:** Lone Star College, Biology department. Tomball, TX 77381

**8:** Escuela de Nutrición y Dietética. Escuela de Medicina. Universidad Andres Bello, Concepción, Chile.

**9:** Grupo de Investigación en Gestión de la Innovación y el Emprendimiento. Universidad Simón Bolívar, Colombia

**10.** Health Sciences Faculty, Research Department. Universidad Simón Bolívar, Colombia.

**Correspondence author:** Rojas, Edward. Er520@rutgers.njms.edu

**Running title:** *Stevia rebaudiana* Bertoni and its effects on human health

## **ABSTRACT**

*Stevia rebaudiana* Bertoni is a perennial shrub with zero calorie content that has been increasing in popularity for its potential use as an adjuvant in the treatment of obesity. We conducted a review of the literature summarizing the current knowledge and role in human disease. Despite its very minimal systemic absorption, studies have been promising regarding its potential benefits against inflammation, carcinogenesis, atherosclerosis glucose control and hypertension. On the other hand, the growing popularity of artificial sweeteners does not correlate with improved trends in obesity. An increased intake of artificial non-caloric sweeteners may not be associated with decreased intake of traditional sugar-sweetened beverages and foods. The effects of Stevia on weight change have been linked to bacteria in the intestinal microbiome, mainly by affecting *Clostridium* and *Bacteroides sp.* populations. A growing body of evidence indicates that *Stevia rebaudiana* Bertoni is protective against malignant conversion by inhibition of DNA replication in human cancer cell growth in vitro. The level of evidence supporting general benefits to human health is insufficient. However, consumption of Stevia has demonstrated to be generally safe in most reports. Further clinical studies are warranted to determine if regular consumption brings sustained benefits for human health.

**Keywords:** *Stevia rebaudiana* Bertoni, obesity, type 2 diabetes, hypertension.

## 1. Introduction

Obesity is a complex disease that represents one of the greatest challenges of the 21<sup>st</sup> century. The prevalence of obesity has tripled since 1975 due to growing Urbanism, sedentary lifestyle, high caloric diets and changes in modes of transportation. In the United States, the prevalence of obesity among adults was 36.5% between 2011 and 2014. Higher prevalence has been observed among women (38.3% vs. 34.3% in men), non-Hispanic white, non-Hispanic black, and Hispanic adults [1]. On the other hand by the year 2016, 39% of the adult population were overweight [2]. Obesity as well as its comorbid conditions has led to significant morbidity and mortality and higher health care costs, especially in the westernized world. Despite having an important genetic component, obesity itself is still a preventable and treatable condition and by reducing its incidence and prevalence we can potentially reduce the burden of other comorbid conditions associated with obesity, such as type 2 diabetes, hypertension, dyslipidemia and coronary artery disease.

A large body of evidence supports that resistance training and aerobic exercise are effective in the prevention and treatment of obesity. Nonetheless, and according to the American Association of Clinical Endocrinologist, reducing total caloric intake *should be the main component* of any weight-loss intervention [3]. *Stevia Rebaudiana* Bertoni (*S. rebaudiana*) is a perennial shrub with zero calorie content that is 100-300 times sweeter than sucrose [4-8]. Despite its relatively recent popularity as an adjuvant of weight loss therapies, it has been used for centuries by the Guarani Indians in Paraguay and Brazil as a natural sweetener for tea and other medicinal purposes. In 1901, the plant and its potential uses as an artificial sweetener were first described by Moises Santiago Bertoni, a Swiss-Italian botanist who migrated to Paraguay [9,10].

There are at least 230 different *Stevia species* that grow spontaneously in subtropical and humid regions of several Latin-American countries (Figure 1) but only *S. rebaudiana* has a sweet essence. *S. rebaudiana* is endemic in Paraguay, growing in the vicinities of the Monday River at a latitude of 25° S between 500 and 1,500 meters above sea level, with an annual average temperature of 75° F and an average rainfall of 55 inches per year. *S. rebaudiana* has been successfully cultivated in several regions of Asia, North-America and Europe, mainly for commercial purposes [11-13].



**Figure 1.** Latin-American countries with spontaneous growth of *Stevia species* and specifically *S. rebaudiana* Bertoni.

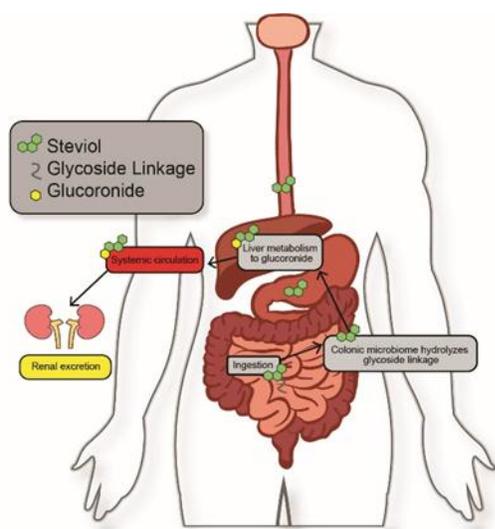
**Legend:** *Stevia species* grow in subtropical and humid regions of Latin-American countries and specifically *S. rebaudiana* is endemic in Paraguay

## 2. *Stevia rebaudiana* Bertoni: extracts and biological activity

At least twelve compounds can be isolated from *S. rebaudiana* Bertoni leaves: stevioside, rebaudioside A to F, steviolbioside, dihydroisosteviol, rubusoside and dulcoside A. [14-20]. In its pure form, stevioside {(4 $\alpha$ )-13-[2-o- $\beta$ -D-glucopyranosyloxy] kaur-16-en-18-oic acid  $\beta$ -D-glucanopyranosyl ester} is 210 times sweeter than sucrose [11]. Steviobioside and rebaudioside A have been isolated by high performance thin layer chromatography methods [21]. The structure and isolation methods to extract steviol and isosteviol were described by Bridel and Lavielle in 20 years of continued works [22]. The plant also contains nutrients such as protein, fiber, monosaccharides, lipids, essential oils, vitamin C, beta-carotene, vitamin B12, antioxidants like apigenin, quercetin, isoquercitrin, luteolin, miocene, kaempferol, chlorogenic acid, and caffeic acid [23].

Initial reports describe that after ingestion, stevioside passes through the digestive system unaltered [12] and it is not cleaved by gastric enzymes or acidity within the stomach nor other digestive enzymes from the gastrointestinal tract [25-27]. However, later reports have shown that these compounds are metabolized by hydrolysis in the gastrointestinal lumen. Bacteria in the colonic microbiome synthesize hydrolases able to cleave steviol extracts, leading to minimal amounts being absorbed into the blood stream (Figure 2).

Geuns *et al.* [28] corroborated these results by demonstrating that low concentrations of stevioside (0.1 lg/ml) were found in plasma. They describe that most stevioside is degraded by colonic bacteria into steviol which is excreted in the feces. Their results are in agreement with those of Simonetti *et al.* who also failed to detect free steviol in blood and urine [27]. Further analysis of Geuns *et al.* studies showed no statistically significant differences in insulin levels, diastolic, and systolic blood pressures in subjects taking 750 mg of stevioside a day compared to a control group. The stevioside group had a 36% higher 24 h urine output than controls, however, this difference was not statistically significant due to interindividual variations nonetheless the sample was composed by 10 patients therefore underpowered [28]. On the other hand, large body of evidence demonstrates that, *S. rebaudiana* Bertonii compounds have not shown any toxic effects to humans, [29] guinea pigs, rabbits or chickens [24].



**Figure 2.** Pharmacokinetics of *S. Bertonii*.

**Legend:** After ingestion and association to glucuronide by glycoside linkages, colonic microbiome hydrolyzes these glycoside linkages permitting minimal amounts of steviol to be absorbed and reach systemic circulation. Posteriorly, steviol is excreted in the urine. Bernadene A. et al [30].

### 3. *S. rebaudiana* Bertonii and Diabetes Mellitus

The American Diabetes Association (ADA) states that the use of non-nutritive sweeteners have the potential of reducing total calorie intake, when substituted for caloric sweeteners and with no compensation of calories from other sources [1]. Several studies have shown some beneficial effects of *S. rebaudiana* and its extracts in the treatment of type 2 diabetes mellitus, essentially by avoiding hyperglycemia [32] and serving as a substitute for sugars with caloric content. Suanarunsawat *et al.* [33] used a model of streptozotocin-induced diabetic rats and found that plasma glucose was not altered in normal rats fed with *S.*

*rebaudiana* but it was significantly reduced in diabetic rats. The researchers propose that an elevation of insulin and suppression of glucagon were responsible for antihyperglycemic effects. These results are similar to those presented by Naveen *et al.* [34] who found that a significant antihyperglycemic effect was seen in streptozotocin-exposed rats. Furthermore, they observed decreased levels of AST, ALT, malondialdehyde and a lesser decrease in the glomerular filtration rate induced by streptozotocin. This demonstrates that *S. rebaudiana* not only has antidiabetic effects in rats, but also has a theoretical antioxidant and renal and liver protective effect.

Assaei *et al.* [35] went further in demonstrating mechanisms behind *S. rebaudiana*'s anti-diabetic effects. They observed that aquatic extract of stevia significantly reduced blood glucose, triglycerides, and malondialdehyde in diabetic Sprague-Dawley rats. They dissected and extracted pancreatic tissue from the rats and found that PPAR $\gamma$  and insulin mRNA levels in treated rats were increased. They therefore hypothesized that the plant's antidiabetic effects were induced by PPAR $\gamma$ . Additionally, in 2017 Philippaert *et al.* [36] demonstrated that stevioside, rebaudioside A and their aglycon steviol enhance glucose-induced insulin secretion by activation of Ca<sup>2+</sup> - activated cation channels (TRPM5) expressed in taste receptors and pancreatic  $\beta$  cells. Their results identify TRPM5 as a potential therapeutic target for type 2 diabetes mellitus.

The effects of *S. rebaudiana* Bertonii extracts on postprandial glucose levels have been studied in type 2 diabetic subjects [37]. One gram of stevioside supplementation added to a standard test meal resulted in an 18% reduction in the glucose response curve when compared to a control group (test meal and 1g of maize starch). Also, the insulinogenic index (AUC, insulin/AUC, glucose) was increased by approximately 40% in the stevioside group ( $p < .001$ ). The use of *S. rebaudiana* Bertonii extracts in the preparation of food and its acceptance of volunteers were explored by Ruiz-Ruiz *et al.* [38]. They added the variant *Morita* to a preparation of bread and tested its feasibility for

human consumption, demonstrating that bread made with *S. rebaudiana* extracts led to inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase in the gastrointestinal tract, therefore decreasing glycemic index. These studies suggest a promising role of *S. rebaudiana* as an adjuvant in the treatment of type 2 diabetes.

#### 4. *S. rebaudiana* Bertoni: obesity, human microbiome and dental caries

##### 4.1. Obesity

The increasing popularity of artificial sweeteners - and especially those with zero or very low caloric content - is mainly due to its potential use for the treatment of obesity. Low caloric intake is the main strategy to promote weight loss and substituting sugar with artificial sweeteners seemed promising. However, this has not been witnessed in clinical practice. From 1999 to 2007, consumption of artificial sweeteners is believed to have increased from 6.1 to 12.5% in children and from 18.7 to 24.1% among adults, though other reports show an increase of as much as 48-56% [39-42]. Nonetheless, there has been neither a corresponding decrease in the consumption of beverages and foods with added sugar [43], nor a decrease in the incidence of obesity [44].

Surprisingly, recent evidence has shown that the use artificial sweeteners do not decrease the risk for hypertension, stroke coronary artery disease and insulin resistance when compared to sugar-containing beverages and foods, but may actually have an equivalent or increased risk [45-48]. A meta-analysis of 11 studies showed that patients consuming sugar-containing soda had a relative risk of obesity by 1.18 (95% CI, 1.10-1.27) and those who consumed artificially sweetened soda had a relative risk of 1.59 (95% CI, 1.22-2.08) [49]. Even after Agüero *et al.* demonstrated an association between stevia consumption and normal weight among Chilean students [50] a larger body of evidence from randomized controlled trials (RCT) and meta-analysis shows opposite results. Azad *et al.* [51] published a meta-analysis showing that published RCTs does not support the intended benefits of nonnutritive sweeteners for weight management and the examined cohort trials actually suggest that it routinely intake may be associated with increased BMI and cardiometabolic risk.

A cohort study of 1454 patients that self-reported regular consumption of artificial sweeteners in the past 10 years showed that participants had a significantly increased BMI compared to nonusers [52]. We can speculate that these patients were obese at baseline and their regular intake of artificial sweetener reflects attempts to lose weight, however, in a number of studies the negative associations between artificial sweeteners and obesity, diabetes and cardiovascular disease cannot be attributed to baseline characteristics [53-56]. Switchers [57] proposes that consumption of artificial sweeteners interferes with learning responses that contribute to energy glucose homeostasis, thus causing a counterintuitive effect of inducing metabolic derangements.

We may also theorize that those who consume artificial sweeteners with zero calorie content would have compensatory eating behaviors hours after. This was studied by Anton *et al.* [58] in 19 healthy lean and 12 obese volunteers. They observe that participants consuming stevia and aspartame preloads did not compensate eating more in the next meals and reported similar levels of satiety levels after eating higher calorie sucrose preloads. The mechanism for these poorer outcomes in people consuming artificial sweeteners remains unclear and specific sub-analyses on *S. rebaudiana* Bertoni's impact on obesity on a large scale are lacking. Further studies are needed to clarify the role of stevia in long-term weight reduction and maintenance [59].

##### 4.2. Microbiome

The human microbiome is a diverse population of bacteria cohabitating in the immense mucosal surface lining the lumen of the gastrointestinal tract (approximately 300-400m<sup>2</sup>) [60]. The number, diversity and virulence of these microorganisms are a result of complex interactions between the host's immune system, intake and the different bacterial species in this microenvironment. Studies in mice and in type 2 diabetes patients have demonstrated that consumption of artificial sweeteners is associated with an increased population of *Bacteroides sp.* and a decreased population of *Clostridia sp.* in the colon [61,62]. Vijay-Kumar [63] and Backhed *et al.* [64] studies demonstrated that changes in intestinal microbiome can trigger inflammatory processes that can promote insulin resistance and fat storage in the host. These results are contrary to those of Gardana *et al.* [65] who reported inconsistent effects of stevioside and rebaudioside A on the populations of *Bacteroidaceae* and *Clostridia sp.* after fecal cultures from healthy volunteers were examined. Later studies by Suez *et al.* [66] showed that four out of seven healthy volunteers exhibited worse glucose tolerance after starting artificial sweeteners, and they attributed this effect to changes in their microbiome. Conversely, and

Renwik and Tarka [67] did not find evidence of changes in the human microbiota after hydrolysis of stevia extracts by gut bacteria. These results do not reflect a clear relationship between the ingestion of *S. rebaudiana* and changes in microbiome; although stevia might change the colonic microenvironment, this will be dependent on the amount and frequency of intake, as well as other dietary elements ingested with the extract that could serve as confounders.

### 4.3. Dental caries

Dental caries arises from acid fermented sugars causing enamel erosion by the action of anaerobic bacteria, *Streptococcus mutans* and *Lactobacillus casei* [68]. Giacaman *et al.* [69] evaluated the effect of different sweeteners in a culture medium for *S. Mutans*. After 5 days, the biomass, bacterial count and intra and extracellular polysaccharides of the biofilm were assessed. They found less enamel demineralization and cariogenic effects for all tested sweeteners except for sucrose. These results correspond with those of Gamboa and Chaves [70] who observed that extracts of *S. rebaudiana* have an antibacterial effect on 16 bacterial strains of *Streptococcus* and *Lactobacillus*. Oral rinses four times a day with a *S. Rebaudiana* extract preparation also reduced dental plaque 57-84% less than with sucrose rinse washes when measured with the Silness-Löe index [71]. Aqueous extracts of *S. rebaudiana* also have non-acidogenic effects [72] that in conjunction with its anti-cariogenic effects makes them a beneficial substitute for frequently consumed beverages [73].

## 5. *S. Rebaudiana* Bertoni: Inflammation, atherosclerosis and blood pressure

Many have touted the anti-inflammatory effects of stevia, especially those looking to promote diets with more naturally occurring substances. Multiple studies have looked to elucidate the exact mechanism of such anti-inflammatory actions. In one study of mice with mastitis, animals that were treated with stevioside showed decreased concentrations of inflammatory markers TNF- $\alpha$ , IL-1 beta, and IL-6 via downregulation of the TLR2, NF- $\kappa$ B, and MAPK pathways [74]. Similarly, a study looking at rats who were injected with a cardiotoxin found that those who received stevioside had decreased activation of NF- $\kappa$ B, though clinical benefits in muscle healing were not seen [75]. The effect seen on NF- $\kappa$ B in rodents supports in vitro studies, which have found that lipopolysaccharide-induced cells that had been treated with stevioside showed decreased levels of inflammatory cytokines, likely via inhibition of NF- $\kappa$ B and its upstream stimulating proteins [76-78]. IL-8 may also be inhibited by stevioside [79]. These findings are in contrast to a study done on horses, where animals given stevia were found to have increased levels of TNF-alpha, IL-6, TLR4, and IFN-gamma compared to horses given corn syrup [80]. Sehar *et al.* demonstrated that stevioside increased phagocytic activity, T and B cell activity, therefore we can argue that stevioside has immunomodulatory effects instead of anti- or pro-inflammatory [81].

Clinically, anti-inflammatory benefits may manifest in the form of decreased cardiovascular disease risk, given studies in mice showing decreased atherosclerosis with more stable plaques in mice receiving stevioside. This benefit was believed to come via increased circulating adiponectin [82]. If proven true in humans, this could make an important point in future research given the significantly increased risk of cardiovascular disease in diabetic patients [83, 84]. Unfortunately, studies looking at the anti-inflammatory effects of stevioside specifically related to atherosclerosis and coronary artery disease are inconclusive.

### 5.1 *S. Rebaudiana* Bertoni and blood pressure

Several studies in animal models and humans have investigated the association between consumption of Stevia and possible decrease in blood pressure level [85,86]. From 1991 to 1999, Melis conducted a series of studies that revealed *S. rebaudiana* extracts caused hypotension by inducing systemic vasodilation, and natriuresis in normal and hypertensive rats [87-91]. Vasodilatory effects of stevioside are at least in part secondary to inhibition of Ca<sup>2+</sup> influx into the myocytes of the muscular layer within the arterial media [85]. Liu *et al.* studied effects of stevioside on hypertensive dogs and found that significant hypotensive effects were dose-dependent [92]. Similar results have been reported in other studies [93-95].

In 2000, Chan *et al.* [96] conducted a randomized, double blind, placebo-controlled study on 106 Asian hypertensive patients in which 60 of them were assigned to receive capsules containing stevioside and the rest received placebo. After 3 months, both the systolic and diastolic blood pressure of the patients who received 750 mg of daily stevioside was significantly decreased compared to the placebo group, and this effect persisted for one year. In 2003, similar

results were observed in a 2-year study in a Chinese population with mild hypertension treated with 1500 mg of stevioside daily vs. placebo [97]. On the other hand, Savita *et al.* [98] studied the effect of the stevia on blood pressure in 8 patients with arterial hypertension and 6 patients type 2 diabetes. After 30 days of consuming stevia leaf powder, they found no significant change in the blood pressure level of participants. However, given the small sample size, this study might be underpowered. Similarly, Barriocanal *et al.* [99] found no significant effect of steviol intake on blood pressure level in normotensive and hypotensive patients.

## **6. *S. rebaudiana* Bertoni and cancer**

In the past two decades, several researchers have investigated anti-carcinogenic effects of Stevia and its metabolites [100,101]. In 1995, Nakamura *et al.* [102] found that stevioside decelerated by the tumor progression induced by tumor promoting agent (TPA) in skin carcinogenesis in mice. Later, Akihisa *et al.* [103] stated that stevioside, the Stevia leaf aglycones, steviol, isosteviol and their metabolites block Epstein Barr virus Early Antigen (EBV-EA) induction, which ultimately inhibits tumor promotion. Stevia leaf extracts were also found to decrease tumor formation in the two-stage mouse skin carcinogenesis model following sequential exposure to 7,12-dimethylbenz[a]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate [104,105]. In 2005, Mizushima *et al.* [106] stated that isosteviol plays a key role in inhibition of DNA replication and human cancer cell growth *in vitro*.

Other studies tested the mutagenicity of Stevia and its metabolites in human and animal cells. In 1984, Salim *et al.* [107] investigated the carcinogenic effects of Stevioside in the urinary bladder and revealed that stevioside has no effect on the development of pre-neoplastic or neoplastic lesions in the organ. In 1993, Suttajit *et al.* revealed that there was no chromosomal effect of stevioside and steviol in cultured blood lymphocytes from healthy donors [108]. Matsui *et al.* [109], found that the Stevioside is nonmutagenic in mutagenicity tests using bacteria, mice and cultured mammalian cells. The results of two studies performed on rats and hamsters given stevioside orally for 2 years and 6 months, respectively, revealed no correlation between stevioside intake and carcinogenicity [110, 111].

## **7. Safety of *S. rebaudiana* Bertoni**

Although the Stevia shrub had been used by Guaraní Indians for centuries, it took more than seven decades since Bertoni described its potential as a sweetener until its commercialization. Originally introduced to Japan in 1970 by the consortium of food-product manufacturers Morita Kagaku Kogyo Co., Ltd. today represents approximately 41% of the market share of potently sweet substances consumed in Japan [112]. Japanese had been using Stevioside as their main sweetener and food additive sweetener in the industry long before western cultures, its regulation became evident in 1996 by Japan's Ministry of Health and Welfare [113].

Stevia glucosides have shown non-toxic effects for humans [99]. This evidence is supported in Brusick's review article, which shows evidence that Steviosides and aglycone steviol do not pose a risk for genetic damage after human consumption [114]. Moreover, there is little substantial evidence to support a warning statement about hypersensitivity reactions secondary to highly purified stevia extracts [115].

After reviewing the available studies on Stevioside, worldwide authorities on food regulation authorized the use of Steviol glycosides as food additive in the first decade of 2000. Nowadays Steviol glycosides are permitted food additives in the Codex Alimentarius General Standard for Food Additives (GSFA), and in many countries, including the USA, the European Union, Canada and many Asian, and Central and South American countries [113,116-123].

The existing Acceptable Daily Intake (ADI) of 0-4 mg/kg bodyweight, which is expressed on the basis of steviol equivalents, was therefore applicable to all steviol glycosides in stevia leaf, reviewed from 2000-2009 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [116]. In the U.S Steviol glycosides preparation are GRAS and FDA authorized the use of stevia as a dietary supplement since 1995 and in 2004, WHO experts approved stevia as a food additive [117]. In 2015, the European Food Safety Authority (EFSA) emitted a scientific opinion report on the safety of Steviol glycosides for the purpose uses as a food additive, concluding that based on available data, are not carcinogenic, genotoxic or associated with any reproductive/developmental toxicity [118].

Recently in 2017, the Food Standards Australia New Zealand (FSANZ) approved to expand the definition of Steviol glycosides for use as an intense sweetener to include all Steviol glycosides present in the *Stevia rebaudiana* Bertoni leaf, definition limited previously to mixtures that comprise not less than 95% Stevioside and/or Rebaudioside A [123].

### Conclusions

*Stevia rebaudiana* Bertoni has a long history of use in health and food, and yet its use and benefits remain controversial. While its potential to replace sugar as a sweetening agent with zero caloric or glycemic content is tempting as a potential way to fight the obesity and diabetes epidemics, we have not seen a significant impact in obesity incidence and prevalence since the popularity of these artificial sweeteners arouses. Studies have been promising regarding its potential benefits to modulate inflammation, carcinogenesis, atherosclerosis, and hypertension, but most studies are either underpowered or have not reached human trials, making it impossible to make definitive statements on its benefits with any degree of confidence. Currently, only a small handful of studies worldwide, looking at the effects of stevia in humans has been listed by the US National Library of Medicine, most of which have looked at the gut microbiome. However, given the extreme popularity of artificial sweeteners in the diet food industry and the potential positive health effects of compounds such as stevia extracts, further clinical studies are could be very helpful to elucidate their true applicability in current practice. This could change the way we approach the treatment of obesity and diabetes worldwide.

### Author contributions:

ER, VB and YM wrote the initial draft. ER designed and drew the illustrations. YM contributed with the section on cancer and blood pressure. JM contributed with the section on inflammation. JF contributed with section on microbiome and obesity. MCdB, JR, JC, LA, PAS, IK reviewed initial draft, proposed pertinent changes and edits. JM, JF and YR read and reviewed the entire manuscript. ER and VB read and made final changes to the manuscript.

### Conflict of interest:

The authors declare no conflict of interest.

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