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CARDIOVASCULAR DISEASE (JHY WU, SECTION EDITOR)

Stevia rebaudiana Bertoni and Its Effects in Human Disease: Emphasizing Its Role in Inflammation, Atherosclerosis and Metabolic Syndrome

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Abstract

Purpose of Review *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. The level of evidence supporting general benefits to human health is insufficient. We conducted a review of the literature summarizing the current knowledge and role in human disease.

Recent Findings Despite stevia's 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.

Summary 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

Introduction

Obesity is a complex disease that represents one of the greatest medical challenges of the twenty-first century. The prevalence of obesity has tripled since 1975 due to growing urbanism, sedentary lifestyle, poor quality diet and changes in modes of transportation. In the USA, the prevalence of obesity in adults was 36.5% between 2011 and 2014. A higher prevalence has been observed in women (38.3 vs. 34.3% in men), non-Hispanic white, non-Hispanic black, and Hispanic adults

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compared to non-Hispanic Asian adults [1]. In a worldwide perspective, by the year 2016, 39% of the adult population was overweight [2]. This trend has led to significantly higher morbidity and mortality and higher health care costs. Despite having an important genetic component, obesity itself is still a preventable disease. Successfully treating obesity can also be treated by aiming to reduce the burden of other associated comorbid conditions such as type 2 diabetes, hypertension, dyslipidemia, and coronary artery disease.

A large body of evidence supports that aerobic exercise is effective in the prevention and treatment of obesity. Nonetheless, 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 in subtropical and humid regions of Latin-America (Fig. 1) but only *S. rebaudiana* has a sweet essence. *S. rebaudiana* is endemic in Paraguay, growing in the vicinities of the Monday River at latitude of 25° S between 500 and 1500 m above sea level, with an annual average temperature of 75 F and an average rainfall of 55 in per year. *S. redaubiana* has been successfully cultivated in several regions of Asia, North America, and Europe, mainly for commercial purposes [11–13].

Stevia rebaudiana Bertoni: Extracts and Biological Activity

At least 12 compounds can be isolated from *S. rebaudiana* Bertoni leaves: stevioside, rebaudioside A to F, steviolbioside, dihydroisosteviol, rubusoside, and dulcoside A [14–20]. In its

Fig. 1 Latin-American countries with spontaneous growth of *Stevia species* and specifically *S.rebaudiana* Bertoni in Paraguay. *Stevia species* grow in subtropical and humid regions of Latin-American countries and specifically *S. rebaudiana* is endemic in Paraguay

pure form, stevioside {(4α)-13-[2-o-β-D-glucopyranosyloxy] kaur-16-en-18-oic acid β-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 Lavieille [22].

The plant also contains nutrients such as protein, fiber, monosaccharaides, 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 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 [24–26]. 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 (Fig. 2).

Geuns et al. corroborated these findings by demonstrating that low concentrations of stevioside (0.1 lg/ml) are found in plasma after ingestion [28]. They described that most stevioside is degraded by colonic bacteria into steviol which is excreted in the feces. Their results are in disagreement with those of Simonetti et al. who failed to detect free steviol in blood and urine [26].

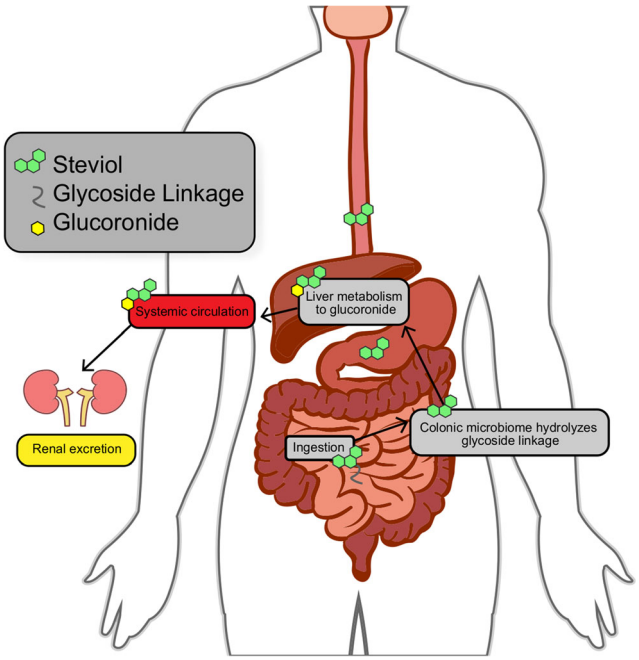

Fig. 2 Metabolism of *S. rebaudiana* Bertoni. After ingestion and association to glucuronide by glycoside linkages, colonic microbiome hydrolyzes these glycoside linkages permitting minimal amounts of steviol to be absorbed and reach systemic circulation. Posteriorly, steviol is excreted in the urine. Bernadene A. et al. [27]

Further analysis of Geuns et al.'s 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. Urine output was 36% higher in the stevioside group compared to the control group. However, this difference was not statistically significant due to interindividual variations and the sample was composed by only ten patients therefore underpowered to look for significant differences [28]. On the other hand, large body of evidence demonstrates that *S. rebaudiana* Bertoni compounds have not shown any toxic effects to humans, [29] guinea pigs, rabbits, or chickens [30].

S. rebaudiana Bertoni and Diabetes Mellitus

The American Diabetes Association (ADA) states that the use of non-nutritive sweeteners has the potential of reducing total calorie intake. This is true only when they substitute caloric sweeteners and there is no compensation of calories from other sources [31]. 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. Recent studies in patients with metabolic syndrome demonstrated that a dietary *Stevia* does not increase HbA1c values [33].

Suanarunsawat et al. [34] used a model of streptozotozin-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. [35] who found that a significant antihyperglycemic effect was seen in streptozotozin-exposed rats. Furthermore, they observed decreased levels of AST, ALT, and malondialdehyde, and a lesser decrease in the glomerular filtration rate induced by streptozotozin. 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. [36] went further in demonstrating mechanisms behind *S. rebaudiana*'s antidiabetic 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 PPARγ and insulin mRNA levels in treated rats were increased. They therefore hypothesized that the plant's antidiabetic effects were induced by PPARγ. Additionally, in 2017, Philippaert et al. [37••] demonstrated that stevioside, rebaudioside A, and their aglycon steviol enhance glucose-induced insulin secretion by activation of Ca2 - activated cation channels (TRPM5) expressed in

taste receptors and pancreatic β cells. Their results identify TRPM5 as a potential therapeutic target for type 2 diabetes mellitus.

The effects of *S. rebaudiana* Bertoni extracts on postprandial glucose levels have been studied in type 2 diabetes subjects [38]. One gram of stevioside supplementation added to a standard test meal resulted in a 18% reduction in the glucose response curve when compared to a control group (test meal and 1 g of maize starch). Also, the insulinogenic index (AUC, insulin/AUC, glucose) was increased by approximately 40% in the stevioside group ($P < 0.001$). The use of *S. rebaudiana* Bertoni extracts in the preparation of food and its acceptance of volunteers were explored by Ruiz-Ruiz et al. [39]. 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 α-glucosidase and α-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.

S. rebaudiana Bertoni: Obesity, Human Microbiome, and Dental Caries

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 theoretical impact has not been seen in clinical practice. From 1999 to 2007, consumption of artificial sweeteners 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% [40–43]. Interestingly, there has been neither a corresponding decrease in the consumption of beverages and foods with added sugar, [44] nor a decrease in the incidence of obesity [45•].

Recent evidence has shown that the use of 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 [46••, 47–49]. 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 [50]. In this study, authors conclude that low-calorie sweetener use is independently associated with higher prevalence and incidence of abdominal obesity, larger waist, and heavier relative weight; however, baseline characteristics show that artificial sweetener users had higher BMI ($p < 0.001$), and higher waist circumference ($p < 0.04$) compared to non-users. This might reflect attempts to lose weight in this group by using artificial sweeteners. On the other hand, in a number of studies, the associations between artificial sweeteners and obesity, diabetes and cardiovascular disease cannot be attributed to increased waist circumference or higher BMIs at baseline [51–54]. Swithers proposes that consumption of artificial sweeteners interferes with learning responses that contribute to energy glucose homeostasis, thus causing a counterintuitive effect of inducting metabolic derangements [55].

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. [56] 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 are lacking.

A meta-analysis of 11 studies showed that patients consuming sugar-containing soda had a relative risk of obesity of 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) [57]. Even after Agüero et al. demonstrated an association between *Stevia* consumption and maintenance of normal weight among Chilean students [58], a larger body of evidence from randomized controlled trials (RCT) and meta-analysis shows opposite results. Azad et al. [59] published a meta-analysis showing that published RCTs does not support the intended benefits of nonnutritive sweeteners for weight management and the examined trials actually suggest that routine consumption may be associated with increased BMI and cardiometabolic risk. Further studies are needed to clarify the role of stevia in long-term weight reduction and maintenance [60].

Microbiome

The human microbiome is a diverse population of bacteria cohabitating in the immense mucosal surface lining the lumen of the gastrointestinal tract (approximately 300–400 m^2) [61]. 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 [62, 63]. These results are contrary to those of Gardana et al. [64] 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.

Vijay-Kumar [65] and Backhed et al.'s [66] studies demonstrated that changes in intestinal microbiome can trigger inflammatory processes that can promote insulin resistance and fat storage in the host. Later studies by Suez et al. [67] 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, Renwik and Tarka [68] did not find evidence of changes in the human microbiota after hydrolysis of *Stevia* extracts by gut bacteria. Summarizing these studies, there is no clear relationship between the ingestion of *S. rebaudiana* and changes in microbiome; although stevia might change the colonic microenvironment, we hypothesize that 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.

Dental Caries

Based on human trials, dental caries arises from acid fermented sugars causing enamel erosion by the action of anaerobic bacteria, *Streptococcus mutans* and *Lactobacillus casei* [69]. Giacaman et al. [70] 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 [71] 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 [72]. Aqueous extracts of *S. rebaudiana* also have non-acidogenic effects [73] that in conjunction with its anti-cariogenic effects make them a beneficial substitute for frequently consumed beverages [74].

S. rebaudiana Bertoni: Inflammation and Atherosclerosis

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-α, IL-1 beta, and IL-6 via downregulation of the TLR2, NF-κB, and MAPK pathways [75]. Similarly, a study looking at rats that were injected with a cardiotoxin found that those who received stevioside had decreased activation of NF-κB, though clinical benefits in muscle healing were not seen [76]. The effect seen on NF-κB 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-κB and its upstream stimulating proteins [77–79]. IL-8 may also be inhibited by stevioside [80]. 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 [81]. 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 [82].

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 stevoside. This benefit was believed to come via increased circulating adiponectin [83]. 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 [84, 85]. Unfortunately, studies looking at the anti-inflammatory effects of stevioside specifically related to atherosclerosis and coronary artery disease are inconclusive.

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 [86, 87]. 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 [88–92]. Vasodilatory effects of stevioside are at least in part secondary to inhibition of Ca^{2+} influx into the myocytes of the muscular layer within the arterial media [86]. Liu et al. studied effects of stevioside on hypertensive dogs and found that significant hypotensive effects were dose-dependent [93]. Similar results have been reported in other studies [94–96].

In 2000, Chan et al. conducted a randomized, double-blind, and 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 [97]. After 3 months, both the systolic and diastolic blood pressure of the patients who received 750 mg of daily stevioside significantly decreased compared to the placebo group, and this effect persisted for 1 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 [98].

On the other hand, Savita et al. studied the effect of the stevia on blood pressure in eight patients with arterial hypertension and six patients with type 2 diabetes [99]. 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. found no significant effect of steviol intake on blood pressure level in normotensive and hypotensive patients [100].

S. rebaudiana Bertoni and Cancer

In the past two decades, several researchers have investigated anti-carcinogenic effects of *Stevia* and its metabolites [101, 102]. In 1995, Nakamura et al. found that stevioside decelerated by the tumor progression induced by tumor-promoting agent (TPA) in skin carcinogenesis in mice [103]. Later, Akihisa et al. 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 [104]. 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 [105, 106]. In 2005, Mizushina et al. stated that isosteviol plays a key role in inhibition of DNA replication and human cancer cell growth in vitro [107].

Other studies tested the mutagenicity of *Stevia* and its metabolites in human and animal cells. In 1984, Salim et al. 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 [108]. In 1993, Suttajit et al. revealed that there was no chromosomal effect of stevioside and steviol in cultured blood lymphocytes from healthy donors [109]. Matsui et al. found that the stevioside is nonmutagenic in mutagenicity tests using bacteria, mice and cultured mammalian cells [110]. 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 [111, 112].

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 [113]. 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 [114].

Stevia glucosides have shown non-toxic effects for humans [100]. 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 [115]. Moreover, there is little substantial evidence to support a warning statement about hypersensitivity reactions secondary to highly purified stevia extracts [116].

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, Central, and South American countries [114, 117–124].

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 to 2009 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [117].

In the US steviol glycosides preparation, 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 [118]. 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 [119].

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, a definition limited previously to mixtures that comprise not less than 95% stevioside and/or rebaudioside A [124].

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 could be very helpful to elucidate their true applicability in current practice.

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Compliance with Ethical Standards

Conflict of Interest Edward Rojas, Valmore Bermúdez, Yasaman Motlaghzadeh, Justin Mathew, Enzamaria Fidilio, Judith Faria, Joselyn Rojas, Mayela Cabrera de Bravo, Julio Contreras, Linda Pamela Mantilla, Lissé Angarita, Paola Amar Sepúlveda, and Isaac Kuzmar declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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