The Effects of Stevia Consumption on Gut Bacteria

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Stevia, a zero-calorie sugar substitute, is recognized as safe by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). In vitro and in vivo studies showed that stevia has antiglycemic action and antioxidant effects in adipose tissue and the vascular wall, reduces blood pressure levels and hepatic steatosis, stabilizes the atherosclerotic plaque, and ameliorates liver and kidney damage.

Stevia rebaudiana | stevioside | gut microbiota | bacteria

1. Introduction

Stevia rebaudiana (Bertoni) is a natural, non-caloric sweetener (~200–400 times higher than sucrose). Its sweet taste occurs from the steviol glycosides, especially stevioside and rebaudioside A (REB-A) together with rebaudioside C and dulcoside A [1][2]. Until now, more than 40 steviol glycosides have been identified, which are classified as ent-kaurene-type diterpenes with sugar fractions attached to the aglycone steviol. Steviol glycosides cannot be broken through enzymes such as pancreatic α-amylase, pepsin, and pancreatin found in saliva and gastric secretions, and pass intact through the upper gastrointestinal tract where finally they are hydrolyzed by intestinal bacteria to steviol [3][4][5]. Bacteroides hydrolyze stevioside and REB-A to steviol, while other bacteria such as *Lactobacilli, Bifidobacteria, Clostridia, Coliforms, and Enterococci* cannot [6]. Absorbed steviol via the portal vein reaches the liver, is metabolized to steviol glucuronide, and is excreted in the urine [3][4]. According to the Commission Regulation (EU) 1131/2011, the acceptable daily intake (ADI) for steviol equivalents is 4 milligrams (mg) per kilogram of body weight [7].

Stevia's superiority against sucrose and artificial sweeteners was confirmed many years ago. Given its safety, studies revealed beneficial properties for human health [8]. In vitro and in vivo, stevia showed anti-viral effects [8][9], immunomodulatory activity, and anti-inflammatory properties by inhibiting the activation of nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK) signaling, and the release of proinflammatory cytokines [10][11] [12][13]. In rats, stevioside showed antiglycemic effects by increasing insulin secretion, decreasing plasma glucose concentrations, and suppressing glucagon levels, although the underlying mechanism has not been clarified yet [14] [15]. Beyond improved insulin signaling and the antioxidant effect in the adipose tissue and the vascular wall, stevia also significantly reduced blood pressure levels (systolic and diastolic), whereas it stabilizes the atherosclerotic plaque and inhibits its further development [15][16]. Other studies on rodents found that stevia-derived compounds reduce hepatic steatosis [17] and ameliorate liver and kidney damage [8][18]. In vitro, steviol glycoside derivatives

were found to possess antiproliferative and anticancer activity, via the mitochondrial apoptotic pathway, in several cancer cell lines, including breast [19][20], prostate [21], gastric [22], and colon cancer cell lines [23].

The gut microbiota is considered an organ that regulates metabolism, cellular immune response, and contributes to the host's health. The human gut microbiota shows a wide variation, but its within-individual variation is relatively stable over time, with Firmicutes and Bacteroidetes representing 90% of the dominant phyla [24].

2. The Effects of Stevia and Steviol Glycosides on Bacterial Growth

2.1. In Vitro Studies

Based on the theory that prophage induction in bacteria may result in the horizontal transfer of genes to other bacterial strains or species, researchers tested three gut bacteria, *B. thetaiotaomicron*, *S. aureus,* and *Enterococcus faecalis*, for their response to stevia as a prophage inductor. They found that stevia increased virus-like particles (VLPs) detected at 410% and 321% from B. thetaiotaomicron and *S. aureus*, respectively [25]. The abundance of terpenes (naturally occurring chemical compounds found mainly in plants) is possibly responsible for the antimicrobial properties of stevia [25], with a potential mechanism of action to be related to the rupture or dysfunction of their cell membrane. Given that previous works showed strain-specific bacteriostatic effects of stevia, it is interesting to note that studies agreed with its effectiveness against *S. aureus* but not against *E. faecalis* [26][27].

Mahalak et al. performed an experiment comparing changes to the gut microbiota in the feces of a healthy donor when exposed to steviol glycosides and erythritol. Results showed that common gut bacteria have a limited growth response to stevia components. The presence of steviol had a statistically significant increase in growth compared with the control only for *Bacteroidetes* thetaiotaomicron. The typical stabilized human gut microbiota remained the same, with the *Bacteroidaceae* family being dominant, followed by *Lachnospiraceae, Fusobacteraceae,* and *Eubacteraceae* [28]. Gerasimidis et al. measured the effect of stevia using human microbiome batch fermentations and observed no significant differences in the growth of *Bacteroides/Prevotella, Bifidobacterium, Blautia coccoides*, *Clostiridium leptum*, and *E. coli* [29]. These results were consistent with the work of Kunová et al., who highlighted the lack of prebiotic effect of REB-A and steviol glycosides. Eight *Bifidobacteria* and seven *Lactobacilli* were cultured and tested for their ability to grow in the presence of REB-A and steviol glycosides. The growth of some *Bifidobacteria* species (*Bifidobacterium bifidum* CCDM 559, *Bifidobacterium* breve CCDM 562, and *Bifidobacterium adolescentis* AVNB3- P1) was higher than others, but no significant changes were detected. Among *Lactobacilli, Lactobacillus* mucosae SP1TA2-P1 grew the most. Overall, neither *Bifidobacteria* nor *Lactobacilli* can substantially use steviol glycosides as a substrate, indicating their very poor fermentation [30].

On the contrary, Denina et al. claimed that stevia glycosides—stevioside and REB-A—inhibit Lactobacillus reuteri growth in a strain-dependent manner [31]. In another prototype trial, researchers evaluated the effects of stevia on the bacterial ability to detect and respond to cell population density by gene regulation (quorum sensing, QS). QS

is an essential communication system (intra- and inter-bacterial) that enables many features of bacterial community behavior to be regulated. Experiments were conducted with a recombinant bioluminescent E. coli K802NR-pSB1075 and the lasRI gene from *Pseudomonas aeruginosa*. Results showed that stevia might lead to microbial imbalance, disrupting the communication between *Gram-negative bacteria* in the gut via either the LasR or RhlR receptor proteins of P. aeruginosa. However, even if stevia inhibits these pathways, it cannot kill off the bacteria [32].

2.2. In Vivo Studies

Researchers hypothesized that stevia could correct high-fat-diet-induced glucose intolerance by altering the gut microbiota, but results in a murine model highlighted no impact on glucose intolerance nor protection from high-fat-diet-induced changes. The significant increase in *Firmicutes/Bacteroidetes* ratio correlated with the high-fat diet and obesity [33]. In contrast to this publication, Yu et al. investigated the effects of different supplementation levels of stevia residues in high-fiber diets on the fecal bacteria of pregnant mammalians. It is known that high-fiber diets can promote the abundance of beneficial bacteria *Bifidobacteria* and *Lactobacilli* and improve intestinal balance. The parallel stevia-residue supplementation significantly increased the beneficial and reduced the harmful bacteria, while the optimal supplementation level of the stevia residue was 30% [34]. Another trial evaluated the dose-dependent effects of REB-A (low (0.5 mg/mL) and high dose (5.0 mg/mL)), and indicated that the different doses did not affect the growth of *Enterobacteria* and *Lactobacilli* nor alter the microbial diversity but might have changed the number of some bacterial genera [35].

Reimer et al. attempted to prove that prebiotic consumption can reverse the potential adverse effects of stevia. REB-A reduces the relative abundance of *Bifidobacteriaceae*—the “health-promoting” bacteria—but increases *B. thetaiotaomicron*, which stimulates Paneth cells and promotes intestinal angiogenesis. A significantly greater abundance of these taxa was induced in rats on prebiotics compared to that in the non-probiotic group. Stevia and prebiotic consumption protected from alterations in gut microbiota composition observed in the group with REB-A consumption only [36]. The increasing evidence that gut microbiota in offspring is shaped in part from maternal diet led the scientific community to investigate the role of stevia during the prenatal period, pregnancy, and lactation. Thus, they observed alterations of fecal microbiota in dams and offspring fed with stevia correlated with a greater risk for metabolic syndrome (increased *Porphyromonadaceae*), and type-2 diabetes (increased *Sporobacter*) [37]. In continuation of studying the possible mechanisms by which maternal consumption of stevia increases the risk of altered gut microbiota in offspring, investigators recently reconstructed the most significant alterations of the cecal microbiome in the offspring of obese dams consuming a high fat/sucrose (HFS) diet with or without stevia. Stevia had limited influence on the overall structure of cecal microbiota in dams but induced significant alterations in offspring. Consequently, maternal consumption contributes to the metabolic changes in the offspring who were never directly exposed to stevia [38].

Given that the gut–brain axis plays a crucial role in the etiology of mental illness and cognitive and memory disorders, de la Garza et al. indicated that maternal gut dysbiosis deteriorates learning procedures and leads to memory loss susceptibility in adult male offspring rats. A maternal high-stevia diet induced the upregulation of

Bacteroidales and *Clostridiales*, leading to memory loss and cognitive problems in offspring lasting up to adulthood, while the changes found in these phyla were independent of their body weight gain [39].

3. The Effects of Stevia and Steviol Glycosides on Microbial Diversity

Species diversity is a measure of "health" in an ecosystem. Total species diversity in a landscape, with regards to spatial scale, is determined by two different indicators: the average species diversity at the local level (alpha diversity) and the differentiation among local sites (beta diversity). More specifically, alpha diversity is defined as "the average species diversity in a particular area or habitat", and beta diversity as "the diversity of species between two habitats or the measure of similarity or dissimilarity of two regions" [40]. Researchers detected eight studies measuring alpha diversity, using a variety of different indices (Shannon index, Simpson index, Pielou's evenness, Operational Taxonomic Units, Chao1 richness, and Faith's Phylogenetic Diversity Index) [28][29][31][34][35][36][37][39]. Furthermore, researchers identified three studies evaluating beta diversity [28][36][37]. Stevia consumption did not change beta diversity significantly in all studies [28][36][37]. The results regarding the effect of stevia in alpha diversity were contradictory. Alpha diversity did not significantly differ in three studies for stevia and control groups [34][36][41]. On the contrary, four studies—including the only study with a sample of human feces fermented in batch cultures [29]—showed significantly higher alpha diversity in the intervention group as compared to the controls [28][29][35][37]. De la Garza et al. assessed feces from male dams fed with a high-stevia diet [39] and reported a significantly higher alpha diversity index in controls than in the stevia group during breastfeeding, but the difference during adulthood was non-significant. The aforementioned studies indicate a potential benefit of stevia consumption in alpha diversity, but the lack of human trials does not allow extractions of safe conclusions.

References

1. Koyama, E.; Sakai, N.; Ohori, Y.; Kitazawa, K.; Izawa, O.; Kakegawa, K.; Fujino, A.; Ui, M. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. Food Chem. Toxicol. 2003, 41, 875–883.
2. Halasa, B.; Walter, P.; Cai, H.; Gonzales, M.; Walter, M.; Shouppe, E.; Kosa, P.; Bielekova, B.; Hui, L.; Rother, K. Stevia Metabolites in Biosamples Ranging from Fetal Life to Adulthood. Curr. Dev. Nutr. 2020, 4, 1126.
3. Renwick, A.G.; Tarka, S.M. Microbial hydrolysis of steviol glycosides. Food Chem. Toxicol. 2008, 46, S70–S74.
4. Ashwell, M. Stevia, Nature's Zero-Calorie Sustainable Sweetener: A New Player in the Fight Against Obesity. Nutr. Today 2015, 50, 129–134.

5. Iatridis, N.; Kougioumtzi, A.; Vlataki, K.; Papadaki, S.; Magklara, A. Anti-Cancer Properties of Stevia rebaudiana; More than a Sweetener. Molecules 2022, 27, 1362.
6. Ruiz-Ojeda, F.J.; Plaza-Díaz, J.; Sáez-Lara, M.J.; Gil, A. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. Adv. Nutr. 2019, 10, S31–S48.
7. Commission Regulation (EU) No 1131/2011 of 11 November 2011 Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council with Regard to Steviol Glycosides. Available online: https://eur-lex.europa.eu/ (accessed on 17 December 2021).
8. Peteliuk, V.; Rybchuk, L.; Bayliak, M.; Storey, K.B.; Lushchak, O. Natural sweetener Stevia rebaudiana: Functionalities, health benefits and potential risks. EXCLI J. 2021, 20, 1412–1430.
9. Takahashi, K.; Matsuda, M.; Ohashi, K.; Taniguchi, K.; Nakagomi, O.; Abe, Y.; Mori, S.; Sato, N.; Okutani, K.; Shigeta, S. Analysis of anti-rotavirus activity of extract from Stevia rebaudiana. Antivir. Res. 2001, 49, 15–24.
10. Fengyang, L.; Yunhe, F.; Bo, L.; Zhicheng, L.; Depeng, L.; Dejie, L.; Wen, Z.; Yongguo, C.; Naisheng, Z.; Xichen, Z.; et al. Stevioside suppressed inflammatory cytokine secretion by downregulation of NF-κB and MAPK signaling pathways in LPS-stimulated RAW264.7 cells. Inflammation 2012, 35, 1669–1675.
11. Sehar, I.; Kaul, A.; Bani, S.; Pal, H.C.; Saxena, A.K. Immune up regulatory response of a non-caloric natural sweetener, stevioside. Chem. Biol. Interact. 2008, 173, 115–121.
12. Alavala, S.; Sangaraju, R.; Nalban, N.; Sahu, B.D.; Jerald, M.K.; Kilari, E.K.; Sistla, R. Stevioside, a diterpenoid glycoside, shows anti-inflammatory property against Dextran Sulphate Sodium-induced ulcerative colitis in mice. Eur. J. Pharmacol. 2019, 855, 192–201.
13. Jiang, J.; Qi, L.; Lv, Z.; Jin, S.; Wei, X.; Shi, F. Dietary Stevioside Supplementation Alleviates Lipopolysaccharide-Induced Intestinal Mucosal Damage through Anti-Inflammatory and Antioxidant Effects in Broiler Chickens. Antioxidants 2019, 8, 575.
14. Mohd-Radzman, N.H.; Ismail, W.I.W.; Adam, Z.; Jaapar, S.S.; Adam, A. Potential Roles of Stevia rebaudiana Bertoni in Abrogating Insulin Resistance and Diabetes: A Review. Evid. Based Complement. Alternat. Med. 2013, 2013, 718049.
15. Jeppesen, P.B.; Gregersen, S.; Rolfsen, S.E.D.; Jepsen, M.; Colombo, M.; Agger, A.; Xiao, J.; Kruhøffer, M.; Orntoft, T.; Hermansen, K. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism 2003, 52, 372–378.
16. Geeraert, B.; Crombé, F.; Hulsmans, M.; Benhabilès, N.; Geuns, J.M.; Holvoet, P. Stevioside inhibits atherosclerosis by improving insulin signaling and antioxidant defense in obese insulin-resistant mice. Int. J. Obes. 2010, 34, 569–577.

17. Holvoet, P.; Rull, A.; García-Heredia, A.; López-Sanromà, S.; Geeraert, B.; Joven, J.; Camps, J. Stevia-derived compounds attenuate the toxic effects of ectopic lipid accumulation in the liver of obese mice: A transcriptomic and metabolomic study. Food Chem. Toxicol. 2015, 77, 22–33.
18. Shivanna, N.; Naika, M.; Khanum, F.; Kaul, V.K. Antioxidant, anti-diabetic and renal protective properties of Stevia rebaudiana. J. Diabetes Complicat. 2013, 27, 103–113.
19. Khare, N.; Chandra, S. Stevioside mediated chemosensitization studies and cytotoxicity assay on breast cancer cell lines MDA-MB-231 and SKBR3. Saudi J. Biol. Sci. 2019, 26, 1596–1601.
20. Gupta, E.; Kaushik, S.; Purwar, S.; Sharma, R.; Balapure, A.K.; Sundaram, S. Anticancer potential of steviol in MCF-7 human breast cancer cells. Pharmacogn. Mag. 2017, 13, 345–350.
21. Martínez-Rojo, E.; Cariño-Cortés, R.; Berumen, L.C.; García-Alcocer, G.; Escobar-Cabrera, J. Stevia eupatoria and Stevia pilosa extracts inhibit the proliferation and migration of prostate cancer cells. Medicina 2020, 56, 90.
22. Chen, J.; Xia, Y.; Sui, X.; Peng, Q.; Zhang, T.; Li, J.; Zhang, J. Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. Oncotarget 2018, 9, 26299–26308.
23. Bessler, H.; Djaldetti, M. The impact of three commercial sweeteners on cytokine expression by mononuclears impelled by colon carcinoma cells. Int. J. Food Sci. Nutr. 2019, 70, 970–976.
24. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019, 7, 14.
25. Boling, L.; Cuevas, D.A.; Grasis, J.A.; Kang, H.S.; Knowles, B.; Levi, K.; Maughan, H.; McNair, K.; Rojas, M.I.; Sanchez, S.E.; et al. Dietary prophage inducers and antimicrobials: Toward landscaping the human gut microbiome. Gut Microbes 2020, 11, 721–734.
26. Pawar, R.S.; Krynitsky, A.J.; Rader, J.I. Sweeteners from plants--with emphasis on Stevia rebaudiana (Bertoni) and Siraitia grosvenorii (Swingle). Anal. Bioanal. Chem. 2013, 405, 4397–4407.
27. Guimarães, A.C.; Meireles, L.M.; Lemos, M.F.; Guimarães, M.C.C.; Endringer, D.C.; Fronza, M.; Scherer, R. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules 2019, 24, 2471.
28. Mahalak, K.K.; Firrman, J.; Tomasula, P.M.; Nuñez, A.; Lee, J.-J.; Bittinger, K.; Rinaldi, W.; Liu, L.S. Impact of Steviol Glycosides and Erythritol on the Human and Cebus apella Gut Microbiome. J. Agric. Food Chem. 2020, 68, 13093–13101.
29. Gerasimidis, K.; Bryden, K.; Chen, X.; Papachristou, E.; Verney, A.; Roig, M.; Hansen, R.; Nichols, B.; Papadopoulou, R.; Parrett, A. The impact of food additives, artificial sweeteners and

domestic hygiene products on the human gut microbiome and its fibre fermentation capacity. Eur. J. Nutr. 2020, 59, 3213–3230.

30. Kunová, G.; Rada, V.; Vidaillac, A.; Lisova, I. Utilisation of steviol glycosides from Stevia rebaudiana (Bertoni) by lactobacilli and bifidobacteria in in vitro conditions. Folia Microbiol. 2014, 59, 251–255.
31. Deniņa, I.; Semjonovs, P.; Fomina, A.; Treimane, R.; Linde, R. The influence of stevia glycosides on the growth of Lactobacillus reuteri strains. Lett. Appl. Microbiol. 2014, 58, 278–284.
32. Markus, V.; Share, O.; Teralı, K.; Ozer, N.; Marks, R.S.; Kushmaro, A.; Golberg, K. Anti-Quorum Sensing Activity of Stevia Extract, Stevioside, Rebaudioside A and Their Aglycon Steviol. Molecules 2020, 25, 5480.
33. Becker, S.L.; Chiang, E.; Plantinga, A.; Carey, H.V.; Suen, G.; Swoap, S.J. Effect of stevia on the gut microbiota and glucose tolerance in a murine model of diet-induced obesity. FEMS Microbiol. Ecol. 2020, 96, fiaa079.
34. Yu, M.; Gao, T.; Liu, Z.; Diao, X. Effects of Dietary Supplementation with High Fiber (Stevia Residue) on the Fecal Flora of Pregnant Sows. Animals 2020, 10, 2247.
35. Li, S.; Chen, T.; Dong, S.; Xiong, Y.; Wei, H.; Xu, F. The Effects of Rebaudioside A on Microbial Diversity in Mouse Intestine. Food Sci. Technol. Res. 2014, 20, 459–467.
36. Nettleton, J.E.; Klancic, T.; Schick, A.; Choo, A.C.; Shearer, J.; Borgland, S.L.; Chleilat, F.; Mayengbam, S.; Reimer, R.A. Low-Dose Stevia (Rebaudioside A) Consumption Perturbs Gut Microbiota and the Mesolimbic Dopamine Reward System. Nutrients 2019, 11, 1248.
37. Nettleton, J.E.; Cho, N.A.; Klancic, T.; Nicolucci, A.C.; Shearer, J.; Borgland, S.L.; Johnston, L.A.; Ramay, H.R.; Noye Tuplin, E.; Chleilat, F.; et al. Maternal low-dose aspartame and stevia consumption with an obesogenic diet alters metabolism, gut microbiota and mesolimbic reward system in rat dams and their offspring. Gut 2020, 69, 1807–1817.
38. Wang, W.; Nettleton, J.E.; Gänzle, M.G.; Reimer, R.A. A Metagenomics Investigation of Intergenerational Effects of Non-nutritive Sweeteners on Gut Microbiome. Front. Nutr. 2021, 8, 795848.
39. de la Garza, A.L.; Romero-Delgado, B.; Martínez-Tamez, A.M.; Cárdenas-Tueme, M.; Camacho-Zamora, B.D.; Matta-Yee-Chig, D.; Sánchez-Tapia, M.; Torres, N.; Camacho-Morales, A. Maternal Sweeteners Intake Modulates Gut Microbiota and Exacerbates Learning and Memory Processes in Adult Male Offspring. Front. Pediatr. 2021, 9, 746437.
40. Willis, A.D. Rarefaction, Alpha Diversity, and Statistics. Front. Microbiol. 2019, 10, 2407.
41. Wang, Q.-P.; Browman, D.; Herzog, H.; Neely, G.G. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. PLoS ONE 2018, 13, e0199080.