

# Artificial Sweeteners: A Systematic Review and Primer for Gastroenterologists

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Artificial sweeteners (AS) are ubiquitous in food and beverage products, yet little is known about their effects on the gastrointestinal (GI) tract, and whether they play a role in the development of GI symptoms, especially in patients with irritable bowel syndrome. Utilizing the PubMed and Embase databases, we conducted a search for articles on individual AS and each of these terms: fermentation, absorption, and GI tract. Standard protocols for a systematic review were followed. At the end of our search, we found a total of 617 eligible papers, 26 of which were included. Overall, there is limited medical literature available on this topic. The 2 main areas on which there is data to suggest that AS affect the GI tract include motility and the gut microbiome, though human data is lacking, and most of the currently available data is derived from in vivo studies. The effect on motility is mainly indirect via increased incretin secretion, though the clinical relevance of this finding is unknown as the downstream effect on motility was not studied. The specific effects of AS on the microbiome have been conflicting and the available studies have been heterogeneous in terms of the population studied and both the AS and doses evaluated. Further research is needed to assess whether AS could be a potential cause of GI symptoms. This is especially pertinent in patients with irritable bowel syndrome, a population in whom dietary interventions are routinely utilized as a management strategy.

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## Key Words

Gastrointestinal tract; Irritable bowel syndrome; Microbiota; Motility; Sweetening agents

## Introduction

Irritable bowel syndrome (IBS), a syndrome that is characterized by recurring episodes of abdominal pain, bloating, and altered bowel habits, is a common problem that is seen in approximately 10-20% of the general population.<sup>1,2</sup> Roughly two thirds of patients with IBS associate the onset or worsening of their symptoms with food intake.<sup>3-6</sup> To date, the greatest attention has focused on diets excluding FODMAPs (fermentable oligo-di-monosaccharides and

polyols) or gluten in treatment of IBS symptoms.<sup>7,8</sup> Many commercially available foods and beverages contain artificial sweeteners (AS), sometimes unbeknownst to the consumer. The prevalence of these nonnutritive sweeteners in products and their resultant ingestion by consumers has been increasing over time.<sup>9,10</sup> The US Food and Drug Administration (FDA) has approved 5 AS to date: acesulfame, aspartame, neotame, saccharin, and sucralose. Other non-nutritive sweeteners, such as stevia, exist but these are not technically considered to fall under the term “artificial sweeteners.” AS are often used for assistance with weight loss by decreasing the amount

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of calories consumed from sugar.<sup>9</sup> Some anecdotal evidence exists of AS triggering gastrointestinal (GI) symptoms in humans, but little has been published in the medical literature on this topic. The purpose of this review is to evaluate the available literature on the effects of AS on the GI tract in order to determine whether they could be playing a role in GI symptoms, especially those experienced by IBS patients.

## Methodology

We first constructed a search strategy for PubMed which combined MeSH terms and free text terms to construct a broad discovery net for articles on individual AS and each of these terms: fermentation, absorption, and GI tract. The searches were then limited to full articles in the English language, and no other filters were applied. The search strategies were adapted to suit the Embase databases as well, and duplicate papers found in both databases were eliminated. All searches were completed by June 8, 2015. We retrieved potentially relevant articles and reviewed their reference lists to identify any studies missed by our search strategy. At the end of our search, we found a total of 617 eligible papers which were reviewed separately by 2 reviewers (MS and AG) and any discrepancies were resolved by a third party (SM); 591 papers were excluded because of irrelevance to the specific questions being asked, not written in English, case reports, letters to the editor, or reviews reporting data of the same original studies. In the end we selected 26 original papers to be included in our systematic review.

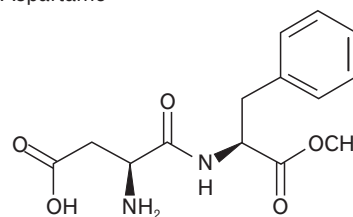
## Results

Given the limited amount of data on the AS acesulfame and neotame, these AS were excluded from our review. Only data on aspartame (4 papers), saccharin (11 papers), and sucralose (14 papers) were included. Background data on the chemical structure (Figure), metabolism, and prevalence in commercially available products for each of the individual AS are presented first, followed by a summary of the evidence on their effects on motility, the microbiome, secretion, absorption/permeability, and GI symptoms. A sample of products that contain aspartame, sucralose, and saccharin are presented in Tables 1-3. Common food and beverage products and the AS they contain are presented in Table 4.

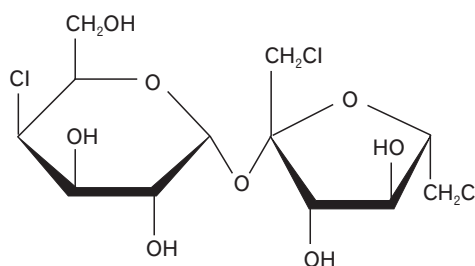
### Structure, Metabolism, and Prevalence

**Aspartame** is a dipeptide methyl ester that is composed of the amino acids aspartic acid and phenylalanine (L-aspartyl-L-phenyl-

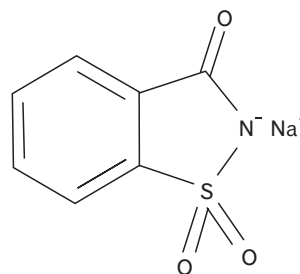
Aspartame



Sucralose



Saccharin



**Figure.** Chemical structures of artificial sweeteners.

alanine methyl ester). It is approximately 200 times sweeter than sucrose.<sup>11</sup> Aspartame is hydrolyzed in the gut to its constituent amino acids, aspartate, and phenylalanine, as well as methanol which are all absorbed into the portal circulation.<sup>12,13</sup> Methanol is then converted to formaldehyde and subsequently oxidized into formic acid.<sup>12</sup> The amount of methanol from aspartame in a soft drink is less than that found naturally in many other foods/beverages.<sup>14,15</sup> An ingredient search on foodfacts.com yields 2254 products that contain aspartame. The FDA's acceptable daily intake (ADI) of aspartame is 50 mg/kg/day.

**Sucralose** (C<sub>12</sub>H<sub>19</sub>Cl<sub>3</sub>O<sub>8</sub>) is a synthesized disaccharide that is formed when 3 hydroxyl groups from sucrose are replaced with chlorine atoms.<sup>16</sup> The resulting compound is around 600 times as sweet as sucrose.<sup>17</sup> Splenda is a combination of sucralose and

**Table 1.** Products Containing Aspartame

Products containing aspartame	Additional non-nutritive sweeteners
Breath Savers Peppermint	Sorbitol
Crystal Light	Acesulfame K
Diet Coke	None
Diet Pepsi	Acesulfame K
JELL-O Sugar Free Gelatin Snacks	Acesulfame K
Mrs. Butterworth's Sugar Free Syrup	Acesulfame K, neotame, sorbitol
Orbitz Gum	Acesulfame K, sorbitol, xylitol, glycerol, mannitol
Snapple Diet Lemon Tea	None
Sprite Zero	Acesulfame K
Trident Gum	Acesulfame K, sucralose, sorbitol, xylitol, glycerin, mannitol

**Table 2.** Products Containing Sucralose

Products containing sucralose	Additional non-nutritive sweeteners
Altoids Smalls Sugar Free Peppermints	Sorbitol
Dasani Drops Flavor Enhancer	Acesulfame K
Diet V8 Splash	None
Fiber One Cereal - Original	None
Gatorade G2 Low Calorie	Acesulfame K
Glacéau FruitWater "naturally flavored sparkling water beverage"	None
Life Savers Sugar Free Hard Candy-Wild Cherry	Isomalt
Powerade Zero	Acesulfame K
Quaker High Fiber Instant Oatmeal Packets	None
Smucker's Sugar Free Strawberry Preserves	Polydextrose
Yoplait Light Yogurt	Acesulfame K

**Table 3.** Products Containing Saccharin

Products containing saccharin	Additional non-nutritive sweeteners
Diet Coke (Fountain soda at Burger King)	Aspartame
Diet Mountain Dew	Aspartame, acesulfame K
Fanta Zero Soda	Aspartame, acesulfame K
Fortuna Foods Pound Cake- Marble Flavor	None
Gold's Low Calorie Borscht (jar of beets)	None
Hainich Sweet Red Peppers	None
IBC Diet Root Beer	Aspartame
Schweppes Diet Tonic Water	None
Shasta Diet Grapefruit Soda	Aspartame
Tab Soda	Aspartame

maltodextran (1% and 99% by dry-weight respectively).<sup>16</sup> Given how potent sucralose is as a sweetener, these fillers are utilized to add volume. Radiolabeled sucralose studies have confirmed that approximately 85% of sucralose is eliminated unchanged in the feces.<sup>18,19</sup> It should be noted that the absolute amount of sucralose eliminated in the feces is small given how potent it is as a sweetener.

The remaining approximately 15% of oral intake is absorbed following consumption. Most is excreted unchanged in the urine, but minor metabolites, likely the result of glucuronidation and not products of metabolism, have also been shown to be present in the urine.<sup>19</sup> These metabolites represent only about 2-3% of the total oral intake.<sup>19</sup> Both sucralose (unchanged) and the suspected gluc-

**Table 4.** Common Products and the Artificial Sweeteners They Contain

Products	Type of artificial sweetener/sugar substitute
Beverages	
7Up Ten	Aspartame, acesulfame K (and HFCS)
Coca-Cola Life	Stevia (and sugar)
Country Time Lemonade On the go packets	Sucralose
Crystal Light	Aspartame, acesulfame K
Crystal Light Pure Fitness	Stevia (rebiana: Truvia brand) (and sugar)
Dasani Drops Flavor Enhancer	Sucralose, acesulfame K
Diet 7Up, Diet 7Up Cherry	Aspartame, acesulfame K
Diet Canada Dry Gingerale	Aspartame, acesulfame K
Diet Coke	Aspartame
Diet Coke Cherry; Diet Coke Lime; Coke Zero	Aspartame, acesulfame K
Diet Coke (Fountain soda at Burger King)	Aspartame, saccharin
Diet Dr. Pepper; Diet Dr. Pepper Cherry	Aspartame
Diet Mountain Dew	Aspartame, acesulfame K, saccharin
Diet Mug Root Beer	Aspartame
Diet Pepsi, Diet Pepsi Wild Cherry	Aspartame, acesulfame K
Dr. Pepper Ten	Aspartame, acesulfame K (and HFCS)
Diet Sierra Mist	Aspartame, acesulfame K
Diet Snapple (Diet Lemon Tea)	Aspartame
Diet Snapple (Diet Half N' Half Tea)	Acesulfame K, sucralose
Diet V8 Splash	Sucralose
Fanta Zero soda	Saccharin, aspartame, acesulfame K
Fresca	Aspartame, acesulfame K
Gatorade G2 Low Calorie	Sucralose, acesulfame K
Glaceau FruitWater "naturally flavored sparkling water beverage"	Sucralose
Glaceau VitaminWater Zero	Erythritol, stevia
Glaceau VitaminWater Zero Drops	Stevia
Honest tea	Stevia (and sugar)
Kool-aid powdered liquid drink mix; singles	Sucralose, acesulfame K
Lipton Diet Citrus Green Tea & Diet Mixed Berry Green Tea	Aspartame, acesulfame K
Minute Maid Just 15 Calories Fruit Punch	Aspartame, acesulfame K (and HFCS)
Minute Maid Light Lemonade	Aspartame, acesulfame K (and HFCS)
Minute Maid Pure Squeezed Light OJ	Sucralose, acesulfame K
Minute Maid Drops (lemonade flavor)	Sucralose, stevia
MiO Liquid Water Enhancer drops- Original	Sucralose, acesulfame K
Mountain Dew Kick Start Orange Citrus	Sucralose, acesulfame K (and HFCS)
Nesquik No Sugar Added Chocolate Low Fat Milk	Sucralose, acesulfame K
Ocean Spray Diet Cranberry	Sucralose, acesulfame K
Pepsi Next	Sucralose, acesulfame K (and HFCS)
Pepsi MAX	Aspartame, acesulfame K
Powerade Zero	Sucralose, acesulfame K
Propel - Berry	Sucralose, acesulfame K
Propel Liquid Enhancer (drops)-grape, Propel Powder Packs - Lemon	Sucralose, acesulfame K
Pure Leaf Iced Tea - Diet Lemon	Sucralose, acesulfame K
Schweppes' Diet Tonic Water	Saccharin
SoBe Lifewater	Stevia (and sugar)
SoBe Lifewater 0 Calories	Erythritol, stevia
Sparkling ICE Waters/Lemonades	Sucralose, mannitol
Sprite Zero	Aspartame, acesulfame K

**Table 4.** Continued

Products	Type of artificial sweetener/sugar substitute
Starbucks DoubleShot Energy - Vanilla Light (in can)	Sucralose
Starbucks Frappuccino Light- Mocha (glass bottle)	Sucralose, acesulfame K
Starbucks Sugar Free Vanilla Beverage Syrup	Sucralose
Sunny D Original	Sucralose, acesulfame K, neotame
Tab Soda	Saccharin, aspartame
Tropicana Trop50 OJ	Stevia
<b>Desserts/Candy</b>	
Brach's Sugar Free Hard Candy - Cinnamon	Sucralose, isomalt, maltitol
Breyer's Ice Cream: No Sugar Added and CarbSmart	Sorbitol, polydextrose, sucralose, Acesulfame K, glycerin
Edy's Slow Churned No Sugar Added Ice Cream	Maltitol, sucralose, sorbitol, glycerin, polydextrose
Hershey's Lite Chocolate Syrup	Acesulfame K
Hershey's Sugar Free Chocolate Syrup	Sucralose, acesulfame K, erythritol, glycerin
Hershey's Sugar Free Chocolates	Maltitol, polyglucitol, polydextrose
JELL-O Sugar Free/Low Calorie Gelatin Snacks and Sugar Free Jello box mixes	Aspartame, acesulfame K
JELL-O Reduced Calorie Pudding Cups - Chocolate	Xylitol, sucralose, acesulfame K
JELL-O Cook and Serve Sugar Free Calorie Pudding Dessert (box)	Aspartame, acesulfame K
Jelly Belly Sugar Free Gummy Bears	Sucralose, maltitol
Jolly Rancher Sugar Free Hard Candy	Acesulfame K, isomalt, polyglucitol syrup
Klondike Bar - Sugar Free	Acesulfame K, sucralose, aspartame, maltitol
Klondike Bar - 100 Calorie	Acesulfame K, sucralose, maltitol
Life Savers Sugar Free Hard Candy - Wild Cherry	Sucralose, isomalt
Pillsbury Sugar Free Frosting - Chocolate Fudge	Sucralose, maltitol, sorbitol, isomalt, polydextrose
Pillsbury Sugar Free Chocolate Brownie Mix	Maltitol, sucralose, acesulfame K
Russell Stover Sugar Free Pecan Delights	Sucralose, maltitol
Skinny Cow Ice Cream Sandwich	Sucralose, acesulfame K, sorbitol, isomalt, polydextrose
Skinny Cow Ice Cream Bar - Chocolate	Sorbitol, polydextrose
Skinny Cow Dreamy Clusters Chocolate Candy	Maltitol, polydextrose, glycerin, erythritol
Tastykake Sugar Free Cream Filled Cupcakes	Maltitol, polydextrose, maltitol Syrup, sucralose
Twizzlers Sugar Free Candy	Acesulfame K, maltitol, sorbitol, glycerin
Weight Watcher's Ice Cream Cones (Vanilla Fudge Sundae)	Sucralose, lactitol
Werther's Original Sugar Free Candy	Isomalt, acesulfame-K
<b>Mints/Gum</b>	
Altoids Smalls Sugar Free Peppermints	Sucralose, sorbitol
Breath Savers Peppermint	Aspartame, sorbitol
Doublemint Gum	Acesulfame K, aspartame, sorbitol, xylitol, maltitol, mannitol, glycerol
Dentyne Ice Peppermint Sugar Free Gum	Sorbitol, maltitol, mannitol, acesulfame potassium, aspartame, glycerin, sucralose
Eclipse Sugar Free Gum	Sorbitol, maltitol, glycerol, aspartame, acesulfame K
Extra Sugar Free Gum	Sorbitol, mannitol, glycerol, aspartame, acesulfame K
Five Sugar Free Gum - Cobalt Cooling Peppermint	Sorbitol, glycerol, mannitol, aspartame, acesulfame K
Ice Breakers Frost Mints	Acesulfame K, aspartame, neotame, sucralose, isomalt, xylitol, glycerin, maltitol
Lifesaver's Sugar Free Mints	Sorbitol, aspartame
Mentos Sugar Free Peppermints	Sucralose, acesulfame K, isomalt, polydextrose, glycerol
Orbit Gum	Acesulfame K, aspartame, sorbitol, xylitol, glycerol, mannitol
Pur Gum - Wintergreen	Xylitol, glycerol
Spry Green Tea Sugar Free Gum	Xylitol

Table 4. Continued

Products	Type of artificial sweetener/sugar substitute
Stride Sugar Free Peppermint Gum	Sorbitol, glycerin, mannitol, xylitol, acesulfame potassium, aspartame, sucralose
Trident Gum	Acesulfame K, aspartame, sucralose, sorbitol, xylitol, glycerin, mannitol
Wrigley's Big Red Chewing Gum	Glycerol, aspartame, acesulfame K
Wrigley's Bubble Tape Gum	Acesulfame K, aspartame, glycerol
Yogurt	
Activia Greek Light Non-Fat Yogurt - Blueberry	Sucralose, acesulfame K
Chobani: Simply Chobani 100 Calories	Stevia (and monk fruit extract)
Dannon: Light and Fit Regular & Greek; Light and Fit Protein Shakes	Sucralose, acesulfame K
Dannon Activia: Light; Greek Light	Sucralose, acesulfame K
Kroger Carbmaster Yogurt	Sucralose, acesulfame K
Yoplait: Light; Greek Light; Greek 100 Calories Whips	Sucralose, acesulfame K
Cereal	
Cinnamon Toast Crunch Reduced Sugar (75% less)	Sucralose
Fiber 1 Original Cereal	Sucralose
Fiber 1 80 Calorie Cereal - Chocolate	Sucralose
Quaker Oatmeal: Low Sugar Instant Oatmeal Packets; High Fiber Instant Oatmeal	Sucralose
Snack bars	
Pure Protein Bars - Chocolate Peanut Butter	Sucralose, maltitol, glycerin
Quaker Chewy Bars: 25% Reduced Sugar	Sucralose, glycerin, polydextrose
Quaker Chewy Bars: 90 Calorie Bars	Sorbitol, glycerin
Special K Bars - Chocolate Pretzel, Protein Meal Bar	Sorbitol, glycerin
Condiments	
Coffee-Mate Creamer - Sugar Free Hazelnut (liquid and powder forms)	Acesulfame K, sucralose
Heinz Reduced Sugar Ketchup	Sucralose
International Delight Coffee Creamer - Fat Free & Sugar Free Toasted Hazelnut	Acesulfame K, sucralose
Mrs. Butterworth's Sugar Free Syrup	Aspartame, acesulfame K, neotame, sorbitol
Smucker's Sugar Free Strawberry Preserves	Sucralose, polydextrose
Smucker's Sugar Free Blueberry Syrup	Sucralose, acesulfame K, sorbitol
Starbucks Sugar Free Caramel Syrup	Sucralose
Shakes/supplements	
Atkins Shakes- Dark Chocolate Royal	Sucralose, acesulfame K
Benefiber Fiber Drink Mix - On the Go (Strawberry Kiwi)	Aspartame, acesulfame K
Citrucel Sugar Free Orange Fiber Powder	Aspartame
Glucerna Shake	Acesulfame K, sucralose, glycerol
Glucerna Nutrition Bar	Acesulfame K, sucralose, glycerol, lactitol, maltitol, sorbitol
GNC brand Pro Performance 100% Whey Protein - Vanilla Cream	Sucralose, acesulfame K
Isopure Low Carb Protein Powder	Sucralose
Metamucil Smooth Texture Orange Sugar Free Fiber Powder	Aspartame
Optimum Nutrition 100% Whey Gold Standard Protein Powder	Acesulfame K
Quaker Breakfast Shake - Chocolate	Sucralose
Special K Protein Breakfast Shake	Sucralose, acesulfame K
Fruit Cups	
Del Monte Fruit Cups - No Sugar Added (Pears, Mandarin Oranges, Mixed fruit)	Sorbitol, sucralose, acesulfame K

uronide conjugates are excreted in urine with no bio accumulation. An ingredient search on foodfacts.com yields 5166 products that contain sucralose. Its higher prevalence compared with other AS is due to its stability in heat, its stability over a range of pH conditions, and its solubility in ethanol, methanol, and water.<sup>17</sup> The FDA's ADI of sucralose is 5 mg/kg/day.

**Saccharin** is a strong organic acid with the chemical formula C<sub>7</sub>H<sub>5</sub>NO<sub>3</sub>S. It is almost completely ionized at physiological pH. Saccharin is 200–700 times sweeter than sucrose. It has nearly complete absorption from the gut (about 3% is recovered in the feces in humans after normal and high doses)<sup>20</sup> and is rapidly eliminated unchanged in the urine. Metabolism of this molecule has not been detected. It is often used with other sweeteners, as it stores well, but is unstable when heated. Therefore, its prevalence in commercial products is much less compared with other AS. An ingredient search on foodfacts.com yields 110 products that contain saccharin. The FDA's ADI of saccharin is 5 mg/kg/day.

### Effects on Gastrointestinal Motility

Evidence that AS may cause alterations in GI motility is mostly indirect, via their effects on the secretion of incretin hormones and serotonin. Certain AS, sucralose being the most studied, have been shown to cause increases in the incretin hormones glucagon-like-peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), Peptide YY (PYY), and cholecystokinin (CCK). GLP-1 decreases motility in the antro-duodeno-jejunal region and inhibits the migrating motility complex in healthy subjects and IBS patients.<sup>21</sup> CCK delays gastric emptying,<sup>22,23</sup> and GIP may also slow gastric emptying.<sup>24,25</sup> PYY has been shown to cause delayed intestinal transit.<sup>26,27</sup> Additionally, sucralose has been shown to increase serotonin, which initiates peristaltic and secretory activity.<sup>28</sup>

### Cellular/animal studies

Five studies done in cell lines showed that sucralose increased GLP1, GIP, PYY, CCK, and serotonin release.<sup>29–33</sup> Another study, however, showed no difference in CCK and GLP-1 levels in duodenal tissue between the sucralose and control groups. However, there was a significant increase in both hormone levels above controls when sucralose was combined with pea protein, suggesting that there may be a synergistic relationship between a macronutrient and sucralose to enhance incretin release.<sup>34</sup> Contrary to studies done at the cellular level, sucralose, saccharin, and acesulfame did not increase GLP-1 or GIP in rats<sup>35</sup> despite the fact that the concentrations of the sweeteners used in the study were 1000 fold in excess of the amount used in diet soda.

### Human studies

A number of human studies have been performed to assess the effect of AS on incretin secretion and thus GI motility indirectly. In 2009 Ma et al<sup>36</sup> performed a controlled cross over trial on 7 healthy subjects (mean age 24 years old, mean body mass index [BMI] 21.6) who were given intragastric infusions of the following radio-labelled substances: 50 g sucrose in water, 80 mg sucralose in 500 mL normal saline (NS), 800 mg sucralose in 500 mL NS, and 500 mL NS. They found that sucralose did not stimulate GLP-1 or GIP release, and did not slow gastric emptying as assessed by carbon dioxide breath samples.

In 2011 Ford and colleagues performed a randomized controlled single-blind cross over study of 8 healthy humans (7 female, aged 22–27 years old, BMI 18.8–23.9).<sup>37</sup> The study participants swallowed either 50 mL of water, sucralose (0.083% sucralose), or maltodextran plus sucralose (Splenda, 0.083% sucralose), followed by a modified sham feeding (swish and spit) of same solution. Blood samples were taken before and up to 2 hours after. They concluded that sucralose ingestion did not increase plasma GLP-1 or PYY levels. Additionally, sham feeding did not elicit a cephalic phase response for GLP-1.

Another randomized double-blinded placebo controlled cross over study looking at aspartame, sucralose, and acesulfame was performed in 2011 in 12 healthy, nonsmoking volunteers (6 female, mean age 23.3 [19–29] with a mean BMI 23 [20.5–24.7]).<sup>38</sup> Subjects received intragastric infusions of 6 substances: aspartame, acesulfame, sucralose, fructose, glucose, and water. These were separated by at least 3–5 days. They found that equisweet loads of AS did not affect secretion of GLP-1 PYY, or ghrelin.

Brown et al<sup>39</sup> performed a randomized controlled cross over trial looking at sucralose and acesulfame. In this study, 22 healthy volunteers (mean age 18.5 [12–25], 45% males mean BMI [25.6]) drank 240 mL of Diet Rite cola or unflavored carbonated water 10 minutes prior to a 75 g oral glucose load. GLP-1 was measured for 180 minutes after the glucose load. They found that GLP-1 levels were significantly higher with diet soda than carbonated water, suggesting that sucralose given with glucose can enhance GLP-1 release, though sucralose in the absence of carbohydrate did not seem to stimulate GLP-1 secretion in prior human studies. Effects on gastric emptying were not assessed as part of this study.

### Effects on the Microbiome

There is a significant amount of evidence from animal studies that AS affect the gut microbiome but data on how specifically the



sweeteners affect the microbiome are conflicting, and to our knowledge only one study has reported human data.

### Animal studies

Anderson and colleagues first performed a controlled trial of male rats who were fed a 7.5% sodium saccharin (NaS) diet or a control diet with no saccharin for 10 days in 1980.<sup>40</sup> NaS resulted in an increase in the total number of aerobic microbes but no change in the number of total anaerobes, resulting in a lower anaerobes/aerobes ratio in the saccharin group. Visual inspection of plates showed that there was deletion of a specific anaerobic microbe in the NaS group, but this organism was not identified. Fifty or 100 mg NaS also inhibited the urease activity of proteus vulgaris likely due to inhibition of microbial growth (reduced numbers of organisms were present in culture tubes after 72 hours).

This same group then performed a follow up study that was published in 1985.<sup>41</sup> Rats were fed a control diet, or a diet that contained NaS at 5% or 7.5%. NaS ingestion caused an increase in the mass of cecal contents and a dose-dependent increase in the fecal content of soluble polysaccharide, which resulted in more carbohydrate available to the gut microflora. NaS also caused inhibition of the hydrolysis of starch by amylase, an increase in the cecal concentration of lactate, and a decreased cecal concentration of volatile fatty acids compared with controls. NaS groups also showed decreased propionate, butyrate, and valerate levels compared with controls, and it was hypothesized that these results were due to effects on the microbiome.

Also in 1985, Mallett et al<sup>42</sup> fed male rats a diet supplemented with 50 g NaS for 4 or 20 weeks, or a control diet with no saccharin.<sup>42</sup> The animals were subsequently sacrificed and their cecal contents were examined and enzyme assays were performed. The concentration and number of bacteria were determined by microscopic count. NaS over 4 weeks had no effect on the total bacterial counts, but after 20 weeks the total bacterial count had significantly increased, although the concentration of bacteria was similar to controls. The mean ammonia concentration in cecal contents increased by 30-50% in the saccharin group and the activity of a number of bacterial enzymes significantly decreased. Direct incubation of saccharin with cecal contents decreased nitrate reductase, B glucuronidase, and endogenous ammonia production. In this study they did not attempt to identify specific bacterial species. They hypothesized that the decreased enzyme activity may be how saccharin exhibits its effects on the microbiome.

A study done by Naim et al<sup>43</sup> in 1985 measured bacterial counts of small intestinal contents of male rats after being incubated

in vitro with 2.5% NaS for up to 20 hours. They showed decreased counts of *Lactobacillus* and *Escherichia coli* compared with controls, and in particular NaS inhibited the growth of 3 *Lactobacillus* strains and 3 *E. coli* strains after their isolation from intestinal contents and incubation in laboratory medium. This study suggested the inhibitory effect of NaS on bacterial growth in pooled small intestinal contents in vitro and in pure culture. However, it should be emphasized that this study was done with small intestinal and not colonic samples, which may account for the different results seen in other studies.

Lawrie et al<sup>44</sup> looked at the effect of NaS on the metabolism of amino acids by bacteria in rats in a study published in 1985. Male rats were given 7.5% NaS in their diet or a control diet for 40 days. Rats in the study group had a 3-4 fold increase in the daily urinary excretion of indican and p-cresol (which are microbial metabolites of tryptophan). Phenol (another metabolite of tryptophan) was almost completely suppressed in the study group. The authors felt that saccharin changes the metabolism of amino acids by gut flora, leading to the formation of products with possible carcinogenic properties. They also hypothesized that saccharin inhibits intestinal protein digestion leading to increased metabolism by bacteria.

In a study done by Pfeffer et al,<sup>45</sup> rats were sacrificed and their anaerobically recovered cecal contents were incubated with glucose. The addition of NaS and acesulfame K with the glucose inhibited anaerobic acid production by cecal bacteria. The authors felt the nutritive relevance of this was probably small because such high concentrations of NaS and acesulfame K were needed to inhibit fermentation.

A randomized controlled trial was performed by Abou-Donia et al<sup>46</sup> in 2008, in which male rats were randomly assigned to 1 of 5 groups: control, or sucralose plus maltodextran in the following doses (mg/kg/day): 1.1, 3.3, 5.5, or 11. Fecal pH and bacterial analysis were conducted weekly, and after 12 weeks half of the rats from each group were sacrificed. Small intestinal tissue was used to evaluate the effects of sucralose and maltodextran on levels of P-glycoprotein (P-gp) and cytochrome P-450 (CYP450). The remainder of the animals underwent a 12 week recovery period after which further assessment of pH/bacteria/P-gp, CYP450 was performed. During the duration of the study, no diarrhea in any of the rats was observed. In terms of the effect on the microbiome, they found that the total number of anaerobes decreased significantly in all sucralose plus maltodextran groups. At the lowest dose, total anaerobes were reduced by approximately 50% compared with controls. *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* were significantly reduced at the lowest dose as well. Aerobic bacteria decreased significantly



in all sucralose plus maltodextran groups except at the lowest dose (decreased by 51-68% of controls). The enterobacteria count was not different from the control population in any group. In the recovery period, the total anaerobes remained significantly lower in all groups, as did bifidobacteria (other specific bacteria were not significantly different from the controls). The mean fecal pH significantly increased in all sucralose plus maltodextran groups at end of 12 weeks and stayed significantly elevated in all groups except the lowest sucralose plus maltodextran group after 12 weeks of recovery. Sucralose plus maltodextran enhanced the intestinal expression of P-gp up to 2.4-fold, and increased CYP3A4 and CYP2D1 intestinal expression (though these effects were not seen in the group that received the lowest dose of sucralose plus maltodextran). In summary, sucralose plus maltodextran significantly decreased beneficial intestinal bacteria (ie, *Bifidobacterium*, *Lactobacillus*, and *Bacteriodes*), but had no effect on enterobacteria. Sucralose plus maltodextran also resulted in elevated fecal pH and enhanced intestinal expression of P-gp and CYP450 enzymes. Several parameters continued to differ after 12 weeks of recovery, including a decrease in the total number of anaerobes and enhanced P-gp and CYP expression.

Suez et al<sup>47</sup> assessed the effect of AS in a multi-faceted study. The sweeteners NaS, sucralose, or aspartame were added to drinking water of mice (control mice drank only water or water supplemented with either glucose or sucrose). Impaired glucose tolerance was significantly increased in mice by consumption of the sweeteners NaS, sucralose, and aspartame after 11 weeks. Given that NaS produced the most profound effect, it was decided to study this AS alone going forward. Changes in glucose tolerance were suppressed with antibiotic treatment, suggesting a direct role of the microbiome. Fecal microbiota composition was then determined by sequencing the 16S ribosomal ribonucleic acid (rRNA) gene. Mice drinking NaS had a distinct microbial composition that clustered separately from their pre-saccharin microbiome and all controls at week 11. Bacteria that increased in abundance in NaS mice included bacteroides and some clostridiales (with other clostridiales under-represented). *Lactobacillus* was under-represented. Shotgun metanomic gene sequencing was performed in order to study the functional consequences of NaS consumption, and similar to the 16S rRNA analysis, NaS treatment induced the largest changes in microbial relative species abundance. Pathways overrepresented in NaS mice included an increase in glycan degradation pathways in which glycans are fermented to form a number of compounds including short chain fatty acids.

They then transferred intestinal microbiota from mice fed NaS

or control mice into germ-free mice, and those given microbiota from NaS group became more glucose intolerant than controls. Similar effects were seen in germ free mice that received microbiota that had been grown in vitro in the presence of NaS. They concluded that NaS directly alters the microbiome composition and metabolism. An in vitro stool culture with saccharin increased *Bacteriodes* and reduced Firmicutes. Additionally, germ-free recipients of cultured configuration had increased *Bacteriodes* and under-representation of several Clostridiales. The fecal microbiota composition of germ-free mice who received stool from saccharin mice showed different microbial composition as well.

### Human studies

In the aforementioned study, Suez et al<sup>47</sup> went on to assess the effect of AS on the microbiome in humans. They performed an observational study of a cohort of 381 non-diabetic humans who reported regular NaS consumption (based on a food frequency questionnaire). This data showed a correlation between NaS consumption and increase in central obesity, fasting blood glucose, Hemoglobin A1c, glucose tolerance test, and alanine aminotransferase. Furthermore, a subgroup analysis comparing high NaS consumers to non-NaS consumers showed a significant increase in Hemoglobin A1c even after correcting for BMI. One hundred and seventy-two randomly selected individuals from this cohort showed intestinal microbial changes characterized by increases in *Enterobacteriaceae*, *Deltaproteobacteria*, and *Actinobacteria phylum*.

This group also performed a smaller trial of 7 healthy volunteers (5 male, 2 female, aged 28-36 years old) who did not normally consume NaS and who were given saccharin for 1 week at a dose of 5 mg/kg. The majority of these (4/7), termed "NaS responders," developed poorer glucose tolerance and had altered intestinal microbiota when compared with the "NaS non-responders." None of the NaS non-responders showed an improvement in glucose tolerance. The microbiome of the NaS responders showed compositional changes by 16S rRNA analysis. Germ-free mice that were given stool samples from NaS responders developed glucose intolerance, while those who were given stool from the same patients pre-NaS or from non-responders did not. Mice given stool from NaS responders showed an increase in *Bacteriodes fragilis* and *Weissella* (of the order Lactobacillales) and a decrease in *Candidatus Arthromitus* (of the order Clostridiales). It is unclear what determined individual human responses to NaS consumption. It is also unclear whether NaS selects against certain microbes by inhibiting their function allowing competitors to flourish, or if it directly stimulates other organisms or both.

## Effects on Secretion

There are only 2 studies that assess the effect of AS on secretion in the GI tract. In a study performed by Kidd et al<sup>48</sup> rat G cells exposed to sucralose showed increased gastrin secretion, which in turn regulates proliferative activity in the GI tract and gastric acid secretion.

Bianchi et al<sup>11</sup> performed a controlled trial of male pylorus-ligated rats who were given 250 mg/kg aspartame intragastrically. After 5 hours the rats were killed and the gastric juice volume and concentrations were determined. Aspartame resulted in no inhibition or stimulation of gastric juice, and no change in concentration of gastric acid. In vitro testing was also performed, and found that aspartame did not affect the proteolytic activity of pepsin or the lipolytic activity of pancreatic lipase.

## Effects on Absorption/Permeability

Similarly, there is minimal data in the literature on the effect of AS on absorption and intestinal permeability. Two studies by Kim-mich et al<sup>49</sup> looked specifically at saccharin. In the first study, they isolated intestinal epithelial cells from chicks and found that saccharin had little direct effect on the sodium (Na) dependent sugar transport system from the lumen to the intracellular space. They did find that passive transportation of sugar through the basolateral membrane was inhibited by saccharin. The inhibition of net efflux by saccharin allowed the active system to establish higher concentration gradients of sugars. There also appeared to be a non-specific effect that compromised the driving forces (Na gradient) of the sugar transport system, which would limit delivery of sugar to the epithelial cell and into circulation. In a follow up study,<sup>50</sup> the same group hypothesized that the nonspecific effect of saccharin on Na coupled sugar transport could be caused by inducing a change in the permeability of the cell membrane to Na, which would diminish the Na gradient and inhibit sugar transport. However, results actually showed the opposite, that saccharin inhibits Na permeability, so therefore it should not inhibit glucose transport into the cell.

## Effects on Gastrointestinal Tract/Gastrointestinal Symptoms

There is minimal data (and no human data) on the effect of AS on GI symptoms, stool form, GI histology, and alterations in the anatomy of the GI tract. Anderson and Kirkland<sup>40</sup> noted that the stool of rats fed a diet of 7.5% NaS for 10 days was softer compared with controls (not the typical pellets that are seen), and was not as odiferous per laboratory technician report. Additionally, in the NaS

group the cecal tissue weight increased by 45% and the weight of cecal contents increased by 80%, which was hypothesized to be due to osmotic effect of NaS. In a follow up study done by the same group, 49 male rats were fed a control diet or a diet with different concentrations of NaS (1%, 3%, 5%, or 7.5% of their chow).<sup>51</sup> They found that stool water increased 4-fold in the group fed NaS at 7.5% compared to the control group, but the stool dry weight remained constant. NaS resulted in a dose-dependent increase in the stool content of an unidentified carbohydrate, and it was hypothesized that this may represent an undigested polysaccharide or polysaccharide synthesized by the microbiota. The cecal enlargement and increased water in the saccharin groups was thought to be secondary to high stool saccharin content and increased stool hydration.

Hibino<sup>52</sup> looked at the effect of NaS on the upper GI tract of rats. Sixty-eight male rats were fed a diet supplemented with 5% NaS and 31 control rats were fed a normal diet without NaS. After 80 weeks, the stomachs were examined in 20 experimental and 11 controls. All 20 in the experimental group had hyperkeratosis and 5 had papillomas of the forestomach. In the experimental group, ulcers were found in the glandular stomach in 4 rats and atypical glands were seen in 4 rats. All 11 controls had normal stomachs. It is unclear what the significance of these findings is in humans.

One study done by Sasaki<sup>53</sup> aimed to assess potential genotoxicity as a result of AS use. They fed rats NaS, sucralose, aspartame, or acesulfame, sacrificed them after 3 or 24 hours, and then ran a comet assay to test for genotoxicity. They found statistically significant DNA damage above controls for both sucralose and NaS in the stomach and colon. The lowest dose that induced DNA damage for NaS was 1000 mg/kg and was 2000 mg/kg for sucralose, which is much higher than the ADIs for these AS in humans. Acesulfame and aspartame did not increase DNA damage.

Abou-Donia<sup>46</sup> found histopathologic changes in the colons of mice fed sucralose plus maltodextran versus control mice, which included lymphocytic infiltrates into the epithelium, epithelial scarring, and mild depletion of goblet cells.

The only study that assessed GI symptoms was performed by Kille et al.<sup>54</sup> They found that rabbits fed sucralose developed GI symptoms (perianal soiling, scouring, and cecal enlargement) at doses of 750 and 1000 mg/kg/day. In lower doses the rabbits experienced little GI distress. However, rabbit elimination of sucralose is prolonged compared to other species and they absorb sucralose to a greater degree than other species, so the significance of these findings in humans is unclear.

## Conclusions

Although AS are widely utilized in commercially available products, there is a remarkable paucity of medical literature addressing their effects on GI physiology and symptoms. The 2 main areas on which there is data to suggest that AS affect the GI tract include motility and the gut microbiome. Most of the currently available data is derived from in vivo studies. Incretin secretion, which may indirectly result in changes in GI motility, was only shown to be increased in one human study where sucralose was combined with glucose (in other studies looking solely at the AS itself, no effect on incretin secretion was found). Additionally, the downstream effect on motility from the increased incretin secretion was not studied, so the clinical relevance of this finding is unknown. Multiple studies in animals suggest that AS exert measurable effects upon the gut microbiome. To date, only one published study has reported data from humans. The specific effects on the microbiome have been conflicting and the available studies have been heterogeneous in terms of the population studied and both the AS and doses evaluated. There is virtually no data in many other areas relevant to IBS including alterations in permeability, immune activation, and visceral sensation. There are also no studies on the effects of AS on GI symptoms in the general population, or more specifically in IBS patients.

Further research is needed to assess whether AS could be a potential cause of GI symptoms. This is especially pertinent in IBS patients, a population in whom dietary interventions are routinely utilized as a management strategy. IBS patients have been shown to have similar rates of lactose/fructose malabsorption as healthy controls,<sup>55</sup> though they are more likely to experience symptoms from malabsorption more commonly than controls, which may be due in part to underlying abnormalities in motility and visceral hypersensitivity.<sup>55-57</sup> Research to characterize whether AS impact upon pathophysiologic factors relevant to IBS and symptoms in affected patients would be of considerable interest and are strongly encouraged.

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