Section A -Research paper

Effect of artificial sweeteners on Pancreas

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Abstract:

Aspartame is the most popular artificial sweetener consumed by many individuals worldwide. Yet, there is still a debate on its consumption as an alternative to sugar. Further studies are warranted to assess the effects of aspartame on pancreas morphodynamics.

Keywords: aspartame on pancreas, artificial sweeteners.

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Introduction:

Artificial sweeteners, (AS) common substitutes for sugar, were first introduced to the food market in the 1800s, but their consumption did not increase dramatically until the 2000s. High sugar intake is associated with weight gain, obesity, and hypertriglyceridemia, which can lead to cardiovascular disease (CVD), diabetes, and other metabolic diseases. As a result, people are opting for low-calorie and sugar-free products; any food marketed as “Sugar-free” or “Diet food” contains artificial sweeteners **(1)**.

Currently, in the U.S. Food and Drug Administration (FDA) has approved six synthetically derived artificial sweeteners, as food additives: aspartame, sucralose, neotame, acesulfame potassium, saccharin, and advantame. Advantame was most recently approved artificial sweetener by the FDA as a broad purpose sweetener and flavor enhancer. There are also two naturally

occurring artificial sweeteners that are also approved by the FDA: stevia leaf extract and monk fruit extract **(2)**.

Increased sugar intake leads to well-established adverse health outcomes such as weight gain, dental caries, diabetes, and cardiometabolic disorders. Thus, the World Health Organization (WHO) has suggested keeping total sugar intake to less than 10% of the total daily calorie intake. But since people all over the world enjoy sweet flavors, the food industry began to employ the use of artificial sweeteners as substitutes to cut added sugar amount and the number of calories associated with them while preserving sweetness at the same time **(3)**.

Sweetener consumption

Sweeteners can help to reduce the positive energy balance for managing BW and blood glucose. However, there are still conflicting data from short- and long-term consumption of both natural and artificial AS, such as aspartame, sucralose, and stevia derivatives. Preclinical and clinical studies report that sweeteners can have negative effects on the intestinal microbiota, appetite control, and glucose metabolism **(4)**.

Specifically, in animal models their assumption for a period longer than 12 months, increased the food consumption, BW gain, the percentage of adiposity and induced hyperinsulinemia, whereas reduced the postprandial thermogenesis when compared to animals exposed to carrier water or even to foods or soft drinks sweetened with calorie **(5)**.

The negative effects of AS were reduced in mice during a restrictive diet and were pronounced among male animals, genetically predisposed to obesity. Preclinical studies, therefore, indicated, on a biological level, likely mechanisms to explain the results of long-term observational studies conducted in humans, in which increases in BW and incidence of overweight and obesity were observed. Finally, some evidence reports that ASs interact with the sweet taste receptors in the mouth and modify the intestinal secretion of molecules, such as glucagon-like peptide-1 (GLP-1), YY peptide (PYY), ghrelin and polypeptide glucose-dependent insulinotropic (GIP), which could affect blood sugar levels following AS consumption.

However, although AS could represent a valid substitute to sugar, according to recent clinical evidence, AS excess consumption can do not correlate with what is observed in preclinical studies. Indeed, as chronic consumption worse obesity, metabolic syndrome, T2D and related CVD **(6)**.

To date, there are few clear recommendations regarding the consumption of artificially sweetened foods and drinks in children. In general, as indicated by the Institute of Medicine and the American Academy for Pediatrics, AS are not recommended for children under 12 months of

age, since there are no studies on the safety of sugar substitutes in infants. Additionally, foods and drinks containing sugar substitutes are generally not recommended even for infants over 12 months of age, as they are nutrient poor and would not allow for optimal growth and development. Indeed, it was demonstrated that substituting sugar-sweetened beverages with water decreases body fatness development in adolescence **(7)**.

Main characteristics

Sweeteners are substances used to give a sweet taste to the food and/or drinks to which they are added. Although the European Food Safety Authority (EFSA) or the US Food and Drug Administration (FDA) do not consider a classification of sweetener based on origin, this report analyzed them, following a classification into two groups: synthetic sweeteners (also called non-calorie sweeteners), with little or no nutritional power and natural sweeteners (also called caloric sweeteners), for a better understanding of the characteristics and an easier reading of the studies conducted **(8)**.

Today, many low- or zero-calorie drinks and foods are available. This section describes the natural and artificial AS marketed or under development most used worldwide. Acesulfame-K, aspartame, cyclamate, saccharin, sucralose, and stevia are the sweeteners approved for consumption. Other sweeteners, such as neohesperidine DC, neotame, and thaumatin were not included because they have more limited use. Sweeteners are widely used, alone or in combination, by the food industry for also sweetening candies, chewing gums, and jams. Their sweetening power is 30 to 500 times higher than sucrose, the common sugar used **(9)**.

However, many consumers prefer products with natural sweeteners. The use and dosage of the different AS in food and soft drinks are established based on the acceptable daily intake (ADI) values assigned after reviewing their safety assessment studies. ADI is calculated according to the following formula: sweetener mg/BW (kg)/day. The numerical value corresponds to the maximum quantity that can be safely taken throughout the day **(10)**.

For this reason, it is also defined as 1% of the NOEL level, as no observable adverse effects, determined by human and animal safety assessment studies. Since non-caloric sweeteners are generally much sweeter than sucrose, therefore, can be used in small quantities. Non-caloric ASs are classified into chemically synthesized sweeteners, including aspartame saccharin, and sucralose; and natural sweeteners extracted from plants, such as stevia glycosides, thaumatin and monellin **(11)**.

Pharmacokinetics

To determine safety of artificial sweeteners the FDA considers probable intake, cumulative effects from all uses, and toxicological data in animals. The European Food Safety Authority (EFSA) evaluates and confirms that the intake of artificial sweeteners, within the acceptable daily intake (ADI), does not cause cancer or other health-related problems, and are therefore safe for human consumption. Although authorities consider artificial sweeteners as safe as they do not pose any health-related problems, when consumed within the ADI, no specific safety claims have been made about the effects of sweeteners on non-communicable diseases, such as obesity and T2DM **(12)**.

Even though several artificial sweeteners are tested for pharmacological and toxicological aspects, the concerns about the effects of unmetabolized compounds on non-communicable diseases still exist. Artificial sweeteners have distinct structures and are metabolized differently as some but not all are digested or fermented **(13)**.

Commonly used artificial sweeteners

Currently, the FDA has approved five artificial sweeteners for consumption: acesulfame-K, aspartame, neotame, saccharin, and sucralose. In addition, the FDA has determined that a new sugar substitute, stevia, is a dietary supplement "generally recognized as safe" (GRAS). Artificial sweeteners are known by several names, which include: low-calorie sweeteners, high intensity sweeteners, non-sucrose sweeteners, intense sweeteners, non-nutritive sweeteners, sugar substitutes, and sugar-free sweeteners. For the purposes of this review, we will use the term "artificial sweeteners." **(14)**.

Mechanism of action of artificial sweeteners

Multiple behavioral mechanisms have been proposed to account for the epidemiologic association between artificial sweetener use and weight gain. It has been suggested that the dissociation of the sensation of sweet taste from caloric intake may promote appetite, leading to greater food consumption and weight gain **(15)**.

In addition, increased consumption of added caloric sweeteners has been associated with lower diet quality in children, perhaps by altering taste preferences toward sweetened foods in place of more healthful foods, such as fruits and vegetables; this mechanism could apply to artificial sweeteners as well **(16)**.

New data from both humans and animal models have provided convincing evidence that artificial sweeteners play an active role in the gastrointestinal tract, thus providing a mechanistic

explanation for observed metabolic effects. Sweet-taste receptors, including the taste receptor T1R family and α-gustducin, respond not only to caloric sugars, such as sucrose and glucose, but also to artificial sweeteners, including sucralose (Splenda™) and acesulfame-K **(17)**.

In both humans and animals, these receptors have been shown to be present not only in lingual taste buds, but also in glucagon-like peptide-1 (GLP-1) secreting L cells of the gut mucosa, where they serve as critical mediators of GLP-1 secretion. It has been shown in rat studies that stimulation of intestinal taste receptors with sucralose led to more rapid absorption of sugars from the intestine into the bloodstream. It has demonstrated in young healthy volunteers that consumption of diet soda before an oral glucose challenge potentiates GLP-1 secretion, thus potentially altering both gastric emptying and insulin secretion **(18)**.

Translating these results into the clinical realm, consumption of an artificial sweetener in conjunction with a sugar containing food or drink could lead to more rapid sugar absorption, as well as increased GLP-1 and insulin secretion, potentially affecting weight, appetite, and glycemia **(19)**.

Artificial sweeteners are associated with an increased risk of metabolic syndrome, a cardiometabolic risk factor that includes hypertension, insulin resistance, excessive blood sugar, abdominal obesity, and dyslipidemia. There are three plausible mechanisms: (1) alteration of gut microbiota, (2) acceleration of senescence and atherosclerosis, and (3) relation with arrhythmogenesis. One potential mechanism through which artificial sweeteners may contribute to cardiovascular disease is by disrupting the balance of gut bacteria by selectively promoting the growth of certain bacterial species while suppressing others **(20)**.

Contribution to the development of metabolic syndrome

AS consumption provides a very low calorie or zero-calorie alternative intake that provides minimal or no carbohydrates or energy. Their dietary consumption can modulate energy balance and may influence feeding and metabolism through a variety of peripheral and central mechanisms. Recent evidence highlighted that AS consumption has been associated with increased risk factors for MetS **(21)**.

In particular, the association between waist circumference and total AS, such as saccharin, sucralose, and acesulfame-K was demonstrated. In addition, it was found positively associated between fasting glucose and triglyceride values with total AS and aspartame consumption **(21)**.

Sweeteners and human gut microbiota

Composition and function of the microbiota were affected by external factors, such as environmental stressors, antibiotics, and diet as aberrations in the gut microbiota have been associated with the development of insulin resistance, obesity, and also MetS. To date, there are conflicting results on the specific roles of AS on the microbiota. In human studies, it was demonstrated that AS consumption may induce changes in microbiota composition. Dysbiosis was observed following AS consumption in animal studies **(22)**.

In several diet-induced animal models of MetS by using AS, changes in microbiota composition (Bacteroidetes to Firmicutes) were positively correlated with reduced glucose tolerance contrarily to observed for overweight people dysbiosis would seem to increase intestinal permeability and thus promote the development of a pro-inflammatory niche that stimulates β-cell autoimmunity. AS consumption modulates gut microbiota composition and associations with an increased risk of MetS, obesity, and T2D were demonstrated **(23)**.

➢ Epigenetic mechanisms and AS consumption

The growing interest in the epigenetic involvement in human disease, on the role of miRNAs changing cell function has focused attention on miRNA implications on gut microbiota function. miRNAs regulate at least 30% of human genes, playing critical roles in cell proliferation, differentiation, apoptosis, and hematopoiesis. miRNA expression can be altered by stress and diet and AS consumption may modify miRNA expression by altering bacterial composition and lead to metabolic changes **(24)**.

MiRNA by acting at the DNA level or directly on RNA in the mitochondria could help to restore gut microbiota composition . Besides, up- or down-regulation of certain specific miRNAs has been correlated with the development of insulin resistance and increased severity of T2D. Regular AS consumption induces changes in the composition of the gut microbiota with a consecutive development of insulin resistance **(25)**.

MiRNAs, as regulators of many metabolic processes, could be useful therapeutic agents. MiR-126 expression that is significantly reduced in diabetic patients, in which an impaired proangiogenic capacity causes diabetic vasculopathy, manipulation of miR-126 expression could induce migration and proliferation of vascular endothelial cells and facilitate their repair. The explanation would be since separation of sweetness from calories interferes with physiological responses and the interaction of AS with sweet-taste receptors in the gut negatively affects glucose absorption provoking inducing fat accumulation and weight gain **(26)**.

➢ Artificial sweeteners and the obesity epidemic

To help curtail the obesity epidemic, small dietary changes to prevent weight gain in children and adolescents have been encouraged. Artificial sweeteners have gained attention as dietary tools that provide sweet taste without the extra energy derived from foods and drinks containing caloric sugars, and thus may assist in weight-loss plan adherence **(15)**.

A key question is whether replacement of sugar-sweetened products with those containing artificial sweeteners is truly beneficial. Since their FDA approval, artificial sweeteners and their benefits on metabolic health have been questioned. An association between artificial sweetener intake and weight gain was first observed in epidemiological studies with adults. Several largescale studies, including the National Health and Nutrition Examination Survey (NHANES) and the San Antonio Heart Study, have shown a positive association between artificial sweetener use and increases in weight and/or BMI **(13)**.

➢ Artificial sweeteners and the metabolic syndrome

Components of the metabolic syndrome have been assessed in two pediatric studies. The previously discussed study of encapsulated aspartame versus placebo in young people found no differences in blood pressure, glucose, or lipid profiles between groups. Similarly, in the study in which teenage girls were permitted either sugar-sweetened or artificially sweetened soda as a snack during weight loss, there were no differences between groups in blood pressure, waist circumference, or lipid profile **(27)**.

➢ Contribution to the prevalence of cardiovascular diseases

It is now known that hyperglycemia is associated with an increased risk of cardiovascular disease (CVDs) that occurs in diabetic patients. Animal studies have suggested that AS consumption may affect glucose or insulin homeostasis; it can alter the intestinal microbiota, increase appetite and promote weight gain however evidence of these associations in humans is limited. Furthermore, studies that have assessed the relationship of AS consumption with incident T2D are also confounding; some studies reported that higher intake of diet soda and/or consumption of AS is associated with a higher risk of T2D, while others find no association **(28)**.

An association study found that although the consumption of diet soda and AS was high, neither was associated with the risk of diabetes. The principal hyperglycemic pathologic complications can be classified as macro-vascular complications, which are the CVDs as acute myocardial infarction (AMI), stroke, and peripheral artery disease (PAD); and micro-vascular complications, such as kidney disease, retinopathy, and neuropathy **(29)**.

Effect of Artificial Sweeteners on pancreas

The consumption of sugar-sweetened beverages has been associated with cardiometabolic complications, driven by an increased energy intake and obesity. Therefore, one common approach to improve energy balance is to refrain from sugars by replacing them with artificial sweeteners. Although the World Health Organization (WHO) recommends free sugar intake of <10% of total energy intake, preferably <5% of total energy intake as a conditional recommendation, a large proportion of the European population appears to exceed this threshold, especially children. For instance, 81% of the Dutch population does not fulfill this recommendation as the intake of free sugars equals ~14% of total energy intake in the Netherlands **(30)**.

As artificial sweeteners offer a sweeter taste without calories, the replacement of sugars with these sweeteners seems promising in reducing sugar and energy intake. Meta-analyses of Randomized Controlled Trials (RCTs) have shown that daily energy intake (after 4 or 10 weeks) and sugar intake (after 4 weeks) were lower in healthy, overweight, and obese individuals receiving artificial sweeteners as a replacement of sugars in the diet. Sweeteners are classified as natural sweeteners and artificial sweeteners **(31)**.

➢ Body Weight and Adiposity

An increased body weight and adiposity develop under conditions of a positive energy balance. The regulation of energy balance is a complex process that involves homeostatic regulation of energy intake and energy expenditure. Although artificial sweeteners are as sweet or even sweeter than natural sugars, the caloric content and the metabolism routes are different **(32)**.

Therefore, it is likely that artificial sweeteners may affect energy balance, and thus body weight, differently compared to natural sugars via underlying physiological processes comprising the gut microbiota, the reward-system, and adipogenesis **(17)**.

Considering specific types of artificial sweeteners, meta-analyses, based on RCTs, showed no effect of aspartame consumption on body weight compared to sugar or water in individuals with either obesity or T2DM. Only studies wherein aspartame was evaluated alone were included in the meta-analyses to clarify the specific effects of aspartame without interference of results obtained due to the consumption of other sweeteners **(33)**.

However, large heterogeneity was found due to different treatment patterns for aspartame and sugar or water. Similarly, meta-analysis, based on RCTs, showed no effect of steviol glycoside consumption on BMI compared to talcum, maize starch, or unspecified matching placebo in healthy individuals and patients with diabetes. Additionally, subgroup analyses showed no

significant effect of steviol glycoside on BMI in either healthy individual and patients with diabetes. The results indicate that these artificial sweeteners do not affect body weight **(34)**.

However, the effects of acesulfame-K and saccharin can still be debated, as there is no consistent evidence, and meta-analyses are lacking. More specifically, one study that used the ADI-dosage for human consumption (15 mg/kg/day) showed no effect on body weight in mice after 8 weeks of acesulfame-K consumption, while another study shows an increase in body weight by exceeding the ADI more than 2-fold (37.5 mg/kg/day) after 4 weeks in mice **(19)**.

Furthermore, saccharin consumption was found to increase body weight in mice compared to water, sucrose, or glucose, whereas other studies in rodents have shown reduced or unchanged body weight compared to mice receiving water, glucose, fructose or sucrose. However, the absorption of saccharin is lower in rodents compared to humans due to a relative higher stomach pH in rodents **(35)**.

Moreover, sucralose consumption has been reported to have no effect on body weight in mice compared to water, and in human studies compared to placebo (calcium carbonate) or control (no-intervention). Notably, contradictory results from rodent studies for the effect on body weight exist only for acesulfame-K and saccharin, which are largely or entirely absorbed in their intact form, thereby being able to reach the peripheral tissues **(16)**.

Consistently, rodent, and human studies found no effect of sucralose on body weight as only a small amount is absorbed in its intact form, thereby reaching the microbiota in a larger amount compared to acesulfame-K and saccharin. As artificial sweeteners have different metabolic fates, differences in physiological effects affecting energy balance and adiposity should be elucidated **(17)**.

The Interaction Between Artificial Sweeteners, and Adiposity

As artificial sweeteners contain no or low amounts of calories, one might expect that these sweeteners may contribute to lower energy intake and thus body weight reduction. Nevertheless, controversies exist whether artificial sweeteners affect appetite, hunger, and eating behavior, and if these effects are beneficial or not **(36)**.

After ingestion of either natural sugars or artificial sweeteners, gustatory information is perceived by sweet taste receptors, which are heterotrimeric G-protein coupled receptors (GPR) consisting of two subunits, namely taste receptor type 1 member 2 (T1R2) and 3 (T1R3). The sweet taste receptors are located in taste buds in the oral cavity and outside the oral cavity, including the intestine and pancreatic β-cells **(37)**.

As artificial sweeteners and natural sugars bind differently to the sweet taste receptors, the gustatory branch is activated differently as well. Thereupon, artificial sweeteners may generate weaker signals that are sent to areas involved in reward and satisfaction, as consistently demonstrated by using functional Magnetic Resonance Imaging (fMRI) in several randomized cross-over trials **(38)**.

Likewise, the ingestion of artificial sweeteners induces a signaling cascade outside of the oral cavity. Within the GI tract, sweet taste receptors are primarily located on enteroendocrine L- and K-cells. The signal transduction pathway is similar as in cells present in the oral cavity. Upon ligand binding of natural sugars to sweet taste receptors, enteroendocrine L-cells secrete glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), whereas K-cells secrete glucose-dependent insulinotropic peptide (GIP) **(39)**.

These hormones can cross the semi-permeable blood-brain barrier, thereby reaching the hypothalamus and affecting food intake by reducing appetite and increasing satiety. However, artificial sweeteners may not be potent secretagogues for GLP-1, PYY, and GIP to the same extent in vivo as natural sugars since the secretion is nutrient-dependent. For instance, aspartame is digested and absorbed before reaching the lower GI tract to bind to the sweet taste receptors. Acesulfame-K, sucralose, steviol glycoside, and saccharin pass through the lower GI tract to be absorbed, digested, or eliminated directly **(40)**.

Consistently, mice studies and human crossover trials in lean and obese individuals have shown no significant effects of artificial sweeteners on incretin secretion. In addition to the lack of an effect on incretin secretion, two human crossover studies showed no effect on appetite upon sucralose or aspartame-sweetened diet coke consumption in healthy and obese individuals **(35)**.

Therefore, it has been suggested that artificial sweeteners do not activate the food reward pathways in the same way as natural sugars. The elimination of the post-ingestive reward holds true for non-caloric artificial sweeteners, whereas the intake of artificial sweeteners in the presence of carbohydrates may elicit post-ingestive incretin responses, as demonstrated using sucralose-sweetened beverages **(41)**.

Based on the above, it can be postulated that artificial sweeteners solely offer less reward compared to natural sugars, although it should be emphasized that the differences in reward response have not been shown in the context of a whole-meal approach or diets, where sugar was replaced by artificial sweeteners **(42)**.

➢ Energy Intake

The lack in complete satisfaction may drive the assumption that artificial sweeteners fuel food seeking behavior, thereby contributing to increased or no differences in energy intake. However, less satisfaction does not necessarily translate into compensatory (excess) energy intake. RCTs have shown that the reduced caloric intake by replacing natural sugars with artificial sweeteners is not completely compensated. As a result, energy intake after the use of artificial sweeteners is still lower compared to natural sugars, even after putative compensatory energy intake. Therefore, the compensatory energy intake does not seem to pose a threat to weight gain and may aid in weight loss (maintenance) **(35)**.

➢ Adipogenesis

Sweet taste receptors are expressed in many organs, including adipose tissue. Not all artificial sweeteners will reach the adipose tissue as some are not absorbed into the systemic circulation. The sweet taste-sensing receptor in adipose tissue differs in comparison to the receptors in sweet taste buds or in the GI tract. In adipocytes, the expression of T1R3 was found to be higher than T1R2, suggesting that a higher percentage of T1R3 is present as a homomer. Nevertheless, increased adipogenesis and reduced lipolysis were found, independent of T1R2 and T1R3, upon *in vitro* stimulation of adipocytes with saccharin **(38)**.

It has been suggested that saccharin act on a protein kinase A-mediated mechanism downstream of cyclic adenosine monophosphate (cAMP). Consequently, hormone sensitive lipase (HSL) phosphorylation is reduced by regulating HSL phosphatase, thereby inhibiting lipolysis. Likewise, acesulfame-K was found to stimulate adipogenesis. However, the active concentrations of saccharin and acesulfame- K in adipocytes (4.5 mM) were higher than expected to be observed in humans as bolus oral doses of maximum daily intake of saccharin, for instance, results in peak plasma concentrations of ~75 μm **(41)**.

➢ Energy Expenditure

Besides affecting the hunger-satiety cycle, SCFA may modulate body weight control by influencing energy expenditure. A previously performed double-blind, crossover study, showed increased lipid oxidation, and thus energy expenditure, upon acute colonic infusions of SCFA in overweight or obese men. Consistently, mice studies have shown increased lipid oxidation by increasing sympathetic activity in brown adipose tissue, via gut-neural signaling, upon SCFA administration **(43)**.

➢ Glucose Homeostasis

Besides potentially affecting body weight control, artificial sweeteners may also affect glycemic control, since glucose absorption may be reduced upon replacement of available carbohydrates. However, this does not necessarily translate into an improved glucose homeostasis, since alterations in intestinal glucose transport and absorption, insulin resistance, and reduced insulin secretory capacity by artificial sweeteners may contribute to impaired glucose homeostasis **(44).**

However, the evidence for a relationship between artificial sweeteners and T2DM is based on prospective cohort studies using only baseline exposure and may be caused by reverse causation. Hence, evidence from systematic and meta-analysis does not consistently show that artificial sweeteners reduce the risk of T2DM in humans **(45)**.

Considering specific types of artificial sweeteners, glucose homeostasis seems to be unaffected by aspartame and steviol glycoside. No significant effect on glucose levels and glycated hemoglobin (HbA1c) levels were found after acute or long-term aspartame consumption. Similarly, a meta-analysis of long-term RCTs showed no effect of steviol glycoside on glucose levels and HbA1c levels in healthy individuals and patients with diabetes **(35)**.

Glucose and HbA1c levels were not affected by acute or long-term sucralose consumption in healthy individuals and patients with diabetes. Remarkedly, short-term sucralose consumption alone showed no effect on insulin sensitivity in healthy individuals, whereas sucralose-sweetened beverages, containing carbohydrates, or sucralose sachets added to carbohydrate-containing beverages or meals, decreased insulin sensitivity in healthy individuals **(46)**.

Therefore, it has been suggested that sucralose may impair glucose metabolism only when co-ingested with carbohydrates. The role of artificial sweeteners in enhancing intestinal glucose absorption, thereby perturbating glucose homeostasis in the presence of carbohydrate content, can be speculated **(47)**.

➢ Insulin Secretion

The intake of nutrients is associated with a large set of sensory cues that enables the human body to prepare for metabolic digestion and utilization. Exposure to sweet-tasting sugars, even before ingestion, triggers physiological responses related to the release of insulin or incretin to reduce blood glucose levels. However, artificial sweeteners are not able to prepare the GI tract for digestion and utilization of nutrients as well as sugars **(34)**.

While natural sugars can stimulate the secretion of incretins, thereby stimulating β-cells to secrete insulin, artificial sweeteners do not directly induce incretin secretion as this appears nutrient-dependent. Moreover, insulin secretion is stimulated upon the interaction of both natural sugars and artificial sweeteners with sweet-taste receptors in pancreatic β-cells by initiating a signal transduction pathway via Ca^{2+} and cAMP-dependent mechanism. Taken together, this may suggest that artificial sweeteners stimulate insulin secretion less compared to natural sugars **(17)**.

➢ Insulin Resistance

Insulin resistance is a major factor in the pathophysiology of T2DM, of which the pathogenesis involves the accumulation of ectopic fat and the activation of innate immune pathways, thereby interfering with insulin signaling and action. The artificial sweetener-induced gut microbiota dysbiosis has been linked to metabolic endotoxemia and the development of an inflammatory state, at least in rodents **(48)**.

Microbiota dysbiosis is related to the loss of gut mucosal integrity as the expression of tight junction proteins is reduced, among other mechanisms. Therefore, LPS may translocate from the gut into the portal or systemic circulation, thereby able to stimulate the activation of pro-inflammatory macrophages and the secretion of pro-inflammatory cytokines **(49)**.

Other studies showed disrupted intestinal epithelial barrier *in vitro* using Caco-2 cells upon saccharin stimulation, whereas aspartame, acesulfame-K, and sucralose did not alter intestinal permeability. Similarly, other rodent studies showed increased LPS concentration, and subsequently enhanced inflammation, in mice upon saccharin consumption by interfering with the gut microbiota **(50)**

Regarding other artificial sweeteners, the intake of acesulfame-K (exceeding the ADI-dosage for humans by more than twice) or sucralose was found to enhance inflammation in mice, whereas steviol glycoside was found to reduce inflammation by attenuating LPS-induced pro-inflammatory cytokine production in Caco-2 cells and by regulating TLR2 and cytokine expression in *S. aureus*-infected mouse mammary gland **(48, 51)**.

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