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The impact of non-nutritive sweeteners on gut microbiota: insulin resistance

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Impacto dos edulcorantes não nutritivos na microbiota intestinal: insulinorresistência

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Abstract

Non-nutritive sweeteners (NNS) are ever more common in the ingredients list of food products. They are being used, by the food industry, to bypass the use of sugar and, yet, retain the sweetness that allures consumers, who, although increasingly health conscious, seek out sweet tasting foods. It is this growing presence in our diet, which raises health concerns in the scientific community. Even though there are studies that demonstrate the usefulness of NNS in weight loss and in glycaemic control, there also exists evidence that chronic usage of these substances can lead to the opposite results intended. While the physiologic pathways that link NNS with weight gain and insulin resistance remains unclear, there is a growing amount of evidence that such a connection does, in fact exist. Gut microbiota has been hypothesised as a possible link between NNS consumption and obesity and insulin resistance, through its own microbe modulation and resulting metabolites.

These studies could shed new light on the possible hazards of NNS usage and, in the future, impact the present recommendations.

Keywords: non-nutritive sweeteners, insulin resistance, gut microbiota

Resumo

Os edulcorantes são elementos cada vez mais comuns na lista de ingredientes dos produtos alimentares. Estão a ser utilizados pela indústria alimentar de forma a evitar o uso de açúcar e, ainda assim, manter o sabor doce que atrai os consumidores, que, apesar de apresentarem cada vez mais preocupação com a saúde, procuram produtos doces. A presença crescente dos adoçantes não nutritivos na nossa dieta leva a que a comunidade científica questione a sua segurança para a saúde. Apesar de haver estudos que demonstram benefícios na utilização destes edulcorantes para a perda ponderal e para o controlo glicémico, existe, também, evidência científica que indica que o uso destas substâncias leva a efeitos contrários àqueles pretendidos. Embora os mecanismos fisiológicos que ligam os edulcorantes ao aumento ponderal e insulinorresistência ainda não sejam claros, há evidência crescente de que tal ligação existe. Pensa-se que a microbiota intestinal possa ser o elo entre o consumo de adoçantes não nutritivos e a obesidade e insulinorresistência, através da modulação da composição microbiológica da mesma e, por consequência, dos seus metabolitos.

Estes estudos podem elucidar-nos quanto aos perigos que derivam da utilização dos adoçantes não nutritivos e, no futuro, ainda ter impacto nas recomendações do presente.

Palavras-chave: adoçantes não nutritivos, insulinorresistência, microbiota intestinal

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Abbreviations

ACC – Acetyl-CoA carboxylase

ADI – Acceptable daily intake

AMPK – AMP-activated kinase

EFSA – European Food Safety Authority

FDA – Food and Drug Administration

GIP – Gastric inhibitor peptide

GLP-1 – Glucagon-like peptide-1

NNS – Non-nutritive sweeteners

PPARγ – Peroxisome proliferator-activated receptor gamma

PYY – Peptide YY

SCF – Scientific Committee on Food

SCFA – Short-chain fatty acid

UCP2 – Uncoupling protein 2

Introduction

When products reach the shelves of the local market, consumers deem them safe, but the additives present in food and drink goods could, later in life, have health damaging effects. This is where organisations such as the EFSA, the FDA and the SCF come in. Additives that must go through a rigorous analysis process before its usage in the food and drink industry are sweeteners. This work will focus on non-nutritive sweeteners (NNS), which provide a sweetening effect without contributing calories to a product. Some NNS are saccharin, sucralose, acesulfame potassium and aspartame (Table 1.).

In the European Union, all sweeteners are exposed to toxicological testing and can be tested several times, leading to new findings and new ADIs.

Table 1. Some non-nutritive sweeteners and their acceptable daily intake as agreed by the FDA and the SCF.

NNS	**ADI FDA[(1)] (per mg/kg/bw)**	**ADI SCF (per mg/kg/bw)**	**E number [a)]**
Saccharin	15	0-5[(2)]	954
Sucralose	5	0-15[(3)]	955
Acesulfame potassium	15	0-15[(4)]	950
Aspartame	50	0-40[(5)]	951

a) E number is a code that represents a food additive in the European Union.

The NNS that will be mentioned throughout this review are saccharin, sucralose and aspartame, of which only the latter is metabolised in humans. Despite the lack

of metabolism, it is not to be assumed that these sweeteners have no impact on the consumers health. There is a growing body of evidence that NNS influence gut microbiota.

Gut microbiota is a term used to describe the bacteria that take up residence in a host's gastrointestinal tract and is, today, considered to have the function of an organ[6, 7]. Alteration of this ecosystem can lead to an imbalance in its metabolism and, consequently its host's. Through the uptake of indigestible carbohydrates, the gut microbiota produces short-chain fatty acids, who also play a role in the host's health[8].

This review will focus on studies that link NNS ingestion with changes in gut microbiota and the onset of insulin resistance and/or type 2 diabetes. Some mechanisms that, in the current literature, have been proposed to explain the link between NNS usage and microbiota shifts and glucose intolerance are explored. Finally, there is also a mention about SCFAs and their possible role in glucose metabolism.

Theme development

NNS' link to obesity and metabolic disease

NNS are substances that are used to attribute sweetness to food and drink, adding little to zero energetic value, while also abiding by the current recommendations for lower sugar intake. There are several studies that investigate the link between NNS ingestion with obesity and metabolic disorders, though there is still no consensus. Sylvetsky et. al. have recently shown, in a randomized clinical trial, that many individuals consume NNS unbeknownst to themselves, through packaged goods and beverages, hence this ever growing exposure should be investigated along with any health risks it might entail[9].

Despite the general idea that artificial sweeteners help control and lose weight, studies such as those conducted by Stellman and Garfinkel, back in 1986, suggest the opposite. Their results from a prospective cohort study which, having followed 78694 women for a year, depicted that those who used saccharin became significantly heavier, compared to their baseline weight[10]. Similarly, in 1990, Colditz, G.A. et. al. reported results of the Nurses' Health Study, indicating a dose-response relationship between the usage of saccharin and weight gain in participants who were followed during 8 years[11]. More recent studies, in humans, tend to use low calorie sweetened beverages to investigate this association and the findings are parallel. Some even present data showing an increased risk in developing a number of diseases in NNS consumers, such as metabolic syndrome, type 2 diabetes and cardiovascular disease[12, 13].

This potential connection has yet to gain relevance due to the lack of a solid physiological model to explain how NNS can promote weight gain and metabolic disorders[14]. Still, three possible mechanisms are suggested as explanations for metabolic dysregulation as a consequence of NNS intake: deregulation of learned responses for energy and glucose control; interference with sweet-taste receptors present in the gut that modulate insulin secretion and glucose absorption; and interference of these substances on gut microbiota induces glucose intolerance[15].

It is established that, in humans, cephalic responses are necessary in order for a normal glucose tolerance to be observed[16]. Cephalic-phase responses are pre-absorptive reflexes, just like hormone secretion and thermogenesis, preparing the gastrointestinal tract for the arrival of nutrients, while maintaining homeostasis[17].

The afore mentioned hypothesis that NNS interferes with learned responses, such as cephalic responses, has not been tested in human subjects. Thus there is no

concrete evidence that artificial sweeteners weaken this mechanism[15]. However, animal studies do provide data that indicate the existence of said interference. Swithers et.al., throughout their research, have suggested the Pavlovian conditioning principles as a basis for the theory that usage of these sweeteners weakens the association between sweet taste signals and caloric intake. This results in the inability of sweet taste being used as a nutritive predictor. In other words, the consequence is a lower energy balance capacity due to lack of physiological responses[14, 18]. The authors found that, in a rat model, there was a significant increase in food intake, followed by weight gain and higher adiposity in the subjects who consumed NNS. These were compared to the control group, who, instead of artificial sweeteners, were privy to glucose in their food and fluids[14].

In a different study, Swithers et. al. also observed that when comparing a control group, whose diet was exempt of artificial sweeteners, with rats consuming water sweetened with NNS, the latter demonstrated relative hyperglycaemia after being subject to a glucose tolerance test. Subsequently, the glucoregulatory response was altered, being associated, by the authors, to decreased incretin hormone glucagon like peptide-1 (GLP-1) plasma levels[19]. Incretins such as GLP-1 and GIP are gut hormones that stimulate pancreatic beta-cells to secrete insulin[20]. Additionally, there was a different response to how the glucose load was administered. When given orally, the afore results were observed; in contrast, when the glucose load was infused directly into the subjects' stomach, there were no significant differences in glucose levels between the control group and NNS group[19]. In this case, the glucoregulatory response might have been spared the previous NNS effect, by bypassing oral sweet taste receptors.

Regarding NNS modulating insulin secretion and glucose absorption through interference with sweet-taste receptors present in the gut, attention has been given to the sweet taste receptor subunit T1R3 along with the taste G protein alpha-gustducin. This is because it is thought that they can be the basis for sugar sensing in the bowels. Jang et. al. conducted a study using a rodent model, whose findings showed that when mice lacked either alpha-gustducin or T1R3 receptor, their incretin response was dulled during a glucose challenge[21]. Therefore, alpha-gustducin and T1R3 receptor are linked to the incretin effect. This last definition has its origin in the 1960s and describes how insulin response is greater when an oral glucose load is taken orally rather than being administered intravenously, even when the loads are measured with the objective of causing equal glucose plasma levels[22]. Similarly, knockout mice for alpha-gustducin demonstrate a disturbed glucose homeostasis not only as a result of glucose tolerance test, but also after feeding on chow[21].

The third hypothesis, mentioned above, on how NNS ingestion might lead to metabolic dysregulations is that artificial sweeteners induce glucose intolerance through the modulation of the host's gut microbiota.

NNS and gut microbiota

The human gut microbiota encodes a substantially larger number of genes than its host and, therefore, has biochemical abilities and metabolic functions that does not coincide with the human organism's capabilities, or surpasses it[23]. Examples of this is vitamin and amino acid synthesis, bile biotransformation and metabolism of oligosaccharides that have alluded digestion, like unabsorbed sugars and alcohols, and polysaccharides that are indigestable, such as pectins, gums, cellulose, hemicellulose and resistant starches[24-26]. This capacity derives from the

60000 glycoside hydrolases and polysaccharides lyases that the human gut microbiota is privy to and is essential for the afore mentioned pathways, seeing as how humans have about 17 of those forms of enzymes[24]. From these functions, a higher energy harvest is obtained. This is because energy and absorbable substrates are recovered for the host and for the gut bacteria themselves, permitting their growth and proliferation[27].

There were early indications that gut microbiota are involved in obesity. In 2005, Ley et.al. found that metabolically obese mice, with a leptin gene mutation, presented significant differences in gut microbiota composition when compared to lean mice. The former had a higher Firmicutes/Bacteroidetes ratio, while Bacteroidetes were favoured in the gut of lean mice[28, 29]. More recently, intestinal microbiota composition has been thought to be involved in the risk of developing insulin resistance, a condition that leads to type 2 diabetes[28, 30]. It might be that an increased monosaccharide uptake, due to differences in the bacterial community in the gut, leads to a higher hepatic production of triglycerides, which is associated with the onset of insulin-resistance[31].

A human case-control study on type 2 diabetes found that bacteria from the phylum Firmicutes and the class Clostridia were significantly reduced in patients with this disease and there was also a decline in butyrate-producing bacteria (see Table 2.)[32]. Furthermore, increase of Bacteroides ssp. was observed in diabetic rats, in an experimental study about the development of type 1 diabetes[31].

Further evidence on this matter has been given by Membrez et. al. when they subjected genetically and diet induced obese and insulin-resistant mice to two weeks of antibiotics, known to suppress cecal bacteria. They found that these rodents displayed improvement in fasting plasma glucose levels and oral glucose

tolerance testes. These improvements were independent from food intake and adiposity, since these factors were equal between the test and control groups[33].

Table 2. Shifts observed in gut microbiota composition in comparison to healthy individuals. Adapted from Clemente Jose C. et. al 2012[34]

Shifts	Increase	Decrease
Bacteria	Betaproteobacteria	Firmicutes
	Bacteroidetes/Firmicutes ratio	Clostridia
	*Bacteroides – Prevotella**	Clostridia coccoides - Eubacterium rectale*

Note - in type 2 diabetes, both the increase and decrease of the mentioned bacteria are observed simultaneously*

There is existing evidence that intake of NNS modulates bacteria population in animal fecal samples (Table 3). Even though NNS are considered inert, that does not rule out the possibility that they interact with the human gut microbiota[35].

In 2014, Suez et. al. shared their findings that indicate that NNS, especially saccharin, strongly affected glycaemic responses in mice and that this metabolic dysregulation was mediated by alterations suffered by the gut microbiota. The rodents were subjected to 11 weeks of exposure to saccharin, sucralose or aspartame and their serum glucose levels were constantly measured. Furthermore, the association with the gut microbiota was made, through fecal transplantation. Germ-free mice, who had never been exposed to saccharin, received fecal microbiota transplant from the rodents who suffered altered glucose response due to their saccharin fuelled diet. They also transferred microbiota incubated in vitro in the presence of saccharin. The outcome of this experiment

was the induction of hyperglycaemic responses to glucose tolerance tests in these germ-free mice after said transplants[36].

Table 3. Impact of some NNS on microbiota; adapted from Suez J et. al. 2015[35]

NNS	**Study subject**	**Increase**	**Decrease**
Aspartame	Rats	Total bacteria (Enterobacteriacease, Clostridiales, Roseburia)	Lactobacilli
Sucralose	Culture and rats		Total anaerobes (Bacteroides, Bifidobacteria), Total aerobes, Lactobacilli
Saccharin	Swine, rats and mice	Total aerobes, Lactobacilli, Enterobacteriaceae, Bacteroides, Clostridiales	Lactobacilli and Clostridiales

This same group of researchers proceeded to study the glycaemic response in humans. Seven volunteers, who did not regularly consume NNS, were subjected to a daily dose of saccharin that matched the FDA's maximum daily intake (Table

1.), during a week. They also performed daily glucose tolerance tests, ingesting 75 grams of glucose. The results suggest that regular saccharin ingestion alters glycaemic response, because most of the subjects showed increased glycaemia. The microbiota fecal transplant was also used with the human subjects and the findings were consistent with those from their animal model, where stools from the human responders induced glucose intolerance in germ-free mice[36]. This study does strongly demonstrate that some NNS change the composition of the host's microbiota and that such alterations can lead to insulin resistance, although the question remains about how this alteration brings about said consequence. Since these sweeteners are seen to modulate the gut bacterial community, it could be that a dysbiotic state resulting from this could also lead to changes in SCFA production.

SCFAs and dysbiosis

Dysbiosis represents an impaired microbiota or, in other words, a disrupted microbiome, and it has been associated with insulin resistance[37]. Gathering from the various studies mentioned in this review, we may conclude that NNS ingestion can, possibly, disrupt the gut microbiota and, consequently, induce insulin resistance and, later on type 2 diabetes. As the bacterial community in a host's intestine shift, so might the production of short-chain fatty acids.

Fermentation of dietary fibres, in the colon, produces metabolites like SCFAs which include, primarily, acetate, propionate and butyrate, who are mostly produced in the caecum and proximal colon and they are found in a faecal molar ratio of 3:1:1, respectively[38, 39]. These metabolites play a role in pathophysiology of obesity and metabolic syndrome since they regulate energy intake, energy harvest and are energy substrates themselves, and therefore influence body

weight[40]. They also inhibit histone deacetylases and activate G-coupled receptors[38]. Additionally, mechanisms that associate SCFAs with type 2 diabetes have been proposed that influence adipose tissue function, lipid storage, inflammatory profile and liver and skeletal muscle energy metabolism[41-43]. This is evidenced by many studies, and an example is the one done by Vrieze et. al.. Here, in a single blinded randomized control trial, obese human subjects were treated with two antibiotics and one of them, vancomycin – attacks gram-positive bacteria – modulated the gut microbiota by decreasing its diversity. This impacted the bile acid and glucose metabolism, since peripheral insulin sensitivity was decreased[44]. Other than that, these same authors, in a double-blinded randomized trial, transferred faecal microbiota from lean donors to individuals with metabolic syndrome and found that there was an increase of butyrate-producing bacteria and improved peripheral insulin sensitivity[45].

Studies such as one conducted by Freeland et. al, in a single-blinded randomised cross-over trial, observed that humans receiving rectal or intravenous perfusions of acetate demonstrated higher serum concentrations of PYY (peptide YY) and GLP-1[46]. In turn, propionate supplementation, when administered to healthy women, reduced fasting glucose levels during oral glucose tolerance tests, as well as increased insulin response[47].

Butyrate is known to be the main energy source for the colonocytes, consequently being the only out of the three SCFAs mentioned to be absorbed locally. Butyrate is the most studied SCFA and, possibly because of that, is the one most linked to health benefits[38]. Such an example is given by Qin et. al. found indication that patients with type 2 diabetes had some degree of dybiosis and decreased number of bacteria who produce butyrate[32].

SCFAs seem to exert benefits and protection of glucose metabolism. Bestens et. al. suggest that these metabolites provide protection against obesity and insulin resistance, when subjects are on a high-fat diet, through downregulation of PPARγ. The authors carried out in vitro and in vivo experiments and they propose that downregulation of PPARγ induced by SCFAs activates a pathway - UCP2-AMPK-ACC – this permits the transition, in adipose and liver tissue, from lipogenesis to fat oxidation, which may reduce body weight and improve insulin sensitivity[41].

Critical analysis and conclusion

The amount of evidence linking NNS consumption, altered gut microbiota and insulin resistance – or type 2 diabetes – is abundant. We must, however, recognize that many studies have their limitations and confounders that warp the findings. The influence of the factors in question, throughout this review, are, in many cases, impacted by the characteristics of the animals or human subjects, such as their sex, diet, genetic predisposition towards obesity and/or type 2 diabetes and, also, their state at the onset of the experiments.

Although the negative impact of NNS ingestion has been emphasized, there do exist numerous studies that show the efficacy of these substances in weight control, though few compare NNS intake with a control group who is not subjected to non-caloric sweeteners. In fact, many studies, actually, rely on the comparison between subjects consuming NNS to those consuming caloric sweeteners[48]. A hypothesis worth mentioning is the possible reverse causality in many studies on NNS and microbiota. This is because human subjects who are already obese or suffer from metabolic syndrome tend to use artificial sweeteners.

The study from Suez et. al. is the most recent about the NNS impact on microbiota and insulin resistance and has the potential to be the stepping stone for further findings. They took their experiment a step farther by subjecting humans to the trials they set on their animal model. However, there is a limitation that can call their results into question. The fact that their human trial lacked a control group for the exposure of saccharin, weakens their conclusions. Because there is no control group, we cannot know if such a group would also display changes in glucose metabolism or even if their stools, transplanted into germ-free mice, would have caused glucose intolerance.

Another study that might warrant attention is the aforementioned trial conducted by Qin et. al.. Although they observed a decline in certain gut bacteria and production of butyrate in patients with type 2 diabetes, they also alert to the fact that the microbiota of said patients only displayed a limited deviation from the control groups. This calls into question whether an altered microbiota plays a role or not in this disease.

In relation to the studies that focus on SCFAs, they tend to measure these metabolites through stool analysis, but it has been shown that SCFAs uptake rather than their caecum concentrations can be correlated with dietary physiological effects[49]. Also related to this topic, long-term exposure to dietary fibre and, therefore, SCFA production has failed to demonstrate benefits on glucose homeostasis[50, 51]. One cannot deny, however, the possibility of a role of SCFA for prevention or counteraction of obesity and insulin resistance, but seeing as how most data comes from animal or *in vitro* studies, the clinical relevance in humans is still not well established.

This chapter is not meant to debunk the evidence mentioned throughout the work, but rather to alert that all and any findings should be interpreted with caution and that there is a need for large scale randomised controlled trials. Biologically relevant doses of sweeteners should be used and the molecular mechanisms should be further investigated so that it can become clearer whether NNS does impact the gut microbiota and if this, through SCFA production or other pathway, can amount to the development of type 2 diabetes.

In conclusion, care must be applied by health professionals when suggesting sucrose to be substituted by NNS. As was mentioned, the food and drink industry are ever more reliant on these substances in order to curtail sugar taxes and health aware consumers. Therefore, in addition to the sweetener applied in homemade drinks, there must be considered the deserts, beverages and snacks that patients will resort to when trying to control their weight and/or diabetes. Another approach might consist on the insistence of taste education, where the patient can lower his/her dependence on sweet tasting foods.

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