A rational review on the effects of sweeteners and sweetness enhancers on appetite, food reward and metabolic/adiposity outcomes in adults

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Abstract

Numerous strategies have been investigated to overcome the excessive weight gain that accompanies a chronic positive energy balance. Most approaches focus on a reduction of energy intake and the improvement of lifestyle habits. The use of high intensity artificial sweeteners, also known as non-caloric sweeteners (NCS), as sugar substitutes in foods and beverages, is rapidly developing. NCS are commonly defined as molecules with a sweetness profile of 30 times higher or more that of sucrose, scarcely contributing to the individual's net energy intake as they are hardly metabolized.

The purpose of this review is first, to assess the impact of NCS on eating behaviour, including subjective appetite, food intake, food reward and sensory stimulation; and secondly, to assess the metabolic impact of NCS on body weight regulation, glucose homeostasis and gut health. The evidence reviewed suggests that while some sweeteners have the potential to increase subjective appetite, these effects do not translate in changes in food intake. This is supported by a large body

of empirical evidence advocating that the use of NCS facilitates weight management when used alongside other weight management strategies. On the other hand, although NCS are very unlikely to impair insulin metabolism and glycaemic control, some studies suggest that NCS could have putatively undesirable effects, through various indirect mechanisms, on body weight, glycemia, adipogenesis and the gut microbiota; however there is insufficient evidence to determine the degree of such effects. Overall, the available data suggests that NCS can be used to facilitate a reduction in dietary energy content without significant negative effects on food intake behaviour or body metabolism, which would support their potential role in the prevention of obesity as a complementary strategy to other weight management approaches. More research is needed to determine the impact of NCS on metabolic health, in particular gut microbiota.

INTRODUCTION

Dietary patterns have changed associated with industrialization and other societal movements[1]. Currently, the overall diet quality and variety has decreased while the caloric content is increasing, thereby contributing to noncommunicable diseases prevalence, including obesity[2], type 2 diabetes mellites (T2DM), cardiovascular disease (CVD), and cancer[3,4]. Obesity rates has planetary risen worldwide by more than twofold in most countries, with more than 1.9 billion adults suffering from overweight and 650 million adults from obesity according to WHO data[5]. To tackle this obesity epidemic, many weight-loss strategies focus on reducing caloric intake and improving dietary habits, thereby inducing a negative energy balance[6]. Although energy intake from sugars is decreasing in some countries, the consumption of added sugar continues to be high[7]. Even though conflicting evidence has been published[8], some harmful effects on dentition, caries, obesity and diabetes incidence have been reported[9]. Also, for this reason, non-caloric sweeteners (NCS) have begun to be increasingly found in the eating habits of consumers, resulting potentially useful in weight control and weight loss[10]. Sugar consumption could create a short-term peak of energy in the body, thereby contributing to the overall energy density of diets and the development of obesity[11–13], which is an effect partly driven by the sugar-induced overconsumption of energy resulting in a positive energy balance[14,15]. In addition, sugar intake increases the risk of developing CVD and T2DM indirectly by promoting body weight and fat deposition[16]. In this context, the use of non-caloric sweeteners and sweetness enhancers seems promising in assisting dietary sugar reduction and weight loss due to their lack of caloric content[17]. Thus, the use of NCS is increasing[18,19], particularly in individuals attempting to control the energy content of their habitual diets[20]. However, there is conflicting evidence regarding the effects of these sweeteners on subjective states and behaviours that influence body weight, including appetite, food intake and

food reward[21]. These outcomes are important when trying to understand energy balance and to identify the effect of NCS ingestion on energy intake whilst maintaining consumer acceptability[22]. Non-caloric sweeteners have the potential to moderate sugar and energy intake while maintaining the sweet palatability. These compounds are generally defined as a substance with a sweetness profile of 30 times or more greater than that of table sugar (sucrose)[23]. Consequently, much smaller amounts are required to achieve the same sweetness intensity, although each sweetener presents a unique intensity, persistence of taste and aftertaste[24]. The American Heart Association categorises all forms of low-calorie, artificial or NCS as non-nutritive sweeteners as they provide no nutritional benefits in the form of vitamins and minerals[25]. The term NCS is often applied to non-nutritive sweeteners as well as bulk sweetening agents such as isomalt and tagatose, which are not sufficiently metabolised to contribute to net energy intake. While the consumption of beverages and foods containing NCS is rising, the controversies surrounding the health effects of sweeteners and sweetness enhancers on human health has been a recurring topic for decades[26]. Longitudinal studies suggest a link between the intake of NCS and obesity and related metabolic disturbances[27–29], however inverse causality cannot be discarded. Moreover, several studies have highlighted a possible cause-effect connection between the intake of NCS and an increase in appetite[30,31], with a correlated increase in food intake, and unfavourable changes in metabolic health, implying the possible onset of problems related to the worsening of insulin secretion, to an accumulation of energy intake with consequent promotion of adipogenesis. However, extensive scientific research has shown that the most common sweeteners, both natural including stevia[32], as well as artificial sweeteners such as acesulfame-K, aspartame, neotame, saccharin and sucralose, are safe in terms of metabolic disturbance, when consumed at moderate and acceptable doses[33,34], whose impact is monitored in Europe by EFSA. Biological and psychological mechanisms have been proposed for explaining these adverse effects[35] including perturbations in eating behaviour, satiety-signalling[36], energy balance, glucose tolerance, microbiota composition, and adipogenesis but so far the mechanistic evidence is mainly based on *in vitro* and animal studies.

The objective of this review is to assess the impact of sweeteners and sweetness enhancers on appetite (eating behaviour) and metabolism/adiposity in healthy subjects as well as in adults suffering of chronic conditions, with emphasis on obesity. Specifically, the impact of sweeteners and sweetness enhancers on the psychobiology of appetite, eating behaviour including subjective food intake, food hedonics/reward, sweet taste perception and the regulation of glucose homeostasis and body weight control was appraised.

METHODS

A comprehensive review was conducted through a rationalized search of the scientific literature to develop a narrative synthesis with a focus on the effect of sweeteners and sweetness enhancers on appetite and metabolism, by analyzing the roles on appetite, metabolic and adiposity markers in adults. Due to the broad thematic field, it was decided to not conduct a formal systematic review, but a structured overview.

Data searching process

A search strategy of published records was driven through MEDLINE, EMBASE, EMBASE CLASSIC and Psychinfo, according to the principles of the Cochrane Handbook for Systematic Reviews of Interventions' guidelines[37].

Keywords related to sweeteners and sweetness enhancers and energy balance, specifically included food intake, subjective appetite, food hedonics, body weight, energy, glucose metabolism/obesity/diabetes and adiposity markers. Study design and testing environment (i.e. lab vs field) were analyzed and distinguished by the presence of sugary products or water (Table 1).

The focus and search pathway (Figure 1) were based on selecting reports characterized by the presence of at least both an intervention group (that means, individuals who receive non-caloric sweeteners in the form of drinks or food), as well as a comparison group (that means individuals who received sugar or water). No restrictions concerning population characteristics or origin were applied, but when available, this information was mentioned (Table 1).

The selection of articles and analysed documents in the current review followed accepted guidelines, whose features are detailed in Table 1. The inclusion criteria for the records, were related to healthy individuals and metabolically healthy obese adults of any sex and age, with no restriction to EU or Caucasian populations. Therefore, studies including animal models or protocols without a comparison or control condition were excluded.

Main metabolic outcomes included eating behaviour, body weight and adiposity and glucose homeostasis/glycaemic control (Table 2). While the former includes food intake, subjective appetite, food hedonics and sweet taste perception, as well as food reward, the latter appraises energy balance, adiposity and weight changes, lipid metabolism and gastrointestinal physiology. In addition, intestinal glucose absorption, microbiome alterations, sensory receptors of insulin secretion, sensitivity to insulin and intestinal inflammation were assessed as a measure of glucose

homeostasis/glycaemic control. To achieve the objectives of the study, both between- and within-subject comparisons were included, to verify not only food intake, subjective appetite, food hedonics, but also body weight, energy, glucose metabolism and adiposity markers.

EATING BEHAVIOUR

Eating behaviour, which involves appetite regulation, food intake control and reward mechanism, haves been related with sweeteners and sweetness enhancers by affecting neural circuits, buccal sensory pathways and diverse biomarkers[38]. Currently, it is important to understand if sweeteners and sweetness enhancers have an impact on appetite and energy intake by verifying whether the use of NCS can promote an increase in appetite or compensatory eating behaviour in response to reduced energy content[39].

Appetite

There is a traditional lack of clarity regarding the effect of NCS use on appetite[40], with some studies highlighting no change in food intake, whereas others demonstrate an increase or a decrease in appetite. Appetite can be measured using subjective ratings (visual analogue scales (VAS) for hunger, fullness, etc.) and/or using blood biomarkers (for example glucose, insulin, ghrelin and other gut peptides)[41]. Early trials demonstrated that there may be a short-lived suppressive effect on subjective appetite ratings upon acute ingestion of NCS (saccharin, aspartame or acesulfame-K), which may be followed by an increase above baseline values[42] – a phenomenon known as rebound hunger - , although further studies challenge this concept. For example, newer data have shown no effect on motivation to eat following regular consumption of a commercially available beverage (aspartame, acesulfame-K and sucralose)[43]. Therefore, it is also important to consider both acute and prolonged exposure effects when analysing NCS impacts and outcomes.

Acute studies have shown that while a glucose load (50 g in 200 ml) suppresses motivation to eat and increases fullness ratings, ingestion of an aspartame load (162 mg in 200 ml) produces depression impairments of hedonic ratings, increasing motivation to eat and decreasing satiety ratings[30]. Another study demonstrated that water sweetened with a 340 mg dose of aspartame resulted in an increase of subjective appetite (hunger, desire to eat, fullness and prospective consumption) relative to an unsweetened water control[44]. In another investigation, 0.44 g of aspartame in 500 ml of water produced higher hunger, desire to eat and prospective consumption ratings relative to a matched-intensity sucrose load (65 g in 500 ml)[31]. However, this subjective response did not result in alterations in food intake at a buffet style meal 65 minutes later as the increase in appetite was short-lived, lasting approximately 30 minutes[44]. On the other hand,

long-term effects on appetite ratings were detected in a study reporting increased mean 24-hour ratings of hunger and desire to eat following daily ingestion of saccharin over a 12-week period[45]. Therefore, concerns remain that some sweeteners have the potential to increase subjective appetite both acutely and following repeated consumption.

The lack of consistency within the scientific literature may be explained by the use of different doses of sweeteners and differences in study designs. For example, a sucralose dose of 500 mg has been shown to increase hunger ratings compared to a sucrose dose of 105 g[46], whereas a dose of 330 mg of sucralose produced lower hunger ratings when compared to ratings provided following water ingestion[47]. Similarly, an aspartame dose of 162 mg has been shown to increase motivation to eat whereas a dose of 320-340 mg decreased ratings of desire to eat[48]. For this reason, it is important to consider the type of non-caloric sweeteners provided as well as the dose and experimental conditions on assessed outcomes related to subjective appetite.

It should be noted that some acute studies investigating the potential effects of NCS on appetite report effects of buccal sweet stimulation, rather than the ingestion of a sweetener *per se*[49]. Evidence demonstrates an appetite inducing effect of oral sweet stimulation when compared to ingestion with no taste stimulation. For example, when examining chewing gum sweetened with aspartame or unsweetened gum, hunger ratings increased in those individuals chewing the sweetened gum compared to those individuals provided with unsweetened gum or nothing[50]. In this way, when oral taste receptors were stimulated but the aspartame was not swallowed and ingested, the outcome was an increase in subjective hunger, whereas the process of mastication lacking a sweet taste did not impact hunger. Similarly, an aspartame dose ingested via a capsule – that is, without a sweet taste – did not result in different appetite ratings compared with a water control[44]. Together, these findings suggest that detection of the sweet taste in the oral cavity can be sufficient to increase appetite, without ingestion of the sweet substance. This phenomenon may be explained by cephalic phase responses (CPRs), which are innate and learned physiological response to sensory signals preparing the G.I. tract for optimal digestion. For example, CPRs may initially increase the perceived palatability of sweet foods and allow for the ingestion of larger portions[51]. However, this phenomenon applies mainly to nutritive sweeteners, as opposed to NCS, as these do not stimulate the same insulin response (see below under Glucose homeostasis).

Regarding blood biomarkers of appetite, in acute studies nutritive sweetener ingestion consistently produces significantly increases in plasma glucose and insulin levels compared to NCS[52], with increased glucose and insulin concentrations starting 5–10 min after the onset of ingestion[53] and with higher concentrations by 30 min[54]. The phenomenon was also evaluated in

repeated consumption studies, where glucose and insulin levels increased by 0.24 ± 0.09 mmol/l and 11.8 ± 4.9 pmol/l respectively after 10 weeks of consumption of sucrose-based drinks and foods. This resulted in higher glucose and insulin values than the group consuming NCS (between 0.09 ± 0.15 mmol/l and -1.2 ± 3.2 pmol/l for glucose and insulin, respectively)[55]. Although glucose is not strictly a satiety biomarker, it plays a role together with insulin, in the cephalic phase satiety response and may modulate the hunger response, where ghrelin/leptin may play a role[56–58].This situation generates concern surrounding the ingestion of sugar-sweetened beverages due to their potential to reduce insulin sensitivity following repeated consumption[16]. A sucrose-rich diet is known to contribute to insulin resistance and consequently the satiating effect of insulin may be lost following chronically elevated levels of plasma insulin[59,60]. Non-caloric sweeteners, however, possessing negligible energy, may not present the same risks in impacting blood glucose levels and therefore overall glycaemic effects[52,61,62] and may allow for wider food choice for those seeking to control energy intake whilst maintaining food palatability[63].

Specific hormones and neuropeptides may mediate appetite functionality[64]. In an acute study, administration of sucralose (62 mg), aspartame (169 mg) or acesulfame-K (220 mg) did not result in any alterations in plasma insulin – nor glucose or glucagon. However, as a relatively low dose of sucralose was used, in a similar acute study, a 330 mg dose of sucralose produced a small yet significant decrease in insulin levels below baseline[47]. This finding illustrates the effect of varying doses of NCS on appetite-related biomarkers, which may partially explain differences across studies. Regarding long-term exposure, daily consumption over 12 weeks of a beverage sweetened with a blend of aspartame (129 mg) and acesulfame-K (13 mg) did not significantly impact insulin sensitivity or secretion[65]. Taken together, these findings suggest that even with varying doses and types of NCS, there appears to be little impact on insulin release and sensitivity in both acute and repeated consumption trials, suggesting that their regular consumption may be a viable alternative to sugar-sweetened beverages.

There is limited evidence for effects of NCS on other appetite-related peptides. The GLP-1 response is greater following acute consumption of sugar-sweetened beverages of varying energy contents (103-215 kcal)[66,67] than beverages using NCS of little (1.7 kcal)[66] or no energy content[67]. This response is also the case following repeated consumption[55] (dose dependent on body weight). Similarly, following a 60 mg sucralose preload, plasma GLP-1 levels did not significantly increase, whereas ingestion of 40 g of glucose resulted in a prompt increase in GLP-1, as described elsewhere[68]. This finding suggests that GLP-1 responds to nutritive sweeteners, whereas sweeteners absent of calories do not influence secretion. However, sucralose ingestion at a dose of 24 mg (absent of energy) in addition to a 75 g oral glucose tolerance test (~307 kcal), resulted in a

significantly higher AUC GLP-1 response in the sucralose condition compared to water[69]. Interestingly, the response to aspartame (72 mg) was not found to be different to the water condition. Sucralose therefore enhanced GLP-1 release in the presence of glucose, reinforcing that GLP-1 release occurs in response to energy, but also suggesting that sucralose provided in conjunction with energy, may result in a higher GLP-1 response. Overall, the evidence on GLP-1 demonstrates differential effects between non-caloric sweeteners types. Consequently, caution must be taken when drawing conclusions due to unsolved interactions. Certain non-caloric sweetener administration appears to result in a lower GLP-1 response than with nutritive sweeteners such as glucose, but when the NCS is combined with a nutritive sweetener there may be an additional effect on GLP-1 release.

Comparable results have been reported when examining other appetite-related biomarkers. For example, gastric inhibitory peptide (GIP) and C-peptide levels were not significantly different from fasting levels following ingestion of sucralose at varying doses[61]. Similarly, increases in GIP were only observed following ingestion of nutritive sweetener preloads (30 mg and glucose), whereas following ingestion of a sucralose or blend of tagatose and isomalt preloads, there was no observable difference from fasting values[68].

A further study showed that the intragastric intake of acesulfame-K dissolved in 250 ml of water was able to stimulate a greater secretion of ghrelin and lower, nearly undetectable, production of CCK compared with equivalent solutions of fructose and glucose (dissolved in 250 ml of water)[67]. Furthermore, an intragastric infusion of NCS such as aspartame (169 mg), acesulfame-K (220 mg), sucralose (62 mg) dissolved in 250 ml of water, do not affect the levels of PYY and ghrelin when compared with a glucose solution (50 g)[52].

From these findings, it can be proposed that NCS do not impact appetite-related biomarkers in the same manner that nutritive sweeteners do, due to the lack of energy content, which ultimately relates to the chemical structure of each compound (Table 3).

From the previous evidence, it would appear that NCS ingestion increases subjective appetite, which may be related to sensory stimulation (sweet taste), with a limited impact of NCS ingestion on appetite-related biomarkers. Further research is required to distinguish the impact of energy and sweetness, but also differences between dose and sweetener type need to be assessed (Table 3). Subsequently, the influence of NCS on food intake and the possibility of using them to reduce or replace the intake of free sugars, remains to be determined. Additional studies are also required to investigate the association between the consumption of NCS and sweet food cravings (and associated potential overconsumption).

Food Intake

Free living food intake usually relies on self-report methods such as retrospective dietary recall or food diaries in order to obtain information regarding participant's habitual dietary intake patterns[70]. Generally, the sweet taste is indicative of an ample energy source[71] and is an extremely potent phenomenon including a powerful hedonic drive capable of driving food seeking behaviours and consumption[72]. At present, it is unclear if this remains true when the associated energy content is removed, as the human brain has demonstrated through neuroimaging studies to discriminate between nutritive and non-nutritive sweet tastes[73–75].

In general, intervention studies have shown that beverages containing NCS have at least a comparable effect on energy intake to water[76,77]. For example, some acute studies have failed to identify differences in energy intake following consumption of nutritive sweeteners (sucrose or glucose) or NCS (aspartame) in liquid or solid form during a test meal[44,78,79]. A preload of 0.25 grams of aspartame in 500 ml of lemon flavoured water was not able to significantly stimulate subsequent food intake compared with plain water[78],In addition, the results of lemonade preloads (20 g of fresh squeezed lemon and 200 g of water) sweetened with sucrose (8/16 oz) or aspartame (8/16 oz) do not support the hypothesis that NCS increase energy intake and that they impact on subsequent food choice[79]. However, generally a sucrose compared to sucralose load reduced the subsequent intake of a test meal in whatever viscous form was provided (drink, jelly and candy). Thus, a reduction in the energy intake of the test meal following sucrose and sucralose preloads in female participants, compared to aqueous preload, was found[46]. A review by Bellisle and Drewnowski points out that although NCS drinks may promote weight loss, they are not found to suppress appetite[17]. Indeed, available results should be analysed with care since a repeated exposure and acute consumption alone vs. with a meal, may influence the outcomes. These responses would suggest that sweet beverages – sweetened via either sucrose or sucralose - had a suppressive effect on energy intake in the test meal in female participants. Despite this observation, when the energy content of the beverage preloads was included alongside the energy intake of the meal, it resulted in an elevated total energy intake for the sucrose condition only[46]. That is, the energy from the sucrose was not compensated for. This evidence would suggest that a sucrose sweetened beverage is capable of reducing a single meal energy intake, but the energy content of the beverage will result in a higher net energy intake than if the beverage was sweetened using a non-caloric sweetener. Moreover, in a study which provided participants with either a high or low calorie food option – with the energy density manipulated through the use of nutritive or NCS– both conditions demonstrated a suppressive effect on hunger, yet there was no difference observed between conditions regarding total energy intake across the day[79]. Taken together, these data suggest that

ingestion of a NCS may result in a reduction in energy intake at the following meal; however, when assessed by daily energy intake there seems to be no clear effects.

Furthermore, in a repeated consumption trial which utilised commercially available beverages over a 4-week intervention period, no difference in self-reported energy intake (7-day diary) was found between commercially available regular or diet beverage conditions[80]. However, this approach relied upon the accuracy of information obtained via the 7-day diary and cannot be used to establish causation due to the disparity and inaccuracy of the collected data. This evidence is contrasted by a 10-week intervention in which participants consumed supplements consisting of sucrose or NCS via a variety of different commercially available products. Within this study it was found that NCS consumption did not stimulate carbohydrate intake; in addition, intake of sucrose and carbohydrates decreased voluntarily across the intervention period[81]. This finding is supported by other long-term trials.In a study looking at the effect of sucrose consumption on inflammatory markers, compared to NCS (a blend of 54% aspartame, 23% cyclamate, 22% acesulfame-K and 1% saccharin) within a diet[82], overweight adults followed a diet containing predominantly drinks with sucrose or NCS for 10 weeks. At the end of this period,, the NCS group decreased weight while the sucrose group gained weight, with inflammatory markers also increasing[82]. Other trials comparing repeated consumption of high fructose corn syrup and aspartame[83], also demonstrated a higher energy intake in the high fructose group. The elevated energy intake observed in sucrose-sweetened diets can be explained by the energy content of sucrose provided via the dietary intervention, as when this energy is removed from the analysis there is no longer a significantly elevated intake of sucrose[81]. This evidence suggests that the use of NCS obtained via various commercially available products may be sufficient to reduce energy intake, particularly by reducing the intake of free sugars. This outcome is particularly relevant given that a large portion of the European population fails to meet the current World Health Organisation (WHO) recommendations to limit free sugars intake to less than 10% of total daily energy intake[84]. However, as a number of long-term studies utilise commercially available products, distinguishing the effects of different NCS, doses or blends remains a challenge.

Food Reward

Common methods of assessing food reward involve the use of self-reported questions (for example a VAS assessing liking or pleasantness), behavioural tasks or neuroimaging techniques (for example fMRI scans whilst being presented a stimulus). These methods can be used both in acute and long-term studies comparing baseline scores to post-intervention scores. It has been suggested that NCS stimulate a preference for sweetness, encouraging sugar cravings precisely because they are sweet[85] and it has also been established that repeated exposure to a specific flavour promotes an

increased preference[86]. In contrast, others have suggested that consumption of a certain taste reduces preference for that taste via an increase of sensory-specific satiety. However, this effect has been shown to be stronger for savoury than sweet tastes[87]. Given that the hedonic value of food is a powerful driver of future food intake[62,72], it is important to understand any impact of NCS on food reward. Additionally, it is necessary to distinguish between the rewards elicited from ingestion of a stimulus from the potential impact on food reward later in the day.

Given that apparently the human brain is capable of discriminating nutritive and non-nutritive sweetness[88], it is important to distinguish the impact of caloric vs. NCS on food reward. Acute ingestion of glucose (23 g) or fructose (23 g) loads produced significant decreases in Blood Oxygen Level Dependent (BOLD) signalling in regions involved in reward – cingulate cortex, insula and basal ganglia – whereas, a sucralose (50 mg) or allulose (23 g), with similar sweetening power than glucose and fructose, load had no effect on BOLD signalling in these regions during ingestion[62]. This evidence would suggest that the hedonic properties of sweetness may be closely linked to the associated energy content of sweet foods, rather than sweetness *per se,* where allulose and sucralose have similar sweetening power than glucose and fructose. However, subjective pleasantness ratings in response to oral stimulation (not ingested) using a sucrose solution did not differ to those provided following ingestion of an aspartame sweetened solution (234 mg) as reported elsewhere[89]. Taken together, this response would suggest that either sweetness is rewarding neurologically due to the associated energy content[90], or the energy content itself is rewarding, and that sweetness is subjectively rewarding regardless of energy content. This finding is supported by a comparable study, which revealed that a glucose load (50 g) led to immediate activation in the ventral tegmental area (VTA), with fructose (50 g) displaying a delayed response, in part due to a longer digestion time, while the effect of sucralose (330 mg) was comparable to that of water[47].

It has also been reported that hedonic properties may differ between types of NCS[31]. Using a number of subjective scales, participant's overall liking ratings provided in response to beverages sweetened using aspartame (440 mg) and sucrose (65 g) are similar; however, responses to both were significantly greater than those provided for beverages sweetened with monk fruit extract (630 mg) and stevia (330 mg)[31]. For this reason, care must be taken when drawing conclusions surrounding the hedonic properties of various NCS, as the reward elicited during ingestion may not always be comparable among sweetener types.

Furthermore, a recent study highlighted that following ingestion of a sucralose-sweetened beverage (4 g) - contrasted to a sucrose-sweetened beverage (31 g) - the motivation to gain access

to sweet snacks turned out to be greater relative to savoury foods[91]. However, this motivation may be affected by cravings for sweet taste in certain individuals. In fact, availability of NCS products may actually result in reduced calorie consumption compared with availability of only sugar-sweetened products amongst frequent consumers of NCS products[92]. Thus, sweetness in the absence of energy may lead to some individuals seeking sweet tasting foods; however, it is important to note that this may not always result in increased consumption; and individuals with elevated cravings for sweet taste may benefit from access to NCS products. The literature regarding any changes in food reward after consumption of NCS is currently not well understood and therefore further work is required to draw firm conclusions[93].

WEIGHT AND ENERGY METABOLISM REGULATION

Sweetener and sweetness enhancers consumption may influence fuel homeostasis and weight gain, affecting inflammation, adipogenesis and microbiota composition, where glucose metabolism and insulin regulation have been involved in addition to the impact on eating behaviour[94,95].

Body Weight and Composition

The evidence regarding the effect of NCS on body weight is presently unclear with some studies showing reductions in body weight with use of NCS while others reported no changes[96]. It is important to understand the impact of regular consumption of NCS on body weight as recent evidence has identified their use to be motivated by weight management goals[97], with a large proportion of habitual consumers being those with overweight or obesity, or individuals that regularly exercise and diet[98].

There are some additional trials revealing reductions in body weight following NCS consumption compared to increases in body weight following consumption of nutritive sweeteners (primarily sucrose)[55,83]. For example, at the end of a 4-week intervention comparing diets supplemented with commercially available beverages (250 ml 4x daily), sweetened with either sucrose or aspartame, there was an increase in body weight in the sucrose condition[99]. This finding is supported by a longer 10-week intervention where reductions in fat mass were observed following a diet using NCS compared to a sucrose-sweetened diet[81]. Furthermore, increases in overall body weight have been shown following a sucrose-sweetened diet relative to a diet composed of reformulated food items using NCS[82].

The change in body weight has been speculated to be due to the differences in energy content of nutritive versus non-caloric sweeteners. Thus, following a 6-month dietary intervention whereby participants consumed regular cola, diet cola or water, there were increases in total fat mass, visceral fat, liver fat, serum triglycerides and serum total cholesterol following regular cola consumption, whereas those in the diet cola condition demonstrated reductions in total fat mass that were comparable to the decreases produced with water consumption[66]. Such evidence indicate that commercially available non-nutritive products sweetened using NCS are comparable to water in their effects on body weight[43]. Subsequently, it is possible that NCS may be used to facilitate a reduction in body fat whilst maintaining a palatable diet.

There is also data demonstrating no change in body weight though. For example, a 12-week cross-over intervention in which participants consumed daily either two 330 ml servings of beverage sweetened using a blend of aspartame (129 mg) and acesulfame-K (13 mg) or water, failed to demonstrate significant reductions to waist circumference, body weight or BMI in either condition[65]. These findings would suggest that NCS consumed regularly have no impact on body weight; however, it also highlights that their effects on body weight are comparable to those of water. In a similar cross-over study which employed the use of regular sugar or sugar-reduced foods and beverages for 8 weeks, no differences in body weight or body fat percentage were found in a sample of healthy normal weight individuals[100] . Examination of energy and macronutrient intake identified that this was due to energy compensation. When individuals consumed the sugar-sweetened foods, the added energy from the intervention products displaced protein and fat[101]. When participants consumed the sugar-reduced items, carbohydrate intake declined, and protein and fat intake increased. Additionally, in a sample of adults with overweight or obesity, replacement of caloric beverages with water or diet beverages resulted in significant reductions to body weight and waist circumference, although there were no differences between diet beverage and water conditions[102]. These findings support the recent report provided by Bonnet and colleagues[65], demonstrating comparable effects between NCS beverages and water. The disagreement between studies in the effect on body weight may be explained by the population's baseline BMI. Thus, in Bonnet et al (2018)[65], the mean BMI was 24.7 kg/m^2 and in Markey et al (2016)[100] it was 23.5 kg/m^2 – both samples were healthy weight individuals. The sample in the Tate et al study (2012)[102] however presented a mean BMI of 36.3 kg/m^2. From these differences, it can be hypothesized that replacement of caloric beverages with NCS beverages produces weight loss that is comparable to water in individuals with overweight or obesity, but not individuals with a healthy weight.

To summarise, examination of the evidence and consideration of the differences in methodology and study populations used points towards a modest reduction in body weight

following non-caloric sweetener consumption, compared to increases in body weight following a sucrose-sweetened diet[103]. As supported by the systematic review and meta-analysis of randomised controlled trials examined by Laviada-Molina et al.[10], body weight/BMI differences were evident, and favouring NCS consumers (-1.27 kg and -0.08 kg/m^2). In addition, this reduction in body weight was more pronounced particularly in participants with overweight and obesity, rather than healthy weight individuals[10].

Glucose homeostasis: mechanistic evidence

Carbohydrate metabolism related to glucose uptake, insulin secretion, inflammation, adipogenesis may be affected by dietary sugar and sweeteners intake[104], where some pioneer studies were carried out in in vitro animal models[105–109]

Intestinal glucose absorption

Upon non-caloric sweetener intake, sweet-taste receptors, located in the enteroendocrine L and K cells, are able to detect the sweet compound[105]. Sweet-taste receptors are involved in intestinal glucose absorption in mice by modulating the expression of sodium-dependent glucose transporter isoform 1 (SGLT1) and glucose transporter 2 (GLUT2), which is also stimulated by SGLT1, to the intestine[106–108]. In turn, SGLT1 stimulates the secretion of GIP and GLP1 in mice[108,109] . Notably, these effects were found for acesulfame-K and saccharin, while not for aspartame as mice do not sense it as sweet, thereby not acting on sweet-taste receptors[106,110]. Furthermore, NCS, acting on sweet taste receptors on enteroendocrine GLUTag cells, were found to stimulate the secretion of incretins implicated in SGLT1 upregulation[106]. These data underline that NCS are able to increase intestinal glucose absorption, and in turn, stimulate gut hormone secretion, via sweet-taste receptors, thereby regulating postprandial hyperglycaemia in mice. Nevertheless, to date no differences in intestinal glucose absorption in humans have been reported. Insufficient research has been devoted to the regulation mechanisms involved in glucose metabolism after NCS administration in humans, but some artificial sweeteners may elicit incretin secretion and activate intestinal glucose absorption through TIR2/3 receptors[111]. Therefore, additional investigation concerning effects of NCS on glycaemia are needed[112].

Insulin secretion

Different doses and types of NCS appear to have little impact on insulin release and sensitivity in acute and repeated consumption trials. Cross-over studies showed no early rise in insulin concentration upon NCS intake in healthy subjects, while this response was found upon intake of natural sugars[53]. Furthermore upon natural sugar intake, the secretion of incretins, in turn, is able to stimulate the β-cells of the pancreas to secrete insulin[113]. As the secretion of incretins is nutrient-dependent, NCS are not able to stimulate the secretion of insulin via incretins[52,114,115]. Nevertheless, insulin secretion is stimulated upon the interaction of NCS with sweet-taste receptors in isolated pancreatic β-cells of mice[116,117]. Consistent with the data on intestinal glucose absorption, this outcome was not found for aspartame as it is not very appealing to rodents whose attraction to the taste of aspartame appears to be low[110,118] as compared to humans. Regarding insulin levels, results in human trials are inconsistent so far. Three studies identified no effect on fasting insulin concentrations after acute or longer-term (1 – 16 weeks) intake of NCS in healthy subjects nor those with diabetes, overweight, or obesity[52,119–121]. However, another study, where participants were required to rate the sweetness and palatability of sucrose or sucralose preloads in either beverage or solid form (gelatin cubes), detected a raise in the cephalic phase insulin response (CPIR) in a sub-set of subjects with overweight and obesity, especially after the solid form[122]. However, this response was short-lived given it was part of the CPIR (2 min). Two other studies showed an increase in insulin levels after acute or long-term (4 weeks) intake of NCS in the form of a water solution, capsule, or diet beverage compared to either water alone, placebo (unspecified), or carbonated water in healthy subjects or those with obesity[61,123]. Notably when replacing the diet or carbonated water beverage with a water solution, no difference in insulin levels was found after consuming water with sucralose compared with water[61]. This indicates that the ingredients within the diet soda or the associated taste may affect the insulin secretion and not the sucralose content per se. Of the two studies showing an increase in insulin levels after NCS intake, one study indicates a decrease in insulin clearance rather than a decrease in insulin secretion, as the insulin secretion remains unaffected[123]. Taken together, the overall human data suggests that NCS do not affect total insulin levels or do not stimulate insulin secretion to the same extent as natural sugars, although the chemical structure may be involved[112]. On the other hand, the CIPR may be impacted but only in certain populations, with likely negligible effects on appetite and food intake[122,124].

Microbiota, body weight control and glucose homeostasis

An important component of metabolic health is the gut microbiome as it plays an important role in metabolic functions and energy balance[125]. In general, a healthy diet, composed of a high intake of fruit, vegetables, fibres, and fish, and a low intake of sugar, is associated with a richer and more diverse gut microbiome[126]. Upon reaching the gut, NCS are able to modulate the ratio and

diversity plus functions of the microbiota, where neuroendocrine effect may be involved[127]. However, not all NCS will reach the microbiota as they follow different metabolic pathways within the body. For instance, neither aspartame or its metabolized components (aspartic acid, phenylalanine and methanol) reach the colon as these are metabolized in the small intestine and rapidly absorbed into the blood stream[128,129]. In contrast, steviol glycoside encounters the microbiota directly as it is degraded by it[130]. Acesulfame-K, saccharin, and sucralose are not metabolized and are absorbed or excreted directly into the faeces in their intact form, being thereby able to reach the microbiota and to elicit bacteriostatic effects[131–134]. Although acesulfame-K is not metabolized, it has been suggested that it is unlikely for this NCS to reach the lower gastrointestinal tract due to a rapid absorption upon normal adequate daily intake and dosage[135].

The intake of NCS, that are able to reach the lower gastrointestinal tract in their intact form, may cause dysbiosis of gut microbiota, with a microbial imbalance or maladaptation of the gut microbiota[136]. Non-caloric sweeteners such as aspartame[137] and others[105] were found to be associated with increased dysbiosis and impairments on the Firmicutes: Bacteroidetes ratio in studies involving individuals with morbid obesity[138], metabolic syndrome[105] or NAFLD[139]. Consistently, Suez et al. demonstrated that NCS are able to induce glucose intolerance in mice and distinct human subgroups by altering the gut microbiome[140]. Saccharin consumption (5 mg/kg/d) for one week was found to increase glycaemic response in 4 of the 7 subjects, clustered as ‘responders’, while no response was found in the ‘non-responders’[140]. Notably, the gut microbiota composition was already distinct prior to saccharin consumption between ‘responders’ and ‘non-responders’, thereby indicating that the gut microbiota may predict susceptibility to NCS. Furthermore, in that study it was demonstrated that saccharin was able to increase the Firmicutes: Bacteroidetes ratio in the gut microbiome of mice, resembling that of individuals with obesity[140]. Along with compositional change, fermentation of glycans was increased, resulting in an increase in short chain fatty acids (SCFA). The authors proposed that an increase in SCFA may promote energy harvest and a positive energy balance as the capacity to extract energy is enhanced[141]. However, human studies indicate a positive or preventive role of SCFA in body weight-and glycaemic control by modulating energy and substrate metabolism, eliciting beneficial effects on hepatic fat and adipose tissue function, and in turn, improving body weight control, insulin sensitivity, and reducing ectopic fat[142,143]. Moreover, human evidence for non-caloric sweetener-induced alterations in microbiota is scarce and in some cases the sample sizes utilised have been small[140]. As more research emerge, the effects of NCS on gut health may become clearer. A recent study with 17 healthy subjects demonstrated that daily repeated consumption (14 days) of pure aspartame or sucralose in doses

reflective of typical high consumption have minimal effect on gut microbiota composition or SCFA production[144].

Whether NCS perturbate the microbiota composition and whether the resulted dysbiosis increases SCFA production in larger populations remains to be determined. In addition, the role of energy harvest in human energy balance is of uncertain significance, whilst SCFA have been associated with overall positive health effects in human studies[142].

Microbiota, inflammation and adipogenesis

Upon non-caloric sweetener-induced gut microbiota dysbiosis, metabolic endotoxemia and the development of insulin resistance occurs. Dysbiosis can disrupt the mucosal integrity of the intestinal barrier, leading to the translocation of endotoxins, including lipopolysaccharide (LPS), from the gut into the circulation[145–148].

Mice studies have shown increased LPS concentration, by gut microbiota modulation, and/or increased inflammation upon consumption of NCS, including saccharin, acesulfame-K, and sucralose[133,147,149,150]. In contrast, steviol glycoside was found to suppress inflammation by regulating the expression of TLR2 and cytokine production by affecting NF-κB signalling pathways in mice and Caco-2 cells[151,152]. Hence, not all NCS have the same metabolic impact mediated by the gut microbiota due to being involved in different metabolic pathways has described elsewhere[153].

As NCS have been associated with weight gain, it remains to be determined whether they may affect adipose tissue function and adipogenesis since sweet taste receptors are also expressed in adipose tissue[154]. Saccharin and acesulfame-K enhance adipogenesis and reduce lipolysis by stimulating Akt and downstream targets involved in adipogenesis and by suppressing hormone-sensitive-lipase phosphorylation, respectively, in mouse adipocytes[154]. Nevertheless, the results were found independently of T1R2 or T1R3 expression. Likewise, another *in vitro* study found an increase in fat accumulation and adipogenesis upon stimulation with sucralose in human mesenchymal stem cells[155]. In contrast, Masubuchi et al. showed reduced adipogenesis upon saccharin or sucralose stimulation in 3T3-L1 cells[156]. Whereas *in vitro* data show inconsistent results, *in vivo* studies are largely lacking.

Non-caloric sweeteners, obesity and type 2 diabetes mellitus (T2DM)

The awareness of the harmful effects of eating too much sugar has contributed to the increasing use of NCS. Undoubtedly, replacing sugars with NCS reduces the energy density of diets contributing thus to reduced dietary energy. Besides the lack of calories, NCS do not contribute to blood glucose levels directly unlike natural sugars[157]. However, whether reduced energy density and

carbohydrate content of the diet translates into improved body weight- and glycaemic control is still debated (Table 4). Evidence from prospective cohort studies suggest that frequent consumers of NCS are at increased risk of excessive weight gain, metabolic syndrome, and T2DM[158]. Similarly, as reported in the review by Carocho et al.[8], systematic reviews and meta-analyses, based on prospective cohort studies, showed an association between NCS and an increased incidence of T2DM, independent of adiposity[159]. However, the majority of systemic reviews and meta-analyses, based on RCTs and prospective cohort studies in healthy and diabetic individuals, showed no relationship between NCS and the risk of developing T2DM[159]. Furthermore, meta-analyses of RCTs showed no significant difference in body weight change between overweight and lean individuals after consumption of NCS (<6 months) compared to natural sugars or placebo (cellulose)[160]. Regarding long-term RCTs, one meta-analysis showed no effect on weight change after non-caloric sweetener consumption for 6 months or longer compared to sugar or water in obese individuals, whereas another meta-analysis showed reduced body weight after non-caloric sweetener consumption (4 weeks to 40 months) compared to sugar or water in overweight and lean individuals[27,77]. Thus, whereas prospective cohort studies suggest that NCS increases the risk of obesity, evidence from meta-analyses, based on RCTs, suggest that NCS do not contribute to obesity and may even be beneficial in body weight control. Part of this controversy may be related to reverse causality, that is, individuals who suffer from overweight or obesity typically resorts to the consumption of NCS in an attempt to manage or control their weight[77]. Thus, a key question to be clarified is whether NCS have a real effect on the risk of developing T2DM, or it is the inverse causality which is the real cause (Table 4).

CONCLUSIONS

While some consensus exists on the potential benefits of NCS to reduce net energy intake and assist in weight management, the mechanisms by which NCS impact on eating behaviour, glucose homeostasis and body weight control remain complex and not fully understood (Figure 2). NCS are linked to appetite, on which food intake and reward depend, and metabolic health, with connections to insulin secretion, energy expenditure and glucose homeostasis. As a whole, the available data suggest that NCS have positive inputs concerning food intake/appetite, food reward and hedonic oral perception, which may benefit a reduction in dietary calories and body weight control. On the other hand, methodological differences may contribute to disagreement in study findings, concerning unexpected adverse effects of NCS on body weight- and glycaemic control via various indirect mechanisms, including effects on gut microbiota, adipogenesis, and glucose homeostasis mainly based in animal models. Despite some research suggesting that the ingestion of

non-calorie sweeteners is related to an increase in food intake for a limited period of time, probably due to the sweet taste in the mouth, further research is needed to distinguish the impact of energy and sweetness interactions. Furthermore, it is unlikely that NCS affect total insulin secretion, and thus glycaemic regulation, as the majority of clinical studies in humans showed no relevant metabolic effects.

Despite some mechanistic evidence in mice, some meta-analysis of RCTs show no effect on glycaemic control or body weight control, whereas other meta-analysis even show a positive effect on body composition [77,159]. Moreover, *in vitro* data regarding the effects of NCS on adipogenesis remain still inconclusive.

NCS effects on human gut microbiota have not yet been clarified and whether effects are linked to an increased energy harvest from the diets or negative effects on insulin sensitivity and metabolic health.

Equally, it is necessary to establish evidence around particular sweeteners more specifically, rather than NCS as a whole[161]. This is an important requirement given the increase in the consumption of NCS in individuals motivated by weight loss goals, as well as the diverse food environment that is currently available to these individuals, including a wide range of products with a wide range of NCSs. Hence, more clinical studies are needed to confirm and expand the existing *in vivo* and *in vitro* data in humans. No concluding findings were achieved from studies combining in parallel measurements of appetite/metabolic outcomes are available; therefore, there is a gap in knowledge that should be addressed in future research. Notably, most systematic reviews and meta-analyses of RCTs in humans show no or a beneficial effect of NCS on body weight control and glucose homeostasis. Taken together, the evidence suggests that NCS may be used to facilitate a reduction in energy content in the diet without compensatory increases in appetite or food intake therefore potentially contributing to weight loss. The impact of NCS on the human gut microbiota remains to be established but potential health effects on appetite and metabolism needs to be investigated.

SUMMARY POINTS

1. The use of NCS as sugar substitutes is rising among individuals with the aim of controlling energy intake and body weight owing to eventual effects on appetite, although some studies show no change in food intake, while others show an increase or decrease in appetite following consumption of NCSs

2. NCS use appears to be subject to controversy regarding their metabolic health effects, despite wide application, which needs to be investigated paying attention on putative effects on microbiota
3. Evidence associate NCS with an increased incidence of T2DM, which has been attributed to a reverse causal effect, since NCSs do not contribute to obesity and may also be helpful in controlling body weight and hyperglycaemia as they facilitate carbohydrate intake reduction
4. Non-caloric sweeteners do not appear to impact insulin levels or stimulate insulin secretion to the same extent as natural sugars, which makes them good candidates as co-adjuvants in the dietary treatment of diabetes and associated complications
5. Non-caloric sweeteners can be used to facilitate a reduction in dietary energy content without compensating for the reduced intake via increased appetite or actual food intake, thereby potentially contributing to weight loss

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REFERENCES

1. WHO, The world health report 2007 - A safer future: global public health security in the 21st century, https://www.who.int/whr/2007/whr07_en.pdf, (accessed September 2020)
2. González-Muniesa P, Mártinez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, et al., Obesity, *Nat. Rev. Dis. Prim.*, 2017, **3**, 17034.
3. Grosso G, Bella F, Godos J, Sciacca S, Del Rio D, Ray S, et al., Possible role of diet in cancer: Systematic review and multiple meta-analyses of dietary patterns, lifestyle factors, and cancer risk, *Nutr. Rev.*, 2017, **75**, 405–19.
4. Ley SH, Hamdy O, Mohan V, Hu FB, Prevention and management of type 2 diabetes: Dietary components and nutritional strategies, *Lancet*, 2014, **383**, 1999–2007.
5. WHO, Obesity and overweight, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight, (accessed September 2020).
6. Hill JO, Wyatt HR, Peters JC, Energy balance and obesity, *Circulation*, 2012, **126**, 126–32.
7. Welsh JA, Sharma AJ, Grellinger L, Vos MB, Consumption of added sugars is decreasing in the United States, *Am. J. Clin. Nutr.*, 2011, **94**, 726–34.
8. Carocho M, Morales P, Ferreira ICFR, Sweeteners as food additives in the XXI century: A review of what is known, and what is to come, *Food Chem. Toxicol.*, 2017, **107**, 302–17.
9. Latti BR, Kalburge J V., Birajdar SB, Latti RG, Evaluation of relationship between dental caries, diabetes mellitus and oral microbiota in diabetics, *J. Oral Maxillofac. Pathol.*, 2018, **22**, 282–3.
10. Laviada-Molina H, Molina-Segui F, Pérez-Gaxiola G, Cuello-García C, Arjona-Villicaña R, Espinosa-Marrón A, et al., Effects of nonnutritive sweeteners on body weight and BMI in diverse clinical contexts: Systematic review and meta-analysis, *Obes. Rev.*, 2020, **21**, 1–13.
11. Powell ES, Smith-Taillie LP, Popkin BM, Added Sugars Intake Across the Distribution of US Children and Adult Consumers: 1977-2012, *J. Acad. Nutr. Diet.*, 2016, **116**, 1543-1550.e1.
12. Martinez JA, Navas-Carretero S, Saris WHM, Astrup A, Personalized weight loss strategies - The role of macronutrient distribution, *Nat. Rev. Endocrinol.*, 2014, **10**, 749–60.
13. Greyling A, Appleton KM, Raben A, Mela DJ, Acute glycemic and insulinemic effects of low-energy sweeteners: a systematic review and meta-analysis of randomized controlled trials, *Am. J. Clin. Nutr.*, 2020, **00**, 1–13.
14. Stanhope KL, Sugar consumption, metabolic disease and obesity: The state of the controversy, *Crit. Rev. Clin. Lab. Sci.*, 2016, **53**, 52–67.
15. Blaak EE, Carbohydrate quantity and quality and cardio-metabolic risk, *Curr. Opin. Clin.*

Nutr. Metab. Care, 2016, **19**, 289–93.

16. Malik VS, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB, Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: A meta-analysis, *Diabetes Care*, 2010, **33**, 2477–83.
17. Bellisle F, Drewnowski A, Intense sweeteners, energy intake and the control of body weight, *Eur. J. Clin. Nutr.*, 2007, **61**, 691–700.
18. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB, Low-calorie sweetener consumption is increasing in the United States, *Am. J. Clin. Nutr.*, 2012, **96**, 640–6.
19. Sylvetsky AC, Rother KI, Trends in the consumption of low-calorie sweeteners, *Physiol. Behav.*, 2016, **164**, 446–50.
20. Catenacci VA, Pan Z, Thomas JG, Ogden LG, Roberts SA, Wyatt HR, et al., Low/no calorie sweetened beverage consumption in the National Weight Control Registry, *Obesity*, 2014, **22**, 2244–51.
21. Pearlman M, Obert J, Casey L, The Association Between Artificial Sweeteners and Obesity, *Curr Gastroenterol Rep.*, 2017, **19**, 64.
22. Raben A, Richelsen B, Artificial sweeteners: A place in the field of functional foods? Focus on obesity and related metabolic disorders, *Curr. Opin. Clin. Nutr. Metab. Care*, 2012, **15**, 597–604.
23. Hutchinson SA, Ho GS, Ho CT, Stability and degradation of the high-intensity sweeteners: Aspartame, alitame, and sucralose, *Food Rev. Int.*, 1999, **15**, 249–61.
24. Mortensen A, Sweeteners permitted in the European Union: Safety aspects, *Scand. J. Food Nutr.*, 2006, **50**, 104–16.
25. American Heart Association, Non-Nutritive Sweeteners (Artificial Sweeteners), https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/sugar/nonnutritive-sweeteners-artificial-sweeteners, (accessed September 2020).
26. Gardner C, Wylie-Rosett J, Gidding SS, Steffen FLM, Johnson FRK, Reader D, et al., Nonnutritive sweeteners: Current use and health perspectives - A scientific statement from the American Heart Association and the American Diabetes Association, *Diabetes Care*, 2012, **35**, 1798–808.
27. Azad MB, Abou-Setta AM, Chauhan BF, Rabbani R, Lys J, Copstein L, et al., Nonnutritive sweeteners and cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials and prospective cohort studies, *Cmaj*, 2017, **189**, E929–39.
28. Laverty AA, Magee L, Monteiro CA, Saxena S, Millett C, Sugar and artificially sweetened beverage consumption and adiposity changes: National longitudinal study, *Int. J. Behav. Nutr.*

Phys. Act., 2015, **12**, 137.

29. Fowler SP, Low-calorie sweetener use and energy balance: Results from experimental studies in animals, and large-scale prospective studies in humans, *Physiol Behav.*, 2016, **164**, 517–23.
30. Blundell JE, Hill AJ, Paradoxical effects of an intense sweetener (aspartame) on appetite, *Lancet*, 1986, **327**, 1092–3.
31. Tey SL, Salleh NB, Henry J, Forde CG, Effects of aspartame-, monk fruit-, stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake, *Int. J. Obes.*, 2017, **41**, 450–7.
32. Rojas E, Bermúdez V, Motlaghzadeh Y, Mathew J, Fidilio E, Faria J, et al., Stevia rebaudiana Bertoni and Its Effects in Human Disease: Emphasizing Its Role in Inflammation, Atherosclerosis and Metabolic Syndrome, *Curr. Nutr. Rep.*, 2018, **7**, 161–70.
33. Tandel KR, Sugar substitutes: Health controversy over perceived benefits, *J. Pharmacol. Pharmacother.*, 2011, **2**, 236–43.
34. Marinovich M, Galli CL, Bosetti C, Gallus S, La Vecchia C, Aspartame, low-calorie sweeteners and disease: Regulatory safety and epidemiological issues, *Food Chem. Toxicol.*, 2013, **60**, 109–15.
35. Burke M V., Small DM, Physiological mechanisms by which non-nutritive sweeteners may impact body weight and metabolism, *Physiol. Behav.*, 2015, **152**, 381–8.
36. Peters JC, Beck J, Low Calorie Sweetener (LCS) use and energy balance, *Physiol. Behav.*, 2016, **164**, 524–8.
37. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane, 2020, www.training.cochrane.org/handbook, (accessed October 2020).
38. Yunker AG, Patel R, Page KA, Effects of Non-nutritive Sweeteners on Sweet Taste Processing and Neuroendocrine Regulation of Eating Behavior, *Curr. Nutr. Rep.*, 2020, **9**, 278–89.
39. Olszewski PK, Wood EL, Klockars A, Levine AS, Excessive Consumption of Sugar: an Insatiable Drive for Reward, *Curr. Nutr. Rep.*, 2019, **8**, 120–8.
40. Mattes RD, Popkin BM, Nonnutritive sweetener consumption in humans: Effects on appetite and food intake and their putative mechanisms, *Am. J. Clin. Nutr.*, 2009, **89**, 1–14.
41. Gibbons C, Finlayson G, Dalton M, Caudwell P, Blundell JE, Metabolic phenotyping guidelines: Studying eating behaviour in humans, *J. Endocrinol.*, 2014, **222**, G1–12.

42. Rogers PJ, Blundell JE, Uncoupling Sweet Taste and Calories Effects of Saccharin on Hunger and Food Intake in Human Subjects, *Ann. N. Y. Acad. Sci.*, 1988, **575**, 569–71.
43. Fantino M, Fantino A, Matray M, Mistretta F, Beverages containing low energy sweeteners do not differ from water in their effects on appetite, energy intake and food choices in healthy, non-obese French adults, *Appetite*, 2018, **125**, 557–65.
44. Black RM, Leiter LA, Anderson GH, Consuming aspartame with and without taste: Differential effects on appetite and food intake of young adult males, *Physiol. Behav.*, 1993, **53**, 459–66.
45. Higgins KA, Mattes RD, A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity, *Am. J. Clin. Nutr.*, 2019, **109**, 1288–301.
46. Gadah NS, Kyle LA, Smith JE, Brunstrom JM, Rogers PJ, No difference in compensation for sugar in a drink versus sugar in semi-solid and solid foods, *Physiol. Behav.*, 2016, **156**, 35–42.
47. van Opstal AM, Kaal I, van den Berg-Huysmans AA, Hoeksma M, Blonk C, Pijl H, et al., Dietary sugars and non-caloric sweeteners elicit different homeostatic and hedonic responses in the brain, *Nutrition*, 2019, **60**, 80–6.
48. Black RM, Tanaka P, Leiter LA, Anderson GH, Soft drinks with aspartame: Effect on subjective hunger, food selection, and food intake of young adult males, *Physiol. Behav.*, 1991, **49**, 803–10.
49. Drewnowski A, Intense Sweeteners and the Control of Appetite, *Nutr. Rev.*, 2009, **53**, 1–7.
50. Tordoff MG, Alleva AM, Oral stimulation with aspartame increases hunger, *Physiol. Behav.*, 1990, **47**, 555–9.
51. Smeets PAM, Erkner A, De Graaf C, Cephalic phase responses and appetite, *Nutr. Rev.*, 2010, **68**, 643–55.
52. Steinert RE, Frey F, Tpfer A, Drewe J, Beglinger C, Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides, *Br. J. Nutr.*, 2011, **105**, 1320–8.
53. Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J, Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories, *Am. J. Clin. Nutr.*, 2005, **82**, 1011–1016.
54. Crézé C, Candal L, Cros J, Knebel JF, Seyssel K, Stefanoni N, et al., The impact of caloric and non-caloric sweeteners on food intake and brain responses to food: A randomized crossover controlled trial in healthy humans, *Nutrients*, 2018, **10**, 615.

55. Raben A, Møller BK, Flint A, Vasilaras TH, Møller AC, Holst JJ, et al., Increased postprandial glycaemia, insulinemia, and lipidemia after 10 weeks' sucrose-rich diet compared to an artificially sweetened diet: A randomised controlled trial, *Food Nutr. Res.*, 2011, **55**, 1–13.
56. Schultes B, Oltmanns KM, Kern W, Fehm HL, Born J, Peters A, Modulation of hunger by plasma glucose and metformin, *J. Clin. Endocrinol. Metab.*, 2003, **88**, 1133–41.
57. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al., Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans, *J. Clin. Endocrinol. Metab.*, 2001, **86**, 4753–8.
58. Lauritzen ES, Voss T, Kampmann U, Mengel A, Vendelbo MH, Jørgensen JOL, et al., Circulating acylghrelin levels are suppressed by insulin and increase in response to hypoglycemia in healthy adult volunteers, *Eur. J. Endocrinol.*, 2015, **172**, 357–62.
59. Davidoff AJ, Mason MM, Davidson MB, Carmody MW, Hintz KK, Wold LE, et al., Sucrose-induced cardiomyocyte dysfunction is both preventable and reversible with clinically relevant treatments, *Am. J. Physiol. - Endocrinol. Metab.*, 2004, **286**, 718–24.
60. Erlanson-Albertsson C, How palatable food disrupts appetite regulation, *Basic Clin. Pharmacol. Toxicol.*, 2005, **97**, 61–73.
61. Sylvetsky AC, Brown RJ, Blau JE, Walter M, Rother KI, Hormonal responses to non-nutritive sweeteners in water and diet soda, *Nutr. Metab.*, 2016, **13**, 1–8.
62. Van Opstal AM, Hafkemeijer A, van den Berg-Huysmans AA, Hoeksma M, Mulder TPJ, Pijl H, et al., Brain activity and connectivity changes in response to nutritive natural sugars, non-nutritive natural sugar replacements and artificial sweeteners, *Nutr. Neurosci.*, 2019, **0**, 1–11.
63. Sharma A, Amarnath S, Thulasimani M, Ramaswamy S, Artificial sweeteners as a sugar substitute: Are they really safe?, *Indian J. Pharmacol.*, 2016, **48**, 237–40.
64. Austin J, Marks D, Hormonal Regulators of Appetite, *Int. J. Pediatr. Endocrinol.*, 2009, **2009**, 1–9.
65. Bonnet F, Tavenard A, Esvan M, Laviolle B, Viltard M, Lepicard EM, et al., Consumption of a carbonated beverage with high-intensity sweeteners has no effect on insulin sensitivity and secretion in nondiabetic adults, *J. Nutr.*, 2018, **148**, 1293–9.
66. Maersk M, Belza A, Holst JJ, Fenger-Gron M, Pedersen SB, Astrup A, et al., Satiety scores and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk and with non-caloric soft drink: a controlled trial., *Eur. J. Clin. Nutr.*, 2012, **66**, 523–9.

67. Meyer-Gerspach AC, Biesiekierski JR, Deloose E, Clevers E, Rotondo A, Rehfeld JF, et al., Effects of caloric and noncaloric sweeteners on antroduodenal motility, gastrointestinal hormone secretion and appetite-related sensations in healthy subjects, *Am. J. Clin. Nutr.*, 2018, **107**, 707–16.
68. Wu T, Zhao BR, Bound MJ, Checklin HL, Bellon M, Little TJ, et al., Effects of different sweeteners on incretin hormone secretion, gastric emptying, intragastric distribution and postprandial glycemia in healthy humans, *Am. J. Clin. Nutr.*, 2012, **95**, 78–83.
69. Temizkan S, Deyneli O, Yasar M, Arpa M, Gunes M, Yazici D, et al., Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes, *Eur. J. Clin. Nutr.*, 2015, **69**, 162–6.
70. Gregersen NT, Flint A, Bitz C, Blundell JE, Raben A, Astrup A, Reproducibility and power of ad libitum energy intake assessed by repeated single meals, *Am. J. Clin. Nutr.*, 2008, **87**, 1277–81.
71. Tan SY, Tucker RM, Sweet taste as a predictor of dietary intake: A systematic review, *Nutrients*, 2019, **11**, 94.
72. Blundell JE, Low-calorie sweeteners: More complicated than sweetness without calories, *Am. J. Clin. Nutr.*, 2019, **109**, 1237–8.
73. Chambers ES, Bridge MW, Jones DA, Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity, *J. Physiol.*, 2009, **587**, 1779–94.
74. Frank GKW, Oberndorfer TA, Simmons AN, Paulus MP, Fudge JL, Yang TT, et al., Sucrose activates human taste pathways differently from artificial sweetener, *Neuroimage*, 2008, **39**, 1559–69.
75. Haase L, Cerf-Ducastel B, Murphy C, Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety, *Neuroimage*, 2009, **44**, 1008–21.
76. Ashwell M, Gibson S, Bellisle F, Buttriss J, Drewnowski A, Fantino M, et al., Expert consensus on low-calorie sweeteners: Facts, research gaps and suggested actions, *Nutr. Res. Rev.*, 2020, **33**, 145–54.
77. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al., Does Low-Energy Sweetener Consumption Affect Energy Intake and Body Weight? A Systematic Review, Including Meta-Analyses, of the Evidence From Human and Animal Studies, *Int J Obes (Lond).*, 2016, **40**, 381–394.
78. Rodin J, Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake, *Am. J. Clin. Nutr.*, 1990, **51**, 428–35.
79. Rolls BJ, Kim S, Fedoroff IC, Effects of drinks sweetened with sucrose or aspartame on

hunger, thirst and food intake in men, *Physiol. Behav.*, 1990, **48**, 19–26.

80. Reid M, Hammersley R, Duffy M, Effects of sucrose drinks on macronutrient intake, body weight, and mood state in overweight women over 4 weeks, *Appetite*, 2010, **55**, 130–6.
81. Raben A, Vasilaras TH, Christina Møller A, Astrup A, Sucrose compared with artificial sweeteners: Different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects, *Am. J. Clin. Nutr.*, 2002, **76**, 721–9.
82. Sørensen LB, Raben A, Stender S, Astrup A, Effect of sucrose on inflammatory markers in overweight humans, *Am. J. Clin. Nutr.*, 2005, **82**, 421–7.
83. Tordoff MG, Alleva AM, Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight, *Am. J. Clin. Nutr.*, 1990, **51**, 963–9.
84. WHO, Guideline: sugars intake for adults and children, https://www.who.int/publications/i/item/9789241549028, (accessed September 2020).
85. Yang Q, Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: Neuroscience 2010, *Yale J. Biol. Med.*, 2010, **83**, 101–8.
86. Liem DG, De Graaf C, Sweet and sour preferences in young children and adults: Role of repeated exposure, *Physiol. Behav.*, 2004, **83**, 421–9.
87. Griffioen-Roose S, Finlayson G, Mars M, Blundell JE, de Graaf C, Measuring food reward and the transfer effect of sensory specific satiety, *Appetite*, 2010, **55**, 648–55.
88. Green E, Murphy C, Altered processing of sweet taste in the brain of diet soda drinkers, *Physiol. Behav.*, 2012, **107**, 560–7.
89. Rogers PJ, Pleming HC, Blundell JE, Aspartame ingested without tasting inhibits hunger and food intake, *Physiol. Behav.*, 1990, **47**, 1239–43.
90. Small DM, DiFeliceantonio AG, Neuroscience: Processed foods and food reward, *Science*, 2019, **363**, 346–7.
91. Casperson SL, Johnson LA, Roemmich JN, The relative reinforcing value of sweet versus savory snack foods after consumption of sugar- or non-nutritive sweetened beverages, *Appetite*, 2017, **112**, 143–9.
92. Maloney NG, Christiansen P, Harrold JA, Halford JCG, Hardman CA, Do low-calorie sweetened beverages help to control food cravings? Two experimental studies, *Physiol. Behav.*, 2019, **208**, DOI: 10.1016/j.physbeh.2019.03.019.
93. Cabral TM, Pereira MGB, Falchione AEZ, Sá DAR de, Correa L, Fernandes D da M, et al., Artificial Sweeteners as a Cause of Obesity: Weight Gain Mechanisms and Current Evidence, *Health (Irvine. Calif).*, 2018, **10**, 700–17.
94. Wiebe N, Padwal R, Field C, Marks S, Jacobs R, Tonelli M, A systematic review on the

effect of sweeteners on glycemic response and clinically relevant outcomes, *BMC Med.*, 2011, **9**, 123.

95. Samuel P, Ayoob KT, Magnuson BA, Wölwer-Rieck U, Jeppesen PB, Rogers PJ, et al., Stevia Leaf to Stevia Sweetener: Exploring Its Science, Benefits, and Future Potential, *J. Nutr.*, 2018, **148**, 1186S-1205S.
96. Benton D, Can artificial sweeteners help control body weight and prevent obesity?, *Nutr. Res. Rev.*, 2005, **18**, 63–76.
97. Pielak M, Czarniecka-Skubina E, Trafiałek J, Głuchowski A, Contemporary trends and habits in the consumption of sugar and sweeteners—A questionnaire survey among poles, *Int. J. Environ. Res. Public Health*, 2019, **16**, 1164.
98. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP, Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain, *Obesity*, 2008, **16**, 1894–900.
99. Reid M, Hammersley R, Hill AJ, Skidmore P, Long-term dietary compensation for added sugar: Effects of supplementary sucrose drinks over a 4-week period, *Br. J. Nutr.*, 2007, **97**, 193–203.
100. Markey O, Le Jeune J, Lovegrove JA, Energy compensation following consumption of sugar-reduced products: a randomized controlled trial, *Eur. J. Nutr.*, 2016, **55**, 2137–49.
101. Bhargava A, Amialchuk A, Added sugars displaced the use of vital nutrients in the National Food Stamp Program Survey, *J. Nutr.*, 2007, **137**, 453–60.
102. Tate DF, Turner-McGrievy G, Lyons E, Stevens J, Erickson K, Polzien K, et al., Replacing caloric beverages with water or diet beverages for weight loss in adults: Main results of the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial, *Am. J. Clin. Nutr.*, 2012, **95**, 555–63.
103. Sørensen LB, Vasilaras TH, Astrup A, Raben A, Sucrose compared with artificial sweeteners: a clinical intervention study of effects on energy intake, appetite, and energy expenditure after 10 wk of supplementation in overweight subjects, *Am J Clin Nutr*, 2014, **100**, 36–45.
104. Heidari-Beni M, Kelishadi R, The Role of Dietary Sugars and Sweeteners in Metabolic Disorders and Diabetes, In: book: Sweeteners, Springer, Cham; 2018. p. 225–43.
105. Liauchonak I, Qorri B, Dawoud F, Riat Y, Szewczu MR, Non-Nutritive Sweeteners and Their Implications on the Development of Metabolic Syndrome, *Nutrients*, 2019, **11**, 644.
106. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KSH, Ilegems E, Daly K, et al., T1R3 and gustducin in gut sense sugars to regulate expression of Na +-glucose cotransporter 1, *Proc.*

Natl. Acad. Sci. U. S. A., 2007, **104**, 15075–80.

107. Mace OJ, Affleck J, Patel N, Kellett GL, Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2, *J Physiol.*, 2007, **582**, 379-392.
108. Gorboulev V, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, et al., Na +-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion, *Diabetes*, 2012, **61**, 187–96.
109. Moriya R, Shirakura T, Ito J, Mashiko S, Seo T, Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice, *Am J Physiol Endocrinol Metab*, 2009, **297**, 1358–65.
110. Sclafani A, Abrams M, Rats Show Only a Weak Preference for the Artificial Sweetener Aspartame, *Physiol Behav.*, 1986, **37**, 253-256.
111. Daniel H, Zietek T, Taste and move: Glucose and peptide transporters in the gastrointestinal tract, *Exp. Physiol.*, 2015, **100**, 1441–50.
112. Chan CB, Hashemi Z, Subhan FB, The impact of low and no-caloric sweeteners on glucose absorption, incretin secretion, and glucose tolerance, *Appl. Physiol. Nutr. Metab.*, 2017, **42**, 793–801.
113. Suzuki K, Jayasena CN, Bloom SR, Obesity and appetite control, *Exp. Diabetes Res.*, 2012, **2012**, 824305.
114. Holst JJ, On the physiology of GIP and GLP-1., *Horm Metab Res.*, 2004, **36**, 747–54.
115. Han P, Bagenna B, Fu M, The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: a review, *Int J Food Sci Nutr.*, 2019, **70**, 125–35.
116. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, et al., Sweet Taste Receptor Expressed in Pancreatic b-Cells Activates the Calcium and Cyclic AMP Signaling Systems and Stimulates Insulin Secretion, *PLoS One*, 2009, **4**, 54–62.
117. Malaisse WJ, Vanonderbergen A, Louchami K, Jijakli H, Malaisse-Lagae F, Effects of artificial sweeteners on insulin release and cationic fluxes in rat pancreatic islets, *Cell. Signal.*, 1998, **10**, 727–33.
118. Sclafani A, Ackroff K, Advantame sweetener preference in C57BL/6J mice and Sprague-Dawley rats, *Chem. Senses*, 2015, **40**, 181–6.
119. Ford HE, Peters V, Martin NM, Sleeth ML, Ghatei MA, Frost GS, et al., Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. European journal of clinical nutrition, *Eur J Clin Nutr*, 2011, **65**, 508–13.
120. Thomson P, Santibañez R, Aguirre C, Galgani JE, Garrido D, Short-term impact of sucralose

consumption on the metabolic response and gut microbiome of healthy adults, *Br J Nutr*, 2019, **122**, 856–862.

121. Maki KC, Curry LL, Reeves MS, Toth PD, McKenney JM, Farmer M V., et al., Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus, *Food Chem. Toxicol.*, 2008, **46**, S47–53.
122. Dhillon J, Lee JY, Mattes RD, The cephalic phase insulin response to nutritive and low-calorie sweeteners in solid and beverage form, *Physiol. Behav.*, 2017, **181**, 100–9.
123. Pepino MY, Tiemann CD, Patterson BW, Wice BM, Klein S, Sucralose Affects Glycemic and Hormonal Responses to an Oral Glucose Load, *Diabetes Care*, 2013, **36**, 2530–5.
124. Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P, et al., Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels, *Appetite*, 2010, **55**, 37–43.
125. Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, Milagro FI, Martinez JA, Diet, Gut Microbiota, and Obesity: Links with Host Genetics and Epigenetics and Potential Applications, *Adv. Nutr.*, 2019, **10**, S17–30.
126. Laitinen K, Mokkala K, Overall Dietary Quality Relates to Gut Microbiota Diversity and Abundance, *Int J Mol Sci.*, 2019, **20**, 1835.
127. Moriconi E, Feraco A, Marzolla V, Infante M, Lombardo M, Fabbri A, et al., Neuroendocrine and Metabolic Effects of Low-Calorie and Non-Calorie Sweeteners, *Front. Endocrinol. (Lausanne).*, 2020, **11**, 444.
128. European Food Safety Authority, Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive., *EFSA J.*, 2013, **11**, 3496.
129. Butchko HH, Stargel WW, Comer CP, Mayhew DA, Andress SE, Aspartame, In: O'Brien Nabors L ed., editor. Alternative Sweeteners, Boca Raton, FL: CRC Press; 2012. p. 41–61.
130. Gardana C, Simonetti P, Canzi E, Zanchi R, Pietta P, Metabolims of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora, *J Agric Food Chem*, 2003, **51**, 6618–22.
131. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG, Biological Fate of Low-Calorie Sweeteners, *Nutr Rev.*, 2016, **74**, 670–689.
132. Renwick AG, The metabolism of intense sweeteners, *Xenobiotica*, 1986, **16**, 1057–1071.
133. Sweatman TW, Renwick AG, The tissue distribution and pharmacokinetics of saccharin in the rat., *Toxicol Appl Pharmacol*, 1980, **55**, 18–31.
134. Knight I, The development and applications of sucralose, a new high-intensity sweetener., *Can J Physiol Pharmacol*, 1994, **72**, 435–9.

135. von Rymon Lipinski GW, Klug C, Acesulfame K, In: O'Brien Nabors L ed., editor. Alternative Sweeteners, 4th ed. Boca Raton, FL: CRC Press; 2012. p. 13–30.
136. Tamboli CP, Neut C, Desreumaux P, Colombel JF, Dysbiosis in inflammatory bowel disease, *BMC Syst Biol*, 2004, **53**, 1–4.
137. Choudhary AK, Lee YY, Neurophysiological symptoms and aspartame: What is the connection?, *Nutr. Neurosci.*, 2018, **21**, 306–16.
138. Farup PG, Lydersen S, Valeur J, Are Nonnutritive Sweeteners Obesogenic? Associations between Diet, Faecal Microbiota, and Short-Chain Fatty Acids in Morbidly Obese Subjects, *J Obes.*, 2019, **2019**, 4608315.
139. Emamat H, Ghalandari H, Tangestani H, Abdollahi A, Hekmatdoost A, Artificial sweeteners are related to non-alcoholic fatty liver disease: Microbiota dysbiosis as a novel potential mechanism, *EXCLI J.*, 2020, **19**, 620–6.
140. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al., Artificial sweeteners induce glucose intolerance by altering the gut microbiota., *Nature.*, 2014, **514**, 181–6.
141. Turnbaugh P, Ley R, Mahowald M, Magrini V, Mardis ER, Gordon JI, An obesity-associated gut microbiome with increased capacity for energy harvest, *Nature*, 2006, **444**, 1027–1031.
142. Canfora EE, Jocken JW, Blaak EE, Short-chain fatty acids in control of body weight and insulin sensitivity, *Nat Rev Endocrinol*, 2015, **11**, 577–591.
143. Canfora EE, Meex RCR, Venema K, Blaak EE, Gut microbial metabolites in obesity, NAFLD and T2DM., *Nat Rev Endocrinol.*, 2019, **15**, 261-273.
144. Ahmad SY, Friel J, Mackay D, The Effects of Non-Nutritive Artificial Sweeteners, Aspartame and Sucralose, on the Gut Microbiome in Healthy Adults: Secondary Outcomes of a Randomized Double-Blinded Crossover Clinical Trial, *Nutrients*, 2020, **12**, E3408.
145. Leung C, Rivera L, Furness JB, Angus PW, The Role of the Gut Microbiota in NAFLD, *Nat Rev Gastroenterol Hepatol*, 2016, **13**, 412–25.
146. Santos PS, Caria CRP, Gotardo EMF, Ribeiro ML, Pedrazzoli J, Gambero A, Artificial sweetener saccharin disrupts intestinal epithelial cells' barrier function in vitro, *Food Funct.*, 2018, **9**, 3815–3822.
147. Bian X, Tu P, Chi L, Gao B, Ru H, Lu K, Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions, *Food Chem Toxicol.*, 2017, **107**, 530–539.
148. André Laugerette, F., Féart, C P, Metabolic Endotoxemia: A Potential Underlying Mechanism of the Relationship between Dietary Fat Intake and Risk for Cognitive

Impairments in Humans?, *Nutrients*, 2019, **11**, 1887.

149. Bian Chi, L., Gao, B., Tu, P., Ru, H., & Lu, K. X, Gut Microbiome Response to Sucralose and Its Potential Role in Inducing Liver Inflammation in Mice, *Front Physiol.*, 2017, **7**, 487.
150. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K, The Artificial Sweetener Acesulfame Potassium Affects the Gut Microbiome and Body Weight Gain in CD-1 Mice, *PLoS One.*, 2017, **12**, e0178426.
151. Boonkaewwan C, Burodom A, Anti-inflammatory and Immunomodulatory Activities of Stevioside and Steviol on Colonic Epithelial Cells, *J Sci Food Agric*, 2013, **93**, 3820–5.
152. Wang T, Guo M, Song X, Zhang Z, Jiang H, Wang W, et al., Stevioside Plays an Anti-Inflammatory Role by Regulating the NF-κB and MAPK Pathways in S. Aureus-Infected Mouse Mammary Glands, *Inflammation*, 2014, **37**, 1837–46.
153. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A, Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials, *Adv. Nutr.*, 2019, **10**, S31–48.
154. Simon BR, Parlee SD, Learman BS, Mori H, Scheller EL, Cawthorn WP, et al., Artificial Sweeteners Stimulate Adipogenesis and Suppress Lipolysis Independently of Sweet Taste Receptors, *J Biol Chem.*, 2013, **288**, 32475–89.
155. Sen S, Rouphael C, Houston S, Abstract P029: Sucralose Promotes Increase in Fat Accumulation in Human Mesenchymal Stem Cells, *Circulation*, 2015, **131**, AP029.
156. Masubuchi Y, Nakagawa Y, Ma J, Sasaki T, Kitamura T, Yamamoto Y, et al., A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells., *PLoS One*, 2013, **8**, e54500.
157. Saraswathy S, Toora BD, M M, Mishra S, Effect of Artificial Sweeteners on the Blood Glucose Concentration, *J. Med. Acad.*, 2018, **1**, 81–5.
158. Swithers SE, Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements, *Trends Endocrinol Metab.*, 2013, **24**, 431–41.
159. Daher MI, Matta JM, Abdel Nour AM, Non-nutritive sweeteners and type 2 diabetes: Should we ring the bell?, *Diabetes Res. Clin. Pract.*, 2019, **155**, 107786.
160. Toews I, Lohner S, Küllenberg de Gaudry D, Sommer H, Meerpohl JJ, Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies, *BMJ*, 2019, **364**, k4718.
161. Mela DJ, McLaughlin J, Rogers PJ, Perspective: Standards for Research and Reporting on Low-Energy (“Artificial”) Sweeteners, *Adv. Nutr.*, 2020, **11**, 484–91.

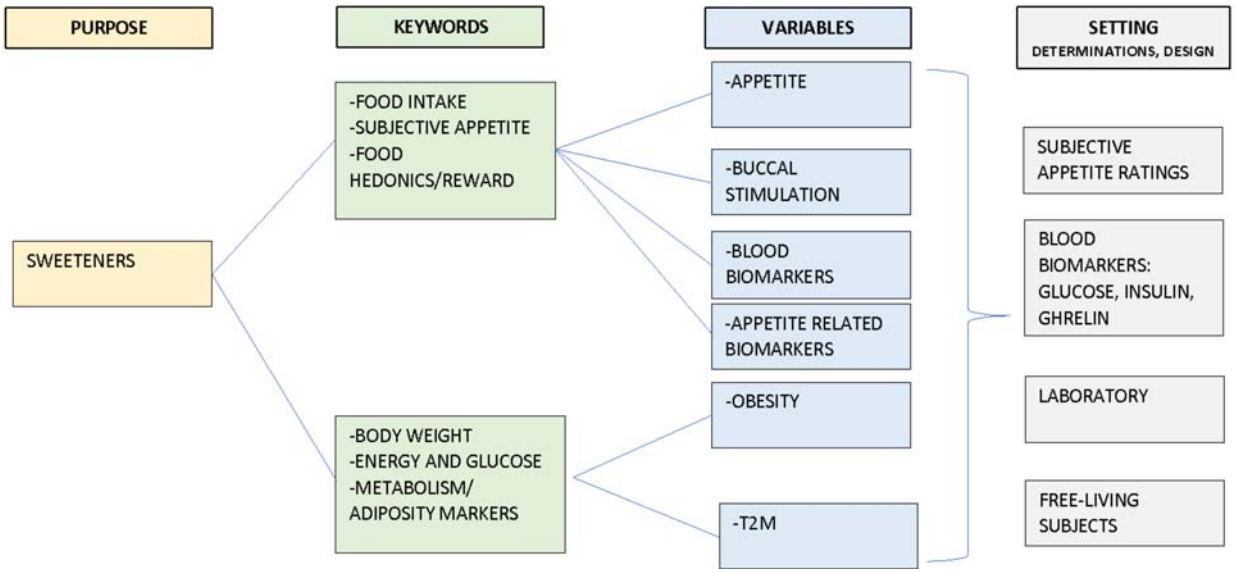

Figure 1. Flow chart of the process carried out for the implementation of the review.

Table 1. Methods section and searching strategy: databases, keywords/MesH terms

Criteria for including studies in the review following PRISMA/PROSPERO Approaches	
Title of review	A systematic review on the effects of sweeteners and sweetness enhancers on appetite, food reward and metabolic/adiposity outcomes in adults.
Population, or participants and conditions of interest	Healthy individuals and metabolically healthy obese adults of any sex and age. No population restrictions were applied
Interventions or exposures	Individuals receiving no-calorie sweeteners in either beverage or food form.
Comparisons or control groups	Individuals receiving sugar or water in direct comparison to the no-calorie sweetener groups. Repeated measures design whereby participants serve as their own comparison will be included.
Outcomes of interest	1. Food intake, Subjective appetite, Food hedonics 2. Body weight, energy and glucose metabolism/adiposity markers
Setting	Laboratory/free-living studies
Study designs	Randomised controlled trials Sugar or water comparison
Criteria for excluding studies in the review	
Excluded studies included; Animal models and protocols without a comparison or control condition	
Search method	
Electronic databases	MEDLINE EMBASE+EMBASE CLASSIC Psychinfo COCHRANE
Method of review	
Details of methods	At least two searchers in every center
Quality assessment	Searches followed the PRISMA/ Cochrane guidelines.
Narrative synthesis	YES: Two parts 1) Appetite issues 2) Metabolic and adiposity markers
Presentation of results	
Additional material	Flow chart of PRISMA search process Protocol

	Data tables

Table 2. Main outcomes to be assessed from literature search

Key concept	**Associated items**
Food intake	Meal onset, frequency, quantity, Snacking/grazing
Subjective appetite	Hunger, phases of satiety (early, late), Specific appetite-related hormones
Food hedonics	Preference/choice, Craving, Reward, fMRI / neural correlates
Sweet taste perception	Sensory perception, Sweet taste receptor function/polymorphism
Food reward	Food hedonics, sweet taste perception
Body weight, adiposity, glucose homeostasis/glycaemic control	
Energy balance	Energy intake, energy expenditure, thermogenesis microbiome
Adiposity / lipid metabolism	Adipogenesis, lipogenesis, sweet taste receptors
Gastrointestinal Physiology	Sweet taste receptors in oral cavity, intracellular Ca2, Neurotransmitters in intestine, Gut brain axis (GLP1, CCK, PYY), reward
Glucose homeostasis/ glycaemic control	
Intestinal glucose absorption	Sweet taste receptors in intestine SGLT1, GLUT 2, Hyperglycaemia, Ectopic fat accumulation, De novo lipogenesis
Insulin secretion sensory receptors	Oral cavity, cephalic phase sweet taste, receptors in intestine, GLP1, beta cells
Alterations gut microbiome microbial changes (composition, function), SCFA, lipogenesis	
Insulin sensitivity hyperinsulinemia, insulin desensitization	
Inflammation intestinal permeability, metabolic endotoxemia, oxidative stress, AT inflammation	

Table 3. Appetite, buccal stimulation and blood biomarkers as affected by sweeteners and sweeteners enhancers intake

Author (ref) year	Trial characteristics/design	Hypothesis / Research question / Aims	Outcomes and remarks
Blundell and Hill[30] **1986**	• Subjects: 95 men and women • Age: 18-22 years • Design: parallel intervention • Treatment: glucose, aspartame or water	Effects of aspartame on measures involved in appetite control	• Aspartame has appetite-stimulating properties in comparison with the ingestion of water • Glucose loads suppressed motivational ratings, in contrast with aspartame • There appears to be a contrast between the effects of aspartame on alliesthesia and the effects on motivation to eat
Rogers and Blundell[42] **1988**	• Subjects: 12 adults, 8 females and 4 males • Age: 19-25 years • BMI: of normal weight • Design: RCT • Treatment: saccharin, aspartame, acesulfame-K, glucose, water • Duration: 5 sessions at weekly interval	Effects of uncoupling the dimensions of taste and calories achieved by intense sweeteners varying, chemical structure and biological properties	• Glucose preload significantly depressed appetitive motivational ratings, increased ratings of fullness, decreased the frequency of items checked on a food preference checklist and reduced food consumption in a test meal • There may be a short-lived suppressive effect on subjective appetite ratings produced by acute ingestion of HIS saccharin, aspartame or acesulfame-K, but is then followed by an increase above baseline values
Rolls et al.[79] **1990**	• Subjects: 42 men • Age: 21-39 years • BMI: of normal weight • Design: within-subjects design • Treatment: sucrose, aspartame, water • Duration: 7 session with at least 3 days between sessions	Effects of commercially available pudding and jelly sweetened with either sucrose or aspartame on appetite ratings and food intake.	• No differences were seen between the effects of the different types of drinks on any of the hunger ratings over the hour after fluid consumption • Data do not support the hypothesis that aspartame-sweetened drinks increase food intake
Tordoff and Alleva[50] **1990**	• Subjects: 120 participants, 60 men and 60 women • Age: 25.5 ± 0.9 years women 26.1 ± 0.9 years men • Design: RCT • Treatment: aspartame • Duration: test day	Receive subjects' subjective ratings of hunger at intervals after they chewed an unflavoured gum base that was sweetened with one of five different	• Hunger ratings increased in those individuals chewing the sweetened gum • The highest concentrations of aspartame tended to have a time-dependent, biphasic effect, producing a transient decrease followed by a sustained increase in hunger ratings.

		concentrations of aspartame.	
Black et al.[48] 1991	• Subjects: 20 men • Age: 19-25 years • BMI: 22-29 Kg/m^2 • Design: randomised trial • Treatment: aspartame, water • Duration: test days	Control timing and size of the breakfast meal on test days and deliver the NCS aspartame in a commercially available soft drink.	• The consumption of aspartame-sweetened beverages did not increase short-term subjective hunger, or food intake, in a meal taken within the following 60 to 90 minutes.
Black et al.[44] 1993	• Subjects: 18 males • Age: 19-25 years • BMI: 21-25 Kg/m^2 • Design: randomised trial • Treatments: carbonated mineral water and aspartame • Duration: 5 test days	Sweeteners like aspartame accounts for the appetite suppression	• Water sweetened with aspartame resulted in increases to subjective appetite, hunger, desire to eat, fullness and prospective consumption, relative to an unsweetened water control. • The increase in appetite was a short-lived effect, lasting approximately 30 minutes
Chambers et al.[73] 2009	• Subjects: -study 1A: 8 men -study 2A: 6 men and 2 women -study 1B: 4 men and 3 women -study 2B: 5 men and 2 women • Age: -study 1A: 29±9 years -study 2A: 22±3 years -study 1B: 23±3 years-study 2B: 24±2 years • BMI: -study 1A: 23.8±2.5 Kg/m^2 -study 2A: 22.3±2.7 Kg/m^2 -study 1B: 22.2±1.0 Kg/m^2	• Observe how rinsing the mouth with solutions containing glucose and maltodextrin, disguised with artificial sweetener, would affect exercise performance. • Examine functional magnetic resonance imaging fMRI to identify the brain regions activated by these substances	• A non-sweet carbohydrate in the human mouth produces a similar central neural response to that obtained with glucose • Both sweet and non-sweet carbohydrate in the human mouth activate a variety of brain areas, some of which may be involved in reward and the regulation of motor activity • Glucose activated the orbitofrontal cortex and the adjoining rostral part of the anterior cingulate cortex

	-study 2B: 22.7±0.7 Kg/m^2 ● Design: RCT ● Treatment: glucose, maltodextrin, saccharin, aspartame ● Duration: 4 visits		
Frank et al.[74] 2009	● Subjects: 12 women ● Age: 20-36 years ● BMI: 20-25 Kg/m^2 ● Design: RCT ● Treatment: sucrose, sucralose	Determine whether human brain activation is different for caloric sucrose compared to an artificial sweetener	● Sucrose and sucralose activate common taste pathways, but the primary taste cortex as well as pleasantness-related brain reward circuitry are activated greater for sucrose. ● Sucralose activates taste reward circuits but may not fully satisfy a desire for natural caloric sweet ingestion.
Raben et al.[55] 2011	● Subjects: 23 participants, 4 men and 19 women ● Age: 20-50 years ● BMI: 25-30 Kg/m^2 ● Design: RCT ● Treatment: sucrose, sweetener ● Duration: 10 weeks	Investigate the effects of a diet high in sucrose versus a diet high in artificial sweeteners on fasting and postprandial metabolic profiles after 10 weeks.	● A sucrose-rich diet resulted in elevations of postprandial glycaemia, insulinemia, and lipidemia compared to a diet rich in artificial sweeteners
Maersk et al.[66] 2012	● Subjects: 24 participants, 12 females and 12 males ● Age: 20-50 years ● BMI: 28-36 Kg/m^2 ● Design: randomised crossover study ● Treatment: sucrose-sweetened regular cola, semi-skimmed milk, aspartame-sweetened diet cola, and bottled still water ● Duration: 4 test days with 2 weeks of washout between them	Investigate the acute effects of two energy containing drinks sucrose-sweetened regular cola and isocaloric semi-skimmed milk and two non-energy-containing drinks aspartame-sweetened diet cola and water on appetite scores, appetite regulating hormones and energy intake EI.	● Milk increased appetite scores and GLP-1 and GIP responses compared with sugar-sweetened soft drinks SSSD. ● The energy containing beverages were not compensated by decreased EI at the following meal ● There were no indications of aspartame-sweetened soft drink ASSD increased appetite or EI compared with water.

Wu et al.[68] **2012**	• Subjects: 10 participants, 7 men and 3 women • Age: 28.8 ± 4.0 y • BMI: 25.5 ± 1.5 Kg/m^2 • Design: RCT • Treatment: glucose, tagatose/isomalt, 3-O-methylglucose, sucralose • Duration: 4 test days at least 3 days apart	Determine the effects of 4 sweet preloads on GIP and GLP-1 release, gastric emptying, and postprandial glycaemia.	• SGLT1 substrates stimulate GLP-1 and GIP and slow gastric emptying, whereas the artificial sweetener sucralose does not. • Following a 60 mg sucralose preload, plasma GLP-1 levels did not significantly increase, whereas ingestion of 40g of glucose resulted in a prompt increase in GLP-1 • Following ingestion of a sucralose or blend of tagatose and isomalt preloads, there was no observable difference from fasting values
Temizkan et al.[69] **2015**	• Subjects: 8 healthy volunteers, 4 men and 4 women and 8 diabetics, 4 men and 4 women • Age: healthy 45.0 ± 4.1 years Diabetic 51.5 ± 9.2 years • BMI: healthy 30.3 ± 4.5 Kg/m^2 Diabetic 33.7 ± 5.4 Kg/m^2 • Design: RCT • Treatment: aspartame, sucralose, water • Duration: 3 settings	Determine the effect of artificial sweeteners aspartame and sucralose on blood glucose, insulin, c-peptide and glucagon-like peptide-1 GLP-1 levels.	• Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in newly diagnosed type 2 diabetic patients. • Sucralose ingestion at a dose of 24 mg absent of energy in addition to a 75g oral glucose tolerance test ~307kcal, resulted in a significantly higher AUC GLP-1 response in the sucralose condition compared to water
Sylvetsky et al.[61] **2016**	• Subjects: 61 healthy adults, 30 arm 1 of which 47% men, 31 arm 2 of which 45% men • Age: 18-45 years • BMI: 25.8 ± 4.2 kg/m^2 arm 1 and 26.3 ± 7.5 kg/m^2 arm 2 • Design: randomised Cross-over study • Treatment arm 1: water+sucralose,	• Test the effects of NCS on glycaemia, insulin, and incretin responses in healthy adults. • Test whether two combinations of NCS increase GLP-1 secretion	• Diet sodas but not NCS in water augmented GLP-1 responses to oral glucose. • Insulin concentrations were nominally higher following all NCS conditions without altering glycaemia. • Sucralose alone at any concentration did not affect metabolic outcomes. • Gastric inhibitory peptide GIP and C-peptide were not significantly different from fasted values following ingestion of sucralose at varying doses

	• Treatment arm 2: selzer water, diet rite cola (sucralose and acesulfame-K), diet mountain dew (sucralose, acesulfame-K and aspartame) or seltzer water (sucralose and acesulfame-K) • Duration: 1 screening + 4 test visits		
Gadah et al.[46] 2016	• Subjects: 144 participants 72 men, 72 women • Age: 18-65 years • BMI: 22.9 ± 3.3 Kg/m^2 • Design: between-subjects (parallel group) • Treatment: 6 combinations of sucralose or sucrose drinks, jelly and candy • Duration: Two test days	• Evaluate energy compensation in the participants who receive jelly and candy as a preload compared to those who receive the drink. • Assess the effect of sweet food intake on appetite reduction for sweet foods	• Consumption of sucrose was found to reduce subsequent energy intake • The cumulative intake preload plus assumption with test-meal was greater in the sucrose conditions. • The compensation was greater when the preload was a drink than when it was in food • The consumption of sweet drinks reduced the relative intake of sweet foods. • Sugar consumed in a drink was no less satiating than the same amount of sugar consumed in realistic semi-solid and solid foods
Tey et al.[31] 2017	• Subjects: 34 men • Age: 21-50 years • BMI: 18.5-25 Kg/m^2 • Design: randomised crossover study • Treatment: aspartame, monk fruit, stevia, sucrose • Duration: 1 screening and 4 test sessions with a minimum of 5-days hiatus between the test days	Compare the effects of consuming NCS and sucrose on energy intake, blood glucose and insulin responses.	• Calorie-free beverages sweetened with NNSs has minimal influences on total daily energy intake, glucose and insulin responses compared with a sucrose sweetened beverage in healthy lean males

Casperson et al.[91] **2017**	• Subjects: 21 participants, 10 men and 11 women • Age: 24 ± 6 years • BMI: < 25 Kg/m^2 • Design: randomised crossover study • Treatment: sucrose, sucralose • Duration: 2 testing sessions separated by a minimum of 7 days	Test the effects of NCS beverages consumption on later appetite and the reinforcing value of foods with sweet or salty/savoury taste profiles	• 4 h after consuming a NCS at lunch, the participants were willing to do more work to gain access to a sweet snack than a salty/savory snack. • NCS consumption may uncouple the relationship between the motivation for a sweet food and eating behavior, at least temporarily • NCSs, specifically those sweetened with sucralose, may play a role in altering eating behavior and food choices
Fantino et al.[43] **2018**	• Subjects: 166 participants, 86 men and 80 women • Age: 18-45 years • BMI: 19-28 Kg/m^2 • Design: RCT • Treatment: acesulfame-K, aspartame, sucralose, water • Duration: 9 weeks	Prove that NCS beverages would not differ from plain water in their impact on mean energy intake, either before or after NCS habituation, in the laboratory or at home	• NCS beverages do not increase total energy intake when compared with water. • The use of NCS in place of sugar led to reduced appetite for sweet-tasting foods and sugars, suggesting a sensory-specific satiety effect • No effect on motivation to eat following regular consumption of a commercially available beverage aspartame, acesulfame-K and sucralose
Meyer-Gerspach et al.[67] **2018**	• Subjects: 12 participants, 6 men and 6 women • Age: 18-28 years • BMI: 19-25 Kg/m^2 • Design: randomised crossover study • Treatment: glucose, fructose, acesulfame-K, water • Duration: 4 test days at least 3 days apart	Determine and compare the effects of caloric and NCS on GI motility and GI hormone secretion, as well as on appetite-related sensations in healthy volunteers.	• Glucose and fructose inhibit motilin secretion and antral motility while increasing CCK secretion but no effect after acesulfame-K • An initial stronger decrease in hunger feelings and stronger increase in satiety after ace-K $P < 0.05$, followed by a steeper return
Van Opstal et al.[62] **2019**	• Subjects: 16 men • Age: 18-25 years • BMI: 20-23 Kg/m^2	Investigate the effects of glucose, fructose, sucrose and sucralose ingestion on the	• Glucose induces a deactivation in the hypothalamus after ingestion • Fructose and sucrose are both associated with a delayed and lesser response from the hypothalamus

	• Design: randomised crossover study • Treatment: glucose, fructose, sucrose, sucralose, water • Duration: five visits	magnitude and trajectory of the hypothalamic and the ventral tegmental area (VTA) blood oxygen level dependent (BOLD) responses	• Sucralose might not have a similar, possibly satiating, effect on the brain as the natural sugars
Higgins and Mattes[45] **2019**	• Subjects: 154 participants • Age: 18-60 years • BMI: 25-40 Kg/m^2 • Design: RCT parallel-arm • Treatment: sucrose, aspartame, saccharin, sucralose, rebaudioside A • Duration: 5 testing days for a total of 12 weeks	Compare the effects of consumption of 4 NCS and sucrose on body weight, ingestive behaviors, and glucose tolerance over a 12-wk intervention in adults	• Sucrose and saccharin consumption significantly increase body weight compared with aspartame, rebA, and sucralose • Weight change was directionally negative and lower for sucralose • Energy intake decreased with sucralose consumption P = 0.02 and ingestive frequency was lower for sucralose than for saccharin P = 0.045. • Glucose tolerance was not significantly affected by the sweetener treatments.
Van Opstal et al.[47] **2019**	• Subjects: 20 men • Age: 18-25 years • BMI: 20-23 kg/m^2 • Design: Randomised Cross-over study • Treatment: glucose, fructose, allulose, sucralose • Duration: Four visits with a week-long wash-out period	Investigate the effects of the ingestion of sweetened nutrient shakes containing fats and protein.	• The type of sweetener can affect brain responses and might thus affect reward and satiety responses and feeding behaviour • Sweet taste without the corresponding energy content of the non-nutritive sweeteners appeared to have only small effects on the brain

Table 4. Body weight, insulin secretion and glucose related metabolic biomarkersas affected by sweeteners and sweeteners enhancers consumption

Author (ref) year	Trial characteristics/design	Hypothesis / Research question / Aims	Outcomes and remarks
Smeets et al.[53] 2005	• Subjects: 5 men • Age: 18-28 years • BMI: 19-25 Kg/m^2 • Design: randomised crossover design • Treatment: water, glucose, maltodextrin, aspartame • Duration: 4 test days	Measure the effects of sweet taste and energy content on the hypothalamic response to glucose ingestion and to measure the concomitant changes in blood glucose and insulin concentrations.	• Sweet taste and energy content are required for a hypothalamic response • The combination of sweet taste and energy content could be crucial in triggering adaptive responses to sweetened beverages • Aspartame did not trigger any insulin response
Maki et al.[121] 2008	• Subjects: 122 participants, 60 in the rebaudioside group (28 females), 62 in the placebo group (32 females) • Age: 18-74 years • BMI: 25-45 Kg/m^2 • Design: RCT • Treatment: rebaudioside A • Duration: 16 weeks	Examine the safety of 16 weeks of rebaudioside A consumption in men and women with type 2 diabetes mellitus, with particular attention to any potential glycaemic and hemodynamic effects	• Consumption of rebaudioside A for 16 weeks did not affect glucose homeostasis or resting blood pressure in men and women with type 2 diabetes mellitus • Rebaudioside A was well-tolerated and generally had no effects on laboratory measurements of safety
Ford et al.[119] 2011	• Subjects: 8 volunteers, 7 females and 1 male • Age: 22-27 years • BMI: 18.8-23.9 Kg/m^2 • Design: randomised crossover study • Treatment: Water, sucralose, maltodextrin + sucralose, cephalic sucralose • Duration: 4 test days with at least 3 days between sessions	Investigate whether oral ingestion of sucralose could stimulate L-cell-derived GLP-1 and peptide YY PYY release in vivo	• Oral ingestion of sucralose does not increase plasma GLP-1 or PYY concentrations and hence, does not reduce appetite in healthy subjects. • Oral stimulation with sucralose had no effect on GLP-1, insulin or appetite. • Sucralose ingestion did not increase plasma GLP-1 or PYY. • Maltodextrin ingestion significantly increased insulin and glucose compared with water • Appetite ratings and energy intake were similar for all groups

Pepino et al.[123] 2013	• Subjects: 17 participants, 15 females and 2 males • Age: 35.1 ± 1.0 years • BMI: 41.0 ± 1.5 Kg/m^2 • Design: randomised crossover design • Treatment: sucralose, water • Duration: 2 test days, 7 days apart	Test the hypothesis that sucralose ingestion alters the glycaemic and hormonal responses to glucose ingestion in obese subjects who are not regular users of NCS.	• Sucralose affects the glycaemic and insulin responses to an oral glucose load in obese people who do not normally consume NCS • Modest reduction in insulin clearance after sucralose was ingested • Sucralose is not metabolically inert but has physiologic effects
Bonnet et al.[65] 2018	• Subjects: 50 individuals 22 men, 28 women • Age: mean age 31 years • BMI: 19-29 Kg/m^2 • Design: randomised crossover study • Treatment: aspartame, acesulfame-K, carbonated water • Duration: 4 visits for a 12-wk intervention period	Compare the effects of regular consumption of a carbonated beverage containing high intensity sweeteners and an unsweetened carbonated beverage on insulin sensitivity and secretion	• Daily consumption over 12 weeks of a beverage sweetened with a blend of aspartame 129 mg and acesulfame-K 13 mg did not produce any significant effect on insulin sensitivity or secretion
Crézé et al.[54] 2018	• Subjects: 18 men • BMI: normal weight • Design: randomised crossover study • Treatment: water, sucrose, NNS (cyclamate, acesulfame-K, aspartame) • Duration: 3 test days with 3 weeks of wash-out	Investigate whether activation of sweet taste receptors with NCS or with sucrose, exert different acute effects on a postprandial brain responses to food viewing, b postprandial gastro-intestinal hormone secretion known to impact hunger and satiety feelings and c subsequent food intake behavior, both in terms	• An acute effect of NCS consumption on immediate food intake in humans who are not frequently drinking NCS beverages wasn't observed • The responsiveness of the brain areas to sweet taste has been shown to 'fade' as a function of longer-term NCS consumption • NCS consumption did not lead to pronounced modulations of glucose, insulin, and ghrelin concentrations

		of quantity and quality of choices.	
Thomson et al.[120] **2019**	• Subjects: 34 men • Age: 18-50 years • BMI: 20-30 Kg/m^2 • Design: RCT • Treatment: sucralose, placebo • Duration: 7 days	Evaluate the short-term effect of sucralose on glycaemic control and its interaction with the microbiota in healthy subjects.	• Consumption of high doses of sucralose for 7 days does not alter glycaemic control, insulin resistance, or gut microbiome in healthy individuals • There were no changes in the gut microbiomes of these subjects with respect to the consumption of sucralose or placebo

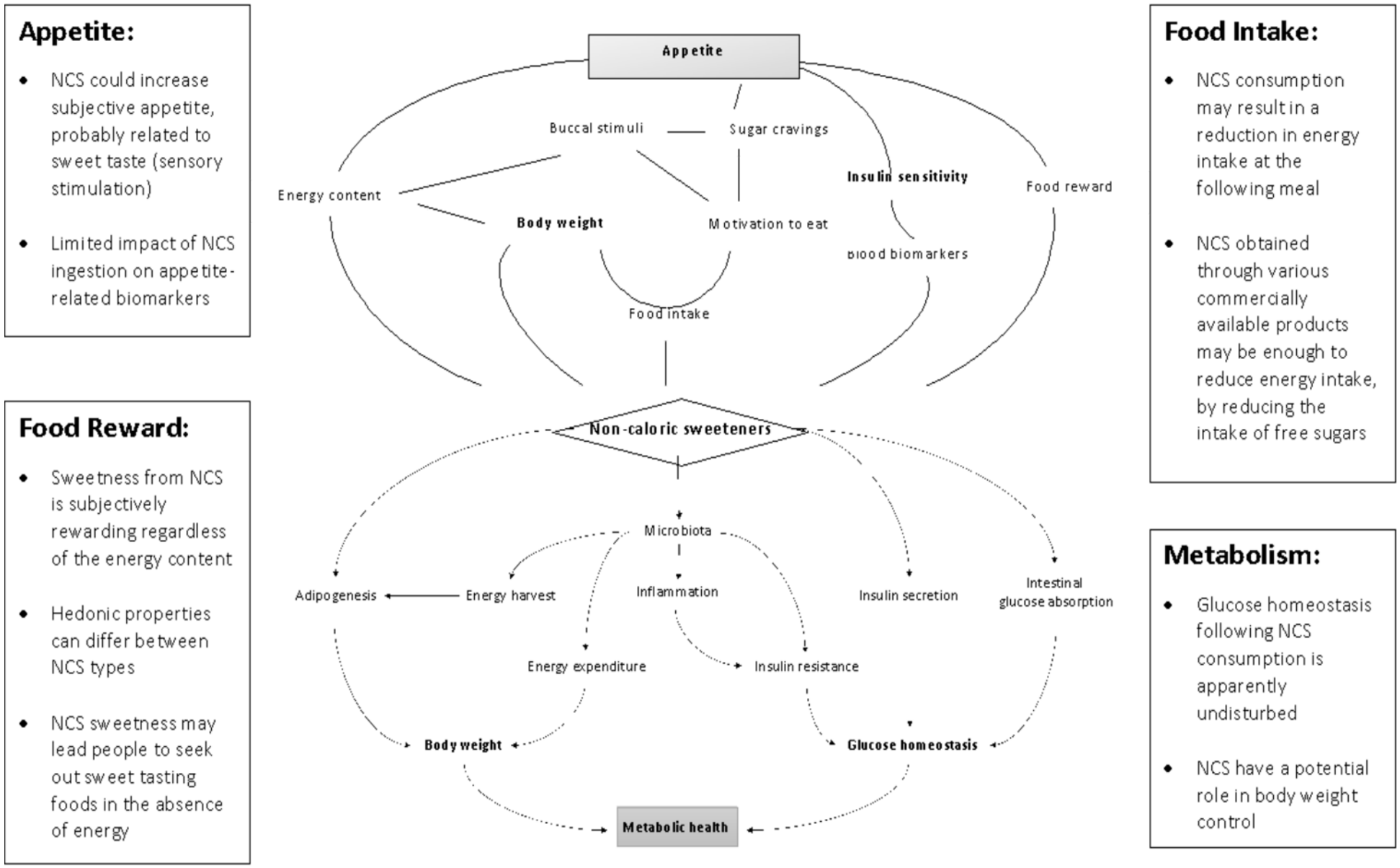

Figure 2: Proposed mechanisms of non-caloric sweeteners on metabolic health. Non-caloric sweeteners may induce gut microbiota dysbiosis. Thereupon, short chain fatty acid levels may increase and enhance energy harvest and energy expenditure. Furthermore, the gut microbiota dysbiosis has been linked to inflammation and insulin resistance. Moreover, non-caloric sweeteners may reach the adipose tissue and affect adipogenesis. In addition, non-caloric sweeteners may affect glucose homeostasis via intestinal glucose absorption and insulin secretion.