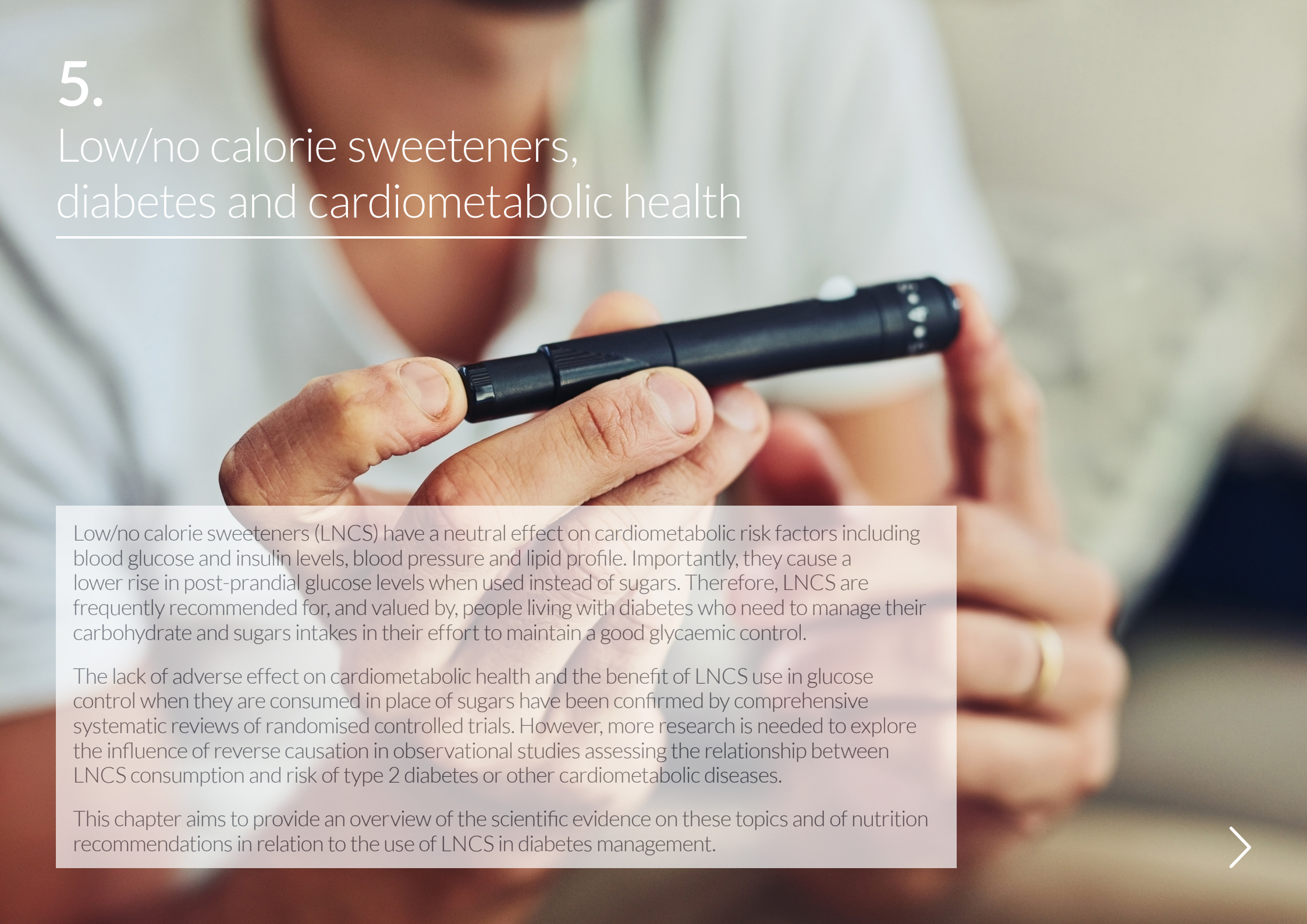
5.
Low/no calorie sweeteners, diabetes and cardiometabolic health



Low/no calorie sweeteners (LNCS) have a neutral effect on cardiometabolic risk factors including blood glucose and insulin levels, blood pressure and lipid profile. Importantly, they cause a lower rise in post-prandial glucose levels when used instead of sugars. Therefore, LNCS are frequently recommended for, and valued by, people living with diabetes who need to manage their carbohydrate and sugars intakes in their effort to maintain a good glycaemic control.

The lack of adverse effect on cardiometabolic health and the benefit of LNCS use in glucose control when they are consumed in place of sugars have been confirmed by comprehensive systematic reviews of randomised controlled trials. However, more research is needed to explore the influence of reverse causation in observational studies assessing the relationship between LNCS consumption and risk of type 2 diabetes or other cardiometabolic diseases.

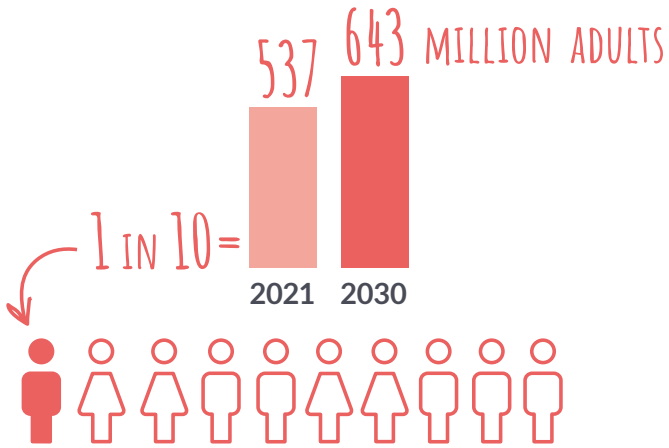
This chapter aims to provide an overview of the scientific evidence on these topics and of nutrition recommendations in relation to the use of LNCS in diabetes management.

Introduction

Cardiometabolic health is a term that refers to a combination of conditions and related risk factors, including insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease (CVD). Common risk factors involve poor glucose control, hypertension, raised blood lipid levels and increased body weight, as well as following an unhealthy lifestyle including smoking, lack of physical activity, inadequate sleep and eating an unhealthy diet *(Vincent et al, 2017)*.

Optimal cardiometabolic health rates are falling as indicated by the increasing prevalence of CVD, including heart disease and stroke, type 2 diabetes, and other cardiometabolic diseases *(World Heart Federation, 2019; International Diabetes Federation, 2021)*. A recent study found that less than 7% of the US adult population had good cardiometabolic health in 2018, declining significantly compared to 2000 *(O'Hearn et al, 2022)*. It is believed that the COVID-19 pandemic has further affected cardiometabolic health, as there is evidence that physical activity decreased and unhealthy habits increased during the lockdown periods *(Freiberg et al, 2021)*.

Diabetes and cardiovascular disease (CVD): Facts and figures



In 2021, **537 million adults** were living with diabetes - 1 in 10 adults globally. By 2030, this number is predicted to further rise to 643 million.

In 2019, **CVD caused 18.6 million deaths worldwide**. This marks a **24% increase** in the global CVD burden compared to 2000.

Following a healthy diet, exercising regularly, maintaining a normal body weight, and avoiding tobacco use are ways to **prevent or delay the onset of cardiometabolic diseases**.

Sources:
International Diabetes Federation (IDF). IDF Diabetes Atlas, 10th edition, 2021. Available at: https://diabetesatlas.org/
World Heart Federation (WHF). World Heart Observatory. Trends in cardiovascular disease. 2019. Available at: https://worldheartobservatory.org/trends/

A healthy diet is key to protecting cardiometabolic health. Eating a balanced diet low in dietary fat, salt and sugars that includes a variety of fruits and vegetables, legumes, nuts, and whole grains, can help prevent or manage cardiometabolic diseases including CVD and type 2 diabetes *(WHO, 2020)*. Limiting excess intake of free sugars is globally recommended as part of a healthy diet *(WHO, 2015; USDA, 2020; EFSA, 2022)*. **LNCS can help individuals reduce excessive sugars intake and be part of an overall healthy diet and lifestyle, including for people with, or at risk of, cardiometabolic diseases.**

Low/no calorie sweeteners and glycaemic control

Evidence from randomised controlled trials

Several systematic reviews including meta-analyses of a large battery of available randomised controlled trials (RCTs) have examined the impact of LNCS on glycaemic control (Table 1). These comprehensive studies that consider the totality of published controlled clinical trials confirm that, as food ingredients, LNCS have no effect on blood glucose levels post-prandially, i.e., after food ingestion *(Romo-Romo et al, 2016; Tucker and Tan, 2017; Nichol et al, 2018; Greyling et al, 2020; Zhang et al, 2023)*, or after longer-term consumption *(Lohner et al, 2020; McGlynn et al, 2022; Rios-Leyvraz and Montez, 2022)*. Similarly, LNCS do not affect insulin secretion and blood insulin levels *(Greyling et al, 2020; Lohner et al, 2020; McGlynn et al, 2022; Rios-Leyvraz and Montez, 2022; Zhang et al, 2023)*. The absence of glycaemic or insulinemic effect of LNCS has been shown for healthy individuals as well as for people living with diabetes *(Greyling et al, 2020; Lohner et al, 2020)*.

In 2022, a systematic review by the World Health Organization (WHO) including a meta-analysis of 21 medium- to long-term RCTs reporting on intermediate markers of type 2 diabetes concluded that LNCS had no significant effects on any measures of glycaemic control (fasting glucose, fasting insulin, HbA1c (glycosylated haemoglobin), HOMA-IR (homeostatic model assessment of insulin resistance) in healthy adults or children *(Rios-Leyvraz and Montez, 2022)*. Similarly, a Cochrane and WHO-supported systematic review and meta-analysis of 9 long-term RTCs also indicated a neutral effect of LNCS on glycaemic control and other health outcomes in people living with type 1 or type 2 diabetes *(Lohner et al, 2020)*. Similar findings were reported for people living with overweight or obesity in a systematic review and network meta-analysis of 17 RCTs with a median duration of 12 weeks, involving 1733 participants *(McGlynn et al, 2022)*. McGlynn and colleagues examined the impact of LNCS beverages on several cardiometabolic risk factors and found no long-term effect on glycaemia or other outcomes.

With the aim to examine the acute effect of LNCS consumption, Greyling and colleagues (2020) conducted a systematic review and meta-analysis of RCTs showing that the ingestion of LNCS, consumed either alone or together with a caloric preload, had no acute effects on postprandial glycaemic (34 trials involving 452 participants) or insulinemic responses (29 trials involving 394 participants) compared with a control intervention. The results did not appreciably differ by the type or dose of LNCS consumed. Interestingly, in patients with type 2 diabetes, results showed a small beneficial effect of LNCS on postprandial glucose response, versus control *(Greyling et al, 2020)*.

Zhang and colleagues (2023) concluded to similar results in a systematic review and network meta-analysis of data from 36 acute feeding trials (involving 472 participants) examining the short-term effect of LNCS beverage consumption on glycaemic and endocrine responses, versus water or sugar-sweetened beverages (SSBs). The study found that, like water, beverages with either single or blends of LNCS had no effect on postprandial glucose or insulin levels, or on endocrine responses (i.e., glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), peptide YY (PYY), ghrelin, leptin, and glucagon), whereas SSBs increased postprandial glucose, insulin, and incretin levels. The results were similar in all tested patterns of intake, i.e., when LNCS beverages were consumed alone, or together with additional energy (calories) from carbohydrates, or when given as a preload, prior to added energy/ carbohydrates *(Zhang et al, 2023)*.

What is glycaemic control?

Glycaemic control is a term referring to the regulation of blood glucose levels. In people with diabetes, many of the long-term complications of diabetes result from many years of elevated levels of glucose in the bloodstream, which is also referred to as hyperglycaemia. Therefore, good glycaemic control is an important goal in diabetes care *(IDF, 2021)*.

Earlier reviews reported similar findings. In their systematic review and meta-analysis of 29 RCTs involving 741 participants, Nichol and colleagues found that the intake of LNCS did not increase glycaemia post-prandially (Figure 1), and that the glycaemic impact did not differ by type of LNCS (Nichol et al, 2018). A year earlier, Tucker and Tan concluded that under acute conditions, when administered without a carbohydrate load, LNCS consumption led to reduced blood glucose levels compared to caloric sweeteners such as sugars *(Tucker and Tan, 2017)*. This was not attributed to a direct effect of the LNCS consumption, but rather to an absence of an effect and a total lower carbohydrate load that led to a lower blood glucose response. The review also found that LNCS did not differ from water in their effects on blood glucose. Romo-Romo and colleagues also suggested that the majority of RCTs reported neutral effects on blood glucose and insulin levels, but a meta-analysis was not conducted in this study *(Romo-Romo et al, 2016)*.

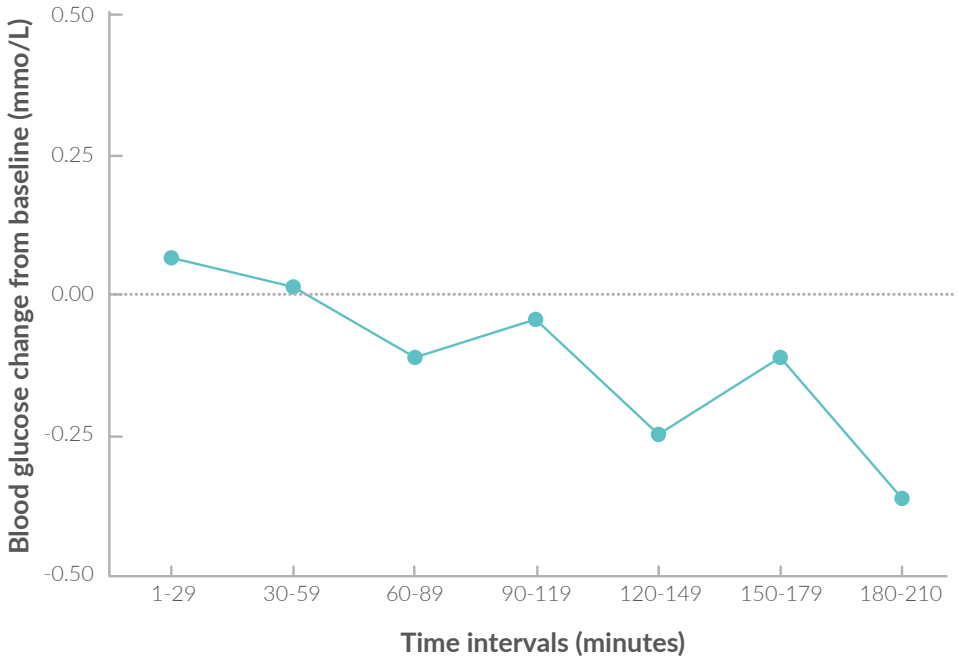


Figure 1: Estimated trajectory for glycaemic impact of low/no calorie sweeteners consumption over 210 minutes following ingestion, as estimated in the meta-analysis by Nichol et al. (2018).

The benefit of LNCS on glucose control when used in place of sugars has been recognised more than a decade ago. Reviewing the collective evidence, the European Food Safety Authority (EFSA) concluded in a scientific opinion that: **"Consumption of foods containing intense sweeteners instead of sugar induces a lower blood glucose rise after their consumption compared to sugar-containing foods"** *(EFSA, 2011)*. This is an authorised health claim in the EU as stated in the Commission Regulation (EU) No 432/2012.

Low/no calorie sweeteners cause a lower spike in post-prandial blood glucose levels when used instead of sugars, without otherwise affecting overall glycaemic control.

Table 1: Systematic reviews and meta-analyses of randomised controlled trials (RCTs) examining the impact of low/no calorie sweeteners on glycaemic control.

Systematic review (first author, year)	Number of included studies	Study characteristics (PICO)				Conclusions
		Population	*Intervention*	*Comparison*	*Outcome*	
Romo-Romo et al, 2016*	28 acute and long-term studies (including non-RCTs)	Adult population of any gender, weight and diabetes status	Any type of LNCS, ingested alone, or with a meal, or as preloads	Water or caloric sweeteners	Glucose, Insulin, HbA1c, GLP-1, GIP, C-peptide	Majority of RCTs reported neutral effects on blood glucose and insulin levels. No possible comparison between trials due to heterogeneity. No meta-analysis.
Tucker & Tan, 2017*	41 RCTs, acute studies	Adult population of any gender, weight and diabetes status	Any type of LNCS, ingested alone, or with a meal, or as preloads	Water or caloric sweeteners or placebo	Fasting blood glucose, Fasting blood insulin, Glucagon, GLP-1, GIP, Glucose absorption rates	No acute effects on measures of glycaemic control when LNCS are administered alone. LNCS lead to reduced blood glucose when compared with caloric sweeteners. No meta-analysis.
Nichol et al, 2018	29 RCTs, acute studies	Population of any age, gender, weight and diabetes status	LNCS under examination included aspartame, saccharin, steviosides, & sucralose	Comparison with baseline (Trajectory over time, from baseline to 210 min after consumption)	Change in blood glucose levels	LNCS consumption did not increase blood glucose level, and its concentration gradually declined following LNCS intake. No difference by type of LNCS.
Greyling et al, 2020	34 RCTs for postprandial blood glucose & 29 RCTs for postprandial insulin response, acute studies	Population of any age >3y, gender, weight and diabetes status	Acute exposure to LNCS alone; in water, diet beverage, or intragastric infusion; or with meal or other nutrient-containing preloads	Same intervention without LNCS	Glucose iAUC, Insulin iAUC	LNCS intake, administered alone or in combination with a nutrient-containing preload, has no effect on mean change in postprandial glycemic or insulinemic responses. No difference by type and dose of LNCS.

LNCS, low/no calorie sweeteners; LNCSB, low/no calorie sweetened beverage; SSB, sugar-sweetened beverage; HbA1c, glycosylated haemoglobin A1c; GLP-1, glucagon-like peptide 1; GIP, gastric inhibitory peptide; PYY, peptide YY; iAUC, incremental area under the curve; HOMA-IR, homeostatic model assessment of insulin resistance.
*Systematic review without meta-analysis
**Systematic review with network meta-analysis

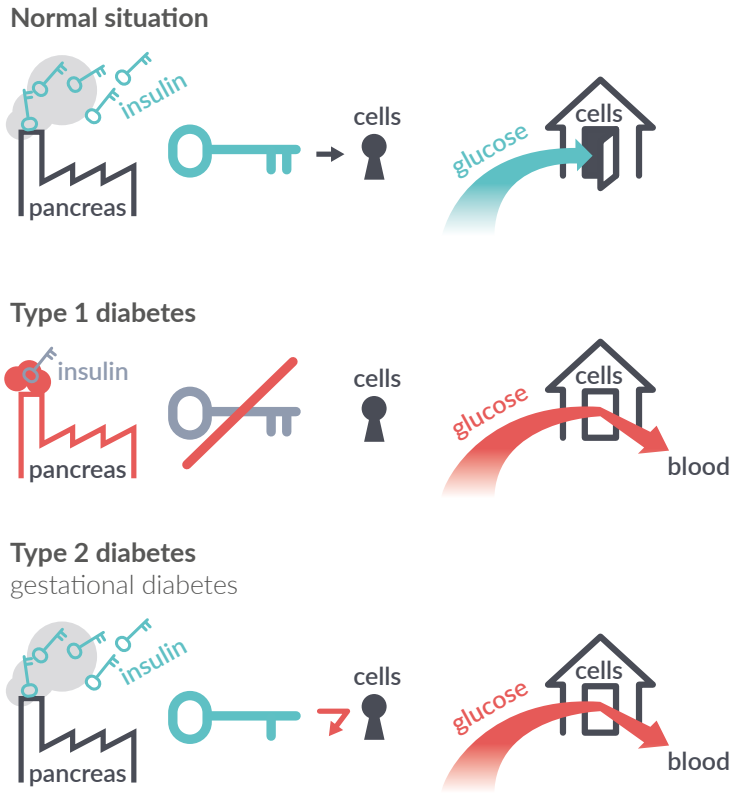
Systematic review (first author, year)	Number of included studies	Study characteristics (PICO)				Conclusions
		Population	*Intervention*	*Comparison*	*Outcome*	
Lohner et al, 2020	9 RCTs with ≥4-wk duration	Individuals with type 1 and type 2 diabetes	Any type of LNCS	Usual diet, or no intervention, or placebo, or water, or a different LNCS, or a caloric sweetener	HbA1c	Results showed no difference between LNCS and sugars, or placebo
McGlynn et al, 2022**	19 RCTs with ≥2-wk duration	Adult population of any gender, with or at risk of obesity and type 2 diabetes	LNCSBs or SSBs or water	LNCSBs vs SSBs, or SSBs vs water, or LNCSBs vs water	Fasting blood glucose, Fasting blood insulin, 2-hour post-prandial glucose, HbA1c, HOMA-IR	LNCSBs did not differ on their effects on any measures of glycaemic control, except for a greater decrease in HbA1c with water vs LNCSBs.
Rios-Leyvraz & Montez, 2022	21 RCTs in adults and 1 RCT in children with ≥7-day duration	Healthy populations of adults, children or pregnant women	Any type of LNCS	No or lower doses of LNCS or any type of sugars, or placebo, or water or no intervention	Fasting blood glucose, Fasting blood insulin, HbA1c, HOMA-IR	No significant effects were observed for any measure of glycaemic control
Zhang et al, 2023**	36 acute feeding trials	Population of any age, gender, weight and health status	LNCSBs with single of LNCS blends or SSBs or water	LNCSBs vs SSBs or vs water	Glucose iAUC, Insulin iAUC, GLP-1 iAUC, PYY iAUC, GIP iAUC, Ghrelin iAUC, Glucagon iAUC	No effect of LNCSBs on glycaemic and endocrine responses, like water. SSBs increased postprandial glucose, insulin, and incretins

LNCS, low/no calorie sweeteners; LNCSB, low/no calorie sweetened beverage; SSB, sugar-sweetened beverage; HbA1c, glycosylated haemoglobin A1c; GLP-1, glucagon-like peptide 1; GIP, gastric inhibitory peptide; PYY, peptide YY; iAUC, incremental area under the curve; HOMA-IR, homeostatic model assessment of insulin resistance.
*Systematic review without meta-analysis
**Systematic review with network meta-analysis

The role of low/no calorie sweeteners in the diet of people living with diabetes

The absence of glycaemic effect, and the lower spike in postprandial blood glucose LNCS cause when used instead of dietary sugars, makes them a useful dietary aid for people living with diabetes who need to manage their carbohydrate and sugars intake.

Living with diabetes often means being constantly concerned about what and how much to eat and feeling deprived, especially when it comes to sweet taste. However, having diabetes shouldn't keep people from enjoying a variety of foods including some favourites in moderation.



Diabetes is a serious, chronic condition that occurs either when the pancreas cannot produce enough insulin or when the body cannot effectively use the insulin it produces. Source: IDF Diabetes Atlas, 10th edition, 2021.

In persons living with diabetes, blood glucose levels are affected by how much carbohydrate is being consumed within each meal *(Evert et al, 2019)*. Therefore, managing carbohydrate intake and reducing excess sugars' consumption are important aspects of glycaemic control in diabetes management *(ElSayed et al, 2023)*. Using LNCS instead of sugars can make meal planning for diabetes management easier. Furthermore, because humans have an innate preference for sweet taste (see Chapter 7), having palatable, good-tasting foods can help improve the compliance in meal planning for diabetes. In addition, a variety of LNCS products can help people with diabetes feel less deprived *(ElSayed et al, 2023)*. There should be no expectation that LNCS, by themselves, would decrease blood glucose levels as they are not substances that can exert pharmacologic-like effects, however, **LNCS can help provide people with diabetes with wider food choices and satisfy their cravings for sweet taste without contributing to raised blood glucose levels or increased insulin needs** *(Fitch et al, 2012)*. In addition, using LNCS in place of sugars in the context of an overall healthy diet can help reduce energy intake and be a useful tool in nutritional strategies for weight management, which is especially important for people living with type 2 diabetes or pre-diabetes who need to lose weight or prevent additional excess weight gain *(Diabetes UK, 2018)*. This strategy may be particularly helpful for people who regularly consume sweet foods and especially SSBs. The role of LNCS in weight control is discussed in Chapter 4.

For individuals with type 1 diabetes, a key element in the nutritional management of their diabetes is carbohydrate-counting meal planning adjustments to insulin doses based on carbohydrate intake. The American Diabetes Association's consensus recommendations on nutrition therapy support that intensive insulin therapy using the carbohydrate counting approach can result in improved glycaemia *(Evert et al, 2019)*. In this context, using LNCS in place of sugars in foods and drinks has the potential to reduce the carbohydrate content in a meal or snack, and thus to reduce the insulin dose required for this eating occasion.

"Any dietary measure that has the potential to limit an excessive rise in blood glucose levels can assist with overall glycaemic control and is therefore likely to promote the maintenance of optimal health. A considerable amount of scientific evidence demonstrates that the substitution of sugars with low/no caloric sweeteners is one of the available means to help achieve this goal as, by themselves, low/no caloric sweeteners do not induce any glycaemic excursion."

Dr Marc Fantino, Emeritus Professor

Diabetes- and nutrition-related organisations support the use of low/no calorie sweeteners in diabetes management

Several health organisations around the world have issued clinical guidelines for the nutritional management of diabetes. Nutritional recommendations aim to serve as a guide for health professionals in educating their patients, and ultimately, to help individuals living with diabetes make more balanced and healthier choices in order to improve their glycaemic control.

Diabetes-related organisations globally, including the American Diabetes Association (ADA), the Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes (EASD), Diabetes UK, Diabetes Canada, and the Latin-American Association of Diabetes (Asociación Latinoamericana de Diabetes – ALAD) recognise that **LNCS can be safely used to replace dietary sugars and be a useful tool in the nutritional management of diabetes**.

In its 2023 update of Medical Nutrition Therapy recommendations, ADA supported that: "The use of nonnutritive sweeteners as a replacement for sugar-sweetened products may reduce overall calorie and carbohydrate intake as long as there is not a compensatory increase in energy intake from other sources. There is evidence that low- and no-calorie sweetened beverages are a viable alternative to water." *(ElSayed et al, 2023)*

In the same year, the Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD) published updated European recommendations for the nutritional management of diabetes with the aim to provide health professionals with evidence-based guidelines *(Reynolds et al, 2023)*.

The European guidelines recommend the use of LNCS to replace sugars in foods and beverages, while the intake of free or added sugars should be below 10% of total energy intake. The latest European recommendations on sweeteners are based on a series of systematic reviews and meta-analyses of RCTs *(McGlynn et al, 2022)* and prospective cohort studies *(Lee et al, 2022)* assessing the impact of LNCS beverages on cardiometabolic health in people with or at risk of developing diabetes. The two studies concluded that LNCS beverages, when replacing SSBs, reduce body weight and cardiometabolic risk factors in people with or at risk for diabetes and are associated with reductions in the risk of obesity and cardiovascular outcomes in participants inclusive of people with diabetes, with reductions similar to those seen with water *(McGlynn et al, 2022; Lee et al, 2022)*.

Similarly, the Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes supported that LNCS may be recommended for diabetes as they are safe and have no effect on glycaemia *(Dyson et al, 2018)*. In its Position Statement about the use of LNCS, Diabetes UK concluded that replacing free sugars with LNCS can be a helpful strategy to aid glucose management and weight control *(Diabetes UK, 2018)*.

In line with the above conclusions, a consensus of the Latin-American Association of Diabetes (ALAD) also acknowledged that LNCS use can have benefits in energy intake reduction, weight loss and glucose control, when used to replace sugars in the context of a structured dietary plan *(Laviada-Molina et al, 2018)*.

Also, in its 2018 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada, Diabetes Canada Clinical Practice Guidelines Expert Committee pointed out that the evidence from systematic reviews and meta-analyses of RCTs, which give a better protection against bias, have shown a weight loss benefit when LNCS are used to displace excess calories from added sugars *(Sievenpiper et al, 2018)*.

Diabetes-related organisations globally recognise that, when used in place of sugars, low/no calorie sweeteners can be a useful dietary strategy in the nutritional management of diabetes

Nutrition-related organisations have reached similar conclusions. For example, the US Academy of Nutrition and Dietetics (AND) recommended that registered dietitians and nutritionists (RDNs) should educate adults living with diabetes that the use of approved LNCS does not significantly affect glucose or insulin levels and has the potential to reduce overall energy and carbohydrate intake if they are used in place of caloric sweeteners, without compensation by intake of additional calories from other food sources *(Franz et al, 2017; MacLeod et al, 2017)*. Likewise, the British Dietetic Association (2016) supported that opting for LNCS may assist in the management of weight and other health conditions such as diabetes mellitus adding that a tailored individualised approach is required.

People living with diabetes consider low/no calorie sweeteners as a useful dietary tool…

- "They help me feel less deprived while still enjoying sweet taste in my diet"
- "Low/no calorie sweeteners can be a quick and easy replacement for sugar"

Source: Patients' focus group as part of ISA activities for World Diabetes Day 2017

Low/no calorie sweeteners and cardiometabolic risk factors beyond diabetes markers

Evidence from randomised controlled trials

Human clinical research shows that, beyond a lack of effect on glycaemic control, LNCS ingestion has a neutral, or even beneficial, impact on other cardiometabolic intermediate markers such as blood pressure and blood lipids, liver enzymes, uric acid and intrahepatocellular lipid *(Onakpoya and Heneghan, 2015; Pham et al, 2019; Toews et al, 2019; Movahedian et al, 2021; McGlynn et al, 2022; Rios-Leyvraz and Montez, 2022; Golzan et al, 2023)*.

The WHO systematic review reported that higher intakes of LNCS did not have a significant effect on systolic or diastolic blood pressure *(meta-analysis of 14 RCTs)*, though a trend to lower systolic blood pressure was observed with LNCS intake *(Rios-Leyvraz and Montez, 2022)*. Furthermore, this study found no significant effects for any blood lipid measure in RCTs (meta-analysis of 14 RCTs), including LDL cholesterol or triglycerides, with the exception of a small, clinically insignificant, increase in total cholesterol:HDL cholesterol.

In their systematic review and network meta-analysis, McGlynn and colleagues reported a neutral effect of LNCS beverages on glycaemia, blood lipid levels, uric acid and liver enzymes, and a beneficial effect of LNCS beverages as an intended substitute for SSBs in Body Mass Index (BMI), percentage of body fat, and intrahepatocellular lipid, which was a result of displacement of calories from SSBs *(McGlynn et al, 2022)*. The study also found that LNCS beverages compared with water were associated with a greater decrease in systolic blood pressure.

Other systematic reviews are in line with these conclusions *(Pham et al, 2019; Toews et al, 2019; Movahedian et al, 2021; Golzan et al, 2023)*. A systematic review and meta-analysis of 10 RCTs, involving 854 participants, showed that LNCS intake had no significant effect on liver enzyme levels in adults *(Golzan et al, 2023)*. Also, Movahedian and colleagues systematically reviewed and meta-analysed data from 14 RCTs, involving 1407 participants, that examined the impact of LNCS on blood triglyceride levels, total cholesterol, LDL- and HDL cholesterol. The results showed non-significant effects of LNCS on lipid profile *(Movahedian et al, 2021)*. Also, Pham et al (2019) concluded that LNCS have demonstrated minimal or no effect on postprandial blood pressure, while Toews et al (2019) reported that data from three RCTs showed that systolic and diastolic blood pressure were lower in people receiving LNCS than in those receiving sugars or placebo, and two other RCTs reported a neutral effect.

Collectively, **evidence from systematic reviews of RCTs, including from the WHO review by Rios-Leyvraz and Montez (2022), does not support a WHO recommendation suggesting against the use of non-sugar sweeteners as a means for reducing the risk of non-communicable diseases** (WHO, 2023). This recommendation was largely based on low certainty evidence from observational studies with important methodological issues, while clinical studies in humans consistently show a neutral or even beneficial impact, and no adverse effect, of LNCS on cardiometabolic intermediate markers and risk factors of non-communicable diseases (NCDs).

Low/no calorie sweeteners and risk of diabetes and cardiovascular disease

Evidence from observational studies

Contrary to evidence from RCTs, which consistently indicates a lack of adverse effect of LNCS on cardiometabolic risk factors, observational research reports inconsistent outcomes. As a result, while some systematic reviews and meta-analyses of observational studies have reported a positive association between higher LNCS intake and risk of diabetes or CVD *(Romo-Romo et al, 2016; Azad et al, 2017; Meng et al, 2021; Rios-Leyvraz and Montez, 2022)*, this was not confirmed in a recent review including meta-analysis of prospective cohort studies that used repeated measures of LNCS intake and substitution analyses to mitigate the influence of reverse causation *(Lee et al, 2022)*. Importantly, systematic reviews of observational studies mainly provide low certainty evidence as a result of the limitations of observational research. **By design, observational studies cannot establish a cause-and-effect relationship due to their inability to exclude residual confounding or attenuate the effects of reverse causality**, as discussed in Chapter 4.

Reverse causation is a major risk of bias in observational research. The term implies that individuals who are already at high risk for disease at baseline (e.g., have elevated risk factors) may have in response turned to, or increased, LNCS intake, thus leading to a spurious association between LNCS intake and increased cardiometabolic risk *(Rios-Leyvraz and Montez, 2022)*. In addition, inaccuracies resulting from the methods used to assess dietary intake of LNCS, usually evaluated only at baseline, raise concerns regarding the reliability and interpretation of associations reported in observational studies *(Gallagher and Logue, 2019)*. Baseline analyses of LNCS intake cannot capture change over time or the intended replacement strategy of the substitution of SSBs with LNCS beverages and are susceptible to reverse causation, resulting in an underestimation of the intended cardiometabolic benefits *(Lee et al, 2022)*.

Prospective observational studies that have used substitution analyses that model the intended replacement strategy for LNCS sweetened beverages (i.e., substitution of SSBs with LNCS beverages) can partly overcome these methodological limitations and provide more consistent results. For example, results from the Harvard Pooling Project of Diet and Coronary Disease Substitution analyses suggested that replacing SSBs with LNCS beverages might be associated with a lower risk of developing coronary events *(Keller et al, 2020)*.

A systematic review and meta-analysis by EASD's Diabetes and Nutrition Study Group included only prospective observational studies that used change analyses of repeated measures of intake and substitution analyses in order to minimize the impact of reverse causality and residual confounding from incomplete adjustment of confounders *(Lee et al, 2022)*. The results of this meta-analysis of 14 prospective cohort studies (416,830 participants) showed that the intended substitution of SSBs with LNCS beverages was associated with lower body weight and lower risk of incident obesity, coronary heart disease, CVD and total mortality, with no adverse associations across other outcomes such as type 2 diabetes. The findings by Lee et al (2022) confirm that LNCS are not associated with higher, but rather, with a lower risk in important cardiometabolic outcomes in the intended substitution for SSBs, comparable with outcomes for water, and are in line with the evidence from systematic reviews and meta-analyses of RCTs of intermediate cardiometabolic risk factors *(McGlynn et al, 2022; Rios-Leyvraz and Montez, 2022)*.

Indeed, the association between the consumption of LNCS and the risk of diabetes that is reported in observational studies is usually attenuated or lost after adjustment for variables, including age, physical activity, family history of diseases, diet quality, energy intake and mainly measures of adiposity such as BMI and waist circumference *(Romo-Romo et al, 2017)*. In a meta-analysis of ten observational studies estimating the risk of type 2 diabetes by consuming LNCS beverages, Imamura et al. found that after adjustment for BMI and the calibration for information and publication bias, the association between LNCS drinks and the development of type 2 diabetes was no longer statistically significant *(Imamura et al, 2015)*. Similarly, links between LNCS intake and CVD reported in some studies *(Mossavar-Rahmani et al, 2019; Debras et al, 2022)* are subject to the same criticism: limitations of observational studies including selection bias, reverse causation and residual confounding may partly or largely explain the reported associations *(Khan et al, 2019; Pyrogianni & La Vecchia, 2019)*.

By design, observational studies cannot establish a causal relationship due to their inability to exclude residual confounding or attenuate the effects of reverse causality

How can we interpret contradictory findings between randomised controlled trials and observational research studying low/no calorie sweeteners' cardiometabolic health effects?

Prof Carlo La Vecchia: Randomised controlled trials (RCTs) provide a more valid and reliable evidence than observational (cohort and case-control) studies essentially since they are not affected by selection bias. Information and other sources of bias can also severely distort the findings of observational studies but are of little or no relevance for RCTs where allocation is randomised. Thus, the evidence from RCTs that LNCS have a favourable – though moderate – effect on cardio-metabolic, and more in general cardiovascular risk factors has to be considered the valid and relevant one on the issue.

Since most RCTs have a limited duration, they cannot provide adequate information on long-term effects of LNCS on the risk of cardiovascular disease and cardiometabolic factors. The apparently inconsistent findings of several observational studies are largely or totally attributable to reverse causation, i.e., in the long term LNCS tend to be more frequently used by subjects with overweight and obesity, hyperglycemia, diabetes or – more in general – an unfavourable cardiometabolic profile. There is no way to overcome such inherent bias of observational studies, and it is also not possible to reliably estimate its possible impact on the outcomes of interest. Other sources of bias and confounding of observational studies may also distort the findings. As a general rule, a change in relative risk estimates of the order of 20% (i.e. RRs 0.80 to 1.20) do not allow inference on causation since bias and confounding cannot be excluded.

In short, LNCS are associated to favourable cardiometabolic patterns in the short term. Assuming adequate compliance, these should be maintained in the long term too, but data on long term effects from RCTs are inadequate for the moment.

Examining proposed mechanisms linking low/no calorie sweeteners to cardiometabolic effects

Several potential mechanisms have been suggested and explored mostly in in-vitro and animal studies in an attempt to explain the positive association reported in some observational studies. Proposed mechanisms include alterations in intestinal glucose absorption, changes in insulin secretory capacity, insulin resistance, and sweetener-induced gut microbiota dysbiosis *(Pang et al, 2021)*. However, a 2018 science advisory from the American Heart Association (AHA) on LNCS beverages and cardiometabolic health warned that caution is required before drawing conclusions about whether these findings, primarily conducted in rodents, are applicable to humans *(Johnson et al, 2018)*. To date none of the proposed mechanisms of how LNCS could affect glucose homeostasis or otherwise increase the risk of cardiometabolic diseases has been confirmed in humans *(O'Connor et al, 2021; McGlynn et al, 2022)*.

Importantly, evidence from RCTs does not confirm these hypotheses and consistently shows no adverse effect on risk factors linked to cardiometabolic health, including blood pressure, blood lipids levels, glucose homeostasis, or body weight *(Nichol et al, 2018; Pham et al, 2019; Toews et al, 2019; Greyling et al, 2020; Movahedian et al, 2021; Rogers and Appleton, 2021; McGlynn et al, 2022; Rios-Leyvraz and Montez, 2022; Golzan et al, 2023; Zhang et al, 2023)*.

Intestinal Glucose Absorption

It has been suggested that LNCS may enhance intestinal glucose absorption by activating sweet taste receptors in the gut, which, in turn stimulates the secretion of incretin hormones, glucagon-like protein-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), known to have a role in regulating glucose absorption and promoting insulin release. Nevertheless, to date no differences in intestinal glucose absorption in humans have been reported *(O'Connor et al, 2021; Pang et al, 2021; Zhang et al, 2023)*

The present hypothesis stems largely from isolated cell or tissue (in vitro) experiments that typically utilised LNCS concentrations that were extraordinarily high *(Fujita et al, 2009)*. Because effects are seen under these testing conditions, however, does not mean they are reliable for interpreting what happens with exposure in the whole human body. Contrary to the findings of these in vitro studies, most clinical human trials have found no effects of LNCS on circulating incretin hormones levels (*Gregersen et al, 2004; Ma et al, 2009; Ma et al, 2010; Ford et al, 2011; Steinert et al, 2011; Maersk et al, 2012a; Wu et al, 2012; Wu et al, 2013; Sylvetsky et al, 2016; Higgins et al, 2018; Ahmad et al, 2020a; Romo-Romo et al, 2020; Orku et al, 2022; Zhang et al, 2023)*.

In a few studies testing the effects of LNCS-containing beverages, results reported a significant increase in GLP-1 in healthy adults with overweight and obesity *(Brown et al, 2009; Temizkan et al, 2015; Sylvetsky et al, 2016; Lertrit et al, 2018)* or in healthy youth with and without type 1 diabetes *(Brown et al, 2012)*, however, these effects have not been found in patients with type 2 diabetes participating in the same studies *(Brown et al, 2012; Temizkan et al, 2015)*. It is unknown whether levels of changes in endogenous GLP-1 secretion as observed in these studies have any clinically relevant consequences *(Brown et al, 2012)*. Importantly, the collective evidence as assessed in a systematic review and network meta-analysis of 36 acute feeding studies showed that LNCS beverages with single or blends of LNCS had no significant effect on endocrine responses including GLP-1 and GIP, similar to water controls, when consumed alone, or together with, or prior to the consumption of a carbohydrate load *(Zhang et al, 2023)*.

Taken together, current evidence from human studies doesn't support a clinically meaningful stimulatory effect of LNCS on the secretion of gut hormones in humans *(Bryant and McLaughlin, 2016; Grotz et al, 2017; Ahmad et al, 2020b; Zhang et al, 2023)*.

Insulin secretion

A large body of evidence, as comprehensively assessed in systematic reviews and meta-analyses of RCTs, confirms that LNCS do not significantly affect blood insulin levels *(Greyling et al, 2020; Zhang et al, 2023)*. Moreover, human data collectively do not confirm proposed mechanisms suggesting that LNCS may affect insulin secretion via eliciting a cephalic phase insulin response (CPIR) or by stimulating the gut sweet taste receptors *(O'Connor et al, 2021; Pang et al, 2021)*.

CPIR is an early low-level increase in blood insulin associated with only oral exposure, i.e., occurring prior to increasing plasma glucose levels typically seen with intake of foods containing carbohydrate. Eliciting CPIR has sometimes been hypothesized as a possible way for LNCS to cause hunger (see Chapter 4) or a later increase in blood glucose levels that is abnormal *(Mattes and Popkin, 2009)*. While a few studies have suggested that exposure to LNCS may elicit a CPIR *(Just et al. 2008; Dhillon et al. 2017)*, most clinical trials did not confirm such an impact *(Teff et al, 1995; Abdallah et al, 1997; Morricone et al, 2000; Ford et al, 2011; Pullicin et al, 2021)*. Additionally, other research has suggested that CPIR is generally not a meaningful determinant in hunger or glucose response *(Morey et al, 2016)*. Recently, a systematic review on endocrine cephalic phase responses to food cues concluded that there was weak evidence for human CPIR and, importantly, the evidence for the existence of a physiologically relevant CPIR seemed minimal *(Lasschuijt et al, 2020)*. Taken together, human data collectively do not support the assertion that LNCS may significantly affect insulin secretion and blood insulin levels, nor confirm an adverse effect of LNCS on either appetite regulation or glucose metabolism *(Tucker and Tan, 2017; Greyling et al, 2020; O'Connor et al, 2021; Pang et al, 2021; Zhang et al, 2023)*.

Insulin sensitivity

The potential effect of LNCS on insulin sensitivity garnered attention primarily following the publication in 2014 of an animal experiment and a small, non-randomised human trial in 7 subjects by Suez and colleagues suggesting that high doses of saccharin at the ADI level might contribute to insulin resistance via effects on the gut microbiota *(Suez et al, 2014)*. Several controlled human clinical studies have been conducted since then. A few RCTs have suggested a potential adverse effect of sucralose on insulin sensitivity *(Lertrit et al, 2018; Romo-Romo et al, 2018; Bueno-Hernández et al, 2020; Romo-Romo et al, 2020)*. However, in one study the effect was not consistent with dose *(Bueno-Hernández et al, 2020)*, and a second study reported an increase in the homeostasis model assessment of insulin resistance only at 1 week postdosing, but not during or after the end of the intervention, which is of unknown clinical significance, if any *(Romo-Romo et al, 2020)*. In contrast, the majority of published RCTs have shown no impact of different doses of LNCS including aspartame alone *(Maersk et al, 2012b; Engel et al, 2018; Higgins and Mattes, 2019; Ahmad et al, 2020a)* or in blend with acesulfame-K *(Bonnet et al, 2018; Kim et al, 2020; Orku et al, 2022)*, saccharin *(Higgins and Mattes, 2019; Serrano et al, 2021; Orku et al, 2022)*, steviol glycosides *(Higgins and Mattes, 2019)*, and sucralose *(Higgins and Mattes, 2019; Thomson et al, 2019; Ahmad et al, 2020a; Orku et al, 2022)* on insulin sensitivity. A meta-analysis of 11 RCTs in the WHO systematic review also confirmed a neutral effect of LNCS on HOMA-IR, a method for assessing insulin resistance *(Rios-Leyvraz and Montez, 2022)*.

Gut microbiota

Some LNCS compounds have been assumed to affect glucose homeostasis and/or insulin sensitivity by modulating the gut microbiota *(Suez et al, 2014; Richardson and Frese, 2022; Suez et al, 2022)*. Most research to date has been studies involving in-vitro and animal experiments, and often, testing has utilized very high doses of LNCS *(Lobach et al, 2019; Ruiz-Ojeda et al, 2020; Plaza-Diaz et al, 2020)*, limiting biological relevance due to differences in the rodent gut microbiome and limitations in extrapolating tested concentrations in vitro to human exposure levels from the diet *(Hughes et al, 2021)*. A few RCTs have investigated potential gut microbiota changes following exposure to different types and doses of LNCS in humans reporting mixed and inconsistent findings *(Thomson et al, 2019; Ahmad et al, 2020c; Serrano et al, 2021; Méndez-García et al, 2022; Suez et al, 2022)*.

Three controlled clinical trials found no impact of aspartame *(Ahmad et al, 2020c)*, saccharin *(Serrano et al, 2021)* or sucralose *(Thomson et al, 2019; Ahmad et al, 2020c)* on gut microbiota, and ultimately on glucose homeostasis or insulin sensitivity. A randomized, double-blind controlled trial in 34 subjects using a parallel study design concluded that consumption of high doses of sucralose for 7 days did not alter glycaemic control, insulin resistance, or gut microbiome in healthy individuals *(Thomson et al, 2019)*. Another RCT of cross-over design in 17 participants found that daily repeated consumption of pure aspartame or sucralose for 14 days in doses reflective of typical high consumption had no impact on gut microbiota composition or the production of short-chain fatty acids (SCFAs), a subset of fatty acids that are produced by the gut microbiota *(Ahmad et al, 2020c)*. Interestingly, a double-blind, placebo-controlled, parallel arm RCT in 23 adults also showed that the consumption of pure saccharin at maximum acceptable levels for 2 weeks did not alter microbial diversity or composition in humans and mice alike, nor caused any changes in fecal metabolites or SCFAs *(Serrano et al, 2021)*. Results also showed no impact of saccharin consumption on glucose tolerance. These findings by Serrano et al, who used a well-controlled trial design, contradicted the results of a small study by Suez et al, which lacked a control group, and suggested that in 4 out of 7 participants saccharin administration at ADI levels for 1 week induced glucose intolerance by altering the gut microbiota *(Suez et al, 2014)*.

In contrast, two human studies reported potential adverse effects of LNCS on gut microbiota *(Méndez-García et al, 2022; Suez et al, 2022)*. An open-label, parallel-design RCT in 40 young adults reported that consumption of 48mg of sucralose for 10 weeks induced gut dysbiosis associated with altered insulin and glucose levels during an oral glucose tolerance test *(Méndez-García et al, 2022)*. However, in the present study, habitual diet was neither controlled nor well-characterised, so any reported changes in the gut microbiota could very likely be due to unreported dietary differences between the sucralose and water groups. Also, an unblinded, parallel-arm RCT testing the impact of four different LNCS, water (control) or glucose, consumed for 2 weeks in doses lower than the ADI (n=20 participants per group) suggested that some LNCS might induce person-specific, microbiome-dependent glycaemic alterations *(Suez et al, 2022)*. The latest study by Suez and colleagues reported a significant effect on the microbiome composition and function linked to elevated glycaemic response in the sucralose and saccharin groups, while aspartame and stevia had no impact on glycaemia despite inducing distinct alterations in microbiome function.

However, participants' diet in this study, while recorded, was also not fully controlled. Indeed, it is well established that, not only energy and nutrients intake, but also differences in the type of food consumed can rapidly alter the human gut microbiome *(David et al, 2014)*. Therefore, it cannot be ruled out that dietary intake aspects, which are known to affect the gut microbiota but have not been recorded in this trial, had an impact on the study results. When conducting dietary intervention studies to assess the effects of ingredients that are added to the diet in small amounts, such as LNCS, the habitual diet of the subjects should be well-characterized and the intervention diets should be carefully controlled *(Lobach et al, 2019)*. Contrary to these findings by Suez et al (2022), numerous clinical trials, and systematic reviews of RCTs, have consistently confirmed that LNCS have no impact on glycaemic response *(Grotz et al, 2017; Tucker and Tan, 2017; Nichol et al, 2018; Greyling et al, 2020; Lohner et al, 2020; Rios-Leyvraz and Montez, 2022; Zhang et al, 2023)*.

Important considerations in evaluating and interpreting research about LNCS and gut microbiota is the different absorption, distribution, metabolism, and excretion (ADME) profiles of each individual sweetener, and the biological plausibility of how the different LNCS could potentially affect the gut microbiota composition or function *(Plaza-Diaz et al, 2020)*. Importantly, extrapolation of the effect of one LNCS on the gut microflora to all LNCS is not appropriate, on the basis of well-documented differences in their chemistry, movement through the body, and the amount of LNCS or their metabolites that reach the gut microbiota *(Magnuson et al, 2016)*.

Aspartame is rapidly hydrolysed and absorbed in the small intestine and neither aspartame as an intact molecule nor its metabolites ever reach the colon or contact gut bacteria *(EFSA, 2013)*. Therefore, a direct effect of aspartame on gut microbiota synthesis or function is not biologically plausible. Similarly, it is extremely unlikely that acesulfame-K could have a direct effect on the colonic microbiota as the concentration that reaches the gut microbiota is negligible. Once ingested, acesulfame-K is absorbed almost completely in the small intestine as an intact molecule and distributed by the blood to different tissues without undergoing any metabolization, with 99% of acesulfame-K excreted in urine and less than 1% being eliminated in the feces *(Magnuson et al, 2016)*. On the other hand, sucralose has a very low level of absorption and is practically not metabolized *(Roberts et al, 2000)*. However, although more than 85% of the ingested sucralose reaches the gut microbiota, between 94% and 99% of this sweetener is recovered in the feces without any structural change, indicating practically no metabolism by the intestinal bacteria. Thus, sucralose does not appear to be a substrate for the colonic microbiota. With regard to saccharin, after its intake, more than 85% is absorbed as an intact molecule and does not undergo gastrointestinal metabolism *(Renwick, 1985; Magnuson et al, 2016)*. Hence, only a small percentage of non-absorbed saccharin is excreted into the feces, indicating that only high doses of this sweetener could lead to changes in the composition of the intestinal microbial population. Finally, steviol glycosides enter the colon as intact molecules and need bacteria for their metabolisation into steviol *(Magnuson et al, 2016)*. However, the resulting steviol is not a substrate for the intestinal microbiota, since it is resistant to bacterial degradation, and is further completely absorbed. So, while steviol glycosides interact with the colonic microbiota, there is no indication that these sweeteners could adversely affect the gut microbiota.

While certain diseases have been associated with abnormal microbiota (ie, dysbiosis), it is unclear what constitutes a "healthy" gut microbiome *(Fan and Pedersen, 2021)*. The role of the gut microbiota in affecting human health is currently an area of extensive research. There are hypotheses that certain types of changes could translate into increased risk of certain health outcomes, however, in general, the meaningfulness of most changes are unknown. There are also no changes known to be reliable biomarkers for increased risk of either becoming overweight or developing diabetes or CVD. There is commonly also a wide variability in the normal gut microbiome profile between one human subject and another, further complicating interpretation of data outcomes even from RCTs *(Lobach et al, 2019)*. Additionally, the gut microbiome profile can change daily just with normal changes in daily food intake *(David et al, 2014)*.

Taken together, there is no clear evidence that LNCS may adversely impact health via effects on the gut microbiota when consumed by humans at approved levels. The clinical significance of reported gut microbiota changes by some LNCS is questioned since, collectively, evidence from RCTs do not confirm adverse effects of LNCS on host physiology.

Considerations in interpreting research on low/no calorie sweeteners and gut microbiota. The role of study design.

Prof Wendy Russell: Dietary change such as replacing sugars with LNCS is likely to have an impact on shaping our gastrointestinal microbiota. To date, these changes are mostly substantiated from feeding trials with animal models and there are still only a handful of studies in humans where the results are contradictory *(Harrington et al, 2022)*. One study has shown that bacterial diversity (but not abundance) differed between consumers and non-consumers of aspartame and/or Acesulfame K *(Frankenfeld et al, 2015)* and another demonstrated positive correlations between high LNCS consumption and several taxonomic entities *(Suez et al, 2014)*. In contrast, three more recent interventional studies have shown no effect of sucralose and/or aspartame, or saccharin, respectively, on the gut microbiome *(Thomson et al, 2019; Ahmad et al, 2020c and Serrano et al, 2021)*. There is also evidence that inter-individual heterogeneity could be an important factor *(Suez et al, 2022)*.

While these outcomes are difficult to interpret, it is important to appreciate that changes in the microbiome do not necessarily indicate an impact on human health. If we are to begin to understand the impact of LNCS on the gut microbiota and more importantly what this means for health outcomes, several factors need to be considered. While there is a need for more well-designed randomized controlled trials, we also need information on the microbiome beyond the genus level, as most studies to date have profiled the microbiota using only 16S rRNA sequencing. Studies exploring microbiome function, which is almost completely unknown for LNCS, will be extremally informative. Intervention studies providing information at a species level, as well functional output will allow for a greater understanding of personalised effects, and this is likely key to recognising the impact of LNCS on human health.

Conclusion

In all, LNCS and foods and drinks containing them can be safely used by people living with, or at risk of developing diabetes or other cardiometabolic diseases since they have a neutral effect on cardiometabolic risk factors including blood glucose and insulin levels, blood pressure and lipid profile. Using LNCS in place of caloric sweeteners can help reduce excess sugars intake and curb cravings for something sweet without risking a spike in blood glucose levels, provided that other ingredients of the food/ drink don't influence blood glucose either. Certainly, there should be no expectation that LNCS, by themselves, would have a glucose lowering effect, but they can be part of an overall healthy diet aiming to help reduce the excess intake of calories and sugars in the diet.

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