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Low- and No- Calorie Sweeteners (LNCS): critical evaluation of their safety and health risks

Hellen Dea Barros MALULY[1]*, Craig JOHNSTON[2], Norberto Damián GIGLIO[3], Ligia Lindner SCHREINER[4], Ashley ROBERTS[5], Eyassu Getachew ABEGAZ[6]

Abstract

The increase of the prevalence of type-2 diabetes as a consequence of overweight and obesity has stimulated public health authorities worldwide to develop strategies for its risk management and prevention. Among them, a reduction on the content of sugar in sugar-sweetened foods and beverages has been suggested, which led the food industry to replace partially sucrose with food additives, such as low- and no-calorie sweeteners (LNCS). As a consequence, there has been an increase of their consumption, which has made regulatory agencies to evaluate their exposure and possible consequences. At the same time, speculations about adverse effects, such as carcinogenicity, preterm delivery and metabolic changes involving appetite, weight increase and glucose intolerance, have been published. This scenario led *ILSI Brasil* to organize a meeting to update scientific knowledge on the safety of LNCS and to promote discussions among academia, regulatory bodies and food industries to clarify currently controversial information. The results of this initiative are presented in this review. It is suggested that LNCS, when used according to recommendations provided by scientific committees and regulatory authorities are considered safe. Further studies are required to evaluate the current level of exposure in general population and specific ones as children.

Keywords: sweeteners; safety; regulation; metabolism; cancer.

Practical Application: Discussions about the safety and exposure to Low- and No- Calorie Sweeteners.

1 Introduction

One of the strongest associations with the development of type-2 diabetes and cardiovascular diseases is excessive weight gain. In 2016, the World Health Organization (World Health Organization, 2010a) estimated that more than 1.9 billion adults (18 years and older) were overweight, and, of these, over 650 million were obese. This fact indicates the urgent need to develop management strategies for the prevention of such noncommunicable diseases, including the decrease of their risk factors. In this regard, the adoption of two main behavioural modifications have been suggested: reduction of the consumption of caloric foods and the encouragement of physical exercise practice (Gregg et al., 2012; Knowler et al., 2002). Related to calorie reduction, strategies such as the replacement of sugar with low- and no- Calorie sweeteners (LNCS) in sugar-sweetened foods and beverages have been adopted by the food industry (European Food Safety Authority, 2019).

Risk assessments performed by scientific committees, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA), have demonstrated that the intake of these additives through the diet is below their respective acceptable daily intakes (ADI) levels and when they are used according to recommendations and limits to achieve their technical purpose, are considered safe (European Food Safety Authority, 2019; Food and Agriculture Organization of The United Nations, 2019a, b; Serra-Majem et al., 2018).

Concerns have been raised regarding the possible relationship between the consumption of LNCS and the development of the following adverse effects: induction of cancer cell formation from aspartame and sucralose consumption (Belpoggi et al., 2006; Soffritti et al., 2007; Soffritti et al., 2016; Soffritti et al., 2010); weight gain (Swithers et al. 2013); and absorption glucose and insulin secretion stimulation (Mace et al., 2007; Renwick & Molinary, 2010). Other findings in human studies suggested that there could be an increase of appetite and, as a consequence, weight gain (Chia et al., 2016; Malik & Hu, 2012). A review, published by Araújo et al. (2014), also reported adverse effects after the consumption by pregnant women and their offspring.

These arguments led *ILSI Brasil* to organize a meeting, held in São Paulo (Brazil), in March, 2017 and also in May, 2019 with the objective to promote a discussion involving scientists, governmental and non-governmental organizations and food industries, about the controversies related to the use of LNCS in foods, taking into consideration available information about safety and health risks of these food additives.

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[1] *Departamento de Ciência de Alimentos, Escola de Engenharia de Alimentos, Universidade de Campinas – UNICAMP, Campinas, SP, Brasil*
[2] *Department of Health and Human Performance, University of Houston, Houston, United States of America*
[3] *Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina*
[4] *Agência Nacional de Vigilância Sanitária – ANVISA, Brasília, DF, Brasil*
[5] *Intertek Scientific & Regulatory Consultancy, Mississauga, Canadá*
[6] *Ajinomoto North America Inc., Itasca, Illinois, United States of America*
*Corresponding author: hdbmaluly@gmail.com

2 International regulation

In 1963, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) established the Codex Alimentarius and the Joint Food Standards Program and the Statutes for the Codex Alimentarius Commission (CAC) (Codex Alimentarius, 2017). Codex Alimentarius is a collection of internationally recognized food standards, guidelines, and codes of practice and recommendations related to foods and adopted by the CAC. These guidelines can be applied voluntarily by member countries, and, in many cases, serve as a basis for national legislations, such as ANVISA (Agência Nacional de Vigilância Sanitária, 2016).

Within the Codex Alimentarius, the Codex Committee on Food Additives (CCFA) has established and endorsed permitted maximum levels of LNCS and other additives. In addition, the CCFA prepares priority lists for risk assessment of food additives, by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This Committee also assigns functional classes for LNCS, recommends specifications of identity and purity, assesses methods for analysis on food products and considers and elaborates standards or codes for related subjects, such as the labelling of LNCS when sold as such. All relevant information about LNCS is published in the "Codex General Standard for Food Additives" database, which sets forth the conditions under which permitted intense sweeteners may be used in all foods, whether or not they have been previously standardized by Codex (Food and Agriculture Organization of The United Nations, 1995).

The Joint FAO/WHO Expert Committee on Food Additives (Food and Agriculture Organization of The United Nations, 2019a) establishes principles for evaluating intense sweeteners safety and for quantifying their risks. JECFA evaluates population exposure assessments and toxicological risks in order to establish acceptable daily intakes (ADIs) of certain LNCS which are or can be marketed in different countries (Food and Agriculture Organization of The United Nations, 1995).

A summary with the main findings and conclusions of the JECFA meetings has been published electronically by the Joint Secretary shortly after each annual meeting. The concise description of the key data used in the assessments has been published in the WHO website (Food and Agriculture Organization of The United Nations, 2019b). The full monographs about the toxicological and exposure assessment are also published in the WHO website, at the JECFA monograph section (World Health Organization, 2019). The specifications of identity and purity of food additives and flavor agents, such as aromas, are available in the Compendium of FAO Food Additive Specification (Food and Agriculture Organization of The United Nations, 2019 a, b, c).

The standard for labelling of LNCS is codified in the Codex General Standard for the Labelling and Prepacked Foods (CODEX STAN 1-1985 - Rev. 1, Codex Stan, 1991). Therefore, LNCS are regulated as food additives and mandatorily included on the "list of ingredients" of the product and the sweetener class must be used with the specific name and the recognized numerical identification, as required by the national legislation of each country.

3 Brazilian regulation

The Health Minister and The Brazilian Health Regulatory Agency (ANVISA) approved the Technical Regulation n° 540 published in 1997 to define, classify and establish guidelines in relation to the use of food additives. Among their main premises, the substances must be safe, possess technological need, and be used at the lowest levels needed to reach the desired effect (Brasil, 1997).

This regulation also defines LNCS as "a substance, other than sugars, which promote sweet taste in foods" (Brasil, 1997). For the term "table top sweetener", the Regulation RDC nº 271 (22/10/2005) considers as "a formulated product for sweet taste promotion in foods and beverages, constituted of LNCS previously regulated". This resolution also approaches the possible vehicles that can be used, as well as the additional requirements to labelling (Brasil, 2005). For the inclusion of sweeteners and other additives in the national market, a petition with ANVISA is needed, always following the agreement established by the Guide to Procedures for Inclusion and Extension Requires of Food Additives and Technological Coadjutants in the Brazilian Regulation (Agência Nacional de Vigilância Sanitária, 2015).

The Resolution RDC nº 18 (24/03/2008), which authorizes the use of food additives and sweeteners, with their respective maximum limits, has considerations in relation to the need for constant improvement of sanitary control actions (Agência Nacional de Vigilância Sanitária, 2008): to be limited to specific foods, in specific conditions and at the lowest level to reach the desirable effect; to have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and to be in the Mercosur General Harmonized List for Additives (GMC n. 11/2006), currently under revision by the Food Commission of SGT-3 (Mercosur, 2006); to consider other references, such as of the European Union, for the proposed uses; not exceed the Acceptable Daily Intake (ADI); to review constantly the legislations that authorizes the use of LNCS in foods; to be in line with the National Policies to Dietary and Nutrition.

Food and beverage categories for weight control, for diets with controlled intake of sugars, for sugar-restricted diets and with complementary nutritional information, have permission for the addition of the LNCS mentioned in Table 1 (Agência Nacional de Vigilância Sanitária, 2008).

ANVISA also approved a *quantum satis* LNCS, such as: sorbitol, mannitol, isomalt, thaumatin, maltitol and maltitol syrup, lactitol, xylitol, erythritol and polyglycitol syrup (Agência Nacional de Vigilância Sanitária, 2008; BRASIL, 2018).

Beyond the RDC 18 (24/03/2008) (Agência Nacional de Vigilância Sanitária, 2008), there are more regulations established: to guarantee the Good Laboratory Practice (Agência Nacional de Vigilância Sanitária, 2010); to Food Additives and Adjuvants authorized for enteral formulae (Brasil, 2017), Food Supplements (Agência Nacional de Vigilância Sanitária, 2018) and other food categories (Brasil, 2019).

In Brazil, at present, concerns have been raised on the use of LNCS in infant foods, since the real exposure of this population to these additives is unknown. The latest survey of food consumption (Consumer Expenditure Survey - POF 2008/2009) did not cover

subjects under 10, which can exceed the safe doses of ingestion (Instituto Brasileiro de Geografia e Estatística, 2011).

4 Sweeteners: do they pose a carcinogenicity risk?

Prior to entering the market, there is a hierarchy of data approach for food additives, which include results from rodent studies of 2-year duration. These studies have been considered the "Gold Standard" for carcinogenicity evaluation, according to guidelines established by the Organization for Economic Co-operation and Development (Organisation for Economic Co-Operation and Development, 1998) and other internationally recognized organizations, i.e., US - Food and Drug Administration Redbook 2000 (Food and Drug Administration, 2007b).

After entering the market and decades of consumption, high quality epidemiological studies, such as randomized and double blind, which are considered as "gold standard", could detect causal associations (with opposing correlations) between food additives and incidence of cancer (Organisation for Economic Co-Operation and Development, 1998).

The carcinogenicity evaluations for sweeteners were conducted either individually, or more commonly, as a group of "high intensity sweeteners". In general, as described below, the large majority of these studies have reported little, if any, association of LNCS with an increased risk of cancer (Knight et al., 2006). Table 2 shows the characteristics of the main LNCS used in the food industry:

Throughout the investigations about carcinogenicity, (Gurney et al., 1997) evaluated a possible association between the consumption of aspartame and the increase of brain tumour incidence in children's brains. A case-control study was conducted and the researchers collected data from aspartame consumption by patients and their mothers, before the disease was diagnosed. The authors noticed that this hypothesis was identified exactly in the moment the sweetener entered the market, around the 80s. For this reason, the analyses were conducted in 56 subjects (case group) and 94 subjects (control group), who were born after 1981 (date corresponding to the approval of the use of aspartame in the United States of America). They suggested that their findings were not consistent to establish an association between the ingestion of aspartame and infant brain tumour.

The EFSA re-evaluation of aspartame has shown that reproductive studies available are still misunderstood, since they did not comply with the Good Laboratory Practices (GLP). However, some data were evaluated and did not specify any alterations in brain tissues, demonstrating that there is no evidence to associate this artificial sweetener with brain tumour (Stanley, 2013).

Araújo et al. (2014), who published a review article about exposure to LNCS during pregnancy and lactation, analysed long-term studies in animals, which related the increase of tumours after aspartame exposure. One of these studies showed that adverse effects, including the presence of lymphomas, leukaemia and breast cancer, were related to an exposure higher than ADI or close to the value. These studies were discussed in further details in the carcinogenicity section of this manuscript. Nevertheless, it was possible to recognize that clinical trials in humans are insufficient to determine certain cause-effect relationship.

Researchers at the Ramazzini Institute (Bologna, Italy) have reported three carcinogenicity studies with aspartame (two in rats and one in mice) and one for sucralose (in mice) (Belpoggi et al., 2006; Soffritti et al., 2007; Soffritti et al., 2010; Soffritti et al., 2016). Each study alleged a carcinogenic effect of these sweeteners using non-standard protocols.

For aspartame, the first study was conducted with a high dose (up to 100,000 ppm in the diet) in rats (100-150/sex/group), treated for their whole lives. The researchers observed an increased incidence of malignant tumour-bearing animals, an increase in lymphomas/leukaemia, an increase in tumours of the renal pelvis/ureter in females, and an increased incidence of tumours of the peripheral nerves, with a positive trend in males (Belpoggi et al., 2006). They repeated the same protocol, but this time with 70-95 males and females/group, exposed in utero, up to 2,000 ppm in the diet for life. It was reported a significant dose-related increase of malignant tumour-bearing males, a significant increase in incidence of lymphomas/leukaemia in higher-dose animals, and a significant dose-related increase in incidence of mammary cancer in females (Soffritti et al., 2007). Another study, conducted in mice 62-122/sex/group, treated prenatally through to end of life at up to 32,000 ppm in the diet, reported a significant dose-related increased incidence of hepatocellular carcinomas and of alveolar/bronchiolar carcinomas, both in males (Soffritti et al., 2010).

For sucralose, mice (457 male and 396 female) were treated prenatally throughout life at up to 16,000 ppm in the diet. It was reported a significant dose-related increase in total malignant

Table 1. Maximum limits to the use of LNCS in Brazil, according to the RDC 18 (24/03/2008) (Agência Nacional de Vigilância Sanitária, 2008).

L&NCS	Maximum limit (g/100g or g/100 mL)
Acesulfame potassium	0.035 (a, b, c, d); 0.026 (e)
Advantame	0.005 (a, b, c, d); 0.00375 (e)
Aspartame	0.075 (a, b, c, d); 0.056 (e)
Cyclamate	0.04 (a, b, c, d); 0.03 (e)
Saccharin	0.015 (a, b, c, d); 0.01 (e)
Sucralose	0,04 (a, b, c, d); 0,03 (e); 0,025 (f, g, h, i); 0,02 (j)
Steviol glycosides	0,06 (a, b, c, d); 0,045 (e)
Neotame	0,0033 (a); 0,0065 (b, c, d); 00,49 (e)

a. Food and beverage for weight control; b. Food and beverage for diets with controlled intake of sugars; c. Food and beverage for diets with sugar restriction; d. Food and beverage with complementary nutritional information with total sugar replacement; e. Food and beverage for complementary nutritional information with partial sugar replacement; f. Non- alcoholic sparkling and no sparkling beverages for weight control; g. Non- alcoholic sparkling and no sparkling beverages for diets with controlled intake of sugars; h. Non- alcoholic sparkling and no sparkling beverages for sugar-restricted diets; i. Non- alcoholic sparkling and no sparkling beverages for complementary nutritional information with total sugar replacement; j. Non- alcoholic sparkling and no sparkling beverages with complementary nutritional information with partial sugar replacement.

Table 2. LNCS characteristics.

LNCS	'x' sweeter than sucrose	Mechanism of absorption	ADI mg/kg bw/day	Number of tabletop L&NCS packets equivalent to ADI	References
Aspartame	200	Rapid hydrolyzed in the small intestine in aspartic acid, phenylalanine and methanol (below than normal dietary exposures from fruits and vegetables) (O'Brien-Nabors, 2016).	0-50[a]	75[a]	21 CFR 172.804 - Food and Drug Administration (2018)
			0-40[b]		World Health Organization (2010b)
					(Stanley, 2013)
Acesulfame K	200	Quickly absorbed as the same molecule and with a rapid excretion, mainly in the urine (O'Brien-Nabors, 2016).	0-15[a,b]	23[a]	21 CFR 172.800 - Food and Drug Administration (2018)
					World Health Organization (2010b)
Neotame	7,000 – 13,000	Rapidly but incompletely absorbed; completely eliminated (O'Brien-Nabors, 2016).	0-0.3[a]	23[a]	21 CFR 172.829 - Food and Drug Administration (2018)
			0-0.2[b,c]		World Health Organization (2010b)
					European Food Safety Authority, 2019, 2007
Advantame	20,000	Rapid hydrolyzed in the small intestine, short plasma half-lives and completed elimination (O'Brien-Nabors, 2016).	1,970[a]	4.92[a]	21 CFR 172.803 - Food and Drug Administration (2018) and Food and Drug Administration (2014)
	(O'Brien-Nabors, 2016)		0-5	0-5[b]	World Health Organization (2015)
Cyclamate	30	Incompletely absorbed from the gastrointestinal tract (~37%), converted or not in cyclohexylamine (O'Brien-Nabors, 2016).	0-11		World Health Organization (2010b)
Saccharin	200 – 700	Saccharin is not metabolized by humans and is excreted in the urine and to a small extent, in the feces, unchanged (O'Brien-Nabors, 2016)	15[a]	45[a]	21 CFR 180.37 (Food and Drug Administration, 2018)
			0-5		World Health Organization (2010b)
Sucralose	600	Not metabolized and poorly absorbed (O'Brien-Nabors, 2016).	5[a]	23[a]	21 CFR 172.831 (Food and Drug Administration, 2018)
Steviol glycosides	200 – 400	Hydrolyzed to a common metabolite (aglycone steviol) by colonic and/or cecal bacteria, and a small amount could pass through the intestinal tract completely or partially intact (O'Brien-Nabors, 2016).	0-4[b]	9[a]	World Health Organization (2010b)
					GRAS Notice Inventory (Food and Drug Administration, 2019)

tumours in males and significant dose-related increase in hematopoietic neoplasia also in males (Soffritti et al., 2016).

Recognized institutions such as the European Food Safety Authority (2011), US-FDA (Food and Drug Administration, 2007a) and NTP (National Toxicology Program, 2011) evaluated possible shortcomings of the Ramazzini studies, which are shown in Chart 1:

The results are uninterpretable and unreliable due to flaws in the study design, conduct, as well as the reporting of the data. In addition, the data in rats and mice with a high and variable incidence of leukaemia/lymphoma may have occurred due to infection or inflammation, making the interpretation more difficult.

The results of the Ramazzini Institute studies need to be placed into context with the results of core guideline studies conducted in accordance to regulatory standards, since there is no evidence of carcinogenicity of aspartame (studies in rats and transgenic mice assays) or of sucralose (2-year cancer studies in both in rats and mice) (Ishii et al., 1981; Iwata, 2006; Mann et al., 2000a, b; Reno et al., 1973a, b; Reno & Ferrell, 1974a, b; Trutter et al., 1974).

The conclusions from regulatory authorities do not consider either aspartame or sucralose as carcinogenic in rodents or posing a carcinogenic risk to humans. Therefore, the Ramazzini Institute studies provide no credible evidence that both aspartame and sucralose are carcinogenic.

Chart 1. Comments from regulatory authorities.

EFSA and US FDA	Not designed to GLP- OECD-compliant protocols or FDA Redbook.
EFSA and US FDA	Misinterpreted results due to rapid increase in neoplasm development beyond the age of 2 years.
EFSA and US FDA	Lifetime is critical – studies were conducted during the whole life.
EFSA	Apparent lack of randomization of animals (size of studies appears to preclude such randomization)
EFSA	Lack of information on the nature of the diets used (not stated in their publications).
EFSA, US EPA/NTP Re: aspartame studies	Rats and mice used by Ramazzini have a high and variable spontaneous incidence of leukaemia/lymphoma that has been related to infection/inflammation (misinterpretation of the results)
FDA	Use of poorly described and/or inadequate statistical tests and incorrect levels of significance (e.g., trend vs pair-wise; significance levels for common vs uncommon tumor [p=0.01 vs 0.05) based on FDA guidance.
EFSA, NTP Re: aspartame studies	Lumping all tumors, malignant, benign, and malignant + benign, rather than by tissue of origin, for analysis it is not appropriate.
US FDA	Dose-response relationships often absent in alleged response.
EFSA Re: aspartame	Effects often reported only in one sex.
EFSA, EPA Re: aspartame studies	Documented inconsistencies in diagnoses (e.g., leukaemia vs infection).
US FDA (e.g., sucralose study)	Tabulated data does not match alleged "tumorigenic effects".
EFSA, US FDA Re: aspartame studies	Limited or no external peer-review of the pathology slides.

5 What is the evidence concerning the effects of LNCS on energy intake, body weight and glucose intolerance?

Intervention programs have been developed around the world, which have involved food habit modifications, including the reduction in the consumption of caloric food, and encouraging patients to practice physical exercise (Oliveira et al., 2017; Gregg et al., 2012; Knowler et al., 2002).

Considering the market demand, food industries seized the technology and have been started the production of diet and light foodstuffs. Nevertheless, to reduce calories in foods, structural alterations in the recipes of formulations are needed. This fact has expanded the use of LNCS, which today are a part of the strategies to help the remission of diabetes incidence (Ley et al., 2014).

As there was a large growth in the incidence of obesity/diabetes, the consumption of these food additives also increased, also in children and teenagers (Sylvetsky et al., 2015, 2017), which generated new questions with regard to their exposures, mostly for possible disturbance in the sweet taste perception and weight gain (Wise et al., 2016; Mattes & Popkin, 2009).

Studies involving implications about childhood weight management were also published and they discussed ideas about multiple causes. For example, von Poser Toigo et al. (2015) evaluated possible effects during pre and postnatal period in animals exposed or not to aspartame. Both male and female rats consumed aspartame chronically until the reproduction period. The female rats kept consuming the artificial sweetener from pregnancy to lactation. After this period, the offspring was submitted to a considerable caloric diet for 112 days. During this period, clinical parameters such as overweight, body fat, among others, were measured comparing them with different consumption data observed before the exposition to aspartame. In a more detailed exploration of the data, it was possible to observe that, in terms of weight gain, only male rats showed significant results. No significant results were observed in relation to body fat, gonadal fat, intraperitoneal fat, neither the total abdominal fat weight. After the measurements of metabolic syndrome parameters, some significant results were detected, mostly in males again, such as total cholesterol, LDL, HDL and triglycerides. According to this study, it was possible to predict a type of tendency that aspartame could cause some effects in males. However, one must consider all limitations to make a decision that is already proposed by a pre-concept.

The prospective cohort study had as a purpose to determine if the ingestion of sugar sweetened and artificial sweetened beverages in pregnant women was associated with the body mass index (BMI) z score of their descendants, under 1-year old. The most significant results have shown that the increase BMI z score and overweight in descendants was associated to the consumption of LNCS by the mothers more than once a day (Azad et al., 2016). The only postulate that is applied in this study is the dose-response principle – the higher the ingestion of LNCS, the higher the BMI z score. In this case, considerations about the biologic plausibility in relation to the taste receptors will be considered, which may perhaps have some implication. On the other hand, after a careful evaluation of the results, it was noted that it is not possible to respond to the question in a decisive manner.

One of the few randomized clinical trials found in the available literature was published by (Ruyter et al., 2012). They conducted an 18-month trial involving normal-weight children (between 4 years and 10 months and 11 years and 11 months), who received 250 ml of sugar (26 g of sucrose per can) or artificial sweetened (34 mg of sucralose and 12 mg of acesulfame potassium per can) beverages at random. The authors clearly observed that the ingestion of sugar sweetened beverage by children increased the score of BMI and the replacement for artificial sweetened beverages decreased the body weight and fat accumulation in normal-weight children.

To look into these possible associations with details, the same authors published another randomized trial, with a similar design of the previous trial, but adding models with physiological basis

of growth and energetic balance to estimate the degree of caloric compensation that each child had to compensate for the loss of the sugar calories from their drinks by increasing their intake of kilocalories from other foods and beverages. Children with overweight (16%) and obesity (3%) also participate in the trial (Katan et al., 2016). It was possible to verify that the children with BMI above the media had a reduction of the tendency to compensate the changes in the intake of calories. The trial confirmed that the reduction of sugar sweetened beverages can be beneficial, especially in children with tendency to gain weight.

To ascertain if the exposure to LNCS is associated to weight gain, (Rogers et al., 2016) carried out a systematic review, which included meta-analyses, about evidence in animals and humans. For the articles that reported studies with animals (68), 22 showed a significant reduction in body weight, when the animals were submitted or not to compulsory or voluntary consumption of LNCS added to food or drink; in 37 there were no-significant differences and 9 reported increased body weight. After comparing a dietary supplementation with LNCS and glucose in learning studies (22), 3 showed no-significant difference and 19 observed weight gains. These results suggested that the intermittent exposure to a dietary supplement sweetened or not with LNCS could result in an increased body weight, when compared with an exposure to a dietary supplement sweetened with glucose. Nevertheless, the relevance for humans seems to be unclear, since the rodents used in the tests have a limited dietary experience and they do not usually have the habit of consuming sweet tasting foods, both with and without inherent energy (Glendinning, 2016).

To analyse human studies, the authors have selected 10 articles that reported 12 observational studies (prospective, cohort), with more than 500 participants followed more than one year. Most of these studies have reported that there is a minimal risk of weight gain associated with LNCS consumption. There is no evidence to confuse the relationship between sweet taste and calories (and thereby increase sugar and energy intake) in humans, since there are logical problems with this argument and the relevant results from animal studies have recently been disputed. For these reasons, future researchers could evaluate the validity of these studies in animals for human eating patterns in order to demonstrate whether people who consume foods with the same taste have energy intake variability or are prone to eat more.

6 Are LNCS safe to pregnant women, breastfeeding / infants and children?

In an attempt to reduce the consequence of obesity and diabetes, pregnant women and children have been consuming LNCS through diet and light foodstuffs, which has made researchers identify other aims to their investigations, mostly concerning the safety of food additives (Sylvetsky et al., 2015, 2017). In this context, published literature was critically evaluated in terms of preclinical studies (animal studies) and human clinical trials to discuss effects on different outcomes related to preterm delivery, paediatric oncology disease, overweight and obesity, considering the limited amount of the evidence in paediatric population, which are called "therapeutic orphans" (Wilson, 1999).

Two clinical trials from Scandinavia region evaluated the association between the ingestion of carbonated beverages (sugar sweetened and LNCS sweetened) and preterm delivery (Englund-Ögge et al., 2012; Halldorsson et al., 2010).

The Danish study, published by Halldorsson et al. (2010), considered the critical preterm delivery the period less than 37 weeks of pregnancy. The results showed that the risk of preterm delivery to the pregnant women who consumed artificially sweetened carbonated beverages 2-4 times a day was statistically significant (OR 1.41 (1.06-1-87) unadjusted and OR 1.35 (1.01-1.80) adjusted) when compared to mothers who consumed sugar sweetened beverages in the same amount and mothers who consumed artificially sweetened less than 2-3 times a day. The authors made the same evaluation with noncarbonated soft drinks, and they reported similar results and also, they did not find statistical differences between artificially sweetened beverages and sugar sweetened beverages when mothers drank less than 2-4 times a day. After assessing the results, it was possible to provide the dose-response relationship. However, appraisals about the biological plausibility are needed to confirm such effect. Some hypothesis supposed a possible deposit of sweeteners or intermediate metabolites in the placenta, but this evidence is still controversial.

In contrast to the Danish study, a Norwegian group, published by (Englund-Ögge et al., 2012) verified that preterm delivery could occur both in women who consumed sugar and artificially sweetened beverages there is no dose-response correlation. For example, mothers who consumed less beverage artificial sweetened had significant risk for preterm delivery than mothers who consumed high amounts of this beverage. A confirmed fact was observed in both groups and the women who ingested more beverages were also those who smoked more - which is considered an important variable, from the point of view of multiple causes to these occurrences. The extreme cases of preterm delivery (less than 32 weeks) were associated with the ingestion of sugar sweetened beverages and there was no association with artificially sweetened beverages, when both were compared. Despite some significant results, this association was considered as moderate, since there are differences between risk of preterm delivery in mothers with overweight (who consumed artificially sweetened beverages) and underweight, demonstrating the needs of more investigations to confirm this proposition (Araújo et al., 2014).

Sylvetsky et al. (2015) analysed the possible occurrence of LNCS in breast milk samples from 20 women, during breastfeeding. Saccharin, sucralose and acesulfame potassium were identified in 13 samples and aspartame was not detectable in mothers who reported LNCS consume. In mothers who reported no LNCS consume acesulfame was detected. After thorough assessment, it was possible to verify that the amounts were extremely low and the measures were expressed in micrograms per millilitre, considered much lower than the ADI. Even if the ADI values are not applicable to this population, it is very difficult to predict an effect to the infant from some substances at very low concentrations. Therefore, more clinical trials are needed to evaluate this exposure and possible future effects.

Abou-Donia et al. (2008) conducted a preclinical trial in which Splenda (mix of sucralose – 1.1%, maltodextrine and glucose) was

orally administered at different doses (100, 300, 500, or 1000 mg/kg), using Sprague-Dawley rats, during 12 weeks, to evaluate possible alterations in the microflora and the intestinal expression of the membrane efflux transporter P-glycoprotein (P-gp) and the cytochrome P-450 (CYP) metabolism system. After evaluation of the results, the authors observed significant changes in the expression of P-glycoprotein (P-gp) and cytochrome P-450 (CYP). It is known that the metabolism of children is different from adults and expression changes in the cytochrome P-450 (CYP) occur during lifetime (Kearns et al., 2003). For these reasons, it is difficult to conclude how the pediatric population, which already have their own ontogenic changes of hepatic enzymes, could be related with these facts. It was discussed that the observed evidence in animals could be applied to give us a preconception about the metabolism; however, there is still a need of evidence from clinical trials for a more adequate decision to be taken.

7 Final considerations

Intense discussions took place and numerous questions were raised during the meeting. All speakers critically analysed a large part of the available literature and explained the methodologies applied and possible health impact of LNCS. The consensus was that these food additives, when used according to recommendations provided by scientific committees and regulatory authorities, can be considered safe. The findings on potential adverse effects related to LNCS are not sufficient to support any change in the regulatory status of these additives, and the available exposure data worldwide has shown intakes below the established ADIs. Nevertheless, it is important to emphasize that countries should constantly monitor the consumption of foods containing LNCS among different age groups, especially infants, in order to guarantee that any change in product formulations, including the total or partial replacement of sugar by LNCS, do not result in intakes that exceed the ADI.

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