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Commentary

The health effects of artificial sweeteners: Towards personalized quantification and prediction through gut microbiome

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Artificial sweeteners (AS) have been widely applied in the food industry as sugar substitutes with reduced calorie content but high sweetening power [1]. The consumption of sugar-sweetened beverages is associated with the incidence of obesity and type 2 diabetes [2,3]. Non-caloric AS (NCAS) have been recommended for weight management and as a treatment strategy for type 2 diabetes [4]. While AS are generally considered safe with acceptable daily intake by regulatory agencies (e.g., European Food Safety Authority, US Food and Drug Administration), mounting epidemiological evidence shows that the consumption of AS is associated with the risk of cardiometabolic disease [2,3,5]. Moreover, due to the large consumption, AS are emerging contaminants in aquatic environments with concentrations exceeding 100 μg/L [6–8]. Therefore, a few controversies about AS need to be addressed, and both short- and long-term health effects of AS should be re-evaluated [2,5].

The ecotoxicity of AS at environmentally relevant concentrations has been investigated in several aquatic organisms [8–10]. For example, both the physiology and locomotive behavior of crustaceans can be affected by sucralose exposure at environmentally relevant concentrations of 0.5–500 μg/L [9]. Further studies in animal models and humans have shown that AS can act as potential endocrine disruptors with various adverse effects by modifying hormone levels and metabolism [8,10]. Thus, the continuous accumulation of AS in the environment requires comprehensive investigations to explore the distribution and fate of AS and their human and environmental risks.

The gut microbiome plays an important role in food digestion, immunomodulation, maintenance of structural integrity of the gut mucosal barrier, and xenobiotic and drug metabolism [11]. The dysbiosis of our normal gut microbiota contributes to the pathogenesis of various metabolic disorders [11]. Some *in vivo* and *in vitro* studies, observational studies, and randomized clinical trials have provided insights into the association of gut microbiota perturbations with the consumption of AS [12–18]. An early study by Suez et al. [13] revealed that NCAS consumption induced glucose intolerance in mice, and the positive correlations between NCAS consumption and several metabolic characteristics (e.g., higher fasting blood glucose, glycosylated hemoglobin) were observed in non-diabetic individuals. Significant correlations exist between microbial taxa (e.g., Enterobacteriaceae family, Deltaproteobacteria class, Actinobacteria phylum) and NCAS consumption. Both the antibiotic treatment and faecal microbiota transplantation (FMT) from NCAS-consuming mice to germ-free mice supported that the effects of NAS were mediated by the alteration of gut microbiota. However, some contradictory results have been observed, especially in human studies [14–19]. For example, Serrano et al. [17] performed a double-blind, placebo-controlled, and parallel-arm study involving 46 healthy adults to investigate the effects of high-dose NCAS (saccharin) on gut microbiota and glucose tolerance. In contrast to the glucose intolerance reported by Suez et al. [13], no altered glucose or hormonal responses were observed during the oral glucose tolerance test in the subjects with NCAS consumption, and no alterations in microbial diversity or composition were observed. As known, the inter-individual variabilities of gut microbiota have been shown to affect host responses to dietary interventions or therapeutics [20,21]. The differences in the study design (e.g., control groups), microbiota at the baseline, dietary patterns, antibiotic treatments, medications, and other related confounding factors could strongly influence the observations of gut microbial diversity and compositions, which requires well-designed and data-rich clinical trials.

Recently, Suez et al. [22] investigated the effects of oral supplementation with non-nutritive sweeteners (NNS) on the microbiome and glycemic responses in 120 healthy subjects in the short term. In contrast to their previous study [13], a stringent screening protocol was applied to only include complete NNS abstainers according to a food frequency questionnaire based on NNS-containing foods or beverages in the Israeli market, which finally recruited 120 participants from 1,375 healthy individuals for eligibility. Four NNS intervention arms with saccharin, sucralose, aspartame, and stevia, as well as a control arm with glucose, were used in this randomized-controlled trial with 20 subjects per arm. Importantly, the consumption of NNS was at a dose lower than the

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acceptable daily intake. Moreover, an additional control group with 20 healthy subjects without supplementation was evaluated. Altogether, the 14-day NNS intervention was preceded and followed by a 7-day observation phase, during which a series of anthropometric (i.e., body mass index [BMI], HbA1c), clinical (i.e., continuous glucose monitor, glucose tolerance test) and biological measurements (i.e., oral and fecal microbiome, plasma metabolome) were performed at pre-determined time points.

Although extensive inter-individual variations of glucose tolerance changes were observed, each NNS intervention showed distinct longitudinal profiles of glucose tolerance. The supplementation of saccharin and sucralose impaired glucose tolerance in the healthy subjects, while aspartame and stevia showed neutral effects on glucose tolerance when compared against the glucose vehicle or non-supplementation groups. Moreover, saccharin and sucralose significantly elevated glycemic response during the intervention. Metagenomic analysis showed significant effects of saccharin and sucralose on the gut microbiota, whereas all NNS had significant effects on microbial functions, such as purine metabolism, glycolysis, polyamine metabolism, and fatty acid biosynthesis. Based on correlations between microbial features at baseline and the glycemic response at the 2nd week of intervention, significant contributions of microbiome to the variation of glucose tolerance, at least for the sucralose group, were revealed. A following plasma metabolomics analysis identified differential metabolites and corresponding enriched pathways, which were in accordance with the correlation between microbial metabolic pathways and elevated glycemic response.

To evaluate the causal roles of the gut microbiome for glucose intolerance, FMT experiments were conducted to transfer stool microbiome from top/bottom responders in each group into germ-free mice. The top responders were classified by the potent glycemic responses, while the bottom responders had the lowest responses in the respective group. Both stool samples from the baseline and the end of the intervention were used for FMT. Impaired glycemic responses were observed in mice humanized with stool samples from NNS consumption top responders (at the end of intervention), while the mice colonized with samples from controls did not show significant effects on glucose tolerance. The following metagenomic analysis in mice showed doner-specific variations in glycemic responses. Together, it provides evidence of the causal links between NNS-mediated microbial alterations and glucose intolerance.

In summary, Suez et al. [22] demonstrated the individual-dependent impacts of NNS on the human gut microbiome in short-term interventions and established the causal roles of the microbiome in glycemic responses by FMT. It advances our understanding of the microbiota–diet interactions in the healthy population and provides the possibility to predict potential glycemic responses at the individual level [22,23]. The participants included in Suez et al.'s study [22] were mainly young and middle-aged adults with a median age of 29.95. It will be particularly interesting to study the effects of AS with a much wider range of age groups since the sweet taste preference and sensitivity are significantly different between age groups [24]. Although no significant baseline differences were found between intervention groups, including weight, BMI, smoking, and other related clinical parameters, it is unknown how subtle variance of the baseline characteristics influences the effects of AS on gut microbiota after intervention at the individual level. Considering the variance of gut microbiota between individuals of different demography, ethnicity, gender, age, dietary patterns, and lifestyle, a well-designed study cohort and the choices for data analysis are particularly important for controlling confounding effects. In the future, scale-up of the study population and enriching the data collection may thus provide foundations for the precision control of gut microbiome.

Current clinical trials examined the long-term effects of AS, showing a strong association between AS and increased risk of cardiometablic disease [2,3,5]. However, the published studies only investigate the short-term effects of AS on human gut microbiota, and clinical trials investigating the long-term effects of AS on human gut microbiome have not been performed [22,24]. The findings in these short-term studies strongly suggest that further explorations studying the long-term effects of AS on the human gut microbiome are recommended. Moreover, most of these gut microbiota studies did not investigate the fate of AS during the intervention, and whether the *in vivo* AS dynamics are associated with the short-term responses remains unknown. The design of clinical trials with *in vivo* AS measurements could be performed to confirm the responses of gut microbiota to AS interventions. In addition, current studies focused on the gut microbiota alternations in healthy populations. The information about the effects of AS on individuals' gut microbiota in populations with diabetes or cardiometabolic diseases are missing. Thus, extrapolation of the AS effects on the gut microbiome to diabetes/cardiovascular disease patients by controlled trials is of great interest.

It should be noted that AS are usually used to replace caloric sugars, which may reduce the overall caloric intake. In several clinical trials, the dose of AS used was lower than the acceptable daily intake with glucose as a bulking agent. In everyday life, AS are usually consumed together with caloric sugars. Moreover, individuals may simultaneously consume multiple types of AS from different foods or beverages. Therefore, investigating the effects of AS with different doses, the combination effects of AS, or the combinations of AS with different types of carbohydrates (e.g., fructose, sucrose, fibers) would give clues of AS effects from a real-life view [25]. In addition, AS are emerging contaminants in aquatic environments. The consequences of long-term exposure to AS with environmental concentrations are not available, which require systematical analysis with animal experiments, observational studies, and controlled trials.

Although current clinical studies have established the causal links between AS, gut microbiota, and health, the mechanisms through which AS can affect gut microbiota or the human body are unknown. Since most non-nutritive AS are not metabolized, whether there are direct interactions between AS and gut microbial organisms is still unclear. The utilization of *in vitro* microbial cultures and synthetic microbiota may provide an efficient/direct way to investigate the responses of gut microbial species to AS. With state-of-the-art organoid methods, the intestinal organoid cocultures with microorganisms will enable the mechanistic study of AS-microbe-host interactions with precise experimental control.

Recent controlled clinical trials have shown personalized responses of humans to AS. Analyzing the personalized data with artificial intelligence may provide a basis to develop computational tools for precise prediction of glycemic responses, which can be integrated into current weight management algorithms for personalized suggestions of AS usage. When the baseline characteristics are integrated with the gut microbiota features, dietary patterns, lifestyle, and medication histories, it may be possible to predict the metabolic dynamics of individuals and provide healthy dietary nutrition recommendations, which will help control diabetes and cardiovascular disease. In the future, the application of such a personalized longitudinal multi-omics approach with artificial intelligence may pave the way to understanding the links between metabolic disease developments and the long-term effects of AS.

Author contributions

Y.J.W.: conceptualization, investigation, writing; B.B.J.: conceptualization, supervision, writing.

Declaration of competing interest

The authors declare no conflicts of interest.

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References

[1] M. Carocho, P. Morales, I.C.F.R. Ferreira, Sweeteners as food additives in the XXI century: a review of what is known, and what is to come, Food Chem. Toxicol. 107 (2017) 302–317, https://doi.org/10.1016/j.fct.2017.06.046.

[2] C. Debras, E. Chazelas, L. Sellem, R. Porcher, N. Druesne-Pecollo, Y. Esseddik, F.S. de Edelenyi, et al., Artificial sweeteners and risk of cardiovascular diseases: results from the prospective NutriNet-Santé cohort, BMJ 378 (2022), e071204, https://doi.org/10.1136/bmj-2022-071204.

[3] F. Imamura, L. O'Connor, Z. Ye, J. Mursu, Y. Hayashino, S.N. Bhupathiraju, N.G. Forouhi, Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction, BMJ 351 (2015) h3576, https://doi.org/10.1136/bmj.h3576.

[4] C. Gardner, J. Wylie-Rosett, S.S. Gidding, L.M. Steffen, R.K. Johnson, D. Reader, A.H. Lichtenstein, Nonnutritive sweeteners: current use and health perspectives, Circulation 126 (2012) 509–519, https://doi.org/10.1161/CIR.0b013e31825c42ee.

[5] M. Witkowski, I. Nemet, H. Alamri, J. Wilcox, N. Gupta, N. Nimer, A. Haghikia, et al., The artificial sweetener erythritol and cardiovascular event risk, Nat. Med. 29 (2023) 710–718, https://doi.org/10.1038/s41591-023-02223-9.

[6] M. Scheurer, H.-J. Brauch, F.T. Lange, Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT), Anal. Bioanal. Chem. 394 (2009) 1585–1594, https://doi.org/10.1007/s00216-009-2881-y.

[7] N.H. Tran, M. Reinhard, K.Y.-H. Gin, Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review, Water Res. 133 (2018) 182–207, https://doi.org/10.1016/j.watres.2017.12.029.

[8] S.M. Praveena, M.S. Cheema, H.-R. Guo, Non-nutritive artificial sweeteners as an emerging contaminant in environment: a global review and risks perspectives, Ecotoxicol. Environ. Saf. 170 (2019) 699–707, https://doi.org/10.1016/j.ecoenv.2018.12.048.

[9] A.-K.E. Wiklund, M. Breitholtz, B.-E. Bengtsson, M. Adolfsson-Erici, Sucralose - an ecotoxicological challenger? Chemosphere 86 (2012) 50–55, https://doi.org/10.1016/j.chemosphere.2011.08.049.

[10] K.I. Rother, E.M. Conway, A.C. Sylvetsky, How non-nutritive sweeteners influence hormones and health, Trends Endocrinol. Metabol. 29 (2018) 455–467, https://doi.org/10.1016/j.tem.2018.04.010.

[11] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. 19 (2021) 55–71, https://doi.org/10.1038/s41579-020-0433-9.

[12] F.J. Ruiz-Ojeda, J. Plaza-Díaz, M.J. Sáez-Lara, A. Gil, Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials, Adv. Nutr. 10 (2019) S31–S48, https://doi.org/10.1093/advances/nmy037.

[13] J. Suez, T. Korem, D. Zeevi, G. Zilberman-Schapira, C.A. Thaiss, O. Maza, D. Israeli, et al., Artificial sweeteners induce glucose intolerance by altering the gut microbiota, Nature 514 (2014) 181–186, https://doi.org/10.1038/nature13793.

[14] C.L. Frankenfeld, M. Sikaroodi, E. Lamb, S. Shoemaker, P.M. Gillevet, High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States, Ann. Epidemiol. 25 (2015) 736–742.e4, https://doi.org/10.1016/j.annepidem.2015.06.083.

[15] K. Gerasimidis, K. Bryden, X. Chen, E. Papachristou, A. Verney, M. Roig, R. Hansen, et al., The impact of food additives, artificial sweeteners and domestic hygiene products on the human gut microbiome and its fibre fermentation capacity, Eur. J. Nutr. 59 (2020) 3213–3230, https://doi.org/10.1007/s00394-019-02161-8.

[16] S.Y. Ahmad, J. Friel, D. Mackay, 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 12 (2020) 3408, https://doi.org/10.3390/nu12113408.

[17] J. Serrano, K.R. Smith, A.L. Crouch, V. Sharma, F. Yi, V. Vargova, T.E. LaMoia, L.M. Dupont, et al., High-dose saccharin supplementation does not induce gut microbiota changes or glucose intolerance in healthy humans and mice, Microbiome 9 (2021) 11, https://doi.org/10.1186/s40168-020-00976-w.

[18] G. Singh, A.J. McBain, J.T. McLaughlin, N.S. Stamataki, Consumption of the non-nutritive sweetener stevia for 12 weeks does not alter the composition of the gut microbiota, 2022, bioRxiv 11 (17) (2022), 516749, https://doi.org/10.1101/2022.11.17.516749.

[19] J. Plaza-Diaz, B. Pastor-Villaescusa, A. Rueda-Robles, F. Abadia-Molina, F.J. Ruiz-Ojeda, Plausible biological interactions of low- and non-calorie sweeteners with the intestinal microbiota: an update of recent studies, Nutrients 12 (2020) 1153, https://doi.org/10.3390/nu12041153.

[20] G.R. Healey, R. Murphy, L. Brough, C.A. Butts, J. Coad, Interindividual variability in gut microbiota and host response to dietary interventions, Nutr. Rev. 75 (2017) 1059–1080, https://doi.org/10.1093/nutrit/nux062.

[21] C.-B. Zhou, Y.-L. Zhou, J.-Y. Fang, Gut microbiota in cancer immune response and immunotherapy, Trends Cancer 7 (2021) 647–660, https://doi.org/10.1016/j.trecan.2021.01.010.

[22] J. Suez, Y. Cohen, R. Valdés-Mas, U. Mor, M. Dori-Bachash, S. Federici, N. Zmora, et al., Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance, Cell 185 (2022) 3307–3328, https://doi.org/10.1016/j.cell.2022.07.016.

[23] K.A. Page, A gut reaction: microbiome-driven glycemic effects of non-nutritive sweeteners, Cell 185 (2022) 3282–3284, https://doi.org/10.1016/j.cell.2022.08.007.

[24] M.D. Pang, G.H. Goossens, E.E. Blaak, The impact of artificial sweeteners on body weight control and glucose homeostasis, Front. Nutr. 7 (2021), 598340, https://doi.org/10.3389/fnut.2020.598340.

[25] A.C. Sylvetsky, J.E. Blau, K.I. Rother, Understanding the metabolic and health effects of low-calorie sweeteners: methodological considerations and implications for future research, Rev. Endocr. Metab. Disord. 17 (2016) 187–194, https://doi.org/10.1007/s11154-016-9344-5.