

The Metabolic Effects of Natural Sugars, Refined Sugars and Low-Calorie Sweeteners

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To Eilan & Ollie

THESIS ABSTRACT

This thesis evaluated the metabolic effects of natural sugars, refined sugars and low-calorie sweeteners in healthy individuals. Two narrative reviews, one comprehensive critical review and one experimental study were conducted. The first narrative review examined the metabolic health effects associated with the consumption of both sugar-sweetened beverages and natural fruit juices. Specifically, it summarized the physiology of glucose and fructose metabolism and their distinct impacts on metabolic health, with particular emphasis on blood lipid profiles. However, the limited evidence on metabolic effects beyond systemic low-grade inflammation, combined with significant discrepancies in study designs and measured outcomes, prevents the formulation of evidence-based recommendations regarding whether fruit juices represent healthier alternatives to sugar-sweetened beverages. The second article reviewed the potential metabolic health effects associated with low-calorie sweetener consumption, also an alternative to sugar-sweetened beverages. Aimed at informing physicians and clinicians, it provided a concise overview of the available evidence in French, thereby contributing to knowledge dissemination and enhancing scientific accessibility within francophone communities. Since the publication of this narrative review, substantial new evidence has emerged examining the effects of low-calorie sweeteners, particularly sucralose, on glucose homeostasis and gut microbiota. To contextualize and deepen these findings, the third article presents a comprehensive and critical synthesis of current evidence specifically addressing the cumulative effects of sucralose consumption on postprandial glycemic responses in humans. Furthermore, it explores the potential role of gut microbiota composition and functionality in mediating metabolic outcomes. Building on the mechanistic insights and study limitations synthesized in this critical review, the fourth article presents a randomized controlled trial designed under real-world conditions to evaluate the cumulative effects of sucralose consumption on postprandial glycemic responses and related physiological mechanisms in healthy adults (NARELO study). These potential mechanisms included alterations in gut microbiota profiles, intestinal barrier

permeability and systemic low-grade inflammation. In this study, we demonstrated that the realistic consumption of sucralose-sweetened beverages for 21 days impairs postprandial glycemic responses in healthy adults compared to sugar-sweetened beverages ($P_{\text{Bonf}} = 0.040$), in the absence of significant changes in insulin concentrations, body composition, gut microbiota composition, fecal concentrations of SCFAs and BCFAs, and plasmatic biomarkers of intestinal permeability and systemic low-grade inflammation. Clinically, postprandial glycemic responses were 8.4% higher following the three-week consumption of sucralose-sweetened beverages, based on estimated marginal means (baseline: 5.00 mmol/L, 95% CI 4.36–5.64; post-intervention: 5.42 mmol/L, 95% CI 4.78–6.06). Further, we also explored whether free sugars derived from natural sources elicit distinct metabolic responses compared with refined sugars. To this end, 100% natural fruit juice was included as an exploratory comparator. No meaningful differences were observed in glucose tolerance when comparing natural fruit juice to sugar-sweetened beverages. Collectively, the findings of this thesis challenge the prevailing assumption that low-calorie sweeteners are physiologically inert, emphasizing the need for compound-specific evaluation as the food industry advances toward the next generation of sweeteners. Although a definitive mechanistic explanation remains elusive, the work presented herein considerably advances our understanding of the potential long-term effects of low-calorie sweeteners on glucose homeostasis. Overall, this thesis provides critical evidence on the metabolic impacts of natural sugars, refined sugars, and low-calorie sweeteners, offering valuable insights to inform public health guidelines.

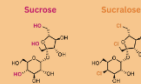
GRAPHICAL ABSTRACT

LITTERATURE REVIEWS

- 1 Are Fruit Juices Healthier Than Sugar-Sweetened Beverages? A Review. *Nutrients*, 2019.
- 2 Les édulcorants de la controverse, *Médecine/sciences*, 2020.
- 3 Is Sucralose Contributing to the Very Problem It Was Designed to Solve? A critical review of the evidence on the effects of chronic sucralose consumption on postprandial glycemic responses and gut microbiota in healthy individuals. *To be published*.

KEY FINDINGS

- Evidence on the metabolic effects of natural fruit juice consumption is very limited.
- Compelling evidence demonstrate that low-calorie sweeteners exert distinct physiological effects based on their individual chemical structures.
- Current evidence on sucralose's effects on glycemic responses is marked by substantial methodological heterogeneity, thereby limiting the generalizability of findings. Although mechanistic pathways remain elusive, modulations in gut microbiota composition and functionality are gaining attention as potential contributors.



- The **NARELO** study provides evidence that the realistic consumption of sucralose-sweetened beverages for 21 days impairs **postprandial glycemic responses** in healthy adults compared to sugar-sweetened beverages, despite the absence of significant changes in insulinemic responses, body composition, gut microbiota composition, SCFAs concentrations, intestinal permeability and biomarkers of systemic inflammation. Continued efforts to explore the underlying mechanisms are essential.

Overall, this thesis challenges the prevailing assumption that all low-calorie sweeteners are physiologically inert, highlighting the need for compound-specific evaluation as the food industry accelerates innovation toward the next generation of low-calorie sweeteners.

ORIGINAL DATA

- 4 A 3-Week Randomized Controlled Trial on the Effects of Natural Sugars, Refined Sugars, and Low-Calorie Sweeteners (Sucralose) on Postprandial Glycemic Responses and Mechanistic Pathways in Real-World Conditions (NARELO study). *To be published*.

Pre-intervention : usual *ad libitum* diet while refraining from 1) consuming sugar-sweetened beverages, fruit juices, and low-calorie sweeteners in any forms; 2) drinking more than two alcoholic beverages/day; 3) using cannabis; 4) taking any vitamin, mineral, dietary, probiotic, natural, herbal (including herbal tea) or sports supplements and 5) exercising more than 3.5 hours of endurance training per week.

- n = 42 (21 women, 21 men)
- Healthy, 18-35 years
- Low-consumers of LCS, SSB, and FJ
- No antibiotics used in the past 3 months
- Controlled for menstrual cycle

DAY -7

DAY 0

Randomized parallel-group design stratified by sex



DAY 22

21 days of consuming either sucralose-sweetened beverages (LCS), sucrose-sweetened beverages (SSB), or natural orange juice (FJ).

FORMULATION :

SSB matched total free sugar concentration of 100% natural orange juice (FJ). LCS matched for sweetness based on the manufacturer's information; All beverages represented realistic comparators to commercially available products.

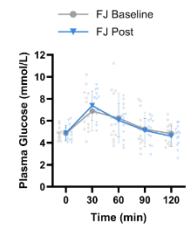
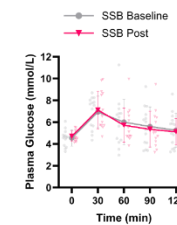
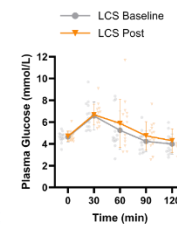
DOSE :

SSB & FJ : Individualized dose of 15% Ereq representing estimated free sugars consumption in Canada.

LCS : Equivalent volume (mL) to SSB group. Average dose of 150 mg (± 30 mg) of pure sucralose per day (2.00 ± 0.23 mg/Kg BW), which is below Acceptable Daily Intake for sucralose (5 mg/Kg BW/day).

DAY 0 and DAY 22

- Standardized meal before overnight fast
- Two serial stool samples collected within 24-72h prior each experimental session
- Body composition assessed by DXA
- Fasting blood samples (LBP, zonulin, IL-6, CRP, TNF- α)
- OGTT (75g), measuring plasma glucose (0, 30, 60, 90, 120 min) and insulin (0, 120 min)



Exploratory comparator

ACKNOWLEDGMENTS

There's a book my son loves titled *Hugo and the Impossible Thing*¹. The story is about a dog who sets out to conquer something everyone says is impossible. Despite the doubts, Hugo decides to try. Along the way, he's joined by others who help him achieve his dream. You probably see where I'm going with this.

To Dr. Pascal Imbeault, I cannot begin to express my gratitude for having you as a thesis supervisor. I knocked on your door in my second year of undergrad, asking to do a PhD, and without any hesitation you welcomed me in. Over the past ten years we have known each other, you have guided me to make the best decisions of my professional career. You pushed me out of my comfort zone when I needed to. You celebrated every win and supported me through every fall. Most importantly, you never stopped believing in me, even when I couldn't believe in myself. You raised the bar so incredibly high for how I intend to mentor the students of my future lab on a professional and human level. Thank you, truly.

I also want to thank my thesis committee and examiners, Dr. Éric Doucet, Dr. Krista Power, Dr. Charlène Roussel, and Dr. Vasanti Malik, for providing valuable insights and feedback throughout the completion of this thesis and for accepting to take part in this special chapter of my life. Dr. Malik, I candidly remember being inspired by your research at the very beginning of my graduate studies. Having you as the external examiner of my thesis is both an honor and a sincere full circle moment. To Dr. Éric Doucet, thank you for being part of my “*challenge system*” and for helping me turn my black-and-white tunnel vision into a hundred shades of gray. Our endless lunchtime debates helped shape the researcher I am today. To Dr. Krista Power and Dawson Livingston, thank you for embarking on this journey with me. The time, energy, and scientific input you both devoted to the NARELO study elevated it to a level I could have only dreamed of without you. It would be my greatest pleasure to work together again in the future. I also want to extend my thanks to Dr. Kimber L. Stanhope for welcoming me as an intern in her lab. Although my time at UC Davis was short, your mentorship made a lasting impact.

To my lab mates at Lees, I am grateful for the memories, the laughs, and even the lows. Your support and friendship truly brightened this experience. Thank you, JF, for keeping up with me during the 300 hours we spent running the NARELO experimental sessions (and for putting up with my many OCDs). While your contribution to this study was invaluable, I also want you to know that it was your teaching during my undergraduate years that sparked my

¹ Felice Smith, R. & Gabriel, C. (2021). *Hugo and the Impossible Thing* (S. Hanson, Illus.). Flamingo Books.

interest in metabolism and physiology, and I am sincerely grateful for that. Alexanne, thank you for your valuable contribution in the wet lab. Nic, thank you for being the great human, colleague, and friend that you are. Without even trying, you have shown me how meaningful scientific collaboration can be. I hope I get the chance to return the favor someday.

To all NARELO participants, I will be forever grateful for your commitment, time, and effort. Next, I would like to thank the Fonds de recherche du Québec for their financial support throughout my doctoral training. In particular, I want to acknowledge the exemplary support the FRQ provides to doctoral candidates on parental leave, which plays a vital role in promoting equal opportunities for women in research.

To my close friends and family who offered encouragement, support, and never dimmed my light, thank you for your love and presence in my life. To Vic and Felix, thank you for welcoming me into your lives and making it so easy to become your stepmother. Most importantly, thank you for being such wonderful siblings to your baby brother. To my mom, Sylvie, thank you for your unwavering support in all my endeavors and for always making me feel like I could achieve anything I put my heart into. To my dad, Serge, who had to give up on his dream of becoming a police officer after one semester because he couldn't afford it. You promised me that you would do whatever it took so I wouldn't have to give up on mine, and you did. While I may never be able to give back what you have both given me, I swear I will do the same for your grandson. Merci pour tout, je vous aime.

Finally, to my husband Guillaume, you are everything a great man, husband, and father should be. Thank you for making my ambitions just as important as yours. Thank you for the nights you slept with the lights on so I could work on this thesis (the joys of having an office space in our bedroom), and for making banana pancakes at 22h00 when I was hungry. The way you show generosity, patience, and understanding is something I strive to live up to every day. Above all, thank you for never making me feel, not even for a second, that I had to choose between my career and our family. You are the pillar of my life. I love you deeply.

Because of all of you, “the Impossible Thing became known as the Extremely Difficult but Absolutely, Positively Possible Thing”.

PREFACE

This thesis includes two published articles and two manuscripts. Authors' contributions by article are as follows:

- **Article 1** – *published*

Pepin A, Stanhope KL, Imbeault P. Are Fruit Juices Healthier Than Sugar-Sweetened Beverages? A Review. *Nutrients*. 2019;11(5):1006. doi:10.3390/nu1105100

A.P. conducted searches, assessed inclusion, extracted data and assessed validity; K.L.S. and P.I. supervised the work of A.P.; all authors contributed to interpreting data and writing the manuscript and read and approved the final draft of the manuscript.

- **Article 2** – *published*

Pepin A, Imbeault P. Les édulcorants de la controverse. *Médecine/sciences*. 2020;36(5) :472-478. doi :<https://doi.org/10.1051/medsci/2020079>

A.P. conceived and designed the review, completed data acquisition, interpreted data and wrote the original manuscript; P.I. supervised, edited and reviewed the final manuscript.

- **Article 3** – *manuscript*

Pepin A, Imbeault P. Is Sucralose Contributing to the Very Problem It Was Designed to Solve? A critical review of the evidence on the effects of cumulative sucralose consumption on postprandial glycemic responses and gut microbiota in healthy adults.

A.P. conceived and designed the review, interpreted data and wrote the original manuscript; P.I. contributed to the conceptual development by providing critical feedback throughout the process, edited and reviewed the manuscript.

- **Article 4** – *manuscript*

Pepin A, Goulet N, Livingston D, Mauger J-F, Larocque A, Power K, Imbeault P. A 3-Week Randomized Controlled Trial on the Effects of NATural Sugars, REfined Sugars, and LOw-Calorie Sweeteners (Sucralose) on Postprandial Glycemic Responses and Mechanistic Pathways under Real-World Conditions (NARELO study).

A.P. Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization, Project administration, Funding acquisition (collaborator). **N.G.** Methodology (statistical approach), Formal Analysis, Data Curation, Writing – Review & Editing. **D.L.** Methodology (gut microbiota), Formal Analysis (gut microbiota), Data Curation (gut microbiota), Writing – Review & Editing. **J-F.M.** Methodology (biological analyses), Investigation, Data Curation, Writing – Review & Editing. **A.L.** Investigation, Data Curation, Writing – Review & Editing. **K.P.** Conceptualization (gut microbiota), Methodology (gut microbiota), Resources, Writing – Review & Editing, Supervision (gut microbiota), Funding acquisition. **P.I.** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding acquisition.

TABLE OF CONTENTS

THESIS ABSTRACT	iii
GRAPHICAL ABSTRACT	v
ACKNOWLEDGMENTS	vi
PREFACE	viii
LIST OF FIGURES	xi
LIST OF TABLES	xiv
LIST OF ABBREVIATIONS	xv
CHAPTER I. Introduction	1
Rationale	1
General Objectives	4
Specific Objectives and Hypotheses (Thesis Article 4)	4
CHAPTER II. Thesis Article 1	6
CHAPTER III. Thesis Article 2	23
CHAPTER IV. Thesis Article 3	31
CHAPTER V. Thesis Article 4	82
CHAPTER VI. Discussion	138
Summary of Findings	138
Limitations	140
Future Research Perspectives	140
Conclusion	141
CHAPTER VII. References	143
APPENDICES	166
<i>Final Words</i>	193

LIST OF FIGURES

▪ Thesis article 1

Figure 1. Absorption of fructose and glucose in the enterocytes. Glucose enters the enterocytes mostly by secondary active transport via sodium-glucose transporters (SGLT1) located in the apical membrane of the enterocytes. Under high concentrations of glucose in the lumen of the intestine, glucose also enters the enterocytes by facilitated diffusion via glucose transporters (GLUT2). Fructose enters the enterocytes through a specific fructose transporter (GLUT5). Then, both glucose and fructose exit the enterocytes to enter the systematic circulation by facilitated diffusion via GLUT2 transporters located in the basolateral membrane of the enterocytes. A small part of dietary fructose will be converted and released in the bloodstream by the enterocytes as glucose.

Figure 2. Metabolic fate of dietary fructose. Dietary fructose is ingested and released by the enterocytes mostly as fructose but also converted and released as glucose, lactate, and fatty acids (in chylomicrons). Fructose spills over to the liver where it is phosphorylated as Fructose 1-Phosphate (F 1-P). The largest part of F 1-P will be metabolized and converted by the hepatocytes as glucose, which can be stored as glycogen or released in the bloodstream. Hepatocytes can also convert F 1-P into lactate and fatty acids. Fatty acids accumulate into the liver, consequently favoring the production and secretion of very low-density lipoproteins (VLDL), which leads to increased levels of circulating triglycerides and dyslipidemia.

Figure 3. Effects of sugar consumption from sugar-sweetened beverages on fasting blood triglycerides (TG) and fasting LDL-cholesterol levels in healthy individuals with normal weight, overweight, or obesity. Mean difference in fasting blood triglycerides and fasting low-density lipoprotein cholesterol (LDL-C) levels in studies that compared higher with lower sugar intakes from sugar-sweetened beverages in healthy individuals with normal weight, overweight, or obesity. (a) Refers to the higher sugar intake intervention. The percentage of energy from the lower sugar intake intervention is detailed in Table I. Studies with ad libitum energy intake controlled for a minimal sugar intake but not for total energy intake. Studies with eucaloric energy intake controlled for a minimal sugar intake and for weight maintenance throughout the studies. (b) Studies that controlled for a minimal sugar intake, for weight maintenance throughout the studies, and for diet macronutrient variations between experimental groups.

- **Thesis article 2**

No figure.

- **Thesis article 3**

Figure 1. Chemical structures of sucrose (molecular weight 342.3, CAS number 57-50-1) and sucralose (molecular weight 397.6, CAS number 56038-13-2). *adapted from* <https://pubchem.ncbi.nlm.nih.gov>¹

- **Thesis article 4**

Figure 1. Plasma glucose concentrations during OGTT, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS) or sugar-sweetened beverages (SSB), in healthy adults.

Data are presented as mean (SD) and individual points. Log-transformed data were analyzed using linear mixed-effects models with group (LCS, SSB), condition (baseline, post-intervention), and time (30, 60, 90, 120 min, excluding 0 min) as fixed effects, and participant ID as a random intercept (group x condition interaction: $P = 0.009$; $P_{Bonf} = 0.040$). **Abbreviations:** post-intervention (Post).

Figure 2. α -diversity metrics (Shannon Index, Pielou's Evenness Index, Observed Features, Faith's PD) of the fecal microbiota, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB), or 100% natural fruit juice (FJ) in healthy adults.

Gut microbiota composition was assessed using 16S rRNA sequencing and analyzed using linear mixed-effects models with group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects, and participant ID as a random intercept (group x condition interaction: all $P \geq 0.123$). **Abbreviations:** post-intervention (Post).

Figure 3. β -diversity (Bray-Curtis Dissimilarity) of the fecal microbiota measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB), or 100% natural fruit juice (FJ) in healthy adults.

Gut microbiota composition was assessed using 16S rRNA sequencing and analyzed using PERMANOVA (group x condition interaction: $q = 0.999$). **Abbreviations:** baseline (B), post-intervention (P).

Figure 4. Mean relative abundance of the (a) six most dominant fecal microbiota phyla-level taxa and (b) 20 most dominant fecal microbiota genus-level taxa, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB) or 100% natural fruit juice (FJ) in healthy adults for three weeks.

Gut microbiota composition was assessed using 16S rRNA sequencing. Group-wise comparisons of taxonomic abundances (mean abundance >0.2% in any group) were performed using Wilcoxon rank-sum tests (all $P \geq 0.161$). **Abbreviations:** baseline (B), post-intervention (P).

Figure 5. Plasma glucose concentrations during OGTT, measured before and after three weeks of consuming 100% natural fruit juice (FJ) or sugar-sweetened beverages (SSB), in healthy adults.

Data are presented as mean (SD) and individual points. Log-transformed data were analyzed using linear mixed-effects models with group (FJ, SSB), condition (baseline, post-intervention), and time (30, 60, 90, 120 min, excluding 0 min) as fixed effects, and participant ID as a random intercept (all $P \geq 0.075$). **Abbreviations:** post-intervention (Post).

LIST OF TABLES

▪ Thesis article 1

Table 1. Characteristics of studies included in Figure 3. Detailed characteristics of studies included in Figure 3 in terms of participants (sample size, sex, weight, age), duration, intervention (% Ereq of sugar intake provided in sugar-sweetened beverages only, sugar-sweetened beverages and food, or unspecified), and control (comparative: low-sugar intervention or baseline values).

▪ Thesis article 2

Tableau I. Comparaison des profils de différents substituts de sucre (édulcorants). *La quantité d'édulcorant (en grammes) diffère en termes de volume et de poids. Les calculs ont été effectués en supposant qu'un sachet d'édulcorant procure un pouvoir sucrant équivalent à 2 cuillères à thé de sucre de canne blanc (sucrose).

▪ Thesis article 3

Table 1. Randomized-controlled trials evaluating the cumulative effects of sucralose consumption on postprandial glycemic responses in humans.

▪ Thesis article 4

Table 1. Participant Characteristics at Baseline.

Table 2. Body weight, BMI, Waist circumference and Fat mass at Post-Intervention.

Table 3. Plasma Concentrations of Sex Hormones and Self-Reported Use of Contraceptives in Women.

Table 4. Plasma Insulin Concentrations during a 75 g OGTT.

Table 5. Fecal Concentrations of Short-Chain Fatty Acids and Branched-Chain Fatty Acids (SCFAs and BCFAs).

Table 6. Plasma Concentrations of Intestinal Permeability and Systemic Low-Grade Inflammation Biomarkers.

LIST OF ABBREVIATIONS

Ace-K : acesulfame potassium
ADI : acceptable daily intake
ANOVA : analysis of variance
AUC : area under the curve
BCFAs : branched-chain fatty acids
BMI : body mass index
CRP : C-reactive protein
DNL : de novo lipogenesis
DV : daily value
DXA : dual-energy X-ray absorptiometry
Ereq : estimated daily energy requirements
FCQ-T-r : food cravings questionnaire – trait reduced
FDA : U.S. Food and Drug Administration
FJ : fruit juice(s)
FRQ : Fonds de Recherche du Québec
FSIVGTT : frequently sampled intravenous-glucose-tolerance test
F 1-P : fructose 1-phosphate
F 6-P : fructose 6-phosphate
GIP : glucose-dependent insulintropic polypeptide
GLP-1 : glucagon-like peptide-1
GLUT2 : glucose transporter 2
GLUT5 : glucose transporter 5
G 3-P : glyceraldehyde 3-phosphate
HbA1c : hemoglobin A1c
HDL-C : high-density lipoprotein cholesterol
HFCS : high-fructose corn syrup
HOMA-IR : homeostatic model assessment of insulin resistance
iAUC : incremental area under the curve
IL-1 β : interleukin-1 beta

IL-6 : interleukin-6
IL-17 : interleukin-17
IQR : interquartile range
IUD : intrauterine device
IVGTT : intravenous glucose tolerance test
LBP : lipopolysaccharide-binding protein
LCS : low-calorie sweeteners
LDL-C : low-density lipoprotein cholesterol
NARELO : **NA**tural sugars, **RE**efined sugars and **LO**w-calorie sweeteners
NF- κ B : nuclear factor kappa-B
NHANES : National Health and Nutrition Examination Survey
NOAEL : no observed adverse effect level
NSAIDs : non-steroidal anti-inflammatory drugs
OGTT : oral glucose tolerance test
PCR : polymerase chain reaction
qPCR : quantitative polymerase chain reaction
PERMANOVA : permutational multivariate analysis of variance
RCF : relative centrifuge force
RCTs : randomized-controlled trials
RPM : revolutions per minute
SD : standard deviation
SGLT1 : sodium-glucose cotransporter 1
SCFAs : short-chain fatty acids
SSB : sugar-sweetened beverage(s)
TG : triglycerides
TNF- α : tumour necrosis factor-alpha
VLDL : very low-density lipoproteins
WHO : World Health Organization

CHAPTER I. Introduction

Rationale

In Canada, the top source of free sugar consumption comes from beverages including sugar-sweetened beverages (SSB) and fruit juices (FJ)². Free sugars are defined as any types of simple sugars added to beverages or food products as part of their transformation by food industries or preparation by the consumer, in addition to sugars naturally present in FJ, FJ concentrates, honey, and various syrups³. While compelling evidence links the consumption of SSB to increased risks of noncommunicable diseases³, the evidence regarding natural FJ remains elusive⁴. Although FJ are often perceived as healthier alternatives, they contain a similar free sugar profile to that of SSB and typically lack the dietary fiber found in whole fruits⁵. Consequently, their consumption could theoretically induce adverse metabolic outcomes comparable to those associated with sugar-sweetened beverages.

Article 1 (narrative review - *published*): This rationale formed the foundation of the first article of this thesis, which examined the metabolic health effects associated with the consumption of both SSB and natural FJ. This narrative review was developed through a collaborative effort with Dr. Kimber L. Stanhope, as part of a doctoral research internship I undertook in her laboratory at the University of California, Davis campus (United States). Specifically, it summarized the physiology of glucose and fructose metabolism and their effects on metabolic health, with a particular focus on blood lipids.

The well-established evidence linking the consumption of SSB to adverse metabolic effects and increased risks of noncommunicable diseases³ prompted public health instances to implement regulations aimed at reducing free sugar consumption^{6,7}. Reflecting these efforts, free sugar intake from beverages has declined in North America over the past decade^{8,9}, although this trend does not necessarily indicate a decline in preference for sweetness. In contrast, the global market for low-calorie sweeteners (LCS) has experienced steady growth, with projected revenues between 2025 and 2030 estimated to

rise by 43% and reach a global market value of 39.4 billion \$USD¹⁰. At present, the North American food industry primarily incorporates LCS into beverages rather than solid food products. However, a shift toward substituting sucrose with LCS in solid food products is anticipated following the implementation of Canada's front-of-package labelling regulations in January 2026⁷. This expectation is based on trends observed in countries that have adopted similar policies¹¹, where LCS consumption increased as the food industry engaged in extensive product reformulation in a strategic effort to mitigate the impact of such regulatory measures. LCS such as aspartame, acesulfame K, saccharin, sucralose and stevia offer the sweetness of sugar with fewer or no calories. For many years following their approval as food additives, the observation that these compounds are not metabolized for energy continued to support the assumption that they were physiologically inert.

Article 2 (narrative review - published): The second article presented herein is a narrative review summarizing key findings on the potential impacts of LCS on metabolic health. Aimed at informing physicians and clinicians, this work consolidated foundational knowledge and offered a concise overview of the evidence available at the time of publication. Beyond its scientific contribution, this French-language publication also reflects my personal commitment to improving access to scientific evidence for health professionals, who play a central role in nutrition-based health interventions.

Since the publication of Article 2, a substantial body of research examining the effects of LCS on glucose homeostasis and gut microbiota has emerged. The hypothesis that certain LCS may impair glucose tolerance through compositional and functional alterations to the gut microbiota originated from the work of Suez et al. (2014)¹² using saccharin in both animal and human models. Building on these foundational observations, the authors conducted a second study to expand their findings in humans. The findings from Suez et al. (2022)¹³ put forward personalized microbiome responses to both saccharin and sucralose, suggesting a causal relationship between microbiome signature and disruptions in glucose tolerance in humans. In parallel, growing evidence in animal models also demonstrated

that sucralose, the most extensively used LCS worldwide^{11,14}, can alter gut microbial composition by selectively promoting or inhibiting the growth of specific bacterial taxa¹⁵⁻¹⁹. Ultimately, these alterations may lead to dysbiosis, which is defined as a disruption of intestinal homeostasis resulting from an imbalance in the structural and/or functional composition of the microbiota's beneficial and protective microorganisms²⁰. Beyond impairments in glucose homeostasis, dysbiosis has been associated with a wide range of diseases and adverse health outcomes, including compromised intestinal barrier integrity^{15,21,22} and increased systemic low-grade inflammation^{15,18,19}.

Article 3 (critical review - *manuscript*): To refine the theoretical framework of this thesis, the third article presents a critical synthesis of current evidence on the cumulative effects of sucralose consumption on postprandial glycemic responses and gut microbiota. This manuscript is intended for submission to a peer-reviewed journal.

Article 4 (original data - *manuscript*): Conflicting findings continue to fuel ongoing debates and hinder the establishment of a clear scientific consensus regarding the influence of sucralose on glucose tolerance and gut microbiota in humans. The substantial heterogeneity in methodological approaches highlighted in article 3 informed the design and objectives of the final article included in this thesis. In this randomized-controlled trial, we evaluated the effects of a three-week consumption of sucralose-sweetened beverages on postprandial glycemic responses in healthy adults, compared to sugar-sweetened beverages, under real-world conditions. In an effort to identify potential underlying mechanisms, this study also investigated whether changes in postprandial glycemic responses are accompanied by alterations in insulin concentrations, gut microbiota composition and functional activity, intestinal permeability, and systemic low-grade inflammation. Finally, it explored whether free sugars derived from natural FJ elicit distinct metabolic responses compared with refined sugars.

General Objectives

The objectives of this thesis were as follows:

1. Examine the mechanisms through which sugar-sweetened beverages contribute to adverse health outcomes and determine whether natural fruit juices might elicit similar metabolic effects. (**Article 1** and **Article 4**)
2. Summarize the potential metabolic health effects associated with low-calorie sweeteners consumption for clinicians. (**Article 2**)
3. Comprehensively synthesize and critically appraise the current evidence on the effects of sucralose on glucose homeostasis, with a particular focus on proposed underlying mechanisms. (**Article 3**)
4. Evaluate the effects of cumulative sucralose consumption on postprandial glycemic responses in healthy adults under real-world conditions and investigate potential underlying mechanisms, including alterations in insulin concentrations, gut microbiota composition and functionality, intestinal permeability, and systemic low-grade inflammation. (**Article 4**).

Specific Objectives and Hypotheses (Thesis Article 4)

The specific objectives and hypotheses for Article 4 (original data) are as follows:

1. The primary objective of this study was to **evaluate** the effects of a three-week consumption of sucralose-sweetened beverages (low-calorie sweetener) on postprandial glycemic responses in healthy adults, compared with sugar-sweetened beverages, under real-world conditions.

It was hypothesized that the three-week consumption of sucralose-sweetened beverages would impair postprandial glycemic responses compared with sugar-sweetened beverages, when consumed under real-world conditions.

2. As a secondary outcome, this study **investigated** whether changes in postprandial glycemic responses are accompanied by alterations in insulin concentrations, gut microbiota composition and functionality, intestinal permeability, and systemic low-grade inflammation.

It was hypothesized that impaired glucose tolerance would be accompanied by alterations in insulin concentrations, gut microbiota composition and functionality, compromised intestinal barrier integrity and elevated levels of systemic low-grade inflammation plasmatic biomarkers.

3. Finally, this project **explored** whether free sugars derived from a natural source (100% natural fruit juice) elicit distinct metabolic responses compared with refined sugars.

100% natural fruit juice was included as an exploratory comparator to represent a natural source of free sugars. Due to the limited empirical evidence directly comparing its metabolic effects with those of sugar-sweetened beverages beyond systemic inflammation, no specific hypothesis was formulated. Therefore, findings related to this group are considered exploratory.

CHAPTER II. Thesis Article 1

Are Fruit Juices Healthier Than Sugar-Sweetened Beverages? A Review

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Review

Are Fruit Juices Healthier Than Sugar-Sweetened Beverages? A Review

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Abstract: Free sugars overconsumption is associated with an increased prevalence of risk factors for metabolic diseases such as the alteration of the blood lipid levels. Natural fruit juices have a free sugar composition quite similar to that of sugar-sweetened beverages. Thus, could fruit juice consumption lead to the same adverse effects on health as sweetened beverages? We attempted to answer this question by reviewing the available evidence on the health effects of both sugar-sweetened beverages and natural fruit juices. We determined that, despite the similarity of fruits juices to sugar-sweetened beverages in terms of free sugars content, it remains unclear whether they lead to the same metabolic consequences if consumed in equal dose. Important discrepancies between studies, such as type of fruit juice, dose, duration, study design, and measured outcomes, make it impossible to provide evidence-based public recommendations as to whether the consumption of fruit juices alters the blood lipid profile. More randomized controlled trials comparing the metabolic effects of fruit juice and sugar-sweetened beverage consumption are needed to shape accurate public health guidelines on the variety and quantity of free sugars in our diet that would help to prevent the development of obesity and related health problems.

Keywords: free sugars; fruit juices; fructose; high-fructose corn syrup; sugar-sweetened beverages; dyslipidemia

1. Introduction

The ingestion of free sugars may favor the overconsumption of energy, thus promoting the development of risk factors associated with metabolic diseases such as hypertriglyceridemia, hypercholesterolemia, and insulin resistance [1–3]. Moreover, the current literature strongly suggests that ingestion of sugar-sweetened beverages increases the cardiometabolic risk and risk factors more than isocaloric amounts of complex carbohydrates [4]. Free sugars are defined as any types of simple sugars (monosaccharides or disaccharides) that have been added to beverages or food products during their transformation or preparation by food industries or by the consumer per se, plus sugars naturally present in fruit juices, fruit juice concentrates, honey, and various syrups [5]. Sugars that occur in the natural structure of entire fruits and vegetables as well as those from milk (lactose) are not categorized as free sugars [5,6]. Added sugars include sugars and syrups that are added during the preparation or the transformation of food and beverages. Therefore, natural fruit juices do not contain added sugars, but on the basis of the above definition, they are a significant source of free sugars.

While it is mainly accepted that the overconsumption of sugar-sweetened beverages may lead to adverse effects on health [3,4], the evidence pertaining to the consumption of fruit juices is a matter of

debate. This is reflected in the inconsistency between dietary guidelines that relate to the consumption of natural fruit juice. The World Health Organization (WHO) recommends reducing the intake of free sugars to less than 10% (and, ideally, less than 5%) of total daily energy intake, thus including sugars naturally present in fruit juices in the category of sugars whose consumption should be reduced. The 2015–2020 Edition of the Dietary Guidelines for Americans, a resource for health professionals and policymakers for the design and implementation of nutrition programs in the United States, recommends consuming less than 10% of calories per day from added sugars, thus not including sugars naturally present in fruit juices in the category of sugars whose consumption should be reduced [7]. However, the American Academy of Pediatrics recommends limiting the consumption of fruit juice for children between the age of 1 and 3 years to 4 oz (120 mL)/day, for those from 4 to 6 years to 4–6 oz (120–180 mL)/day, and for those from 7 to 18 years to 8 oz (240 mL)/day [8,9].

There are currently no data estimating the average consumption of free sugars in the United States. Still, fruit juices are commonly consumed on a daily basis by many American adults and children. This review aims to summarize the effects of free sugar consumption, especially from sugar-sweetened beverages, on metabolic health and to consider whether fruit juices may lead to similar health outcomes.

2. Added Sugars: Sucrose and High-Fructose Corn Syrup

Simple sugars have been a part of the human diet for millennia. They were provided mainly by fruits and honey until white sugar (sucrose) became a common consumer product in the 19th century [10]. Nowadays, the worldwide consumption of sucrose is widespread, to the extent that it has tripled over the past 50 years [11]. In the United States, 77% of all calories purchased from 2005 to 2009 contained sweeteners, of which corn syrup, cane sugar, High-Fructose Corn Syrup (HFCS), and fruit juice concentrate were listed as the most commonly used [12]. Sucrose is naturally occurring in sugar cane and sugar beet and therefore extracted and purified directly from sugar cane or beet sap. In contrast, HFCS, which replaces sucrose in 40% of processed foods and beverages, is not naturally occurring [13].

In 1864, the Union Sugar Company generated corn syrup for the first time by treating cornstarch with enzymes to break down the complex carbohydrate into glucose and glucose polymers [14]. Although corn syrup was more affordable than basic cane or beet sugar, it tasted less than half as sweet as sucrose. The isomerization of corn glucose to fructose using microbial enzymes was discovered in the late 1950s by the Clinton Corn Processing Company [14]. This product was, however, not economically viable at that point, mainly because of its instability. Yoshiyuki Takasaki, a Japanese scientist from the Japanese Agency of Industrial Science and Technology, was the first to develop HFCS by isolating a heat-stable glucose isomerase (xylose isomerase) derived from *Streptomyces* sp. in the late 1960s [14]. HFCS can be labeled under many different appellations such as isoglucose or glucose-fructose. The increasing popularity of HFCS amongst food industries was principally due to a more favorable price (the USA being the world's largest producer of corn) and to the fact that its liquid form both inhibits crystallization and helps to maintain the moisture content of baked goods [15], while also being easily diluted in sugar-sweetened beverages. A few years later, HFCS-55 (45% glucose, 55% fructose) was marketed, and HFCS largely replaced sucrose in sugary drinks made by major brands of cola beverages (1984) [14]. Amongst 43 countries from which the HFCS consumption per capita was analyzed in 2000, 2004, and 2007, the United States was ranked the highest with a yearly HFCS consumption of 24.78 kg/per capita [16]. Nevertheless, HFCS consumption has been steadily decreasing since 2009 in the United States, as opposed to sucrose consumption which tends to increase [17]. This observed shift in sweetener choice by the food industry is probably due to the fact that HFCS consumption has been badly publicized in the public media in recent years. The 2017 per capita supply of caloric sweeteners in the United States was estimated to be 158 g [17], of which 86 g was derived from sucrose (cane or beet sugar), 50 g from HFCS, and 22 g from glucose corn syrup, dextrose, honey, and other syrups [17]. The actual intake can be estimated by assuming losses due to spoilage and wastage of 11% at the retail and institutional level and of 20% by the consumer [18].

The sucrose molecule is composed of one unit of glucose covalently bonded to one unit of fructose, the fructose concentration of sucrose therefore being 50%. In contrast, glucose and fructose occur in their free forms in HFCS [19]. This makes it possible to vary the fructose-to-glucose ratio in the syrup mixture and create different formulations. According to the Corn Refiners Association, there are two primary compositions of HFCS which are HFCS-42 and HFCS-55, respectively produced with 42% and 55% of fructose [20]. HFCS-55 is considered as the most commonly used form amongst food industries, but there are currently no obligations for manufacturers to disclose what formulation of HFCS they use on the ingredient list of the food labels. Thus, the fructose content of most processed foods and sugar-sweetened beverages remains uncertain [21]. Ventura et al. (2011) measured the sugar content of 23 popular sugar-sweetened beverages purchased at the grocery store with a focus on fructose. The results from this study revealed that 15 of those beverages had a fructose-to-glucose ratio exceeding 55%, with a mean fructose content of 59%. Moreover, several renown-brand beverages appeared to be made with a formulation of HFCS containing 65% of fructose instead of 55% [21]. However, this study was criticized for the methods used, which were designed to identify sucrose, glucose, and fructose, but were not sensitive enough to precisely measure other traces of sugars (such as maltose) if they were present in the beverages [22]. In response, a study funded by the International Society of Beverage Technologists randomly selected 80 beverages known to be sweetened with HFCS-55 by Coca-Cola Company, PepsiCo, and Dr Pepper Snapple Group in order to validate the fructose content. The mean fructose content of those beverages resulted to be 55.58%, with a 95% confidence interval of 55.51–55.65% [23]. It should be noted that this study sampled amongst beverages known to be sweetened with HFCS-55 only, thereby not including the ones that are potentially sweetened with HFCS-65, such as the ones distributed in the Coca-Cola Freestyle dispensers [24]. Walker et al. (2014) [25] selected 10 of the 23 sweetened beverages (based on popularity) that were previously analyzed by Ventura and colleagues (2011) [21] for further analysis using two alternative methods to determine the fructose content. In beverages listing HFCS as an ingredient, the mean fructose concentration was 59.4 ± 8.9 g/L, which corroborates the results of Ventura et al. (2011) [21]. Three of the selected beverages had a free fructose concentration exceeding 60 g/L. Maltose was detected in 8 of the 10 beverages, and its concentration was less than 2%, whereas galactose and lactose were not detected in any of the beverages [25]. The results of these two studies [21,25] provide evidence that many popular beverages sweetened with HFCS have a fructose content greater than 55%, thus suggesting the use of a higher concentration of HFCS. From a biological standpoint, it is unlikely that a meaningful difference exists between sucrose and HFCS-55 based on their fructose content. However, there may be a significant biological difference between fructose-to-glucose ratios of 50:50 and 60:40. Thus, the use of HFCS at a fructose concentration of more than 55% by the sweetened beverage industries, without any obligation to disclose the formulation to consumers, is concerning.

3. How Does Fructose Can Alter Lipemia?

3.1. Glucose Metabolism

Concerns about the ratio of fructose to glucose in beverages relate to the well-established differences between glucose and fructose metabolism. Glucose enters the enterocytes mostly by secondary active transport via sodium–glucose transporters (SGLT1) located in the apical membrane of the enterocytes. SGLT1 transporters have a high-affinity for glucose, but a low transportation capacity. Thus, under high concentrations of glucose in the lumen of the intestine, glucose also enters the enterocytes by facilitated diffusion via low-affinity, but high-capacity glucose transporters (GLUT2) [26]. GLUT2 transporters are expressed to a lesser extent in the apical membrane of the enterocytes but can be rapidly translocated from the basolateral membrane to the apical membrane to enhance glucose uptake under high concentrations of intestinal glucose [26]. Then, glucose exits the enterocytes to enter the bloodstream by facilitated diffusion via GLUT2 transporters located in the basolateral membrane (Figure 1). Glucose is then transported to the liver by the portal vein. Hepatic glucose metabolism

is regulated by insulin and hepatic energy needs. This allows much of ingested glucose arriving via the portal vein to bypass the energy-replete liver and to rapidly reach the systemic circulation [27]. The first step of glycolysis, the responsible pathway for glucose metabolism, is the phosphorylation of glucose on its 6th carbon by the enzyme glucokinase (hexokinase). This step is then followed by an isomerization reaction resulting in fructose 6-phosphate (F6P). The major limiting step of glycolysis is the phosphorylation of F6P to fructose 1,6-biphosphate (catalyzed by phosphofructokinase), which will allow the molecule to be cleaved in two three-carbon units that can later be used to generate ATP [28].

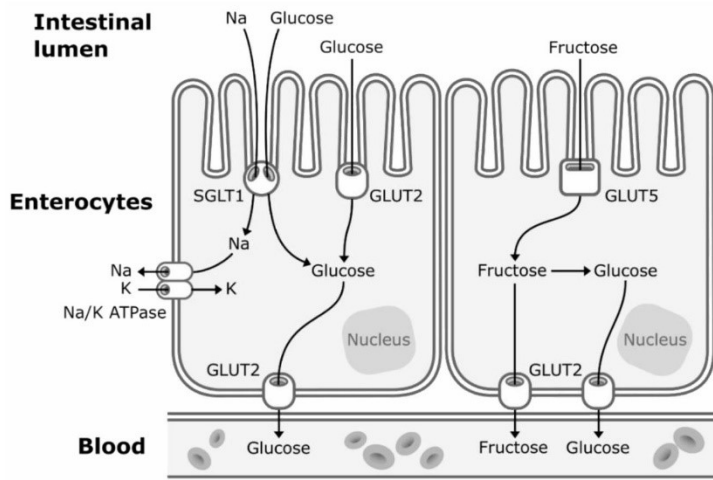


Figure 1. Absorption of fructose and glucose in the enterocytes. Glucose enters the enterocytes mostly by secondary active transport via sodium-glucose transporters (SGLT1) located in the apical membrane of the enterocytes. Under high concentrations of glucose in the lumen of the intestine, glucose also enters the enterocytes by facilitated diffusion via glucose transporters (GLUT2). Fructose enters the enterocytes through a specific fructose transporter (GLUT5). Then, both glucose and fructose exit the enterocytes to enter the systematic circulation by facilitated diffusion via GLUT2 transporters located in the basolateral membrane of the enterocytes. A small part of dietary fructose will be converted and released in the bloodstream by the enterocytes as glucose.

3.2. Fructose Metabolism

When fructose is ingested, it enters enterocytes through a specific fructose transporter (GLUT5), which is independent of sodium-glucose linked transporters and does not require ATP hydrolysis as opposed to SGLT1 [1]. Fructose will then enter the systematic circulation in a similar way to glucose, that is by facilitated diffusion via GLUT2 transporters (Figure 1). A small part of dietary fructose will be converted and released in the bloodstream by the enterocytes as glucose [1,29], lactate [1,29], and fatty acids in chylomicrons [30–34] (Figure 2). Yet, the role of the enterocytes in determining the metabolic fate of fructose has not been clearly established.

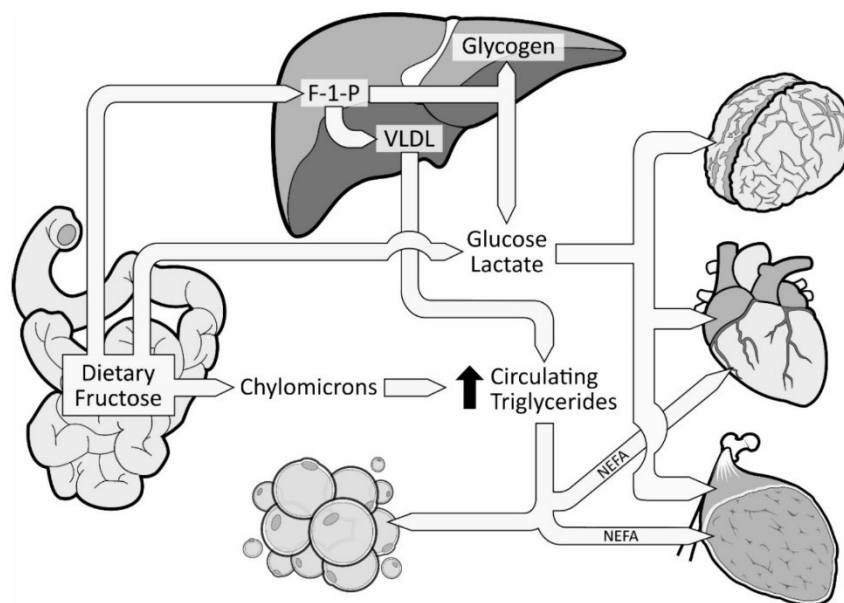


Figure 2. Metabolic fate of dietary fructose. Dietary fructose is ingested and released by the enterocytes mostly as fructose but also converted and released as glucose, lactate, and fatty acids (in chylomicrons). Fructose spills over to the liver where it is phosphorylated as Fructose 1-Phosphate (F 1-P). The largest part of F 1-P will be metabolized and converted by the hepatocytes as glucose, which can be stored as glycogen or released in the bloodstream [35]. Hepatocytes can also convert F 1-P into lactate and fatty acids. Fatty acids accumulate into the liver, consequently favoring the production and secretion of very low-density lipoproteins (VLDL), which leads to increased levels of circulating triglycerides and dyslipidemia.

The excessive amount of fructose will spillover to the liver where it is nearly all cleared from the portal blood after its first pass. Fructose will be rapidly phosphorylated (catalyzed by fructokinase C, a key enzyme in the metabolism of fructose [36–38]) on its first carbon, resulting in fructose 1-phosphate (F 1-P) instead of F6P [28] (Figure 2). F 1-P has the capacity to bypass the first limiting step of glycolysis [28] without being regulated by insulin nor inhibited by ATP production. F 1-P will mostly be metabolized by aldolase B into glyceraldehyde 3-P (G 3-P). Subsequently, G 3-P can be: (1) converted into pyruvate (resulting in acetyl-CoA production); (2) converted and released as lactate; or (3) converted to glucose (gluconeogenesis) [1]. The largest part of G 3-P will be converted to glucose, which can be stored as glycogen or released as glucose 6-phosphate in the bloodstream [35]. In fact, in the 1990s, isotope tracing with intravenously infused ^{13}C -labeled fructose in humans showed that ~50% of a fructose load was converted and recirculated as ^{13}C -labeled glucose [39]. When released in the systematic circulation, glucose and lactate can be utilized as an energy substrate by the brain, heart, and muscle tissue [35] (Figure 2). As mentioned previously, a large part of F 1-P will be metabolized in G 3-P. Nonetheless, the excessive supply of fructose spilled to the liver has also been shown to simultaneously inhibit lipid oxidation [40] and to enhance hepatic de novo lipogenesis (DNL) [41,42]. DNL has the capacity to convert fructose, more precisely F 1-P, into fatty acids [43], thus consequently increasing the intrahepatic lipid supply. Elevated levels of intrahepatic lipids content favor very low-density lipoproteins (VLDL) production and secretion [44], which also leads to increased levels of postprandial triglycerides and dyslipidemia [1,42] (Figure 2). Increased levels of intrahepatic lipids are associated with hepatic insulin resistance [45,46]. Of note, a systematic review indicated that fructose consumption in an energy-matched exchange for other carbohydrates (mostly glucose) induces hepatic insulin resistance [47]. This suggests that the promotion of hepatic insulin resistance by fructose could

not be attributed only to the excess of energy intake under hypercaloric diet conditions. Knowing that DNL is more strongly activated in the insulin-resistant liver [48], fructose consumption has the potential to generate a vicious cycle that would further increase the intrahepatic lipid supply, thus amplifying VLDL-triglyceride production and secretion [2]. Continued exposure to triglycerides promotes muscle lipid accumulation [49], which may also promote whole-body insulin resistance [50,51].

4. Sugar-Sweetened Beverages and Their Implication in Metabolic Health

Free Sugars Consumed from Sugar-Sweetened Beverages Induce Metabolic Perturbations

Epidemiological studies have shown that the consumption of sugar-sweetened beverages is associated with increased energy intake, long-term weight gain, and prevalence of metabolic and cardiovascular diseases [3,52,53]. Experimental evidence strongly suggests the fructose component of HFCS and sucrose promotes metabolic perturbations such as dyslipidemias [41,42,49,54–64] and insulin resistance [35,42,57,65]. The consumption of free sugars at the level that is currently consumed by Americans may adversely alter lipemia. It has indeed been shown that sucrose, when consumed at 13% of estimated daily energy requirements (Ereq) (80 g/day) as a beverage along with a usual ad libitum diet for three weeks, led to increased low-density lipoprotein cholesterol (LDL-C) and decreased hepatic insulin sensitivity in healthy young men compared to the consumption of glucose [65]. It should be noted that daily energy requirements refer to the calorie intake needed to balance energy expenditure in order to maintain a healthy individual's body weight stable. These results are in line with recent findings showing that a 12-week intervention where 13% of diet energy as fructose was served in the habitual diet of 71 men with abdominal obesity led to enhanced DNL and increased body weight, liver fat content, and postprandial triglyceride levels [56]. Young men and women consuming beverages containing 0, 10, 17.5, and 25% of Ereq as HFCS along with ad libitum diets exhibited a dose-dependent increase of LDL-C, apolipoprotein B (the protein backbone of VLDL), apoCIII, uric acid, and postprandial triglycerides [66]. Importantly, even the group consuming the 10% dose exhibited significantly increased concentration of LDL-C, apolipoprotein B, and postprandial triglycerides compared with their baseline concentration [66]. In a six-month dietary intervention study, subjects consuming one liter of sucrose-sweetened beverages/day exhibited increased triglycerides, cholesterol, and liver fat [49]. A recent meta-analysis revealed that the dose and overall caloric intake of free sugars have the strongest deteriorating effect on blood lipids as compared to interventions where isocaloric substitution of free sugars with complex carbohydrates is provided [67]. However, most of these studies are limited in that free sugars were consumed along with the subjects' own usual ad libitum diets during most of the intervention, thus the total amount of free sugars consumed daily are unknown. This prevents attributing adverse effects to precise doses of free sugars. Another limitation to the above studies is that many of the subjects exhibited increases in body weight. This makes it difficult to separate the direct effects of fructose from those indirectly mediated by increased adiposity.

There are several studies that compared sugar (sucrose, HFCS, and/or fructose) consumption from sugar-sweetened beverages with isocaloric substitutions for complex carbohydrates [41,57] or glucose [59,65], or from low-sugar diets [54,55,61,66,68,69] in healthy individuals on health outcomes (Figure 3 and Table 1 summarizes these studies and their effects on fasting triglycerides and fasting LDL-C levels). However, to our knowledge, there are only three studies [41,68,69] in which the effects of sugar consumption from sugar-sweetened beverages at levels less than 30% Ereq were investigated in healthy subjects utilizing a controlled dietary protocol that prevented body weight gain (eucaloric) and diet macronutrient variations between experimental groups. Black et al. (2006) [69] conducted a six-week crossover study with healthy men who consumed eucaloric diets containing high (25% Ereq) or low (10% Ereq) amounts of sucrose. The 25% Ereq sucrose diet increased the levels of total cholesterol by 15% and of LDL-C by 24% as compared to the 10% Ereq sucrose diet; the authors suggested this could have been caused by the high-sucrose diet containing more saturated fats [69]. More recently, Lewis et al. (2013) [68] conducted a randomized six-week crossover study in which

individuals with obesity consumed low- (5%) or high- (15%) sucrose diets for six weeks. While no differences in lipid levels were observed, the subjects displayed increased glucose (5.0 ± 0.2 vs. 5.4 ± 0.2 mmol/L, $p < 0.01$) and insulin responses (59.0 vs. 109.2 mU/L, $p < 0.01$) during the oral glucose tolerance test (OGTT) when consuming the high-sucrose diet [68]. Likewise, Schwarz and colleagues (2015) [41] investigated eight healthy men consuming crossover diets containing eucaloric amounts of either fructose or complex carbohydrates (25% of Ereq) for nine days, while maintaining their body weight stable and providing the same macronutrient distributions. The subjects exhibited elevated DNL (average, $18.6 \pm 1.4\%$ vs $11.0 \pm 1.4\%$; $p = 0.001$), liver lipids (median, +137%, $p = 0.016$), and postprandial triglycerides (in seven of eight participants: average, 172 ± 29 vs. 140 ± 28 mg/dL; $p = 0.002$) when consuming the high-fructose diet compared with the complex-carbohydrate diet [41]. Overall, these results suggest that sucrose or fructose consumption at levels as low as 18% Ereq, increases the risk factors for metabolic diseases, even when consumed with a diet that does not promote weight gain. However, this conclusion could be confounded by the vulnerability of subjects with obesity [68], the different saturated fat content between diets [69], and the use of pure fructose instead of HFCS or sucrose [41]. Therefore, because of the limited number of studies and the limitations of these studies, it is difficult to establish firm conclusions regarding the weight-independent effects of sugar consumption on health outcomes.

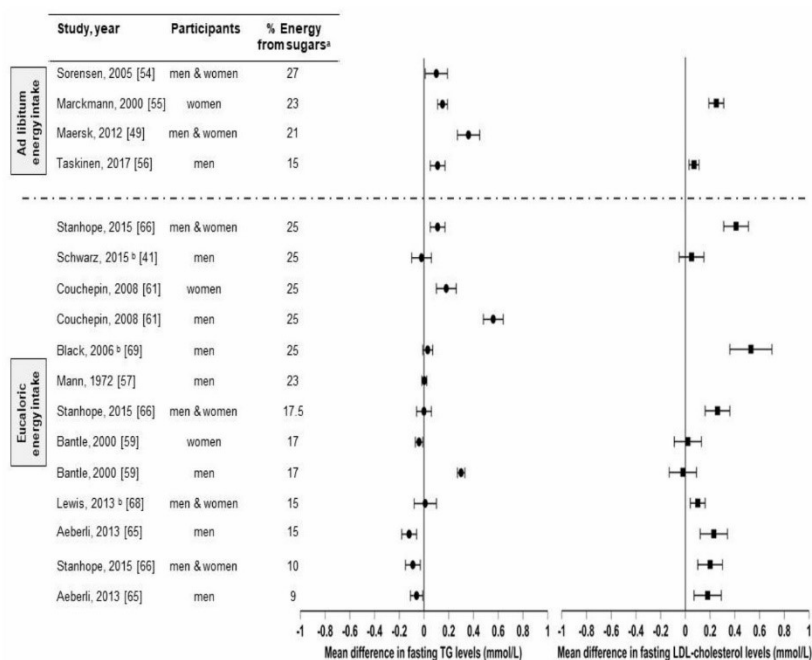


Figure 3. Effects of sugar consumption from sugar-sweetened beverages on fasting blood triglyceride (TG) and fasting LDL-cholesterol levels in healthy individuals with normal weight, overweight, or obesity. Mean difference in fasting blood triglyceride and fasting low-density lipoprotein cholesterol (LDL-C) levels in studies that compared higher with lower sugar intakes from sugar-sweetened beverages in healthy individuals with normal weight, overweight, or obesity. ^a Refers to the higher sugar intake intervention. The percentage of energy from the lower sugar intake intervention is detailed in Table I. Studies with ad libitum energy intake controlled for a minimal sugar intake but not for total energy intake. Studies with eucaloric energy intake controlled for a minimal sugar intake and for weight maintenance throughout the studies. ^b Studies that controlled for a minimal sugar intake, for weight maintenance throughout the studies, and for diet macronutrient variations between experimental groups.

Table 1. Characteristics of studies included in Figure 3. Detailed characteristics of studies included in Figure 3 in terms of participants (sample size, sex, weight, age), duration, intervention (% Ereq of sugar intake provided in sugar-sweetened beverages and food, or unspecified), and control (comparative: low-sugar intervention or baseline values).

	First Author, Year of Publication [ref]	Participants	Duration	Intervention	Control
Ad libitum energy intake with usual diets	Sorensen, 2005 [54]	6 healthy men with overweight 35 healthy women with overweight Age: 24–47 year	10 weeks	27% Ereq sucrose; Sweetened beverages and food	4% sucrose; Food
	Marckmann, 2000 [55]	20 healthy women with normal weight Age: 21–52 year	2 weeks	23% Ereq sucrose; Sweetened beverages and food	3% Ereq sucrose; Sweetened beverages and food
	Maersk, 2012 [49]	17 healthy men with overweight 30 healthy women with overweight Age: 33–45 year	6 months	21% Ereq sucrose; Sweetened beverages (1000 mL)	Compared to baseline values
	Taskinen, 2017 [56]	82 healthy men with obesity Age: 20–45 year	12 weeks	15% Ereq fructose; Sweetened beverages (3 × 330 mL)	Compared to baseline values
Eucaloric energy intake with isocaloric diets	Stanhope, 2015 [66]	42 healthy men with normal weight to obesity 43 healthy women with normal weight to obesity Age: 18–40 year	19 days	10% Ereq HFCS 17.5% Ereq HFCS 25% Ereq HFCS; Sweetened beverages	Artificially sweetened beverages (aspartame)
	Schwarz, 2015 [41]	8 healthy men with normal weight to obesity Age: 18–45 year	9 days	25% Ereq fructose; Sweetened beverages	Isocaloric substitution of fructose for complex carbohydrates; Sweetened beverages
	Couchepin, 2008 [61]	8 healthy men with normal weight 8 healthy women with normal weight Age: 21–23 year	2 × 6 days 4 weeks washout	25% Ereq fructose; (Eucaloric low fructose diet + overfeeding with 25% Ereq fructose); Unspecified medium	Eucaloric low fructose diet; Unspecified medium
	Black, 2006 [69]	13 healthy men with normal weight Age: 30–36 year	2 × 6 weeks unspecified washout period	25% Ereq sucrose; Sweetened beverages and food	10% Ereq sucrose; Sweetened beverages and food
	Mann, 1972 [57]	9 healthy men with normal weight Age: 30–40 year	3 × 14 days	23% Ereq sucrose; Unspecified medium	Isocaloric substitution of sucrose for complex carbohydrates; Unspecified medium
	Bantle, 2000 [59]	12 healthy men with normal weight 12 healthy women with normal weight Age: <40 year (6 men, 6 women) >40 year (6 men, 6 women)	2 × 6 weeks 4 weeks washout	17% Ereq fructose; Sweetened beverages and food	17% Ereq glucose; Sweetened beverages and food
	Lewis, 2013 [68]	13 healthy men and women with overweight or obesity Age: 35–56 year	2 × 6 weeks 4 weeks washout	15% Ereq sucrose; Sweetened beverages and food	5% Ereq sucrose; Sweetened beverages and food
	Aeberli, 2013 [65]	9 healthy men with normal weight Age: 21–25 year	4 × 3 weeks 4 weeks washout	9% Ereq fructose 14% Ereq fructose 15% Ereq sucrose; Sweetened beverages (3 × 200 mL)	15% Ereq glucose; Sweetened beverages (3 × 200 mL)

5. What about the Effects of Natural Fruit Juices on Blood Lipids?

It is well accepted that fruit intake is protective for human health, but there is no clear consensus about the effects of consuming the juices that are extracted from them [70]. The primary component of fruit juices, apart from water, is free sugars in a concentration of about 100–120 g/L depending on the variety and the quality of fruits [71]. The fructose content of most natural fruit juices is quite similar to that of beverages sweetened with HFCS-55. For instance, orange juice has an average total fructose concentration (including free fructose and fructose from sucrose) of 51–57 g/L, which represents 52 to 54% of its total sugar content [25,72]. However, it is important to note that, despite their similarity to sugar-sweetened beverages in terms of fructose composition, fruit juices are also a source of various bioactive compounds such as phytonutrients, whose consumption has been shown to be beneficial to human health and the prevention of chronic diseases [73]. Given the comparable free sugar and fructose content of natural fruit juices and HFCS-55-sweetened beverages, could their consumption potentially lead to similar metabolic effects, despite their different composition in vitamins, minerals, and antioxidants?

5.1. Population Studies

A meta-analysis and systematic review from 17 cohort studies examined the prospective associations between the consumption of different types of beverages containing free sugars such as sugar-sweetened beverages and fruit juices in subjects with type 2 diabetes [74]. The daily consumption of 250 mL of sugar-sweetened beverages was associated with an increased incidence risk of type 2 diabetes by 13% (95% confidence interval 6% to 21%, I^2 for heterogeneity = 79%), whereas the same consumption of fruit juices elevated the incidence risk by 7% (1% to 14%, I^2 = 51%), both independently of adiposity [74]. Another recent population study showed that the consumption of more than five servings/week (200 mL/serving) of either natural fruit juices or sugar-sweetened beverages was associated with an increased risk of metabolic syndrome, more specifically of abdominal obesity and hypertriglyceridemia [75]. Surprisingly, the consumption of 1–5 servings of natural fruit juices weekly (without any sugar-sweetened beverages) was inversely associated with the same risk factors [75]. Furthermore, the substitution of 250 mL of natural fruit juices per day by the same amount of water for four years was associated with an 8% abated risk of type 2 diabetes [76]. On the other hand, other cohort studies that reported an association of sugar-sweetened beverage consumption with the risks of chronic diseases, such as metabolic syndrome [77], cardiovascular diseases [78,79], and type 2 diabetes [80–84], failed to find the same association with natural fruit juices. One major limitation of population studies is that they rely on self-reported food intake data. Additionally, there are many confounding factors. For example, in three of the aforementioned studies, participants who reported consuming fruit juices on a daily basis also reported being more physically active than the non-consumers or the consumers of sugar-sweetened beverages [80] or were shown to have general healthier diets [83,84], which can both have an impactful effect on lipid metabolism and insulin resistance.

5.2. Intervention Studies

5.2.1. Absence of Effect

A meta-analysis of 19 randomized controlled trials investigating the effects of various fruit juices, concentrated fruit juices, and fruit juice powders suggested that fruit juices had a borderline significant effect on the reduction of diastolic blood pressure and did not affect total or LDL-C [85]. However, nearly all these studies investigated a dose of free sugars provided by fruit juices well below 10% Ereq, whereas adverse effects in relation to the consumption of sugar-sweetened beverages and blood lipids have never been observed at doses below 10% Ereq. Moreover, very few studies included in the meta-analysis directly compared the effects of fruit juices to sugar-sweetened beverages. The interventions were all conducted with usual diets with or without restrictions of consuming other fruits juices, alcohol, food sources of antioxidants, and specific fruits and vegetables. None of the

19 studies included in the meta-analysis controlled for energy intake nor for macronutrient distribution. In a 12-week trial, Simpson et al. (2016) showed that the consumption of 250 mL/day of natural orange juice in overweight hypercholesterolemic men did not affect body weight, insulin sensitivity, and circulating lipids when compared to the consumption of energy-matched orange sugar-sweetened beverage [86]. Nevertheless, a 250 mL dose of fruit juice provides 25 g of total free sugars [25], which represents an average of only 5% Ereq.

5.2.2. Detrimental Effects

In a four-week dietary controlled crossover study, 23 healthy men and women ingested a daily intake of various apple supplementations, including 550 g of whole fresh apples or 500 mL of clear or cloudy apple juice [87]. The consumption of 500 mL/day of clear apple juice was shown to significantly increase LDL-C by 6.9% as compared to whole apples, despite no adverse effects on high-density lipoprotein cholesterol (HDL-C) and plasma triglycerides concentrations. Interestingly, elevation in LDL-C was not observed in the group consuming cloudy apple juice. The lack of soluble fibers (pectin) and lower polyphenols levels in clear apple juice compared to cloudy apple juice have been suggested to explain the different metabolic responses observed [87]. At a dose closer to 15% Ereq, Kurowska et al. (2000) provided 750 mL/day of orange juice for four weeks to 25 subjects (men and women) with hypercholesterolemia and observed a reduction of the LDL to HDL cholesterol ratio by 16% due to an elevation of HDL-C, without, however, affecting LDL-C levels [88]. While an elevation in HDL-C could be interpreted as beneficial for cardiovascular health, this intervention also led to a 30% increase in triglyceride levels (from 1.56 ± 0.72 to 2.03 ± 0.91 mmol/L; $p < 0.02$) without any weight gain being observed [88].

5.2.3. Beneficial Effects

In contrast with the results reported by Kurowska et al. (2000) [88], Cesar et al. (2006) [89] showed that the effects of 750 mL orange juice combined with an ad libitum diet for 60 days in men with normal cholesterol levels non-significantly decreased LDL-C (-0.08 mmol/L). When the same dose was given to men with high cholesterol levels, a significant decrease of LDL-C (-0.47 mmol/L) was observed, while triglyceride levels remained unchanged in both groups [89]. More recently, 78 participants living with obesity were provided individualized weight-loss diet prescriptions that included randomization into two groups, one of which including 500 mL/day of orange juice. After 12 weeks, both groups exhibited similar body mass index (BMI) and fat mass loss. Subjects in the orange juice group showed a reduction of homeostasis model assessment-insulin resistance by 33% ($p = 0.04$), of LDL-C by 24% ($p \leq 0.001$), and of the inflammation biomarker C-reactive protein by 33% ($p = 0.001$) compared with the placebo group [90].

A plausible explanation as to why fruit juices may lead to different health effects from those of sugar-sweetened beverages is that fruit juices are a source of various bioactive compounds, such as vitamins (vitamin C, folate, etc.), minerals (mostly potassium), and antioxidants [73], while sugar-sweetened beverages generally have none. Antioxidants were shown to decrease oxidative stress [91], which plays an important role in the prevention of cardiovascular disease, including atherosclerosis, diabetes, and dyslipidemia [92]. Hesperidin and naringenin are the most important citrus flavanones (type of antioxidant) present in orange juice [93], which is the most studied type of fruit juice due to its popularity amongst consumers. A limited number of human studies [94,95] suggest that the anti-inflammatory effects [96] induced by the ingestion of those flavanones could contribute to the prevention of chronic diseases and therefore potentially counteract the detrimental effects of free sugars when consumed in limited amounts [97]. It is also hypothesized that fruit juices may differently affect the gut microbiota as compared to sugar-sweetened beverages and therefore lead to different metabolic effects [98]. Notwithstanding, a recent meta-analysis concluded that there is weak evidence to suggest that blood lipid levels may be improved with consumption of fruit juices [99]. The authors indicate that factors such as the variety of natural fruit juices, the dose, the length of exposure, the

study design (randomized vs cross-sectional studies), as well as the dietary and exercise patterns of participants must be considered in future studies to obtain a clearer picture of the relationship between fruit juice consumption and blood lipid levels in humans.

6. Conclusions

There is ample evidence that links sugar-sweetened beverage consumption to adverse effects on metabolic risk factors, such as dyslipidemia [35,41,42,58–65] and insulin resistance [35,41,42,58,61,65]. Despite the similarity of fruit juices to sugar-sweetened beverages in terms of free sugar and fructose content, it remains unclear in the current literature whether they lead to the same metabolic consequences if consumed in equal doses. More randomized controlled trials comparing the metabolic effects of fruit juice and sugar-sweetened beverage consumption under dietary conditions that will eliminate confounding differences due to diet are needed in order to shape clear evidence-based public health guidelines. These future studies should also address important aspects such as individuals' gene variants, metabolic status, medical treatment, and physical activity levels, but also more qualitative characteristics such as eating habits and behaviors. Most consumers do not ingest as much fruit juice as sugar-sweetened beverages. Indeed, a systematic assessment of beverage intake (from annual food balance information) has estimated the average daily consumption of fruit juices in American adults over 20 years old in 2010 to be approximately 80 mL and the consumption of sugar-sweetened beverages to be 237 mL [100]. Would it be beneficial to their health if Americans reversed this pattern and consumed 237 mL/day of fruit juices and only 80 mL of sugar-sweetened beverages? The question still needs to be answered: Are fruit juices healthier than sugar-sweetened beverages?

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CHAPTER III. Thesis Article 2

Les édulcorants de la controverse

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Les édulcorants de la controverse

Alexandra Pepin, Pascal Imbeault

► La littérature révèle un lien important entre la consommation de boissons sucrées et des effets néfastes sur la santé humaine, comme la prévalence de l'obésité et des maladies métaboliques. Différentes stratégies ont été adoptées par de nombreux pays afin d'inciter la population à réduire leur consommation de boissons sucrées. Les industries alimentaires ont, quant à elles, promu les substituts de sucre (ou édulcorants), les présentant comme une saine alternative qui permettrait de réduire l'apport calorique sans avoir à compromettre l'agréable goût sucré des aliments et des boissons. Toutefois, l'utilisation des substituts de sucre comme moyen de limiter ou de réduire l'apport énergétique est-elle sans conséquence pour la santé ? Cette revue vise à discuter des différents effets associés à la consommation d'édulcorants sur la santé et à élucider si leur utilisation devrait être recommandée par les professionnels de la santé à leurs patients dans le cadre de la gestion de leur poids. ◀



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alimentaires englobent à la fois les édulcorants artificiels et les édulcorants d'origine naturelle, tels que les extraits purifiés de stévia¹ et de fruit du moine² [5]. Substituts des sucres ajoutés, ils procurent la sensation de goût sucré tout en ne contenant peu ou pas de calories et sont communément utilisés comme additifs dans les produits alimentaires, tels que les boissons, les bonbons, les yogourts, les fruits en conserve et les céréales à déjeuner [5]. Ils peuvent également être obtenus en vente libre par les consommateurs [5]. Dans cette revue, nous examinerons si l'utilisation des édulcorants présente un risque pour la santé métabolique. Plus précisément, nous discuterons des effets potentiels de la consommation de substituts de sucre sur l'apport énergétique, sur le microbiote intestinal et sur le métabolisme du glucose. Finalement, nous analyserons les édulcorants comme outil nutritionnel et s'ils méritent d'être recommandés par les professionnels de la santé à leurs patients.

L'innocuité des substituts de sucre : qui la détermine et comment ?

À l'échelle mondiale, une panoplie de substituts de sucres sont approuvés pour l'utilisation alimentaire, dont les suivants : l'acésulfame-potassium (Ace-K), l'advantame, l'aspartame, le néotame, la saccharine, le sucralose, la thaumatococine, les glycosides de stéviol, les extraits de fruit du moine, ainsi que les sucres alcool (polyalcools ou polyols), tels que le lactitol, l'isomalt, le mannitol, le sorbitol et le xylitol [6]. Leur avantage principal repose sur leur pouvoir sucrant

La littérature récente révèle un lien important existant entre la consommation de boissons sucrées et des effets néfastes sur la santé, comme la prévalence de l'obésité et des maladies métaboliques qui y sont associées [1-3]. Les lignes directrices nutritionnelles émises par les instances de santé publique, comme celles de l'Organisation mondiale de la Santé, recommandent à la population de limiter leur consommation de sucres libres et ajoutés, notamment en réduisant leur consommation de boissons sucrées [4]. De leur côté, les industries alimentaires font de plus en plus la promotion des substituts de sucre, comme étant une judicieuse alternative qui permettrait de réduire l'apport calorique sans compromettre l'agréable goût sucré des aliments... L'utilisation de substituts de sucre comme moyen de limiter l'apport calorique suscite toutefois des débats controversés. Aussi appelés édulcorants, ces additifs

Vignette (Photo © Fédération française des diabétiques).

¹ Il s'agit d'extrait naturel de plante utilisé comme édulcorant naturel par les communautés indigènes d'Amérique du Sud depuis plusieurs siècles. Les glycosides de stéviol sont réglementés comme additifs alimentaires. Cependant, les feuilles de Stévia sont considérées comme un ingrédient alimentaire, et non comme un additif alimentaire. Peu de réglementations existent donc sur l'utilisation des feuilles de stévia dans les aliments et les boissons emballés.

² Ce petit fruit ovale (Luo Han Guo) poussant au sud de la Chine et dans le nord de la Thaïlande est utilisé comme édulcorant et ingrédient fonctionnel dans la médecine traditionnelle chinoise. Les moines bouddhistes le cultivaient pour ses vertus médicinales, d'où son nom de fruit du moine.



très élevé comparé au sucrose, soit le traditionnel sucre blanc [7]. De minimes quantités d'édulcorants sont donc suffisantes pour obtenir le même goût sucré. Par exemple, le pouvoir sucrant de l'aspartame et du sucralose est perçu respectivement 200 fois et 600 fois plus sucré que celui du sucrose pour une même quantité. Bien que les sucres alcool soient également des substituts de sucre, ils ne sont pas considérés comme des édulcorants de haute intensité, étant donné que leur pouvoir sucrant est similaire ou inférieur à celui du sucrose [8]. Le *Tableau 1* présente différents substituts de sucre et permet de comparer leurs profils en termes de pouvoir sucrant, d'apport quotidien acceptable et la façon dont ils sont métabolisés par notre organisme. Les substituts de sucre sont soumis à des réglementations très strictes dans le but d'assurer qu'ils ne sont pas nocifs pour la santé humaine [5]. Les étapes qui précèdent l'approbation d'un substitut de sucre par un organisme de réglementation fédéral ou gouvernemental sont mal comprises du public et méritent d'être mises en lumière. Bien avant son approbation, des études précliniques rigoureuses sont entreprises afin de déterminer la toxicocinétique (examen de l'absorption, de la distribution, du métabolisme et de l'excrétion d'une substance donnée) de l'édulcorant et d'en assurer son innocuité [9]. Ces études précliniques d'innocuité sont réalisées chez l'animal et utilisent un large éventail de concentrations afin de parvenir à évaluer la relation dose-effet qui pourrait engendrer des effets néfastes pour la santé. La dose la plus élevée qui ne provoque pas d'effets biologiques, lorsqu'elle est consommée dans le cadre d'études à doses répétées de longue durée, est appelée *Dose Sans Effet Indésirable Observé* [9]. Afin de pouvoir extrapoler les résultats de ces études conduites chez l'animal, à l'humain, la valeur de la *Dose Sans Effet Indésirable Observé* est ensuite divisée par un facteur de sécurité (pour les substituts de sucre, ce facteur est souvent de 100) afin de garantir une marge de sécurité conservatrice [9]. Ce calcul est utilisé pour établir un apport quotidien admissible (AQA) chez l'humain, étant défini comme « la quantité d'additif alimentaire [dans ce cas d'un substitut de sucre X], exprimée en mg/kg de poids corporel/jour, pouvant être ingérée quotidiennement dans une vie sans aucun risque appréciable pour la santé » [10]. Il est très peu probable de dépasser ou même d'atteindre l'AQA de la plupart des édulcorants, principalement en raison des quantités minimales d'édulcorants requises pour obtenir un goût sucré appréciable, mais aussi parce que les industries utilisent souvent une combinaison de plusieurs édulcorants dans le but de réduire la quantité de chacun dans la formulation de leurs produits. Ainsi, pour atteindre l'AQA de l'aspartame, soit 50 mg/kg de poids corporel/jour, il serait nécessaire de consommer environ 18 cannettes de boissons gazeuses « diète » contenant de l'aspartame comme seul édulcorant, et ce en une journée [11]. Cependant, cette quantité, qui peut paraître élevée, ne l'est pas forcément pour tous les édulcorants. En effet, l'AQA du sucralose est de 5 mg/kg de poids corporel/jour [7]. Sachant qu'une cannette de 355 mL de boisson gazeuse sans caféine « diète » d'une marque populaire contient 68 mg de sucralose et 41 mg d'acésulfame-potassium [12], il suffirait donc de 5 cannettes pour qu'un individu ayant un poids corporel de 70 kg atteigne l'AQA du sucralose. Bien que des réglementations strictes et des études

rigoureuses assurent l'innocuité des substituts de sucre au préalable à leur approbation pour utilisation comme additif alimentaire, les études toxicocinétiques ne permettent cependant pas de tirer des conclusions quant aux conséquences métaboliques qui pourraient être associées à la consommation de ces substituts. En d'autres termes, le simple fait que les substituts de sucre sont en effet considérés comme étant « sans danger » pour la santé humaine (absence de toxicité ou de risque de causer des maladies, telles que le cancer) ne signifie pas qu'ils constituent une option « saine » pour le consommateur (état physiologique optimal pour maintenir une bonne santé) [13]. De ce fait, un grand nombre de professionnels de la santé hésitent donc à recommander la prise d'édulcorants à titre d'outil nutritionnel favorisant la gestion du poids corporel, et ce principalement en raison des incertitudes quant aux effets de leur consommation à long terme sur la santé métabolique [14].

Les substituts de sucre et la gestion du poids corporel : ce qu'en dit la science...

Ce n'est pas d'hier que des préoccupations en lien avec les effets de la consommation d'édulcorants sur le métabolisme et la régulation des mécanismes de l'appétit ont été exprimées. Des études observationnelles ont en effet révélé que l'augmentation substantielle de la consommation d'édulcorants au cours des quatre dernières décennies coïncide avec l'accroissement de la prévalence et de l'incidence de l'obésité [15-17], ouvrant ainsi la porte à suspecter que la prise de substituts de sucre pourrait altérer certains mécanismes régulant l'apport énergétique. Néanmoins, des essais randomisés contrôlés, qui ont un pouvoir statistique plus fort que les études observationnelles, ne supportent pas cette idée selon laquelle l'apport en substituts de sucre favoriserait une augmentation de l'apport énergétique. Une méta-analyse récente, incluant des études conduites chez des populations d'adultes et d'enfants pendant une durée de 3 à 78 semaines, révèle que la substitution du sucre par des édulcorants entraîne une perte de poids modeste inférieure à 1 kg [18]. À la lumière de ces résultats, il fut conclu que l'utilisation d'édulcorants pouvait donc être recommandée chez les individus visant une perte ou un maintien de poids corporel [18]. Cependant, comme les études comprises dans cette méta-analyse sont toutes des interventions ayant comparé la consommation d'édulcorants à celle de sucre, on ne peut déterminer si les effets observés au niveau du poids corporel sont directement reliés à la

Substituts de sucre (édulcorants)	Pouvoir Sucrant (Comparé au sucrose)	Apport quotidien admissible (AQA) en mg/kg de poids corporel	Nombre de sachets d'édulcorant équivalent à l'AQA*	Métabolisme : digestion, absorption et excrétion
Acésulfame-potassium (Ace-K)	200 × [7]	15 [7]	23 [7]	Rapidement et presque complètement absorbé dans l'intestin grêle pour atteindre plus tard la circulation systémique. Il est ensuite excrété principalement dans l'urine dans les 24 heures suivant sa consommation [9].
Aspartame	200 × [7]	50 [7]	75 [7]	Rapidement digéré en ses 3 composants principaux: la phénylalanine, l'acide aspartique et le méthanol, qui sont absorbés dans l'intestin grêle et ensuite métabolisés. Aucune molécule d'aspartame intacte (non hydrolysée) n'atteint la circulation périphérique [9].
Saccharine	200-700 × [7]	15 [7]	45 [7] (pouvoir sucrant 400 × celui du sucrose)	Absorbée dans l'intestin grêle (taux d'absorption de ± 85 %) et rapidement éliminée de la circulation systémique. Elle est ensuite excrétée principalement dans l'urine [9].
Sucralose	600 × [7]	5 [7]	23 [7]	Faiblement absorbé au niveau intestinal (taux d'absorption de ± 15 %) et n'est peu ou pas métabolisé. Il est excrété quasi exclusivement sous sa forme intacte dans les matières fécales [9].
Glycosides de stéviol (Extrait purifié de feuilles de <i>Stévia rebaudiana</i> ou Bertoni)	200-400 × [7]	4 [7]	9 [7] (pouvoir sucrant 300 × celui du sucrose)	Non absorbés dans l'intestin grêle, mais sont plutôt hydrolysés lentement par les bactéries du côlon (<i>Bacteroides</i>) qui hydrolysent la liaison glycosidique de la molécule. Le stéviol est progressivement absorbé dans le gros intestin, puis transporté vers le foie où il sera glucuroconjugué afin de faciliter son excrétion. Ceci entraîne une augmentation lente et régulière des taux plasmatiques de glucuronides de stéviol. Ces derniers sont ensuite majoritairement excrétés dans l'urine [9].
Extrait de fruit du moine (<i>luo han guo</i>)	100-250 × [7]	Non disponible	Non disponible	

Tableau 1. Comparaison des profils de différents substituts de sucre (édulcorants). *La quantité d'édulcorant (en grammes) diffère en termes de volume et de poids. Les calculs ont été effectués en supposant qu'un sachet d'édulcorant procure un pouvoir sucrant équivalent à 2 cuillères à thé de sucre de canne blanc (sucrose).



consommation d'édulcorants. Autrement dit, il est possible qu'une perte de poids similaire soit observée en réduisant la consommation de sucre sans nécessairement avoir à substituer le sucre dans l'alimentation par des édulcorants. Il faut également noter que cette méta-analyse a reçu un soutien financier de la branche nord-américaine de l'*International Life Sciences Institute* (Institut International des Sciences Humaines), une organisation internationale à but non lucratif, mais financée et fortement influencée par les industries alimentaires [19-21]. Une revue systématique d'auteurs ayant bénéficié de subventions de recherche par l'industrie du sucre n'a également pas relevé d'évidence chez l'humain supportant l'augmentation de l'apport énergétique et du poids corporel en lien avec la consommation de substituts de sucre [22]. Fait intéressant, les auteurs de cette étude ont également stipulé que l'utilisation de boissons sucrées à base d'édulcorants semblait neutre ou même bénéfique à la gestion du poids corporel, et ce même en comparaison à de l'eau [22]. Toutefois, cette revue systématique a regroupé les interventions d'une durée de plus de 24 heures comme étant des « interventions prolongées », cette durée étant nettement insuffisante pour observer les effets chroniques de l'utilisation de substituts de sucre au niveau de la gestion du poids corporel. Ainsi, les conclusions issues des résultats de cette revue systématique ne reposent pas sur des données représentatives d'une consommation à long terme de substituts de sucre et ne peuvent donc pas être interprétées comme telles.

Les effets paradoxaux des substituts de sucre

Bien que les résultats issus d'études observationnelles ne permettent pas d'établir une relation de cause à effet entre la consommation de substituts de sucre et le gain de poids corporel, certains mécanismes par lesquels les édulcorants pourraient favoriser un gain pondéral à long terme ont été proposés. Il est d'ailleurs suggéré que la prise de substituts de sucre pourrait maintenir et renforcer le désir pour les aliments sucrés, pouvant ainsi compromettre la perte ou le maintien du poids corporel à long terme. La consommation d'édulcorants pourrait également mener à la production de signaux d'appétit contradictoires dû au fait qu'ils sont perçus comme une source de calories par nos papilles gustatives, alors qu'ils en sont exempts. Ces « faux messages » pourraient en fait résulter en une augmentation ultérieure de l'appétit et subséquemment l'augmentation de l'apport énergétique, phénomène présenté comme un des effets paradoxaux des substituts de sucre [23].

Les stimulations orosensorielles qui sont induites lorsque nous consommons des aliments ou des boissons qui procurent à la fois un goût sucré et un contenu calorique initient des mécanismes impliqués dans la régulation de l'apport énergétique. Parmi ceux-ci, l'alliesthésie alimentaire négative est une sensation qui prévient la surconsommation des aliments sucrés et/ou gras en diminuant le plaisir perçu lorsqu'on les consomme, ou après les avoir ingérés [23]. Ainsi, l'alliesthésie alimentaire négative est susceptible d'être affaiblie par la consommation d'édulcorants si l'on prend en considération le fait que le goût sucré de ces derniers n'est pas associé à un apport énergétique [23-25]. De plus, l'ingestion répétée de substituts de sucre, qui fournit le stimulus

orosensoriel du goût sucré sans toutefois être une source d'énergie considérable, pourrait également perturber l'équilibre énergétique en diminuant la capacité du goût sucré à prédire le contenu calorique d'un aliment ou d'une boisson, conduisant ainsi à une diminution des réponses de la phase céphalique [26]. Les réponses de la phase céphalique sont une variété de réponses hormonales, neuronales et métaboliques induites par les stimulus orosensoriels (par exemple, la salivation, la sécrétion d'acide gastrique, la thermogénèse et la sécrétion d'hormones telles que l'insuline, la ghréline et la leptine) qui facilitent le transit des aliments ingérés dans le tractus gastro-intestinal [27,28] et qui sont également impliquées dans la régulation de l'appétit et de la satiété [29]. Selon cette approche, l'altération des réponses de la phase céphalique par les édulcorants aurait ainsi pour effet de stimuler l'apport énergétique spécifiquement pour les aliments sucrés et caloriques, ceci pouvant contribuer à un gain de poids non désiré [26]. Quoique prometteur, il est important de souligner que ces hypothèses ont été confirmées par des études réalisées chez le rongeur [30]. Il reste maintenant à savoir si ces résultats peuvent être transposés à l'humain.

Les substituts de sucre : ennemis du microbiote ?

En plus de fournir très peu de calories, les substituts de sucre sont également intéressants pour les patients diabétiques puisqu'ils n'ont aucun effet sur la glycémie... du moins c'est ce que nous pensions. La plupart des édulcorants ne sont pas digérés dans l'intestin grêle (les détails quant au métabolisme de digestion, d'absorption et d'excrétion de divers édulcorants sont présentés dans le *Tableau 1*) et peuvent par conséquent rencontrer le microbiote intestinal. La composition du microbiote intestinal est une signature unique qui peut pratiquement être comparée à une empreinte digitale. En tant qu'écosystème complexe et dynamique, le microbiote diffère grandement entre les individus et est directement influencé par certains facteurs propres à l'hôte qui l'héberge, comme la génétique [31] et l'âge [32], mais aussi par des facteurs environnementaux, tels que l'exposition aux antibiotiques [33], les habitudes de vie [33] et de manière encore plus importante, par l'alimentation [34,35]. Des preuves convaincantes démontrent que les altérations du microbiote intestinal ont des effets néfastes sur la physiologie de l'hôte et contribuent, comme facteur environnemental, au développement de l'obésité, de l'inflammation de faible grade et de la résistance à l'insuline [13,36-38] (→).

(→) Voir la Nouvelle de B. Chassaing, m/s n° 6-7, juin-juillet 2015, page 586

Selon ses interactions avec l'environnement, ce microbiote est sujet à une dysbiose, soit une perturbation de l'homéostasie intestinale causée par un déséquilibre dans la configuration structurelle et/ou fonctionnelle des microorganismes bénéfiques et protecteurs qui le composent [39]. Comme cette voie de recherche est très récente, les mécanismes qui sous-tendent l'implication du microbiote dans la modulation des réponses glycémiques chez l'humain n'ont pas encore été clairement établis [37]. Toutefois, plusieurs mécanismes ont été proposés et sont au cœur des recherches actuelles dont la production de peptides intestinaux qui stimuleraient la sécrétion d'insuline par les cellules bêta du pancréas et la production par les bactéries intestinales d'acide gras à chaîne courte présentant des effets bénéfiques au niveau de l'inflammation et de l'intégrité de la barrière muqueuse intestinale [37].

La relation entre le microbiote intestinal et le métabolisme du glucose a notamment été examinée par le transfert du microbiote intestinal, c'est-à-dire la greffe de matières fécales, de donneurs sains à des sujets atteints du syndrome métabolique. Celle-ci a montré une amélioration significative de la sensibilité à l'insuline des patients transplantés, après six semaines d'intervention [36].

Les travaux de Suez et al. en 2014 [40] réalisés chez le rongeur ont permis de démontrer que la consommation de saccharine, de sucralose, ou d'aspartame, à des doses équivalentes à l'AQA durant 11 semaines, produisait une dysbiose et une diminution significative de la tolérance au glucose, celle-ci étant associée avec un état prédiabétique [41]. Parallèlement, la greffe de matières fécales provenant de souris ayant consommé une solution commerciale de saccharine transférée à des souris microbiologiquement stériles a également résulté en une altération de la tolérance au glucose, suggérant ainsi un rôle important du microbiote dans l'intolérance au glucose [40]. En poursuivant leurs observations chez l'humain, la consommation de saccharine à des doses équivalentes à l'AQA a résulté en une dysbiose, en plus d'une intolérance au glucose chez la moitié des sujets étudiés en sept jours seulement, alors que l'autre moitié des sujets n'ont pas présenté d'altération [40]. Les auteurs ont d'ailleurs souligné qu'il semble exister des différences interindividuelles importantes dans les réponses glycémiques et le profil du microbiote suivant la consommation de substituts de sucre, suggérant ainsi que la consommation d'édulcorants n'affecterait pas tous les individus de la même façon et que les réponses métaboliques pourraient être influencées par la composition initiale de leur microbiote [40]. Afin d'identifier les réponses métaboliques pouvant être propres à chaque variété de substituts de sucre, une intervention visant à étudier les effets sur 7 jours de doses de sucralose équivalentes à approximativement 75 % de l'AQA sur le microbiote intestinal et le métabolisme du glucose a été réalisée chez l'humain. Aucune différence significative n'a été détectée entre les résultats du groupe expérimental et ceux du groupe contrôle en ce qui concerne la diversité du microbiote intestinal et de la résistance à l'insuline [42]. Dans l'ensemble, ces résultats suggèrent que les effets métaboliques associés à la consommation de substituts de sucre peuvent varier en fonction de l'individu, de la variété d'édulcorant utilisée et du temps d'exposition. Les deux études précédentes font partie

des très rares interventions qui ont examiné les effets métaboliques de la consommation d'édulcorants pendant plus d'une semaine. Une méta-analyse récente a par ailleurs conclu que la consommation d'édulcorants (aspartame, saccharine, sucralose et stévia) n'avait pas d'effet significatif sur les réponses glycémiques chez l'humain [43]. Néanmoins, seuls 3 des 29 essais randomisés contrôlés inclus dans cette méta-analyse ont exposé les participants aux édulcorants durant plus d'une journée (deux interventions avec une durée d'exposition de 3 jours [44,45] et une avec une durée d'exposition de 2 semaines [46]). Les résultats issus de cette méta-analyse doivent donc être interprétés avec prudence puisqu'ils ne sont pas représentatifs d'une consommation à long terme de substituts de sucre.

Ce qu'il faut en retenir...

Ne compromettant pas le goût sucré des aliments et des boissons, les substituts de sucre semblent être une alternative prometteuse pour une utilisation par des individus qui visent une restriction calorique ou qui désirent maintenir une perte de poids à long terme. Cette affirmation suscite la controverse dans la sphère scientifique puisque la consommation de substituts de sucre a été impliquée dans la perturbation de mécanismes de régulation de l'appétit, pouvant ainsi conduire à un apport calorique accru et à une prise de poids ultérieure. La plupart des mécanismes par lesquels cela pourrait se produire ne sont cependant pas supportés par des preuves convaincantes à ce jour. Bien qu'il soit incertain que les substituts de sucre favorisent l'apport alimentaire, on ne peut non plus conclure qu'ils favorisent la perte de poids. Des études récentes suggèrent que la consommation de certaines variétés d'édulcorants altère la diversité et la richesse du microbiote intestinal, ayant pour effet d'entraîner une perturbation des réponses glycémiques. Une question persiste : quelle option est la meilleure pour la santé, le sucre ou les substituts de sucre ? La réponse est : *aucune*. L'idée de considérer les substituts de sucre comme une alternative saine aux sucres libres et ajoutés, sous prétexte qu'ils ne contiennent peu ou pas de calories, constitue une solution trop simple à un problème beaucoup plus complexe. La surconsommation de sucres libres et ajoutés est clairement associée à des effets néfastes pour la santé. Cependant, il n'y a pas suffisamment de preuves supportant que l'utilisation d'édulcorants soit sans conséquence sur la santé métabolique et physiologique. Sur la base des preuves disponibles, il est donc difficile d'endosser la recommandation des substituts de sucre comme outil



nutritionnel dans le contexte de la gestion du poids corporel. L'utilisation d'édulcorants n'est pas nécessaire pour réduire l'apport en sucre ni pour réduire l'apport énergétique. Il serait plus bénéfique de mettre l'accent sur une réduction graduelle de la consommation quotidienne de sucres libres et ajoutés, tout en faisant valoir les avantages pour la santé de consommer des aliments et des boissons non transformés. Il existe un dicton célèbre voulant qu'il suffise de 21 jours pour briser une habitude [47] (en fait, scientifiquement parlant, il faudrait en moyenne 66 jours pour en former une nouvelle [48]). Nous croyons que cela vaut la peine d'essayer. ♦

SUMMARY

The controversial effects of low-calorie sweeteners

Substantial reviews of the most recent evidence report a strong relationship between the consumption of sugar-sweetened beverages and adverse health effects, such as the prevalence of obesity and metabolic diseases. Various public policies were recently undertaken by many countries in the hope of reducing sugar-sweetened beverage consumption amongst their population. This has led the food industries to promote low-calorie sweeteners (sugar substitutes) as a healthy alternative that would limit caloric intake without compromising the sweet taste of food and beverages. However, is the use of low-calorie sweeteners as a means of limiting or reducing energy intake without consequences for our health? This review aims to discuss the effects of low-calorie sweeteners consumption on health and to elucidate whether their use should be recommended by health professionals to their patients as part of weight management. ♦

LIENS D'INTÉRÊT

Les auteurs déclarent n'avoir aucun lien d'intérêt concernant les données publiées dans cet article.

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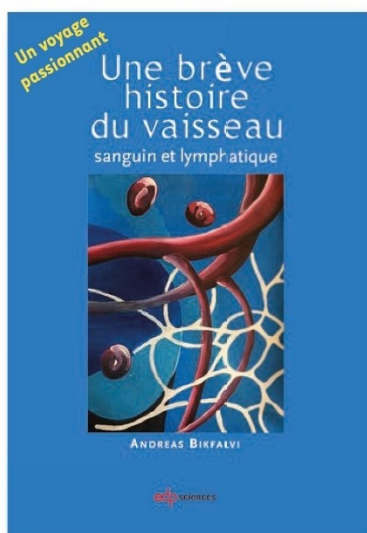
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TIRÉS À PART

A. Pepin



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Ce livre, intéressant et lisible à la fois pour le spécialiste et le grand public, apporte des observations originales et nouvelles concernant l'angiogenèse, et notamment l'histoire des différentes découvertes, et discute les aspects et les concepts plus généraux en les plaçant dans le contexte de la philosophie des sciences.

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CHAPTER IV. Thesis Article 3

Is Sucralose Contributing to the Very Problem It Was Designed to Solve? A critical review of the evidence on the effects of cumulative sucralose consumption on postprandial glycemic responses and gut microbiota in healthy adults.

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Abstract

Low-calorie sweeteners have long been regarded as physiologically inert due to their non-nutritive nature. In response to emerging evidence suggesting potential effects of certain LCS on glucose homeostasis, we conducted a critical review examining the cumulative impact of sucralose consumption on postprandial glycemic responses in healthy individuals. The review begins by describing patterns of sucralose consumption and its metabolic fate, and emphasizes the importance of adopting sweetener-specific hypotheses when investigating the metabolic effects of low-calorie sweeteners. We then contrast the rationale underlying studies of acute exposure using preload designs with those assessing repeated consumption, highlighting why single-dose protocols do not capture the cumulative effects of habitual intake and are inadequate for evaluating the long-term impact of dietary compounds on glucose dynamics. Mechanisms proposed to explain the sucralose-induced modulation of glycemic outcomes are discussed, with particular attention to those involving the gut microbiota. To assess the robustness and consistency of the evidence, we conducted a critical appraisal of randomized controlled trials that met specific inclusion criteria, including a minimum sucralose exposure duration of seven days in healthy adults and the assessment of postprandial glycemic responses. We focused on methodological approaches, interpretation of findings, and their ecological validity. Through this critical appraisal, our aim is to shed light on the methodological and mechanistic sources of variability in the current evidence and to support the development of more targeted research capable of generating conclusive insights into the metabolic implications of long-term sucralose intake in healthy populations.

Introduction

Low-calorie sweeteners (LCS) have long been regarded as physiologically inert, based on the assumption that they are not metabolized for energy and must therefore exert no meaningful impact on metabolic health. This view prevailed until prospective cohort studies revealed associations between LCS consumption and an elevated risk of type II diabetes, prompting a shift in research focus toward potential mechanisms of action, including interactions with the gut microbiota. Despite growing research interest, substantial heterogeneity in methodological designs and research outcomes across randomized controlled trials (RCTs) has hindered clear interpretation of the evidence linking LCS consumption and glucose homeostasis, particularly in the context of systematic reviews and meta-analyses. The inconsistencies in research outcomes have contributed to ongoing debate within the scientific community. Interpretation of existing data is further complicated by the predominance of RCTs focusing on acute metabolic responses to single exposures, typically using preload designs, rather than assessing the cumulative effects of LCS intake under realistic conditions.

To clarify this complex and often contradictory body of research, we employed a sweetener-specific approach to synthesize current evidence on the cumulative effects of sucralose—the most widely used LCS globally^{11,14}—focusing exclusively on postprandial glycemic responses in healthy adults rather than broader glycemic outcomes. Notably, findings from studies assessing the effects of various LCS on fasting plasma glucose concentrations are frequently interpreted and cited as evidence that sucralose exerts no impact on glycemic responses^{23–25}. However, this interpretation overlooks the limitation of fasting glucose measurements in detecting early or subtle impairments in glucose tolerance, particularly in healthy individuals. Fasting glucose levels are precisely regulated by homeostatic mechanisms, resulting in relatively stable levels in such populations. In contrast, postprandial glycemic responses exhibit greater interindividual variability^{26–28}. Epidemiological evidence indicates that postprandial glycemia is a stronger predictor of cardiometabolic outcomes than fasting glucose. For instance, data from the DECODE

Study Group and the European Diabetes Epidemiology Group (10 prospective cohorts; n = 22,514) revealed that isolated postprandial hyperglycemia, despite normal fasting glucose, doubled cardiovascular mortality risk in non-diabetic individuals^{29,30}. Similarly, Nakagami et al. (2004)³¹ demonstrated that adding 2-hour plasma glucose to a model containing fasting glucose significantly improved prediction of cardiovascular mortality, whereas adding fasting glucose to a model containing 2-hour plasma glucose did not (5 prospective cohort studies, n = 6817). Beyond epidemiological associations, mechanistic evidence associated postprandial hyperglycemia with oxidative stress and endothelial dysfunction, both early events in the development of microvascular damage. Importantly, these effects occur independently of HbA1c and fasting glucose, indicating that postprandial glycemic excursions can impair vascular integrity even when conventional glycemic markers remain within normal ranges³². Together, these results suggest that postprandial measures capture critical metabolic disturbances that may be missed by fasting glucose, underscoring their greater relevance for mechanistic insight. In addition to glycemic outcomes, we also consider potential underlying mechanisms, particularly those involving the gut microbiota. Importantly, this review does not aim to evaluate the efficacy of sucralose and other LCS as a replacement strategy for sugar-sweetened beverages (SSB) in individuals living with diabetes, nor does it address their effects on body weight regulation or sweet taste preferences. Instead, this critical review focuses on the metabolic implications that could be associated with repeated sucralose consumption in healthy individuals, a population of high relevance given the widespread use of LCS across all demographic groups³³.

We begin by providing a comprehensive overview of sucralose and its potential mechanisms of action on glucose homeostasis, with emphasis on interactions involving the gut microbiota and the intestinal sweet taste receptors. We then critically appraise key evidence from RCTs, with a particular focus on distinguishing between studies that assessed acute versus repeated exposures. Particular attention is given to the methodological rigor of experimental designs, interpretation of findings, and their ecological validity. Where appropriate, readers are directed to specialized reviews for in-

depth discussion of specific subtopics. By synthesizing and critically reviewing current evidence, we aim to disentangle the methodological and mechanistic complexities underlying the observed variability in findings. Ultimately, we hope this review will inform the design of high-quality RTCs capable of generating definitive conclusions about the metabolic effects of sucralose and other LCS, including those yet to be developed.

Overview of Low-Calorie Sweeteners: Focus on Sucralose

LCS (also referred to as low-energy sweeteners, non-nutritive sweeteners or sugar substitutes) are food additives that include both artificial sweeteners and naturally derived sweeteners such as purified stevia and monk fruit extracts³⁴. These compounds offer a sweet, low- or zero-calorie alternative for added sugars. Commonly used as additives in food products such as beverages, candy, yogurt, canned fruit, and breakfast cereals, some LCS are also available over the counter for direct consumer use in tabletop formats³⁵. Several sweeteners have been approved for use in Canada, namely acesulfame potassium (Ace-K), advantame, aspartame, neotame, saccharin, sucralose, thaumatin, steviol glycosides, monk fruit extract and sugar alcohols or polyols^b (e.g., xylitol, sorbitol, mannitol, erythritol)³⁵. Most LCS have a significantly higher relative sweetness intensity. For example, on a weight-for-weight basis, aspartame and sucralose are perceived, 200 and 600 times sweeter than sucrose, respectively^{23,36,37}. Thus, minimal amounts of pure LCS are needed to achieve the same level of sweetness. To facilitate consumer use, most commercially available LCS products contain a bulking agent such as maltodextrin, allowing their volume to be measured comparably to sugar.

The food industry rapidly innovates to find the “ next generations ” of sweeteners, with a primary focus on those derived from natural sources. This trend includes rare sugars such as D-allulose and D-tagatose, which have drawn scientific attention over the last three years^{38–41}. These two rare sugars occur naturally in small amounts in the heat process of

^b Sugar alcohols are not classified as high-intensity sweeteners given that their relative sweetness is comparable to or lower than that of sucrose²⁴⁰.

fructose-containing foods such as dried fruits and fruit juices³⁹. However, their scarcity and low extraction yields render large-scale isolation economically unfeasible, prompting production via genetically engineered enzymes and microbes³⁸. Currently, both D-allulose and D-tagatose are approved as food ingredients in the United States^{42,43}. However, other agencies including Health Canada have approved D-tagatose⁴⁴, while D-allulose remains classified as a novel food ingredient requiring a comprehensive safety assessment prior to market entry⁴⁵. Nonetheless, rare sugars represent a promising avenue for replacing existing sweeteners in food products.

Consumption of low-calorie sweeteners in North America

The global LCS market has experienced steady growth over the past decade. By 2030, the market is expected to reach an all-time high of 39.4 billion USD, marking a 43% increase over five years. This rapid expansion is largely driven by increasing consumer demand for alternatives to refined sugars, alongside the implementation of public health regulations aimed to reduce total sugar intake, such as tax policies and front-of-package labelling^{6,7}. Quantifying the average daily consumption of LCS in mg/day is exceedingly challenging, as manufacturers are currently not required to disclose the amounts used in their products, nor are they obligated to provide this information to federal agencies, even upon request⁴⁶. LCS are often combined in formulations of beverages and food products to mask the bitter aftertaste associated with certain sweeteners, further complicating efforts to accurately estimate individual sweetener consumption. To date, no study has precisely quantified the daily intake of LCS in North America. However a cross-sectional analysis of data from the National Health and Nutrition Examination Survey (NHANES) conducted in the United States from 2009 to 2012 (n = 16,942) reported that 25.1% of children and 41.4% of adults consumed LCS, with the majority doing so on a daily basis³³. At present, the North American food industry primarily uses LCS in beverages rather than solid food products. This trend is expected to change following the implementation of Canada's front-of-package labelling regulations in January 2026, which will require warning symbols on products high in total sugars⁷. A shift toward the substitution of sucrose with LCS in solid

food products is anticipated, mirroring trends observed in countries that have implemented similar front-of-package labelling policies. A notable example is Chile, being the first country to implement mandatory front-of-package labels for food products exceeding established thresholds for total sugars⁶. In a strategic effort to circumvent the impact of these regulations, Chile's food industry undertook extensive product reformulation, partially or fully replacing sucrose with LCS. This shift led to a marked increase in the prevalence of LCS in food products, suggesting a potential rise in population-level exposure. Specifically, a detailed analysis conducted three years after the policy's implementation in 2016 examined approximately 1,500 food products (including beverages) available on the Chilean market and found that 55.5% contained at least one LCS. Sucralose was the most prevalent LCS, present in 73.5% of LCS-containing products¹¹.



In North America, low-calorie sweeteners are primarily used in beverages, but a shift toward substituting sugar (sucrose) with LCS in solid food products is anticipated in the coming years. Sucralose remains the most widely used LCS globally. As the food industry continues to innovate, there is growing interest in developing the next generation of LCS, particularly those derived from natural sources.

Sucralose : Safety and Physiological Properties

Sucralose, commercially known as Splenda®, was developed in 1976 by scientists funded by Tate & Lyle (historically one of the largest sugar companies), who designed it by selectively chlorinating sucrose, replacing three hydroxyl groups (-OH) with chlorine atoms (Cl)^{47,48} (**Figure 1**). This structural modification prevents digestive enzymes from recognizing and hydrolyzing sucralose for energy. In 1991, Canada was the first country to approve the use of sucralose as a food additive⁴⁷, followed later by other countries including the United States in 1998⁴⁹. Because of its high solubility and exceptional stability under extreme pH and temperature conditions¹⁴, sucralose is considered ideal for use in baking, cooking, and use in sweetening processed foods and beverages, making it the most extensively used LCS by both the consumers and the food industry^{11,14}.

Unfortunately, these physicochemical properties come at the cost of environmental persistence, as sucralose is resistant to natural degradation and wastewater treatment processes, ultimately leading to its accumulation as a pollutant in surface waters^{50,51}. In North America, LCS are subject to strict regulations to ensure that they do not pose a risk to human health³⁴. Long before approval by regulatory agencies, extensive preclinical studies are undertaken to elucidate the toxicology of the sweetener and determine its safety³⁷. These preclinical safety studies are conducted in animal models and use a wide range of concentrations to characterize dose-response relationships of potential adverse health effects. The highest dose that produces no observable biological effects in long-term repeated-dose studies is referred to as the “no observed adverse effect level” (NOAEL)³⁷. To extrapolate the results from animal studies to humans while ensuring an adequate safety margin, the NOAEL value is divided by a safety factor (often 100-fold for LCS)³⁷. This calculation is then used to establish an Acceptable Daily Intake (ADI) in humans, defined as “the amount of food additive expressed in mg/Kg BW/day, that can be ingested daily over a lifetime without any appreciable risk to health”⁵². It is unrealistic for consumers to exceed or even reach the ADI for most LCS. This is due to the minimal amounts needed to achieve a sweet taste and the common practice of manufacturers using a combination of LCS in their food products. For instance, an individual would need

to consume approximately 19 cans of Diet Mountain Dew™ (18mg sucralose, 18mg Ace-K, 57mg aspartame)⁵³ in a single day in order to reach the ADI for sucralose of 5 mg/Kg BW/day³⁶. But this number varies significantly among different beverage brands, as some contain much higher concentrations of sucralose. For example, a single 355 mL can of caffeine-free Diet Rite Cola™ contains 68 mg of sucralose and 41 mg of acesulfame-K⁵³. Therefore, a person weighing 70 kg would only need to consume ~ 5 cans to reach the ADI for sucralose. The safety of using sucralose as a food additive has been thoroughly reviewed by Magnuson et al. (2017)²³. Although strict regulations and extensive research demonstrated the safety of LCS prior to their approval, this does not constitute evidence that they are *physiologically inert*.

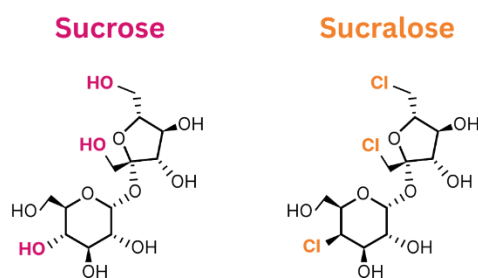


Figure 1. Chemical structures of **sucrose** (molecular weight 342.3, CAS number 57-50-1), and **sucralose** (molecular weight 397.6, CAS number 56038-13-2). *Adapted from* <https://pubchem.ncbi.nlm.nih.gov>¹

A key aspect of assessing the safety of LCS is understanding their toxicokinetics—a comprehensive examination on how a compound is absorbed, distributed, metabolized, and excreted in various animal species that closely resemble human anatomy. For sucralose, the toxicokinetic studies that supported federal regulatory approval of its use as a food additive comprised of four animal interventions and one human trial. Of note, human clinical studies are not typically required for the approval of novel food ingredient

or food additive⁵⁴. In the sole human trial, Roberts et al. (2000)⁵⁵ found that 78.3% of orally ingested sucralose was primarily recovered structurally unchanged in feces, while 14.5% was recovered in urine, also structurally unchanged. Two minor sucralose metabolites, glucuronide conjugates, were also found in urine, but accounted for less than 3% of the administered dose. These findings confirm that a small fraction of ingested sucralose is absorbed and entered systemic circulation⁵³, although absorption varied significantly among individuals, ranging from 9% to 22%⁵⁵. The metabolic fate of the remaining 7.2% of the orally administered sucralose was not examined and remains unknown⁵³. Of particular note, the study involved eight men participants administered a single dose of 1 mg/kg BW of ¹⁴C-labeled sucralose, while two other men participants were administered a higher dose of 10 mg/kg BW. The absence of women from this foundational investigation represents a significant methodological limitation, especially given the importance of accounting for sex-based biological differences⁵⁴. A compelling example of why such inclusion is essential emerged 15 years later, when studies by Sylvetsky et al. (2015)⁵⁶ and Rother et al. (2018)⁵⁷ demonstrated that sucralose could be recovered intact in breast milk. Pharmacokinetic analyses showed that sucralose was detectable in breast milk as early as two hours after the ingestion of a commercially available diet beverage containing sucralose⁵⁷. These findings underscore the critical need for inclusive study designs that consider both sexes, particularly when research outcomes inform public health policies affecting vulnerable populations such as infants.

Twenty years after the U.S. Food and Drug Administration (FDA) approved sucralose as a food additive, Bornemann et al. (2018)²² identified two acetylated sucralose metabolites in the urine and feces of rodents administered sucralose at doses consistent with those used in regulatory toxicology studies. These metabolites were detected throughout the 40-day dosing period and remained present at low levels for up to 11 days following cessation of sucralose intake. Building on this, one of the co-authors raised concerns regarding the safety of sucralose-6-acetate, a byproduct formed during the manufacturing of sucralose. In this process, sucrose undergoes multiple chemical transformations, including the formation of sucralose-6-acetate, which is subsequently deacetylated to produce

sucralose as the final product. Schiffman et al. (2023)⁵⁸, reported that trace amounts of sucralose-6-acetate persist as residual byproducts, with commercially available sucralose samples containing approximately 0.67% sucralose-6-acetate⁵⁸. Furthering their investigation on the toxicological and pharmacokinetic properties of sucralose-6-acetate, the authors reported that this residual byproduct exhibited genotoxicity through a mechanism classified as clastogenic (i.e., inducing DNA stand breaks). Moreover, in vitro exposure of human intestinal epithelium cells to both sucralose and sucralose-6-acetate in the absence of bacteria was found to compromise intestinal barrier integrity via damages to tight junctions, suggesting a direct interaction with epithelial structures. While these findings raise important safety considerations at first glance, it is worth noting that the Heartland Food Products Group (TC Heartland LLC) stated that routine impurity testing of the sucralose used in Splenda® products consistently detected no measurable levels of sucralose-6-acetate, down to the lowest detection limit which is 10 ppm (0.001%)⁵⁹.

Based on findings from Roberts et al. (2000)⁵⁵ and tolerance studies such as McLean Baird et al. (2000)⁶⁰, which reported no bioaccumulation of sucralose in blood following daily consumption of 5 mg/kg for 12 weeks in 108 individuals including women, sucralose was initially assumed to be **physiologically inert**. Now, accumulating evidence consistently suggests that both acute and cumulative consumption of sucralose exerts physiological effects in humans. For instance, functional magnetic resonance imaging of the human brain indicated that sucralose activates taste-related pathways associated with behavioral pleasantness and reward responses that differs from sucrose^{61,62}. Hypothalamic activity was also shown to increase in response to the immediate sweet taste of sucralose, but not to the taste of water⁶². Interestingly, this effect was stronger in individuals living with obesity, especially women⁶³. In a rodent study, sucralose has also been shown to bioaccumulate in adipose tissue, with detectable levels persisting two weeks after cessation of a 40-day sucralose supplementation at doses comparable to those used in toxicology studies²². Because nearly all sucralose found in feces is structurally unchanged, it was also assumed that this sweetener could not exert any effects on the gut microbiota²³. While its resistance to microbial degradation clearly shows that sucralose

cannot be fermented by mutualist bacteria, environmental studies published in 2013^{50,51} revealed that sucralose can exert a bacteriostatic effect on certain bacterial species due to its inevitable bioaccumulation in surface waters. Specifically, the inhibitory effect on the growth of six bacterial strains was found to be directly proportional to the concentration of sucralose present in water and soil. This phenomenon was hypothesized to result from a reduction in sucrose uptake by the bacteria^{50,51}. Hence, dismissing any potential metabolic effects of sucralose or other LCS in humans through alternative mechanisms should not be accepted without the supporting evidence. Collectively, these findings challenge the notion of sucralose's physiological inertness and underscore the need for further investigation into its biological and ecological impacts.



LCS, such as sucralose, have long been regarded as physiologically inert, based on the assumption that they are not metabolized for energy and therefore exert no meaningful impact on metabolic health. However, accumulating evidence consistently suggests that consumption of sucralose exerts metabolic effects in humans.

Acute Effects of Sucralose on Postprandial Glycemic Responses in Humans (single-dose exposure/preload design)

In humans, the effects of LCS on glycemic responses are most investigated using a preload design, consisting of a single LCS dose. This approach, which isolates the acute effects of LCS on glucose tolerance, is problematic as any metabolic outcome is more likely to result from habitual dietary patterns, rather than one isolated exposure to a dietary component. The collective evidence on the acute effects of LCS on postprandial glucose and insulin responses has been synthesized by Greyling et al. (2020)⁶⁴ in a systematic review and

meta-analysis. Their analysis revealed no statistically significant differences in glycemic or insulinemic responses between LCS and control groups, independently of the type or dose of LCS. However, several factors limit the interpretability of these findings, the first being the absence of a sweetener specific hypothesis. For example, of the 34 RCTs included in the analysis, only nine specifically investigated sucralose alone; the remaining studies examined other LCS either administered alone or in combination. Knowing that each LCS has a distinct chemical structure that influences how they will be physiologically handled, pooling multiple LCS in systematic reviews and meta-analyses may compromise statistical validity (i.e., potential intervention effects may not be detected due to reduced statistical power). Moreover, substantial heterogeneity in experimental designs further complicates the generalizability of the review's findings. These include differences in doses (ranging from 24mg to 800 mg per day for sucralose), comparators (i.e., water, saline, unsweetened beverages, placebo capsules, isocaloric meal), form of administration (i.e., in beverages or solid foods, capsules, intragastric infusions, with or without carbohydrates), and study populations (i.e., healthy individuals vs. individuals living with type 2 diabetes). Two other systematic reviews and meta-analyses have reported comparable findings, indicating that acute sucralose exposure does not induce significant changes on postprandial glycemic responses^{65,66}.



In human studies, the effects of LCS on glycemic responses are most commonly investigated using a preload design. This approach typically assesses the acute effects of a single LCS dose (or exposure) on postprandial glycemic responses. While useful for isolating immediate physiological responses, this approach does not account for the cumulative effects associated with their regular daily consumption.

Cumulative Effects of Sucralose on Postprandial Glycemic Responses in Humans (exposure $\geq$ 7 days)

A major contributor to inconsistencies in LCS research is the tendency to treat LCS as a “class of compounds” rather than individual molecules. Each LCS possesses a distinct chemical structure that determines its specific properties related to digestion, absorption, metabolism and excretion³⁷. Growing evidence indicates that LCS exert distinct physiological effects and should therefore be studied using sweetener-specific hypotheses^{13,67–69}. This need is increasingly important as new LCS continue to be discovered and introduced into the food supply. However, the current paucity of RCTs investigating the cumulative effects of individual LCS consumption on postprandial glycemic responses limits the feasibility of conducting systematic reviews and meta-analyses for each sweetener separately. As a result, existing systematic reviews and meta-analyses have relied on pooled analyses grouping multiple sweeteners together (singles or blends). While using LCS blends enhances ecological validity (i.e., the degree to which findings can be generalized to real-world settings) by reflecting commercially available products, the practice of pooling diverse LCS types (single or blends) within meta-analyses introduces methodological limitations, assuming homogeneity where it may not exist. This approach can potentially dilute or mask LCS-specific effects, further contributing to inconsistencies in LCS research. Therefore, the combined use of LCS should be examined only after the distinct physiological effects of each sweetener have been clearly characterized, as potential interactions may modify their individual effects. Two recent systematic reviews and meta-analyses illustrate this trend and underscore the critical need for more targeted research focused on individual LCS to disentangle the current body of evidence and draw robust, generalizable conclusions about individual LCS.

McGlynn et al. (2022)⁷⁰ conducted a systematic review and network meta-analysis to evaluate the effects of replacing SSB with water or LCS-sweetened beverages in individuals living with overweight or obesity who were at risk for, or living with, type II diabetes. The analysis included 17 RCTs with a minimum duration of two weeks. Notably,

only eight trials reported the type of LCS used, and only one investigated sucralose. The primary outcome was body weight, while secondary outcomes included various markers of cardiometabolic health, including glycemic control. The authors found no statistically significant improvements in glycemic control when SSB were replaced with either water or LCS, although pooled effect estimates consistently favored water or LCS beverages across most comparisons. A closer look at postprandial glycemic outcomes, more precisely 2-hour postprandial blood glucose and, to a lesser extent, hemoglobin A1c (HbA1c)^c, revealed more nuanced findings. When LCS-sweetened beverages replaced SSB, the direction of the effect favored SSB (moderate certainty of evidence). Similarly, when LCS-sweetened beverages replaced water, the effect favored water (moderate to low certainty of evidence). Finally, when water replaced SSB, the results were mixed. Postprandial glycemic responses appeared to favor SSB, whereas HbA1c outcomes, reflecting average glycemia over the preceding 2–3 months, favored water. Both findings were supported by low-certainty evidence. The authors concluded that LCS-sweetened beverages were a comparable alternative to water for replacing SSB within the studied population. While this interpretation is well supported for body weight and most cardiometabolic outcomes, it appears less definitive for postprandial glycemic responses. To build upon their previous findings, the same group of authors conducted a second systematic review and network meta-analysis examining the acute effects of LCS on postprandial glycemic and endocrine responses in adults without considering health status. Departing from their earlier work on repeated exposure to LCS-sweetened beverages, Zhang et al. (2023)⁶⁶ examined the acute responses following a single exposure to LCS. The analysis included 36 randomized and non-randomized trials that assessed outcomes including postprandial plasma concentrations of glucose, insulin, and incretins; precisely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). These outcomes were presented as incremental area under the curve (iAUC) over 120 minutes following a single exposure to various types or blends of LCS, with water and SSB as comparators. The findings indicated

^c HbA1c, or glycated hemoglobin, serves as a biomarker of average blood glucose levels over the preceding 2–3 months²⁴¹.

that LCS-sweetened beverages (single or blends) did not significantly affect glycemic, insulinemic or incretin responses. Consistent with their earlier interpretation, the authors concluded that these findings support the use of LCS-sweetened beverages as viable alternatives to SSB as their metabolic effects were comparable to those of water. Nevertheless, as previously discussed in the present review, evaluating LCS under single-dose protocols does not capture the potential cumulative effects of regular LCS consumption on metabolic outcomes, such as glucose dynamics.

Proposed Mechanisms

Several key mechanisms have been proposed to explain how sucralose may influence glucose homeostasis. These mechanisms are central to ongoing research and include:

1. Alterations in cephalic phase responses (uncoupling sweet taste from energy).
2. Interactions with intestinal sweet taste receptors, leading to increases release of incretin hormones, particularly GLP-1, and the subsequent modulation of intestinal glucose absorption.
3. Changes in gut microbiota composition and function, specifically through the induction of dysbiosis, a mechanism currently receiving the most attention.

Uncoupling sweet taste from energy

Davidson (2004)⁷¹ and Swithers (2008)⁷² originally hypothesized that repeated intake of LCS could undermine the ability of sweet taste receptors to predict caloric content, a mechanism now referred to as “ **uncoupling sweet taste from energy** ”^{71,72}. The conditioned association between orosensory cues as signals for post-ingestive responses is among the most robust form of Pavlovian conditioning^{d73}. Orosensory signals can independently elicit a variety of hormonal, neural and metabolic responses (i.e., salivation,

^d Pavlovian conditioning is a learning process in which a stimulus becomes associated with a biologically significant event, leading to a conditioned response²⁴².

gastric acid secretion, thermogenesis and hormone secretion including insulin and glucagon), collectively referred to as cephalic phase responses. Thus, the perception of sweetness signals the presence of calories from carbohydrates, triggering cephalic phase responses in preparation to use carbohydrates for energy⁷⁴. According to the uncoupling hypothesis, frequent LCS consumption may reduce the effectiveness of sweet taste as a conditioned stimulus, potentially leading to impaired glycemic regulation over time via disruption of the cephalic phase insulin response.

The hypothesis that uncoupling sweet taste from caloric content impairs metabolic regulation was recently challenged by findings from Dalenberg et al. (2020)⁷⁵. In a study involving 39 healthy young adults, glucose tolerance, taste perception as well as brain responses to sweet, sour, salty, and umami (measured with functional magnetic resonance imaging) were assessed after consuming a total of 7 cans of 355 mL beverages over 10 days. These beverages contained either pure sucralose (60 mg), sucrose (30 g), or a combination of sucralose and maltodextrin (60 mg + 32 g, respectively, similar to commercially available sucralose products like Splenda®). The authors hypothesized that if uncoupling sweet taste from caloric intake impaired cephalic phase responses, it would result in reduced glucose tolerance and attenuated brain responses to the sweet taste of sucrose in the pure sucralose group, but not in the sucrose or sucralose-maltodextrin groups. Contrary to this hypothesis, they found elevated insulin response after 30 minutes of the oral glucose tolerance test (OGTT) in the sucralose-maltodextrin group compared with the two other groups, with no statistically significant differences in postprandial glycemic responses. Additionally, brain responses to sweet taste were inversely associated with insulin sensitivity only in the sucralose-maltodextrin group. No differences in sweet taste perception or preference were observed between groups. To further investigate whether maltodextrin alone could account for the observed changes in insulin sensitivity, the authors conducted a follow-up experiment. The results indicated that maltodextrin alone did not alter insulin sensitivity, suggesting that the metabolic effects were specific to the combination of sucralose and carbohydrates.

Overall, the findings of Dalenberg and colleagues rejected the hypothesis that *uncoupling sweet taste from energy* lessen the ability of sweet taste as a conditioned stimulus. Rather, they suggested that short-term consumption of sucralose impairs insulin sensitivity only when combined with a carbohydrate source, potentially via alterations in central regulation of insulin secretion. A key limitation of the study pertains to the exposure conditions. The short duration of exposure and the low sucralose dosage, 420 mg over 10 days, equivalent to less than one can of LCS-sweetened beverages per day⁵³, may have been insufficient to detect significant changes in glucose tolerance. Notably, the authors acknowledged that the study was initially powered to detect changes in neuroimaging outcomes, which may have resulted in an insufficient sample size for detecting statistically significant glycemic-insulinemic responses. Relatedly, although regular consumers of LCS were excluded, participants were not instructed to avoid other sources of LCS or SSB before and during the intervention, which may have influenced glycemic outcomes.

Interaction with the intestinal sweet taste receptor

Another proposed mechanism by which sucralose may alter glucose homeostasis involves its interaction with the intestinal sweet taste receptor, which may, in turn, stimulate the secretion of incretin hormones and modulate intestinal glucose absorption. Nelson et al. (2001)⁷⁶ first identified the mammalian sweet taste receptor in gustatory papillae as a heterodimer composed of two subunits, T1R2 and T1R3, now formally referred to as TAS1R2 and TAS1R3, respectively^{77e}. Together, these subunits form a single functional receptor capable of detecting a wide range of sweet-tasting compounds, including LCS. Upon activation by sweet compounds, the sweet taste receptor initiates a cascade of neural signalling events, beginning with the stimulation of peripheral gustatory nerves and extending to central gustatory pathways in the brain, ultimately contributing to the hedonic perception of sweet stimuli. A few years after its initial identification in the gustatory

^e TAS1Rs are members of the class C family of G protein-coupled receptors⁷⁷.

papillae, Margolskee et al. (2007)⁷⁸ demonstrated that the mammalian sweet taste receptor was also expressed in the small intestines' enteroendocrine cells^f. Notably, the structure of the intestinal sweet taste receptor binding to sucralose was just recently revealed by Juen et al. (2025)⁷⁷. Activation of the intestinal sweet taste receptor elicits physiological responses independently of *conscious* sweet taste perception, indicating that these effects are not mediated by cephalic phase responses⁷⁹. Margolskee and colleagues presented convincing evidence that the TAS1R2+TAS1R3 complex can detect glucose and other sweeteners, such as sucralose, and induce the release of GLP-1 and GIP via an α -gustducin-mediated signalling process, which in turn enhances the expression of sodium-glucose cotransporter 1 (SGLT1) responsible for active intestinal glucose transport. Based on these findings, variations in the metabolic fate of LCS may stem from their differential binding affinities for the sweet taste receptor, indirectly modulating enterocyte glucose uptake and ultimately increasing circulating glucose availability.

In agreement with these observations, Mace et al. (2007)⁸⁰ found that the translocation of the passive glucose transport 2 (GLUT2) from the basal to the apical membrane of enterocytes is also stimulated by LCS like ace-K, sucralose, and saccharin, thereby resulting in increased intestinal absorption of glucose in the enterocytes. Recent evidence from Rose et al. (2024)⁸¹ further supports this proposed mechanism. In a murine model, the two-week supplementation with Ace-K and sucralose significantly increased jejunal glucose absorption and GLP-1 secretion following glucose infusion. These results suggest that sucralose and/or Ace-K may enhance intestinal glucose uptake through pathways potentially involving sweet taste receptor activation. While the authors concluded that these effects were gut microbiota-independent based on the persistence of increased glucose absorption in mice treated with both LCS and antibiotics, the reduction in the richness and evenness of fecal bacterial communities observed in LCS-supplemented

^f The expression of functional sweet taste receptor TAS1R2+TAS1R3 was later found in numerous other extragustatory tissues, including the pancreas, bladder, adipose tissues, bones, lymphocytes, heart and brain²⁴³.

mice indicates that microbiota-mediated contributions cannot be fully excluded. This highlights the complexity of disentangling direct receptor-mediated effects from microbiota interactions in the metabolic responses to LCS.

The capacity of the intestinal sweet taste receptor to directly stimulate the release of GLP-1 into the bloodstream was demonstrated by Krokrashvili et al. (2014)⁸² using an animal model. GLP-1, along with GIP, are a gut-derived incretin hormones that upregulate insulin release by pancreatic β -cells, slows down gastric emptying, and contribute to appetite regulation and satiety⁸³. GLP-1 also plays a well-established role in glucose homeostasis via insulin-independent pathways, including the modulation of glucose transporters (SGLT1 and GLUT2) expression, the suppression of glucagon release, and the indirect optimization of glucose metabolism in muscle, liver, and adipose tissue⁸⁴. Moreover, evidence from animal and in vitro studies suggests that GLP-1 improves the integrity of the intestinal barrier by downregulating the release of pro-inflammatory cytokines by macrophages^g, such as IL-1 β and TNF- α ⁸³. Although sucralose-induced alterations in glycemic responses—mediated by chronically elevated GLP-1 secretion and subsequent increases in intestinal glucose uptake—represent a promising mechanistic hypothesis, it is important to note that this effect has been primarily observed in culture studies with acute exposure to sucralose⁸⁵, in animal models with saccharin⁸⁶, and in a small number of human studies^{53,87–89} that failed to be consistently replicated^{23,90–94}.

Gut Microbiota

Understanding the gut microbiota: a microbial signature as unique as a fingerprint

The composition of the gut microbiota is a complex and dynamic ecosystem that greatly differs among individuals as it is directly influenced by the host's genotype, diseases, age^h

^g Macrophages are a type of innate immune cells.

^h In individuals over 60 years old, an age-related decline of the gut microbiota's richness occurs. This decline is characterized by a shift in the ratio of *Bacteroidota* to *Firmicutes* species and is also associated with a decline in immune system functions⁹⁹.

sex⁹⁵ and environment^{96–98}. Approximately 100 microbial species inhabit an infant's microbiota, acquired from the mother during intrauterine development and postnatal environment, including birth delivery method, breastfeeding, and epigenetic influences⁹⁹. By adulthood, the gut microbiota is estimated to harbour trillions of intestinal microorganisms (including bacteria, archaeaⁱ, fungi, viruses, and parasites) consisting of both mutualist species^j that live in synergy with the host in the gastrointestinal tract and pathogenic ones that may disrupt host's health under specific conditions. The microbial communities tend to increase from the stomach to the colon, the latter being the most concentrated place in the entire digestive system¹⁰⁰ (noteworthy, up to 60% of fecal mass consists of microbial matter¹⁰¹).

The core assemblage of the gut microbiota is believed to remain essentially stable throughout adulthood⁹⁹, but the interindividual variability is tremendous. The shaping of its composition continues through the lifespan as it responds to environmental factors such as frequent antibiotic exposure (particularly in early childhood), lifestyle (i.e., exercise, sleep, and stress) and most significantly, **dietary patterns**, the latter being the most influential determinant of the gut microbiota's composition, diversity, and functional capabilities^{98,102–104}. This relationship has been thoroughly reviewed by Sanz et al. (2025)⁹⁸, who detail how dietary patterns influence the gut microbiota environment. Notably, dietary fiber intake stands out as a major determinant of gut microbiota composition. Specifically, dietary fibers significantly affect both α -diversity and β -diversity by modulating the production of short-chain fatty acids (SCFAs) through the fermentation activity of mutualistic bacteria⁹⁸. SCFAs serve as key mediators of host–microbe interactions, playing critical roles in maintaining intestinal barrier^{98,99,105,106}, regulating inflammation^{106,107},

ⁱ Archaea are single-celled microorganisms structurally similar to bacteria.

^j The relationship between the bacteria of the gut microbiota and the host is often described as commensal (where one organism benefits while the other is unaffected). However, this definition is inaccurate for many bacteria inhabiting the human gut, for which both the bacteria and the host derive mutual benefits from each other. This mutualistic relationship is exemplified by the numerous roles bacteria play, such as fermenting dietary fibers, synthesizing vitamins, and contributing to the host's immunological responses, while receiving nutrients necessary for their survival in return. Nonetheless, there are some commensal bacteria in our microbiota from which we neither benefit nor suffer harm, although these are not dominant²⁴⁴.

facilitating gut–immune system crosstalk^{108,109}, and modulating glucose and lipid metabolism^{98,109,110}. In contrast, saturated fats also influence gut microbiota structure but tend to foster a less favourable microbial environment. High-fat diets have been associated with reduced expression of intestinal tight junction proteins, such as occludins, which are crucial to the intestinal barrier integrity¹⁸. Similarly, nutrients such as L-carnitine and choline found in animal-based proteins can result in gut-microbiota-derived metabolites^k associated to adverse health outcomes¹¹¹.

To date, more than 4000 bacterial and archaeal genomes have been identified in the human gut microbiota, with many more yet to be discovered through advancements in sequencing techniques^{112–114}. Over 90% of these numerous species belong to just two dominant bacterial phyla^l, *Bacteroidota* and the *Firmicutes*^{112,115,116}. Other prevalent, though less abundant, phyla include *Actinomycetota*, *Proteobacteria*, *Verrucomicrobiota*, and *Fusobacteriota*^{117–119}. In addition to taxonomic classification into phyla, genera, and species, gut microbial communities also exhibit broader ecological patterns in how they assemble and cluster into distinct community types known as **enterotypes**. These enterotypes are defined by the dominance of specific bacterial genera and appear to be largely independent of host genetics¹²⁰. Instead, they are shaped primarily by long-term environmental factors, with dietary patterns playing a particularly influential role¹²¹. While enterotypes are relatively stable and resilient to disturbances, drastic dietary changes can induce rapid and measurable shifts in microbiota structure on the taxa level. For instance, early work on the gut diet–microbiota interplay demonstrated that initiating a high-fat/low-fiber vs. low-fat/high-fiber diet¹²¹ or switching from an omnivorous to a complete animal- or plant-based diet¹²², led to significant changes in microbiota composition detected by 16S rRNA within as little as 24 hours to a few days. Although these acute changes are generally insufficient to transition individuals between enterotype clusters¹²¹, they may still have

^k L-carnitine and choline can be hydrolyzed by a microbial enzyme of the gut microbiota (trimethylamine lyase) into trimethylamine, the precursor of trimethylamine N-oxide (TMAO).

^l Taxonomic rank grouping bacteria with broad structural and genetic similarities. Forty-one phyla have been identified to date²⁴⁵.

functionally relevant implications for human health. Over longer durations, however, sustained adherence to such dietary patterns can induce enterotype transitions, as observed in dietary acculturation studies conducted over several months following immigration, and across first- and second-generation immigrant populations¹²³.

The Insurance Hypothesis and the Resilience Phenomenon

In most natural ecosystems, high biodiversity shields the ecosystem against perturbations that could disrupt community structure and function, a concept referred to as “**the insurance hypothesis**”. This resilience arises primarily from the presence of multiple species competing for the same limited resources, thereby preventing the overgrowth of any single species at the expense of others. Applied to the gut microbiota, this translates into competition for energy substrates and colonization space along the intestinal mucosal barrier. Therefore, the insurance hypothesis would suggest that a rich diversity of microbial communities confers a protective effect by maintaining equilibrium among microbial species, thereby preserving the stability and functional dynamics of the intestinal environment. In line with this, a complementary hypothesis proposes that bacterial communities possess recovery capabilities, enabling them to restore equilibrium following environmental disturbances. This concept is referred to as “**the resilience phenomenon**”. In response to acute disturbances, such as acute infections or antibiotic treatments, the gut microbiota typically adapts by reorganizing its microbial communities to establish a new equilibrium. However, it is important to note that a new equilibrium does not necessarily equate to a healthy one. Both of these concepts were comprehensively discussed in a perspective article by Sommer et al. (2017)¹²⁴. **Drawing together the insights discussed above, an important question arises: if repeated sucralose consumption induces perturbations in microbiota structure and function, are these changes substantial enough to drive a shift in host microbial enterotypes, or will the protective and resilient properties of the gut microbiota prevail?**



The gut microbiota is a complex and dynamic ecosystem. Its composition, as unique as a fingerprint, varies widely across individuals and is influenced by factors such as host genotype, disease status, age, and sex. Although the core microbial assemblage is believed to remain relatively stable throughout adulthood, the microbiota continues to adapt in response to environmental factors. Among these, dietary patterns have emerged as the most influential determinant, shaping its composition, species richness, and functional capacity.

Gut Microbiome vs. Microbiota: Interpretation of the Data

The growing body of evidence describing the composition, structure, and function of the microbial communities harbouring the human body has led to confusion in the terminology used to describe these communities and their environment^{125,126}. Although often used interchangeably in the literature, there is a subtle distinction between what defines the **microbiome** and the **microbiota**. By definition, the microbiota refers to the communities of microorganisms residing in a specific environment and is typically assessed using 16S rRNA gene sequencing¹²⁵. On the other hand, the microbiome refers to the collective genomes of all microorganisms (i.e., bacteria, fungi, and viruses) and their associated metabolites, characterized using one or a combination of techniques such as metagenomics, metabolomics, metatranscriptomics, and metaproteomics¹²⁵. For the purposes of this review, studies investigating the gut microbiota and/or microbiome were both considered, given the frequent misuse of the term “microbiome” in the literature that identifies bacterial communities through culture-based methods or 16S rRNA gene sequencing, rather than through whole-genome sequencing.

Marker gene sequencing, such as **16S rRNA** (for bacteria and archaea) and internal transcribed spacer (for fungi), relies on primers that target specific gene regions to

determine the microbial communities of a sample¹²⁷. The 16S rRNA approach involves PCR amplification of sequences from the 16S ribosomal RNA gene, which is universally present in bacteria and archaea. This technique enables taxonomic profiling of bacterial communities, typically at the genus^m level resolution (in rare cases at the species level), providing insights into their diversity, richness, and evenness^{128,129}. Importantly, if no difference in bacterial composition is detected using 16S rRNA, this does not preclude the presence of differences in the functional potential of bacterial communities (i.e., functional gene pathways and microbially produced metabolites) or variations in non-bacterial members of the microbiome (i.e., fungi and viruses). Due to sequence variability within targeted regions, primers may bind with differing affinities, introducing amplification bias during PCR¹²⁷. Therefore, using consistent reagent kits and PCR primers across all study samples is critical, as such variability can exceed the interindividual differences observed in healthy individuals' microbiota profiles^{112,118}. Overall, 16S rRNA sequencing is a validated and cost-effective method that offers a broad, albeit relatively low-resolution, overview of bacterial and archaeal community composition¹²⁷.

In contrast, **metagenomics** techniques sequence all genomic DNA present in a sample (bacteria, archaea, fungi, viruses, and others), down to the strain-levelⁿ resolution of microorganisms. This approach offers a detailed and comprehensive view of the microbial communities, including their functional gene content (i.e., the genes and pathways they encode), which cannot be captured from 16S rRNA sequencing¹²⁸. However, one technical limitation of metagenomic sequencing in fecal samples is the co-extraction of host DNA from shed epithelial cells, which can potentially reduce microbial sequencing depth¹²⁷. A complementary approach is **metabolomic profiling**, which involves the quantitative and qualitative analysis of metabolites produced by both the microbiota and the host¹¹⁸. This method provides insights into the actual biochemical activity and functional interactions within the gut ecosystem, going beyond the genetic potential inferred from metagenomic data. For these reasons, combining metagenomic sequencing and metabolomic profiling

^m Taxonomic rank grouping closely related species with shared characteristics.

ⁿ Subtype or genetic variant within a species.

is widely regarded as the gold-standard method for comprehensive characterization of the human gut microbiome^{128,129}, but accessibility remains limited due to costs that are typically two to three times higher than those of 16S rRNA gene sequencing. Nevertheless, such precise taxonomic resolution and functional data are not essential for all studies investigating the gut microbiota, particularly those focused on broader community structure or compositional shifts. For instance, Durazzi et al. (2021)¹²⁸ compared the accuracy between 16S rRNA and shotgun metagenomic sequencing and confirmed that the latter has more power to map the complete taxonomic composition of a sample, more specifically for the less abundant genera of bacteria. However, 16S rRNA was quantitatively and qualitatively robust to identify the most abundant genera (top 31%) of bacteria in a similar manner to shotgun sequencing.

Building on the ecological complexity and interindividual variability of the gut microbiota signature described earlier, it is nearly impossible (yet) to define a microbiota profile consistently shared by most healthy individuals^{112,115,116}. Administering the same diet across individuals often elicits highly personalized microbial responses, highlighting the need to account for baseline differences when analyzing microbiota data across individuals or populations. This also emphasizes the need for caution when interpreting and generalizing findings from small cohort studies, typically those involving fewer than several hundred participants¹¹⁸. Furthermore, the dynamic shifts in microbial communities and functionalities that can rapidly occur in response to changes in diet or lifestyle emphasize the necessity for standardized fecal sampling and analysis protocols in RCTs design, as transient but meaningful shifts in microbial composition may otherwise be undetected. In this context, an insightful review by Knight et al. (2018)¹²⁷ offers comprehensive guidelines for designing, conducting, and analyzing microbiome/microbiota studies. Among the key considerations for human RCTs they outline the following:

- RCTs investigating the gut microbiota should be powered based on studies specifically designed to assess microbiota outcomes, not on trials powered for

unrelated endpoints where microbiota effects were secondary. Rigorous control of confounders is also essential; for example, participants who received antibiotics in the past six months should be excluded due to their lasting impact on microbial composition.

- Serial baseline samples should be collected to assess intrinsic variability. Recent evidence suggests that aggregating data from three to five serial fecal samples provides an optimal balance for capturing microbiota dynamics using 16S rRNA sequencing in real-world research settings, with no additional benefit observed beyond seven serial samples¹³⁰.
- When using 16S rRNA sequencing, it is essential to use the same reagent kits and PCR primers across all study samples, as primer-induced variability can far exceed the interindividual differences observed in healthy individuals' microbiota profiles^{112,118}.
- Proper documentation and transparency in reporting procedures for sample collection, transport and storage, DNA extraction, PCR (if applicable), sequencing and data analyses are essential to ensure reproducibility of the findings.
- Finally, characterizing microbial composition alone is no longer sufficient; uncovering microbial function is now essential. Future microbiota research should aim to integrate multi-omics approaches for a comprehensive view of both the structure and function of microbial communities harbouring the gut microbiome.

Potential Influences on Glucose Homeostasis: Dysbiosis and Short-Chain Fatty Acids

To our knowledge, the first study to report adverse effects of sucralose on gut microbiota composition was published by Abou-Donia et al. (2008)¹³¹. In this 12-week study, rats were administered Splenda® by oral gavage at doses corresponding to 1.1 to 11 mg/kg BW in pure sucralose content, with weekly fecal samples collected for analysis. The authors reported significant reductions in the abundance of *Bifidobacteria*, *Lactobacilli*,

Bacteroides, and *Clostridia*. Of particular note, the study has faced substantial criticism over the years regarding the scientific integrity and transparency of its data. In 2024, the journal issued an official expression of concern, citing unresolved questions and the authors' failure to provide the necessary supporting materials for verification¹³². More recently, growing evidence in animal models has shown that sucralose can alter gut microbial composition by selectively promoting or inhibiting the growth of specific bacterial taxa^{13,15–19}, ultimately leading to **dysbiosis**. This condition, defined as a disturbance of the intestinal homeostasis caused by an imbalance in the structural and/or functional configuration of the microbiota's beneficial and protective microorganisms²⁰, is widely associated with susceptibility to a wide range of diseases and health altering conditions such as inflammatory bowel disease, Crohn's disease, celiac disease, irritable bowel syndrome, type I and type II diabetes, obesity, asthma, psoriatic arthritis, atopic eczema, and mood disorders^{99,133}. In addition, **dysbiosis has been linked to compromised intestinal barrier integrity^{15,21,22}, and subsequent increased systemic low-grade^{15,18,19,134}** characterized as a persistent state of inflammation occurring in the absence of an inflammatory disease¹³⁵. The intestinal barrier consists of a single layer of epithelial cells sealed with tight junctions' complexes, junction adhesion molecules and an overlying mucus layer. Together, these components precisely regulate selective permeability to nutrients, water and electrolytes, while hindering the translocation of pathogens into systemic circulation through coordinated innate and adaptive mucosal immune responses^{136–138}. Disruption of any component of the intestinal barrier can result in altering its selective permeability and expose the circulatory system to gut-derived bacterial products such as endotoxins^{139–143}, also referred to as **lipopolysaccharides (LPS)**. LPS are the major glycolipid component of the outer membrane of gram-negative bacteria¹⁴⁴. In a homeostatic state, circulating levels of LPS are either undetectable or very low¹⁴⁵. When intestinal permeability is increased, LPS can infiltrate the enterocytes and reach the lymphatic and circulatory systems¹⁴⁶, bind to its receptor on the membrane of

^o The accumulation of circulating LPS can lead to metabolic endotoxemia, characterized by LPS concentrations levels approximately 10 to 50 times lower than those observed in septic conditions²⁴⁶.

both immune and nonimmune cells^{134p}, and finally trigger the release of pro-inflammatory adipokines and cytokines (including TNF- α ^{147–150}, IL-1 β , and IL-6^{148,150}). Evidence strongly supports the role of immune responses and low-grade inflammation as key contributors to insulin resistance and, as a result, impaired glucose tolerance. Through a bidirectional endocrine-immune crosstalk, pro-inflammatory cytokines can interfere with insulin signaling pathways and downregulate insulin receptor expression resulting in decreased sensitivity in insulin sensitive tissues^{151,152}. While these outcomes are typically associated with obesity and metabolic disorders, it has been shown that LPS-induced low-grade inflammation can lead to the development of insulin resistance in rats, even in the absence of weight gain or obesity¹⁵³. In line with this, another study involving both animal and human models demonstrated that LPS exposure rapidly increased plasma levels of GLP-1 as a compensatory response mediated by enteroendocrine cells¹⁵⁴. These observations reinforce the role of low-grade inflammation as a modulator of glycemic control. While these findings in animal models reinforce the role of low-grade inflammation as a modulator of glycemic control, evidence in humans remains inconsistent. Furthermore, the mechanistic pathways by which the gut microbiota may modulate glycemic regulation in humans remain in the early stages of investigation. Nevertheless, ongoing advances in sequencing technologies are expected to provide greater clarity in the years ahead.

Mutualists bacteria within the gut microbiota contribute to numerous biological processes through enzymes not encoded by the human genome, including the synthesis of vitamin K, the conversion of dietary choline, the degradation of bile acids (~5-10%), and the fermentation of dietary fibers into **short-chain fatty acids** (SCFAs). These SCFAs serve as energy substrates for enterocytes and help reinforce the integrity of the intestinal barrier⁹⁹. Acetate, propionate, and butyrate are the primary SCFAs resulting from the bacterial fermentation of dietary fibers by the gut microbiota. Their production occurs in the

^p Immune cells: macrophages, monocytes, Kupffer cells and preadipocytes; nonimmune cells: adipocytes, hepatocytes and endothelium cells.

approximate ratio of 60:20:20¹⁵⁵ and they are found in millimolar concentrations in the large intestine, where they are readily absorbed by passive and active transport through the epithelium¹⁵⁶, with only 5-10% being excreted in feces¹⁵⁷. Once absorbed, SCFAs follow different metabolic pathways: 1) colonocytes oxidize butyrate as a major energy substrate, resulting in the production of ketone bodies and carbon dioxide^{99,110}; 2) hepatocytes metabolize most of acetate, as well as residual butyrate and propionate for gluconeogenesis¹¹⁰; and finally 3) myocytes oxidize residual acetate¹¹⁰. Acetate is the most abundant SCFA in systemic circulation, while butyrate and propionate are found in smaller amounts¹¹⁰. SCFAs have well-documented anti-inflammatory properties¹⁰⁶ and play a key role in gut-immunity crosstalk^{108,109}. They were also shown to contribute to reinforcing the colonic intestinal barrier functions by increasing the expression of tight junctions^{98,105,106} and to suppress LPS-induced release of TNF- α from human neutrophils in cell culture¹⁰⁷. The mechanisms underlying how SCFAs can decrease the expression of pro-inflammatory cytokines are not yet fully understood, but one of the proposed mechanisms is via the inhibition of nuclear factor kappa-B (NF- κ B) pathway, which plays a central role in activating the transcription of genes encoding proteins involved in immune and inflammatory responses¹⁵⁸. In addition, SCFAs play a crucial role in various energy metabolic processes. They are involved in glucose metabolism by promoting the secretion of insulin, GLP-1 and peptide YY through the activation and upregulation of G protein-coupled receptors in intestinal epithelial cells. SCFAs also contribute to lipid metabolism, including fatty acid and cholesterol biosynthesis^{98,109,110}. While acetate, propionate, and butyrate have been extensively studied, other SCFAs present at lower fecal concentrations are now receiving increased research attention. Branched short-chain fatty acids (BCFAs), primarily isovalerate and isobutyrate, are of particular interest, as they are suggested markers of protein fermentation, a process that can lead to the production of microbial metabolites potentially harmful to the colonic epithelium¹⁵⁹.

Randomized-Controlled Trials

Herein, we critically examine RCTs that have investigated the **cumulative effects of sucralose consumption on postprandial glycemic responses in humans**, defined as exposures lasting seven days or longer. Given the diversity in study methodologies and outcomes, we adopted an individualized review strategy to critically assess each study's design and contribution. Each study is separately reviewed to evaluate its experimental design, statistical analyses, interpretation of findings, and its contribution to the broader scientific understanding of sucralose's metabolic effects. This approach was chosen due to the substantial heterogeneity across study designs, including major differences in dosage, form of administration (i.e., beverages, capsules), duration of exposure, comparator selection (i.e., water, SSB with or without matching for relative sweetness, other type of LCS-sweetened beverage), methods for assessing glucose tolerance, statistical analyses, and transparency in reporting outcomes. In our view, this variability precludes meaningful synthesis through systematic reviews or meta-analyses and instead necessitates individual critical appraisal of relevant RCTs. Highlighting these methodological inconsistencies is essential to understanding why, to date, no clear consensus has emerged regarding the effects of sucralose on postprandial glycemic responses in humans. To identify relevant studies, we conducted a literature search using PubMed. The search strategy included combinations of the following keywords: A) *low-calorie sweeteners, artificial sweeteners, non-nutritive sweeteners, sucralose*; B) *glycemic control, glycemic responses, glycemic, glycemia, glucose tolerance, glucose homeostasis*. Each query paired at least one term from Group A with one from Group B to ensure that retrieved articles addressed both the exposure and the outcome of interest. We focused on meta-analyses, systematic reviews, and randomized controlled trials conducted in healthy adults. Narrative reviews were also consulted to identify additional references that may not have appeared in the initial search. Only studies that specifically examined sucralose, with a minimum exposure duration of seven days, and that assessed postprandial glycemic responses were included. In addition to the database search, we manually screened the reference lists of all included publications to identify further

relevant studies. This process yielded a total of 11 studies that met the inclusion criteria. While the employed search strategy was extensive, we did not perform a systematic review. Therefore, some studies may have been missed. A summary of the included randomized controlled trials is provided in [Table 1](#).



The current body of evidence on the effects of sucralose on postprandial glycemic responses is marked by considerable heterogeneity in study design. Key differences include dosage, form of administration (i.e., beverages, capsules), duration of exposure, comparator selection (i.e., water, SSB with or without matching for relative sweetness, other type of LCS-sweetened beverage), methods for assessing glucose tolerance, statistical analyses, and transparency in reporting outcomes. This variability precludes meaningful synthesis through systematic reviews or meta-analyses and instead calls for individual critical appraisal of relevant RCTs. Highlighting methodological inconsistencies is essential to understanding why, to date, there is no clear consensus regarding the effects of sucralose on postprandial glycemic responses in humans.

Table 1. Randomized-controlled trials evaluating the cumulative effects of sucralose consumption on postprandial glycemic responses in humans.

Year	Authors	Study design	Participants characteristics	Duration of exposure	Follow-up	Sucralose type	Form	Dose	Comparator(s)	Measurement technique	GLYCEMIC RESPONSES	INSULIN RESPONSES	INSULIN SENSITIVITY	GLP-1
2017	Grotz et al.	parallel double-blind	47 healthy men	12 weeks	4-week	commercial	capsules	1000 mg	placebo (cellulose)	75g OGTT	No effect	No effect	N/A	N/A
2018	Lertrit et al.	crossover double-blind	15 healthy adults (11W, 4M) LCS low consumers	4 weeks 1 week washout	none	pure	capsules	200 mg/day	placebo (empty capsules)	75g OGTT IVGTT	No effect	↑	↓	↑
2018	Romo-Romo et al.	parallel open-label	61 healthy adults (49W, 17M) LCS low consumers	14 days	none	commercial	sachets	15 mg/kg BW/d	no intervention	FSIVGTT	No effect	↑	↓	N/A
2019	Thomson et al.	parallel double-blind	30 healthy men	7 days	none	commercial	capsules	780 mg/day	placebo calcium carbonate (210 mg/day)	75g OGTT	No effect	No effect	No effect	N/A
2019	Higgins et al.	parallel single-blind	123 healthy adults living with overweight or obesity (50.0-64.3% women)	12 weeks	none	not specified	beverages	160 mg/day	sucrose (400-560 kcal)	75g OGTT	No effect	No effect	N/A	N/A
2020	Ahmad et al.	crossover double-blind	17 healthy adults (10W, 7M) LCS low consumers	2 weeks > 4-week washout	none	pure	beverages	136 mg	aspartame (425 mg/day)	75g OGTT	No effect	No effect	No effect	No effect
2020	Dalenberg et al.	parallel double-blind	39 healthy adults (21W, 18M) no control for menstrual cycle LCS low consumers	7 cans over 10 days	none	pure commercial	beverages	60 mg/can 60 mg + 32 g maltodextrin/can	sucrose (30 g)	75g OGTT	No effect No effect	No effect ↑	No effect ↓	N/A
2020	Bueno-Hernández et al.	parallel double-blind	95 healthy adults (58W, 37M)	10 weeks	none	not specified	beverages	48 mg/day 96 mg/day	water	75g OGTT	↑ No effect	↑ No effect	↓ No effect	N/A
2022	Méndez-García et al.	parallel open-label	40 healthy adults (26W, 14M) sucralose low consumers	10 weeks	none	pure	beverages	48 mg/day	water	75g OGTT	↑	No effect	No effect	N/A
2022	Suez et al.	parallel open-label	120 healthy adults (78W, 42M) strict LCS non-consumers	2 weeks	2-week	commercial	sachets	102 mg/day	glucose (5 g) no supplement	at-home OGGTs using a continuous glucose monitor	↑	No effect	N/A	No effect
2023	Orku et al.	parallel single-blind	42 healthy women	4 weeks	none	not specified	beverages	66 mg/day	water	75g OGTT	No effect	No effect	No effect	No effect

Abbreviations: body weight (BW), frequently sampled intravenous-glucose-tolerance test (FSIVGTT), intravenous glucose tolerance test (IVGTT), low-calorie sweeteners (LCS), men (M), oral glucose tolerance test (OGTT), women (W).

Grotz et al. (2017)

Grotz et al. (2017)¹⁶⁰ evaluated the effects of sucralose on glucose and insulin homeostasis in 47 healthy men who consumed either 1000 mg of sucralose (administered in three 333.3 mg capsules per day) or a placebo with meals over a 12-week period, followed by a 4-week follow-up phase. Postprandial glycemic and insulinemic responses were assessed using repeated OGTTs, including two tests during screening to establish individualized baseline area under the curve (AUC), two during the intervention (weeks 6 and 12) and one during the follow-up visit (week 16). It should be noted that this study was originally conducted in 1995–1996 as part of the Sucralose Food Additive Petition 7A3987 and submitted to the FDA by the Heartland Food Products Group (manufacturer of Splenda®) in support of sucralose's approval as a food additive. After 12 weeks of sucralose supplementation via capsules, no statistically significant differences were observed between the sucralose and placebo groups in changes from baseline for postprandial mean glucose and insulin AUC. Notably, the absence of significant differences in baseline glucose and insulin AUC further supports the comparability of metabolic responses attributable to the intervention. A major strength of the study is the absence of prior sucralose exposure among participants, as sucralose had not yet been approved in Europe at the time of data collection (1995–1996). This eliminated potential confounding from tolerance or accumulation effects and allowed for accurate compliance assessment through urine analysis, based on the presence or absence of sucralose. In addition, the 12-week duration remains among the longest exposure periods in RCTs on sucralose, and the inclusion of a 4-week follow-up phase is particularly valuable, as it allows for the evaluation of the persistence of any metabolic effects, an aspect rarely addressed in LCS research.

This study is often cited as key evidence supporting the lack of sucralose effects on glycemic responses. However, important limitations constrain the generalizability of its findings. First, the study population consisted exclusively of men. The authors justified the exclusion of women as a means “to avoid the potential effect of menstruation-related hormonal changes on blood glucose and/or insulin measures”. While this rationale

reflects a historically common practice in clinical research, it aligns with a broader pattern of excluding women from studies, a practice that persisted in North America until regulatory reforms by the U.S. National Institute of Health in 1993¹⁶¹ and the Canadian Institutes of Health Research in 2010¹⁶². Although this practice was generally accepted at the time the study was conducted (1995-1996), the article was published in 2017, and the exclusion of women was not acknowledged as a limitation despite current knowledge of the role of sex hormones in modulating metabolic outcomes. Further, sucralose was administered in capsule form, thereby bypassing orosensory perception and associated cephalic phase responses, mechanisms that may contribute to alterations in glucose tolerance observed with sucralose consumption. This mode of administration does not reflect typical intake patterns, where sucralose is consumed in foods and beverages. Consequently, the study's findings may not accurately represent the metabolic effects of sucralose under ecological conditions. Another concern involves the reporting of within-group changes. While the authors report that none of the between-group differences in postprandial glucose and insulin AUC were statistically significant, they also mention that some within-group changes reached statistical significance, albeit without being “clinically meaningful”. However, they do not specify which outcomes were affected or provide corresponding p-values. This hinders independent evaluation of the data and limits the reader's ability to assess the internal consistency and robustness of the findings. Although the study demonstrates methodological rigour in several respects, these limitations warrant cautious interpretation of its conclusions.

Lertrit et al. (2018)

Lertrit et al. (2018)⁸⁹ examined the effects of repeated sucralose exposure provided as 200 mg daily capsules for 4 weeks on glycemic and insulinemic responses in 15 healthy adults (11 women, 4 men) using a randomized, double-blind, crossover study. All participants were initially low consumers of LCS, had a body mass index (BMI) within normal range ($23.1 \pm 3 \text{ kg/m}^2$), and were confirmed to be normoglycemic after an OGTT prior to enrollment. Following each 4-week intervention, which were separated by a one-week

washout period, plasma glucose, insulin, and active GLP-1 levels were measured after a 75 g OGTT. The next day, acute insulin responses were assessed using an intravenous glucose tolerance test (IVGTT). No significant differences in glycemic responses were observed between conditions. However, sucralose exposure significantly reduced whole-body insulin sensitivity (Matsuda index of 4.69 ± 1.67 vs 5.31 ± 2.56) and the acute insulin response (i.e., first phase of insulin secretion, 0-10 min) (58.9 ± 48.61 vs 69.94 ± 73.81 mU/mL) compared to placebo. Moreover, an elevation in AUC of active GLP-1 was observed (23.16 ± 18.86 vs 18.5 ± 22.22 pmol/L · 120 min). The study design presents two key limitations that constrain the applicability of the results to real-world LCS consumption. First, the absence of baseline measurements limits the interpretation of the observed changes. Second, administering sucralose in capsule form bypasses cephalic phase responses linked to the orosensory perception of sweetness, as previously discussed in section *Grotz et al. (2017)*.

Romo-Romo et al. (2018)

In a study similar to that of Lertrit and colleagues⁸⁹, Romo-Romo et al. (2018)¹⁶³ assessed glucose effectiveness, defined as the ability of glucose to promote its own uptake and suppress endogenous glucose production independently of insulin¹⁶⁴, and insulin sensitivity in 61 healthy adults (predominantly women). Participants consumed commercial sucralose in beverages daily for 14 days at a dose equivalent to 15% of the ADI for sucralose, as defined by the WHO (15 mg/kg body weight/day¹⁶⁵). Notably, this value exceeds the ADI established by the FDA of 5 mg/kg body weight/day³⁶. Specifically, the actual sucralose dose received by the participants was reported as 123 ± 12.9 mg/d for women and 157.7 ± 12.9 mg/d for men. Following the intervention, participants exposed to sucralose showed a significant 17.7% reduction (interquartile range (IQR): -29.3% to -1.0%) in insulin sensitivity, compared to a 2.8% reduction (IQR: -30.7% to 40.6%) in the no intervention group. In contrast, glucose effectiveness^q remained unchanged in both

^q Glucose effectiveness is the ability of glucose to promote its own uptake and suppress endogenous production in the absence of an incremental insulin effect^{247,248}.

groups. Notably, glycemic-insulinemic responses were assessed using the frequently sampled intravenous-glucose-tolerance test (FSIVGTT), a gold-standard method that provides detailed insights into beta-cell function and insulin dynamics^r. However, it is important to note that intravenous glucose administration bypasses incretin responses, particularly the secretion of GLP-1 and GIP, which is stimulated by oral glucose ingestion. Emerging evidence indicates that oral and intravenous glucose challenges may elicit opposed effects on skeletal muscle blood flow¹⁶⁶, supporting the physiological relevance of the OGTT in assessing postprandial glucose tolerance in a research setting¹⁶⁶. Therefore, while this study offers robust evidence regarding sucralose's impact on insulin sensitivity, its conclusions about broader aspects of glucose metabolism should be interpreted with caution, considering the limitations inherent to the FSIVGTT method for assessing glycemic responses specifically.

Thomson et al. (2019)

In a cohort of 30 healthy men, Thomson et al. (2019)¹⁶⁷ investigated the short-term effects of sucralose consumption on glucose homeostasis and gut microbiota composition using a randomized, parallel, double-blind design. Over a seven-day intervention period, individuals received either sucralose capsules (780 mg/day) or a placebo. Post-intervention analyses revealed comparable postprandial glycemic and insulinemic responses to a 75 g OGTT across groups and conditions, along with similar measures of insulin sensitivity (Matsuda Index) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Similarly, supplementation with sucralose or placebo capsules did not result in statistically significant alterations in gut microbiota composition, as assessed by 16S rRNA sequencing. To contextualize the study's conclusions and facilitate comparison with other RCTs discussed in this review, some limitations should be considered. First, the study did not report whether individuals were low consumers of LCS prior to enrollment, nor whether they were instructed to avoid LCS consumption outside of

^r The gold standard for measuring both glycemic and insulinemic responses in a research setting is euglycemic-hyperinsulinemic clamp (EHC).

the intervention capsules. Additionally, the exclusion of women, justified by the authors to “avoid potential menstrual cycle-related changes in insulin sensitivity”, is difficult to defend in the context of a seven-day intervention. While cyclical fluctuations in estrogen levels are known to influence glycemic responses^{168,169}, the average menstrual cycle spans 21 to 35 days, making it both feasible and necessary to control for cycle phases in short-term trials to enhance the generalizability of findings. Second, the study’s short duration may have constrained its ability to detect meaningful changes in glucose homeostasis, particularly in healthy individuals. The use of sucralose in capsule form further limits ecological validity, as it does not reflect typical consumption patterns (see section *Grotz et al., (2017)* for more details). Third, both the sucralose and placebo capsules contained calcium carbonate (210 mg/day), a compound recently shown to influence gut microbiota composition and function¹⁷⁰. Finally, the study was powered to detect differences in glycemic responses, which may have educed its sensitivity to detect changes in gut microbiota composition, a limitation acknowledged by the authors. This limitation is compounded by the collection of only one fecal sample before and after the intervention, an approach that has been shown to be insufficient for capturing intrinsic variability of fecal microbial communities¹²⁷.

Higgins et al. (2019)

Higgins et al. (2019)⁶⁹ compared the effects of daily sucrose consumption to four commonly used LCS (including sucralose) on metabolic outcomes over a 12-week period in 123 healthy adults living with overweight or obesity. Participants were weight stable and reported low consumers of LCS. In a parallel-arm design, sucrose and LCS were provided in beverages matched for sweetness, with daily volumes ranging from 1.25 to 1.75 L, depending on body weight. The daily energy provided by the sucrose-sweetened beverages ranged from 400 to 560 kcal/day, although the authors did not report how this intake related to participants’ total energy requirements. In contrast, LCS doses were fixed regardless of beverage volume. Specifically, the dose for sucralose was set at 160 mg/day. Across all groups, mean glucose concentrations following OGTT increased from baseline

to week 12, but no significant treatment effect was observed. Moreover, glycemic and insulinemic iAUC values were similar between groups, although the effect of sweetener on glucose iAUC approached statistical significance. Interestingly, body composition assessed via dual-energy X-ray absorptiometry (DXA) revealed divergent changes; the sucrose group experienced a slight increase in total fat mass ($+1.35 \pm 0.25$ kg), while the sucralose group showed a modest reduction (-0.31 ± 0.24 kg). These opposing changes in fat mass may have attenuated between-group differences in glycemic responses, potentially masking treatment effects. This study demonstrates key methodological strengths that enhance the contextual relevance of its findings. These include a 12-week exposure duration, a realistic sucralose dose, study beverages matched for relative sweetness, control for prior LCS consumption, and the use of a urinary biomarker to objectively assess compliance. However, some limitations also warrant consideration. Participants were not given dietary instructions beyond the consumption of the study beverages, allowing for potential intake of additional LCS, free sugars, and nutritional supplements, thereby introducing potential confounding factors.

Ahmad et al. (2020)

Using a randomized crossover approach, Ahmad et al. (2020)¹⁷¹ assessed the two-week effects of consuming sucralose- and aspartame-sweetened beverages at realistic doses (136mg/day and 425 mg/day, respectively) on glycemic outcomes in 17 healthy adults with a low habitual intake of LCS. Glycemic outcomes included postprandial glycemic-insulinemic responses, as well as active GLP-1 concentrations, measured following a 75 g OGTT. Compared to baseline, no significant changes were observed in the total AUC for plasma glucose, insulin, or active GLP-1 following the two-week intervention with sucralose or aspartame. Insulin sensitivity, assessed using HOMA-IR, also remained unchanged compared to baseline values. There are several strengths in the experimental design of this study. The double-blind crossover design with a minimum four-week washout period was robust and the adequacy of the washout period was demonstrated to be sufficient for reinstating baseline fasting concentrations of plasma glucose, insulin, and

GLP-1. Potential confounders such as LCS consumption, both prior to and during the intervention period, as well as substantial changes in dietary intake and physical activity were carefully controlled. Also, efforts were made to account for the menstrual cycle, including the exclusion of participants with irregular menstrual cycles or those using contraceptive pills. However, the study did not specify whether other hormonal contraceptive methods, such as hormonal IUDs, were controlled for despite their increasing use among women. The study reported that OGTTs were performed after the cessation of menses, but the timing relative to the follicular phase was not specified. Standardizing the administration of the OGTT to a consistent phase of the menstrual cycle (preferably the follicular phase, when female sex hormone levels are at their lowest)¹⁶⁸ would have allowed for more accurate comparisons of treatment effects. As previously noted in this review, this consideration remains particularly relevant when assessing postprandial glycemic responses in women, given the influence of cyclical estrogen fluctuations on glucose metabolism^{168,169}. Nonetheless, efforts to account for menstrual cycle-related metabolic effects represent a methodological improvement over several other RCTs and warrants acknowledgment. While the study design is methodologically sound, two notable limitations warrant consideration. The first relates to the absence of a non-LCS comparator. The study compared two LCS-sweetened beverages, sucralose and aspartame, without including a non-LCS comparator, such as SSB. As previously emphasized in this review, each LCS possesses a distinct chemical structure, which underscores the importance of evaluating their metabolic effects using 1) sweetener-specific hypotheses^{13,67–69}, and 2) comparators that are neither other LCS nor water^{70,172–175} (see section *cumulative effects of sucralose on postprandial glycemic responses in humans* for more details). The second limitation concerns the analytical approach to postprandial glycemic responses. These were reported using total AUC values, therefore not adjusted for fasting glucose levels. Because total AUC is strongly correlated with fasting glucose¹⁷⁶, this approach introduces variability due to interindividual differences at time 0 of the OGTT. In contrast, iAUC specifically measures the rise in glycemic responses

above the fasting glucose level¹⁷⁶. By subtracting fasting values, iAUC more accurately captures postprandial glucose dynamics in nutritional interventions¹⁷⁶.

In a follow-up analysis, the authors examined the effects of the intervention on gut microbiota using fecal samples collected during the original trial¹⁷⁷. 16s rRNA sequencing revealed no significant changes in the relative abundance of the four dominant bacterial phyla (*Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*), nor in α -diversity (Shannon index) or β -diversity (Bray-Curtis Dissimilarity). In addition, fecal concentrations of six SCFAs (acetate, propionate, butyrate, isovaleric acid, valeric acid, hexanoic acid) were measured and found to be unaffected by the intervention. These findings suggest that neither sucralose nor aspartame measurably altered gut microbiota composition or SCFA concentrations when consumed at realistic doses for two weeks. Interestingly, a significant sequence effect was detected in β -diversity, indicating that the order in which participants received the LCS in the crossover design influenced microbial community structure. However, how the order of LCS administration affected β -diversity remains unclear. It should be noted that these findings were reported to be exploratory by the authors, as the original study was powered to detect differences in glycemic responses rather than changes in gut microbiota outcomes.

Dalenberg et al. (2020)

To prevent repetition, the findings of Dalenberg et al. (2020)¹⁷⁸ are discussed in the section titled *Uncoupling sweet taste from energy*.

Bueno-Hernández et al. (2020)

In this parallel-group, double-blind RCT, 95 healthy young adults consumed either water, 48 mg or 96 mg of sucralose daily, over a 10-week period to assess the cumulative effects of sucralose consumption on glucose tolerance and insulin sensitivity. Participants were instructed to avoid other sources of LCS during the study, although prior LCS consumption was not considered an exclusion criterion. To assess glycemic and insulinemic responses,

a three-hour 75 g OGTT was administered at week 0 and week 10. Each OGTT was preceded by a preload of the assigned intervention beverage, ingested 15 minutes prior to glucose. Bueno-Hernández et al. (2020)¹⁷⁹ found that 10 weeks of sucralose consumption significantly increased glycemic and insulinemic responses at specific times points during the OGTT (glucose concentrations: -15, 0, and 120 minutes; insulin concentrations : 0, 30, 105, and 120 minutes) in the 48 mg group compared to baseline. However, no statistically significant changes were observed in the 96 mg group, suggesting that the observed effects were not consistently replicated at a higher dose. The study's strengths include its double-blind design and the use of weekly food frequency questionnaires to monitor dietary intake. However, the findings should be interpreted with caution due to a key methodological limitation that affects internal validity. Specifically, the inclusion of a sucralose preload immediately before each OGTT introduces an acute exposure, which complicates the interpretation of cumulative metabolic effects. Without a separate test to isolate cumulative responses, it is unclear whether the observed changes in glucose and insulin dynamics reflect the cumulative impact of repeated sucralose intake or an acute response to the sucralose preload. Additional limitations, though less critical, concern the study beverages. These include the lack of sweetness matching across intervention beverages, the sucralose concentration substantially higher than commercially available beverages (i.e., 48 mg or 96 mg per 60 mL) and the absence of flavoring, which may have compromised blinding, particularly for participants in the control group who received plain water.

Méndez-García et al. (2022)

This study investigated the effects of 10-week sucralose consumption on the abundance of selected bacterial species within the Actinobacteria, Bacteroidetes, and Firmicutes phyla, and explored potential associations between gut microbiota profiles and glycemic-insulinemic responses in 40 healthy adults. Participants included women and men who were low consumers of LCS. The daily sucralose dose was provided in a 60 mL solution (48 mg/day), while the control group received plain water. At baseline and post-intervention,

glycemic and insulinemic responses were assessed using a three-hour OGTT and one fecal sample was provided to evaluate microbiota composition via quantitative polymerase chain reaction. Méndez-García et al. (2022)¹⁸⁰ found that sucralose intake significantly altered the abundance of specific species from the Firmicutes phyla, with a threefold increase in *Blautia coccooides* and a 0.66-fold decrease in *Lactobacillus acidophilus* compared to baseline; while no significant changes were observed in the abundance of *Bifidobacterium longum* or *Bacteroides uniformis*, representing Actinobacteria and Bacteroidetes, respectively. While the study abstract reported that sucralose increased serum insulin and glucose AUC compared to water, a closer examination of the full dataset reveals a more nuanced interpretation. Notably, no statistically significant differences were observed in plasma glucose AUC between groups following the 10-week intervention. The increase in glucose AUC was only significant within the sucralose group when comparing post-intervention to baseline values ($8 \pm 1.7\%$), whereas the water group showed no change. However, when analyzing plasma glucose concentrations during the OGTT within the sucralose group, no significant differences were detected. Regarding insulinemic responses, the sucralose group exhibited a significant $32 \pm 3.5\%$ increase in the insulin peak at 30 minutes during the OGTT, yet no significant differences in insulin AUC were observed between groups or across intervention conditions. Taken together, while the abstract suggests that sucralose alters postprandial glycemic-insulinemic responses, the full dataset indicates that these effects were limited to within-group changes and were not consistently supported by between-group comparisons.

The study demonstrates commendable design features. Notably, it appears to have been specifically powered and structured to detect changes in gut microbiota composition, although this is not explicitly stated in the methods section. The exclusion of individuals who had received antibiotic treatment within six months prior to enrolment, along with weekly monitoring of macronutrient intake and physical activity, and dietary instructions to avoid all sources of LCS throughout the intervention, further strengthen the internal validity of the findings. Notwithstanding methodological strengths, a notable limitation concerns the narrow scope of the microbiota analyses. While the study concluded that long-term

sucralose consumption induces gut dysbiosis, this interpretation may be overstated given the restricted taxonomic resolution and the limited scope of microbial profiling. Specifically, the analysis focused on only four bacterial species using qPCR, rather than employing broader profiling techniques such as 16S rRNA gene sequencing. Although qPCR is highly sensitive for detecting and quantifying preselected bacterial species¹⁸¹, it remains limited to the species included in the assay; more precisely, those targeted by the primer design¹⁸¹. Therefore, the qPCR approach limited the ability to detect broader shifts in bacterial community structure and diversity, potentially overlooking changes in non-targeted taxa that may have contributed to the observed metabolic effects. Of particular note, recent evidence supports the complementary use of qPCR alongside 16S rRNA gene sequencing to obtain a more comprehensive understanding of microbiota dynamics¹⁸². Another limitation of the microbiota study design is the collection of only one stool sample per condition, which restricted the ability to capture temporal microbiota dynamics and intra-individual variability. Other noteworthy limitations should also be acknowledged regarding the assessment of postprandial glycemic responses. Although participants were characterized as low sucralose consumers prior to the study, intake of other LCS was not assessed or included as an exclusion criterion. Lastly, the sucralose concentration in the study beverage was higher than that found in commercially available products, which may limit the ecological validity of the findings.

Suez et al. (2022)

The pioneering work of Suez et al. (2014)¹² provided foundational evidence that LCS intake, at doses within the ADI, can impair glucose homeostasis by inducing compositional and functional alterations in the gut microbiota, as demonstrated across multiple experimental models. Saccharin was the only LCS included in the human component of the experimental design. To address the limitations of their original pilot study, Suez et al. (2022)¹³ expanded their investigation to include other types of LCS in a two-week study involving 120 healthy adults who were randomized to receive commercially available sachets containing either aspartame (240 mg/day), saccharin (180 mg/day), sucralose

(102 mg/day), stevia (180 mg/day), or glucose (5 g/day; control group) to be dissolved in water by participants. All sachets contained 5 g of glucose as a bulking agent. To complement the glucose control, a second control group that received no supplement was also included. Among the four LCS tested, only saccharin and sucralose were found to significantly impair postprandial glycemic responses compared to both control groups (glucose and non-supplemented controls). To further characterize these effects, within-group analyses were conducted by comparing each participant's glycemic response during exposure to their own average baseline iAUC. Sucralose supplementation led to a significant increase in glucose iAUC from the first week of exposure (mean difference: 855.8; 95% CI: 134.8–1,577), which persisted into the second week (mean difference: 976.3; 95% CI: 231.3–1,721), but subsided after discontinuation. Saccharin showed a similar pattern, with significant increases from baseline in both exposure weeks that did not persist during follow-up. Interestingly, sucralose was associated with greater interindividual variability in glycemic responses than saccharin, suggesting individual-specific metabolic responses. In addition, no significant alterations in circulating levels of insulin and GLP-1 were detected, suggesting that the impairments in glycemic control were not mediated by changes in these hormones. No significant changes in glycemic outcomes were observed in either the glucose vehicle or non-supplemented control (NSC) groups across the study period. To our knowledge, this is the first study to assess glycemic responses with multiple OGTTs (nine OGTTs performed on predetermined days; baseline = 2, intervention = 5, and follow-up = 2) at home with continuous glucose monitoring. This innovative design enabled high-resolution, real-world monitoring of glycemic dynamics, enhancing both the ecological validity and temporal resolution of the findings.

To investigate whether changes in gut microbiome composition and function could underlie the altered glycemic responses observed with saccharin and sucralose consumption, shotgun metagenomic sequencing was performed on serial fecal samples collected throughout the study (baseline = 3, intervention = 6, follow-up = 2). Baseline microbiome composition and function were comparable across all groups. Notably, only saccharin and sucralose significantly altered the gut microbiome at genus and species

resolution compared to the no supplement control. No significant compositional changes were observed in both control groups, suggesting that the observed effects were specific to LCS exposure rather than the glucose bulking agent. Functional profiling of the gut microbiome revealed that all four LCS induced distinct shifts in microbial metabolic pathways, with sucralose showing the most pronounced effects. More precisely, sucralose was associated with changes in purine metabolism, while saccharin affected pathways related to glycolysis and glucose degradation. Building on the observed microbiome shifts, the authors next investigated whether these changes could causally influence glycemic control through fecal microbiota transplantation in germ-free mice. Fecal samples were collected from four top and three bottom responders (based on glycemic responses) from each intervention and control group, both at baseline (days 0–7) and at the end of the intervention period (day 21). Mice colonized with microbiota from day 21 of top responders in the sucralose and saccharin groups exhibited significantly elevated glycemic responses compared to those receiving baseline microbiota from the same donors, indicating a significant donor group effect. This effect was not observed in mice colonized with microbiota from top responders in the glucose or no supplement control groups. When the same protocol was applied using microbiota from bottom responders, no significant glycemic effects were observed in any group, except for saccharin, which induced elevated glycemia. In the sucralose group, baseline microbiota from top and bottom responders elicited comparable glycemic responses in mice but differed markedly in microbial composition and function. Specifically, mice colonized with microbiota from top responders showed enrichment in pathways associated with the biosynthesis of menaquinol (previously linked to type I and type II diabetes), purines, and pyrimidine. In contrast, microbiota from bottom responders were enriched in pathways associated with purine degradation as well as SCFAs biosynthesis, oxidation, and gluconeogenesis. Interestingly, SCFA-related pathways were either attenuated or undetectable in mice colonized with microbiota from top responders at baseline and day 21, respectively, highlighting the potential role of bacteria that either produce or utilize SCFAs in mediating the glycemic disruptions induced by sucralose. Interestingly, mice colonized with post-

intervention microbiota from top responders also exhibited increased α -diversity (observed features); a pattern not observed in bottom responders. This finding is somewhat paradoxical, as reduced α -diversity of microbial species is typically considered a hallmark of dysbiosis and associated with various disease states^{99,124,133}. Conversely, higher α -diversity is generally thought to confer resilience to environmental perturbations and is often interpreted as indicative of a “healthier” gut microbiome^{124,183}. The authors suggest that this increase in α -diversity may reflect a selective overgrowth of microbial taxa capable of using sucralose as a carbon source, rather than a shift toward a healthier microbiome ecosystem. Of note, α -diversity was assessed using a single metric evaluating richness only. Recent evidence emphasizes that α -diversity analyses should incorporate complementary metrics evaluating richness, evenness, information, and phylogenetic diversity to provide a more comprehensive and robust characterization of gut microbiota diversity¹⁸⁴. Finally, the glycemic responses observed in colonized mice largely mirrored those of their human donors, providing experimental support for a causal role of sucralose-induced microbiome alterations in impairing glycemic regulation. Taken together, the findings from Suez and colleagues highlight an individualized microbiome response to sucralose consumption and support a causal relationship between specific microbial signatures and disruptions in glucose tolerance.

This study is distinguished by several methodological strengths. First, LCS consumption was assessed over the preceding six months using a detailed food frequency questionnaire that captured both well-known sources, like diet beverages, and less apparent sources of LCSs including yogurt and chewing gum. A rigorous screening of 1,375 individuals ensured that only participants who had completely abstained from consuming LCS during this period were enrolled. Throughout the intervention, participants recorded all food intake and physical activity using a custom-developed smartphone application, enabling real-time monitoring and feedback from the research team. This approach contributed to maintaining similar macronutrient intake (including dietary fiber and sugars) and physical activity levels across groups throughout the intervention. Lastly, the study included a 2-week follow-up to assess whether changes in glucose tolerance persisted after cessation

of exposure. Notwithstanding the robust design of the study, a notable limitation lies in the omission of reporting absolute glucose iAUC values at baseline and post-intervention in the human model. Instead, only changes in glucose iAUC for the sucralose and saccharin groups are explicitly reported, limiting the ability to assess the magnitude of the observed effects. Without access to the absolute values, it becomes challenging to contextualize individual variability or evaluate the clinical relevance of glycemic impairments across groups.

Orku et al. (2023)

In a single-blind, parallel-arm RCT, Orku et al. (2023)¹⁸⁵ investigated the effects of various LCS on glucose tolerance and GLP-1 release in 42 healthy young women. Over a 10-week period, participants consumed 330 mL of LCS-sweetened beverages daily, containing either sucralose (66 mg), saccharin (140 mg), aspartame (88 mg), or a combination of aspartame and acesulfame K (88 mg each), while the control group consumed plain water. Postprandial glycemic and insulinemic responses to a 75 g OGTT, along with fasting GLP-1 concentrations, were measured at baseline and week 4. The study revealed no statistically significant differences in mean glucose concentrations between groups at 60, 120, and 180 minutes during the OGTT. In addition, no within-group changes from baseline were observed. Similarly, mean glucose AUC values during the OGTT were similar across groups and intervention conditions. Following the intervention, significant differences in insulin concentrations at 60 and 120 minutes during the OGTT were observed between the sucralose and the aspartame/Ace-K group. Specifically, the sucralose group exhibited lower plasma insulin concentrations, although this difference was not statistically significant when compared to the water control group. Changes in insulin sensitivity, evaluated using the Matsuda Index, did not differ significantly between or within groups over the intervention period. Key strengths of the study include its 10-week duration, the exclusion of individuals with habitual LCS consumption, and contribution to the body of evidence on the effects of LCS on glucose tolerance in women. While the inclusion of only one sex may be viewed as a limitation, it is important to emphasize that a substantial

portion of existing research on the metabolic effects of LCS has been conducted exclusively in men, as already outlined in this review. Nonetheless, certain methodological and statistical limitations may have influenced the findings. The initial sample size targeted 12 participants per group, but dropouts led to an unequal group distribution (11 participants in each experimental group and 9 in the control group). Combined with the reduced overall sample size, this may have reduced the study's statistical power below the intended threshold. Glycemic responses were evaluated with total AUC, which does not account for interindividual differences in fasting glucose levels, unlike iAUC (see section *Ahmad et al. (2020)* for more details). Finally, the use of one-way ANOVA with Bonferroni correction to analyze postprandial glucose and insulin AUC values at baseline and week 4 separately does not account for the repeated-measures nature of the data. This approach overlooks within-subject correlations and may limit the ability to detect nuanced temporal effects and individual variability, potentially underestimating the true impact of the intervention.

Conclusion

To date, existing evidence has not resolved the question of whether sucralose, when consumed under real-world dietary conditions, adversely impacts postprandial glycemic responses in humans. The current body of evidence is marked by substantial methodological heterogeneity, including major differences in dosage, duration of exposure, form of administration, comparator selection, methods for assessing glucose tolerance and statistical approaches. Strikingly, of the 11 RCTs critically evaluated in this review, only one accounted for the menstrual cycle¹⁷¹, while two excluded women on the premise that controlling for hormonal fluctuations was too complex^{160,186}. Taken together, these inconsistencies undermine the generalizability and ecological validity of current findings. It is crucial for future research to adopt sweetener-specific hypotheses to elucidate the distinct physiological effects of LCS. Accordingly, until sufficient human data from RCTs is available for each compound, conducting systematic reviews and meta-analyses on combined LCS should be considered scientifically unsound. Despite

advances in mechanistic insights, the extent to which alterations in the gut microbiota profile might contribute changes in postprandial glycemic responses associated with sucralose consumption remains highly contested, with findings that are both inconsistent and inconclusive. This is largely attributable to studies that were not originally designed to detect microbiota-related changes as a primary outcome, thereby limiting their ability to capture subtle shifts in the composition and function of bacterial communities. In light of the evidence presented in this review, there is a clear need for future studies to adopt standardized protocols for fecal sample collection and data analysis, and to ensure transparent and detailed reporting of microbiota outcomes to enhance the reproducibility and comparability of results. Although the current literature frequently implicates the gut microbiota as a potential mediator of sucralose-induced alterations in glucose tolerance, it remains plausible that these microbial shifts are reflective of broader, underlying physiological processes that are not yet fully elucidated. Despite many unanswered questions, one key takeaway emerges from this review: sucralose is not physiologically inert. Continued research is essential to disentangle the current evidence on its metabolic effects of sucralose on glucose homeostasis, particularly under realistic dietary conditions, to inform evidence-based dietary guidelines and clinical recommendations.

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Authors Contributions

A.P. conceived and designed the review, interpreted data and wrote the original manuscript; P.I. contributed to the conceptual development by providing critical feedback throughout the process, edited and reviewed the manuscript.

Declaration of interests

Authors declare no conflict of interest.

CHAPTER V. Thesis Article 4

A 3-Week Randomized Controlled Trial on the Effects of NATural Sugars, REfined Sugars, and LOw-Calorie Sweeteners (Sucralose) on Postprandial Glycemic Responses and Mechanistic Pathways under Real-World Conditions (NARELO study)

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ABSTRACT

Background : Low-calorie sweeteners have long been assumed to be metabolically and physiologically inert. Conflicting findings regarding the potential adverse effects of sucralose on glucose homeostasis have fueled ongoing debate, while substantial methodological variability across studies continue to hinder the establishment of a clear consensus.

Objectives : This study aimed to evaluate the effects of three-week cumulative consumption of sucralose-sweetened beverages, compared with sugar-sweetened beverages, on postprandial glycemic responses in healthy adults under real-world conditions. To provide mechanistic context, we assessed insulin concentrations, gut microbiota composition and functionality, and plasma biomarkers of intestinal permeability and systemic low-grade inflammation. Additionally, we explored whether 100% natural fruit juice (exploratory comparator) elicits distinct metabolic responses compared with sugar-sweetened beverages.

Methods : Using a randomized parallel-group design stratified by sex, 42 individuals were randomly assigned to consume either sucralose-sweetened beverages (LCS), sucrose-sweetened beverages (SSB), or 100% natural fruit juice (FJ) at dosage approximating real-world consumption. The beverages were matched for sweetness and reflected commercially available products. At pre- and post-intervention, two serial fecal samples were collected, body composition was assessed by DXA, and a 75 g oral glucose tolerance test was performed. Plasma concentrations of glucose (0, 30, 60, 90, 120 min), insulin (0, 120 min) as well as plasma biomarkers of intestinal permeability and systemic low-grade inflammation (0 min) were measured. Gut microbiota was analyzed for composition using 16S rRNA sequencing (α -diversity and β -diversity metrics) and for short-chain fatty acids (SCFA) and branched-chain fatty acid (BCFA) concentrations.

Results : Consuming sucralose-sweetened beverages for three weeks led to a significant increase in postprandial glycemic responses from baseline ($P_{\text{Bonf}} = 0.040$), despite no

change in insulin concentrations, an effect not observed in the SSB group ($P_{Bonf} = 1.000$). Clinically, this represented an 8.4% increase in postprandial glycemic responses in the LCS group, based on estimated marginal means (baseline: 5.00 mmol/L, 95% CI 4.36–5.64; post-intervention: 5.42 mmol/L, 95% CI 4.78–6.06). These findings occurred in the absence of statistically significant differences in body composition, gut microbiota composition, fecal concentrations of SCFAs and BCFAs, and plasmatic biomarkers of intestinal permeability and systemic low-grade inflammation (all group $\times$ condition $P \geq 0.083$). In exploratory analyses, consumption of FJ led to similar postprandial glycemic responses to those observed with SSB (group $\times$ condition $P = 0.099$).

Conclusion : This study provides evidence that realistic sucralose consumption over 21 days impairs glucose tolerance in healthy adults, challenging the prevailing assumption that all LCS are physiologically inert. Continued investigation into the underlying mechanisms driving these metabolic effects remains essential.

INTRODUCTION

Over the past decade, free sugar^s intake from beverages has decreased in North America^{8,9}. This decline is hardly coincidental; it reflects mounting evidence implicating sugar-sweetened beverages (SSB) in the pathogenesis of noncommunicable diseases and the resulting wave of public health regulatory measures, such as front-of-package labelling^{6,7}, aimed at reducing free sugar intake. Accordingly, this led the food industry to shift its marketing strategies towards promoting low-calorie sweeteners (LCS) as healthier alternatives¹⁸⁷. LCS such as sucralose, saccharin, aspartame, acesulfame K, and stevia offer the sweetness of sugar with fewer or none of the calories. Further, the food industry is rapidly innovating, with a primary focus on discovering new forms of LCS derived from natural sources¹⁸⁸, which tend to be more favorably received by consumers. By 2030, the global market for LCS is projected to reach an all-time high of 39.4 billion \$USD, mirroring the increasing demand for sugar alternatives worldwide¹⁰. As part of their most recent guidelines “*Use of non-sugar sweeteners*” released in 2023⁶⁸, the World Health Organization (WHO) conducted a systematic review and meta-analysis¹⁷⁵ of 283 studies. These studies assessed the effects of LCS intake on health outcomes including body weight, adiposity, type II diabetes, cardiovascular diseases, eating behaviors and sweet preference in healthy adults, children, and pregnant women. Based on the evidence from 97 prospective cohort studies, 47 case-control studies and 50 randomized-controlled trials (RCTs), the WHO issued a conditional recommendation that LCS should not be used as a mean of achieving weight control or reducing the risk of noncommunicable diseases⁶⁸. This conclusion stems from inconclusive evidence that substituting LCS for free sugars confers long-term health benefits beyond those achieved by simply reducing free sugar intake, rather than on evidence indicating potential harm associated with their consumption.

^s Free sugars are defined as any types of simple sugars added to beverages or food products as part of their transformation by food industries or preparation by the consumer, in addition to sugars naturally present in fruit juices, fruit juice concentrates, honey, and various syrups³.

For a long time, LCS were assumed to be metabolically and physiologically inert. Today, growing research interest regarding the potential metabolic effects of certain LCS has sparked heated debates within the scientific community as conclusions drawn by the WHO challenged many expert reviews^{67,70,172,174,189–192}. This is particularly evident when examining the effects of sucralose, the most extensively used LCS in food products and beverages^{11,14}, on glucose homeostasis. This lack of consensus is exemplified by conflicting findings, largely attributable to substantial variability in study outcomes and experimental designs across RCTs. Among the RCTs included in the recent WHO systematic review and meta-analysis, 13 studies involved sucralose, of which only eight assessed glycemic outcomes^{60,69,75,89,179,193–195}. Findings from studies assessing the effects of various LCS on fasting plasma glucose concentrations are frequently interpreted and cited as evidence that sucralose has no effect on glycemic responses^{23–25}. However, this interpretation overlooks the limitation of fasting glucose measurements in detecting early or subtle impairments in glucose tolerance, particularly in healthy individuals. Epidemiological evidence indicates that postprandial glycemia is a stronger predictor of cardiometabolic outcomes than fasting blood glucose^{29–31}. Specifically, postprandial hyperglycemia has been shown to double cardiovascular mortality risk in non-diabetic individuals despite normal fasting glucose³⁰. Beyond epidemiological associations, mechanistic evidence indicates that postprandial hyperglycemia was associated with oxidative stress and endothelial dysfunction independently of HbA1c and fasting glucose³². This suggests that postprandial glycemic excursions may contribute to microvascular damage even when conventional glycemic markers remain within normal ranges. Another source of heterogeneity in study outcomes stems from the methodological approach adopted to investigate LCS consumption under either acute or cumulative exposure, each of which may yield distinct metabolic effects. In humans, the effects of LCS on postprandial glycemic responses have been most extensively studied under acute conditions^{64–66}. Conversely, the WHO's systematic review and meta-analysis¹⁷⁵ focused exclusively on repeated exposure, including RCTs with a minimum intervention duration of seven days for glycemic outcomes. Acute exposure typically involves using a preload

design, which assesses the acute impact of a single LCS dose on glucose tolerance. However, from a clinical perspective, evaluating the cumulative effects of frequent consumption is more relevant as any potential adverse or beneficial metabolic effects are more likely to arise from long-term dietary patterns rather than isolated exposures. Further adding to the complexity of LCS research is the consistent evidence that each LCS exerts distinct physiological effects and therefore, must be examined using sweetener specific hypotheses^{13,67–69}. Each LCS possesses a unique chemical structure that influences how it is digested, absorbed, metabolized, and excreted³⁷. Nevertheless, systematic reviews and meta-analyses continue to draw inference on LCS as a collective “class of compounds” rather than analyzing them individually. Finally, recent narrative reviews, commentaries, and expert consensus^{67,189–192}, systematic reviews and meta-analyses^{70,172–175} emphasized the importance of the comparator in RCT design. In case of LCS, direct comparisons between LCS and SSB provide more real-world relevance than comparisons between LCS and water since LCS-sweetened beverages are marketed as alternatives to SSB, not as alternatives to water.

To address limitations in the current literature, we evaluated the effects of a 3-week consumption of sucralose-sweetened beverages on postprandial glycemic responses, compared with sugar-sweetened beverages, in healthy adults under real-world conditions. As secondary outcomes, we investigated parameters identified in previous research as potential mechanisms influencing glucose homeostasis, including insulin concentrations, gut microbiota profile, intestinal permeability, and systemic low-grade inflammation. These measures were incorporated to provide mechanistic context for interpreting glycemic outcomes, should differences be observed. Finally, we explored whether free sugars derived from a natural source (100% natural fruit juice) elicit distinct metabolic responses compared to refined sugars provided in SSB. This group was included as an exploratory arm, given the severely limited empirical evidence comparing fruit juice consumption with SSB on metabolic outcomes⁴.

METHODS

Ethics Approval

This study consisted of a single-blind, parallel arm, randomized, stratified by sex, controlled trial conducted at the University of Ottawa's Behavioural and Metabolic Research Unit. The University of Ottawa's Health Sciences and Science Research Ethics Board approved the protocol (ethics file number: H-04-20-5636), agreeing with the Declaration of Helsinki, except for registration in a publicly accessible database before the start of recruitment. This trial was registered at clinicaltrials.gov *a posteriori* (ClinicalTrial.gov ID: NCT07194772). All individuals provided written and informed consent before participating.

Participants

Recruitment

A total of 222 screening questionnaires were reviewed to determine the eligibility of potential participants. Forty-seven individuals met all inclusion criteria and were invited to a preliminary session, where final eligibility was confirmed. Among the 47 individuals who underwent a preliminary session, two did not meet inclusion criteria and two declined to participate and were therefore not enrolled in the study. As a result, 43 individuals (22 women and 21 men) were recruited with this purposive sampling approach. Of the 43 enrolled participants, one woman withdrew from the study for personal reasons after completing the baseline experimental session and undergoing seven days of study beverages. Data from this participant was not included in the analyses. The recruitment process continued until 42 participants (21 men and 21 women). Recruitment began in December 2021 and the last participant was recruited in June 2024. Data collection took place from March 16, 2022, to June 27, 2024, with a one-year interruption from September 2022 to September 2023 due to the first author's parental leave.

Screening Process

Inclusion criteria included ages 18-35 y, body mass index (BMI) between 18.0 and 34.9 kg/m², waist circumference < 102cm (men) and < 88cm (women) and a self-reported stable weight during the prior 6 months ($\pm$ 2 kg) of beginning the study. Exclusion criteria consisted of (fasting glucose $\geq$ 6.1 mmol/L), blood pressure (> 140/90 mmHg), self-reported medical history of anemia, autoimmune diseases, diabetes (type I and II), dyslipidemias (including hypercholesterolemia and hypertriglyceridemia), heart disease, hypertension, kidney disease, liver disease, thyroid disease, and surgery for weight loss. In addition, individuals who smoke tobacco (including vaping), habitually ingest > 2 alcoholic beverages/day, use cannabis or cannabis-derived products > 1 time/month^t, exercise > 3.5 h/week at a level more vigorous than walking, or use thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant/antianxiety and weight-loss medications as well as antibiotics in the prior 3 months were excluded. Considering the multitude of medications known to indirectly affect inflammation, participants were additionally required to report any other medications to allow evaluation of potential interactions with inflammatory biomarkers and determine whether these might compromise study eligibility. Finally, individuals susceptible to exhibiting clinically relevant levels of food cravings as indicated by a score > 50 on the *Food Cravings Questionnaire – Trait reduced* (FCQ-T-r)¹⁹⁶, were also excluded. FCQ-T-r (15 items) is a validated questionnaire¹⁹⁷ that assesses four factors: (1) preoccupation with food (i.e., obsessive thought about food and eating), (2) loss of control (i.e., difficulty regulating eating behavior when exposed to food cues), (3) positive outcome expectancy (i.e., believing eating to be positively reinforcing), and (4) emotional craving (i.e., tending to crave food when experiencing negative emotion). All eligibility criteria were carefully chosen to select healthy individuals and to control for confounding factors that might have a direct or indirect influence on metabolic parameters measured in this study. All women who participated in this study were premenopausal. Because the inclusion of women is essential for generating representative and high-quality

^t Participants who reported using cannabis or cannabis derived products less than once per month were asked to refrain from using these products for the whole duration of the study to meet inclusion criteria.

data, additional criteria related to menstrual cycle phase and the use of hormonal or non-hormonal contraceptives were applied to meet inclusion requirements. Details are provided in the supplementary files.

Additional Inclusion Criteria for Women Related the Menstrual Cycle and the use of Hormonal and Non-Hormonal Contraceptives.

All women who participated in this study were premenopausal. Because the inclusion of women is essential for generating representative and high-quality data, additional considerations were applied to meet inclusion criteria: **women using no form of hormonal contraception or using copper intrauterine device (IUD)^u** were required to report having a regular menstrual cycle to be included; **women using anovulants** were advised to take their daily anovulant continuously for a period of 28 days to ensure stable hormone levels throughout the study (informed consent was obtained). Oral contraceptives release a low and stable daily amount of progestins, which were reported to have minor or no effects on glycemic responses of pre-menopausal women in a recent meta-analysis¹⁹⁸; **women using hormonal contraception such as hormonal intrauterine device (IUD)^v and subdermal contraceptive implant^w** were included regardless of their menstrual cycle. Like anovulants, hormonal IUD release a low and stable amount of a progestin (levonorgestrel) into the uterus, therefore preventing periodic fluctuations in hormonal levels during the menstrual cycle. Since Levonorgestrel was included as one of the progestins in the aforementioned meta-analysis¹⁹⁸, it was therefore assumed that hormonal IUD would also not have a meaningful impact on glycemic responses, especially as its effect is local rather than systemic; **women using medroxyprogesterone acetate**

^u Copper IUD release copper ions into the uterus which make the uterus and the fallopian tubes inhospitable to sperm, thus preventing fertilization of an egg.

^v Hormonal IUD releases a low and stable amount of a progestin, levonorgestrel, into the uterus. Progestins are used as a long-term contraceptive by thickening the cervical mucus, thus preventing sperm from entering the uterus for fertilization of an egg.

^w Subdermal contraceptive implants release a low and stable amount of a progestin, etonogestrel, from a small rod placed under the skin. Progestins are used as a long-term contraceptive by thickening the cervical mucus, thus preventing sperm from entering the uterus for fertilization of an egg.

injections (Depo-Provera shot)* were excluded given the pro-inflammatory effects associated with high doses of progestin; **women experiencing pregnancy or lactation** were excluded.

Protocol

Timeline

Each participant completed a preliminary session and two experimental sessions (baseline and post-intervention). Participants were asked to come to the laboratory at ~8h00 AM after a 12h overnight fast and were instructed to 1) refrain from consuming alcoholic beverages and using nonsteroidal anti-inflammatory drugs (e.g., ibuprofen (Advil™), Diclofenac diethylamine (Voltaren™ topical gel), Naproxen (Aleve™)) for 24 h preceding each session; 2) refrain from exercising for 12 h before each session; 3) drink plenty of water (~500 mL) to promote euhydration during the session. In addition, participants were given a standardized meal (President's Choice Meat lasagna or Blue Menu Three-cheese macaroni, 365 kcal, 45% carbohydrates, 25% proteins, 30% lipids) to eat before starting the 12 h overnight fast for both experimental sessions. The baseline experimental session marked the beginning of the study on day 0. The post-intervention session occurred on day 22 after drinking 21 days of study beverages.

Preliminary Session

Height was measured without shoes using a stadiometer (Perspective Enterprises, USA). Body weight was measured without shoes using a beam scale (Tanita, USA). Waist circumference was measured following the WHO's protocol and using a measuring tape¹⁹⁹. Blood pressure was measured using an automatic sphygmomanometer on the left arm (OMCRON Automatic blood pressure monitor, model: BP755CAN-CD6, USA), and fasting blood glucose was measured using a blood glucometer (Bayer Contour next, Canada), a

* Medroxyprogesterone acetate contains a progestin injected every 3 months in a higher dosage than hormonal IUDs or subdermal contraceptive implants, which suppresses ovaries from releasing an egg and thickens cervical mucus, thus preventing ovulation and fertilization.

lancing device, and lancets (TouchSense, USA). Supplementary information was obtained through an in-person screening questionnaire which included a comprehensive list of prescribed medications, dietary supplements, along with detailed information of participants' menstrual cycle if applicable. Participants were then asked to complete three online questionnaires about their dietary habits (*NIH DHQ II month modified questionnaire*) and their relationship with food cravings (FCQ-T-r) accessible with a QR code. Upon confirmation of eligibility, the consent form was carefully reviewed with the participant in person. All individuals provided informed written consent before taking part to the study. Resting metabolic rate was measured using indirect calorimetry (VIASYS Healthcare, USA) in a thermoneutral dark room for 30 min²⁰⁰. Daily energy expenditure was estimated by multiplying resting metabolic rate by a physical activity factor of 1.55, which corresponds to light to moderate intensity exercise performed three to five days per week²⁰¹. Finally, participants were provided with instructions and material for proper collection of two 10 mL fecal samples at home on separate days, within three days prior to their baseline experimental session (day 0). They also received specific dietary instructions to follow for the total duration of the study (21 days) and for a 7-day pre-intervention period preceding the baseline experimental session (day 0). More precisely, participants were asked to 1) maintain their usual *ad libitum* diet while refraining from consuming sugar-sweetened beverages, fruit juices, and foods and beverages that contain LCS (education and documentation were provided to participants by a registered dietitian) and 2) refrain from drinking more than two alcoholic beverages per day, using cannabis^y and taking any vitamin, mineral, dietary, probiotic, natural, herbal (including herbal tea) or sports supplements, and 3) maintain their usual physical activity and lifestyle habits.

Experimental Design

This study employed a single-blind, randomized, stratified by sex, parallel-arm design. Following the preliminary session, participants were randomly assigned to one of the three experimental groups (n = 14 per group; seven women and seven men per group): **LCS :**

^y Cannabis is known to influence appetite regulation and inflammatory processes^{249,250}.

sucralose-sweetened beverages (Splenda® Original granulated sweetener), **SSB: sucrose-sweetened beverages** or **FJ: 100% natural orange juice** (Simply Orange Pulp free, Coca-Cola™). The primary aim was to investigate the metabolic effects of consuming sucralose-sweetened beverages in a real-world context, using sucrose-sweetened beverages as the main comparator; not to assess the effects of substituting free sugars with LCS in terms of energy displacement. Within this framework, this study was powered for a two-group comparison between the LCS and the SSB group using a sweetener-specific hypothesis for sucralose. To provide insight into the metabolic effects of naturally occurring free sugars relative to free sugars derived from refined sources, the FJ group was then added as an additional exploratory arm. In light of the severely limited empirical evidence comparing FJ consumption with SSB⁴ or to any kinds of LCS on metabolic outcomes, we deemed the formulation of a specific hypothesis scientifically unwarranted. Consequently, findings for this group are presented as exploratory. (see *Sample size determination* for details).

Experimental Sessions: Day 0 and Day 22

Height, body weight, waist circumference, body composition, and blood pressure were measured during the baseline (day 0) and post-intervention (day 22) experimental sessions. Body composition analysis was performed using dual-energy X-ray absorptiometry (General Electric Lunar Prodigy, USA) preceding a 75 g oral glucose tolerance test (OGTT). To perform the OGTT, an intravenous catheter was inserted via an antecubital venipuncture and a fasting blood sample of 12 mL was drawn. This fasting sample was used for time 0 of OGTT, but also for measuring systemic inflammatory biomarkers and female sex hormones. Participants were given 5 min to drink a solution containing 75 g of glucose (Trutol™, Thermo Scientific). Blood draws of 12 mL were collected in EDTA-coated tubes (BD Vacutainer, USA) at 30, 60, 90 and 120 min following the ingestion of the glucose solution. Plasma was immediately separated from whole blood by centrifugation at 3,600 RPM (2,000 RCF) for 10 min at 4 °C. Plasma was then aliquoted and stored at -80 °C until analysis. Participants drank water *ad libitum*

throughout the session. Light snacks were provided before the participants were sent home with a one-week supply of study beverages. The study beverages were stored frozen in reusable bottles until they were distributed to participants in insulated lunch bags, with instructions to keep them refrigerated. Participants were instructed to consume their individualized daily dose in a minimum of three separate servings spread throughout the day (see *Formulation and Dosage of Study Beverages* for more details). Weekly visits were scheduled for participants to return their empty bottles and receive their next week's supply of beverages. On the third week, participants were also provided with a standardized meal and materials for collecting two 10 mL fecal samples at home on separate days, within three days prior to their post-intervention session (day 22). Participants were explicitly instructed not to wash or rinse the bottles, as any remaining liquid was weighed during their weekly visits (average of remaining liquid < 5.0 g/day per participant). The reusable bottles were then cleaned and sanitized in a commercial dishwasher.

Standardization of Menstrual Cycle Phase Across Experimental Sessions

Women using no form of hormonal contraception or using copper IUD self-reported their last menstruation to predict the next two cycles. Experimental sessions were scheduled during the early follicular phase (i.e., first 7 days after the start of menstruation) of each cycle to minimize any potential confounding effect of female steroid sex hormones (e.g., β -estradiol, progesterone) on glycemic and inflammatory responses. To do so, participants with longer cycle duration were instructed to delay consumption of study beverages by a few days after their baseline experimental session to ensure that post-intervention sessions consistently took place following 21 days of study beverages consumption (on day 22) for all participants.

Formulation and Dosage of Study Beverages

SSB were formulated to achieve a total sugar concentration equivalent to that of 100% natural orange juice, specifically 99.47 g/L⁵. To match the sweetness of SSB, LCS were formulated according to Splenda® manufacturer information provided on product packaging, yielding a concentration of 11.94 g/L^z. This formulation resulted in a pure sucralose concentration of 143.3 mg/L^{aa}, which represents a realistic comparator to commercially available beverages such as Diet Mountain Dew™ (50.7 mg/L) and Diet Rite Cola™ (191.5 mg/L)^{53bb}. SSB and LCS were of similar color and taste, flavored with Kool-Aid™ orange unsweetened drink mix supplemented with vitamin C (10% DV)^{cc} (Kraft Foods Group, INC.) for blinding and palatability. All components of study beverages are detailed in a STAR Methods key resource table available in the *Supplementary Files*. Participants in the SSB and FJ groups consumed a daily volume of beverages to provide 15% of their estimated total energy expenditure in the form of free sugars. This dosage was based on the average free sugar consumption of Canadian adults at the time of the study's conceptualization, which was estimated to be 15% of energy requirements (Ereq) with 11-13% of Ereq coming from added sugars²⁰², and another estimated 2.2% of Ereq coming from fruit juice²⁰³. Recent studies using the 2015 Canadian Community Health Survey estimated that free sugar consumption in Canada ranges from 12-13.3% Ereq^{2,204}, aligning closely with the dose used in the present study. Since sucralose does not contain free

^z More precisely, 3.1 g of Splenda® Original granulated Sweetener (containing 37.2 mg of pure sucralose) was used to achieve the sweetness provided by 100 kcal of sucrose resulting in a relative sweetness ratio of 1:694, whereas 3.6 g (containing 43.1 mg of pure sucralose) would have been needed for the ideal ratio of 1:600 (sucralose is perceived to be 600 times sweeter than sucrose on a weight-for-weight basis^{23,36,37}).

^{aa} According to the manufacturer's website (as of 2025), Splenda® Granulated Sweetener contains 1.2% pure sucralose by weight²⁵¹. In contrast, an independent analysis conducted in 2008 reported a pure sucralose concentration of 1.1%²⁵². Given the more recent and product-specific nature of the manufacturer's data, the 1.2% value was used in this study to reflect the current formulation.

^{bb} Manufacturers are not required to disclose the amount of LCS used in their products⁴⁶. These specific beverages are cited here as their formulation was made available in the scientific literature.

^{cc} As of January 2020, the daily value for vitamin C in the United States increased from 60mg to 90 mg. The nutrition facts table on the Kool-Aid™ orange unsweetened drink mixes has a DV reference of 60mg.

sugars, the daily volume of beverages for participants in the LCS group corresponded to the volume they would have received if they had been part of the SSB or FJ groups.

Plasma Analyses: Glucose-Insulin Dynamics, Intestinal Barrier Permeability, Systemic Low-Grade Inflammation, and Sex Hormones Profiling

All biological samples were anonymized and analyzed by qualified personnel who were blinded to group allocation. Plasma glucose levels were measured in duplicate at 0, 30, 60, 90, and 120 min following the 75 g OGTT, while plasma insulin levels were analyzed in duplicate at 0 and 120 min. Analyses were performed using colorimetric assays according to the manufacturer's instructions, following previously published methods from our group²⁰⁵. Out of 112 fasting samples, 21 insulin values (LCS = 11; SSB = 10) fell below detection range of the assay kits, which is a common occurrence when measuring fasted insulin levels. These missing values were retained in the linear mixed-effects models by substituting them with the lowest detectable value (1 μ U/mL), in accordance with standard practice for handling left-censored data. Plasma lipopolysaccharide-binding protein (LBP) (i.e., an indirect measure of endotoxemia and intestinal barrier permeability), zonulin (i.e., a mediator of intestinal permeability), as well as interleukin-6 (IL-6), C-reactive protein (CRP), and tumour necrosis factor-alpha (TNF- α) (i.e., systemic low-grade inflammation biomarkers) were measured in fasting plasma samples (0 min) by commercially available ELISA kits according to the manufacturer's instructions. Plasma levels of sex hormones β -estradiol and progesterone were analyzed (in women only) in duplicate in fasting plasma samples (0 min) using commercially available ELISA kits according to the manufacturer's instructions, following previously published methods from our group²⁰⁵. Additional details regarding all biological sampling are provided in the STAR Methods key resource table available in the *Supplementary Files*.

Gut Microbiota Composition and Functionality: DNA Extraction, 16S rRNA Sequencing, and Short-Chain Fatty Acids Quantification

Serial Sampling

Participants collected two 10 mL fecal samples on separate days within the three days preceding both their pre- and post-experimental sessions. Collecting two serial samples per time points allowed for subsequent pooling of samples for analyses to obtain a more representative profile of the gut microbiota composition¹³⁰ (see *Supplementary Files* for more details on aliquoting methods). Each participant received a standardized sample collection kit, which included a 2 (+ 2 extras) disposable paper liners, two pre-labeled sample vials with integrated spatulas, labelling materials, a cold transport container, a biohazard-labeled transparent plastic bag, and a detailed bilingual instruction booklet (English/French). All collection steps were demonstrated in person using the provided materials to ensure participants understood the sampling procedure. Participants were instructed to label each vial with the date and time of collection and to wash their hands prior to handling materials. Feces were collected using a disposable paper liner affixed to the toilet seat. Approximately 10 mL of fecal material was transferred into a single vial using the integrated spatula, ensuring the sample reached the 10 mL fill line. The filled vial was immediately placed into the pre-frozen cold transport container (frozen for a minimum of 12 h), sealed in a biohazard bag, and stored in the participant's home freezer at -20 °C until the day of their experimental session (within a 3-day window). For transport to the laboratory, participants were instructed to place the sealed biohazard bag containing the frozen transport container into a Styrofoam case enclosed in a resealable plastic bag immediately before leaving home. They were explicitly advised not to allow the samples to remain at room temperature. Upon arrival at the research facility, all labeled fecal samples were promptly transferred to a -80 °C freezer for storage until processed for analyses. Frozen samples were aliquoted into smaller tubes and pooled within each time point for subsequent analyses. Additional details are provided in the STAR Methods key resource table (see the *Supplementary Files* for more details).

Aliquoting

Fecal samples were removed from the -80°C and promptly placed on dry ice, along with labeled 2.0 mL polypropylene corning screw cap tubes (Axygen, USA). A ceramic tile covered in 2 layers of tin foil on dry ice was used as a cutting board to prepare fecal samples aliquots for downstream applications. Each fecal sample tube was dipped halfway into a beaker filled with lukewarm water for approximately 10 seconds to allow easier removal of the fecal sample from the sample vial. The bottom of the sample vial was then cut using a heavy-duty razor blade and sample was pushed out of the vial using a disposable, sterile 10cc syringe piston. Stainless steel knives cleaned with RNase Away were used to cut fecal samples into smaller fragments. These fragments were then transferred to four pre-chilled 2.0 mL cryovials kept on dry ice until each tube was half-full. The process was repeated using the second sample from the same time point for the same participant, which fragments were transferred to the 4 cryovials already containing aliquots of the first fecal sample so that each cryovials contained a roughly 50-50 mixture of both fecal samples collected at the same time point for each participant. To expedite the process and minimize degradation of fecal samples, aliquoting was carried out by two individuals working in tandem, each performing specific tasks. Aliquots were promptly stored at -80°C for subsequent analysis. The tin foil covering the ceramic tile and the gloves worn were replaced after processing each fecal samples while all other equipment was cleaned with bleach solution (12%) and RNase Away between samples. Additional details are provided in the STAR Methods key resource table (see *Supplementary Files* for more details).

DNA Extraction

DNA was extracted using the QIAamp Fast DNA stool mini kit (Qiagen) according to manufacturer's instructions. Briefly, 1mL of InhibitX buffer and five ceramic beads were added to the tube which was subsequently loaded into a homogenizer (TissueLyser II, Qiagen). The samples were homogenized for 45 seconds, followed by a 1 min break. This cycle was repeated 2 times for a total of three cycles. The homogenized mixture was then

transferred to a labeled 1.5 mL tube with a locking cap and incubated at 95 °C for six min on a dry bath (Bio-Rad). Tubes were then briefly vented to release pressure, vortexed for 15 seconds and centrifuged for 2 min at 14,000 RPM (18,000 RCF). 400 µl of the supernatant was then transferred to a 2.0 mL tube containing 25 µl of protein kinase. Tubes were then vortexed for 15 seconds, 400 µl of Buffer AL was added tubes, vortexed again for 15 seconds and then incubated at 70 °C for 10 min. After adding 400 µl of 100% ethanol to each tube and vortexing for 15-30 seconds, the sample mixture was transferred to a Qiagen spin column and centrifuged at 14,000 RPM (18,000 RCF) for 1 min and the filtrate was discarded. The spin column was then washed sequentially with 500 µl of Buffer AW1 and 500 µl of Buffer AW2 and centrifuged to dryness. The spin column was finally transferred to a labeled 1.5 mL tube, and 100 µl of Buffer ATE applied directly to the membrane. The column was incubated for 1 min at room temperature and was then centrifuged to elute the DNA. DNA concentration and quality were measured using the Gen5 package on a Synergy HT plate reader. Additional details are provided in the STAR Methods key resource table (see the *Supplementary Files* for more details).

16S rRNA Sequencing

The 16S rRNA library (V3-V4 region) was prepared as described previously²⁰⁶ using the primers outlined in the STAR Methods Key resource table (see *Supplementary Files* for more details). The resultant product was sequenced using an Illumina Miseq (Illumina, San Diego, USA) following the manufacturer's guidelines. The resultant FASTQ files were imported and analyzed using Qiime2 (version 2023.5)²⁰⁷, following previously published methods from our group²⁰⁶. To elaborate, following demultiplexing, sequences were denoised and chimera-filtered using DADA2²⁰⁸. Features were aligned with MAFFT²⁰⁹ and the phylogenetic tree was generated with FastTree²¹⁰. Taxonomy was classified using GreenGenes classifier 13.8²¹¹ and the sklearn naïve Bayes classifier²¹². Finally, the features were filtered to include only those with a frequency of 5000 and present in at least 20 samples.

Short-Chain Fatty Acids Extraction and Quantification

Short-chain fatty acids (SCFAs: acetate, propionate, butyrate, valerate) and branched short-chain fatty acids (BCFAs: isobutyrate, isovalerate) were extracted from fecal samples as described previously²⁰⁶ with minor modifications. Fecal aliquots were removed from the -80 °C and placed into a benchtop Freeze Dryer for 48 h to ensure complete removal of moisture. The dried feces were weighed, mixed with 10 times the dry weight in water, and incubated at room temperature for 3 h with vortexing every 30 min for 15 seconds. The samples were then centrifuged three times at 14,000 RPM (18,000 RCF) with supernatant collected after each centrifugation step, and 10ul of internal standard (5.5 mM 2-ethylbutyric acid in 100% Formic acid) was then added for each 100ul of resulting supernatant. The mixture was then filtered using 0.20 µm filters, then placed into glass inserts inside 2 mL glass snap top amber vials with 11mm Snap Caps. SCFA concentrations were assessed by standard curve on the gas chromatograph (GC) (Shimadzu, Kyoto, Japan; Nexis GC-2030), using a serial dilution of Volatile Free Fatty Acid Mix. The GC was equipped with a 0.25 µm column (Restek, #RSK-10226), a 10 µL syringe with 0.63mm OD (Shimadzu, Kyoto, Japan; SZ-221-34618-00), and a wool deactivated split glass insert (Shimadzu, Kyoto, Japan; SZ-220-90784-5). The samples were injected in triplicate, with a 1 µL volume. The column flow rate was set at 1.5 mL/min at a 20:1 split ratio, with helium as the carrier gas. The initial oven temperature of 100 °C/min was increased to 200 °C, and the detector temperature was set to 300 °C. The concentrations of two to three clean injections were averaged and used as the final value for each sample. Additional details are provided in the STAR Methods key resource table (see the *Supplementary Files* for more details).

Statistical Analyses

Differences in participant characteristics between experimental groups (LCS, SSB, FJ) at study enrolment were assessed using a one-way analysis of variance (ANOVA). Changes in participant characteristics between baseline and post-intervention were compared using

linear mixed-effects models with group (LCS, SSB, FJ) and experimental condition (baseline, post-intervention) as fixed effects, and participant ID as a random intercept. The effect of consuming LCS vs SSB on glycemic responses and insulin concentrations to a 75 g OGTT was evaluated using linear mixed-effects models. For glycemic responses, the model included group, condition, and time (30, 60, 90, and 120 min) as fixed effects, participant ID as a random intercept, and fasting plasma glucose concentrations as a continuous covariate. The exclusion of the fasting time point (0 min) as a categorical predictor in all glycemic responses models was an explicit design choice as the three-week intervention was hypothesized to affect postprandial plasma glucose concentrations rather than fasting glucose concentrations. Therefore, the current approach allows for a better evaluation of changes to the postprandial glycemic response to the OGTT, while also considering the interindividual variability in fasting glycemia. The inclusion of fasting glucose as a continuous covariate improved model fit, as indicated by a lower Akaike's Information Criterion (AIC) value. To assess potential improvement in model performance, additional participant characteristics (age, sex, body weight, BMI, waist circumference, fat mass percentage, and ethnicity) were individually incorporated as covariates. None of these inclusions produced a reduction in AIC relative to the model with fasting glucose. For insulin concentrations, the model included group, condition, and time (0 and 120 minutes) as fixed effects and participant ID as a random intercept. In exploratory analyses, the same modeling approach used for glycemic responses and insulin concentrations was applied to compare FJ to SSB and to LCS in two distinct pairwise comparisons. A three-group comparison including FJ, SSB, and LCS was then conducted to further explore differences in postprandial responses across all conditions. All subsequent secondary outcome analyses were conducted using both a three-group comparison (LCS, SSB, FJ) and pairwise comparisons (LCS, SSB – FJ, SSB – FJ, LCS), to maintain consistency with the modeling approach applied to glycemic responses and insulin concentrations. Changes in plasma concentrations of β -estradiol, progesterone, LBP, zonulin, IL-6, CRP, and TNF- α were assessed using linear mixed-effects model with group and condition as fixed effects and participant ID as a random intercept. For all linear mixed-effects models, assumptions

such as homoskedasticity and normality of residuals were evaluated through visual inspection of diagnostic plots. If data were not normally distributed, as determined by the Shapiro–Wilk test, a logarithmic transformation was applied prior to analysis. For ease of interpretation and to maintain clinical relevance, all data are presented in their original units rather than in log-transformed form. All normally distributed data are presented as mean (standard deviation) and non-normally distributed data are presented as medians (interquartile range), unless stated otherwise. Reported *P*-values were adjusted for multiplicity using the Bonferroni technique. A two-sided $P < 0.050$ was considered statistically significant. The statistical analyses were conducted using *jamovi* (version 2.3.28 with the *gamlj* module, version 2.6.6). Figures were created using GraphPad Prism (v.9.51; GraphPad Software, USA).

Gut microbiota α -diversity metrics (i.e. within-sample communities) were analyzed using the Qiime2 core-metric method, with a sampling depth of 9794. These included Shannon index (captures both species richness (number of distinct taxa) and evenness of their abundance distribution), Pielou's evenness index (evenness), observed features (richness), and Faith's phylogenetic diversity (richness and evolutionary breadth)¹⁸⁴. Differences in α -diversity metrics, as well as differences in fecal concentrations of SCFAs and BCFAs were assessed using linear mixed-effects model with group and condition as fixed effects and participant ID as a random intercept. Gut microbiota β -diversity was assessed with the Bray–Curtis dissimilarity, which quantifies differences in microbial community structure (species presence and relative abundances) between samples (i.e., within an individual across two conditions, here baseline and post-intervention). Bray–Curtis dissimilarity was first analyzed using Qiime2 core-metrics imported into R studio (R core team, V4.4.2). The principal coordinate analysis (PCoA) was performed using the *ape* package²¹³ and visualized using *ggplot2*²¹⁴, followed by permutational multivariate analysis of variance (PERMANOVA) to assess differences between groups. The analysis was conducted using the *adonis2* function from the *vegan* package in R (version 4.4.2), with 999 permutations²¹⁵. Taxonomic differences at the phylum and genus levels were evaluated using centered log-ratio transformed abundances to account for the compositional nature

of microbiome data²¹⁶. Group-wise comparisons were conducted using Wilcoxon rank-sum tests, and *p*-values were adjusted for multiple testing using the Benjamini–Hochberg procedure to control the false discovery rate at 10%. Taxonomic bar plots representing mean relative abundances were generated from mean-filtered reads using the *dplyr*²¹⁷ and *ggplot2* packages²¹⁴.

Sample Size Determination

This study was powered to detect a weight-independent change (Δ) in postprandial blood glucose in the LCS (sucralose) group of at least 0.6 mmol/L higher ($\uparrow$ 12%) than the Δ blood glucose in the SSB group in response to a 75 g OGTT, with a probability of a type I error (α) of 0.05. Due to the scarcity of human RCTs examining the effects of sucralose-sweetened beverages on postprandial glycemic responses at doses within the acceptable daily intake (ADI) at the time of study conceptualization, the sample size calculation was based using the means and the standard deviations of the incremental peak for blood glucose from Pepino et al. (2013)²¹⁸, despite substantial differences in study design. Specifically, Pepino and colleagues assessed acute glycemic responses to a single sucralose exposure using a preload design, whereas our study evaluated the impact of daily consumption over a three-week period. In their study, metabolically healthy participants living with obesity received a fixed dose of 48 mg of sucralose dissolved in 60 mL of water, resulting in a relative sweetness intensity much higher than that of commercially available beverages. Additionally, water was used as a comparator, whereas SSB were used in the present study. Despite these major differences, Pepino et al. (2013)²¹⁸ provided the most relevant available estimates of variability in postprandial glucose responses to inform the power analysis at the time. It was estimated that a sample size of 14 participants per group would be sufficient to detect a significant difference between the LCS and the SSB group at $P < 0.050$, 80% power in a two-group comparison using G*Power (v.3.1.9.7)²¹⁹.

RESULTS

Participant Characteristics

Participant characteristics at baseline are presented in [Table 1](#). Age, body weight, BMI, waist circumference, fat mass percentage, and fasting plasma glucose concentrations were similar across experimental groups (all $P \geq 0.231$) and fell within clinically normal ranges. No statistically significant changes in body weight, BMI, waist circumference, or fat mass percentage were detected after the three-week intervention (all $P \geq 0.198$).

Corresponding post-intervention characteristics are presented in [Table 2](#). Responses to the validated *NIH DHQ II month modified questionnaire*, completed during the preliminary session, indicated that nearly all participants either never consumed or consumed the following beverages less than once per week: 100% natural orange juice (92.9%), 100% other natural fruit juices (85.7%), fruit drinks (95.2%), soft drinks (81.0%), sports drinks (92.9%) and energy drinks (88.1%). When they did consume these beverages, approximately one third of participants reported “*sometimes*” opting for diet or low-calorie versions. We considered this frequency of intake to reflect low consumption of LCS. Plasma concentrations of sex hormones and self-reported use of contraceptives in women are presented in [Table 3](#). β -estradiol and progesterone concentrations were similar in women across groups and conditions (all $P \geq 0.234$). However, plasma concentrations of β -estradiol and progesterone exceeded the normal ranges for the early follicular phase of the menstrual cycle (5th to 95th percentile: 80–266 pmol/L for β -estradiol; 0.32–1.91 nmol/L for progesterone)²²⁰ in 8 and 14 participants, respectively, during at least one of two experimental sessions. Potential contributors to these elevations included continuous hormonal contraception and the occurrence of some sessions outside the intended early follicular phase. Despite careful planning, the inherent unpredictability of menstrual cycles resulted in 14 of 42 sessions falling outside this phase due to earlier or delayed menstruation. Specifically, seven sessions occurred during the late luteal phase, three between day 8 and 10 of the follicular phase, and four on or after

day 12. These deviations were primarily attributable to longer-than-estimated cycle lengths or missed menstruation.

Sucralose Consumption for 21 Days Impairs Postprandial Glycemic Responses Under Real-World Conditions, While Sugar-Sweetened Beverages Do Not.

Postprandial plasma glucose concentrations during OGTT, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS) or SSB are presented in **Figure 1**. There were no statistical differences observed in fasting plasma glucose concentrations across groups, conditions and their interaction (all $P \geq 0.213$). At baseline, a statistically significant group $\times$ time interaction ($P = 0.017$) was detected in plasma glucose concentrations during the OGTT. However, Bonferroni-corrected post hoc comparisons between groups at each corresponding time points (30, 60, 90, and 120 min) revealed no statistically significant differences (all $P_{Bonf} \geq 0.160$). This suggests that the interaction effect was driven by the overall trajectory rather than by differences at corresponding time points, supporting the interpretation that glycemic responses to OGTT were comparable between the LCS and SSB groups at baseline. Although not statistically significant, a consistent trend toward slightly lower plasma glucose concentrations was observed in the LCS group across all time points. When comparing baseline and post-intervention conditions between the LCS and SSB groups, significant interactions were observed for group $\times$ condition ($P = 0.009$) and group $\times$ time ($P = 0.006$). Post hoc analyses revealed that glycemic responses significantly increased from baseline to post-intervention in the LCS group ($P_{Bonf} = 0.040$), whereas no statistically significant change was detected in the SSB group ($P_{Bonf} = 1.000$). Based on estimated marginal means, plasma glucose concentrations were 8.4% higher following the three-week consumption of LCS-sweetened beverages (baseline: 5.00 mmol/L, 95% CI 4.36–5.64; post-intervention: 5.42 mmol/L, 95% CI 4.78–6.06). Although a group $\times$ time interaction was found between the LCS and SSB groups, post hoc analyses did not reveal any statistically significant differences for the same time point between groups (all $P_{Bonf} \geq 0.401$). There were no significant differences in insulin concentrations between baseline and post-intervention,

with no statistically significant main effects of group or condition ($P \geq 0.155$), and no group $\times$ condition interaction ($P = 0.629$). Plasma insulin concentrations at time 0 and 120 min of the 75 g OGTT are presented in [Table 4](#).

No Alteration in Gut Microbiota Composition and Functional Activity Despite Sucralose-Induced Changes in Postprandial Glycemic Responses.

α -diversity metrics including Shannon index, Pielou's evenness index, Observed features and Faith's phylogenetic diversity did not statistically differ across all comparisons, whether in the three-group model (LCS, SSB, FJ) or in any two-group models (LCS,SSB – FJ,SSB – FJ,LCS)(all $P \geq 0.075$). No detectable variation was observed in fecal microbiota β -diversity assessed by Bray-Curtis dissimilarity (PERMANOVA; all $q = 0.999$). α -diversity metrics and Bray-Curtis dissimilarity of the fecal microbiota, measured before and after three weeks of consuming LCS, SSB, or FJ, are presented in [Figure 2](#) and [Figure 3](#), respectively. No meaningful change in Mean relative abundance of the six most dominant phyla-level taxa [Figure 4 \(a\)](#) and the 20 most dominant genus-level taxa [Figure 4 \(b\)](#) of the fecal microbiota were observed (all $P \geq 0.161$). As part of a targeted metabolomics approach to assess the gut microbial functional microbial activity, fecal concentrations of SCFAs (acetate, propionate, butyrate, and valerate) and BCFAs (isobutyrate, and isovalerate) were also measured at baseline and post-intervention and are presented in [Table 5](#). No statistically significant differences in fecal concentrations of SCFAs were detected in the three-group model or in any two-group models (all $P \geq 0.127$). For fecal concentrations of BCFAs, a significant main effect of condition was observed for isovalerate, indicating an increase following the three-week intervention in the three-group model ($P = 0.025$) and in the LCS-SSB two-group model only ($P = 0.005$). Similarly, a significant main effect of condition was observed for isobutyrate, with an increase in fecal concentration detected only in the LCS-SSB two-group model ($P = 0.010$). No main effect of group (all $P \geq 0.538$), and no group $\times$ condition interaction was observed in any model (all $P \geq 0.115$).

No Meaningful Differences in Intestinal Barrier Permeability and Systemic Biomarkers of Low-Grade Inflammation.

Plasma concentrations of intestinal permeability biomarkers LBP and zonulin are presented in [Table 6](#). A statistically significant main effect of group was observed ($P = 0.014$) for LBP concentrations when comparing all three experimental groups (LCS, SSB, FJ). Post hoc analyses revealed that the FJ group exhibited lower LBP concentrations compared to the SSB group across conditions ($P_{Bonf} = 0.017$). In pairwise comparisons, similar results were observed when comparing the FJ to SSB groups and the FJ to LCS groups ($P_{Bonf} = 0.014$), while no statistical difference was detected when comparing the LCS to the SSB groups (all $P \geq 0.101$). There were no main effect of condition (all $P \geq 0.123$) and no group $\times$ condition interaction (all $P \geq 0.057$) detected across all comparisons. Zonulin concentrations were not statistically different in any comparisons (all $P \geq 0.201$). Plasma concentrations of IL-6, CRP, and TNF- α were measured as biomarkers of systemic low-grade inflammation and are presented in [Table 6](#). No statistical differences were observed between groups, conditions, and their interaction, for IL-6 and TNF- α levels in any comparisons (all $P \geq 0.201$). A statistically significant main effect of group was detected for CRP concentrations ($P = 0.049$) when comparing all three experimental groups; however, post hoc analyses did not reveal any difference between groups (all $P_{Bonf} \geq 0.092$). Pairwise comparisons revealed no statistically significant differences in the main effects of group, condition, or their interaction between LCS and SSB (all $P \geq 0.515$). However, a statistically significant main effect of group was observed when comparing the FJ to SSB groups ($P = 0.046$) and the FJ to LCS groups ($P = 0.019$). Post hoc analyses revealed that the FJ group exhibited lower CRP concentrations than the SSB group ($P_{Bonf} = 0.046$) and the LCS group ($P_{Bonf} = 0.019$) across conditions.

No Metabolic Advantage of Fruit Juice Over Sugar-Sweetened Beverages in Glucose Tolerance (Exploratory Analyses).

To explore whether free sugars derived from natural sources elicit metabolic responses similar to those from refined sources, FJ was included as an exploratory comparator group.

Postprandial plasma glucose concentrations during OGTT, measured before and after three weeks of consuming FJ or SSB are presented in [Figure 5](#). At baseline, plasma glucose concentrations were similar between the FJ and the SSB groups when analyzing glycemic responses during the OGTT (main effect of group: $P = 0.297$). Following the three-week intervention, no statistical differences were observed across groups, conditions, and their interaction when comparing FJ to SSB (all $P \geq 0.075$). In additional exploratory analyses, FJ was compared to LCS. Glycemic responses to the OGTT were similar at baseline (main effect of group: $P = 0.181$), and no statistically significant differences were observed following the three-week intervention (all $P \geq 0.096$).

Sensitivity Analyses

To further evaluate the robustness of our primary glycemic findings for sucralose, we conducted exploratory sensitivity analyses beyond the original two-group design (LCS, SSB). Given the addition of an exploratory comparator (FJ), we assessed whether glycemic responses differed among the three groups (LCS, SSB, FJ) across conditions. There was no main effect of group or condition (all $P \geq 0.233$), but a group $\times$ condition interaction approached statistical significance ($P = 0.052$). Post hoc comparisons revealed no significant differences (all $P_{Bonf} \geq 0.201$), consistent with expectations as the study was underpowered for three-group comparisons. We next compared LCS against a combined non-LCS group (SSB + FJ), reasoning that both comparators provide free sugars and could serve as a unified control. A statistically significant group $\times$ condition interaction was observed ($P = 0.019$). Post hoc analyses indicated that glycemic responses significantly increased from baseline to post-intervention in the LCS group ($P_{Bonf} = 0.024$), while no significant change was observed in the non-LCS group ($P_{Bonf} = 0.379$), consistent with the primary two-group findings (LCS, SSB). Insulin concentrations during the OGTT remained statistically similar across all comparisons, whether between two groups (FJ vs SSB, FJ vs LCS) or across all three experimental groups (LCS, SSB, FJ) (main effects of group, condition, and their interaction: all $P \geq 0.145$).

DISCUSSION

Postprandial Glycemic Responses and Gut Microbiota Profiles

This study demonstrates that sucralose, when consumed in a real-world context over three weeks, impairs postprandial glycemic responses in healthy adults. In contrast, SSB and FJ did not elicit such effects. Importantly, these findings occurred despite any statistically significant changes in body weight, BMI, waist circumference, fat mass percentage, and fasting plasma glucose concentrations. From a clinical standpoint, the 8.4% increase in postprandial glycemia observed following sucralose consumption in healthy individuals is highly relevant, given the well-established role of postprandial hyperglycemia in cardiovascular risk^{29–31} and microvascular damage³². This underscores the significance of our findings, particularly in the context of unchanged fasting plasma glucose and body composition. We hypothesized that changes in postprandial glycemic responses would be associated with alterations in gut microbiota profile, consistent with the mechanisms proposed by Suez et al. (2022)¹³, who reported a “personalized microbiome-driven effect” of sucralose on glucose tolerance. Contrary to this hypothesis, **we found no detectable differences in gut microbiota composition, specifically α -diversity and β -diversity metrics assessed by 16S rRNA sequencing, or functionality characterized by fecal concentrations of SCFAs and BCFAs, suggesting that the changes in glucose tolerance observed following three weeks of sucralose consumption are not related to major microbiota modulations, at least within this study’s experimental design and statistical power.** Although our microbiota findings differ from with those of Suez et al. (2022)¹³, our results support their observations of altered glucose tolerance following sucralose consumption. Building on their pilot study in 2014²²¹, Suez and colleagues conducted a two-week intervention in healthy adults, who were provided with ready-to-dissolve sachets of either aspartame, saccharin, sucralose, or stevia (at doses within ADI of 8%(240mg), 20%(180mg), 34%(102mg), and 75%(180mg) respectively) containing glucose as a bulking agent, compared with controls receiving either nothing (no supplement control) or glucose (n = 20 per group). Only saccharin and

sucralose significantly increased postprandial glucose iAUC when compared to both control groups, with effects evident from the first week of exposure and persisting into the second week. Notably, this was the first study to assess postprandial glycemic responses with multiple OGTTs performed at home with continuous glucose monitors, enhancing ecological validity. Glucose tolerance, gut microbiome and metabolome features returned to baseline within two weeks after sucralose cessation. Our study did not include a follow-up phase, making it uncertain whether this reversibility would be observed. A potential explanation for the discrepancy in microbiota-related outcomes between our study and that of Suez et al. (2022) may lie in the differences in sequencing methods. In our study, we characterized microbiota composition using 16S rRNA marker gene sequencing and assessed functionality through a targeted metabolomics approach quantifying SCFAs and BCFAs production. In contrast, Suez and colleagues employed a combination of metagenomic sequencing and metabolomic profiling in serial fecal samples, an approach widely recognized as the gold standard for comprehensive evaluation of human gut microbiome^{dd} dynamics^{128,129}. The 16S rRNA sequencing approach used in our study enables taxonomic profiling at the genus level, offering insights into the diversity, richness, and evenness of gut bacterial communities^{128,129}. This method relies on PCR amplification of the 16S ribosomal RNA gene, which is universally present in bacteria and archaea^{127ee}. In contrast, metagenomic sequencing captures all genomic DNA in a sample, including bacteria, archaea, fungi, and viruses, allowing strain-level resolution and functional gene analysis¹²⁸. This broader scope makes metagenomics a more powerful tool for characterizing microbial communities and their functional potential. When paired with metabolomic profiling, it provides a more comprehensive view of host–microbiota interactions¹¹⁸. Therefore, the use of 16S rRNA sequencing in our study, while methodologically sound, might partly explain the differences in microbiota-related outcomes. Notwithstanding the limitations of using 16S rRNA sequencing in our study,

^{dd} The terms microbiome and microbiota are often used interchangeably in the literature, although they differ by definitions. The gut microbiome refers to the collection of genomes of all microorganisms and their metabolites¹²⁵, whereas populations of bacterial and archaeal communities measured using 16S rRNA gene sequencing are defined as the gut microbiota¹²⁵.

^{ee} Archaea are single-celled microorganisms structurally similar to bacteria.

we implemented several methodological strengths aligned with best practices in microbiome research¹²⁷. These include the collection of two serial fecal samples per timepoint to account for intra-individual variability in gut microbiota profiles (three to five samples are considered optimal when using 16S rRNA sequencing in a real-world research settings¹³⁰), the consistent use of reagent kits and PCR primers across all samples (as primer-induced variability has been shown to exceed interindividual differences in microbiota profiles^{112,118}) and statistical analyses incorporating complementary α -diversity metrics—richness, evenness, information, and phylogenetic diversity—which were shown to offer a comprehensive characterization of microbiota α -diversity¹⁸⁴. Notably, despite its limited taxonomic resolution and reduced sensitivity for detecting low-abundance bacterial genera, 16S rRNA sequencing has been shown to reliably identify the top 31% most abundant genera, with performance comparable to metagenomic approaches¹²⁸. Therefore, while some uncertainty remains, it is unlikely that the use of metagenomic sequencing in our study would have yielded substantially different results for these dominant taxa.

Conversely, the microbiota-related outcomes shown in our study align with those of Ahmad et al. (2020)¹⁷⁷. Using a randomized crossover design, Ahmad and colleagues reported no changes in gut microbiota α -diversity (Shannon index) and β -diversity (Bray-Curtis Dissimilarity) assessed by 16S rRNA sequencing, nor in SCFAs concentrations, following the consumption of pure sucralose-sweetened beverages (136 mg/day) for two weeks in 17 healthy individuals, using aspartame as a comparator. However, unlike our results on glucose tolerance, they found no significant differences in total AUCs for postprandial glycemic responses. Moreover, no statistically significant alterations on insulinemic responses, active GLP-1 concentrations and insulin sensitivity (HOMA-IR) were observed. While their double-blinded crossover design offers methodological advantages over our parallel-group design, differences in analytical approaches may account for the divergent findings. Specifically, the total AUCs were not adjusted for fasting values. Adjusting for interindividual variability in fasting glucose levels has been shown to better capture postprandial dynamics in nutritional interventions^{176,222}.

Accordingly, the model used in our study specifically measured the rise in glycemic responses above fasting glucose levels. Our statistical approach also employed linear mixed models using individual OGTT time points rather than collapsing them into a single AUC value. In the context of assessing postprandial glycemic responses, analyzing all time points with mixed models has been demonstrated to be more robust and statistically powerful because it preserves the full trajectory of glycemic responses²²². Furthermore, the comparator used in their study was an aspartame-sweetened beverage, another low-calorie sweetener, whereas our design employed SSB as the primary comparator. As previously discussed in the Introduction section, compelling evidence indicates that each LCS exerts distinct physiological effects and should therefore be evaluated using sweetener-specific hypotheses^{13,67–69}. Systematic reviews and meta-analyses^{70,172–175} support the use of SSB as a more reliable comparator for assessing metabolic outcomes, compared to water or other LCS. It is important to acknowledge that, like Ahmad et al. (2020), our study was not powered to detect changes in gut microbiota endpoints, but rather to detect differences in postprandial glycemic responses. Given the substantial interindividual variability in human gut microbiota profiles shaped by factors including host genotype, disease status, age, sex environmental influences, and dietary patterns^{95–98,102–104}, the sample size in both studies may have considerably limited statistical power for microbiota analyses thereby reducing the ability to detect meaningful changes in composition and function.

Postprandial Insulin Concentrations, Insulin Sensitivity, and Gut Incretins Hormones

Our data showed comparable insulin concentrations across all groups and conditions, suggesting that alternative mechanisms may underlie the observed sucralose-induced impairment in glucose tolerance. However, insulin concentrations were measured only at 0 and 120 min during the OGTT, precluding assessment of intermediate time points (30–90 min) that capture early-phase insulin dynamics, namely the cephalic and intestinal phases of secretion. As a result, potential differences in these mechanisms may have been missed. One well-established mechanism contributing to

impaired glucose tolerance is insulin resistance. This condition is defined as a decreased physiological response to insulin in insulin-sensitive tissues (particularly skeletal muscle, liver, and adipose tissue), resulting in impaired glucose uptake and metabolism²²³. Although hyperinsulinemia is a common compensatory response to insulin resistance, tissue-specific reductions in insulin sensitivity can also occur under normoinsulinemic conditions, both in fasting and postprandial states. This may be due to impaired insulin signaling pathways or downregulation of insulin receptors²²⁴, preceding the activation of compensatory mechanisms that elevate insulin secretion. To assess whether insulin resistance could explain the 8.4% increase in postprandial glycemic responses observed in the LCS group following our three-week intervention, we could have employed indices relying on insulin concentrations at 0 and 120 during OGTT such as HOMA-IR²²⁵ or the Matsuda Index^{226ff}. However, due to several fasting insulin values falling below the detection threshold, these assessments were deemed inappropriate. Although insulin sensitivity could not be evaluated in our study, others have investigated the effects of repeated sucralose exposure on insulin sensitivity, yielding inconsistent results. For instance, a crossover design study in which sucralose was provided as 200 mg daily capsules over four weeks found significantly decreased whole-body insulin sensitivity (Matsuda index) without significant change observed in postprandial glycemic responses (n = 11, women)⁸⁹. In contrast, a randomized-controlled trial in which sucralose was also provided in capsule form for one week, found no significant changes in HOMA-IR and Matsuda indexes, nor in postprandial glycemic responses (n = 30, men)¹⁶⁷, despite providing higher daily doses (780 mg/day). However, it is important to highlight that administering sucralose in capsules results in bypassing cephalic phase responses associated with orosensory sweetness perception and therefore does not reflect typical patterns of LCS consumption through beverages and food products. Another study, which more closely reproduced real-world conditions by delivering sucralose in beverages (~110–171 mg/day based on body weight) over two weeks, reported decreased insulinemic

^{ff} Homeostatic model assessment of insulin resistance (HOMA-IR) quantifies the dynamic between fasting blood sugar and insulin response²²⁵. Matsuda index is an approximation of whole-body insulin sensitivity derived from data obtained during an OGTT²²⁶.

responses measured via the frequently sampled intravenous glucose tolerance test (FSIVGTT)^{gg}, while HOMA-IR remained unchanged (n = 61, women and men)¹⁶³.

Another proposed mechanism by which sucralose may alter glycemic responses involves its interaction with the intestinal sweet taste receptor, composed of two subunits TAS1R2 and TAS1R3⁷⁷ (formally known as T1R2 and T1R3⁷⁶). Notably, the structure of the intestinal sweet taste receptor binding to sucralose was just recently revealed by Juen et al. (2025)⁷⁷. The intestinal sweet taste receptor has been shown to detect a wide range of sweet-tasting compounds within the gut, triggering the release of incretin hormones, primarily glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP)⁷⁸. GLP-1 and GIP are well-established modulators of glucose homeostasis through several mechanisms^{83,84}. These include the insulin-independent upregulation of intestinal glucose transporters, specifically sodium-glucose cotransporter 1 (SGLT-1) and GLUT2 (glucose transporter 2), in terms of both expression and function^{78,80,81}. Although increased intestinal glucose uptake, driven by chronically elevated secretion of gut incretin hormones, may represent a promising mechanism to explain the altered glycemic responses associated with sucralose consumption, this pathway has been supported by a limited number of human studies involving acute exposure to sucralose^{53,87–89}, which failed to be consistently replicated^{23,90–94}. To our knowledge, no studies have yet demonstrated a sustained increase in GLP-1 or GIP secretion in response to frequent sucralose intake in humans under real-world conditions^{13,171}.

Intestinal Barrier Permeability and Systemic Low-grade Inflammation

Evidence strongly supports the role of immune responses and systemic low-grade inflammation (defined as a persistent state of inflammation occurring in the absence of an inflammatory disease¹³⁵) as key contributors to insulin resistance and impaired glucose tolerance. Recent animal studies have shown that sucralose can induce dysbiosis by

^{gg} FSIVGTT provides precise and detailed information about insulin sensitivity and beta-cell function, making it one of the gold standard for measuring insulin sensitivity, while the gold standard for measuring both insulinemic and glycemic responses is euglycemic-hyperinsulinemic clamp (EHC).

selectively promoting or inhibiting the growth of specific bacterial taxa^{15–19}. Emerging evidence suggests that dysbiosis—a disturbance of the intestinal homeostasis caused by an imbalance in the structural and/or functional microbial configuration of the gut microbiota²⁰—can compromise the integrity of the intestinal barrier^{15,21,22} and alter its selective permeability. Increased intestinal permeability exposes the circulatory system to gut-derived bacterial products such as endotoxins^{139–143}, also referred to as lipopolysaccharides (LPS), which are potent triggers for the release of pro-inflammatory adipokines and cytokines (including TNF- α ^{147–150}, IL-1 β , and IL-6^{148,150}) by both immune and nonimmune cells. Through a bidirectional endocrine-immune crosstalk, pro-inflammatory cytokines can interfere with insulin signaling pathways and downregulate insulin receptor expression, thus resulting in decreased sensitivity in insulin sensitive^{151–153}. **Even so, our microbiota outcomes did not indicate dysbiosis, and our results do not support the hypothesis that sucralose consumption increases intestinal permeability or induces systemic low-grade inflammation; at least not at realistic doses consumed over a three-week period, and when assessed using the plasmatic biomarkers selected for this current study.**

Exploratory Findings on 100% Natural Fruit Juice

Although fruit juices are often perceived as healthier alternatives to SSB due to their content in vitamins and antioxidants, they share a similar profile of free sugars and typically lack the dietary fiber found in whole fruits^{4,5}. Dietary fiber plays a critical role in maintaining intestinal health by serving as a fermentable substrate for the gut microbiota and contribute to glycemic control by slowing intestinal glucose absorption⁹⁸. Consequently, the consumption of free sugars without the presence of dietary fiber, such as in FJ, may theoretically lead to adverse metabolic outcomes comparable to those associated with SSB^{3,4}. In partial alignment with this hypothesis, we demonstrated that FJ and SSB elicited similar effects on glucose tolerance. However, these effects were not adverse, as no changes in postprandial glycemic responses were observed following the three-week consumption of either beverage at doses representative of the average free

sugar intake in Canada. It remains plausible that longer-term exposure or the assessment of additional metabolic outcomes beyond glucose tolerance could reveal adverse effects for which FJ and SSB may be similarly implicated. Interestingly, despite the theoretical expectation that FJ and SSB would elicit comparable metabolic effects, our findings revealed that 100% natural orange juice did not differ from sucralose-sweetened beverages in terms of glycemic response, whereas SSB did. We explored the potential impact of FJ on systemic low-grade inflammation, as its vitamins and antioxidants may modulate inflammatory responses and, consequently, influence insulin signalling pathways. Prior evidence from recent systematic reviews and meta-analyses have reported improvements in pro-inflammatory biomarkers such as CRP and IL-6 following the consumption of 100% natural orange juice^{227,228}. Although our analyses revealed that the FJ group exhibited lower LBP and CRP concentrations, the lack of a condition effect suggests that these differences are attributable to group-level characteristics rather than the intervention. Therefore, these findings do not support a differential effect of FJ on inflammation within the context of our study. Nevertheless, our exploratory findings on FJ warrant careful interpretation. The empirical evidence from RCTs directly comparing FJ consumption with SSB on metabolic outcomes, aside from systemic low-grade inflammation, is extremely limited. To address this important knowledge gap, the FJ group was added in this study as an exploratory comparator representing a natural source of free sugars. Given that our study was powered specifically to detect differences between two parallel groups—those consuming LCS-sweetened beverages with sucralose and those consuming SSB—on postprandial glycemic responses over a three-week intervention, no predefined hypotheses were formulated for the FJ group. Consequently, this may have limited the study's ability to detect more subtle effects associated with FJ consumption. Moreover, the FJ group consumed 100% natural orange juice. Therefore, these findings should not be generalized to all fruit juices, as their composition varies widely in free sugar concentrations, vitamins, antioxidants, and other bioactive compounds. As with sweetener-specific hypotheses in LCS research, future studies on FJ should incorporate hypotheses tailored to the specific type of fruit juice. **Taken together, although**

exploratory, our findings suggest that FJ may not confer metabolic advantages over SSB, highlighting the need for further research specifically designed to evaluate FJ-related endpoints and inform dietary recommendations.

Strengths of Experimental Design

The robustness of our findings is strengthened by rigorous control of potential confounding factors before and during the study. Measures were implemented to prevent antibiotic exposure, cannabis use, intake of medications affecting glycemic control or systemic inflammation, and consumption of dietary supplements and probiotics. In addition, endurance exercise, alcohol intake, and oral and topical NSAID use were carefully regulated. Moreover, participants were low consumers of LCS, SSB and FJ based on their responses to a validated diet history questionnaire. Pepino et al. (2013)²¹⁸, Lertrit et al. (2018)⁸⁹ and Ahmad et al. (2020)²⁴ defined low intake of LCS as less than one can of LCS-sweetened beverages or one spoonful of LCS per week, while Romo-Romo et al. (2018)¹⁹⁵ defined it as less than five servings per week. Both definitions are less conservative than our approach. Additionally, to minimize hormonal variability in women, the intervention was scheduled so that experimental sessions occurred during the early follicular phase of the menstrual cycle. It should be emphasized that fluctuations in female sex hormone levels during the menstrual cycle, particularly in β -estradiol, are known to impact glycemic responses¹⁶⁸. This underscores the importance of measuring blood parameters during the same menstrual phase, ideally during the follicular phase where hormone levels are stable and at their lowest¹⁶⁸. Another notable strength of our experimental design is in the meticulous formulation of the study beverages, which were matched for sweetness and designed to reflect the free sugar or sucralose content of commercially available products, thereby enhancing ecological validity. Complementing this, dosages for the SSB and FJ groups were individualized according to each participant's total energy expenditure, set at 15% of Ereq, in alignment with estimated free sugar intake among Canadian adults (see *Methods* section for details). This individualized approach, rather than a fixed dose, was implemented to more accurately reflect real-world substitution of SSB with sucralose-

sweetened alternatives. Specifically, participants in the LCS group ingested ~150 mg (± 30 mg) of sucralose per day (2.00 ± 0.23 mg/Kg BW), with individual daily intake ranging from 110mg to 200mg. This amount is below the ADI for sucralose established by the United States Food and Drug Administration of 5 mg/Kg BW/day³⁶. Moreover, our dosage was comparable to those used in previous studies, 102 mg/day by Suez et al. (2022)¹³, 136 mg/day by Ahmad et al. (2020)¹⁷¹ and 160mg/day by Higgins et al. (2019)⁶⁹, which reported opposing effects of treatment on glycemic responses. Overall, this level of attention to beverages formulation substantially strengthened the ecological validity of our research setting. Lastly, other aspects of our protocol further reinforce the overall methodological rigor of our study. These include: 1) an equal distribution of women and men in each experimental group; 2) quantification of female sex hormones; and 3) in-person nutritional education provided by a registered dietitian on how to identify sources of LCS, free sugars and probiotics in common food products.

Limitations of Experimental Design

While primary limitations related to gut microbiota outcomes have already been discussed, the following additional considerations warrant acknowledgment. Notably, the use of commercially available sucralose (Splenda®) instead of pure sucralose might have introduced confounding effects due to the presence of maltodextrin as a bulking agent. Dalenberg et al. (2020)⁷⁵ demonstrated that the combination of sucralose with a carbohydrate source reduces insulin sensitivity, while neither pure sucralose nor maltodextrin alone exerted this effect. This finding is often cited to challenge studies using Splenda® in study beverages, as most (but not all^{hh}) commercially available sucralose-sweetened beverages contain the pure form. Nonetheless, sweetened beverages are frequently consumed alongside meals, which likely contain carbohydrates. Thus, investigating the isolated effects of pure sucralose may have limited ecological validity for informing dietary recommendations given that pure sucralose is rarely consumed in

^{hh} Splenda® is currently used in the formulation of commercially available beverages like “Diet Coke® Sweetened with Splenda®”²⁵³.

isolation. Regarding gut microbiota effects, Splenda® contains digestible matodextrin²²⁹, generally considered to have a neutral impact on microbial composition as opposed to resistant maltodextrin which is fermented by the gut microbiota²³⁰. However, some studies suggest digestible maltodextrin may still modulate microbial composition. For example, daily supplementation with 10 g of digestible maltodextrin for 12 weeks in healthy adults increased *Bifidobacterium* species while inhibiting the growth of *Lactobacillus* species, both recognized for their immunomodulatory roles²³¹. Another study found that while 15 g/day of resistant maltodextrin for three weeks tended to increase *Bifidobacterium* populations without affecting SCFAs levels, digestible maltodextrin significantly elevated fecal propionate and acetate concentrations²³². In our study, participants consumed 8.9 - 16.1 g/day of digestible maltodextrin from Splenda®, an intake range that could be sufficient to influence gut microbiota. However, because dietary fiber intake was not controlled in our study design, variability in fiber consumption likely had a greater influence on gut microbiota composition and functionality than the modest dose of maltodextrin. Another limitation of our study is the reliance on plasmatic biomarkers to assess intestinal permeability, which warrants caution due to their indirect nature. We opted to assess intestinal permeability using two plasma biomarkers, lipopolysaccharide-binding protein (LBP) and zonulin. While LPS is a direct indicator of microbial translocation and systemic endotoxin exposure, its measurement is technically challenging due to low circulating concentrations and high susceptibility to contamination and degradation. In contrast, LBP is an acute-phase protein synthesized by the liver in response to LPS exposure. Although LBP reflects host response rather than LPS presence itself, its reliable quantification via standard immunoassays makes it more practical for clinical studies investigating intestinal barrier permeability and endotoxemia^{137,233}. We acknowledge, however, that the gold-standard non-invasive method for assessing intestinal permeability involves five sugar probes and 24-h urine collection, as described by Wijck et al. (2013)²³⁴. Notwithstanding, this method poses logistical challenges that may hinder participant compliance, such as extended stays at the research facility or difficulties with sample transport accessibility. Given the demands of our protocol—including daily beverage

consumption, serial stool sampling, and two three-hour experimental sessions—we prioritized minimizing participant burden to reduce attrition risk. Zonulin, a human analog of the bacterial enterotoxin zonula occludens¹³⁷, is widely used to assess intestinal barrier integrity due to its association with tight junction proteins regulation²³⁵. However, concerns have been raised regarding the reliability of commercially available ELISA kits for zonulin quantification^{236,237}. Therefore, caution is warranted when interpreting findings based solely on zonulin measurements¹³⁷. In addition to the limitations discussed above, two other minor aspects of our study design warrant brief mention. First, variation in vitamin C intake across experimental groups may have introduced a modest confounding factor. Both the LCS-sweetened beverages and SSB were flavored with Kool-Aid™ orange unsweetened drink mix, enriched with vitamin C (24 mg/L). Based on individualized beverage volumes, participants in these groups consumed ~24mg/day of vitamin C (range: 17-40 mg). In contrast, those in the FJ group, who received 100% natural orange juice, consumed a substantially higher dose of ~225 mg/day (range : 157-374 mg), exceeding the Canadian recommended dietary allowance of 75 mg for women and 90 mg for men aged 19-50 years²³⁸. However, all intakes remained well below the established upper limit of 2000mg per day²³⁸. Second, while the use of nonsteroidal anti-inflammatory drugs (NSAIDs) was restricted during the 24 hours preceding each experimental session, complete avoidance throughout the study period may have more effectively minimized potential confounding effects on systemic inflammatory biomarkers.

Improving Future Research: Methodological Considerations

To enhance the design and interpretability of future studies, we offer methodological strategies informed by our findings and current literature:

- 1) **Once the unique physiological effects of each LCS are well documented and understood, future studies should investigate their combinations, as the effects of one sweetener may be amplified or attenuated when paired with another.** This is particularly relevant given that most commercially available

beverages contain blends of LCS, formulated to improve taste and reduce undesirable aftertastes. Investigating the combined effects of LCS would therefore enhance the ecological validity of future studies and better reflect real-world consumption patterns. However, replicating these blends in research settings presents notable challenges. First, manufacturers are not required to disclose the specific quantities or ratios of LCS used in their products⁴⁶, making it difficult to design interventions that accurately mirror commercial formulations. Second, the current body of evidence remains too limited to support nuanced interpretation of combined LCS effects. The common practice of drawing inference on LCS as a single “class of compounds”, despite their distinct chemical properties, had obscured our understanding of individual physiological responses and hindered the development of evidence-based guidelines for their use. Establishing robust, compound-specific data is therefore a necessary step before investigating LCS in combination.

- 2) **To accurately assess microbiota-related outcomes, future studies should employ power calculations specifically designed for these endpoints.** This recommendation is particularly relevant given that powering based on metabolic outcomes has been identified as a key reason for the inconsistent and inconclusive findings often observed in microbiome research¹²⁷.
- 3) **Given the strong influence of diet in shaping the gut microbiota, future studies should consider implementing standardized dietary protocols during both the intervention and preliminary phases to more accurately isolate the effects of sucralose (and other LCS) on microbial composition and functionality.** The gut microbiota is a complex and dynamic ecosystem that varies markedly among individuals, influenced by factors such as host genotype, disease status, age and sex^{95–98}. Although the core microbial assemblage is believed to remain relatively stable throughout adulthood⁹⁹, the microbiota continues to adapt in response to environmental factors^{96–98}. Among these, dietary patterns (particularly dietary fiber

intake) have emerged as the most influential determinant, shaping its composition, species richness, and functional capacity^{98,102–104}. This inherent variability in environment complicates the detection of significant microbiota changes in human nutrition RCTs, as baseline microbial profiles differ markedly between individuals. We believe the standardized-diet approach could substantially reduce dietary confounding and improve interpretability of microbiota findings. Nevertheless, it is also important to acknowledge the possibility that a homeostatic gut microbiota (i.e. eubiosis) may exhibit resilience to sucralose exposure. This concept is supported by “the insurance hypothesis”, which proposes that a rich diversity of microbial communities would confer a protective effect by maintaining ecological equilibrium, thereby preserving the stability and functional dynamics of an environment (here, the gut microbiota)¹²⁴. Consequently, the magnitude of perturbations induced by sucralose may be insufficient to elicit meaningful shifts in microbial communities, particularly in individuals with a well-balanced and diverse microbiota. Taken together, these considerations highlight that while elucidating the effects of LCS on the gut microbiome, it is equally important to recognize that the microbiome itself may modulate individual metabolic responses to dietary interventions.

CONCLUSION

This study demonstrates that the realistic consumption of sucralose-sweetened beverages for 21 days impairs postprandial glycemic responses in healthy individuals, without inducing significant changes in insulin concentrations, body composition, gut microbiota composition, fecal concentrations of SCFAs and BCFAs, and plasmatic biomarkers of intestinal permeability and systemic low-grade inflammation. Although the precise mechanisms remain unclear, the lack of an identified pathway does not negate the clinical relevance of these findings. Future research that is specifically designed and adequately

powered to assess mechanistic endpoints, rather than treating them as secondary to glycemic outcomes, is critical. Importantly, our findings challenge the prevailing assumption that all low-calorie sweeteners are metabolically and physiologically inert, underscoring the need for compound-specific evaluation.

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Authors' Contributions

A.P. Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Project administration, Funding acquisition (collaborator). **N.G.** Formal Analysis, Data Curation, Writing - Review & Editing, Visualization. **D.L.** Methodology (gut microbiota), Formal Analysis (gut microbiota), Data

Curation (gut microbiota), Writing - Review & Editing, Visualization (gut microbiota). **J-F.M.** Methodology (biological analyses), Investigation, Data Curation, Writing - Review & Editing. **A.L.** Investigation, Data Curation, Writing - Review & Editing. **K.P.** Conceptualization (gut microbiota), Methodology (gut microbiota), Resources (gut microbiota), Writing - Review & Editing, Supervision (gut microbiota), Funding acquisition. **P.I.** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Declaration of interests

Authors declare no conflict of interest.

Table 1. Participant Characteristics at Baseline.

	All groups (n = 42)	LCS (n = 14)	SSB (n = 14)	FJ (n = 14)	P-value
Age (years)	24 (20 - 27)	25 (20 - 28)	24 (20 - 27)	24 (21 - 27)	0.859
Women	21 (20 - 27)	25 (20 - 27)	20 (20 - 24)	23 (21 - 26)	
Men	25 (22 - 28)	24 (23 - 29)	25 (24 - 27)	24 (22 - 27)	
Sex (n)					n/a
Women	21 (50%)	7 (50%)	7 (50%)	7 (50%)	
Men	21 (50%)	7 (50%)	7 (50%)	7 (50%)	
Body weight (kg)	69.5 (59.4 - 78.6)	69.7 (63.4 - 87.0)	70.8 (57.6 - 77.7)	63.9 (58.8 - 71.9)	0.432
Women	61.7 (57.4 - 69.4)	62.6 (60.7 - 69.6)	64.0 (55.8 - 72.4)	58.6 (57.2 - 62.6)	
Men	76.6 (69.6 - 89.3)	85.4 (70.4 - 89.8)	76.6 (65.2 - 84.2)	72.7 (66.0 - 87.4)	
Body mass index (kg/m²)	24.1 ± 3.44	25.5 ± 3.41	23.5 ± 3.49	23.4 ± 3.28	0.231
Women	23.4 ± 3.26	24.7 ± 3.86	23.0 ± 3.89	22.6 ± 1.60	
Men	24.9 ± 3.55	26.2 ± 2.98	24.1 ± 3.25	24.2 ± 4.38	
Waist circumference (cm)	74.8 (69.9 - 81.1)	77.0 (73.6 - 86.0)	76.4 (70.1 - 81.1)	72.3 (69.1 - 74.0)	0.377
Women	70.3 (67.8 - 74.0)	73.0 (70.0 - 75.9)	70.3 (66.8 - 76.5)	69.0 (67.9 - 71.8)	
Men	79.0 (75.5 - 88.0)	83.0 (78.5 - 89.0)	79.5 (76.4 - 84.8)	74.0 (72.3 - 85.8)	
Fat mass (%)	27.4 ± 7.84	28.2 ± 7.69	27.1 ± 8.79	26.9 ± 7.50	0.901
Women	32.2 ± 6.46	32.1 ± 9.07	33.5 ± 5.77	31.1 ± 4.50	
Men	22.6 ± 5.96	24.3 ± 3.22	20.7 ± 6.25	22.8 ± 7.83	
Fasting plasma glucose (mmol/L)	4.68 ± 0.601	4.63 ± 0.467	4.55 ± 0.754	4.86 ± 0.545	0.382
Women	4.59 ± 0.456	4.54 ± 0.237	4.44 ± 0.548	4.79 ± 0.510	
Men	4.77 ± 0.718	4.73 ± 0.628	4.65 ± 0.951	4.93 ± 0.609	
Ethnicity (n)					n/a
Caucasian	32 (76%)	12 (86%)	10 (71%)	10 (71%)	
Asian	5 (12%)	2 (14%)	1 (7%)	2 (14%)	
African	3 (7%)	0 (0%)	1 (7%)	2 (14%)	
Indian	1 (2%)	0 (0%)	1 (7%)	0 (0%)	
Middle Eastern	1 (2%)	0 (0%)	1 (7%)	0 (0%)	

Note: Values are presented as means ± SDs, medians (interquartile range) or number of participants (%). Age, sex, and ethnicity were self-reported. Body mass index was calculated as weight in kilograms divided by height in square meters. Waist circumference was measured following the World Health Organization's protocol. Fat mass was assessed via dual-energy X-ray absorptiometry. Fasting plasma glucose concentrations were determined after a 12-h overnight fast using colorimetric assays. *P*-values were derived from a one-way analysis of variance with the experimental group (LCS, SSB, FJ) as the grouping variable; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB), fruit juice (FJ).

Table 2. Body weight, BMI, Waist circumference and Fat mass at Post-Intervention.

	All groups (n = 42)	LCS (n = 14)	SSB (n = 14)	FJ (n = 14)	Group P-value	Condition P-value	Interaction P-value
Body weight (kg)	68.4 (59.5 - 78.3)	68.7 (63.7 - 87.0)	69.8 (58.3 - 77.7)	64.2 (58.0 - 72.2)			
Women	61.7 (57.4 - 69.4)	62.7 (60.2 - 68.7)	65.1 (55.7 - 72.0)	57.9 (57.8 - 62.4)	0.371	0.644	0.358
Men	76.6 (69.6 - 89.3)	85.0 (69.7 - 88.5)	77.1 (64.6 - 84.8)	72.7 (66.2 - 87.8)			
Body mass index (kg/m²)	24.2 ± 3.52	25.4 ± 3.41	23.7 ± 3.60	23.5 ± 3.49			
Women	24.6 ± 3.26	24.7 ± 3.77	23.1 ± 3.81	22.6 ± 1.70	0.219	0.388	0.310
Men	26.3 ± 3.55	26.2 ± 3.07	24.4 ± 3.53	24.4 ± 4.63			
Waist circumference (cm)	74.5 (69.9 - 81.9)	78.0 (72.9 - 85.1)	75.1 (70.0 - 81.5)	72.8 (69.0 - 74.4)			
Women	70.3 (67.8 - 74.0)	72.6 (69.6 - 75.3)	73.0 (67.0 - 76.3)	69.0 (68.8 - 71.3)	0.254	0.331	0.951
Men	79.0 (75.5 - 88.0)	82.0 (80.0 - 88.3)	81.0 (75.1 - 84.3)	75.5 (73.3 - 85.5)			
Fat mass (%)	27.4 ± 7.78	27.7 ± 7.47	27.1 ± 9.14	27.4 ± 7.17			
Women	32.2 ± 6.46	32.0 ± 9.07	33.7 ± 5.77	31.0 ± 4.50	0.926	0.855	0.198
Men	22.6 ± 5.96	23.5 ± 3.22	20.6 ± 6.25	23.8 ± 7.83			

Note: Values are presented as means ± SDs, medians (interquartile range) or number of participants (%). Body mass index was calculated as weight in kilograms divided by height in square meters. Waist circumference was measured following the World Health Organization's protocol. Fat mass was assessed via dual-energy X-ray absorptiometry. *P*-values were derived from linear mixed-effects models with the experimental group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects, participant ID as a random effect and sex as a continuous covariate; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB), fruit juice (FJ).

Table 3. Plasma Concentrations of Sex Hormones and Self-Reported Use of Contraceptives in Women.

	All groups (n = 21)	LCS (n = 7)	SSB (n = 7)	FJ (n = 7)	Group <i>P</i> -value	Condition <i>P</i> -value	Interaction <i>P</i> -value
β-estradiol (pmol/L)							
<i>Baseline</i>	126 (82 - 261)	261 (201 - 354)	117 (72 - 193)	97 (60 - 152)	0.234	0.390	0.480
<i>Post-intervention</i>	150 (82 - 341)	295 (183 - 360)	196 (70 - 276)	135 (114 - 148)			
Progesterone (nmol/L)							
<i>Baseline</i>	1.06 (0.75 - 14.21)	1.35 (1.02 - 15.11)	0.80 (0.62 - 12.05)	1.14 (0.83 - 7.70)	0.999	0.559	0.556
<i>Post-intervention</i>	2.83 (0.78 - 6.26)	2.62 (0.94 - 2.85)	4.87 (0.67 - 19.94)	3.21 (1.58 - 6.26)			
Contraceptive method							
Copper IUD	5 (24%)	3 (43%)	0 (0%)	2 (29%)	n/a	n/a	n/a
Hormonal IUD	1 (5%)	0 (0%)	0 (0%)	1 (14%)			
Birth control pill	5 (24%)	2 (29%)	3 (43%)	0 (0%)			
None	10 (48%)	2 (29%)	4 (57%)	4 (57%)			

Note: Values are presented as medians (interquartile range) or number of participants (%). Plasma β-estradiol and progesterone concentrations were determined using enzyme-linked immunosorbent assays. *P*-values were derived from linear mixed-effects models with the experimental group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects and participant ID as a random effect; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB), fruit juice (FJ), intrauterine device (IUD).

Table 4. Plasma Insulin Concentrations during a 75 g OGTT.

		LCS + SSB (<i>n</i> = 28)		LCS (<i>n</i> = 14)		SSB (<i>n</i> = 14)	
Baseline							
<i>0 min</i>		2.05 (1.00 - 5.29)		1.59 (1.00 - 4.41)		2.94 (1.00 - 6.82)	
<i>120 min</i>		33.7 (22.2 - 56.4)		27.9 (17.6 - 37.9)		50.9 (24.1 - 81.2)	
Post-intervention							
<i>0 min</i>		3.02 (1.00 - 6.02)		1.39 (1.00 - 3.78)		3.36 (1.10 - 7.31)	
<i>120 min</i>		31.6 (12.4 - 61.4)		31.6 (6.61 - 54.0)		34.5 (14.6 - 65.6)	
Time	Group	Condition	Group x Time	Group x Condition	Condition x Time	Group x Condition x Time	
<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	
≤ 0.001	0.225	0.580	0.900	0.806	0.069	0.845	

Note: Values are presented as medians (interquartile range). Plasma insulin concentrations were determined using enzyme-linked immunosorbent assays. Undetectable values were substituted with the lowest concentration that can be detected (1 µU/mL). In total, 21 out of 112 samples fell below the detection range (LCS = 11; SSB = 10). *P*-values were derived from linear mixed-effects models with the experimental group (LCS, SSB), condition (baseline, post-intervention), and time (0 min, 120 min) as fixed effects and participant ID as a random effect; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB).

Table 5. Fecal Concentrations of Short-Chain Fatty Acids and Branched-Chain Fatty Acids (SCFAs and BCFAs).

SCFAs & BCFAs concentrations (mmol/L feces)	All groups (n = 42)	LCS (n = 14)	SSB (n = 14)	FJ (n = 14)	Group P-value	Condition P-value	Interaction P-value
Acetate							
Baseline	77.0 (50.5 - 123.0)	83.5 (51.0 - 125)	67.1 (58.3 - 140)	72.2 (36.1 - 112)	0.339	0.833	0.522
Post-intervention	81.7 (54.4 - 108.0)	93.5 (72.6 - 133)	74.7 (56.0 - 96.0)	64.2 (39.6 - 99.1)			
Propionate							
Baseline	22.2 (14.5 - 36.7)	22.2 (18.1 - 34.7)	21.6 (15.0 - 35.0)	24.9 (11.9 - 37.8)	0.522	0.744	0.695
Post-intervention	22.0 (14.6 - 31.2)	28.1 (17.2 - 35.8)	20.7 (14.9 - 31.1)	18.6 (12.6 - 26.2)			
Butyrate							
Baseline	28.4 (15.8 - 38.4)	29.7 (17.5 - 38.6)	28.6 (16.4 - 39.4)	25.6 (14.1 - 35.7)	0.377	0.440	0.746
Post-intervention	24.1 (15.3 - 33.3)	28.9 (18.5 - 43.1)	27.2 (15.4 - 40.1)	18.2 (12.9 - 30.2)			
Valerate							
Baseline	4.05 (2.77 - 5.30)	3.87 (3.13 - 5.30)	2.86 (2.20 - 4.11)	4.73 (3.43 - 5.84)	0.499	0.511	0.579
Post-intervention	3.84 (2.76 - 5.71)	4.08 (3.05 - 4.83)	3.44 (2.50 - 5.51)	3.75 (3.13 - 6.28)			
Isobutyrate							
Baseline	3.04 (2.37 - 4.24)	2.81 (2.39 - 3.71)	2.53 (2.32 - 3.25)	4.35 (2.99 - 5.42)	0.839	0.080	0.170
Post-intervention	3.54 (2.91 - 4.67)	3.83 (2.97 - 4.62)	3.34 (2.62 - 4.09)	3.37 (3.14 - 4.67)			
Isovalerate							
Baseline	4.07 (2.85 - 5.24)	3.75 (2.88 - 4.79)	2.85 (2.63 - 4.13)	5.04 (3.77 - 5.98)	0.921	0.025	0.254
Post-intervention	4.70 (3.59 - 5.91)	5.58 (3.92 - 5.96)	3.72 (2.77 - 5.55)	4.73 (4.12 - 5.85)			

Note: Values are presented as medians (interquartile range). Fecal concentrations of SCFAs (acetate, propionate, butyrate, valerate) and BCFAs (isobutyrate, isovalerate) were determined using gas chromatography. *P*-values were derived from linear mixed-effects models with the experimental group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects and participant ID as a random effect; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB), fruit juice (FJ), short-chain fatty acids (SCFAs), branched short-chain fatty acids (BCFAs).

Table 6. Plasma Concentrations of Intestinal Permeability and Systemic Low-Grade Inflammation Biomarkers.

	All groups (n = 42)	LCS (n = 14)	SSB (n = 14)	FJ (n = 14)	Group P-value	Condition P-value	Interaction P-value
LBP (ng/mL)							
Baseline	3937 ± 922	3964 ± 605	4295 ± 1105	3551 ± 890	0.014	0.463	0.083
Post-intervention	3877 ± 922	4112 ± 698	4277 ± 1065	3242 ± 626			
Zonulin (ng/mL)							
Baseline	3.70 (3.20 - 5.59)	4.00 (2.68 - 5.40)	4.40 (3.46 - 5.74)	3.59 (3.20 - 5.29)	0.708	0.293	0.256
Post-intervention	4.11 (3.11 - 5.63)	4.08 (2.57 - 5.55)	4.06 (3.31 - 5.10)	4.16 (3.31 - 5.69)			
IL-6 (pg/mL)							
Baseline	0.581 (0.470 - 0.877)	0.579 (0.449 - 0.842)	0.577 (0.483 - 0.819)	0.590 (0.510 - 1.17)	0.766	0.836	0.280
Post-intervention	0.588 (0.455 - 0.967)	0.633 (0.455 - 0.954)	0.679 (0.431 - 0.926)	0.540 (0.478 - 0.919)			
CRP (ng/mL)							
Baseline	489 (267 - 1279)	617 (488 - 1157)	459 (272 - 2098)	337 (168 - 540)	0.049	0.882	0.379
Post-intervention	412 (207 - 1177)	727 (286 - 2643)	685 (313 - 3985)	221 (169 - 371)			
TNF-α (pg/mL)							
Baseline	0.582 (0.511 - 0.719)	0.609 (0.515 - 0.719)	0.573 (0.517 - 0.650)	0.582 (0.511 - 0.742)	0.951	0.512	0.569
Post-intervention	0.624 (0.519 - 0.696)	0.657 (0.568 - 0.692)	0.574 (0.524 - 0.636)	0.612 (0.478 - 0.710)			

Note: Values are presented as means ± SDs or medians (interquartile range). Plasma concentrations of intestinal permeability and systemic low-grade inflammation biomarkers were determined using enzyme-linked immunosorbent assays. *P*-values were derived from linear mixed-effects models with the experimental group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects and participant ID as a random effect; data from models that did not meet normality assumptions (per the Shapiro-Wilk test) were log-transformed. **Abbreviations:** low-calorie sweetener (LCS), sugar-sweetened beverage (SSB), fruit juice (FJ), interleukin-6 (IL-6), C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), lipopolysaccharide-binding protein (LBP).

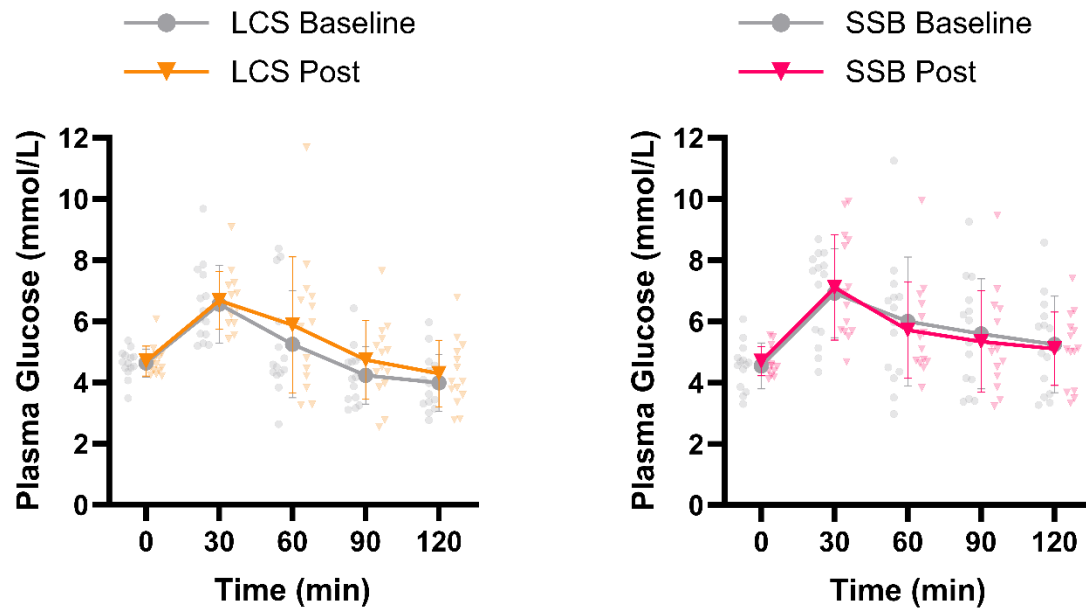


Figure 1. Plasma glucose concentrations during OGTT, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS) or sugar-sweetened beverages (SSB), in healthy adults.

Data are presented as mean (SD) and individual points. Log-transformed data were analyzed using linear mixed-effects models with group (LCS, SSB), condition (baseline, post-intervention), and time (30, 60, 90, 120 min, excluding 0 min) as fixed effects, and participant ID as a random intercept (group x condition interaction: $P = 0.009$; $P_{Bonf} = 0.040$). **Abbreviations:** post-intervention (Post).

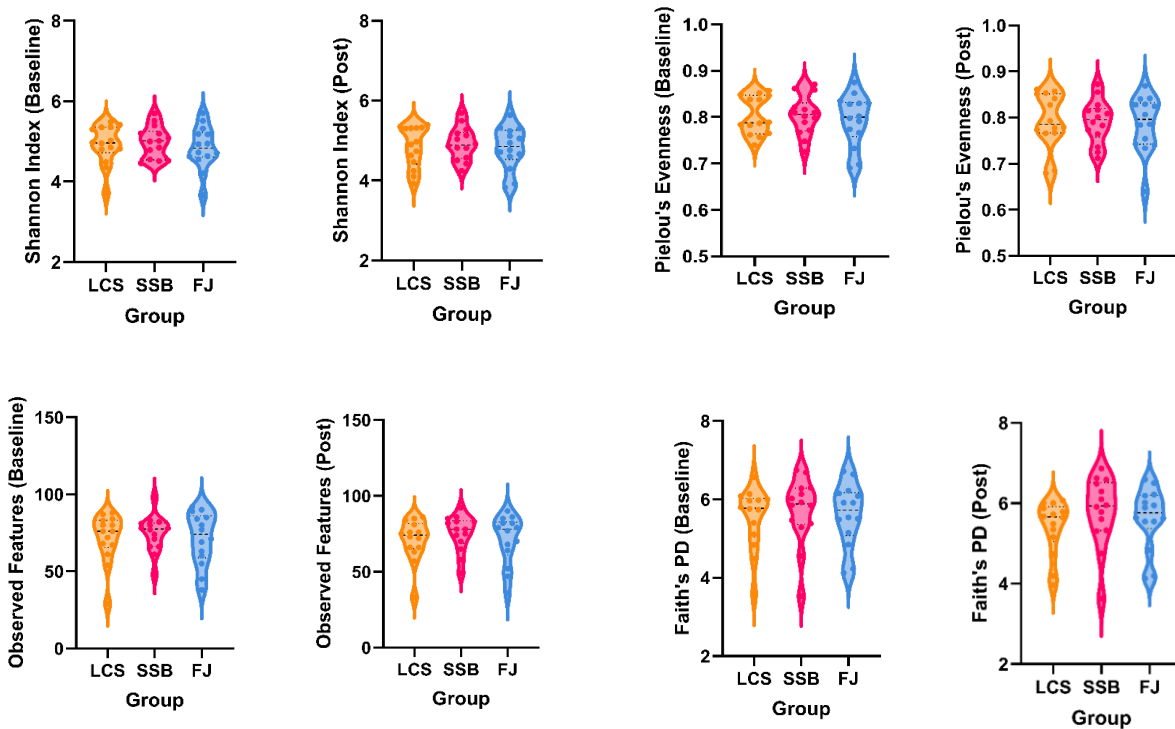


Figure 2. α -diversity metrics (Shannon Index, Pielou's Evenness Index, Observed Features, Faith's PD) of the fecal microbiota, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB), or 100% natural fruit juice (FJ) in healthy adults.

Gut microbiota composition was assessed using 16S rRNA sequencing and analyzed using linear mixed-effects models with group (LCS, SSB, FJ) and condition (baseline, post-intervention) as fixed effects, and participant ID as a random intercept (group $\times$ condition interaction: all $P \geq 0.123$). **Abbreviations:** post-intervention (Post).

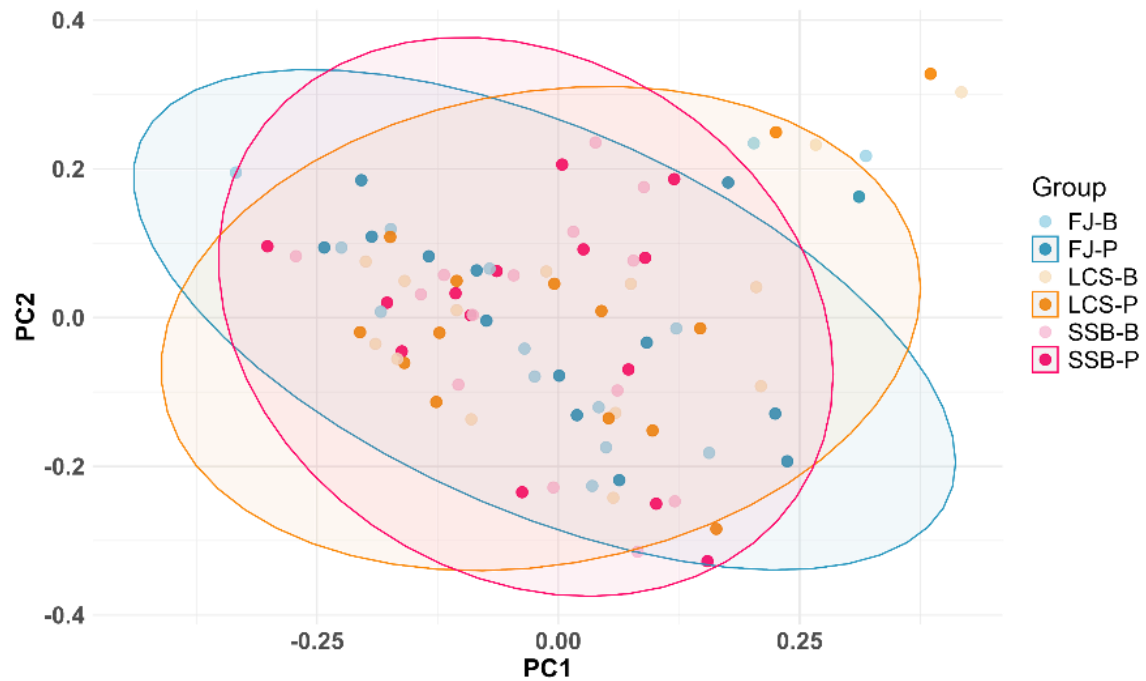
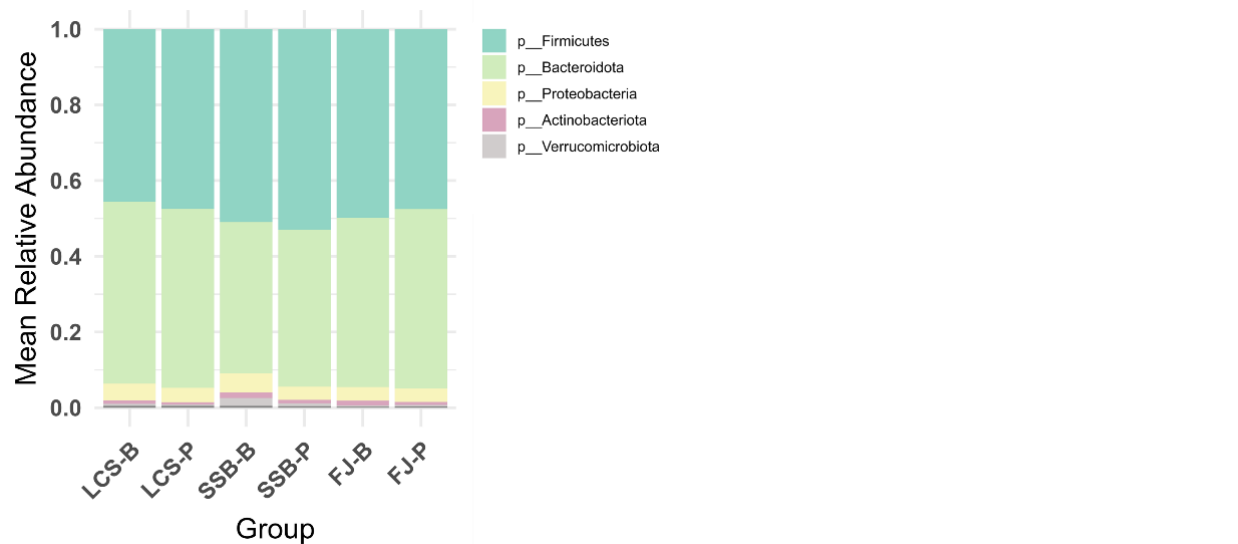
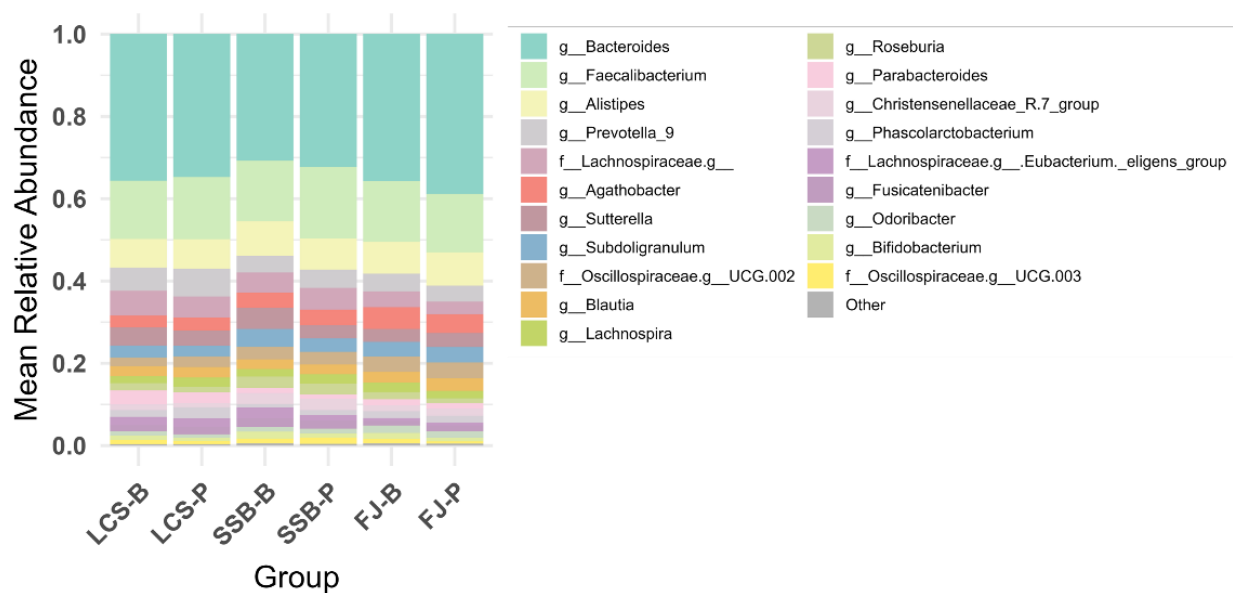


Figure 3. β -diversity (Bray-Curtis Dissimilarity) of the fecal microbiota measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB), or 100% natural fruit juice (FJ) in healthy adults. Gut microbiota composition was assessed using 16S rRNA sequencing and analyzed using PERMANOVA (group $\times$ condition interaction: $q = 0.999$). **Abbreviations:** baseline (B), post-intervention (P).



(a)



(b)

Figure 4. Mean relative abundance of the (a) six most dominant fecal microbiota phyla-level taxa and (b) 20 most dominant fecal microbiota genus-level taxa, measured before and after three weeks of consuming sucralose-sweetened beverages (LCS), sugar-sweetened beverages (SSB) or 100% natural fruit juice (FJ) in healthy adults for three weeks.

Gut microbiota composition was assessed using 16S rRNA sequencing. Group-wise comparisons of taxonomic abundances (mean abundance >0.2% in any group) were performed using Wilcoxon rank-sum tests (all $P \geq 0.161$). **Abbreviations:** baseline (B), post-intervention (P).

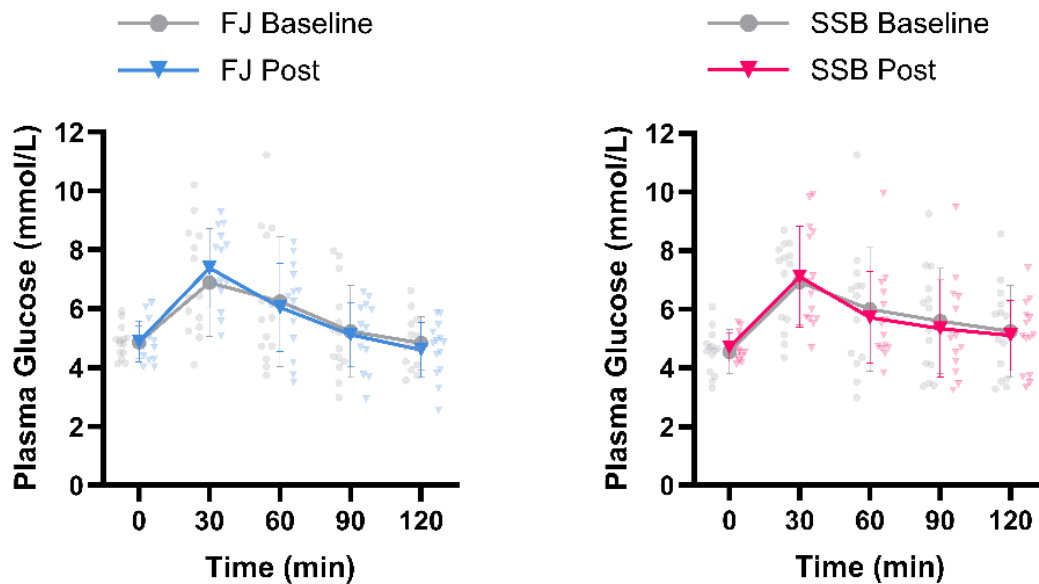


Figure 5. Plasma glucose concentrations during OGTT, measured before and after three weeks of consuming 100% natural fruit juice (FJ) or sugar-sweetened beverages (SSB), in healthy adults.

Data are presented as mean (SD) and individual points. Log-transformed data were analyzed using linear mixed-effects models with group (FJ, SSB), condition (baseline, post-intervention), and time (30, 60, 90, 120 min, excluding 0 min) as fixed effects, and participant ID as a random intercept (all $P \geq 0.075$). **Abbreviations:** post-intervention (Post).

STAR Methods Key resource table – Supplementary File

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Human Insulin ELISA	Millipore	Cat#EZHI-14K
Progesterone ELISA	Eagle Biosciences	Cat#PRG31-K01
Total Estradiol ELISA	Eagle Biosciences	Cat#ESD31-K01
Human LBP Sandwich ELISA	ProteinTech	Cat#KE00134
Human Zonulin ELISA	Cusabio	Cat#CSB-EQ027649HU
Quantikine CRP ELISA	R & D Systems	Cat#DCRP00B
Quantikine HS Human IL-6 ELISA	R & D Systems	Cat#HS600C
Quantikine HS Human TNF- α ELISA	R & D Systems	Cat#HSTA00E
Biological samples		
Bacterial and virus strains		
Chemicals, peptides, and recombinant proteins		
Beverage component : commercial sucralose	Splenda™ Original granulated sweetener, Heartland Food Products Group	N/A
Beverage component : sucrose	Redpath Sugar Ltd	N/A
Beverage component : orange artificial flavor	Kool-Aid™ orange unsweetened drink mix, Kraft Foods Group, INC.	N/A
Beverage component : 100% natural fruit juice	Simply Orange Pulp free, Coca-Cola™	N/A
OGTT (75 g glucose)	Trutol™, Thermo Scientific	N/A
2-ethylbutyric acid	Sigma-Aldrich	Cat#109959-100ML
Formic acid	Sigma-Aldrich	Cat#33015-1L
Volatile Free Fatty Acid Mix	Millipore Sigma	Cat#46975-U
RNase Away	Molecular BioProducts	Cat#7002
Critical commercial assays		
Autokit Glucose (Mutarotase-GOD)	Fujifilm	Cat#997-03001
QIAamp Fast DNA stool mini kit	Qiagen	Cat#51306
Quant-iT PicoGreen dsDNA Assay Kit	ThermoFisher	Cat#P11496
KAPA HiFi HotStart ReadyMix PCR Kit	Roche Diagnostics (Fisher Scientific)	Cat#50-196-5299
Miseq Reagent Kit V3 600 Cycle Cartridge	Illumina	Cat#MS-102-3003
Nextera XT Index Primers	Illumina	Cat#FC-131-1001
PhiX Control v3	Illumina	Cat#FC-110-3001
AMPure XP Reagent, 60 mL	Beckman Coulter	Cat#A63881
Experimental models: Organisms/strains		

Oligonucleotides		
16S rRNA forward primer (5' - 3'): TCGTCGGCAGCGTCAGATGTGTATAAGAGAC AGCCTACGGGNGGCWGCAG	IDT	Custom
16S rRNA reverse primer (5' - 3'): GTCTCGTGGGCTCGGAGATGTGTATAAGAGA CAGGACTACHVGGGTATCTAATCC	IDT	Custom
Recombinant DNA		
Software and algorithms		
Jamovi	Jamovi	v.2.3.28
Jamvovi gamlj module	Jamovi	v.2.6.6
Gen5	Bio-Tek Instruments	v.1.11.5
Qiime2	Qiime2	
Prism 10	Graphpad	v.10.1.2
R and RStudio	R Developers	v.4.4.2
Other		
OM-AC1 Toilet Accessory	DNA Genotek	684
Cold transport container	SARSTEDT	95.1123
Faeces tube, Screw cap, (LxØ): 101 x 16.5 mm, transparent, sterile (with spatula)	SARSTEDT	80.623
2.8 mm Ceramic Bulk Beads (Zirconium oxide)	OMNI International	19-646
FreeZone 2.5 L Benchtop Freeze Dryer	Labconco	#10400000
0.20 µm filters	BioBasic	#HP141
SureSTART glass inserts	ThermoFisher Scientific	#6PME03C1SP
SureSTART 2 mL glass snap top amber vials	ThermoFisher Scientific	#6ARV11-2P
SureSTART 11mm Snap Caps	ThermoFisher Scientific	#6ERC11PEC

CHAPTER VI. Discussion

Summary of Findings

Excessive consumption of free sugars, particularly through beverages, is widely recognized as a contributor to adverse metabolic health outcomes. This raises a critical question: beyond water, is there a healthier alternative that allows consumers to enjoy sweetness without incurring its metabolic consequences? This thesis aimed to address this question through a series of four interrelated articles.

The first article was a narrative review examining the metabolic health effects of SSB and natural FJ, with a particular focus on lipid metabolism. A key finding was that, although natural FJ contain beneficial bioactive compounds such as vitamins and antioxidants alongside free sugars, current evidence remains insufficient to determine whether they represent a healthier alternative to SSB. Beyond FJ, LCS have emerged as the leading alternative to refined sugars in beverages. These have long been regarded as physiologically inert, based on the assumption that they are not metabolized for energy and therefore exert no meaningful impact on metabolic health. This assumption served as the conceptual foundation for the subsequent articles in this thesis, which critically evaluated its validity.

The second article concisely contextualized key findings on the potential metabolic effects of LCS, emphasizing on clinical relevance for physicians and health professionals. This work highlighted two limitations in the existing literature that constrained the generalizability of current evidence: 1) a predominance of studies examining acute exposure to LCS, rather than the cumulative effects of frequent consumption. This is a critical gap considering that metabolic outcomes are more likely to result from habitual dietary patterns; 2) a tendency of drawing inference on LCS as a collective “class of compounds”, rather than evaluating them through sweetener-specific hypotheses, despite

convincing evidence that LCS exert distinct physiological effects based on their distinct chemical structures.

To address these gaps and refine the thesis framework, the third article focused on sucralose, the most widely used LCS in the global food industry. This comprehensive critical review synthesized and evaluated findings from RCTs in humans on the cumulative effects of sucralose consumption on postprandial glycemic responses and gut microbiota as a proposed underlying mechanism. The review revealed substantial methodological heterogeneity across studies, including variations in dose, duration of exposure, comparator selection (i.e., water, SSB or a different LCS), form of administration (i.e., beverages, capsules), and approaches to assessing glucose tolerance and microbiota-related outcomes. These inconsistencies drastically limit the generalizability of current findings. Importantly, this comprehensive critical review also addressed the considerable interindividual variability in gut microbiota and its potential influence on glucose homeostasis, offering key considerations for interpreting microbiota data and designing future studies.

Building on these insights, the final article of the thesis presented findings from a randomized controlled trial (NARELO study) evaluating the effects of a three-week consumption of sucralose-sweetened beverages, compared with SSB, on postprandial glycemic responses in healthy adults under real-world conditions. The study also investigated whether changes in postprandial glycemic responses were accompanied by alterations in insulinemic responses, gut microbiota profile, as well as plasmatic biomarkers of intestinal permeability and systemic low-grade inflammation; mechanisms previously proposed in the literature. To address gaps identified in the first article of the thesis, an exploratory comparator group consuming natural FJ was included to assess whether free sugars from a natural source elicit distinct metabolic responses compared to SSB. We demonstrated that realistic sucralose consumption impairs postprandial glycemic responses in healthy young adults compared to SSB. Interestingly, these effects occurred independently of the mechanistic pathways investigated, underscoring the need for

continued efforts to uncover the underlying mechanisms explaining alterations in glucose tolerance. Reflecting on the journey that led to the completion of NARELO, this clinical trial was the product of five grant applications, relentless participant recruitment during a global pandemic, and data collection carried out through pregnancy and early motherhood. More importantly, it is a story of sincere scientific collaboration and unwavering grit, lessons that transcend the findings of this thesis and continue to shape my approach to research.

Limitations

Since the limitations of this thesis stem from the original experimental study (Thesis Article 4), these are addressed in detail in Chapter V to avoid redundancy.

Future Research Perspectives

As part as their most recent guidelines “*Use of non-sugar sweeteners*” released in 2023⁶⁸, the WHO emphasized the lack of evidence supporting long-term health benefits from substituting LCS for free sugars, beyond those achieved by simply reducing free sugar intake. While this conclusion is well founded, the process of writing this thesis has led me to adopt a more nuanced perspective. Across the thesis articles presented herein, the need for sweetener-specific hypotheses was identified as a critical consideration. Additionally, concerns were raised regarding the substantial methodological heterogeneity in existing studies, which severely limited the generalizability of current findings. Therefore, while the NARELO study demonstrated that sucralose impairs glucose tolerance in humans, generalizing these findings to all LCS would remain a conjecture. Instead, our results challenge the prevailing assumption that the absence of caloric contribution implies metabolic neutrality, underscoring the need to re-evaluate the metabolic effects of each LCS individually. Looking ahead, it is plausible that a LCS, either currently known or yet to be discovered, could provide sweet taste without adverse metabolic consequences. Among the most promising candidates are rare sugars, a class of monosaccharides found

in trace amounts in nature, including in honey, maple syrup, and various fruits, vegetables, and grains⁴⁰. While direct extraction from these sources is economically unfeasible, novel enzymatic and microbial fermentation techniques are being explored to enable scalable production³⁸. Unlike most LCS, rare sugars such as D-allulose and D-tagatose have a relative sweetness comparable to sucrose²³⁹, yet are only partially metabolized⁴⁰. Although data on their metabolic and physiological effects is still in its early stages, a small number of animal trials suggest potential benefits, including improved glycemic control^{39,40} and favorable modulation of the gut microbiota⁴¹. Currently, both D-allulose and D-tagatose are approved as food ingredients in the United States^{42,43}. In Canada, however, only D-tagatose has received approval from by Health Canada⁴⁴, while D-allulose remains classified as a novel food ingredient requiring a comprehensive safety assessment prior to market entry⁴⁵. In light of these developments, rare sugars represent a promising avenue for replacing refined sugars and conventional LCS in food products. Accordingly, I believe that future studies should prioritize investigating their implications for metabolic health, particularly in relation to glucose homeostasis and the gut microbiota. It is my sincere hope that this thesis contributes to laying the groundwork for such research, a path I humbly intend to pursue as a future researcher.

Conclusion

This thesis provides novel evidence challenging the prevailing assumption that all LCS are physiologically inert. Moreover, it underscores the importance of compound-specific evaluation as the food industry innovates toward the next generation of LCS. Specifically, we demonstrated that cumulative sucralose consumption under real-world conditions can impair glucose tolerance in healthy adults over a three-week period. Notably, these changes were not observed following the consumption of SSB or natural FJ. Although several mechanistic pathways have been proposed in the literature to explain the metabolic effects of sucralose—including alterations in insulinemic responses, gut microbiota composition, SCFAs concentrations, intestinal barrier permeability, and systemic low-grade inflammation biomarkers—our findings did not support these

hypotheses under the experimental conditions tested. Furthermore, we highlighted critical gaps and limitations in current LCS and gut microbiota research that justify continued efforts to elucidate potential mechanisms in future studies to fully understand the metabolic implications of sucralose. More broadly, this thesis contributes to a complex and evolving area of research on the metabolic effects associated with the consumption of natural sugars, refined sugars, and LCS. Ultimately, I hope that the insights gained within this thesis, particularly regarding the metabolic effects of sucralose, will inform future research directions and public health recommendations. While there is still no clear consensus regarding which sweeteners, whether currently known or yet to be developed, may serve as suitable alternatives to refined sugars, the findings presented in my thesis suggest that sucralose may not be the answer we are looking for.

CHAPTER VII. References

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APPENDICES

23/07/2020

Université d'Ottawa

Bureau d'éthique et d'intégrité de la recherche

University of Ottawa

Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-04-20-5636
Titre du projet / Project Title	The effects of consuming natural sugars, refined sugars and low-calorie sweeteners on the gut microbiota and risk factors for cardiovascular and metabolic diseases.
Type de projet / Project Type	Thèse de doctorat / Doctoral thesis
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	23/07/2020
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	22/07/2021

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Alexandra PÉPIN	École des sciences de l'activité physique / School of Human Kinetics	Chercheur Principal / Principal Investigator
Pascal IMBEAULT	École des sciences de l'activité physique / School of Human Kinetics	Superviseur / Supervisor
Jean-François MAUGER	École des sciences de l'activité physique / School of Human Kinetics	Collaborateur / Collaborator
Krista POWER	École des sciences de la nutrition / School of Nutrition Sciences	Collaborateur / Collaborator

Conditions spéciales ou commentaires / Special conditions or comments

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Université d'Ottawa

Bureau d'éthique et d'intégrité de la recherche

University of Ottawa

Office of Research Ethics and Integrity

Le Comité d'éthique de la recherche (CÉR) de l'Université d'Ottawa, opérant conformément à l'*Énoncé de politique des Trois conseils* (2014) et toutes autres lois et tous règlements applicables, a examiné et approuvé la demande d'éthique du projet de recherche ci-nommé.

L'approbation est valide pour la durée indiquée plus haut et est sujette aux conditions énumérées dans la section intitulée "Conditions Spéciales ou Commentaires". Le formulaire « Renouvellement ou Fermeture de Projet » doit être complété quatre semaines avant la date d'échéance indiquée ci-haut afin de demander un renouvellement de cette approbation éthique ou afin de fermer le dossier.

Toutes modifications apportées au projet doivent être approuvées par le CÉR avant leur mise en place, sauf si le participant doit être retiré en raison d'un danger immédiat ou s'il s'agit d'un changement ayant trait à des éléments administratifs ou logistiques du projet. Les chercheurs doivent aviser le CÉR dans les plus brefs délais de tout changement pouvant augmenter le niveau de risque aux participants ou pouvant affecter considérablement le déroulement du projet, rapporter tout événement imprévu ou indésirable et soumettre toute nouvelle information pouvant nuire à la conduite du projet ou à la sécurité des participants.

The University of Ottawa Research Ethics Board, which operates in accordance with the *Tri-Council Policy Statement* (2014) and other applicable laws and regulations, has examined and approved the ethics application for the above-named research project.

Ethics approval is valid for the period indicated above and is subject to the conditions listed in the section entitled "Special Conditions or Comments". The "Renewal/Project Closure" form must be completed four weeks before the above-referenced expiry date to request a renewal of this ethics approval or closure of the file.

Any changes made to the project must be approved by the REB before being implemented, except when necessary to remove participants from immediate endangerment or when the modification(s) only pertain to administrative or logistical components of the project. Investigators must also promptly alert the REB of any changes that increase the risk to participant(s), any changes that considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project or the safety of the participant(s).

Kim THOMPSON

Responsable d'éthique en recherche / Protocol Officer

Pour/For **Daniel LAGAREC** Président(e) du/ Chair of the **Comité d'éthique de la recherche en sciences de la santé et sciences / Health Sciences and Sciences Research Ethics Board**

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CONSENT FORM

Metabolic Effects of Natural Sugars, Refined Sugars and Low-Calorie Sweeteners

Researcher (Primary Investigator):

Alexandra Pepin, RD., PhD candidate.
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Thesis supervisor:

Pascal Imbeault, PhD.
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Faculty of Health Sciences, University of Ottawa
School of Human Kinetics,
Behavioral and Metabolic Research Unit (BMRU/URCM)

Why am I being invited to take part in a research study?

We invite you to take part in a research study conducted by Alexandra Pepin, doctoral candidate at the Behavioral and Metabolic Research Unit (BMRU) of the University of Ottawa because you have answered a telephone screening interview and reported that you...

Are between the age of 18-35 years

Are non-smoker (including vaping)

Have a stable body weight over the prior 6 months

Do not have diabetes mellitus or liver, kidney or thyroid disorders, or take hypolipidemic, anti-diabetic, antihypertensive or anti-depression medication

Purpose of the research: *Why is this research being done?*

We hope to learn more about the effects of consuming natural fruit juices, sugar-sweetened beverages and beverages with low-calorie sweeteners (sugar substitutes) on your blood glucose concentrations, your body's sensitivity to the hormone insulin and your inflammation

markers. This project is unique because we also hope to learn more about the impacts of these different sweeteners on your intestinal health and your intestinal microbiota.

How long will the research last?

We expect that you will be in this research study for approximately 28 days, including a 7-day run-in period and 21 days of intervention.

How many people will be studied?

We expect to study about 40 people, including women and men.

Whom can I talk to?

If you have any questions or concerns, you can talk to the research team:

Researcher (Primary Investigator):

Alexandra Pepin, RD., PhD candidate.

alexandra.pepin@uottawa.ca ; (613) 562-5800 ext. 2655

Thesis supervisor:

Pascal Imbeault, PhD.

imbeault@uottawa.ca ; (613) 562-5800 ext. 7290

What happens if I want to participate to this study?

During a preliminary session (which includes an in-person screening interview), we will explain this research study to you, including: the nature and purpose of the research study, the procedures to be followed, any common or important discomforts and risks and any benefits you might expect.

Whether or not you take part is up to you.

You can agree to take part now and later change your mind.

You can ask all the questions you want before you decide.

If you agree to take part, you will be given a signed and dated copy of this document.

You will need to pass the following “in-person screening” tests or procedures to determine if you are eligible to participate to the study. For the tests to be valid, it will be necessary for you to follow these dietary guidelines:

Do not consume **alcoholic beverages** or use **cannabis** the day before the screening visit.

Do not eat or drink anything except water and do not engage in exercise/sport

The tests will occur at the Behavioral and Metabolic Research Unit (BMRU) located at the Lees Campus of the University of Ottawa and will last a total of 75 minutes.

- **Body measurements:** Your height, weight, and waist circumference will be measured using a measuring tape (wearing a hospital gown without shoes).
- **Blood pressure:** Your blood pressure will be measured using an automatic sphygmomanometer, the same kind of equipment used by family physician. Please wear a t-shirt/short sleeve shirt.
- **Fasting blood glucose:** Your fasting blood glucose will be measured using a glucometer with a capillary blood droplet collected on the tip of your finger. This method is the one used multiple times a day by individuals with diabetes.
- **Food questionnaires:** You will be asked to complete two online questionnaires about your dietary habits and your relationship with food cravings. If possible, please bring your smartphone as the questionnaires can be accessed using a QR code.

If the screening tests and procedures show that you can participate in the study, and you choose and are selected to take part, you will be offered to stay at the laboratory to continue the preliminary session.

PRELIMINARY SESSION

During the second part of the preliminary session (60 minutes / tests and procedures), you will be asked to complete one brief questionnaire about your dietary habits and their relationship with food cravings. Then, your body composition and your resting metabolic rate will be measured. These two procedures are detailed below. For the tests to be valid, it is important that you...

*Do not consume **alcoholic beverages** or use **cannabis** the day before the screening visit.*

Do not eat or drink anything except water and do not engage in exercise/sport

- **Resting Metabolic Rate (RMR):** Your daily energy needs will be determined by measuring the amount of oxygen and carbon dioxide in the air that you breathe in and out (indirect calorimetry). To do so, you will be wearing a mask that covers your nose and mouth and you will be asked to stay awake, motionless, in a semi-recumbent position for about 30 minutes.

During the third part of the preliminary session (30 minutes / information session), you will receive essential information to prepare you for your participation in the study. The first thing you need to know is that this 3-week study is preceded by a 7-day run-in period to ensure that you are comfortable with the dietary protocol and to “reset” your gut microbiota for the study. During this meeting, you will...

- Receive specific dietary instructions to follow during the 7-day run-in period. More specifically, you will be asked to maintain your usual diet while refraining from consuming sugar-sweetened beverages, fruit juices and foods and beverages that contain low-calorie sweeteners (a detailed list of foods and beverages to avoid will be provided to you). You will also receive training from the Primary Investigator, who is also a registered dietitian, on how to read and understand nutrition labels. In addition, you will be asked to refrain from drinking more than 2 alcoholic beverages/d, using cannabis (due to cannabis-induced alterations to appetite) and taking any vitamin, mineral, dietary, probiotic, natural, herbal (including herbal tea) or sports supplement.
- Provided with instructions on proper collection methods and given material to collect your stool samples at home. You will need to collect a very small quantity of stool samples (2 tubes of 10 mL each) at two points during this study, at the beginning and at the end of the study. You will need to bring your frozen stool samples to our research facility at both of your experimental sessions.

Following the 7-day run-in period, an appointment for your first experimental session at the BMRU will be scheduled. The baseline experimental session marks the beginning of the 3-week study. You must bring your first frozen stool sample for this session.

IT IS IMPORTANT THAT YOU...

*Do not consume **alcoholic beverages** or use **cannabis** the day before the experimental session.*

*Eat the **standardized meal** that will be provided to you for your **supper** on the evening before the experimental session.*

***Do not eat or drink anything except water** and **do not engage in exercise/sport** before the experimental session starting **12 hours** before your scheduled appointment time.*

*Do not use **nonsteroidal anti-inflammatory drugs** (such as ibuprofen Advil™ or Diclofenac diethylamine (Voltaren™ topical gel)) for 24h prior to the experimental*

FIRST EXPERIMENTAL SESSION

You will be asked to come to the laboratory at ~8h00 AM after a 12-h overnight fast. You will need to bring your first frozen stool sample for this session. The following tests will occur during the first experimental session, that is right before the beginning of the study, and will be repeated 3 weeks later during the second experimental session, that is at the very end of the study. Each experimental session will be of a duration of 3h00. Please wear a t-shirt/short sleeve shirt and comfortable clothing.

Tests and Procedures:

- **Body measurements:** Your height, weight, and waist circumference will be measured using a measuring tape (wearing a hospital gown without shoes).
- **Blood pressure:** Your blood pressure will be measured using an automatic sphygmomanometer, the same kind of equipment used by family physician.
- **Body composition:** The composition of your body (bone density, lean and fat body mass) will be measured using dual energy X-ray absorptiometry (DEXA). You will lie on a padded table while a low-intensity X-ray tube scans your entire body for 5 to 10 minutes. The radiation level for each DEXA scan is lower than what you would be exposed to during a transcontinental airplane flight.
- **Food questionnaires:** You will be asked to fill out two online questionnaires about your dietary habits and your relationship with food cravings. This should take about 15 minutes. If possible, please bring your smartphone as the questionnaires can be accessed using a QR code.
- **Blood samples:** Blood samples will be collected through a catheter. You will have an intravenous line placed in a vein in your forearm by a certified phlebotomy technician. More precisely, a needle will be inserted into a vein in your arm through which a small, flexible plastic tubing (catheter) is placed. The needle will be removed leaving the tubing taped in place. This tubing will allow us to collect small blood samples throughout the procedures without multiple venous punctures. A fasting blood sample will be drawn (2 samples of 6mL). Then, you will be given 5 minutes to drink a measured dose of sugar solution (75g of glucose). Blood draws (2 samples of 6mL) will be collected at 30, 60, 90 and 120 min following the ingestion of the solution to measure your blood glucose concentrations and your body's sensitivity to insulin (a hormone naturally produced by your body). A total of ~60mL of blood will be collected for this procedure, which is a minimal amount. In comparison, a regular blood donation is around 470mL of blood.

You will leave the first experimental session with a 1-week supply of study beverages.

Study beverages and Dietary Protocol:

For 3 weeks, you will drink **study beverages**. These beverages will be provided to you as 3 servings/day, approximately 1 cup (250 mL)/serving, and are to be consumed one at each meal. You will be provided with either 100% natural fruit juice or flavored beverages that contain sugar or sucralose which is low-calorie sweetener branded under the name of Splenda™ and used in several diet beverages available on the market. The beverage that you get will be chosen by chance, like flipping a coin. Neither you nor the researcher will choose what beverage type you get. You will be informed which beverage you received only at the end of the study. In order for this study to produce scientifically useful information, it is necessary that you consume all of the beverages provided each day for the 3 weeks of the study.

In addition to consuming study beverages daily, you will be required to comply to the following **dietary protocol** : For the duration of the 3-week study, you will be asked to maintain your usual diet while refraining from consuming sugar-sweetened beverages, fruit juices and foods and beverages that contain low-calorie sweeteners **other than the study beverages provided by our research team** (a list of specific foods and beverages to avoid during the study will be provided to you). In addition, you will be asked to refrain from drinking more than 2 alcoholic beverages/d, using cannabis (as cannabis affects some of the variables measured in this study) and taking any vitamin, mineral, dietary, probiotic, natural, herbal (including herbal tea) or sports supplement. At any time during the study, you may request to meet in person with the registered dietitian if you have any questions or if you are experiencing difficulties with the dietary protocol.

WEEKLY VISITS

Each week, you will need to schedule a 20-minute visit to pick up your weekly supply of beverages and return your used beverage bottles at the BMRU (total of 2 visits). You will receive 7 frozen bottles of study beverages (1 bottle per day to be consumed in 3 doses with meals) in a cooler bag, which you will keep at home in your refrigerator to defrost. It is important that you do not wash or rinse the containers as they must be weighed by our research staff beforehand. At each pick-up/return visit, you will be asked to fill out a brief online questionnaire (10 questions) on cravings for savory and sweet foods and beverages.

SECOND EXPERIMENTAL SESSION

On the last day of the 3-week study, an appointment for your second experimental session will be scheduled. The post-intervention experimental session marks the end of the 3-week study. You will be asked to bring your second stool sample for this session.

You will be asked to come to the laboratory at ~8h00 AM after a 12-h overnight fast. The same tests and procedures that occurred during your first experimental session will be repeated. This session will be of a duration of 3h00.

IT IS IMPORTANT THAT YOU...

*Do not consume **alcoholic beverages** or use **cannabis** the day before the experimental session.*

*Eat the **standardized meal** that will be provided to you for your **supper** on the evening before the experimental session.*

***Do not eat or drink anything except water** and **do not engage in exercise/sport** before the experimental session starting **12 hours** before your scheduled appointment time.*

*Do not use **nonsteroidal anti-inflammatory drugs** (such as ibuprofen Advil™ or*

What are my responsibilities if I take part in this research?

If you take part in this research, you will be responsible for:

- Consuming the beverages (1 bottle per day) provided by our research unit as required for 3 weeks (21 days).
- Follow the dietary instructions provided to you for the entire duration of the run-in period and the study, i.e. refraining from consuming sweetened beverages, fruit juices and foods and beverages containing low-calorie sweeteners other than the study beverages provided by our research team for the entire duration of this study.
- Picking up, once a week, new supplies of your study beverages at the BMRU.
- Returning all beverage bottles to our research unit on a weekly basis. It is important that you do not wash or rinse the containers as they must be weighed by our research staff beforehand.
- Reporting any deviations from the dietary protocol to the Primary Investigator.
- Participating in the study procedures and showing up on time to appointments. If you fail to keep your appointments for beverage pickup/return and do not make other arrangements in time to allow for uninterrupted study as required by the protocol, you will unfortunately be dismissed from the study.

All provided study supplies and materials are property of the Behavioral and Metabolic Research Unit (BMRU) of the University of Ottawa and it is your responsibility to return them upon completion of the study.

What happens if I say yes, but I change my mind later?

It is your right to leave the research at any time without any consequences.

Is there any discomforts and risks that could occur if I participate in this study?

Study beverages and Dietary Protocol: During the 7-day run-in period and the 3 weeks of this study, you will receive specific dietary instructions to follow. This study will require you to exclude most of sugars and low-calorie sweeteners from your diet with the exception of the study beverages that will be provided to you. We ask you to consider carefully whether this restriction is one that you can accommodate. You may experience gastrointestinal discomforts due to changes in your normal eating habits. If you do experience significant uncomfortable symptoms, please do not hesitate to discuss them with our research staff.

Body weight: Minimal weight variations may occur due to changes in normal eating habits over the course of 3 weeks. You may request to meet with the study registered dietitian for counseling if they do not return to your original body weight within one month after the study.

Catheter: Sometimes proper insertion of the catheter is not accomplished on the first attempt and additional insertion attempts are required. The insertion of the catheter will cause temporary discomfort and may cause bruising. The certified phlebotomy technician will need to flush the catheter line with saline solution in order to be able to draw blood. It is possible that flushing the catheter line may cause some discomfort at the area where the catheter is inserted. Blood sampling poses low risks ranging from simple redness of the skin to a local inflammation of the vein.

Radiological risks (DEXA scan): This study involves a low radiation exposure that is less than other diagnostic tests using ionizing radiation (for example, X-rays of a bone fracture). The radiation exposure received in this study is below levels that result in a significant health risk. Over the course of this study, you will go through 3 DEXA scans. The radiation level for each DEXA scan is lower than what you would be exposed to during a transcontinental airplane flight.

Will being in this study help me in any way?

You will receive your body composition (DEXA) results and your estimated daily energy expenditure results free of charge. This information could be used to estimate the risk of developing health problems. You will also receive training on how to read and understand nutrition labels. Moreover, the results from this study will provide essential knowledge that may have a positive impact on the development of public health guidelines by clarifying the effects of natural sugars, refined sugars and low-calorie sweeteners consumption on intestinal health and risk factors for cardiovascular and metabolic diseases in the Canadian population.

Is there a financial compensation to take part in this study?

To thank you for your contribution to the research project, you will be given the option to enter your name in a draw to win an iPad Air (256GB) including AppleCare+ valued at 979\$ + tx.

The draw is open to all research participants who enter their name in the draw, regardless of whether they decide to withdraw from further participating in the research project. Participants who enroll in the study will receive 1 participation coupon at the end of the preliminary session. A second participation coupon will be given to participants who complete the first experimental session. In addition, participants will be given 2 participation coupons for every week completed of the 3-week study. Finally, participants who complete the study will be given an additional 12 participation coupons on the day of their second experimental session. Each participant can thus cumulate a maximum of 20 coupons to participate in the draw upon completion of all the stages of study. At the end of the full study data collection, a name will be randomly selected amongst those who have entered and the person whose name is drawn will be informed by email and/or telephone. To win the prize, the person must correctly answer a skill-testing question. If the person cannot be reached within 14 days from the date of the draw, the prize will be awarded to the second name that is randomly selected and so on until the prize has been awarded. The odds of winning a prize are approximately 1/42. The prize must be accepted as awarded or forfeited and cannot be redeemed for cash. Your name and contact information that you will provide when entering the draw will be collected for the purposes of contacting the participant selected in the draw. Participants' name and contact information will be kept confidential and then destroyed once the prize has been awarded.

Confidentiality, Anonymity & Conservation of Data: What happens to the information collected to the research?

In order to guarantee confidentiality and anonymity, all precautions and necessary measures will be taken to ensure that your results and personal information are kept under the strictest of confidentiality.

- Only the primary investigator (Alexandra Pepin, registered dietitian. PhD candidate) and her thesis supervisor (Pascal Imbeault, PhD.) will have access to your personal information and results. Any other individuals involved in the study will not have access to your personal information and/or results.
- Your name will not appear on any reports. A unique number code will be used to identify you on all research documents.
- All material and information which can be linked to you will not be made public and will be kept under the strictest confidentiality.
- You will not be identified in any way in publications or reports.
- The data collected will be kept in a locked cabinet in our research facility with restricted access where all participants' folders are kept. In addition, the computer files will be protected by a password.

- Following measurements of metabolic parameters in blood samples by a laboratory technician from our research team, remaining blood samples will be placed in biohazard waste containers. The discarded samples will then be collected and destroyed according to the biosafety guidelines of the office of the risk management of the University of Ottawa.
- The measurements of gut microbiota composition will be done by Dr. Krista Power's team, one of our collaborators at the School of Nutrition Sciences of the University of Ottawa, using methods routinely performed by her group. Only the coded stool samples will be transferred to Dr. Power's laboratory. It will not be possible for lab technicians to identify participants with the codes. Once the analyses are completed, the discarded stool samples will be destroyed.
- Data will be destroyed five years after publication of study results.

During the course of the research, we will collect blood and stool samples. As the complex relationship between nutrition and the intestinal microbiota is a very new and rapidly evolving area of research, we would like to retain some of the remaining samples for future research purposes. The remaining of your blood and stool samples could be used for the analysis of new markers that will be discovered in the coming years. If you agree, these biological specimens will be stored in the laboratory of Dr. Pascal Imbeault and retained for up to 10 years. Only Pascal Imbeault's study staff will have access to the specimens. Paper copies of some of your data will be stored in your participant file, located in a locked cabinet in a locked office. All information about your identity will be removed from the participant file 5 years after publication of the study results. Electronic copies of data will not contain information about your identity and will be stored on a secure server for up to 10 years.

If you agree that we keep some of your remaining biological specimens for future research purposes, they will become the property of the University of Ottawa. The specimens may be used in this study or other related studies and may be shared with other institutions (for example, to measure a blood parameter for which we do not have the necessary equipment). Under no circumstances will the specimens be used for DNA or RNA research.

If you agree to share all or part of the biological specimen(s) collected from you, please check the corresponding boxes and initial below.

- ☐ Blood samples
- ☐ Stool samples

I agree to share biological specimen(s) collected from me :

Your initials: _____

If you **do not agree** to share any of the biological specimen(s) collected from you for future research purposes, please initial below.

I do not agree to share biological specimen(s) collected from me :

Your initials: _____

VOLUNTARY PARTICIPATION

I am under no obligation to participate and if I choose to participate, I can withdraw from the study at any time and/or refuse to answer any questions, without suffering any negative consequences. If I withdraw from the study, my data can only be used if I give permission. At any moment during this study, my best interests will always prevail upon the objectives of the study. I will be made aware of new findings that might influence my decision to take part in the present study.

Acceptance: I, _____, agree to participate in the above research study conducted by Alexandra Pepin of the School of Human Kinetics, Faculty of Health Sciences, University of Ottawa, which research is under the supervision of Professor Pascal Imbeault, PhD.

If I have any questions about the study, I may contact the researcher or her supervisor (contact information on page 1).

If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research of the Office of Research Ethics and Integrity of the University of Ottawa. Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5
Tel.: (613) 562-5387 Email: ethics@uottawa.ca

There are two copies of the consent form, one of which is mine to keep.

Signature of participant

Date

Printed name of participant

Signature of person obtaining consent

Date

Printed name of person obtaining consent

Université d'Ottawa | University of Ottawa

Faculté des Sciences de la Santé | Faculty of Health Sciences

École des Sciences de l'Activité Physique | School of Human Kinetics

FORUMULAIRE DE CONSENTEMENT

Les Effets Métaboliques des Sucres Naturels, des Sucres Raffinés et des Édulcorants.

Chercheuse (Investigatrice Principale):

Alexandra Pepin, Dt.P., candidate PhD.

alexandra.pepin@uottawa.ca ; (613) 562-5800 ext. 2655

Superviseur de thèse:

Pascal Imbeault, PhD.

imbeault@uottawa.ca ; (613) 562-5800 ext. 7290

Faculté des Sciences de la Santé, Université d'Ottawa

École des Sciences de l'Activité Physique,

Unité de Recherche sur le Comportement et le Métabolisme (URCM/BMRU)

Pourquoi suis-je invité à participer à une étude de recherche ?

Nous vous invitons à participer à une étude de recherche menée par Alexandra Pepin, candidate au doctorat à l'Unité de Recherche sur le Comportement et le Métabolisme de l'Université d'Ottawa parce que vous avez répondu à une entrevue de sélection téléphonique et que vous...

Êtes âgés de 18 à 35 ans

Êtes non-fumeurs (incluant les vapoteuses i.e. vaping)

Rapporté un poids corporel stable au cours des 6 mois précédents

N'êtes pas atteint de diabète ou de troubles hépatiques, rénaux ou thyroïdiens, ou ne prenez pas de médicaments hypolipidémiques, hypoglycémifiants, antihypertenseurs ou antidépresseurs.

Objectif de la recherche : *Pourquoi cette recherche est-elle effectuée?*

Nous espérons en apprendre davantage sur les effets associés à la consommation de jus de fruits naturels, de boissons sucrées et de boissons contenant des édulcorants (substituts du sucre) sur vos concentrations sanguines de glucose, la sensibilité de votre corps à l'insuline (une hormone naturellement produite par votre corps) et vos marqueurs d'inflammation dans le sang. Ce projet est unique, car nous espérons également en apprendre davantage sur les effets de ces différents sucres et substituts de sucre sur votre santé intestinale et votre microbiote intestinal.

Quelle sera la durée de l'étude?

Votre participation à ce projet de recherche sera d'une durée d'environ 28 jours, incluant une période préparatoire (" *run-in* ") de 7 jours et 21 jours d'intervention.

Combien d'individus seront étudiés?

Nous prévoyons étudier environ 40 individus, incluant des femmes et des hommes.

À qui puis-je parler?

Si vous avez des questions ou des préoccupations, vous pouvez vous adresser à l'équipe de recherche :

Chercheuse (Investigatrice Principale):

Alexandra Pepin, Dt.P., candidate PhD.

alexandra.pepin@uottawa.ca ; (613) 562-5800 ext. 2655

Superviseur de thèse:

Pascal Imbeault, PhD.

imbeault@uottawa.ca ; (613) 562-5800 ext. 7290

Que se passe-t-il si je souhaite participer à cette étude?

Durant une session préliminaire (comportant une entrevue de sélection en personne), nous vous expliquerons en détail en quoi consiste ce projet de recherche, notamment : la nature et le but de l'étude, les procédures à suivre, les inconforts et les risques pouvant survenir et les avantages que vous pourriez retirer de cette étude.

Il vous appartient de décider si vous voulez y participer ou non.

Vous pouvez accepter de participer maintenant et changer d'avis plus tard.

Vous pouvez poser toutes les questions que vous désirez avant de prendre votre décision.

Si vous acceptez de participer, une copie signée et datée de ce document vous sera remise.

Vous devrez passer les tests et procédures de “ présélection en personne ” suivants afin de déterminer si vous êtes éligible à participer à l'étude. Pour que ces tests soient valides, il vous faudra suivre les directives nutritionnelles suivantes :

*Ne consommez pas de **boissons alcoolisées ou de cannabis** la veille de votre séance préliminaire.*

Ne mangez et ne buvez rien d'autre que de l'eau et ne faites pas d'exercice ou de sport 12 heures avant l'heure prévue de votre rendez-vous.

Les tests se dérouleront à l'Unité de Recherche sur le Comportement et le Métabolisme (URCM) situé sur le campus Lees de l'Université d'Ottawa et seront d'une durée totale de 75 minutes.

- **Mesures corporelles** : Votre taille, votre poids, votre tour de taille seront mesurés à l'aide d'un ruban à mesurer (portant une blouse d'hôpital sans chaussures).
- **Tension artérielle** : Votre tension artérielle sera mesurée à l'aide d'un sphygmomanomètre automatique, le même type d'équipement utilisé par les médecins de famille. Veuillez porter un chandail à manches courtes.
- **Glycémie à jeun** : Votre glycémie à jeun sera mesurée à l'aide d'un glucomètre avec une goutte de sang capillaire prélevée sur le bout de votre doigt. Cette méthode est celle utilisée plusieurs fois par jour par les personnes diabétiques.
- **Questionnaires sur l'alimentation** : Il vous sera demandé de remplir deux questionnaires sur vos habitudes alimentaires et sur votre relation avec l'alimentation et les envies alimentaires (“ *food cravings* ”). Si possible, veuillez apporter votre téléphone intelligent puisque les questionnaires sont accessibles à l'aide d'un code QR.

Si les tests et procédures de sélection montrent que vous pouvez participer à l'étude, et que vous choisissez et êtes sélectionné pour y participer, vous serez invité à rester au laboratoire pour continuer la session préliminaire.

SESSION PRÉLIMINAIRE

Au cours de la deuxième partie de la session préliminaire (60 minutes / tests et procédures), vous serez invité à remplir un bref questionnaire sur vos habitudes alimentaires et sur votre relation avec l'alimentation et les envies alimentaires (“ *food cravings* ”). Ensuite, votre composition corporelle et votre taux métabolique au repos seront mesurés. Ces deux procédures sont détaillées ci-dessous. Pour que les tests soient valides, il est important que vous...

*Ne consommez pas de **boissons alcoolisées ou de cannabis** la veille de votre séance préliminaire.*

Ne mangez et ne buvez rien 'autre que de l'eau et ne faites pas d'exercice ou de sport 12 heures avant l'heure prévue de votre rendez-vous.

- **Taux métabolique au repos (RMR) :** Vos besoins énergétiques quotidiens seront déterminés en mesurant la quantité d'oxygène et de dioxyde de carbone dans l'air que vous inspirez et expirez (calorimétrie indirecte). Pour ce faire, vous porterez un masque qui couvre votre nez et votre bouche et on vous demandera de rester éveillé, immobile, en position semi-couchée pendant environ 30 minutes.

Lors de la troisième partie de la session préliminaire (30 minutes / session d'information), vous recevrez des informations essentielles pour vous préparer à votre participation à l'étude. La première chose que vous devez savoir est que cette étude de 4 semaines est précédée d'une période préparatoire (" *run-in* ") de 7 jours servant à s'assurer que vous êtes à l'aise avec le protocole nutritionnel de l'étude et à " réinitialiser " votre microbiote intestinal pour l'étude. Au cours de cette session, vous...

- Recevrez des directives nutritionnelles spécifiques à suivre pendant la période préparatoire de 7 jours. Plus précisément, il vous sera demandé de maintenir vos habitudes alimentaires quotidiennes tout en vous abstenant de consommer des boissons qui contiennent des sucres ajoutés, des jus de fruits ainsi que des aliments et boissons qui contiennent des édulcorants (substituts de sucre) (une liste détaillée des aliments et des boissons à éviter vous sera fournie). Vous recevrez également une formation de la chercheuse principale de l'étude, qui est également une diététiste professionnelle, sur la manière de lire et de comprendre les étiquettes nutritionnelles. Finalement, il vous sera demandé de vous abstenir de boire plus de 2 boissons alcoolisées par jour, de consommer du cannabis et de prendre tout supplément vitaminique, minéral, nutritionnel, probiotique, naturel, herbal/à base de plantes (incluant la tisane) ou pour sportifs.
- Recevrez des instructions sur les méthodes de collecte appropriées ainsi que le matériel nécessaire pour prélever vos échantillons de selles à la maison. Vous devrez collecter une très petite quantité d'échantillons de selles (2 tubes de 10 mL chacun) à deux moments au cours de cette étude, soit au début et à la fin de l'étude. Vous devrez apporter vos échantillons de selles congelés à notre unité de recherche lors de vos deux sessions expérimentales.

Après la période préparatoire de 7 jours, un rendez-vous sera fixé pour votre première session expérimentale à l'URCM. La session expérimentale de base marque le début de l'étude de 3 semaines. Vous devez apporter votre premier échantillon de selles congelées pour cette session.

IL EST IMPORTANT QUE VOUS...

*Ne consommez **pas de boissons alcoolisées ni de cannabis** la veille de votre session expérimentale.*

*Mangez le **repas standardisé** qui vous sera fourni pour votre **souper** le soir précédant la session expérimentale.*

***Ne mangez et ne buvez rien d'autre que de l'eau et ne faites pas d'exercice ou de sport** 12 heures avant l'heure prévue de votre rendez-vous.*

*Ne pas utiliser d'**anti-inflammatoires non stéroïdiens** (comme l'ibuprofène Advil™ ou du Diclofenac diéthylamine (Voltaren™ gel topique)) pendant les 24h précédant la session expérimentale (l'acétaminophène peut être utilisé si nécessaire Tylenol™).*

PREMIÈRE SESSION EXPÉRIMENTALE

Vous devrez vous rendre à jeun au laboratoire à ~8h00am. Vous devrez apporter votre premier échantillon de selles congelées pour cette session. Les tests suivants auront lieu pendant la première session expérimentale, c'est-à-dire juste avant le début de l'étude, et seront répétés 3 semaines plus tard pendant la deuxième session expérimentale, c'est-à-dire à la toute fin de l'étude. Chaque session expérimentale aura une durée de 3h00. Veuillez porter un chandail à manches courtes et des vêtements confortables.

Tests et Procédures :

- **Mesures corporelles** : Votre taille, votre poids, votre tour de taille seront mesurés à l'aide d'un ruban à mesurer (portant une blouse d'hôpital sans chaussures).
- **Tension artérielle** : Votre tension artérielle sera mesurée à l'aide d'un sphymomanomètre automatique, le même type d'équipement utilisé par les médecins de famille.
- **Composition corporelle** : La composition de votre corps (densité osseuse, masse corporelle maigre et masse adipeuse (grasse)) sera mesurée par absorptiométrie biphotonique à rayons X (DEXA). Vous serez allongé sur une table coussinée, vêtu d'une blouse d'hôpital, tandis qu'un tube à rayons X de faible intensité scanner votre corps entier pendant 5 à 10 minutes. Le niveau de radiation pour chaque procédure

DEXA est inférieur à celui dont vous seriez exposé durant un vol transcontinental en avion.

- **Questionnaires sur l'alimentation** : Il vous sera demandé de remplir deux questionnaires sur vos habitudes alimentaires et sur votre relation avec l'alimentation et les envies alimentaires (" *food cravings* "). Si possible, veuillez apporter votre téléphone intelligent puisque les questionnaires sont accessibles à l'aide d'un code QR.
- **Prélèvements de sang** : Les échantillons de sang seront prélevés au moyen d'un cathéter. Un technicien certifié en phlébotomie placera une ligne intraveineuse dans une veine de votre avant-bras. Plus précisément, une aiguille sera insérée dans une veine de votre avant-bras par laquelle un petit tube en plastique souple (cathéter) sera placé. L'aiguille sera retirée, laissant le tube en place. Ce tube nous permettra de prélever de petits échantillons de sang tout au long de la procédure sans avoir à effectuer de multiples ponctions veineuses. Un échantillon de sang à jeun sera prélevé (2 échantillons de 6mL). Ensuite, vous aurez 5 minutes pour boire une dose mesurée d'une solution sucrée (75g de glucose). Des prélèvements sanguins (2 échantillons de 6mL) seront effectués à 30, 60, 90 et 120 min après l'ingestion de la solution afin de mesurer vos concentrations sanguines de glucose et la sensibilité de votre corps à l'insuline (une hormone naturellement produite par votre corps). Un total de ~60mL de sang sera prélevé pour cette procédure, ce qui représente une quantité minime. En comparaison, un don de sang régulier représente ~470mL de sang.

Vous quitterez la première session expérimentale avec un approvisionnement d'une semaine en boissons d'intervention.

Boissons d'intervention et Protocole nutritionnel :

Pendant 3 semaines, vous boirez les **boissons d'intervention**. Ces boissons vous seront fournies à titre de 3 portions par jour, soit environ 1 tasse (250 ml) par portion, et devront être consommées avec les repas. Vous recevrez soit des jus de fruits 100 % naturels, soit des boissons aromatisées contenant du sucre ou du sucralose, un édulcorant (substitut de sucre) commercialisé sous le nom de Splenda™ et utilisé dans plusieurs boissons diète/zéro calorie disponibles sur le marché. La boisson que vous recevrez sera choisie au hasard. Ni vous ni la chercheuse principale ne choisirez le type de boisson que vous recevrez. Vous ne serez informé de la boisson que vous avez reçue qu'à la fin de l'étude. Pour que cette étude produise des informations scientifiquement utiles, il est nécessaire que vous consommiez toutes les boissons d'intervention fournies chaque jour pendant les 3 semaines de l'étude.

En plus de consommer les boissons d'intervention quotidiennement, il vous sera demandé de respecter le **protocole nutritionnel** suivant : Pendant les 3 semaines de l'étude, il vous sera demandé de maintenir vos habitudes alimentaires quotidiennes tout en vous abstenant de consommer des boissons qui contiennent des sucres ajoutés, des jus de fruits ainsi que des

aliments et boissons qui contiennent des édulcorants (substituts de sucre) (une liste détaillée des aliments et des boissons à éviter vous sera fournie). Il vous sera également demandé de vous abstenir de boire plus de 2 boissons alcoolisées par jour, de consommer du cannabis (car le cannabis affecte certaines des variables mesurées) et de prendre tout supplément vitaminique, minéral, nutritionnel, probiotique, naturel, herbal/à base de plantes (incluant la tisane) ou pour sportifs. À tout moment durant l'étude, vous pouvez demander à rencontrer en personne la diététiste professionnelle si vous avez des questions ou si vous rencontrez des difficultés avec le protocole nutritionnel.

VISITES HEBDOMADAIRES

Chaque semaine, vous devrez prévoir une visite de 20 minutes pour venir chercher votre approvisionnement hebdomadaire en boissons d'intervention et rapporter vos bouteilles de boissons utilisées à l'URCM (2 visites au total). Vous recevrez 7 bouteilles de boissons d'intervention congelées (1 bouteille par jour à consommer en 3 doses avec les repas) dans un sac réfrigérant, que vous garderez à la maison dans votre réfrigérateur pour les décongeler. Il est important de ne pas laver ni rincer les bouteilles utilisées, car celles-ci doivent être pesées au préalable par notre personnel de recherche. Lors de chacune de vos visites hebdomadaires, vous serez invité à remplir un bref questionnaire en ligne (10 questions) sur les envies ("cravings") d'aliments et de boissons sucrés et salés.

DEUXIÈME SESSION EXPÉRIMENTALE

Le dernier jour de l'étude de 3 semaines, un rendez-vous pour votre deuxième session expérimentale sera fixé. La session expérimentale post-intervention marque la fin de l'étude de 3 semaines. Il vous sera demandé d'apporter votre deuxième échantillon de selles pour cette session. Vous serez invité à vous rendre au laboratoire à jeun à ~7h30am. Les mêmes tests et procédures qui ont eu lieu lors de votre première session expérimentale seront répétés. Cette session sera d'une durée de 3h00.

IL EST IMPORTANT QUE VOUS...

*Ne consommez pas de **boissons alcoolisées ni de cannabis** la veille de votre session expérimentale.*

*Mangez le **repas standardisé** qui vous sera fourni pour votre **souper** le soir précédant la session expérimentale.*

***Ne mangez et ne buvez rien d'autre que de l'eau et ne faites pas d'exercice ou de sport** 12 heures avant l'heure prévue de votre rendez-vous.*

*Ne pas utiliser d'**anti-inflammatoires non stéroïdiens** (comme l'ibuprofène Advil™ ou du Diclofenac diéthylamine (Voltaren™ gel topique)) pendant les 24h précédant la session expérimentale (l'acétaminophène peut être utilisé si nécessaire Tylenol™)*

- Consommer les boissons (1 bouteille par jour) qui vous seront fournies par notre unité de recherche tel que demandé pendant 3 semaines (21 jours).
- Suivre les directives nutritionnelles qui vous seront indiquées pour la durée complète de la période préparatoire et de l'étude, i.e. s'abstenir de consommer des boissons qui contiennent des sucres ajoutés, des jus de fruits ainsi que des aliments et des boissons qui contiennent des édulcorants autres que les boissons d'intervention qui vous seront fournies par notre unité de recherche pendant toute la durée de cette étude.
- Récupérer, une fois par semaine, votre approvisionnement de boissons d'intervention à l'URCM.
- Assurer le retour de toutes les bouteilles de boissons d'intervention chaque semaine à notre unité de recherche. Il est important de ne pas laver ou rincer les bouteilles, car celles-ci doivent être pesées au préalable par notre personnel de recherche.
- Signaler à la chercheuse principale tout écart au protocole nutritionnel qui aurait pu survenir.
- Participer aux tests et procédures de l'étude et se présenter à l'heure convenue aux rendez-vous. Si vous ne vous présentez pas à vos rendez-vous pour récupérer/retourner vos boissons d'intervention et ne prenez pas d'autres dispositions à temps pour permettre une étude ininterrompue, comme l'exige le protocole, vous serez malheureusement renvoyé de l'étude.

Tout matériel d'étude fourni est la propriété de l'Unité de Recherche sur le Comportement et le Métabolisme (URCM) de l'Université d'Ottawa et il est de votre responsabilité de les retourner une fois l'étude terminée.

Que se passe-t-il si je dis oui, mais que je change d'avis plus tard ?

Vous avez le droit de quitter la recherche à tout moment, sans aucune conséquence.

Y'a-t-il des inconforts ou des risques qui pourraient survenir si je participe à cette étude?

Boissons d'intervention et Protocole nutritionnel : Pendant la période préparatoire de 7 jours et les 3 semaines de cette étude, vous recevrez des directives nutritionnelles spécifiques à suivre. Dans le cadre de cette étude, vous devrez exclure la plupart des sucres et des édulcorants de votre alimentation, à l'exception des boissons de l'étude qui vous seront fournies. Nous vous demandons de considérer attentivement si vous êtes en mesure de respecter cette restriction. Vous pourriez ressentir des inconforts gastro-intestinaux dus aux changements dans vos habitudes alimentaires normales. Si vous ressentez des symptômes inconfortables importants, n'hésitez pas à en discuter avec notre personnel de recherche.

Variation du poids corporel : Des variations minimales du poids corporel peuvent survenir en raison des changements dans les habitudes alimentaires au cours des 3 semaines de l'étude.

Vous pouvez demander à rencontrer la diététiste de l'étude pour recevoir des conseils si vous ne retrouvez pas votre poids initial dans le mois suivant la complétion l'étude.

Cathéter : Il arrive que l'insertion appropriée du cathéter ne soit pas réalisée du premier coup et que des tentatives d'insertion supplémentaires soient requises. L'insertion du cathéter provoque un inconfort temporaire et peut provoquer des ecchymoses. Le technicien certifié en phlébotomie devra rincer la ligne du cathéter avec une solution saline afin de pouvoir prélever du sang. Il est possible que le rinçage de la ligne du cathéter puisse causer un certain inconfort à l'endroit où le cathéter est inséré. Les prélèvements sanguins présentent de faibles risques allant d'une simple rougeur de la peau à une inflammation locale de la veine.

Risques radiologiques (scan DEXA) : Cette étude implique une faible exposition aux rayons X, inférieure à celle des autres tests de diagnostic utilisant des rayonnements ionisants (par exemple, des radiographies d'une fracture d'un os). L'exposition aux rayonnements reçue dans cette étude est inférieure aux niveaux qui entraînent un risque significatif pour la santé. Au cours de cette étude, vous passerez 3 numérisations DEXA. Le niveau de radiation pour chaque numérisation DEXA est inférieur à celui auquel vous seriez exposé lors d'un vol transcontinental en avion.

Le fait de participer à cette étude m'aidera-t-il d'une manière ou d'une autre ?

Vous recevrez gratuitement les résultats de votre composition corporelle (DEXA) et les résultats de votre estimation de la dépense énergétique quotidienne. Ces informations pourraient être utilisées pour estimer le risque de développer des problèmes de santé. Vous recevrez aussi une formation sur l'analyse des étiquettes nutritionnelles dispensée par la diététiste professionnelle de l'étude. Également, les résultats de cette étude fourniront des connaissances essentielles qui pourraient avoir un impact positif sur l'élaboration de lignes directrices en matière de santé publique en clarifiant les effets de la consommation de sucres naturels, de sucres raffinés et d'édulcorants sur la santé intestinale et les facteurs de risque de maladies cardiovasculaires et métaboliques dans la population canadienne.

Y a-t-il une compensation financière pour participer à cette étude ?

Afin de vous remercier de votre contribution au projet de recherche, vous aurez la possibilité de participer à un tirage au sort pour gagner un iPad Air (256GB) comprenant AppleCare+ d'une valeur totale de 979\$ + tx.

Le tirage au sort est ouvert à tous les participants d'étude qui s'inscrivent au tirage, qu'ils décident ou non de se retirer de leur participation au projet de recherche. Les participants qui s'inscrivent à l'étude recevront 1 coupon de participation au tirage à la fin de la session

préliminaire. Un deuxième coupon de participation au tirage sera remis aux participants qui auront terminé la première session expérimentale. De plus, les participants recevront 2 coupons de participation au tirage pour chaque semaine complétée de l'étude de 3 semaines. Enfin, les participants qui terminent l'étude recevront 12 coupons de participation au tirage supplémentaires le jour de leur dernière session expérimentale. Chaque participant pourra ainsi cumuler un maximum de 20 coupons pour participer au tirage au sort à l'issue de toutes les étapes de l'étude. À la fin de la collecte complète des données de l'étude, un nom sera choisi au hasard parmi les participants et la personne dont le nom aura été tiré au sort sera informée par courriel et/ou par téléphone. Pour gagner le prix, la personne devra répondre correctement à une question réglementaire. Si la personne ne peut être jointe dans les 14 jours suivants la date du tirage au sort, le prix sera attribué au deuxième nom tiré au sort, et ainsi de suite jusqu'à ce que le prix soit attribué. Les chances de gagner un prix sont d'approximativement 1/42. Le prix doit être accepté tel quel ou refusé et ne peut être échangé contre de l'argent. Votre nom et les coordonnées que vous fournirez lors de votre participation au tirage au sort seront recueillis afin de contacter le participant sélectionné pour le tirage au sort. Le nom et les coordonnées des participants seront gardés confidentiels et seront ensuite détruits une fois le prix attribué.

Confidentialité, anonymat et conservation des données : Qu'advient-il des informations recueillies dans le cadre de l'étude?

Afin de garantir la confidentialité et l'anonymat, toutes les précautions et mesures nécessaires seront prises pour que vos résultats et vos informations personnelles soient conservés dans la plus stricte confidentialité.

- Seuls la chercheuse (Alexandra Pepin, diététiste professionnelle, candidate au doctorat) et son directeur de thèse (Pascal Imbeault, PhD.) auront accès à vos informations personnelles et à vos résultats. Toute autre personne impliquée dans l'étude n'aura pas accès à vos informations personnelles et/ou à vos résultats.
- Votre nom n'apparaîtra sur aucun rapport. Un code numérique unique sera utilisé pour vous identifier sur tous les documents de recherche.
- Tous les documents et informations qui peuvent être liés à vous ne seront pas rendus publics et seront conservés dans la plus stricte confidentialité.
- Vous ne serez en aucun cas identifié dans les publications ou les rapports issus de cette étude.
- Les données recueillies seront conservées dans une armoire fermée à clé de notre unité de recherche dont l'accès est restreint et où sont conservés les dossiers de tous les participants. De plus, les fichiers informatiques seront protégés par un mot de passe.
- Après la mesure des paramètres métaboliques dans les échantillons de sang par un technicien de laboratoire de notre équipe de recherche, les échantillons de sang restants seront placés dans des conteneurs de déchets à risques biologiques. Les échantillons jetés seront ensuite collectés et détruits conformément aux directives de biosécurité du bureau de gestion des risques de l'Université d'Ottawa.

- Les mesures de la composition du microbiote intestinal seront effectuées par l'équipe du Dr Krista Power, l'une de nos collaboratrices à l'École des sciences de la nutrition de l'Université d'Ottawa, à l'aide de méthodes couramment utilisées par son groupe. Seuls les échantillons fécaux codés seront transférés au laboratoire du Dr Power. Il ne sera pas possible pour les techniciens de laboratoire d'identifier les participants à l'aide des codes. Une fois les analyses terminées, les échantillons fécaux rejetés seront détruits.
- Les données seront détruites cinq ans après la publication des résultats de l'étude.

Au cours de cette étude, nous prélèverons des échantillons de sang et de selles. Comme la relation complexe entre la nutrition et le microbiote intestinal est un domaine de recherche très nouveau et en évolution rapide, nous aimerions conserver certains des spécimens restants à des fins de recherches futures. Le reste de vos échantillons de sang et de selles pourrait être utilisé pour l'analyse de nouveaux marqueurs qui seront découverts dans les années à venir. Si vous acceptez, ces échantillons seront stockés dans le laboratoire du Dr Pascal Imbeault et conservés pendant une période maximale de 10 ans. Seul le personnel de recherche de Pascal Imbeault aura accès aux échantillons. Des copies papier de certaines de vos données seront conservées dans votre dossier de participant, situé dans une armoire fermée à clé dans un bureau verrouillé. Toutes les informations relatives à votre identité seront supprimées de votre dossier 5 ans après la publication des résultats de l'étude. Les copies électroniques des données ne contiendront pas d'informations sur votre identité et seront stockées sur un serveur sécurisé pour une durée maximale de 10 ans.

Si vous acceptez que nous conservions certains de vos spécimens restants pour des recherches futures, ils deviendront la propriété de l'Université d'Ottawa. Les spécimens pourront être utilisés pour cette étude ou pour d'autres études connexes et pourront être partagés avec d'autres institutions (par exemple, dans le but de mesurer un paramètre sanguin pour lequel nous ne disposons pas de l'équipement nécessaire). Dans aucun cas les spécimens ne seront utilisés pour la recherche sur l'ADN ou l'ARN.

Si vous acceptez de partager la totalité ou une partie des échantillons biologiques prélevés sur vous, veuillez cocher les cases correspondantes et initialer ci-dessous.

- ☐ Échantillons de sang
- ☐ Échantillons de matières fécales

J'accepte de partager le(s) spécimen(s) biologique(s) prélevé(s) sur moi:

Vos initiales: _____

Si vous n'acceptez **pas** de partager aucun des spécimens biologiques prélevés sur vous à des fins de recherches futures, veuillez apposer vos initiales ci-dessous.

Je n'accepte pas de partager aucun des spécimens biologiques prélevés sur moi:

Vos initiales: _____

PARTICIPATION VOLONTAIRE

Je ne suis pas obligé de participer à cette étude et si je choisis de participer, je peux me retirer de l'étude à tout moment et/ou refuser de répondre aux questions, sans en subir les conséquences négatives. À tout moment au cours de cette étude, mes intérêts personnels prévaudront toujours sur les objectifs de l'étude. Je serai informé des nouveaux résultats qui pourraient influencer ma décision de participer à la présente étude.

Acceptation : Je, _____, accepte de participer à l'étude susmentionnée menée par Alexandra Pepin de l'École des Sciences de l'Activité Physique, Faculté des Sciences de la Santé, Université d'Ottawa, dont la recherche est sous la supervision du Professeur Pascal Imbeault, PhD.

Si j'ai des questions concernant l'étude, je peux contacter la chercheuse ou son superviseur de thèse (coordonnées à la page 1).

Si j'ai des questions concernant la conduite éthique de cette étude, je peux contacter le/la responsable du protocole pour l'éthique de la recherche du Bureau d'éthique et d'intégrité de la recherche de l'Université d'Ottawa.

Pavillon Tabaret, 550 rue Cumberland, salle 154, Ottawa, ON K1N 6N5

Tél : (613) 562-5387 Courriel : ethique@uottawa.ca

Il y a deux exemplaires du formulaire de consentement, dont l'un est à moi.

Signature du participant

Date

Nom du participant en lettres majuscules détachées

Signature de la personne obtenant le consentement

Date

Nom de la personne obtenant le consentement en lettres majuscules détachées

Final Words

I would like to conclude this thesis with the words that carried me through this journey, in the hope of inspiring fellow underdogs, who like me, dare to dream beyond the limits of the opportunities laid out for them.

“ Here's to the crazy ones. The misfits. The rebels. The troublemakers. The round pegs in the square holes. The ones who see things differently. They're not fond of rules. And they have no respect for the status quo. You can quote them, disagree with them, glorify or vilify them. About the only thing you can't do is ignore them. Because they change things. And while some may see them as the crazy ones, we see genius. Because the people who are crazy enough to think they can change the world, are the ones who do. ”

Rob Siltanen

If I could do this, so can you.