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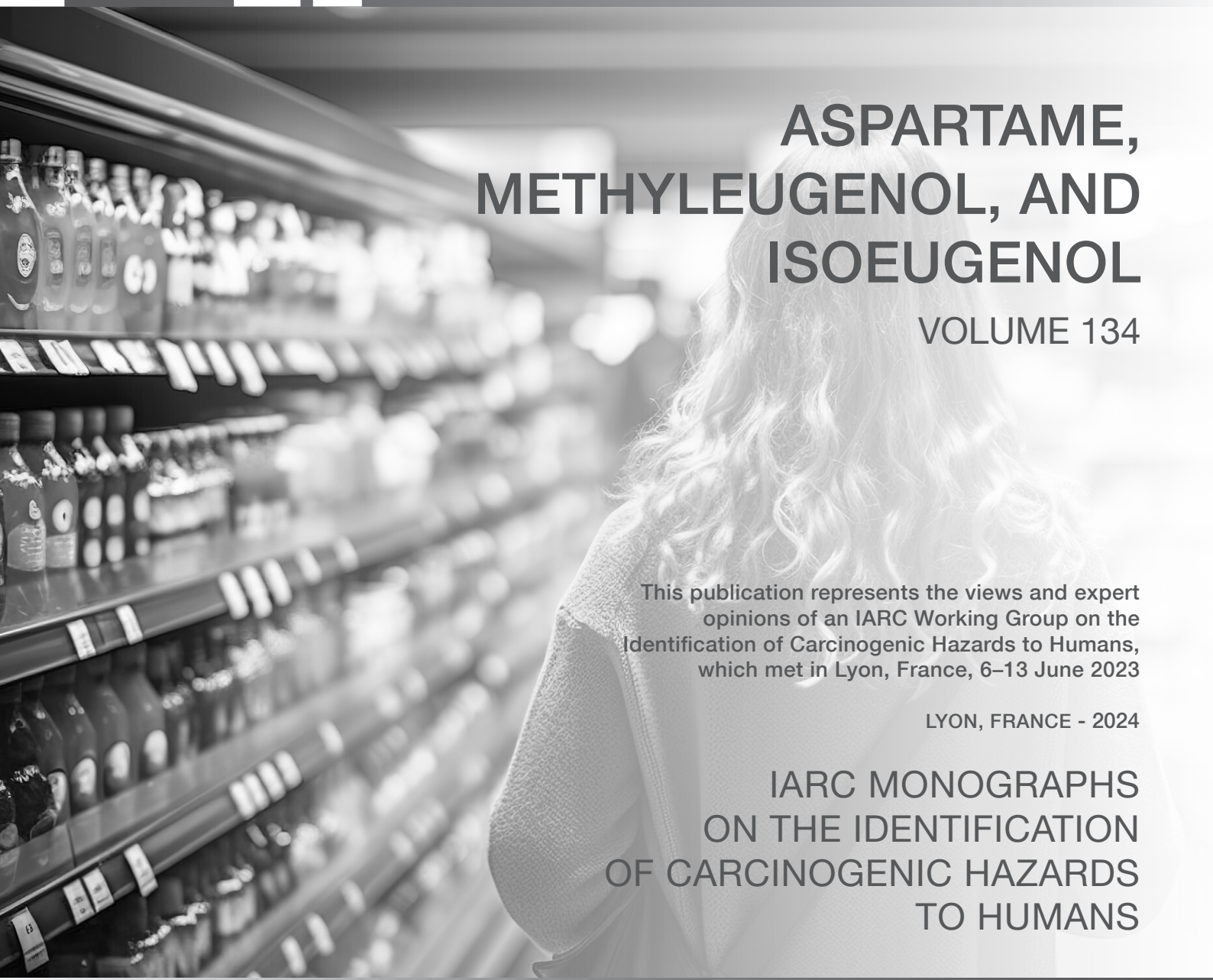

ASPARTAME, METHYLEUGENOL, AND ISOEUGENOL

VOLUME 134

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International Agency for Research on Cancer

World Health Organization

Table S1.3 Exposure assessment review and critique for mechanistic studies in humans exposed to aspartame

Reference and outcome[a]	**What was the study design?**	**What methods were used for the exposure assessment? (incl. data source, environmental and biological measurements, etc.)**	**What was the exposure context?** **Specify period over which exposure data gathered, and how historical exposures were accounted for (if relevant)** What was the agent under investigation?	**Was exposure assessment qualitative, semiquantitative, or quantitative?**	**Which exposure sources were assessed?**	**What exposure metrics were derived for use in analyses (e.g. average exposure, exposure duration, cumulative exposure etc.)?** (specify units)	**What was the timing of exposure relative to the outcome?**	**Was there potential for co-exposures to other carcinogens?** **Which ones were measured?**	**Was there potential for differential exposure misclassification?** **Was there potential for non-differential exposure misclassification?** **(Likely/unlikely)**
Baraniuk et al. (1988) KC6, induces chronic inflammation	Double-blinded placebo-controlled crossover challenge study Location and time not reported	Added dose of aspartame in challenge study. Food matrix not stated Incidence of headache, immunophysiological correlates of cutaneous histamine reactivity: circulating concentrations of IgG, IgA, IgM, IgD, IgE, C1q, C3, C4, factor B, glucose, histamine, adrenaline, noradrenaline, histamine-induced cutaneous flare responsiveness	Potential relationship aspartame and headaches. *n* = 40 predominantly overweight 30 mg/kg aspartame	Quantitative	Additional to dietary intakes	mg/kg	Preceded. Single challenge study (not clear)	Limited information available. Population was selected for self-reported headaches arising from aspartame consumption	Differential unlikely as exposure allocated Non-differential: possible as no detail on background diet
Hall et al. (2017) KC6, induces chronic inflammation – plaque burden, inflammation in HIV	Matched control. Unclear if open trial or blinded in any manner Location: Boston, Massachusetts, USA Timing: unclear	Aspartame: 4-day food diary and Minnesota Nutrition Data System for Research CT angiography, physical activity questionnaire, standard blood clinical chemistry and immune markers, e.g. CD4+ T-cell counts	Intake of nutritive and artificial sweeteners from total food diary intakes in HIV patients matched with healthy controls. Aspartame intakes were recorded as 48 mg/day and 24 mg/day in the HIV group and control group respectively rising to 164 mg/day vs 89 mg/day in consumers only (29% and 27% consumers respectively). Assessed relationship of sweetener consumption with immune and inflammatory markers and coronary plaque characteristics	Quantitative	Total dietary intake. Unclear if any from medicines factored	Mean daily intake mg/day	Preceded. 4 days (3 weekday, 1 weekend)	Potentially yes, but only intakes of aspartame reported arising from 4-day diary and linked intake software (Minnesota Nutrition Data System version 2015) Potential carcinogens not described	Differential: unlikely Non-differential: unlikely as background dietary intakes assessed
Sørensen et al. (2005) KC6, induces chronic inflammation	2-arm parallel design RCT unblinded Location: Denmark Timing: 10-wk intervention (2000)	Added dose of sweeteners consumed as foods and beverages (54% aspartame) at 3 levels depending on body weight for 10 wk (caloric benchmark). (Foods listed are soft drinks, fruit juices, yogurt, marmalade, ice creams, stewed fruits but exact compositions are not stated) Anthropometrics, 7-d food diaries, blood insulin, glucose, triacylglycerol, CRP, haptoglobin, transferrin; 24 h urinary protein	Control arm (n=20) of an intervention testing whether increased intake of SSB and foods increased inflammatory markers (CRP, haptoglobin) and decreased transferrin in 21 overweight adults.	Semiquantitative	Additional to dietary intakes	Energy linked	Preceded. Daily ingestion	Potentially Unclear	Differential unlikely as exposure allocated. Non-differential unlikely as assessed background diets
Tamez et al. (2018) KC6, induces chronic inflammation No effect (diet sodas in general)	Cross-sectional analysis of a prospective cohort study Location: Mexico Timing: cross-sectional analysis (2007)	138-item FFQ, extracted 3 questions relating to intake of beverages (colas, other sodas, and diet soda). Serum CRP, c-peptide, leptin, adiponectin. Questionnaire analysis of covariates	Comparing intake of sugar-containing or diet soft drinks over previous year among 825 Mexican female teachers. Not specific to aspartame	Semiquantitative	Beverages (diet and sugar-containing)	Intakes of beverages as tertiles (diet or sugar) rather than aspartame per se.	Preceded	Yes. Multiple sweeteners and other potential carcinogens. Not clarified or quantified	Differential: Unlikely as low potential for recall bias Non-differential: Likely as no specific assessment of aspartame.
Hess et al. (2018) KC8, modulates receptor-mediated effects	Short-term assessment (over 2 wk) of intakes compared with biomarkers of metabolic syndrome	3 × 24-h dietary recalls to identify consumers of artificially sweetened foods or beverage to which standard intake of 4 sweeteners applied. Physical activity (questionnaire) and healthy eating index scores	2 wk 3 × 24-h recalls (2 weekdays, one weekend) Adults, *n* = 125	Semiquantitative	Food and beverages	Exposure (mg). Participants characterized as consumers or not consumer (average exposure)	Aligned. Within same 2-week period	Yes, potential for exposure to other carcinogens but this was not quantified. Cohorts similar, only significant difference	Differential: unlikely as outcome unknown at time of assessment Non-differential: likely and intake of aspartame

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	Location: South-west Virginia, USA Timing: 2016	Markers of metabolic syndrome: waist circumference, weight, height, fasting blood glucose, triglycerides, HDL						being NNS consumers having a higher BMI and a higher percentage falling into the obese category vs non-consumers	attributed at a standard dose.
Hieronimus et al. (2020) KC8, modulates receptor-mediated effects	Double-blinded parallel assignment intervention study Location: California, USA; Timing: 2008–2014	Commercial aspartame-containing beverages used as a control vs varieties sweetened with various sugar forms. Triglycerides, non-HDL-C, apo B, LDL-C, uric acid AUC, apo CIII, postprandial levels of LDL-C, non-HDL-C, apo B, fasting oxidized LDL, 24-h plasma glucose and insulin, body weight, amplitudes of post-meal glucose and insulin peaks.	2 wk – beverages 3 times on each day, healthy young adults, *n* = 145 Habitual consumption not measured	Not characterized	Beverages	Added dose Concentration not provided Drink: Market Pantry®, Target, Minneapolis (3 beverages/day)	Aligned	Not reported, potential exposure to other carcinogens but this was not quantified. Experimental groups matched for sex, BMI, fasting triglyceride, cholesterol, HDL, insulin concentrations	Differential: unlikely as exposure allocated Non-differential: possible as background diet not assessed
Higgins et al. (2018) KC8, modulates receptor-mediated effects	Randomized 3-parallel-arm study Location: West Lafayette, Indiana, USA Timing: 2016–2017	Beverages with and without added aspartame. Insulin, glucose, HDL, total cholesterol, LDL, TAG, GGT, alanine transaminase, aspartate transaminase, GIP, GLP-1, leptin, HbA1c 24-h urine (PABA, creatinine) Plethysmography. Blood pressure Subjective appetite ratings	500 ml of beverages over 12 wk. 0 mg /day aspartame (680 mg dextrose) 350 mg/day aspartame (beverage) 1050 mg/day aspartame (consisting of 350 mg beverage as above plus capsule of 700 mg aspartame plus 680 mg dextrose) 93 lean adults	Quantitative	beverages	mg/day	Exposure daily for 12 wk; outcome measurement at week 4, 8 and 12	Yes Not reported No difference in baseline characteristics between groups (sex, age, BMI, waist circumference, blood pressure, HbA1c, fasting serum glucose).	Differential: unlikely as exposure allocated. Non-differential: possible as no information on background diets
Hwang et al. (2019) KC8, modulates receptor-mediated effects	GWAS of a twin study Location: Brisbane, Queensland, Australia Timing: 2003–2014	3 cohorts studied but information on aspartame limited to one Australian cohort. Taste test analysis of aspartame at age 14–16 yr, *n* = 1757	GWAS study 1.4×10^{-3} M aspartame	Quantitative	Additional, no mention of habitual intakes	Molar	Not reported	Likely, not reported	Differential: Unlikely for Australian cohort as objective taste test. Non-differential: unlikely as objective taste test
Kim et al. (2020) KC8, modulates receptor-mediated effects	Randomized crossover study Location: Adelaide, Australia Timing: 2018–2019	Added daily dose of water or artificially sweetened soft drink for 2 wk with 4-wk washout period AUC for oral glucose tolerance test for glucose and insulin, incremental AUC for glucose and insulin, HOMA-IR, Matsuda index	Relationship between ASBs and glucose control in normal-weight adults. Added dose. 0.6 L/day of beverage (144 mg/L: aspartame and 211 mg/L: acesulfame-K) equates to 86.4 mg/ 0.6 L aspartame	Quantitative	Additional dose	mg/L	Concurrent – crossover RCT	Possible co-exposures - drink contained acesulfame-K plus aspartame No differences in baseline characteristics between groups indicated	Differential: Unlikely as exposure allocated. Non-differential: possible as background diet not assessed but recruitment criteria included no use of NNS in previous 2 wk
Nguyen et al. (1998) KC8, modulates receptor-mediated effects Effect	Randomized crossover acute study Location: Besancon, France	Added dose consumed as a beverage compared with glucose as a control Serum glucose, insulin, calcium, phosphate, creatinine; U-Ca, U-Pi, U-Oxal	Key outcomes related to calcium-oxalate metabolism assed in acute challenge studies after overnight fast in four men and three women (all healthy), *n* = 7	Quantitative	Additional dose	250 mg aspartame in 250 mL water consumed on two occasions	Single challenge study	No. Crossover study	Differential: Unlikely as exposure allocated. Non-differential: likely as background diet not assessed
Sigala et al. (2020)	Parallel, double-blinded intervention study	Added dose	Potential relationship between SSBs and changes in circulating leptin.	Quantitative	Additional dose	Added dose. Concentration not provided	Parallel intervention group 2 wk	Potential	Differential: unlikely as exposure allocated.

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KC8, modulates receptor-mediated effects	Location: Davis, California, USA Timing: 2014	Leptin AUC, ad libitum food intake and body weight 24-h dietary intake recall at week 0 and week 2	Normal and overweight young adults, *n* = 131 Aspartame sweetened beverage used as control arm			Fruit flavoured Market Pantry™ drink mix		Not stated, emphasis on 24 h recall was on energy intake. Groups matched for sex, BMI, fasting insulin, triglyceride, LDL, HDL	Non-differential: possible, dietary intake data focused on energy rather than aspartame intakes
Sigala et al. (2021) KC8, modulates receptor-mediated effects	Parallel, double-blinded intervention study Location: Davis, California, USA Timing: 2014	Added dose, 3 times per day % hepatic lipid, Matsuda insulin sensitivity index (MISI), predicted MISI, uric acid, blood lipids	Potential relationship SSBs and changes in % hepatic lipids; normal and overweight young adults, *n* = 75 Aspartame sweetened beverage used as control arm	Quantitative	Additional	Added dose Concentration not provided Fruit flavoured Market Pantry™ drink mix	Parallel intervention group 2 wk	Potential Not stated. Groups matched for sex, BMI, fasting triglyceride, LDL, HDL, insulin	Differential: unlikely as exposure allocated. Non-differential: possible, no information on background diet
Sigala et al. (2022) KC8, modulates receptor-mediated effects	Parallel, double-blinded intervention study Location: Davis, California, USA Timing: 2014	Added dose MRI lipid content, oral glucose tolerance test (glucose and insulin), Matsuda and predicted Matsuda insulin sensitivity index	Potential relationship between SSBs and hepatic lipid content and insulin sensitivity. Normal and overweight young adults, *n* = 85 Aspartame sweetened beverage used as control arm	Quantitative	Additional	Added dose Concentration not provided Fruit flavoured Market Pantry™ drink mix	Parallel intervention group 15 days	Potential Not stated Groups matched for sex, BMI, fasting triglyceride, LDL, HDL, insulin	Differential: unlikely as exposure allocated. Non-differential possible, no information on background diet
EFSA_UN07 (2011) KC6, induces chronic inflammation	Multi-centre, randomized, double-blind crossover trial. Location: USA, Canada. Timing: 1988–1991	3 additional doses of aspartame and/or its conversion products on 2 occasions over five days with a single washout day. Allergic reactions: urticaria, angioedema	Recruited individuals self-reporting urticaria and/or angioedema within 12 h of ingestion of an aspartame-containing product. 3 doses of aspartame with a total daily dose chosen to represent the amount one would consume in approximately 1–2 L of degraded aspartame –sweetened beverage (5–6 times P90 consumption at that time). *n* = 21 mix of males and females including 2 children	Quantitative	Additional doses: Aspartame, aspartylphenylalanine diketopiperazine, beta-aspartame vs placebo excipient only	Cumulative dose response exposure to aspartame and/or breakdown products: Body weight > 40 kg (daily dose of 950 mg) Half of the below if body weight < 40 kg 8.00 am – 50 mg, 10.00 am – 300 mg 12.00 pm – 600 mg	2 × challenge studies with a 1-day wash out	No	Differential: unlikely as added dose. Non-differential: possible as no information on background diet
EFSA_UN08 (2011) KC8, modulates receptor-mediated effects	Randomized double-blind placebo-controlled parallel group study. Location: USA Timing: 1985–1986	Long-term study of safety of ingestion of an additional dose of aspartame Key parameters related to routine clinical chemistry tests, serum folate, blood formate & methanol, urine calcium, creatinine & formate, plasma amino acid provides, plasma lipid profile, vital signs, body weight, adverse experiences	Additional dose consumed over 24 wk. Deemed equivalent to amount in 10 L/day of aspartame –sweetened beverages for a 70 kg person. *n* = 108 adults	Quantitative	Additional dose of aspartame	75 mg/kg per day in a capsule consumed at 3 timepoints each day for 24 wk by healthy adults 75 mg/kg per day	Concurrent	Potentially as over 24 wk. Not clearly stated	Differential: unlikely as added dose. Non-differential: unlikely as told to avoid aspartame-containing products
Garriga et al. (1991)	Combined single blind, double-blind placebo-controlled study. Location: Washington, USA Timing: 1986–1989	Study to identify subjects with hypersensitivity followed by single and double challenge study with additional doses up to 2000 mg aspartame. Key parameters related to hypersensitivity and allergy: skin prick tests, histamines along with blood glucose, electrolytes, glutamic oxaloacetic transaminase, glutamic	Study 1: characterized self-reported incidence of aspartame associated hypersensitivity. Study 2: challenge studies on normal and atopic volunteers and individuals with suspected hypersensitivity reactions to aspartame. *n* = 12 adults.	Quantitative	Additional doses of aspartame	Study 1: self-reported hypersensitivity. Study 2: increasing doses: 0, 10, 100, 500, 1000, 2000 mg aspartame at 30-minute intervals or at intervals that exceed the reaction time reported by history	Concurrent	No, acute challenge	Differential: unlikely as added dose. Non-differential: unlikely as additional dose

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		pyruvic transaminase, calcium, blood urea, nitrogen, creatinine, cholesterol, IgG, IgE	Control was lactose, aspartame capsules used but also a diet soda containing aspartame						
Okuno et al. (1986)	Two studies: Study 1: single dose administration Study 2: daily dose for 2 wk (short-term administration). Location: Japan Timing: not stated	Study 1: single dose of aspartame (500 mg) on blood glucose, insulin, glucagon in normal controls and untreated diabetics. Study 2: daily administration of aspartame (125 mg) for 2 wk on fasting and postprandial blood glucose, glucose tolerance, fasting cholesterol, HDL, triglycerides, GGT, blood count, renal and liver function tests	Study 1: 500 mg aspartame in 300 ml water. 7 normal controls and 22 untreated diabetics Study 2: jelly cake with 125 mg aspartame (deemed equivalent in sweetness to mean daily sugar consumption for Japanese adults aged 20–50 yr (20–30 g)) given as a dessert nightly. *n* = 9 diabetics in a steady state of glycaemic control)	Study 1: quantitative Study 2: quantitative	Added doses	mg	Concurrent	Possible in Study 2. Not reported. Study 1: groups differed as one group was normal controls and the other untreated diabetics. Study 2: entire cohort was diabetics with controlled glycaemic control)	Differential: unlikely as added doses. Non-differential: likely in study 2 as background intakes not assessed
Bishop et al. (2002) Cytokines (Interleukin-6, TNF-α) and neutrophil degranulation responses	Randomized, counter-balanced, crossover trail Location: UK Timing: not reported	Known experimental treatment/exposure allocated. CHO solution vs artificially sweetened placebo. Consumption of 5 mL per kg body weight at start of trial. 5 rest periods during exercise trial, consumed an additional 2 ml per kg body weight in each rest period Body weight: mean ± SE 71.7 ± 1.2 kg	ASB. Type of beverage not reported. Background diet was assessed for 2 days prior to trial, but not reported. Same diet for 2 days prior to second trial, but not reported. No assessment of long-term exposure	Quantitative	ASB	ml/kg body weight	Two exercise trials, 7 days apart	Not stated	Unlikely
Auerbach and Garfinkel (1989) KC10	Retrospective case analysis	Retrospective recall by family member as proxy. Frequency of use of artificial sweeteners in soft drinks or added to coffee or tea or other beverages or foods	New Jersey, Ohio, New York USA 149 mainly adult cases autopsied between 1976 and 1984	Qualitative	Artificial sweeteners	Frequency of use (None, regular use, rarely or only occasionally).	Preceded	Smoking	Differential likely: retrospective assessment, by proxy (Family member) Non-differential likely: no specific assessment of aspartame, only total artificial sweeteners
Leon et al. (1989) KC10	Randomized, double-blind, placebo-controlled, parallel-group design	Blood and urine testing with emphasis on the products of aspartame metabolism, i.e. aspartic acid, phenylalanine, and methanol, 5–6 times/24 wk. Unused capsules were returned and capsule counts were done at each 3-week visit	Minneapolis, USA 1987 108 adults; 24 wk	Quantitative	Aspartame	75 mg/kg of aspartame per day	Concurrent. Three times daily for 24 wk	Not reported	Differential unlikely as exposure allocated non-differential possible: background diet not assessed
Ahmad et al. (2020a) KC8, glucose metabolism	Randomized, controlled, double-blinded, crossover design	Known experimental treatment/exposure allocated. Background diet assessed by a 3-day food diary for 2 weekdays and 1 weekend day over the 14-day intervention period and daily checklist to verify beverage consumption.	Winnipeg, Canada 2016–2018 17 young healthy adults, not regular users of NNS	Quantitative	Aspartame	14% (0.425 g) of the ADI for aspartame	Every day for 2 wk		Differential unlikely as exposure allocated non-differential unlikely: background diet and compliance during trial assessed

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		Participants were screened prior to inclusion for use of NNS (i.e. consuming less than 1 using a web-based FFQ) (Canadian Diet History Questionnaire II)							
EFSA_UN01 (1988) KC8	Randomized open-label crossover controlled trial	Known experimental treatment/exposure allocated; plasma glucose, insulin, glucagon	USA 1985–1986 10 middle-aged overweight diabetics, 12 young normal-weight female adults	Quantitative	Aspartame; single dose, aspartame was added to an unsweetened beverage (cherry flavoured Kool-Aid), (400 mg aspartame to 300 mL beverage)	mg	Single dose	Saccharin	Differential unlikely as exposure allocated
Higgins and Mattes (2019) KC8	Parallel-arm design	Known experimental treatment/exposure allocated. Food and energy intake were measured on 3 d (2 non-consecutive weekdays and 1 weekend day) during baseline and weeks 4, 8, and 12 using the Automated Self-Administered 24-h Dietary Recall (ASA24). Brief questionnaire to assess habitual beverage intake measured habitual beverage intake over the past month, completed at baseline, and weeks 4, 8, and 12. It included diet beverages and tea/coffee with sweeteners. PABA was added to the beverages supplied to measure urinary PABA for compliance with beverage consumption	USA 2016–2018	Quantitative	Beverages sweetened with 1 of 5 sweeteners (sucrose, saccharin, aspartame, rebA, or sucralose) daily for 12 wk	g	Daily consumption of beverages sweetened with 1 of 5 sweeteners for 12 wk	Not reported	Differential unlikely as exposure allocated. Non-differential possible: only some aspects of background diet assessed
Kashima et al. (2019) KC8	Randomized crossover design	Known experimental treatment/exposure allocated	Japan Date study conducted not reported, published 2019	0.09% aspartame in water (4 doses of 50 g over 80 minutes)	Aspartame	mg	Within 80 minutes	None reported	Differential unlikely as exposure allocated non-differential possible: background diet not assessed
Ahmad et al. (2020b) Gut microbiome	Randomized, double-blind crossover and controlled clinical trial	Known experimental treatment/exposure allocated. Background diet assessed by a 3-day food diary for 2 weekdays and 1 weekend day over the 14-day intervention period and daily checklist to verify beverage consumption. Participants were screened prior to inclusion for use of NNS (i.e. consuming less than 1 using a web-based FFQ) (Canadian Diet History Questionnaire II)	Winnipeg, Canada 2016–2018 17 young healthy adults, not regular users of NNS	Quantitative	Aspartame	14% (0.425 g) of the ADI for aspartame	Every day for 2 wk		Differential unlikely as exposure allocated non-differential unlikely: background diet and compliance during trial assessed
Frankenfeld et al. (2015) Gut microbiome	Cross-sectional design	Food record for 4 consecutive days Food composition database used: Nutrition Data System for Research for nutrient analysis (version 2010)	USA Data collected prior to 2012 (see reference to methods paper)	Qualitative	Aspartame from all foods	Aspartame non-consumers vs consumers	Four days prior to outcome measure		Differential unlikely as low potential for recall bias as outcome unknown at time of assessment

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									Non-differential unlikely as all sources in diet considered.
Ramne et al. (2021) Gut microbiome	Cross-sectional analysis	4-day food records, short FFQ covering the past 6 months. Consumption frequencies addressing SSB and ASB intakes ranged from never/seldom to several times/day on an 8-level scale; urinary sugars biomarker, gut microbiota	Malmö, Sweden 2013–2017 1371 non-diabetic adults	Qualitative	ASBs	Reported intakes of ASB from the 4DFR were also cross-tabulated from data on 4DFR and FFQ: non-consumer, medium consumers and high consumers	Previous 4 days (4DFR) Last 6 months (FFQ) Data combined to reflect habitual consumption	Smoking, physical activity level, and BMI	Differential unlikely as low potential for recall bias as outcome unknown at time of assessment Non-differential likely: no specific assessment of aspartame, ASB used as a proxy
Suez et al. (2014) Glucose tolerance	Cross-sectional analysis	Long-term NAS consumption was quantified directly from question in FFQ	Israel 2013 381 non-diabetic adults	Qualitative	NAS	Non-consumers, consumers, high consumers	Not reported		Differential unlikely as low potential for recall bias as outcome unknown at time of assessment Non-differential likely: no specific assessment of aspartame, only total artificial sweeteners
Suez et al. (2022) Microbiome and glycaemic response	Randomized controlled trial	Known experimental treatment/exposure allocated. Participants logged all food intake in real time using a dedicated smartphone application, only participants that had at least 20 days with at least 1000 kcal logged per day were included	2018–2020 120 healthy adults. who were complete NNS abstainers according to a detailed FFQ based on NNS-containing products on the Israeli market (identified through screening FFQ)	Quantitative	NNS intervention arms: aspartame, saccharin, sucralose, and stevia	2 sachets/3 times a day), corresponding to 8%, 20%, 34%, and 75% of the ADI of each NNS	2-wk exposure period	BMI, smoking, and habitual diet	Differential unlikely as exposure allocated Non-differential unlikely: study only included previous non-consumers, background diet during trial assessed with 20 days of assessment
Yu et al. (2018)	Nurses' Health Study Cohort, Location: USA Timing: 1989–1990 and 2000–2001	Validated FFQ every 4 yr ASBs consisted of all types of low-energy or artificially sweetened carbonated beverages, such as diet colas and other diet carbonated beverages Dietary intake data used represented a cumulative average of intakes from the last two FFQs before blood collection Fetuin A, alanine transferase, gamma-glutamyl transferase, TAG, total cholesterol: HDL, HDL, LDL, total cholesterol, CRP, ICAM-1, VCAM-1, adiponectin, insulin HbA1c. Covariates controlled by questionnaire	Dietary data was obtained from the last two FFQ before blood collection for each cycle: - 1986 and 1990 for cycle 1 (blood, 1989-1990) - 1994 and 1998 for cycle 2 (blood, 2000–2001) ASB Diet assessed from 1980–1986 to 2010, follow-up until 2014 USA	Semiquantitative	Low-energy or artificially sweetened carbonated beverages, such as diet colas and other diet carbonated beverages	ASBs Participants were asked to report how often, on average they consumed a standard portion of foods and beverages (one standard glass, can, or bottle), using nine possible responses ranging from 'never or less than once per month' to '6 or more times per day' Collapsed respondent responses into 5 categories ranging from never/almost never to ≥ 1/day	Preceded (Average dietary assessment partially reflecting time period before blood sample)	Yes. Other dietary sources of aspartame, presence of other sweeteners and other potential carcinogens. Not clarified or quantified	Differential: possible potential for recall bias Non-differential: Likely as no specific assessment of aspartame

ADI, acceptable daily intake; ASB, artificially sweetened beverage; AUC, area under the curve; BMI, body mass index; CHO, carbohydrate solution; CRP, C-reactive protein; CT, computed tomography; 4DFR, 4-day food record; FFQ, food frequency questionnaire; GWAS, genome-wide association study; h, hour(s); HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; ICAM-1, intracellular adhesion molecule 1; Ig, immunoglobulin; KC, key characteristic of carcinogens; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; MRI, magnetic resonance imaging; NAS, non-caloric artificial sweetener; NHANES, National Health and Nutrition Examination Survey; NNS, non-nutritive sweetener; PABA, *para*-aminobenzoic acid; RCT, randomized controlled trial; SD, standard deviation; SE, standard error; SSB, sugar-sweetened beverage; US, United States; VCAM-1, vascular cell adhesion molecule 1; vs, versus; wk, week(s); yr, year(s).

[a] Key characteristics of carcinogens (KCs): KC6, "induces chronic inflammation"; KC8, "modulates receptor-mediated effects"; KC10, "alters cell proliferation, cell death, or nutrient supply".

References

Ahmad SY, Friel J, Mackay D (2020b). The effects of non-nutritive artificial sweeteners, aspartame and sucralose, on the gut microbiome in healthy adults: secondary outcomes of a randomized double-blinded crossover clinical trial. Nutrients. 12(11):3408. https://doi.org/10.3390/nu12113408 PMID:33171964

Ahmad SY, Friel JK, MacKay DS (2020a). The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized, double-blinded, crossover clinical trial. Appl Physiol Nutr Metab. 45(6):606–12. https://doi.org/10.1139/apnm-2019-0359 PMID:31697573

Auerbach O, Garfinkel L (1989). Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. Cancer. 64(5):983–7. https://doi.org/10.1002/1097-0142(19890901)64:5<983::AID-CNCR2820640502>3.0.CO;2-9 PMID:2758391

Baraniuk JN, Follett Joseph V, Schiffman Susan S, Massey Edward W, Sampson Hugh A, Warick ZS, et al. (1988). Immunophysiologic correlates of cutaneous histamine reactivity in patients with alleged aspartame sensitivity. J Allergy Clin Immunol. 81(1):187. https://doi.org/10.1016/0091-6749(88)90311-9

Bishop NC, Gleeson M, Nicholas CW, Ali A (2002). Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise. Int J Sport Nutr Exerc Metab. 12(2):145–56. https://doi.org/10.1123/ijsnem.12.2.145 PMID:12187615

EFSA_UN01 (1988). Effects of aspartame ingestion without food on serum glucose, insulin, and glucagon concentrations in normal subjects and subjects with diabetes. Protocol No. N04-84-02-088. Final clinical research report. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531.

EFSA_UN07 (2011). Multi-center clinical study to evaluate allergic reactions allegedly due to aspartame consumption. Protocol No. N22-86-02-002. Final clinical research report. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531.

EFSA_UN08 (2011). Safety of long-term aspartame administration in normal subjects. Protocol No. N07-84-02-093. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531.

Frankenfeld CL, Sikaroodi M, Lamb E, Shoemaker S, Gillevet PM (2015). High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. Ann Epidemiol. 25(10):736–42.e4. https://doi.org/10.1016/j.annepidem.2015.06.083 PMID:26272781

Garriga MM, Berkebile C, Metcalfe DD (1991). A combined single-blind, double-blind, placebo-controlled study to determine the reproducibility of hypersensitivity reactions to aspartame. J Allergy Clin Immunol. 87(4):821–7. https://doi.org/10.1016/0091-6749(91)90128-B PMID:2013676

Hall LN, Sanchez LR, Hubbard J, Lee H, Looby SE, Srinivasa S, et al. (2017). Aspartame intake relates to coronary plaque burden and inflammatory indices in human immunodeficiency virus. Open Forum Infect Dis. 4(2):ofx083. https://doi.org/10.1093/ofid/ofx083 PMID:28695142

Hess EL, Myers EA, Swithers SE, Hedrick VE (2018). Associations between nonnutritive sweetener intake and metabolic syndrome in adults. J Am Coll Nutr. 37(6):487–93. https://doi.org/10.1080/07315724.2018.1440658 PMID:29601264

Hieronimus B, Medici V, Bremer AA, Lee V, Nunez MV, Sigala DM, Keim NL, Havel PJ, Stanhope KL. Synergistic effects of fructose and glucose on lipoprotein risk factors for cardiovascular disease in young adults. Metabolism 2020; 112:154356.

Higgins KA, Considine RV, Mattes RD (2018). Aspartame consumption for 12 weeks does not affect glycemia, appetite, or body weight of healthy, lean adults in a randomized controlled trial. J Nutr. 148(4):650–7. https://doi.org/10.1093/jn/nxy021 PMID:29659969

Higgins KA, Mattes RD (2019). A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. Am J Clin Nutr. 109(5):1288–301. https://doi.org/10.1093/ajcn/nqy381 PMID:30997499

Hwang LD, Lin C, Gharahkhani P, Cuellar-Partida G, Ong JS, An J, et al. (2019). New insight into human sweet taste: a genome-wide association study of the perception and intake of sweet substances. Am J Clin Nutr. 109(6):1724–37. https://doi.org/10.1093/ajcn/nqz043 PMID:31005972

Kashima H, Taniyama K, Sugimura K, Endo MY, Kobayashi T, Fukuba Y (2019). Suppression of sweet sensing with glucose, but not aspartame, delays gastric emptying and glycemic response. Nutr Res. 68:62–9. https://doi.org/10.1016/j.nutres.2019.06.005 PMID:31421394

Kim Y, Keogh JB, Clifton PM (2020). Consumption of a beverage containing aspartame and acesulfame K for two weeks does not adversely influence glucose metabolism in adult males and females: a randomized crossover study. Int J Environ Res Public Health. 17(23):9049. https://doi.org/10.3390/ijerph17239049 PMID:33291649

Leon AS, Hunninghake DB, Bell C, Rassin DK, Tephly TR (1989). Safety of long-term large doses of aspartame. Arch Intern Med. 149(10):2318–24. https://doi.org/10.1001/archinte.1989.00390100120026 PMID:2802896

Nguyen UN, Dumoulin G, Henriet MT, Regnard J (1998). Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. J Clin Endocrinol Metab. 83(1):165–8. https://doi.org/10.1210/jcem.83.1.4511 PMID:9435435

Okuno G, Kawakami F, Tako H, Kashihara T, Shibamoto S, Yamazaki T, et al. (1986). Glucose tolerance, blood lipid, insulin and glucagon concentration after single or continuous administration of aspartame in diabetics. Diabetes Res Clin Pract. 2(1):23–7. https://doi.org/10.1016/S0168-8227(86)80025-0 PMID:3522147

Ramne S, Brunkwall L, Ericson U, Gray N, Kuhnle GGC, Nilsson PM, et al. (2021). Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study. Eur J Nutr. 60(4):2087–97. https://doi.org/10.1007/s00394-020-02392-0 PMID:33030577

Sigala DM, Hieronimus B, Medici V, Lee V, Nunez MV, Bremer AA, et al. (2021). Consuming sucrose- or HFCS-sweetened beverages increases hepatic lipid and decreases insulin sensitivity in adults. J Clin Endocrinol Metab. 106(11):3248–64. https://doi.org/10.1210/clinem/dgab508 PMID:34265055

Sigala DM, Hieronimus B, Medici V, Lee V, Nunez MV, Bremer AA, et al. (2022). The dose-response effects of consuming high fructose corn syrup-sweetened beverages on hepatic lipid content and insulin sensitivity in young adults. Nutrients. 14(8):1648. https://doi.org/10.3390/nu14081648 PMID:35458210

Sigala DM, Widaman AM, Hieronimus B, Nunez MV, Lee V, Benyam Y, et al. (2020). Effects of consuming sugar-sweetened beverages for 2 weeks on 24-h circulating leptin profiles, ad libitum food intake and body weight in young adults. Nutrients. 12(12):3893. https://doi.org/10.3390/nu12123893 PMID:33352724

Sørensen LB, Raben A, Stender S, Astrup A (2005). Effect of sucrose on inflammatory markers in overweight humans. Am J Clin Nutr. 82(2):421–7. https://doi.org/10.1093/ajcn/82.2.421 PMID:16087988

Suez J, Cohen Y, Valdés-Mas R, Mor U, Dori-Bachash M, Federici S, et al. (2022). Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. Cell. 185(18):3307–3328.e19. https://doi.org/10.1016/j.cell.2022.07.016 PMID:35987213

Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 514(7521):181–6. https://doi.org/10.1038/nature13793 PMID:25231862

Tamez M, Monge A, López-Ridaura R, Fagherazzi G, Rinaldi S, Ortiz-Panozo E, et al. (2018). Soda intake is directly associated with serum C-reactive protein concentration in Mexican women. J Nutr. 148(1):117–24. https://doi.org/10.1093/jn/nxx021 PMID:29378052

Yu Z, Ley SH, Sun Q, Hu FB, Malik VS (2018). Cross-sectional association between sugar-sweetened beverage intake and cardiometabolic biomarkers in US women. Br J Nutr. 119(5):570-80. https://doi.org/10.1017/S0007114517003841 PMID:29508692