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Xylitol is prothrombotic and associated with cardiovascular risk

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Abstract

Background and Aims The pathways and metabolites that contribute to residual cardiovascular disease risks are unclear. Low-calorie sweeteners are widely used sugar substitutes in processed foods with presumed health benefits. Many low-calorie sweeteners are sugar alcohols that also are produced endogenously, albeit at levels over 1000-fold lower than observed following consumption as a sugar substitute.

Methods Untargeted metabolomics studies were performed on overnight fasting plasma samples in a discovery cohort ($n = 1157$) of sequential stable subjects undergoing elective diagnostic cardiac evaluations; subsequent stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses were performed on an independent, non-overlapping validation cohort ($n = 2149$). Complementary isolated human platelet, platelet-rich plasma, whole blood, and animal model studies examined the effect of xylitol on platelet responsiveness and thrombus formation *in vivo*. Finally, an intervention study was performed to assess the effects of xylitol consumption on platelet function in healthy volunteers ($n = 10$).

Results In initial untargeted metabolomics studies (discovery cohort), circulating levels of a polyol tentatively assigned as xylitol were associated with incident (3-year) major adverse cardiovascular event (MACE) risk. Subsequent stable isotope dilution LC-MS/MS analyses (validation cohort) specific for xylitol (and not its structural isomers) confirmed its association with incident MACE risk [third vs. first tertile adjusted hazard ratio (95% confidence interval), 1.57 (1.12–2.21), $P < .01$]. Complementary mechanistic studies showed xylitol-enhanced multiple indices of platelet reactivity and *in vivo* thrombosis formation at levels observed in fasting plasma. In interventional studies, consumption of a xylitol-sweetened drink markedly raised plasma levels and enhanced multiple functional measures of platelet responsiveness in all subjects.

Conclusions Xylitol is associated with incident MACE risk. Moreover, xylitol both enhanced platelet reactivity and thrombosis potential *in vivo*. Further studies examining the cardiovascular safety of xylitol are warranted.

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Structured Graphical Abstract

Key Question

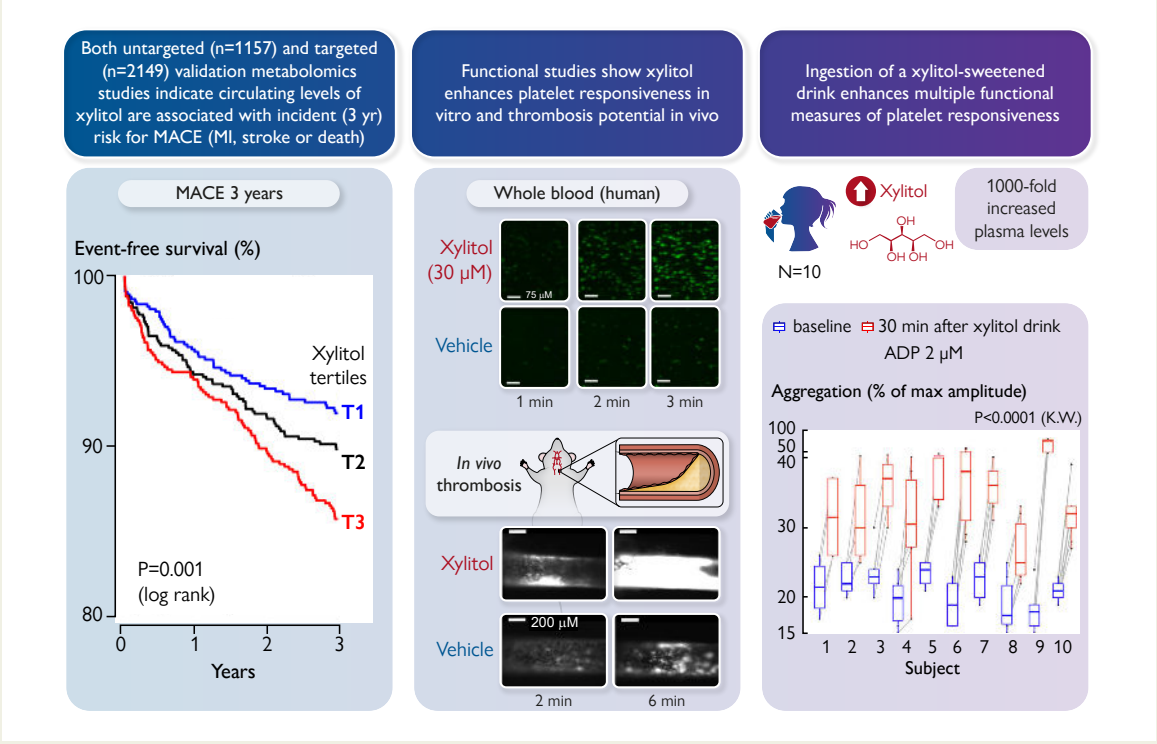
Artificial sweeteners, including xylitol, are widely used to replace dietary sugar in patients with cardiometabolic disease. However, their long-term effects on vascular complications are unclear. This study examines clinical and mechanistic links of xylitol and thrombotic risk.

Key Finding

- Xylitol was associated with major adverse cardiovascular events at 3 year follow up in a derivation and validation cohort.
- Xylitol enhanced multiple indices of platelet reactivity ex vivo and thrombus formation in a mouse model.
- Xylitol ingestion enhanced platelet responsiveness in humans.

Take Home Message

Further studies examining the cardiovascular safety of xylitol and other artificial sweeteners are warranted because of the potential impact on public health due to their broad use.



Role of the artificial sweetener xylitol in cardiovascular event risk. In initial untargeted metabolomics studies (discovery cohort) and subsequent stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) studies (validation cohort), fasting levels of xylitol are associated with incident major adverse cardiovascular events (MACE). Using human whole blood, platelet-rich plasma, and washed platelets, xylitol enhances multiple indices of platelet reactivity *in vitro*. Xylitol also was shown to enhance thrombosis formation in a murine arterial injury model *in vivo*. In human intervention studies, when subjects ingested a typical dietary amount of xylitol in an artificially sweetened food, multiple functional measures of platelet responsiveness were significantly increased. Xylitol is both clinically associated with cardiovascular event risks and mechanistically linked to enhanced platelet responsiveness and thrombosis potential *in vivo*. ADP, adenosine diphosphate; MI, myocardial infarction.

Keywords Artificial sweetener • Low-calorie sweetener • Cardiovascular disease • Platelet • Thrombosis • Heart attack • Stroke • Sugar alcohol • Polyol • Nutrition

Translational perspective

Plasma levels of xylitol, both an endogenous metabolite in glucose metabolism and a commonly used low-calorie sweetener, are associated with incident thrombotic event risks in observational cohort studies and mechanistically linked with heightened thrombosis potential *in vitro* and in animal studies. In a human invention study, consumption of a typical portion size of a xylitol-sweetened drink significantly enhanced multiple indices of platelet responsiveness in healthy volunteers. Thus, xylitol is both clinically associated with and mechanistically linked to heightened atherothrombotic and cardiovascular disease risks. Long-term safety studies and reappraisal of labelling mandates for the sugar alcohol xylitol are warranted.

Introduction

In light of the obesity pandemic worldwide,[1] measures to reduce sugar intake have become a public health priority.[2] One such measure is the proliferation of artificial sweeteners including non-nutritive and low-calorie sweeteners (which collectively are also often called 'artificial sweeteners') in processed foods that are promoted as healthy sugar alternatives. While artificial sweeteners have generally recognized as safe (GRAS) status and are approved by public health authorities [e.g. US Food and Drug Administration (FDA)[3] and European Union (EU)[4]], the World Health Organization recently advised against the use of non-sugar sweeteners for weight control in newly released guidelines.[5] Over the past decade, the use of artificial sweeteners has markedly increased in near-universal fashion across all subject categories including gender, age, race/ethnicity, weight status, and geographic and socio-economic subgroups.[6] Of note, the use of artificial sweeteners is recommended for subjects that suffer from cardiometabolic diseases including obesity, diabetes, and cardiovascular disease (CVD) by multiple guideline organizations (e.g. American Heart Association and diabetes associations in the USA, UK, Canada, and Australia),[7–11] while their potential long-term cardiovascular adverse effects are rarely studied.[12]

Contrary to their intended benefits, a history of artificial sweeteners in a number of epidemiological studies has been associated with cardiometabolic adverse effects including insulin resistance, type 2 diabetes, and CVD, including atherothrombotic complications and death.[13–18] Moreover, in recent studies, the endogenous sugar alcohol erythritol was both clinically and mechanistically linked to CVD.[19] Only a limited number of randomized controlled trials have examined the short-term effects of sweeteners on metabolic indices with results that generally fail to support the promoted benefits,[20–23] though some studies have suggested potential metabolic improvements.[24,25] The limited randomized controlled trials available on artificial sweeteners have been criticized for not reflecting real-life exposure[26] and for typically being conducted over a short period of time, making them unable to recapitulate long-term exposures appropriately for CVD endpoints.

Xylitol is a 5-carbon sugar alcohol (polyol) that is commonly used as a low-calorie sweetener. It can be found in small quantities in fruits and vegetables[27] but is highly enriched (often over 1000-fold higher than found in nature) in numerous artificially sweetened foods and beverages. Because of its anti-cariogenic properties,[28] xylitol is also commonly used in candy, gum, and oral care products.[29] Xylitol is also produced endogenously as a low abundance intermediate side product of human glucose metabolism (part of the glucuronate pathway) and has negligible impact on blood sugar or insulin secretion.[30,31] In contrast to traditional high-intensity sweeteners, xylitol has comparable sweetness to sucrose.[29] Thus, when used as a sugar substitute, it is added in much larger amounts to processed food (i.e. levels equal to sucrose with up to 45 g per serving in some products such as artificially sweetened pie fillings).[32] It is therefore appreciated by the food industry as a bulking sugar substitute that confers texture, moisture, and increases shelf life of processed foods without aftertaste.[33] Consequently, xylitol is sold in grocery stores in bulk (like sugar) where its 1:1 replacement is suggested. It is marketed as a 'natural sweetener', 'keto-friendly', or 'low-carb' and is generally recommended as a sugar substitute for patients with diabetes to improve glycaemic control.[34] Despite the growing market share and use of xylitol in processed foods and oral care products, the impact of xylitol on cardiovascular event risk has not been reported. Here, we provide human clinical observational, interventional, and mechanistic studies linking xylitol to CVD event risk and both heightened platelet reactivity and a pro-thrombotic state *in vivo*.

Methods

More extensive details for all methods can be found in Supplementary data online, *Methods*.

Human subjects

Study approvals

We performed three distinct clinical studies with non-overlapping subjects. All human subjects provided written informed consent, and all human studies abided by the Declaration of Helsinki. The Institutional Review Board of the Cleveland Clinic approved all human study protocols [GeneBank IRB 4265; IRB 21-005 (xylitol ingestion related studies), healthy volunteer blood donors for platelet-related studies IRB 09-506]. Detailed information about the clinical observational cohorts (discovery cohort and validation cohorts) and the xylitol intervention cohort can be found in Supplementary data online, *Methods*.

Animals

Mice were used to causally test whether elevation in xylitol plasma levels results in enhancement in thrombosis potential. All mice were C57BL/6J and 12–14 weeks of age. All animal studies were approved by the Institutional Animal Care and Use Committee at the Cleveland Clinic (IRB 2019-2251).

Aggregometry studies in platelet-rich plasma

Aggregometry in platelet-rich plasma (PRP) was performed as previously described.[35]

Intracellular calcium measurements

Measurement of intracellular calcium release in washed platelets was performed as previously described.[36]

Platelet flow cytometry assay

Antibody staining of washed platelets for flow cytometry was performed as described previously.[36]

Imaging flow cytometry in whole blood

Imaging flow cytometry was performed on an Amnis ImageStreamX MK II two-camera system using anti-CD45 AF488, anti-CD41 AF647, and anti-CD62P-PE. Details and the gating strategy are described in Supplementary data online, *Methods*.

Whole blood *in vitro* thrombosis assay

Shear flow experiments of whole blood were performed with a Cellix Microfluidics System (Cellix, Dublin, Ireland) as previously described and outlined in Supplementary data online, *Methods*.[36–38] The extent of platelet activation and adhesion to the collagen matrix was quantified using computer-assisted tomographic analyses, as previously described.[35]

Carotid artery $FeCl_3$ injury model

The $FeCl_3$-induced carotid artery injury model was performed using intravital fluorescence microscopy with continuous image capture monitoring, as previously described.[39] Time to cessation of blood flow through thrombus formation for all experiments was determined by visual inspection by two independent investigators.

Untargeted and targeted mass spectrometry analyses of human plasma

For untargeted mass spectrometry analyses, subject plasma samples were derivatized and run on gas chromatography–mass spectrometry (GC-MS) analyses as previously described.[19] Raw data files were processed using the metabolomics BinBase database.[40]

Stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed to specifically quantify xylitol in human and mouse plasma, urine, and faeces (faecal pellets were dissolved in water and supernatant subjected to LC-MS/MS). The chromatographic separation of xylitol from its structural isomers is shown in Supplementary data online, *Figure S1*. Accuracy and precision of the method to quantify xylitol (separated from its structural isomers arabitol and ribitol) were assessed by a standard addition method[41] of pure synthetic standard into three different plasma pools. Accuracy was 2.4%–2.8%, and intra- and inter-day coefficients of variation (CVs), were 1.2%–8.9% and 10.9%–11.5%, respectively. Urine creatinine was similarly analysed by stable isotope dilution LC-MS/MS using D_3-creatinine as internal standard as previously described.[42] Erythritol was quantified by stable isotope dilution LC-MS/MS as previously described.[19] Further details for all assays can be found in Supplementary data online, *Methods*.

Statistical analysis

Continuous variables are summarized as median [interquartile range (IQR)], and categorical variables are presented as %. Difference between groups (e.g. tertiles) was examined using Kruskal–Wallis test for continuous variables and χ^2 test for categorical variables. Kaplan–Meier analysis with Cox proportional hazards regression was used for time-to-event analysis to determine hazard ratios (HR) and 95% confidence intervals (CI) for incident major adverse cardiovascular events (MACE). Adjustments included traditional cardiovascular risk factors (age, sex, diabetes mellitus, systolic blood pressure, low-density lipoprotein cholesterol levels, high-density lipoprotein cholesterol levels, triglyceride levels, smoking status) and high-sensitivity C-reactive protein (hsCRP). In some analyses, estimated glomerular filtration rate (eGFR) was used in addition to the aforementioned variables in the adjustment. We confirmed that the proportionality hazards assumptions were met using the Schoenfeld residuals against the transformed time. Mann–Whitney *U*-test or Wilcoxon matched-pairs signed rank test were applied to continuous variables to examine differences between two groups. Kruskal–Wallis test with Dunn's *post hoc* test was used for pairwise multiple comparisons of ranked data. Two-way analysis of variance (ANOVA) with Šidák's multiple comparison *post hoc* test was used for multiple-group comparisons of aggregometry responses using different concentrations of agonists. For analysis of collagen-dependent platelet adhesion in whole blood, a two-way repeated measures ANOVA with Sidák's multiple comparison *post hoc* test was used. All reported measurements represent distinct samples. Data analyses were performed with R software (version 4.2.2) and GraphPad Prism software (version 9.1.2). A two-sided $P < .05$ was considered statistically significant.

Results

Untargeted metabolomics analyses in a discovery cohort suggested xylitol was associated with adverse cardiovascular outcomes

We have previously utilized untargeted metabolomics as a discovery platform to identify metabolites and pathways linked to residual CVD risks beyond traditional established risk factors.[19,36,43,44] During the conduct of those untargeted mass spectrometry studies, we examined plasma from sequential stable subjects undergoing elective diagnostic cardiac evaluations (discovery cohort, $n = 1157$) and observed that numerous polyols, including a metabolite with presumed pre-derivatization elemental composition of $C_5H_{12}O_5$ and tentatively classified as xylitol, showed significant difference in level between those who experienced an incident (3-year) adverse cardiovascular event vs. those who did not.[19] In new studies, we performed further analyses of the raw untargeted metabolomics data from this discovery cohort (*Table 1* and *Table S1* show baseline characteristics), this time utilizing a fragment ion more selective for xylitol as quantifying ion (Methods). As shown in *Figure 1A*, left, levels of the analyte tentatively annotated as xylitol showed enhanced relative abundance in subjects who experienced (vs. not) a MACE during the ensuing 3 years of follow-up. Further examination showed subjects with increased levels of the analyte [tertile 3 (T3) vs. either T1 or T2] displayed both poorer event-free survival in Kaplan–Meier survival analyses (*Figure 1A*, middle) and significant increased risk for incident (3-year) MACE [T3 vs. T1, HR (95% CI), 1.63 (1.04–2.53), $P < .05$] in Cox proportional hazards regression analyses, including following adjustments for traditional CVD risk factors and hsCRP [adjusted(a)HR (95% CI), 1.64 (1.05–2.56), $P < .05$; *Figure 1A*, right].

Stable isotope dilution tandem mass spectrometry analyses of xylitol in the validation cohort

Untargeted metabolomics studies are both semi-quantitative and not designed to necessarily distinguish between structural isomers. For example, xylitol and its isomers arabitol and ribitol all share identical elemental composition ($C_5H_{12}O_5$) and overall structure (see Supplementary data online, *Figure S1*) and have similar physicochemical properties; the isomers also have similar mass spectrometry fragmentation patterns and often co-chromatograph under routine separation protocols employed during untargeted metabolomics (*Methods*). We therefore developed a stable isotope dilution LC-MS/MS assay with conditions that provided baseline separation of xylitol from its structural isomers (see Supplementary data online, *Figure S1*) and then applied the method to unequivocally quantify xylitol in an independent (non-overlapping) validation cohort ($n = 2149$) comprised of stable subjects undergoing elective diagnostic cardiac evaluation (*Table 1* and *Table S2*). Higher plasma levels of xylitol were again observed among subjects who experienced MACE (*Figure 1B*, left). Kaplan–Meier analysis similarly revealed higher levels of circulating xylitol were associated with poorer event-free survival over the follow-up period (*Figure 1B*, middle). In time-to-event Cox proportional hazards analysis, subjects with higher plasma xylitol levels had significantly increased risk of incident (3-year) MACE [HR (95% CI), 1.81 (1.29–2.53), $P < .001$], including following adjustments for traditional cardiovascular risk factors and hsCRP [aHR (95% CI), 1.57 (1.12–2.21), $P < .01$; *Figure 1B*, right]. Elevated levels of xylitol were similarly associated with incident risk in males and females alike, with no sex-specificity noted (P interaction for sex .51). Further, in Cox regression models where xylitol was treated as a continuous variable, xylitol remained independently associated with MACE following adjustments for traditional cardiovascular risk factors and hsCRP [aHR 1.06 (1.01–1.11) per SD of xylitol, $P = .023$]. In further analyses, elevated xylitol levels (T3 vs. T1) were also associated with incident thrombotic event risks [aHR 1.80 (1.05–3.08), $P = .03$]. In parallel analyses, we looked at long-term risk of MACE amongst validation cohort subgroups defined by multiple platelet-related phenotypes (see Supplementary data online, *Figure S2*). The significant association between xylitol levels with incident MACE risk was observed amongst subjects on antiplatelet drugs and, in general, amongst all subgroups examined where subjects were stratified by multiple different platelet functional metrics (P for interaction in all subgroups >.35; Supplementary data online, *Figure S2*). When kidney function (eGFR) was included into the fully adjusted model, the association of xylitol with MACE remained significant [aHR 1.50 (1.06–2.11), $P = .02$].

Table 1 Baseline characteristics of the discovery and validation cohorts

Characteristics	Discovery cohort ($n = 1157$)	Validation cohort ($n = 2149$)
Age (years)	65 (56–72)	63 (55–72)
Male (%)	64	64
BMI (kg/m^2)	28.4 (25.4–32.1)	28.4 (25.5–32.2)
Diabetes mellitus (%)	22	22
Hypertension (%)	72	70
Current smoking (%)	14	13
Systolic blood pressure (mmHg)	132 (119–146)	132 (119–146)
History of cardiovascular disease (%)	76	78
History of coronary artery disease (%)	76	75
History of myocardial infarction (%)	46	40
Coronary vessels ≥ 50% stenosis (%)		
1	22	19
2	19	20
3 or more	30	32
History of heart failure (%)	17	19
eGFR (mL/min/1.73m^2)	89.8 (75.7–99.1)	90.4 (75.4–100.3)
LDL cholesterol (mg/dL)	96 (80–116)	96.0 (77.0–117)
HDL cholesterol (mg/dL)	34 (29–41)	34.3 (28.2–41.7)
Total cholesterol (mg/dL)	163 (143–188)	161 (139–187)
Triglycerides (mg/dL)	122 (84–171)	114 (84.0–163)
hsCRP (mg/L)	2.31 (0.97–5.39)	2.30 (1.01–5.42)
Baseline medication		
Statins (%)	61	59
Aspirin (%)	77	72
Anti-diabetic drugs (%)	9	12
ACE inhibitors/ARBS (%)	50	50
Calcium channel blockers (%)	19	19
Diuretics (%)	19	21

The baseline characteristics of participants in the discovery cohort and validation cohort are shown. Continuous variables are summarized as median [interquartile range (IQR)], and categorical variables are presented as %.
ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Examination of post-prandial circulating levels of xylitol following ingestion of a xylitol-sweetened drink

Plasma levels monitored in both the discovery cohort and the validation cohort were following overnight (>12 h) fast. Since 'naturally sweetened' or 'keto-friendly' processed foods and beverages can contain large amounts of xylitol and thus might substantially increase circulating levels in the post-prandial setting, before performing platelet function studies, we assessed how high circulating levels of xylitol reach following a commonly observed dietary exposure. Post-prandial levels of xylitol were measured in healthy volunteers ($n = 10$) following ingestion of a xylitol-sweetened (30 g) water, an exposure comparable with a pint of numerous xylitol-sweetened ice creams, a xylitol-sweetened bakery good, or several pieces of xylitol-sweetened candy (clinicaltrials.gov identifier NCT04731363; Methods, Supplementary data online, *Table S3*). At baseline (following overnight fast), plasma levels of xylitol were low [median (IQR), 0.30 (0.27–0.34) μM] and comparable with quartile 1 (Q1) values observed in the validation cohort (*Figure 1B*, right). However, 30 min following ingestion, 1000-fold increases in plasma levels were noted [median (IQR), 312 (134–629) μM], with concurrent and subsequent excretion in the urine (*Figure 2A* and Supplementary

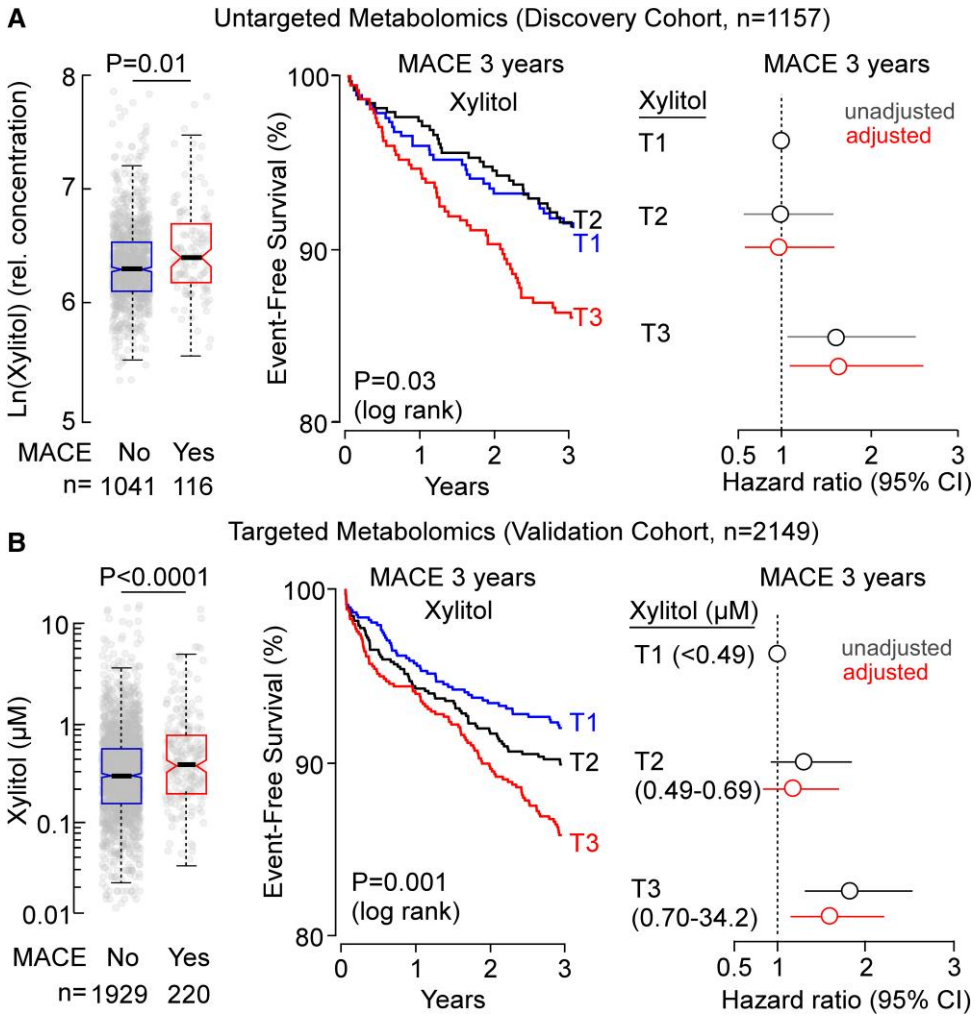


Figure 1 Xylitol levels are associated with higher risks of major adverse cardiovascular events (MACE) in the discovery and validation cohorts. (*A*, left) Circulating levels of a polyol tentatively assigned as xylitol (from untargeted metabolomics) in discovery cohort subjects. Boxes represent IQR with the notch indicating the median. Lower whiskers represent the smallest observation (≥25% quantile − 1.5 × IQR) and upper whiskers the largest observation (≤75% quantile + 1.5 × IQR). Two-tailed Mann–Whitney *P*-values are indicated. (Middle) Kaplan–Meier plot for 3-year MACE stratified by tertiles (T) of relative levels of xylitol in discovery cohort subjects. *P*-values were calculated with log rank test. (Right) Hazard ratios (HR) for incident 3-year MACE based on univariable and multivariable Cox proportional hazards regression analysis. Data points indicate HR, and 95% confidence intervals are represented by line length. Multivariable adjustments include age, sex, smoking, diabetes, systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, and hsCRP. (*B*, left) Circulating xylitol levels (from quantitative stable isotope dilution LC-MS/MS analysis) in discovery cohort subjects. Boxes represent IQR with the notch indicating the median. Lower whiskers represent the smallest observation (≥25% quantile − 1.5 × IQR) and upper whiskers the largest observation (≤75% quantile + 1.5 × IQR). Two-tailed Mann–Whitney *P*-values are indicated. (Middle) Kaplan–Meier plot for 3-year MACE stratified by tertiles (T) of plasma xylitol levels in the validation cohort. *P*-values were calculated with log rank test. (Right) HR for incident 3-year MACE based on univariable and multivariable Cox proportional hazards regression analysis. Data points indicate HR, and 95% confidence intervals are represented by line length. Multivariable adjustments include age, sex, smoking, diabetes, systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, and hsCRP

data online, *Figure S3*). Plasma xylitol levels returned to low-micromolar levels within 4 to 6 h [median (IQR), 1.87 (1.43–2.80) μM and 0.67 (0.57–1.25) μM, respectively], with a plasma half-life of ∼13 (± 4) min. The rapid rate of xylitol excretion observed in healthy volunteers with return to near baseline (fasting) levels within hours following ingestion of a significant dietary exposure suggests that the plasma levels observed in our observational (validation) cohort represent variations in endogenous production/levels and not food intake.

Physiological levels of xylitol augment platelet responsiveness

The observed positive association between xylitol and incident thrombotic event risk (*Figure 1*) suggested a potential impact of xylitol on platelet function. In initial studies, we assessed whether xylitol can impact platelet responsiveness using physiological concentrations of xylitol (i.e. at levels observed in overnight fasted subjects from our validation cohort, which is comprised of subjects with relatively

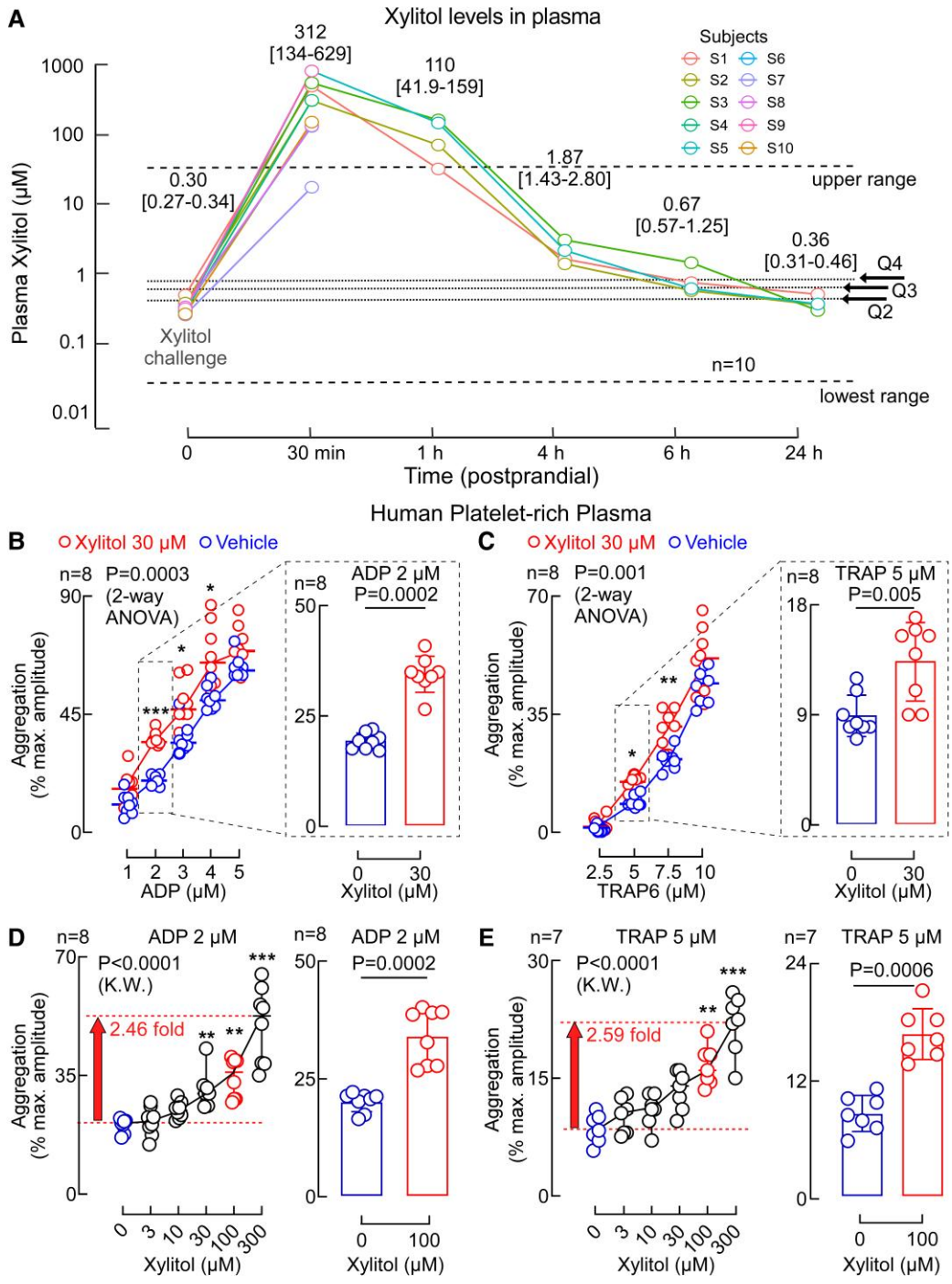


Figure 2 Xylitol levels following oral challenge and effect of xylitol platelet responsiveness. (*A*) Study participants ($n = 10$) were given 30 g of xylitol dissolved in water to ingest. Xylitol levels were quantified via LC-MS/MS in the blood before and at the indicated times after the xylitol challenge in the first four subjects. In the remainder of subjects, xylitol levels were measured before and 30 min after xylitol challenge. Values listed above data points at each time point represent median (IQR). The distribution of fasting (≥12 h) plasma xylitol levels observed in the validation cohort is also shown: the dashed lines represent the upper and lower range, and the dotted lines indicate the bottom boundaries at quartiles (Q) 2, 3, and 4 in the validation cohort. (*B* and *C*) PRP was isolated from healthy volunteers and used to study the effects of varying levels of xylitol on agonist-induced platelet aggregometry. Scatter plots show aggregometry responses for fixed concentrations of xylitol (30 μM, red circles) vs. vehicle (blue circles) with different concentrations of ADP (*A*) or thrombin receptor activator peptide (TRAP6, *B*) with line representing medians. Global *P*-values (for xylitol effect) were calculated with two-way ANOVA and Šídák's multiple comparisons test to compare groups. *$P < .05$, **$P < .01$, ***$P < .001$. Bar graphs (magnified areas) show submaximal ADP-stimulated (2 μM, *A*) and TRAP6-stimulated (5 μM, *B*) platelet aggregometry responses of human PRP following incubation with xylitol (30 μM, red) vs. normal saline (vehicle, blue), with line and whiskers representing means (±SD). *P*-values were calculated by two-tailed Mann–Whitney test. (*D* and *E*) Aggregometry responses of human PRP with varying concentrations of xylitol and fixed submaximal concentration of ADP (2 μM, *D*) and TRAP6 (5 μM, *E*) with lines and whiskers representing medians (IQR). *P*-values were calculated by two-sided Kruskal–Wallis (K.W.) test with Dunn's *post hoc* test. *$P < .05$, **$P < .01$, ***$P < .001$. Bar graph data are represented as means (±SD). *P*-values were calculated by two-tailed Mann–Whitney test

preserved renal function; *Table 1*). Brief incubation of human PRP recovered from healthy volunteers with a fixed physiological (Q4) level of xylitol (30 μM) showed no effect. However, when platelets were exposed to the same fixed xylitol concentration in the presence of submaximal levels of known agonists such as adenosine diphosphate (ADP) or the thrombin receptor activator peptide TRAP6, marked enhancement in stimulus-dependent platelet activation and aggregation was observed (*Figure 2B and C*). Employing a different study design, we next instead used a fixed submaximal level of each of the platelet agonists (ADP or TRAP6) and varying levels of pre-incubation with xylitol. Notably, a dose-dependent enhancement in the extent of platelet responsiveness was observed across both the physiological range of xylitol in fasting subjects in our clinical (validation) cohort (e.g. 30 μM) and extending to the post-prandial levels observed in healthy volunteers following consumption of xylitol [e.g. we also examined 300 μM (*Figure 2D and E*), though post-prandial levels of up to ~1000 μM were observed; *Figure 2A*]. Collectively, these data show that within the range of fasting plasma (endogenous) levels of xylitol observed in our cohort, xylitol augments stimulus-dependent platelet responsiveness (i.e. shifts the dose response curve for known agonists to the left). Moreover, post-prandial levels of xylitol were observed to enhance platelet reactivity to an even greater extent.

Platelet aggregation responses can involve factors beyond direct interaction with platelets. We therefore isolated washed human platelets from healthy volunteers to directly test whether xylitol interacts with the platelets and impacts agonist-induced intracellular cytosolic calcium release. Brief (15–30 min) pre-incubation of Fura 2, loaded platelets with varying levels of xylitol, dose-dependently enhanced submaximal (0.02 U) thrombin-evoked intracellular Ca^{2+} release in multiple different platelet preparations (*Figure 3A and B*). In parallel experiments, exposure of washed human platelets to physiological levels of xylitol caused dose-dependent enhancement in multiple examined platelet activation phenotypes including ADP-stimulated P-selectin surface expression and glycoprotein α2β3 (GP IIb/IIIa) activation (*Figure 3C and D*). Activated platelets bind to leucocytes—a process that leads to mutual changes in cellular effector functions and is associated with various CVD phenotypes.[45] We therefore also examined the effect of xylitol on platelet–leucocyte aggregates. For these studies, we used imaging flow cytometry since it is optimized for cell–cell interaction and, in contrast to conventional flow cytometry, distinguishes between tethered platelets (genuine platelet–leucocyte aggregates) from coincidental, untethered platelets near leucocytes.[46] Following brief pre-incubation with a range of physiological levels of xylitol, whole blood from healthy volunteers showed a dose-dependent increase in TRAP6-stimulated platelet–leucocyte aggregates (*Figure 3E*).

Xylitol enhances platelet clotting in whole blood and thrombosis potential *in vivo*

To investigate the effect of xylitol on the initial step in thrombus formation, platelet adhesion to a collagen surface, we examined platelet adhesion in whole blood under physiological shear flow using a microfluidics device.[35] Xylitol substantially accelerated the rate of collagen-dependent platelet adhesion and spreading under physiological shear flow (*Figure 4A*). In additional studies, the impact of xylitol on *in vivo* thrombosis potential was assessed using a $FeCl_3$-induced carotid artery injury model.[39] In preliminary studies, mice showed poor oral absorption of xylitol (in comparison with erythritol) in both feeding studies (when provided in drinking water or food) and in studies where the sugar alcohol was delivered by gastric gavage (see Supplementary data online, *Figure S4*). Thus, an *i.p.* injection model was performed to recapitulate physiological plasma levels of xylitol in mice that paralleled those observed in humans. Compared with vehicle (saline) control, mice with elevated plasma levels of xylitol exhibited both a marked increase in the rate of clot formation (*Figure 4B*, left) and a significant reduction of the time to cessation of blood flow following arterial injury (*Figure 4B*, right).

Xylitol dietary challenge in subjects enhances multiple indices of platelet reactivity

Since our studies with isolated washed human platelets, PRP, whole blood, and murine *in vivo* thrombosis models all suggested that xylitol can impact platelet responsiveness, we next sought to examine whether dietary exposure to xylitol impacted platelet phenotypes in humans following ingestion of xylitol-sweetened water. For these studies, we rapidly isolated PRP from healthy volunteers ($n = 10$) before and 30 min after ingestion of xylitol (clinicaltrials.gov identifier NCT04731363, *Methods*) and assessed agonist-induced aggregation responses. As noted before, xylitol ingestion significantly increased post-prandial (30 min) plasma levels [median (IQR), 312 (134–629) μM]. In parallel, platelet functional analyses revealed xylitol exposure provoked a substantial (multiple-fold) increase in aggregation responses to either ADP or TRAP6 ($P < .0001$ at all doses examined; *Figure 5*), in line with our *in vitro* studies using similar concentrations of xylitol (*Figure 2D and E*). When responses of individual subjects to submaximal agonist (ADP and TRAP6) concentrations were examined, a significant increase in platelet responsiveness was observed following xylitol ingestion in every subject (*Figure 6*, Supplementary data online, *Figures S5 and S6*). Further, subjects showing the largest increases in platelet responsiveness post-xylitol challenge also tended to have the highest post-prandial xylitol levels, and a strong positive correlation was noted between post-prandial xylitol levels and either ADP-induced or TRAP-induced aggregation responses (Spearman rho 0.71 and 0.74, respectively; $P < .0001$ for each; Supplementary data online, *Figure S7*).

Discussion

Many studies, including data analyses from the National Health and Nutrition Examination Survey (NHANES), have confirmed a dramatic increase of low-calorie sweetener use over the past decades.[47] Meanwhile, the consumption of sweeteners is likely underestimated because of the lack of itemized listing of specific low-calorie sweeteners on labels in many reduced- and low-calorie processed foods and the lack of disclosure requirements (e.g. for the quantity used) in policies for food labelling by both the FDA and EU.[48] Remarkably, artificial sweeteners have even been detected in presumed 'non-consumers' who were counselled extensively to avoid artificial sweetener exposure before they were enrolled into randomized clinical trials.[49] Moreover, the increases in artificial sweetener use have even reached levels where they are readily detected within ground water and waste effluent, where their detection and quantification has been recommended as 'ideal chemical markers of domestic wastewater in groundwater'.[50] Although non-nutritive and low-calorie sweeteners have historically been GRAS by public agencies (e.g. EU[4] and the FDA,[3]) several cohort studies,[13–17] but not all,[51,52] have linked ingestion of artificially sweetened foods with cardiometabolic adverse phenotypes. This recently prompted authorities, including the European Food Safety Authority (EFSA) and the World Health Organization, to re-evaluate exposure

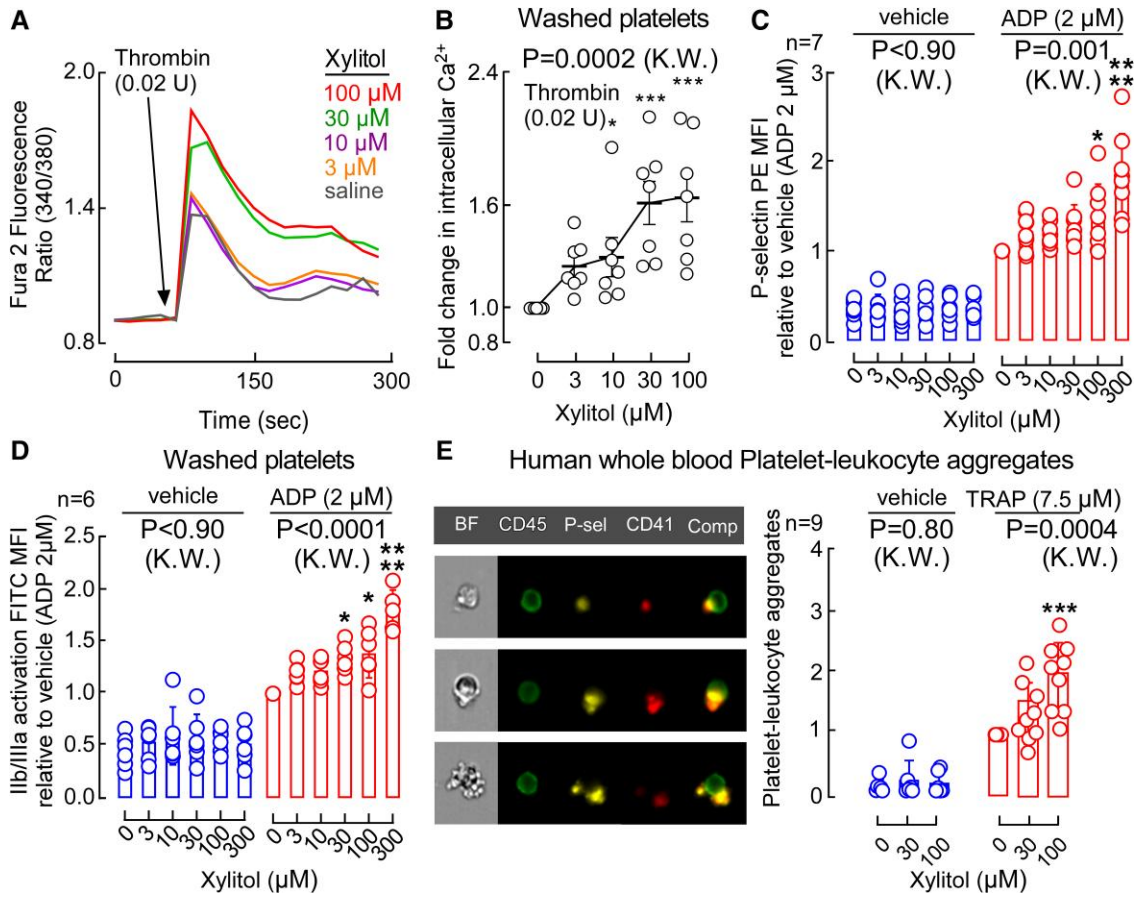


Figure 3 Xylitol increases stimulus-dependent intracellular calcium release and markers for activation in human platelets. (*A*) Representative fluorescent signal showing thrombin (0.02 U)-induced changes in intracellular calcium release in Fura 2-filled washed human platelets incubated with xylitol. (*B*) Fold change (relative to vehicle) in peak Fura 2 fluorescence following submaximal (0.02 U) thrombin stimulation at the indicated concentrations of xylitol in washed human platelets. Bars show mean with SEM indicated by whiskers. *P*-values were calculated by two-sided Kruskal–Wallis test with Dunn's *post hoc* test. **P* < .05; ***P* < .01; ****P* < .001. (*C*) ADP-induced changes in *P*-selectin surface expression in washed human platelets pre-incubated with the indicated concentrations of xylitol. Plotted are interquartile ranges (boxes). The line in the box is the median, and whiskers represent minimum and maximum values. *P*-values were calculated by two-sided Kruskal–Wallis test with Dunn's *post hoc* test. **P* < .05; ***P* < .01; ****P* < .001, **** *P* < .0001. (*D*) ADP-induced changes in GP IIb/IIIa (PAC-1 antibody staining) in washed human platelets pre-incubated with the indicated concentrations of xylitol. Plotted are interquartile ranges (boxes). The line in the box is the median, and whiskers represent minimum and maximum values. *P*-values were calculated by two-sided Kruskal–Wallis test with Dunn's *post hoc* test. **P* < .05; ***P* < .01; ****P* < .001, *****P* < .0001. (*E*, left) Representative fluorescent images of platelet–leucocyte aggregates (BF, bright field, CD45 in green, *P*-selectin in yellow, CD41 in red, merged image) in human whole blood stimulated with TRAP6 (7.5 µM). (Right) Numbers of platelet–leucocyte aggregates (CD45+, *P*-selectin+, CD41+) quantified by image stream in human whole blood incubated with indicated concentrations of xylitol at baseline (blue circles) and stimulated with 7.5 µM TRAP6 (red circles) relative to vehicle control with TRAP6. *N* numbers shown for donors for TRAP-stimulated blood samples, for unstimulated samples *n* = 6–8. *P*-values were calculated by two-sided Kruskal–Wallis test with Dunn's *post hoc* test. **P* < .05; ***P* < .01; ****P* < .001

and toxicity of sweeteners, in particular with respect to potential long-term effects on health.[53,54] In light of the substantial rise in the incorporation of artificial sweeteners into our food chain and the present studies identifying both clinical and mechanistic links between xylitol and CVD risks and relevant phenotypes, further studies that assess their long-term cardiovascular safety seem warranted.

Polyol sweeteners like xylitol are difficult to quantify since they possess multiple structural isomers that differ only in the spatial orientation of the hydroxyl groups on the molecule. These difficulties, coupled with limited regulatory requirements for disclosure, have hampered their quantification in epidemiological studies to explore links between levels of sweeteners in blood and both metabolic and CVD risks. In the present studies, an unambiguous link between plasma levels of xylitol and incident MACE risks was observed in our validation cohort, where xylitol was separated from its structural isomers and quantified using isotope dilution LC-MS/MS. Further, comparisons between xylitol levels that elicit increases in platelet reactivity and *in vivo* thrombosis potential suggest even at elevated plasma levels among overnight fasted subjects (e.g. Q4), significant increases in platelet responsiveness are observed. By performing human clinical intervention studies exploring the physiological effects of xylitol ingestion, a marked enhancement in multiple indices of platelet responsiveness was observed in every subject examined (*Structured Graphical Abstract*). Collectively, the body of evidence accrued, including *in vitro*, *in vivo* (animal model), and xylitol

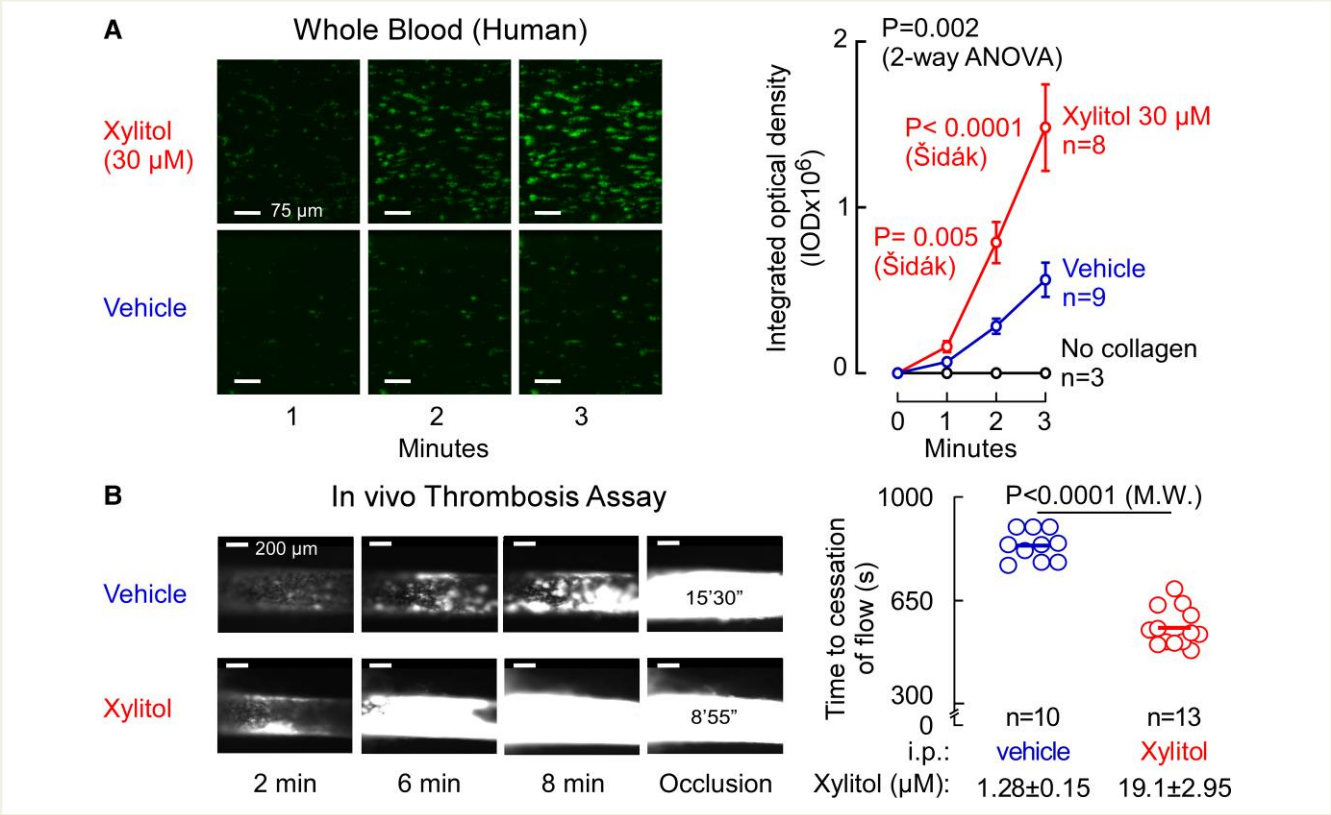


Figure 4 Xylitol enhances *in vivo* clot formation. (*A*) Human platelet adhesion in whole blood to a collagen-coated microfluidic chip surface under physiological shear conditions ± xylitol. Representative images of platelet (green) adhesion at the indicated times (scale bar, 50 µm). *P*-values were calculated by two-way repeated measures ANOVA with Šídák's *post hoc* test. Overall, *P*-value (xylitol effect) is shown in black, and Šídák's *post hoc* test *P*-values are shown in red over the three follow-up times. Data is represented as means (±SEM). (*B*) Representative micrographs of carotid artery thrombus formation at the indicated time points following $FeCl_3$-induced carotid artery injury (scale bar, 200 µm) and time to cessation of blood flow in mice from indicated groups i.p. injected with vehicle or xylitol. Bars represent means, and two-sided *P*-values were calculated by Mann–Whitney test. Plasma xylitol concentrations in both groups are indicated as means (±SEM)

human ingestion studies, argues for a direct effect of the low-calorie sweetener xylitol on platelet function and thrombosis potential *in vivo*.

Interestingly, during the conduct of these and prior studies, we note inter-species differences in the absorption and metabolism of different sugar alcohols, arguing for both the necessity of monitoring circulating levels of specific molecular species in studies (to confirm what is ingested is actually absorbed) and the need for human clinical investigations. For example, we found xylitol, like erythritol,[19] is readily absorbed following oral ingestion by humans, with plasma levels increasing over 1000-fold in the post-prandial setting. However, our present studies also revealed that in mice, in contrast to erythritol, which is rapidly absorbed by oral route,[19] xylitol is poorly absorbed, with the majority of the ingested sugar alcohol recovered in faeces (see Supplementary data online, *Figure S4*). We therefore used intraperitoneal injection to elevate xylitol plasma levels in mice to the concentrations observed in humans, allowing for testing the effect of systemic exposures in rodent models analogous to those experienced in humans (*Methods*). Another example of inter-species differences in metabolism of sugar alcohols like xylitol within mammals occurs with dogs. While xylitol does not induce glucose or insulin secretion in humans or rodents,[55,56] it is highly toxic to dogs, where it provokes extreme insulin secretion,[57] and numerous reports list the extensive use of xylitol in human processed foods as a warning for pet (dog) owners.[58]

Despite the clear evidence for xylitol inducing a direct physiological effect on isolated human platelets, the molecular participants involved (i.e. receptor) transmitting the effect on platelet function remain unknown. Indeed, little is known about how polyol sweeteners are recognized by cells. In the present studies, when added at submaximal levels, xylitol enhanced stimulus-induced platelet aggregation responses to multiple agonists (e.g. ADP, thrombin, collagen) and *in vivo* thrombosis formation. Collectively, when coupled with recent observations with erythritol,[19] our data suggest that xylitol and erythritol (and possibly other sugar alcohol polyol sweeteners) act on converging pathways to enhance platelet responsiveness and *in vivo* thrombosis potential. They thus argue for a potential adverse class effect of some sugar alcohol sweeteners, a finding that is highly relevant for both the processed food industry and the diets of the more vulnerable subjects most likely to consume multiple artificial sweeteners (diabetics, obese, those with CVD).

One topic that deserves further discussion is the relationship in the large-scale clinical observational studies performed associating plasma levels of xylitol with incident risks of MACE and dietary exposures to xylitol. The present studies, we believe, argue that the observed associations noted in the discovery and validation cohorts reflect endogenous xylitol levels (steady state between endogenous production and excretion) and not recent dietary exposure. First, while a

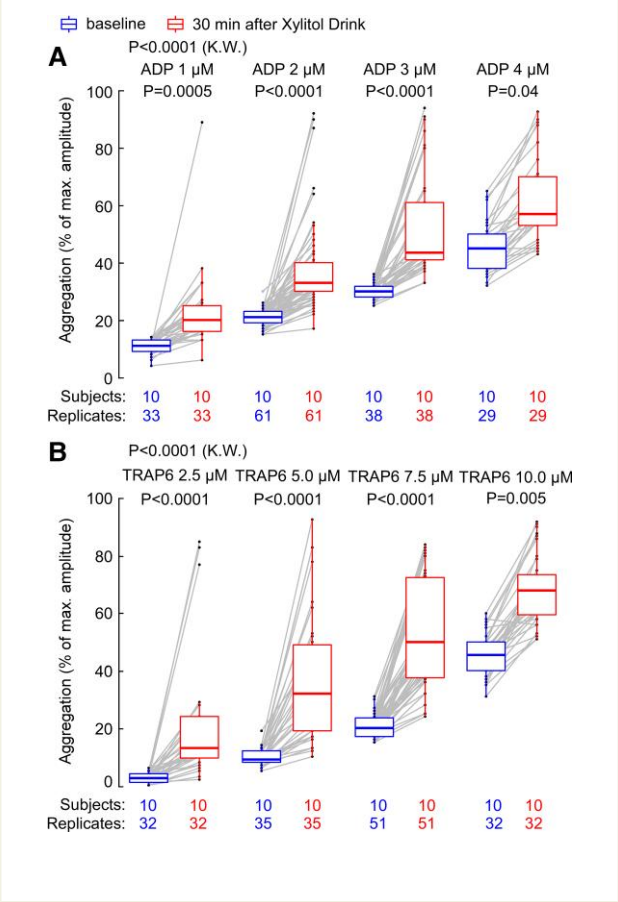


Figure 5 Effect of routine dietary xylitol challenge on platelet responsiveness in healthy subjects. (*A* and *B*) Study participants ($n = 10$) were given 30 g of xylitol in a drink. Before and 30 min after the xylitol challenge, PRP was rapidly isolated, and platelet aggregometry was performed using different concentrations of ADP (*A*) and TRAP6 (*B*). Shown are aggregation responses of paired samples (baseline and post-xylitol) that were analysed together. Boxes represent interquartile ranges (IQR) with the notch indicating the median. Lower whiskers represent the smallest observation (≥25% quantile − 1.5 × IQR) and upper whiskers the largest observation (≤75% quantile + 1.5 × IQR). The total number of replicates (multiple replicates per donor) and the total number of individual donors for each agonist concentration are indicated. *P*-values were calculated with the Kruskal–Wallis (K.W.) test with a Dunn *post hoc* analysis

limitation of the present studies is the lack of any information on xylitol dietary exposure in the clinical observational cohorts, given the speed with which we observe xylitol is excreted (i.e. half-life of elimination was ∼15 min, and post-prandial plasma levels in healthy volunteers, despite reaching 1000-fold increases, return to near baseline levels within 6 h; *Figure 2A*), the fasting (>12 h) plasma levels monitored in the discovery and validation cohorts likely represent endogenous levels of xylitol production. Second, the enrolment of subjects in the validation cohort largely predates the more recent increases in dietary exposure to xylitol in processed foods. We also note that it is across variations in endogenous levels [measured in overnight (>12 h) fasting samples] that our initial clinical observational studies observe heightened MACE risks in subjects with higher xylitol levels (e.g. Q4 vs. Q1). Xylitol is endogenously produced within cells where it serves as an intermediate of the glucuronic acid pathway—an alternative route of glucose utilization that provides biosynthetic precursors and involves detoxification through glucuronidation with an estimated endogenous production of 15 g daily in subjects.[31,59,60] As far as we are aware, the glucuronic acid pathway has not yet been linked with CVD pathogenesis. However, we note that in recent studies, glucuronic acid levels were reported to be associated with health span and longevity in both population-based cohort studies and in mice.[61] Yet, other studies have linked glucuronic acid pathway intermediates with cell migration and metastasis.[62] Finally, we also think it important to mention that throughout the evolution of humans, *Homo sapiens* have not experienced millimolar levels of xylitol in plasma, i.e. until its introduction into our food chain within the past decade or so. The present studies show, however, that even at plasma xylitol levels as low as 19 µM in animal models of thrombosis (*Figure 4B*), well within the fourth quartile of our fasting plasma levels from a cohort (validation) of subjects with largely preserved renal function, we observe significant heightened thrombosis potential *in vivo*. Based on our additional human intervention study data (*Figures 2*, *5*, and *6*), much higher post-prandial levels of xylitol (driven by consumption of dietary xylitol from artificially sweetened processed foods) can be superimposed on endogenous levels, substantially further enhancing thrombotic risk (especially in the post-prandial setting).

Our studies have several limitations. We only measured baseline fasting levels of xylitol in our clinical cohort studies; thus, whether serial measures of xylitol provide enhanced clinical prognostic value for CVD risk assessment remains unknown. In addition, as noted, our observational cohorts do not have dietary information. Moreover, our subjects have a high burden of CVD risk factors, and whether the results can be translated to a community-based setting needs to be determined. However, we note that in countries like the USA, most adults in their 40s have at least one chronic disease and in their 60s two or more chronic diseases, with cardiometabolic diseases including obesity, diabetes, and CVD among the most prevalent. A further limitation of our studies is that the initial observational cohort results are only associative in nature and there is the possibility for residual confounding. However, we also note that we used the observational cohort findings as hypothesis generating and supplemented these findings with numerous *in vitro* and *in vivo* studies, as well as an interventional human xylitol challenge study. Collectively, all results point to xylitol exerting a direct effect on platelet function and thrombosis potential *in vivo*, not only at levels observed in the post-prandial setting but also across the distribution of overnight fasting (and presumed endogenous) plasma xylitol levels observed. Our mechanistic studies also focused on a relatively brief exposure of xylitol, and the effects of chronic exposures need to be explored in future studies. We also note that the receptors involved in recognizing xylitol remain unknown. However, our studies show that xylitol directly elicited enhancement in stimulus-dependent intracellular calcium release and activation in isolated washed human platelets, including for various receptor systems (those recognizing ADP, thrombin, and collagen). Finally, beyond direct platelet effects of xylitol, further studies on different processes that contribute to coagulation are of interest, given the strong clinical association of xylitol levels with thrombotic event risk.

With increased availability and reduced production costs, artificial sweetener use in processed food has expanded in general and xylitol consumption specifically. Industrial production of xylitol has recently been advanced through new biotechnological methods that reduce cost and energy requirements [e.g. microbial and enzymatic fermentation with the substrate (xylose) obtained from abundant sources, such

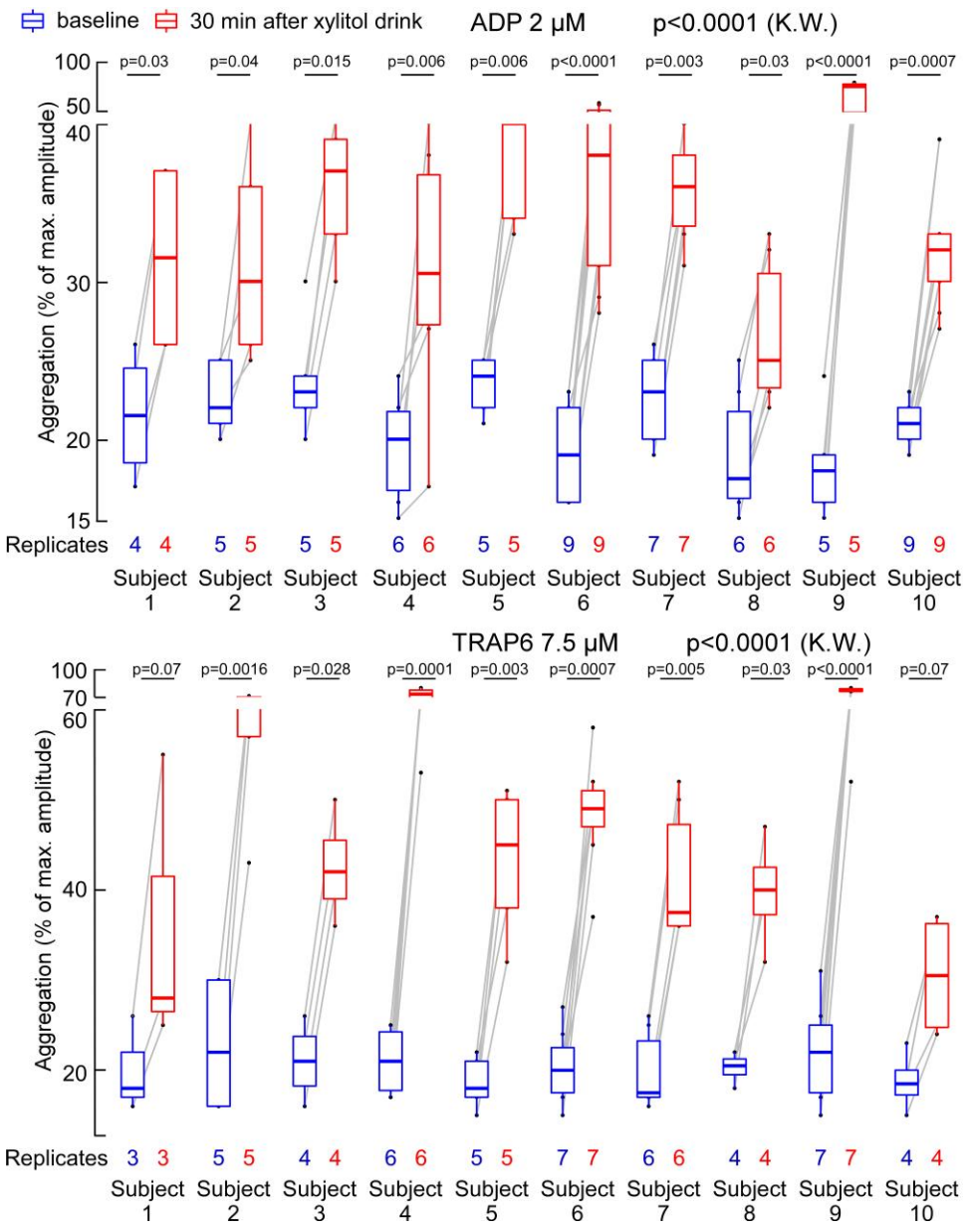


Figure 6 Effect of dietary xylitol exposure on platelet responsiveness in individual subjects. (*A* and *B*) Platelet aggregation responses in PRP from each subject in response to submaximal concentration of ADP (2 µM, *A*) and TRAP6 (7.5 µM, *B*) before and after xylitol exposure. Shown are aggregation responses of paired samples (baseline and post-xylitol) that were analysed together. Boxes represent interquartile ranges (IQR) with the notch indicating the median. Lower whiskers represent the smallest observation (≥25% quantile − 1.5 × IQR) and upper whiskers the largest observation (≤75% quantile + 1.5 × IQR). The total number of replicates per individual donor is indicated. All subjects showed significant differences in agonist-induced aggregation ($P < .05$) for pairwise comparison (pre- vs. post-xylitol exposure) except for Subject 1 with only three replicates, and Subject 10 with four replicates showed $P = .07$ for TRAP6 stimulation. *P*-values were calculated with the Kruskal–Wallis (K.W.) test with a Dunn *post hoc* analysis

as wood and agriculture waste].[63] These advances contribute to increasing global production rates and market penetration with more than a 40-fold increase of xylitol production over the last four decades.[64] For example, xylitol has even been proposed by some as a public health intervention for use in children to prevent dental caries, with recommendation of at least 6–10 g of xylitol daily.[32] Large amounts of xylitol are typically found in numerous processed foods designed for a diabetic diet,[65] like baked pastries, keto ice cream, and confectioneries, often exceeding 30 g per serving size.[32,58] Despite the recent guidance from the WHO recommending avoidance of artificial sweeteners for weight reduction,[5] the acceptable daily xylitol intake assigned by the WHO/FAO Expert Committee on Food Additives remains 'not specified'.[66] Our studies suggest that xylitol will likely confer heightened thrombosis potential in the same vulnerable patients that it is marketed towards and intended to protect (e.g. subjects with diabetes, obesity, and CVD).

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Supplementary Data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

S.L.H. and Z.W. report being named as co-inventors on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. S.L.H. and Z.W. also report having received royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland Heart Lab, a fully owned subsidiary of Quest Diagnostics, and Procter & Gamble. S.L.H. is a paid consultant for Zehna Therapeutics and formerly for Proctor & Gamble; has received research funds from Zehna Therapeutics, Proctor & Gamble, Pfizer, and Roche Diagnostics; and is eligible to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and therapeutics from Zehna Therapeutics. W.H.W.T. reports being a consultant for Sequana Medical A.G., Owkin Inc., Relypsa Inc., and PreCardiac Inc. and having received honorarium from Springer Nature for authorship/editorship and American Board of Internal Medicine for exam writing committee participation—all unrelated to the subject and contents of this paper. The other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Data Availability

Where permissible, the data sets generated and/or analysed during the present studies are available from the corresponding author on request. There are restrictions to the availability of some of the clinical data generated in the present study, because we do not have permission in our informed consent from research subjects to share data outside our institution without their authorizations. Custom R codes used in this manuscript can be accessed at https://doi.org/10.5281/zenodo.6780497. Where permissible, source data are made available in the public data sharing repository (https://doi.org/10.5281/zenodo.10106620). Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

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Ethical Approval

We performed three distinct clinical studies. All human subjects provided written informed consent, and all human studies abided by the Declaration of Helsinki. The Institutional Review Board of the Cleveland Clinic approved all human study protocols [GeneBank IRB 4265; IRB 21-005 (xylitol ingestion related studies), healthy volunteer blood donors for platelet-related studies IRB 09-506]. All animal studies were approved by the Institutional Animal Care and Use Committee at the Cleveland Clinic (IRB 2019-2251).

Pre-registered Clinical Trial Number

The pre-registered clinical trial number for GeneBank at the Cleveland Clinic is NCT00590200. The trial number for the xylitol intervention study is NCT04731363.

References

1. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, *et al.* Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* 2017;**390**:2627–42. https://doi.org/10.1016/S0140-6736(17)32129-3
2. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obes Rev* 2013;**14**:606–19. https://doi.org/10.1111/obr.12040
3. Roberts A. The safety and regulatory process for low calorie sweeteners in the United States. *Physiol Behav* 2016;**164**:439–44. https://doi.org/10.1016/j.physbeh.2016.02.039
4. Mortensen A. Sweeteners permitted in the European Union: safety aspects. *Scand J Food Nutr* 2006;**50**:104–16. https://doi.org/10.1080/17482970600982719
5. World Health Organization. *Use of Non-Sugar Sweeteners: WHO Guideline*: World Health Organization, 2023.
6. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. *Am J Clin Nutr* 2012;**96**:640–6. https://doi.org/10.3945/ajcn.112.034751
7. Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D, *et al.* Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2012;**126**:509–19. https://doi.org/10.1161/CIR.0b013e31825c42ee
8. British Dietetics Association. Policy statement–the use of artificial sweeteners. https://www.bda.uk.com/static/11ea5867-96eb-43df-b61f2cbe9673530d/policystatementsweetners.pdf (18 January 2024, date last accessed).
9. Australian Diabetes Society. The Australian Obesity Management Algorithm. https://www.diabetessociety.com.au/documents/ObesityManagementAlgorithm18.10.2016FINAL.pdf (18 January 2024, date last accessed).
10. Diabetes Canada Clinical Practice Guidelines Expert Committee. Diabetes Canada 2018 clinical practice guidelines for the prevention and management of diabetes in Canada. *Can J Diabetes* 2018;**42**:S1–325. https://doi.org/10.1016/j.jcjd.2017.10.001
11. Johnson RK, Lichtenstein AH, Anderson CAM, Carson JA, Després JP, Hu FB, *et al.* Low-calorie sweetened beverages and cardiometabolic health: a science advisory from the American Heart Association. *Circulation* 2018;**138**:e126–40. https://doi.org/10.1161/CIR.0000000000000569
12. Lohner S, Toews I, Meerpohl JJ. Health outcomes of non-nutritive sweeteners: analysis of the research landscape. *Nutr J* 2017;**16**:55. https://doi.org/10.1186/s12937-017-0278-x
13. Ruanpeng D, Thongprayoon C, Cheungpasitporn W, Harindhanavudhi T. Sugar and artificially sweetened beverages linked to obesity: a systematic review and meta-analysis. *QJM* 2017;**110**:513–20. https://doi.org/10.1093/qjmed/hcx068
14. Imamura F, O'Connor L, Ye Z, Mursu J, Hayashino Y, Bhupathiraju SN, *et al.* Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. *BMJ* 2015;**351**:h3576. https://doi.org/10.1136/bmj.h3576
15. Chazelas E, Debras C, Srour B, Fezeu Léopold K, Julia C, Hercberg S, *et al.* Sugary drinks, artificially-sweetened beverages, and cardiovascular disease in the NutriNet-Santé cohort. *J Am Coll Cardiol* 2020;**76**:2175–7. https://doi.org/10.1016/j.jacc.2020.08.075
16. Malik VS, Li Y, Pan A, Koning D, Schernhammer L, Willett E, *et al.* Long-term consumption of sugar-sweetened and artificially sweetened beverages and risk of mortality in US adults. *Circulation* 2019;**139**:2113–25. https://doi.org/10.1161/CIRCULATIONAHA.118.037401
17. Mullee A, Romaguera D, Pearson-Stuttard J, Viallon V, Stepien M, Freisling H, *et al.* Association between soft drink consumption and mortality in 10 European countries. *JAMA Intern Med* 2019;**179**:1479–90. https://doi.org/10.1001/jamainternmed.2019.2478

18. Bhagavathula AS, Rahmani J, Vidyasagar K, Tesfaye W, Khubchandani J. Sweetened beverage consumption and risk of cardiovascular mortality: a systematic review and meta-analysis. *Diabetes Metab Syndr* 2022;**16**:102462. https://doi.org/10.1016/j.dsx.2022.102462
19. Witkowski M, Nemet I, Alamri H, Wilcox J, Gupta N, Nimer N, *et al.* The artificial sweetener erythritol and cardiovascular event risk. *Nat Med* 2023;**29**:710–8. https://doi.org/10.1038/s41591-023-02223-9
20. Toews I, Lohner S, Küllenberg de Gaudry D, Sommer H, Meerpohl JJ. Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. *BMJ* 2019;**364**:k4718. https://doi.org/10.1136/bmj.k4718
21. Azad MB, Abou-Setta AM, Chauhan BF, Rabbani R, Lys J, Copstein L, *et al.* Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. *CMAJ* 2017;**189**:E929–e39. https://doi.org/10.1503/cmaj.161390
22. Suez J, Cohen Y, Valdés-Mas R, Mor U, Dori-Bachash M, Federici S, *et al.* Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell* 2022;**185**:3307–28.e19. https://doi.org/10.1016/j.cell.2022.07.016
23. Romo-Romo A, Aguilar-Salinas CA, Brito-Córdova GX, Gómez-Díaz RA, Almeda-Valdes P. Sucralose decreases insulin sensitivity in healthy subjects: a randomized controlled trial. *Am J Clin Nutr* 2018;**108**:485–91. https://doi.org/10.1093/ajcn/nqy152
24. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. *Am J Clin Nutr* 2014;**100**:765–77. https://doi.org/10.3945/ajcn.113.082826
25. McGlynn ND, Khan TA, Wang L, Zhang R, Chiavaroli L, Au-Yeung F, *et al.* Association of low- and no-calorie sweetened beverages as a replacement for sugar-sweetened beverages with body weight and cardiometabolic risk: a systematic review and meta-analysis. *JAMA Netw Open* 2022;**5**:e222092. https://doi.org/10.1001/jamanetworkopen.2022.2092
26. Sylvetsky AC, Blau JE, Rother KI. Understanding the metabolic and health effects of low-calorie sweeteners: methodological considerations and implications for future research. *Rev Endocr Metab Disord* 2016;**17**:187–94. https://doi.org/10.1007/s11154-016-9344-5
27. Washuttl J, Riederer P, Bancher E. A qualitative and quantitative study of sugar-alcohols in several foods. *J Food Sci* 1973;**38**:1262–3. https://doi.org/10.1111/j.1365-2621.1973.tb07257.x
28. Scheinin A, Mäkinen KK, Tammisalo E, Rekola M. Turku sugar studies XVIII. Incidence of dental caries in relation to 1-year consumption of xylitol chewing gum. *Acta Odontol Scand* 1975;**33**:269–78. https://doi.org/10.3109/00016357509004632
29. Grembecka M. Sugar alcohols—their role in the modern world of sweeteners: a review. *Eur Food Res Technol* 2015;**241**:1–14. https://doi.org/10.1007/s00217-015-2437-7
30. Livesey G. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. *Nutr Res Rev* 2003;**16**:163–91. https://doi.org/10.1079/NRR200371
31. Wamelink MM, Struys EA, Jakobs C. The biochemistry, metabolism and inherited defects of the pentose phosphate pathway: a review. *J Inherit Metab Dis* 2008;**31**:703–17. https://doi.org/10.1007/s10545-008-1015-6
32. Milgrom P, Rothen M, Milgrom L. Developing public health interventions with xylitol for the US and US-associated territories and states. *Suom Hammaslaakarilehti* 2006;**13**:2–11.
33. Mussatto SI. Application of xylitol in food formulations and benefits for health. In: da Silva SS, Chandel AK (eds.), *D-Xylitol: Fermentative Production, Application and Commercialization*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2012, 309–23.
34. Wölnerhanssen BK, Meyer-Gerspach AC, Beglinger C, Islam MS. Metabolic effects of the natural sweeteners xylitol and erythritol: a comprehensive review. *Crit Rev Food Sci Nutr* 2020;**60**:1986–98. https://doi.org/10.1080/10408398.2019.1623757
35. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, *et al.* Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* 2016;**165**:111–24. https://doi.org/10.1016/j.cell.2016.02.011
36. Nemet I, Saha PP, Gupta N, Zhu W, Romano KA, Skye SM, *et al.* A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell* 2020;**180**:862–77.e22. https://doi.org/10.1016/j.cell.2020.02.016
37. Gupta N, Li W, McIntyre TM. Deubiquitinases modulate platelet proteome ubiquitination, aggregation, and thrombosis. *Arterioscler Thromb Vasc Biol* 2015;**35**:2657–66. https://doi.org/10.1161/ATVBAHA.115.306054
38. Scavone M, Bozzi S, Mencarini T, Podda G, Cattaneo M, Redaelli A. Platelet adhesion and thrombus formation in microchannels: the effect of assay-dependent variables. *Int J Mol Sci* 2020;**21**:750. https://doi.org/10.3390/ijms21030750
39. Witkowski M, Witkowski M, Friebel J, Buffa JA, Li XS, Wang Z, *et al.* Vascular endothelial tissue factor contributes to trimethylamine N-oxide-enhanced arterial thrombosis. *Cardiovasc Res* 2022;**118**:2367–84. https://doi.org/10.1093/cvr/cvab263
40. Fiehn O, Wohlgemuth G, Scholz M. Setup and annotation of metabolomic experiments by integrating biological and mass spectrometric metadata. In: Ludäscher B, Raschid L (eds.), *Data Integration in the Life Sciences*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2005, 224–39.
41. Whitmire ML, Ammerman J, Pd L, Killmer JD, Kyle D, Mainstone E, *et al.* LC-MS/MS bioanalysis method development, validation, and sample analysis: points to consider when conducting nonclinical and clinical studies in accordance with current regulatory guidances. *J Anal Bioanal Techniques* 2011;**2011**:S4. https://doi.org/10.4172/2155-9872.S4-001
42. Wang Z, Bergeron N, Levison BS, Li XS, Chiu S, Jia X, *et al.* Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. *Eur Heart J* 2019;**40**:583–94. https://doi.org/10.1093/eurheartj/ehy799
43. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;**472**:57–63. https://doi.org/10.1038/nature09922
44. Li XS, Obeid S, Wang Z, Hazen BJ, Li L, Wu Y, *et al.* Trimethyllysine, a trimethylamine N-oxide precursor, provides near- and long-term prognostic value in patients presenting with acute coronary syndromes. *Eur Heart J* 2019;**40**:2700–9. https://doi.org/10.1093/eurheartj/ehz259
45. Schrottmaier WC, Mussbacher M, Salzmann M, Assinger A. Platelet-leukocyte interplay during vascular disease. *Atherosclerosis* 2020;**307**:109–20. https://doi.org/10.1016/j.atherosclerosis.2020.04.018
46. Hui H, Fuller KA, Erber WN, Linden MD. Imaging flow cytometry in the assessment of leukocyte-platelet aggregates. *Methods* 2017;**112**:46–54. https://doi.org/10.1016/j.ymeth.2016.10.002
47. Sylvetsky AC, Jin Y, Clark EJ, Welsh JA, Rother KI, Talegawkar SA. Consumption of low-calorie sweeteners among children and adults in the United States. *J Acad Nutr Diet* 2017;**117**:441–8.e2. https://doi.org/10.1016/j.jand.2016.11.004
48. EU European Union. Regulation (EU) 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending regulations (EC) no 1924/2006 and (EC) no 1925/2006 of the European parliament and of the council, and repealing commission directive 87/250/EEC, council directive 90/496/EEC, commission directive 1999/10/EC, directive 2000/13/EC of the European parliament and of the council, commission directives 2002/67/EC and 2008/5/EC and commission regulation (EC) no 608/2004 (text with EEA relevance). Offic J Eur Union 2011;**L304**:18–63. https://eurlex.europa.eu/eli/reg/2011/1169/oj.
49. Sylvetsky AC, Walter PJ, Garraffo HM, Robien K, Rother KI. Widespread sucralose exposure in a randomized clinical trial in healthy young adults. *Am J Clin Nutr* 2017;**105**:820–3. https://doi.org/10.3945/ajcn.116.144402
50. Buerge IJ, Buser HR, Kahle M, Müller MD, Poiger T. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. *Environ Sci Technol* 2009;**43**:4381–5. https://doi.org/10.1021/es900126x
51. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. *Circulation* 2012;**125**:1735–41, S1. https://doi.org/10.1161/CIRCULATIONAHA.111.067017
52. de Koning L, Malik VS, Rimm EB, Willett WC, Hu FB. Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. *Am J Clin Nutr* 2011;**93**:1321–7. https://doi.org/10.3945/ajcn.110.007922
53. European Food Safety Authority. *Outcome of the Public Consultation on a Draft Protocol for Assessing Exposure to Sweeteners as Part of Their Safety Assessment Under the Food Additives Re-Evaluation Programme*: EFSA Supporting Publications, 2020.
54. Rios-Leyvraz M, Montez J. *Health Effects of the Use of Non-Sugar Sweeteners: A Systematic Review and Meta-Analysis*. Geneva: World Health Organization, 2022.
55. Salminen S, Salminen E, Marks V. The effects of xylitol on the secretion of insulin and gastric inhibitory polypeptide in man and rats. *Diabetologia* 1982;**22**:480–2. https://doi.org/10.1007/BF00282594
56. Islam MS. Effects of xylitol as a sugar substitute on diabetes-related parameters in non-diabetic rats. *J Med Food* 2011;**14**:505–11. https://doi.org/10.1089/jmf.2010.0015
57. Piscitelli CM, Dunayer EK, Aumann M. Xylitol toxicity in dogs. *Compend Contin Educ Vet* 2010;**32**:E1–4; quiz E4.
58. Which products contain xylitol? : Preventive Vet. https://www.preventivevet.com/xylitol-products-toxic-for-dogs (18 January 2024, date last accessed).
59. Hollmann S, Touster O. An enzymatic pathway from L-xylulose to D-xylulose. *J Am Chem Soc* 1956;**78**:3544–5. https://doi.org/10.1021/ja01595a080
60. Winkelhausen E, Kuzmanova S. Microbial conversion of d-xylose to xylitol. *J Ferment Bioengineer* 1998;**86**:1–14. https://doi.org/10.1016/S0922-338X(98)80026-3
61. Ho A, Sinick J, Esko T, Fischer K, Menni C, Zierer J, *et al.* Circulating glucuronic acid predicts healthspan and longevity in humans and mice. *Aging* 2019;**11**:7694–706. https://doi.org/10.18632/aging.102281
62. Wang X, Liu R, Zhu W, Chu H, Yu H, Wei P, *et al.* UDP-glucose accelerates SNAI1 mRNA decay and impairs lung cancer metastasis. *Nature* 2019;**571**:127–31. https://doi.org/10.1038/s41586-019-1340-y
63. Umai D, Kayalvizhi R, Kumar V, Jacob S. Xylitol: bioproduction and applications-a review. *Front Sustainability* 2022;**3**. https://doi.org/10.3389/frsus.2022.826190
64. Delgado Arcaño Y, Valmaña García OD, Mandelli D, Carvalho WA, Magalhães Pontes LA. Xylitol: a review on the progress and challenges of its production by chemical route. *Catal Today* 2020;**344**:2–14. https://doi.org/10.1016/j.cattod.2018.07.060
65. Msomi NZ, Erukainure OL, Islam MS. Suitability of sugar alcohols as antidiabetic supplements: a review. *J Food Drug Anal* 2021;**29**:1–14. https://doi.org/10.38212/2224-6614.3107
66. World Health Organization. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA): Xylitol. 1983. https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/2620 (18 January 2024, date last accessed).