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Associations of saccharin intake with all-cause, cardiovascular and cancer mortality risk in USA adults

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Abstract

Saccharin is a widely used sugar substitute, but little is known about the long-term health effects of saccharin intake. Our study aimed to examine the association between saccharin intake and mortality in diabetic and pre-diabetic population and overweight population from NHANES 1988–1994. Cox proportional hazard models were used to evaluate the association between saccharin intake and CVD, cancer and all-cause mortality. After multivariable adjustment, increased absolute saccharin intake was associated with the risk of all-cause mortality (hazard ratio (HR): 1·41, 95 % CI: 1·05, 1·90), CVD mortality (HR: 1·93, 95 % CI: 1·15, 3·25) and cancer mortality (HR: 2·26, 95 % CI: 1·10, 4·45) in diabetic and pre-diabetic population. Among overweight population, higher absolute saccharin intake was associated with the risk of cancer mortality (HR: 7·369, 95 % CI: 2·122, 25·592). Replacing absolute saccharin intake with total sugar significantly reduced all-cause mortality by 12·5 % and CVD mortality by 49·7 % in an equivalent substitution analysis in the diabetic and pre-diabetic population. Aspartame substitution reduced all-cause mortality by 29·2 % and cancer mortality by 30·2 %. Notably, the relative daily intake of saccharin also had similar effects as the absolute intake on all-cause, cardiovascular and cancer mortality in all analyses. This was despite the fact that the relative daily intake in our study was below the Food and Drug Administration limit of 15 mg/kg. In conclusion, our study showed a considerable risk of increased saccharin intake on the all-cause, CVD mortality and cancer mortality.

Keywords: **Cancer: CVD: Diabetic and pre-diabetic population: Mortality: Overweight: Saccharin intake**

Saccharin, the first used artificial sweetener, is widely used in soft drinks, jams, canned food, dessert, chewing gums and other low-energy and sugar-free products. It does not produce any energy content because of its inability of digestion by human gastrointestinal tract[1]. Although saccharin has been approved by the USA Food and Drug Administration as a safe sweetener for processed foods and beverages under certain conditions, there has been controversy about its health effects[2]. Some studies have shown adverse effects of saccharin on insulin release in obese rats[3] and glucose intolerance in C57BL/6 mice and healthy adults[4]. Others have shown no association between saccharin and blood insulin levels or glucose intolerance in animals[5] and fasting glucose or insulin levels or glucose intolerance in diabetic/non-diabetic population[6–8]. In addition, saccharin was originally banned because it caused bladder cancer in animals[9,10], but was subsequently allowed to use due to no evidence in human research. To sum up, although the safety of saccharin was still in debate by scientists and officials, it is continuously permitted in more than ninety countries[11].

Diabetes is a severe, chronic metabolic disease characterised by high glucose levels in blood and impaired glucose regulation. It was estimated to have caused 4 200 000 deaths globally in adults in 2019, with having contributed to 11·3 % of deaths in the world[12]. Besides, prediabetes (or 'intermediate hyperglycaemia'), which is the high-risk status for diabetes, has a conversion rate of 5–10 % annually and was confirmed to show the associations with vascular-related disease[13]. Although the development of insulin resistance or obesity is driven by a

Abbreviation: NHANES, National Health and Nutrition Examination Survey.

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complex set of physiological and lifestyle interactions, excessive consumption of added sugars has long been recognised as a prominent dietary regulatory factor. Given the well-established evidence between added sugars and diabetes[14] or other metabolic disorders[15–17], the 2020 Dietary Guidelines for Americans recommend added sugar should be limited to less than 10 % of total energy intake within a day[18]. The WHO has provided similar guidance[19]. In consideration of added sugar intake limiting, a common strategy is to substitute non-energetic artificial sweeteners of added sugars owing to reduce the caloric intake and have the same taste of nutritive sweeteners. Health-conscious consumers and people with diabetes or obesity often take non-energetic artificial sweeteners as an effect instrument for their health benefits for weight reduction and blood glucose level control.

The long-term health effects of saccharin are still the subject of debate, and there is limited evidence that saccharin has any effect on blood sugar levels. Therefore, our study aimed to examine the association between saccharin intake and mortality in diabetic and pre-diabetic population and overweight population using survey data from the 1988–1994 National Health and Nutrition Examination Survey.

Methods

Study population

National Health and Nutrition Examination Survey (NHANES) is conducted as a representative multistage stratified sampling health survey of the USA. The NHANES is the main source of dietary surveillance data in the USA. The dietary recall component currently uses a multiphase method to measure all foods consumed midnight-to-midnight during the day prior to data collection[20]. Respondents reported all foods and beverages consumed for the previous 24-h time period. An automated, microcomputer-based dietary interview and coding system known as the NHANES III Dietary Data Collection System was used to collect all NHANES III dietary recall data (including saccharin recall data, https://wwwn.cdc.gov/nchs/data/nhanes3/2a/EXAMDR-acc.pdf). The Dietary Data Collection system was developed for use in the survey by the University of Minnesota's Nutrition Coordinating Center.

The study population consisted of all adults (aged over 18) in the NHANES III (1988–1994) (*n* 19 598), excluding those with missing information on saccharin intake (*n* 2902) and those who did not consume saccharin (*n* 15 111). Participants who met the diagnostic criteria of diabetes and pre-diabetes were selected for our study (*n* 936)[21]: (1) glycated Hb ≥ 5·7 %; (2) fasting plasma glucose ≥ 100 mg/dl and (3) oral glucose tolerance test ≥ 140 mg/dl. Individuals with BMI ≥ 25 were selected as overweight population (*n* 1091) (the flow chart is shown in Fig. S1). The whole subject has been approved by the National Centre for Health Statistics Ethics Review Board.

Study procedure

Identify the endpoint of death from a specific disease and start the study. The risk of all-cause, cardiovascular and cancer mortality in diabetic and pre-diabetic populations with different levels of saccharin intake was compared by quartiles of dietary saccharin intake in the study populations. The population was from 1988 to 1994 with a median follow-up of 21 year.

Main outcome

The outcome was the final mortality status ascertained by the National Death Index. The National Death Index is a highly reliable resource for death identification. We use the National Centre for Health Statistics recoded codes to identify the type of death, which can be found at https://www.cdc.gov/nchs/data-linkage/mortality-public.htm. In our study, deaths were recorded up to 31 December 2019[22]. Finally, 625 deaths were recorded, including 223 deaths from CVD and 102 deaths from cancer.

Statistical analysis

All statistical analyses take into account a complex, multi-stage, stratified sampling design by using sample weight. Baseline characteristics were presented as mean (95 % CI) for continuous variables and percentage (95 % CI) for categorical variables. Univariate relationships among groups were assessed by general linear models adjusting for age and sex (for continuous variables) or χ^2 tests (for categorical variables). Cox proportional hazard models were used to evaluate the association between saccharin intake and CVD, cancer and all-cause mortality. Survival time was months between NHANES interview date and death or census date. The proportional hazards assumption of Cox models was tested using the Schoenfeld partial residuals method, and *P* values over 0·05 were considered to be consistent with the null hypothesis. Potential covariates are as follows: age (years), sex (man/women), race (non-Hispanic white/non-Hispanic black/Mexican American/other) (model 1); model 1 plus body mass index (BMI) (kg/m^2), family poverty income ratio (%), current smoking (yes/no), current drinking (yes/no), regular exercise (yes/no), family history of diabetes (yes/no), health status (excellent/very good/good/fair/poor), dietary consumption including energy (kcal/d), carbohydrate (% kcal), SFA (% kcal), MUFA (% kcal), PUFA (% kcal), animal protein (% kcal), vegetable protein (% kcal), fibre (g/d), alcohol (% kcal) and HEI score (model 2); model 2 plus fasting glucose (mg/dl), glycated Hg (%), serum cholesterol (mg/dl), serum triglycerides (mg/dl), serum HDL cholesterol (mg/dl), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), C-reactive protein (mg/dl), aspartate aminotransferase/ alanine aminotransferase (model 3). We further adjusted for another widely used NNS aspartame (mg/d) in a separate model (model 4).

Equivalent substitution model

An equivalent aspartame and total sugars substitution model were carried out to evaluate the alterations in the risk of CVD, cancer and all-cause mortality for the change of saccharin intake. A substitution analysis is mainly conducted by replacing 1-SD change with aspartame or total sugars and adjusting the total energy and other food intake constant.

Sensitivity analysis

We re-examined the association between saccharin intake and various mortality in diabetic and pre-diabetic population by sex (man/women), total sugar intake (stratification criteria: two strata according to median, > 75·35 g/d and < 75·35 g/d) and glycated Hb (stratification criteria: two strata according to median, > 5·9 % and < 5·9 %). The potential covariates used in the Cox model are described above.

All data were analysed using R (version 3.5.3), and *P* values were two-tailed at a significance level of $\alpha = 0{\cdot}05$.

Results

Baseline characteristics

Of the 19 598 NHANES III participants, 18 038 completed at least one dietary questionnaire about saccharin intake. Of these, only 1567 individuals had a non-zero intake of saccharin. Preliminary analysis revealed that 59·73 % of these participants were diabetic and pre-diabetic ($P < 0{\cdot}001$) (online Supplementary Table S1) and 69·62 % were overweight ($P < 0{\cdot}001$) (online Supplementary Table S2), which suggests that saccharin intake is concentrated among consumers with high risk factors. Therefore, our study focused on the diabetic and pre-diabetic population and overweight population to clarify the health effects of saccharin intake.

After screening protocol, a total of 936 diabetic and pre-diabetic participants were included in our study, with a median follow-up of 196·50 months. Table 1 shows the demographic and baseline lifestyle characteristics of participants with different levels of absolute saccharin intake. Those with higher absolute saccharin intake exhibited a higher proportion of family history of diabetes ($P = 0{\cdot}008$) and higher daily energy intake ($P = 0{\cdot}028$). In addition, online Supplementary Table S3 showed the baseline distribution of health-related risk factors of participants. Those with higher absolute saccharin intake had higher levels of TAG ($P = 0{\cdot}038$), systolic blood pressure ($P = 0{\cdot}032$) and a higher proportion of hypertriglyceridemia ($P = 0{\cdot}027$), but there were no significant differences in other variables.

In the second part of our study, 1091 overweight participants were included, with a median follow-up of 205·02 months. Table S4 and S5 described characteristics of the overweight population by absolute saccharin intake. Briefly, participants who derived a higher saccharin intake were more likely to be non-Hispanic white ($P = 0{\cdot}009$) and smokers ($P = 0{\cdot}009$). In addition, those with higher saccharin intake was had higher levels of C-peptide ($P = 0{\cdot}032$) and TAG ($P = 0{\cdot}001$), but lower levels of HDL ($P = 0{\cdot}037$).

Associations of saccharin intake with all-cause, CVD and cancer mortality

To investigate the potential long-term health effects of saccharin in diabetic and pre-diabetic population, we examined the association between absolute saccharin intake and specific disease mortality. As presented in Table 2, after multivariable adjustment for demographics, lifestyle characteristics, major CVD risk factors and NNS aspartame intake, increased absolute saccharin intake was associated with 41 % increased risk of all-cause mortality in model 4 (Q4 HR: 1·41, 95 % CI: 1·05, 1·90, $P_{trend} = 0{\cdot}025$). Additionally, higher absolute saccharin intake was associated with a significant increase in the risk of CVD mortality (raw model, Q4 HR: 1·70, 95 % CI: 1·00, 2·88, $P_{trend} = 0{\cdot}032$) compared with the lowest quartile. After multivariable adjustment, we found a similar effect in the risk of CVD mortality as in all-cause mortality, with the risk increasing by 93 % for CVD mortality (model 4, Q4 HR: 1·93, 95 % CI: 1·15, 3·25, $P_{trend} = 0{\cdot}010$). Although detailed cancer information was not available, our results for total cancer mortality showed that people in the highest quartile of absolute saccharin intake had an increased risk compared with those in the lowest quartile (raw model, Q4 HR: 2·39, 95 % CI: 1·15, 4·96, $P_{trend} = 0{\cdot}013$). Further adjustment remained robust, with only a modest attenuation in 13 %
(model 4, Q4 HR: 2·26, 95 % CI: 1·10, 4·45, $P_{trend} = 0{\cdot}013$).

It should be noticed that the USA Food and Drug Administration has established a body weight-based Acceptable daily intake (ADI) for saccharin of 15 mg per kilogram of body weight per day (mg/kg bw/d), which is considered safe for humans[23]. Therefore, we further investigated the relationship between relative saccharin intake (saccharin intake per kilogram of body weight per day) and mortality. Briefly, participants with higher baseline saccharin intake per weight had a greater risk of mortality due to CVD (raw model, Q4 HR: 1·824, 95 % CI: 1·000, 3·326, $P_{trend} = 0{\cdot}050$) and cancer (raw model, Q4 HR: 2·006, 95 % CI: 1·006, 3·998, $P_{trend} = 0{\cdot}048$) in raw model (Fig. S2). After adjusting for multiple variables, we still observed a significant association with saccharin intake per weight and all-cause mortality (Model 4, Q4 HR: 1·419, 95 % CI: 1·043, 1·932, $P_{trend} = 0{\cdot}027$). Further analysis within the same model showed that the risk of CVD mortality increased by 120·8 % (model 4, Q4 HR: 2·208, 95 % CI: 1·184, 4·117, $P_{trend} = 0{\cdot}014$). As for cancer mortality, the risk increased 100·3 % (model 4, Q4 HR: 2·003, 95 % CI: 1·041, 3·854, $P_{trend} = 0{\cdot}038$) (Fig. S2). In conclusion, our results suggested that both absolute and relative saccharin intakes increased risk of specific disease mortality, especially CVD mortality.

Among overweight population, higher absolute saccharin intake was associated with the risk of cancer mortality (model 4, Q4 HR: 7·369, 95 % CI: 2·122, 25·592, $P_{trend} < 0{\cdot}001$) compared with the lowest quartile after multivariable adjustment (Table S6). When the association between relative saccharin intake and mortality was further analysed, higher saccharin intake was also significantly associated with all-cause mortality (model 4, Q4 HR: 2·165, 95 % CI: 1·194, 3·928, $P_{trend} = 0{\cdot}022$) and cancer mortality (model 4, Q4 HR: 7·369, 95 % CI: 2·122, 25·592, $P_{trend} < 0{\cdot}001$) in model 4 (Table S7).

Equivalent substitution analysis

To examine whether total sugar and aspartame substitution for saccharin reduced the risk of mortality, we performed equivalent substitution analyses in diabetic and pre-diabetic population. In Fig. 1, the results showed the association between the intake of saccharin and specific disease mortality in two sets of substitution models: (1) replacing 1-SD change with aspartame

Table 1. Baseline demographic and lifestyle characteristics of the 936 people from the NHANES III according to the absolute intake of saccharin† (Weighted means, weighted percentages and 95 % confidence intervals)

	Quartiles of absolute saccharin intake (mg/d)								
	1(< 35·30)		2·(35·30–70·49)		3·(70·50–105·80)		4 (> 105·80)		
	Weighted means	95 % CI	Weighted means	95 % CI	Weighted means	95 % CI	Weighted means	95 % CI	*P*‡
All participants, No.	344		262		130		200		
Age at recruitment, years	57·81	55·06, 60·56	56·83	53·51, 60·15	57·31	53·26, 61·36	56·30	52·30, 60·30	0·916
BMI, kg/m^2	28·28	27·54, 29·03	29·56	28·62, 30·50	29·01	27·19, 30·83	29·35	27·97, 30·73	0·320
	Weighted percentages	95 % CI	Weighted percentages	95 % CI	Weighted percentages	95 % CI	Weighted percentages	95 % CI	
Women, no. (%)	59·6	49·9, 68·6	60·0	49·8, 69·4	56·1	42·0, 69·2	53·1	41·9, 64·1	0·790
Non-Hispanic White, no.%	80·6	75·3, 85·0	83·0	77·74, 87·3	86·8	79·9, 91·6	85·3	80·3, 89·2	0·790
Current smoking, no. (%)	17·5	12·0, 24·9	15·8	10·0, 24·1	20·2	11·2, 33·6	29·0	19·5, 40·8	0·254
Current alcohol drinking, no. (%)	35·5	27·5, 44·3	39·8	30·6, 49·8	38·7	26·2, 52·9	27·2	18·5, 38·1	0·554
Poverty Income Ratio, %	3·46	3·14, 3·77	3·40	3·02, 3·78	3·00	2·52, 3·47	3·19	2·75, 3·63	0·269
Regular exercise, no. (%)	77·5	10·6, 83·2	68·3	58·8, 76·5	68·4	54·0, 80·0	70·1	60·6, 78·1	0·288
Family history of diabetes, no. (%)	54·5	45·7, 63·0	54·2	43·1, 64·9	42·9	33·3, 53·0	60·4	51·1, 69·0	0·008*
	Weighted means	95 % CI	Weighted means	95 % CI	Weighted means	95 % CI	Weighted means	95 % CI	
Health status by physician's impression									0·222
Excellent	23·5	16·2, 32·8	20·4	12·7, 31·2	30·9	18·8, 46·5	20·0	11·2, 33·1	
Very good	26·2	19·7, 33·9	31·5	22·8, 41·8	15·0	9·1, 23·8	20·7	13·2, 31·1	
Good	32·8	25·3, 41·3	30·8	21·9, 41·4	35·0	23·1, 49·0	39·9	28·5, 52·5	
Fair	12·9	8·3, 19·4	14·9	9·2, 23·4	15·2	8·6, 25·4	12·2	6·9, 20·7	
Poor	2·5	1·0, 5·9	0·7	0·3, 2·2	1·5	0·4, 5·1	6·3	1·6, 21·1	
Dietary consumption									
Energy, kcal/d	1778·95	1671·99, 1885·91	1831·78	1718·33, 1945·23	2112·78	1819·51, 2406·05	1943·93	1800·72, 2087·14	0·028*
Carbohydrate, % of energy	46·38	43·84, 48·91	48·40	46·08, 50·71	48·33	45·97, 50·69	47·23	45·94, 48·51	0·403
SFA, % of energy	12·68	11·85, 13·51	11·74	10·80, 12·69	11·34	10·58, 12·09	11·62	10·87, 12·36	0·091
MUFA, % of energy	14·12	13·16, 15·08	13·82	12·69, 14·96	13·53	12·69, 14·37	14·57	13·78, 15·36	0·534
PUFA, % of energy	7·04	6·53, 7·55	7·31	6·72, 7·90	7·18	6·37, 7·98	7·46	7·05, 7·87	0·693
Animal protein, % of energy	11·75	10·97, 12·53	10·91	10·10, 11·73	11·00	9·87, 12·14	11·19	10·33, 12·06	0·426
Vegetable protein, % of energy	4·95	4·56, 5·34	4·99	4·67, 5·32	5·25	4·90, 5·61	5·09	4·78, 5·39	0·545
Total sugars, g/d	85·65	74·92, 96·38	94·27	86·58, 101·96	105·32	78·58, 132·06	84·38	74·98, 93·79	0·170
Fibre, g/d	16·80	15·48, 18·12	16·46	14·87, 18·06	18·55	15·75, 21·34	18·32	16·48, 20·16	0·383
Alcohol, % of energy	1·50	0·84, 2·15	1·43	0·61, 2·24	1·72	0·16, 3·28	1·08	0·41, 1·75	0·723
Healthy Eating Index (HEI)	63·78	61·03, 66·53	66·35	63·61, 69·08	66·91	63·89, 69·93	64·58	61·95, 67·21	0·280

† Values are weighted means (95 % CI) or weighted percentages (95 % CI).
‡ *P* value was assessed with General linear models adjusting for age and sex (continuous variables) or χ^2 test (bivariate relationships).
*$P < 0.05$.
All data analyses conducted in the table with sample weights provided by NHANES.

Table 2. Associations of absolute saccharin intake with all-cause, cardiovascular and cancer mortality in NHANES III† (Weighted hazard ratios and 95 % confidence intervals)

	Quartiles of absolute saccharin intake (mg/d)								
	1 (< 35·30)	2 (35·30–70·49)		3 (70·50–105·80)		4 (> 105·80)			Proportional hazard assumption $P^{§}$
		Weighted HR	95 % CI	Weighted HR	95 % CI	Weighted HR	95 % CI	P_{trend}‡	
All-cause mortality									0·981
Death/Person-months	227/64,503	170/49,416		87/25,406		141/34,894			
Unadjusted	1(ref)	1·01	0·65, 1·55	1·03	0·67, 1·56	1·30	0·86, 1·95	0·180	
Model 1	1(ref)	1·02	0·69, 1·50	0·94	0·65, 1·37	1·31	0·99, 1·73	0·066	
Model 2	1(ref)	1·09	0·72, 1·66	1·02	0·73, 1·48	1·40	1·06, 1·86	0·020*	
Model 3	1(ref)	0·86	0·70, 1·38	0·98	0·66, 1·45	1·41	1·05, 1·89	0·026*	
Model 4	1(ref)	0·98	0·70, 1·37	0·98	0·66, 1·46	1·41	1·05, 1·90	0·025*	
CVD mortality									0·211
Death/Person-months	80//64,503	52/49,416		29/25,406		62/34,894			
Unadjusted	1(ref)	0·77	0·41, 1·43	0·87	0·40, 1·92	1·70	1·00, 2·88	0·032*	
Model 1	1(ref)	0·78	0·41, 1·48	1·23	0·37, 1·79	1·71	1·16, 2·52	0·005*	
Model 2	1(ref)	0·85	0·45, 1·61	0·85	0·38, 1·91	1·87	1·24, 2·83	0·003*	
Model 3	1(ref)	0·87	0·46, 1·64	0·94	0·42, 2·11	1·97	1·19, 3·24	0·007*	
Model 4	1(ref)	0·89	0·48, 1·66	0·94	0·42, 2·10	1·93	1·15, 3·25	0·010*	
Cancer mortality									0·072
Death/Person-months	37/64,503	26/49,416		14/25,406		25/34,894			
Unadjusted	1(ref)	1·72	0·73, 4·09	1·55	0·80, 3·01	2·39	1·15, 4·96	0·013*	
Model 1	1(ref)	1·74	075, 4·02	1·47	0·75, 2·86	2·42	1·21, 4·86	0·007*	
Model 2	1(ref)	2·14	0·79, 5·77	1·67	0·75, 3·69	2·69	1·18, 6·12	0·013*	
Model 3	1(ref)	1·13	0·44, 2·89	1·26	0·58, 2·74	2·34	1·19, 4·58	0·006*	
Model 4	1(ref)	1·10	0·43, 2·86	1·27	0·57, 2·85	2·26	1·10, 4·45	0·013*	

Model 1: adjusted for age, sex and race. Model 2: further adjusted BMI, family poverty income ratio, current smoking, current drinking, regular exercise, family history of diabetes, health status and dietary consumption including energy (kcal/d), carbohydrate (%), SFA (%), MUFA (%), PUFA (%), animal protein (%), vegetable protein (%), fibre (g/d), alcohol (%) and HEI score. Model 3: further adjusted CVD risk factors including fasting glucose, HbA1c, serum cholesterol, Serum triglycerides, serum HDL cholesterol, systolic blood pressure, diastolic blood pressure, C-reactive protein and AST/ALT. Model 4: further adjusted another widely used NNS aspartame (mg/d).
† Values are *n* or weighted HR (95 % CI). CVD: cardiovascular disease.
‡ P_{trend} was calculated by median within each group.
§ Proportional hazard assumption P for saccharin intake was calculated by conducting Kaplen–Meier test.
*$P < 0.05$.

and (2) replacing 1-SD change with total sugars. When 1-sd changes of aspartame were used to replace 1-SD changes of absolute intake of saccharin, a significant reduction in all-cause mortality by 29·2 % (HR 0·708, 95 % CI 0·647, 0·774) and cancer mortality by 30·2 % (HR 0·698, 95 % CI 0·569, 0·855). Similarly, when relative intake of saccharin was there was a decrease in all-cause mortality by 11·8 % (HR 0·882, 95 % CI 0·828, 0·939) and cancer mortality by 29·0 % (HR 0·710, 95 % CI 0·578, 0·872). Furthermore, substituting 1-SD changes with total sugars for 1-SD changes of absolute intake of saccharin resulted in a reduction in all-cause mortality by 12·5 % (HR 0·875, 95 % CI 0·819, 0·936), CVD mortality by 49·7 % (HR 0·503, 95 % CI 0·420, 0·603). Replacing relative intake of saccharin led to a reduction in all-cause mortality by 29·1 % (HR 0·709, 95 % CI 0. 597, 0·842) and CVD mortality by 49·8 % (HR 0·502, 95 % CI 0·418, 0·602). To conclude, our results suggested that the use of saccharin to increase sweetness in order to avoid an excessive total intake of natural sugars is not desirable.

Sensitivity analysis

We conducted a further analysis by excluding participants who died prematurely to eliminate any confounding effects of premature death (< 3 years) in diabetic and pre-diabetic population. Overall, we found a slight attenuating effect of absolute saccharin intake on the risk of all-cause mortality (model 4, Q4 HR 1·41, 95 % CI 1·05, 1·90, $P_{trend} = 0·025$) and CVD mortality (model 4, Q4 HR 1·88, 95 % CI 1·06, 3·31, $P_{trend} = 0·026$). However, the risks for cancer mortality remained high (model 4, Q4 HR 2·65, 95 % CI 1·18, 5·92, $P_{trend} = 0·007$ for cancer mortality) (Table S8). In addition, the risk of relative saccharin intake on cancer mortality showed the similar result as previous analysis (Table S9).

After conducting further analysis, stratified by sex, the results showed that women but not man had a significant risk of all-cause mortality (Q4 HR 1·663, 95 % CI 1·144, 2·418, $P_{trend} = 0·019$) and CVD mortality (Q4 HR 2·455, 95 % CI 1·469, 4·102, $P_{trend} = 0·002$) with an increased intake of saccharin. Furthermore, when the results were divided into high and low groups by median total sugar intake, the higher total sugar intake group had a higher risk of CVD mortality with higher saccharin intake (Q4 HR: 2·540, 95 % CI: 1·021, 6·318, $P_{trend} = 0·046$). However, the group with low sugar intake surprisingly showed higher risk when consuming more saccharin for all-cause mortality (Q4 HR 1·420, 95 % CI 1·003, 2·011, $P_{trend} = 0·046$) and cancer mortality (Q4 HR 3·321, 95 % CI 1·318, 8·368, $P_{trend} = 0·012$). Given that poor glycaemic control is associated with higher mortality than in individuals with better glycaemic control, we additionally investigated whether saccharin could have an effect in both poorly and better glycemic control individuals. As expected, when the analysis was stratified by glycaemic control capacity, groups with higher serum glycated Hb had an increased risk of all-cause mortality and CVD mortality with an increased intake of saccharin (Q4 HR for

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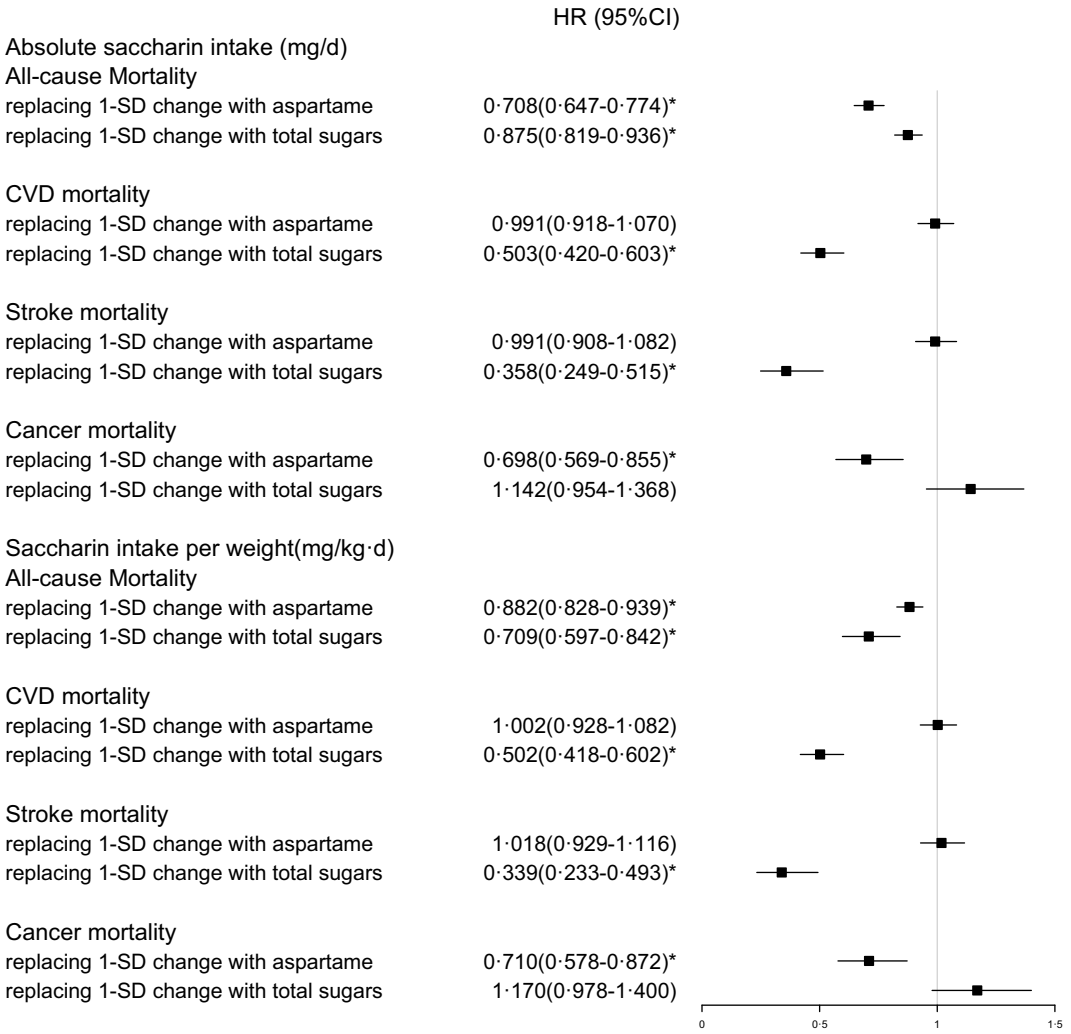

Fig. 1. Forest plot depicting the effect on mortality risk in diabetic and pre-diabetic population after replacing absolute and relative saccharin intake with aspartame and total sugar intake, respectively. The models were adjusted by using covariates in model 3.

all-cause mortality was 1·667, 95 % CI: 1·136, 2·446, $P_{trend} = 0·015$; for CVD mortality was 1·919, 95 % CI: 1·019, 3·616, $P_{trend} = 0·044$) (Fig. 2). The relative intake of saccharin also had a similar effect in these different subgroups (Fig. S3). Notably, there was no increase in mortality with saccharin consumption in people with better glycaemic control. Taken together, our sensitivity analysis suggested that women and diabetic or pre-diabetic population with poor glycaemic control should be aware of saccharin intake, as they are more susceptible to its risky effects on mortality.

Discussion

Our findings suggested that saccharin intake, both absolute and relative, had significant long-term health effects on all-cause, CVD and cancer mortality in the diabetic and pre-diabetic population. In addition, higher saccharin intake was also associated with the risk of cancer in overweight population. Notably, the relative daily intakes of saccharin in our study were all below the acceptable daily intake of 15 mg/kg according to the Food and Drug Administration[23], but these significant effects were still observed. Taken together, these findings suggested that saccharin intake was an important, largely overlooked, risk factor for death in the USA, particularly in diabetic and pre-diabetic population and overweight population.

Saccharin is rarely absorbed by the body and most of it is excreted in its original form in urine after ingesting[1]. Moreover, it has no energetic value after ingestion[24] thus is added to a wide range of foods as a substitute for dietary sugar[25]. However, there is little evidence that replacing dietary sugars or carbohydrates in drinks and foods with artificial sweeteners has any beneficial effect on appetite, energy balance or body weight[26,27]. The results of our substitution analysis suggest that saccharin is a more dangerous additive than natural sugars and aspartame. The use of saccharin to sweeten taste is undesirable to avoid excessive total natural sugar intake. In addition, since there is still a lack of sufficient data to determine the health effects of saccharin in humans, recommendations for its use by various health organisations and institutions are often inconclusive.

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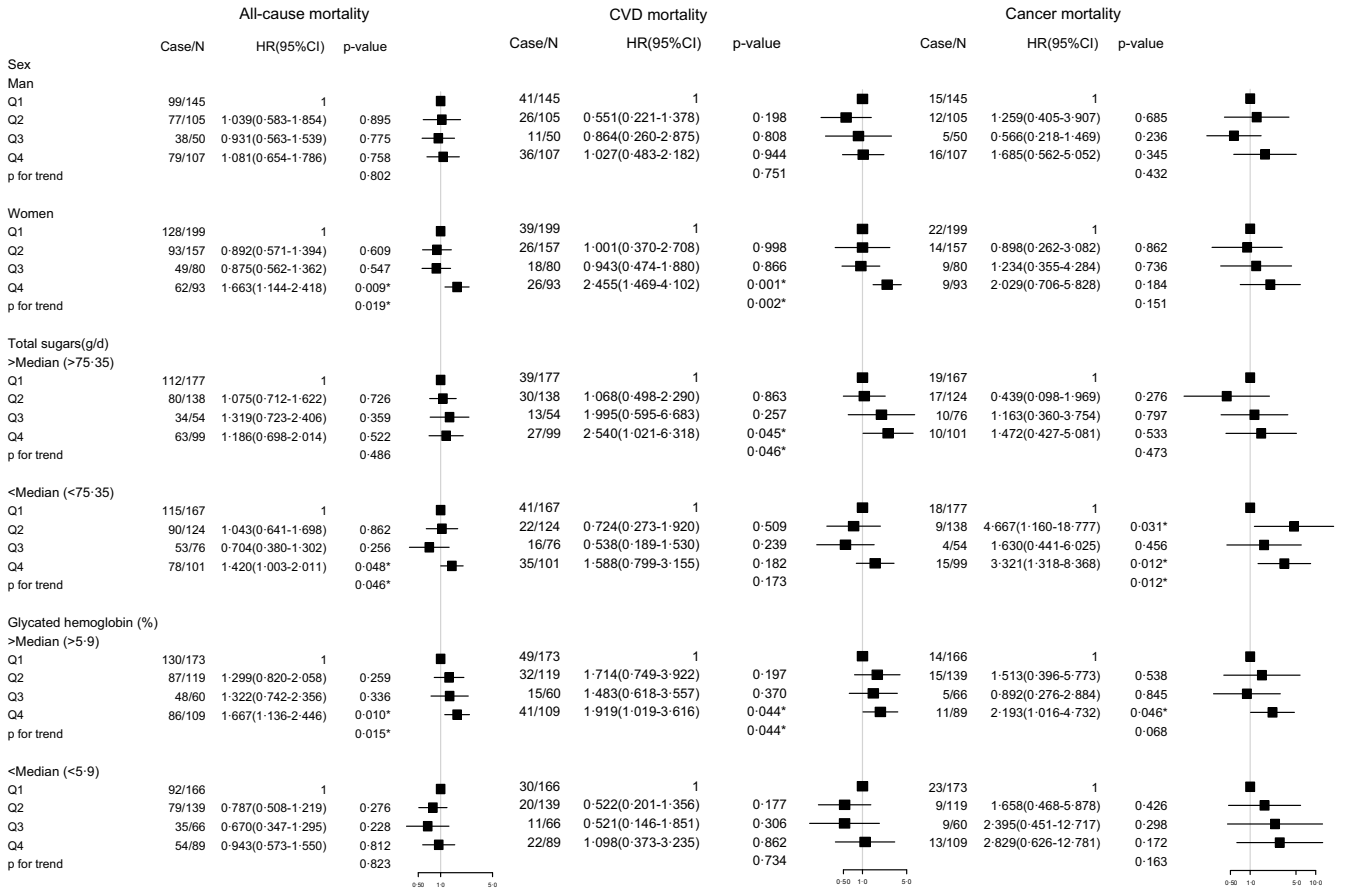

Fig. 2. Multivariable adjusted HR for association between absolute saccharin intake and all-cause, CVD and cancer mortality in diabetic and pre-diabetic population stratified by sex, total sugar intake and glycated Hb levels, respectively. The models were adjusted by using covariates in model 3.

Nowadays, with the increasing consumption of artificial sweeteners[28], saccharin intake is also accumulating in the body. Diabetic and pre-diabetic population, who need to control their total energy and sugar intake due to abnormal blood glucose, often satisfy their sweet taste needs by choosing more non-energetic sweeteners. Consistent with previous studies[29,30], our study did not find any beneficial effects on blood glucose and body weight with increasing saccharin intake in diabetic and pre-diabetic population. Furthermore, our study showed an increased risk for CVD mortality with increased saccharin intake, particularly in women. Although a prospective study showed that the intake of artificially sweetened beverages during pregnancy in women with prior gestational diabetes mellitus was not associated with cardiometabolic profiles[31]. Our results still suggested a need to raise awareness of the effects of prolonged saccharin consumption and adverse health effects. Moreover, our study reported a significant increase in the risk of CVD mortality with increasing saccharin intake in people with poor glycemic control. Many studies have found that sweeteners such as saccharin and aspartame could lead to the release of insulin or glucagon-like peptide 1 by activating the heterodimeric sweet taste receptor T1R2/T1R3[32]. These studies showed that excessive consumption of low-energy sweeteners could be a cause of insulin resistance and subsequent cardiovascular damage, as could dietary sugar. In addition, recent studies have shown that artificial sweeteners, long considered healthy and widely used, are not inert in the human body. They have a significant impact on the human gut microbiome to affect blood glucose levels[33]. Collectively, these findings suggest that artificial sweeteners may not be what we initially thought. Using them as a substitute for dietary sugar may lead to the same adverse outcomes, especially for people with blood glucose control.

The claim that artificial sweeteners may have a cancer risk actually stems from a 1978 study which found bladder cancer in experimental rats fed large amounts of saccharin[34]. However, the study later reiterated that this only happened in rats and that saccharin does not cause cancer in humans[35]. Another study using data from the NHANES (1988–2018) cohort also found no association between low-calorie sweetener use and total cancer risk in the whole population[36]. Unlike previous studies, our study found a significantly increased risk of total cancer mortality with saccharin intake in diabetic and pre-diabetic population. Furthermore, this risk increased particularly in those consuming low amounts of dietary sugar. Our study highlights the importance of focusing on the safety of saccharin consumption in diabetic patients, as this is the first report on the adverse effects of saccharin consumption on cancer mortality in diabetic and pre-diabetic population. There is growing epidemiological evidence that cancer deaths have replaced CVD deaths as the

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major cause of death in type 2 diabetes[37]. Type 2 diabetes has been found to be strongly associated with the development of many cancers, including liver, thyroid, colorectal, gallbladder, breast, endometrial, bladder and pancreatic cancers[38,39]. Potential biological mechanisms for this association include prolonged exposure of the body to hyperglycaemia, hyperinsulinaemia, insulin resistance and chronic inflammation[40,41]. Based on these findings, we suggest that the increased consumption of saccharin, which makes the organism more susceptible to exposure to these pathological conditions, may be responsible for the increase in cancer mortality in diabetic and pre-diabetic population. However, the detailed molecular mechanisms behind this association remained to be elucidated by future research.

It needs to be admitted that our study has several limitations. First, the number of individuals included in our study was relatively small. Second, the causal relationship explored in our study still needs further validation in animal or cellular experiments. Cox regression models may introduce a degree of bias in the analysis of this relationship when performed in a competing-risks scenario. Next, we acknowledge that dietary data on saccharin intake were derived from self-reported 24-h dietary recalls, which may have recall bias. Finally, this association could be further explored in any other populations to further clarify the long-term adverse effects of saccharin intake.

Conclusion

In conclusion, our study found a significant association between absolute and relative saccharin intake and the risk of mortality, particularly CVD mortality and cancer mortality, in diabetic and pre-diabetic population. Among overweight population, higher saccharin intake was also associated with the risk of cancer. Importantly, this association was observed when saccharin intakes below the Food and Drug Administration approved acceptable daily intake in human. Our study highlights the long-term risks of saccharin intake for hyperglycaemic and overweight consumers.

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Y.G., L.Y., Y.Z., X.L. and L.L. were involved in the conception, design and conduct of the study and the analysis and interpretation of the results. Y.G. and Y.Z. wrote the first draft of the manuscript, and all authors edited, reviewed and approved the final version of the manuscript. X.L. and L.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

All the authors declare no competing interests.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524002034

References

1. Byard JL & Goldberg L (1973) The metabolism of saccharin in laboratory animals. *Food Cosmet Toxicol* **11**, 391–402.
2. Bowman SA, Friday JE & Moshfegh A (2008) *MyPyramid Equivalents Database, 2.0, for USDA Survey Foods, 2003–2004*. Beltsville, MD: Food Surveys Research Group, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture.
3. Ionescu E, Rohner-Jeanrenaud F, Proietto J, *et al.* (1988) Taste-induced changes in plasma insulin and glucose turnover in lean and genetically obese rats. *Diabetes* **37**, 773–779.
4. Suez J, Korem T, Zeevi D, *et al.* (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **514**, 181–186.
5. Whitehouse CR, Boullata J & McCauley LA (2008) The potential toxicity of artificial sweeteners. *AAOHN J* **56**, 251–259.
6. Cooper PL, Wahlqvist ML & Simpson RW (2010) Sucrose *v.* saccharin as an added sweetener in non-insulin-dependent diabetes: short- and medium-term metabolic effects. *Diabetic Med* **5**, 676–680.
7. Serrano J, Smith KR, Crouch AL, *et al.* (2021) High-dose saccharin supplementation does not induce gut microbiota changes or glucose intolerance in healthy humans and mice. *Microbiome* **9**, 11.
8. Horwitz DL, McLane M & Kobe P (1988) Response to single dose of aspartame or saccharin by NIDDM patients. *Diabetes Care* **11**, 230–234.
9. Whysner J & Williams GM (1996) Saccharin mechanistic data and risk assessment: urine composition, enhanced cell proliferation, and tumor promotion. *Pharmacol Ther* **71**, 225–252.
10. Arnold DL (1984) Toxicology of saccharin. *Fundam Appl Toxicol Offic J Soc Toxicol* **4**, 674–685.
11. Wang Y, Xu Z-L, Xie Y-Y, *et al.* (2011) Development of polyclonal antibody-based indirect competitive enzyme-linked immunosorbent assay for sodium saccharin residue in food samples. *Food Chem* **126**, 815–820.
12. IDA (2019) IDF Diabetes Atlas. Available at https://www.diabetesatlas.org/en/ (accessed December 2021).
13. Tabák AG, Herder C, Rathmann W, *et al.* (2012) Prediabetes: a high-risk state for diabetes development. *Lancet* **379**, 2279–2290.
14. Palmer JR, Boggs DA, Krishnan S, *et al.* (2008) Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. *Arch Intern Med* **168**, 1487–1492.
15. Malik VS, Popkin BM, Bray GA, *et al.* (2010) Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* **121**, 1356–1364.
16. Vos MB & Lavine JE (2013) Dietary fructose in nonalcoholic fatty liver disease. *Hepatology* **57**, 2525–2531.
17. Malik VS, Pan A, Willett WC, *et al.* (2013) Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *Am J Clin Nutr* **98**, 1084–1102.
18. U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015) 2015 – 2020 Dietary

British Journal of Nutrition

Guidelines for Americans. 8th Edition. https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015 (accessed March 2022).
19. World Health Organization (2015) Guidelines for Sugar Intake in Adults and Children 2015. https://www.who.int/news/item/04–03–2015-who-calls-on-countries-to-reduce-sugars-intake-among-adults-and-children (accessed March 2022).
20. Centers for Disease Control and Prevention (CDC) & National Center for Health Statistics (NCHS) (1997) National Health and Nutrition Examination Survey NHANES Questionnaires, Datasets, and Related Documentation. https://wwwn.cdc.gov/nchs/nhanes/Default.aspx (accessed May 2022).
21. American Diabetes Association (2013) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **36**(Suppl 1), S67–S74.
22. National Center for Health Statistics (2019) *Public Use Linked Mortality File*. Atlanta, GA: Centers for Disease Control and Prevention. https://ftpcdcgov/pub/Health_Statistics/NCHS/datalinkage/linked_mortality/ (accessed July 2021).
23. Administration FaD (2023) Aspartame and Other Sweeteners in Food. https://www.fda.gov/food/food-additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-united-states (accessed July 2023).
24. Agullo V, Dominguez-Perles R, Moreno DA, *et al.* (2020) Alternative sweeteners modify the urinary excretion of flavanones metabolites ingested through a new maqui-berry beverage. *Foods* **9**, 41.
25. Huentupil Y, Pena L, Novoa N, *et al.* (2019) New sulfonamides containing organometallic-acylhydrazones: synthesis, characterisation and biological evaluation as inhibitors of human carbonic anhydrases. *J Enzyme Inhib Med Chem* **34**, 451–458.
26. Mazarati A, Siddarth P, Baldwin RA, *et al.* (2008) Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. *Brain* **131**, 2071–2083.
27. 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**, 1288–1301.
28. Malek AM, Hunt KJ, DellaValle DM, *et al.* (2018) Reported consumption of low-calorie sweetener in foods, beverages, and food and beverage additions by US Adults: NHANES 2007–2012. *Curr Dev Nutr* **2**, nzy054.
29. Hasan HM, Alkass SY & de Oliveira DSP (2023) Impact of long-term cyclamate and saccharin consumption on biochemical parameters in healthy individuals and type 2 diabetes mellitus patients. *Medicina (Kaunas)* **59**, 698.
30. Bayindir Gumus A, Keser A, Tuncer E, *et al.* (2022) Effect of saccharin, a non-nutritive sweeteners, on insulin and blood glucose levels in healthy young men: a crossover trial. *Diabetes Metab Syndr* **16**, 102500.
31. Hinkle SN, Rawal S, Bjerregaard AA, *et al.* (2019) A prospective study of artificially sweetened beverage intake and cardiometabolic health among women at high risk. *Am J Clin Nutr* **110**, 221–232.
32. Jang HJ, Kokrashvili Z, Theodorakis MJ, *et al.* (2007) Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A* **104**, 15069–15074.
33. Suez J, Cohen Y, Valdes-Mas R, *et al.* (2022) Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell* **185**, 3307–3328. e19.
34. Saccharin and Bladder Cancer. (1980) *Lancet (London, England)* **1**, 855–856.
35. Weihrauch MR & Diehl V (2004) Artificial sweeteners–do they bear a carcinogenic risk? *Ann Oncol* **15**, 1460–1465.
36. Fulgoni VL 3rd & Drewnowski A (2022) No association between Low-Calorie Sweetener (LCS) use and overall cancer risk in the nationally representative database in the US: analyses of NHANES 1988–2018 Data and 2019 public-use linked mortality files. *Nutrients* **14**, 4957.
37. Pearson-Stuttard J, Bennett J, Cheng YJ, *et al.* (2021) Trends in predominant causes of death in individuals with and without diabetes in England from 2001 to 2018: an epidemiological analysis of linked primary care records. *Lancet Diabetes Endocrinol* **9**, 165–173.
38. Ling S, Brown K, Miksza JK, *et al.* (2020) Association of type 2 diabetes with cancer: a meta-analysis with bias analysis for unmeasured confounding in 151 cohorts comprising 32 million people. *Diabetes Care* **43**, 2313–2322.
39. Pearson-Stuttard J, Papadimitriou N, Markozannes G, *et al.* (2021) Type 2 diabetes and cancer: an umbrella review of observational and Mendelian randomization studies. *Cancer Epidemiol Biomarkers Prev* **30**, 1218–1228.
40. Giovannucci E, Harlan DM, Archer MC, *et al.* (2010) Diabetes and cancer. *Diabetes Care* **33**, 1674–1685.
41. Lega IC & Lipscombe LL (2020) Review: diabetes, obesity, and cancer-pathophysiology and clinical implications. *Endocr Rev* **41**, 33–52.

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