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Diet soft drink is associated with increased odds of proliferative diabetic retinopathy

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ABSTRACT

Importance: While consumption of soft drink may increase risk of cardiovascular disease, the relationship between soft drink consumption and diabetes complications is unknown.

Background: To explore the association between regular and diet soft drink consumption, and diabetic retinopathy (DR) and diabetic macular edema (DME).

Design: Clinical, cross-sectional study

Participants: Adult patients with diabetes recruited from a tertiary eye hospital (Melbourne, Australia) answered a Food Frequency Questionnaire.

Methods: None, moderate and high soft drink consumption was defined as <1, 1-4 and >4 cans/bottles (375ml) per week, respectively. Due to missing data, data were imputed using the multiple imputation chained equations procedure. Multivariable logistic regression models determined the associations between soft drink consumption, and presence and severity of DR/DME.

Main outcome measures: Presence and severity of DR/DME.

Results: Of the 609 participants (mean age±standard deviation 64.6±11.6 years; males=210), 285 (46.8%) and 190 (31.2%) consumed diet and regular soft drink, respectively. A total of 230 (37.8%), 36 (5.9%), 154 (25.3%), 28 (4.6%), and 146 (24.0%) had no DR, mild NPDR, moderate NPDR, severe NPDR and PDR, respectively. High diet soft drink consumption was independently associated with increased likelihood of having PDR (OR=2.51, 95%CI [confidence interval]=1.05-5.98), compared to no consumption. In contrast, regular soft drink was not associated with DR or DME.

Conclusions and relevance: Consuming >4 cans (1.5 litres)/week of diet soft drink is associated with a more than twofold risk of having PDR in patients with diabetes.

Longitudinal studies are needed to further elucidate the association and its underpinning mechanisms.

Keywords: Diet, Diabetic retinopathy, Soft drink, Nutrition, Risk factors

INTRODUCTION

The consumption of sugar sweetened beverages (soft drinks) has long been associated with increased risk of diabetes,[1, 2] other cardiovascular risk factors, and outcomes, such as generalized and abdominal obesity, hypertension, hyperlipidemia, metabolic syndrome,[3] and stroke[4, 5] associated with the high-fructose corn syrup content, lack of nutrients and a tendency to mask satiety.[6, 7] For example, in the Nurses Health Study II, a large prospective cohort study conducted in the US, women consuming one or more sugar-sweetened soft drinks per day had an almost twofold relative risk of developing type 2 diabetes compared to occasional consumers.[1] Soft drink consumption has also been shown to increase HbA1c and lipid levels in those with diabetes.[8, 9]

To address the health consequences associated with regular soft drink consumption, artificially sweetened "diet" soft drinks have been marketed as a healthier alternative due to their lack of sugar. However, several studies including a recent systematic review and meta-analysis,[10] have linked diet soft drinks with poor cardiovascular outcomes.[3, 11-16] The population-based Multi-Ethnic Study of Atherosclerosis (MESA), for example, found that a daily consumption of diet soda was associated with 36% and 67% greater relative risks of incident metabolic syndrome and type 2 diabetes mellitus, respectively, compared with non-consumption.[12] Although plausible biological mechanisms explaining these associations are limited, it is possible that artificial sweeteners in diet soft drink increase the need for sweet, energy-dense foods or beverages[17] and disrupt people's ability to accurately estimate energy intake and energy needs,[18] both of which may lead to increased consumption of calories.[12]

It is less clear, however, how soft drink consumption influences the risk of microvascular complications in patients with diabetes, such as diabetic retinopathy (DR), a leading cause of vision impairment blindness worldwide.[19, 20] Such information

would add to the limited knowledge base on dietary intake and DR and may contribute to evidence-based dietary recommendations to assist patients and clinicians in better managing DR. Therefore, we explored the association between regular and diet soft drink consumption and severity of DR in a well-defined clinical sample of Australian adults with type 1 and type 2 diabetes mellitus. We hypothesize that both regular and diet soft drink consumption is associated with increased risk of DR, particularly severe DR.

METHODS

This study included participants from the Diabetes Management Project (DMP),[21] a cross-sectional clinical study of 609 English-speaking adults (≥18 years) with type 1 or type 2 diabetes mellitus. Participants were excluded if they had significant hearing and/or cognitive impairment (determined using the 6-item cognitive impairment test[22]), or were living residential care, nursing homes or other assisted living environments. The DMP was conducted in Melbourne, Australia between 2009 and 2010[21, 23] at the Centre for Eye Research Australia (CERA) which is located at the Royal Victorian Eye and Ear Hospital (RVEEH). Questionnaires on socio-demographic, health-related, lifestyle, psychosocial and behavioural factors were interviewer-administered to participants following provision of written informed consent. Ethical approval for the study was provided by the Royal Victorian Eye and Ear Hospital (RVEEH) Human Research and Ethics Committee (08/815H) and all study procedures adhered to the tenets of the Declaration of Helsinki.

Soft drink consumption

Soft drink consumption was assessed using a 145-item food frequency questionnaire (FFQ). The validity reliability and reproducibility of the FFQ has been shown using

correlations between nutrient data and 3, four-day weighed food records spaced evenly over one year.[24] As part of the FFQ, participants were asked how often in the past 12 months they had drunk one 375ml can (or equivalent) of Coke, Pepsi or other Cola and other soft drinks (e.g. lemonade), and how often they had consumed low caloric cola (e.g. Diet Coke) and other low caloric soft drinks (e.g. Diet lemonade).

Frequency of soft drink consumption was categorized in the FFQ as follows: 1) Never; 2) Less than 1 per month; 3) 1-3 per month; 4) 1 per week; 5) 2-4 per week; 6) 5-6 per week; 7) 1 per day; 8) 2-3 per day; 9) 4+ per day. To standardize our data, we converted all frequency categories to units consumed per week. We then added participants' results for 'Coke, Pepsi or other Cola' and 'other soft drinks' to calculate a total amount of regular soft drink consumed, and added 'low caloric cola' and 'other low caloric soft drinks' together to calculate a total amount of diet soft drink consumed. We then defined regular and diet soft drink consumption as: No (<1 can per week), moderate (1-4 cans per week) and high (>4 cans per week). This definition was based both on the distribution of the data and consultation with a dietician on the clinical relevance of the categories. Because the number of people consuming high levels of regular soft drink was very low (n=6), the moderate and high levels were subsequently combined for analyses and renamed 'any consumption'.

Assessment of DR and DME

Our procedures for DR and DME assessment and grading have been described in detail previously.[21, 23] For the current study, we categorized DR severity as no DR (Early Treatment of Diabetic Retinopathy Study level 10-15), mild non-proliferative DR [NPDR] (level 20), moderate NPDR (level 31-43), severe NPDR (level 53-60), and proliferative DR [PDR] (level 61-80) using the eye with the most severe grading. The severity of DME was classified using the American Academy of Ophthalmology scale[25] as no, mild, moderate, and severe DME. Presence of DR and DME (any DR/any DME) and severity

of DR (none, mild NPDR, moderate NPDR, severe NPDR and PDR) and severity of DME (mild, moderate and severe DME) were the four main outcome variables.

Blood collection and blood pressure (BP) measurements

We assessed glycosylated haemoglobin (HbA_{1c}) levels, fasting glucose and lipids (total cholesterol [TC], triglyceride [TG], low-density lipoproteins [LDL] and high-density lipoproteins [HDL]) using a fasting blood sample analysed at the Melbourne Pathology Laboratory, Australia. BP was assessed using an automated BP machine. The average of two separate measurements was recorded for systolic (SBP) and diastolic (DBP). If there was a difference of at least 10mmHg for SBP or 5mmHg for DBP, a third measurement was taken and the closest two BP measurements were averaged.

Height, weight and energy intake

Participants' height and weight were measured using a wall-mounted adjustable measuring scale and a digital scientific weight scale (calibrated daily), respectively. Using the formula weight (kg) divided by height in meters squared (kg/m^2), body mass index (BMI) was calculated. Total energy intake (kcal/day) was calculated using nutrient data from the NUTTAB 2010 electronic nutrient database (Australia).[26]

Statistical Analysis

Statistical analyses were performed using Stata version 14.2 (StataCorp, College Station, TX). We used mean and standard deviation (SD) for normally distributed continuous data, median and inter-quartile range for skewed data, and counts and percentages for categorical data. Key covariables included age (years), gender, education (primary school or below/secondary school/≥14 years), annual income (<AUD$30,000/≥AUD$30,000), smoking status (non-smoker/current or past smoker), insulin use (yes/no), change in eating habit in the last five years (yes/no; determined using the following item extracted from the FFQ "In the last 5 years, have you changed your eating habits in any way "), consumption of alcohol (yes/no), use of hypertensive

medication (yes/no), hyperlipidemia (yes/no), presence of at least one comorbidity (angina, heart attack, irregular heartbeat, stroke, asthma, anaemia, migraine, arthritis or osteoporosis: yes/no), diabetes type, presence of at least one other self-reported diabetes complication (renal, peripheral vascular disease, neuropathy: yes/no), birth country (Australian/other), total energy intake (kcal/day), BMI (kg/m^2), duration of diabetes (years), SBP and DBP (mmHg), HbA1c (%), fasting glucose (mmol/L), TC (mmol/L), LDL (mmol/L); HDL (mmol/L), and TG (mmol/L).

Due to the high number of missing cases for diet soft drink consumption and several other covariates (n=285), we performed multiple imputation using the method of chained equations (MICE), which allowed for a separate conditional distribution for each variable.[27-29] We built the imputation model using the above-mentioned covariables, the outcomes DR and DME severity, and the consumption frequency of various alcohol types (auxiliary variables relating to diet soft drink consumption), assuming data was missing at random (MAR) conditional on these variables. No interactions were found important and included. Missing soft drink consumption frequencies were imputed at their original FFQ categories using an ordinal logistic regression model. The other binary and continuous variables were imputed using logistic regression and predictive mean matching, respectively. We compared the distribution of variables between observed and imputed data, and visually examined convergence in trace plots of model coefficients to check that the imputation performed satisfactorily. A conservative number of 50 imputed datasets were generated based on the fraction of missing information (FMI) estimated for diet soft drink consumption, to ensure that primary effects were estimated with adequate precision. A burn-in of 70 iterations was used per chain as we observed stationarity from around the 50th cycle.

Having imputed all variables with missing data, multivariable logistic regression analysis was then used, incorporating data from all participants in the analysis, to examine the

relationship between regular and diet soft drink consumption and any DR, adjusted for age, gender, all covariables that were significant in univariable analysis and others that were regarded as clinically important or were potential confounders of the relationship. To determine the association between regular and diet soft drink consumption and severity of DR, we used multinomial logistic regression which yielded estimates of ORs for each severity level of DR as compared to no DR. Finally, for diet soft drink, we further conducted a trend analysis by treating the three consumption categories as equally-spaced, ordinal values, i.e. 0 cans/week = 0, ≤4 cans/week = 1 and >4 cans/week = 2, to assess whether there was a pattern of increasing risk of DR with each category of diet soft drink consumption. We only examined regular soft drink consumption as a binary variable as consumption frequency was too low and led to very large standard errors when divided into moderate and high consumption categories, even after imputation. Associations were considered statistically significant if $p<0.05$.

RESULTS

A total of 609 people with type 1 (n=73, 12.5%), type 2 (n=510, 87.5%) or unknown type (n=26, 4.3%) diabetes mellitus participated in this study (mean age±SD [standard deviation] 64.6±11.6 years old; 210 were male; **Table 1**). A total of 230 (37.8%), 36 (5.9%), 154 (25.3%), 28 (4.6%), 146 (24.0%) and 15 (2.5%) had no DR, mild NPDR, moderate NPDR, severe NPDR, PDR and unknown DR severity, respectively. Of those who consumed regular soft drink (n=190, 31.2%), 176 (28.9%) and 14 (2.3%) were moderate and high consumers, respectively. Two hundred and eighty-five (46.8%) people consumed diet soft drink, comprising 195 (32.0%) moderate consumers and 90 (14.8%) high consumers. 127 (20.9%) and 129 (21.2%) people did not provide any information on diet and regular soft drinks consumption, respectively. Those who

consumed regular soft drink were more likely to have lower SBP compared to non-consumers ($p<0.05$). Those who consumed diet soft drink were more likely to be younger, born in Australia, use insulin, have a secondary education (vs. primary or tertiary), higher BMI, type 1 diabetes mellitus, DR, and lower HDL compared to non-consumers (all $p<0.05$, **Table 1)**.

Table 1: Sociodemographic and clinical characteristics by diet soft drinks consumption (n=482*)

Characteristic	**Non-consumers (n=197)**		**Diet soft drink consumers (n=285)**		
Categorical variables	**n**	**%**	**n**	**%**	**p-value**
Gender					
Male	120	60.9	191	67.0	0.169
Female	77	39.1	94	33.0	
Smoking status					
Non-smoker	91	46.2	122	42.8	0.396
Current/past smoker	105	53.3	158	55.4	
Unknown	1	0.5	5	1.8	
Income					
<AUD$30,000	131	66.5	170	59.6	0.290
≥AUD$30,000	50	25.4	90	31.6	
Unknown	16	8.1	25	8.8	
Education					
Primary or below	34	17.3	28	9.8	**<0.001**
Secondary	93	47.2	179	62.8	
≥14 years	69	35.0	69	24.2	
Unknown	1	0.5	9	3.2	
Country of birth					
Australia	72	36.5	134	47.0	**0.048**
Others	125	63.5	150	52.6	
Unknown	0	0.0	1	0.4	
Insulin use					
No	129	65.5	156	54.7	**0.038**
Yes	68	34.5	127	44.6	
Unknown	0	0.0	2	0.7	
Hyperlipidemia†					
No	52	26.4	75	26.3	0.984

Yes	145	73.6	210	73.7	
Lipid-lowering medication					
No	141	71.6	195	68.4	0.734
Yes	47	23.9	77	27.0	
Unknown	9	4.6	13	4.6	
Hypertension					
No	38	19.3	53	18.6	0.848
Yes	159	80.7	232	81.4	
Hypertension medication					
No	126	64.0	178	62.5	0.940
Yes	62	31.5	94	33.0	
Unknown	9	4.6	13	4.6	
At least one diabetes complication‡					
No	134	68.0	190	66.7	0.756
Yes	63	32.0	95	33.3	
At least one comorbidity§					
No	53	26.9	80	28.1	0.931
Yes	143	72.6	204	71.6	
Unknown	1	0.5	1	0.4	
DR severity					
No DR	89	45.2	98	34.4	**<0.001**
Mild NPDR	14	7.1	13	4.6	
Moderate NPDR	48	24.4	75	26.3	
Severe DR	10	5.1	11	3.9	
PDR	29	14.7	86	30.2	
Unknown	7	3.6	2	0.7	
DME severity					
No DME	131	66.5	164	57.5	0.243
Mild DME	16	8.1	36	12.6	
Moderate DME	14	7.1	25	8.8	
Severe DME	18	9.1	36	12.6	
Unknown	18	9.1	24	8.4	
Diet soft drink consumption					
No (0 cans/week)	197	100.0	0	0.0	**<0.001**
Moderate (≤4 cans/week)	0	0.0	195	68.4	
High (>4 cans per week)	0	0.0	90	31.6	

Regular soft drink consumption						
No (0 cans/week)	125	63.5	165	57.9	0.209	
Moderate (≤4 cans/week)	67	34.0	108	37.9		
High (>4 cans per week)	5	2.5	7	2.5		
Unknown	0	0.0	5	1.8		
Change in dietary habit in last 5 years						
No	91	46.2	111	38.9	0.087	
Yes	97	49.2	167	58.6		
Unknown	9	4.6	7	2.5		
Consumes alcohol						
No	94	47.7	144	50.5	0.792	
Yes	102	51.8	139	48.8		
Unknown	1	0.5	2	0.7		
Diabetes type						
					0.020	
Type 1	18	9.1	42	14.7		
Type 2	166	84.3	236	82.8		
Unknown	13	6.6	7	2.5		
Continuous variables	**Mean /median**	**SD /IQR**	**Mean /median**	**SD /IQR**	**p-value**	**Number missing**
Age (years)	69.0	16.0	63.0	15.0	**<0.001**	0
Systolic blood pressure (mm Hg)	141.0	19.3	138.7	18.2	0.186	10
Duration of diabetes (years)	12.0	13.5	15.0	13.6	0.393	11
Body mass index (kg/m2)	29.1	8.4	30.2	7.5	**0.008**	22
HbA1c (%)	7.3	1.4	7.6	1.9	0.089	17
Fasting glucose (mmol/L)	7.6	3.6	7.7	3.6	0.295	28
Total cholesterol (mmol/L)	4.5	1.5	4.4	1.6	0.362	25
HDL cholesterol (mmol/L)	1.4	0.6	1.3	0.5	**0.026**	30
LDL cholesterol (mmol/L)	2.3	1.3	2.2	1.2	0.519	36
Triglycerides (mmol/L)	1.5	1.3	1.5	1.1	0.680	26
Total energy intake (kcal/day)	1717.0	884.3	1782.0	878.6	0.648	49

*Only individuals with data on diet soft drinks were included for this comparison (127 had missing data)

†Defined as total cholesterol ≥6.2 or lipid medication use and/or self-reported hyperlipidemia.

‡Includes: nephropathy, peripheral vascular disease, neuropathy

§Includes angina, heart attack, irregular heartbeat, stroke, asthma, anaemia, migraine, arthritis or osteoporosis

AUD=Australian dollars; DR=Diabetic retinopathy; HbA1c=Haemoglobin A1c; IQR=Interquartile range; SD=Standard Deviation.

Bolded values indicate significant results

In multivariable models adjusted for age, gender, HbA1c, SBP, diabetes duration, insulin use, presence of at least one other diabetes complication, diabetes type, BMI, education, use of anti-hypertensive medication, hyperlipidemia, presence of a comorbidity, smoking, alcohol consumption, total energy intake, and regular soft drink consumption, diet soft drink consumption was not associated with increased odds for any DR compared to non-consumers (**Table 2**). There was also no association between any level of diet soft drink consumption and mild, moderate or severe NPDR. However, compared to no consumption, high diet soft drink consumption was significantly associated with increased odds of having PDR (OR=2.62, 95% CI=1.14-6.06, p=0.024; **Table 2**). Similarly, compared to no consumption, moderate diet soft drink consumption was associated with increased odds of having PDR, although the result was of borderline significance (OR=1.92, 95% CI=0.98-3.76, p=0.058; **Table 2**). The plot in **Figure 1** displays all significant risk and protective factors for the association between diet soft drink consumption and PDR in the multivariable regression model.

In contrast, diet soft drink consumption was not significantly associated with presence or severity of DME (**Supplementary Table 1**). Similarly, regular soft drink consumption was not significantly associated with presence or severity of DR (**Supplementary Table 2**), or presence or severity of DME (**Supplementary table 3**) in the adjusted models.

Table 2: Multivariable association between diet soft drink consumption and presence and severity of diabetic retinopathy

DR severity	*Diet soft drink consumption* n=473*	**No. (%) of DR cases**	**Age-/gender-adjusted OR (95% CI)**	**p**	**Fully adjusted† OR (95% CI)**	**p**
Any DR	No (0 cans/week), n=190	101 (53.2)	1.00		1.00	
	Moderate (≤4 cans/week), n=193	120 (62.2)	1.23 (0.81 to 1.85)	0.328	1.28 (0.77 to 2.13)	0.344
	High (>4 cans/week), n=90	65 (72.2)	1.58 (0.93 to 2.71)	0.093	1.44 (0.71 to 2.90)	0.311
	Ordinal (p-trend)		1.25 (0.97 to 1.62)	0.086	1.21 (0.86 to 1.70)	0.265
Mild NPDR	No (0 cans/week), n=190	14 (7.4)	1.00		1.00	
	Moderate (≤4 cans/week), n=193	12 (6.2)	1.01 (0.45 to 2.29)	0.979	1.07 (0.43 to 2.62)	0.889
	High (>4 cans/week), n=90	1 (1.1)	0.45 (0.10 to 2.11)	0.310	0.45 (0.08 to 2.38)	0.344
	Ordinal (p-trend)		0.79 (0.45 to 1.37)	0.395	0.79 (0.42 to 1.49)	0.474
Moderate NPDR	No (0 cans/week), n=190	48 (25.3)	1.00		1.00	
	Moderate (≤4 cans/week), n=193	52 (26.9)	1.16 (0.70 to 1.92)	0.563	1.20 (0.67 to 2.15)	0.544
	High (>4 cans/week),	23 (25.6)	1.29 (0.66 to	0.464	1.28 (0.57	0.558

	n=90		2.52)		to 2.88)	
	Ordinal (p-trend)		1.14 (0.83 to 1.56)	0.426	1.14 (0.78 to 1.69)	0.495
Severe NPDR	No (0 cans/week), n=190	10 (5.3)	1.00		1.00	
	Moderate (≤4 cans/week), n=193	5 (2.6)	0.52 (0.17 to 1.55)	0.239	0.67 (0.21 to 2.15)	0.498
	High (>4 cans/week), n=90	6 (6.7)	0.97 (0.31 to 3.03)	0.961	1.20 (0.32 to 4.44)	0.787
	Ordinal (p-trend)		0.92 (0.50 to 1.71)	0.800	1.05 (0.53 to 2.09)	0.878
PDR	No (0 cans/week), n=190	29 (15.3)	1.00		1.00	
	Moderate (≤4 cans/week), n=193	51 (26.4)	1.69 (0.97 to 2.95)	0.064	1.92 (0.98 to 3.76)	0.058
	High (>4 cans/week), n=90	35 (38.9)	2.75 (1.43 to 5.29)	**0.002**	2.62 (1.14 to 6.06)	**0.024**
	Ordinal (p-trend)		1.66 (1.20 to 2.29)	**0.002**	1.64 (1.09 to 2.47)	**0.018**

*Out of 609 study participants, only 482 individuals provided information on diet soft drink consumption and DR severity was further present for 473 individuals

All variables with missing observations were imputed so the analysis included data from all participants (n=609)

†Adjusted for age, gender, HbA1c, systolic BP, diabetes duration, insulin use, presence of at least one other diabetes complication, diabetes type, BMI, education, use of anti-hypertensive medication, hyperlipidemia, presence of a comorbidity, smoking, alcohol consumption, total energy intake, and regular soft drink consumption

CI=Confidence interval; OR=Odds ratio

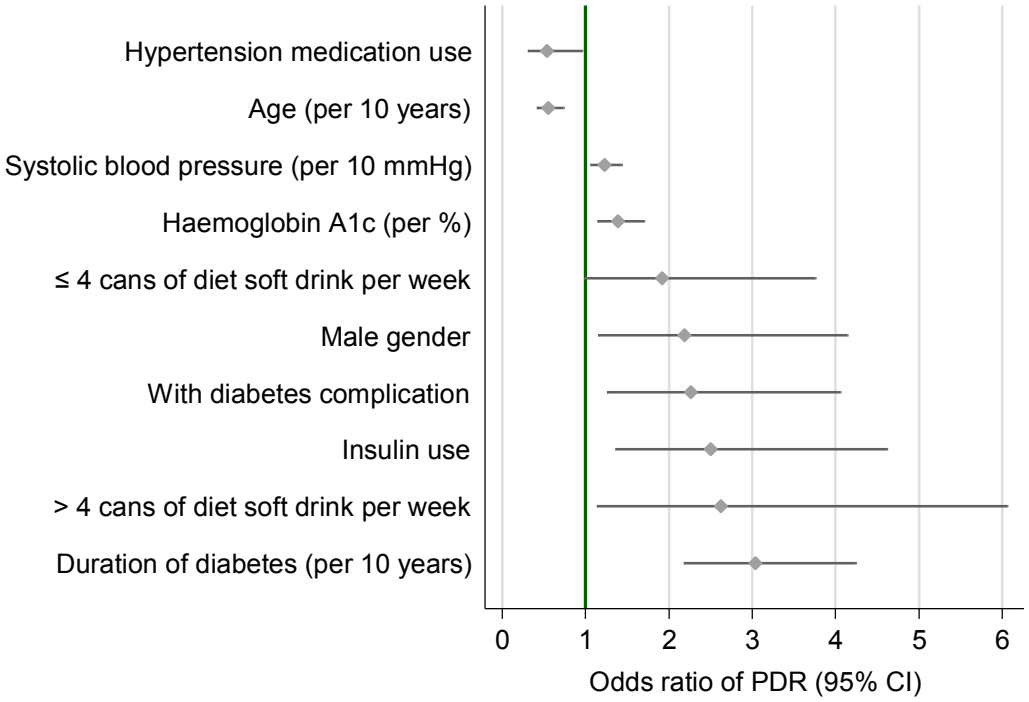

Figure 1: Odds ratios (95% confidence intervals) of our multivariable regression model exploring the association between consumption of diet soft drink (exposure) and PDR (outcome). This plot shows that high consumption of diet soft drink is an independent risk factor for PDR. Notes: PDR=Proliferative diabetic retinopathy.

DISCUSSION

In our clinical sample of people with diabetes attending a tertiary eye centre, we found that people who consumed more than four cans of diet soft drink a week had more than a 2-fold increased risk of having PDR. This finding was independent of traditional risk factors for DR, including diabetes control parameters and duration of diabetes. In contrast, consumption of diet soft drink was not associated with higher odds of having

less severe DR or any level of DME. Similarly, consumption of regular soft drinks was not associated with an increased risk for DR or DME in multivariable-adjusted analyses. Overall, these findings support the growing body of evidence suggesting that regular and frequent consumption of artificially sweetened beverages may have detrimental vascular outcomes. In particular, our findings indicate that a dietary pattern whereby low caloric soda is consumed on most days of the week may be associated with proliferative DR in people with diabetes.

To the best of our knowledge, no prior studies have explored the association between soft drink consumption and DR or any other microvascular complications of diabetes. As such, there are few studies to directly compare our findings. In a recent systematic review and meta-analysis on the topic of artificial sweeteners and cardiometabolic health, the authors conclude that routine consumption of non-nutritive sweeteners may be associated with increased BMI and cardiometabolic risk.[10] Similarly, previous publications have reported an association between high diet soft drink consumption and worse cardiovascular profile in patients with diabetes.[8, 9] For instance, in a large, cross-sectional study of youths with type 1 diabetes mellitus in the US, consumption of at least one glass of diet soft drink per day was independently associated with 0.4% higher HbA1c level, 4mg/dL higher total cholesterol, and 6mg/dL higher triglycerides, compared to consumption of <1 glass per day.[8] Evidence for the harmful effect of diet beverages on a range of health outcomes is also mounting, including increased risk of metabolic syndrome and diabetes,[3, 12-15] vascular events,[16] arthritis, and chronic bronchitis. For instance, in the population-based Northern Manhattan Study, those who drank diet soft drinks daily (vs. none) had a 1.45 increased risk of vascular events (e.g. stroke, myocardial infarction, vascular death), even after controlling for metabolic syndrome, peripheral vascular disease, diabetes, cardiac disease, hypertension, and hypercholesterolemia. Interestingly, there was no increased risk of vascular events

associated with light diet soft drink consumption,[16] which is similar to our study where moderate diet soft drink consumption was not associated with risk of DR severity. Interestingly, we found no association between soft drink consumption and any level of DME. DME may occur at any stage of DR[30] and, as such, is commonly classified as a sight-threatening type of DR,[31-36] with both conditions assumed to share the same underlying pathophysiological microvascular dysfunction.[31, 37] However, the differential associations of diet soft drink consumption with DR and with DME may suggest different pathophysiologic processes underlying the development of these two conditions.[38, 39]

We also did not find an association between consumption of regular soft drink and presence and severity of DR. This could be because we lacked numbers in the high consumption category and had to merge this with moderate consumption, which may have masked the true relationship. Our findings are consistent with the Northern Manhattan Study, which also found an association between consumption of diet but not regular soft drinks and risk of vascular events.[16] However, our findings contrast with other studies showing a relationship between routine consumption of regular soft drinks and increased risk of cardiovascular risk factors and outcomes.[3-5]

Several biological mechanisms to explain the harmful effect of diet soft drink on cardiovascular outcomes have been proposed. For example, consumption of artificial sweeteners in rats was found to weaken the ability to anticipate the amount of calories in food, leading to increased intake of calories and increased body weight.[17] Similarly, it has been proposed that artificial sweeteners may increase people's desire for sweet, and more energy-dense foods.[40-42] People consuming diet beverages may also over-consume other foods/beverages by overestimating the calories saved by substituting diet beverages for sugar-sweetened beverages.[18] However other studies have failed to show that artificial sweeteners increase hunger or subsequent food intake.[43] It is also possible that the caramel coloring of both diet and regular soft drinks may increase the

levels of proinflammatory advanced glycation end products.[1] Unfortunately, randomized, controlled trials addressing how the range of available artificial sweeteners affect metabolic dysfunction are lacking.[12] Therefore, well-designed, longitudinal studies to determine whether diet soft drinks are healthy substitutes for regular soft drinks are required.[10, 16]

Finally, previous prospective analyses of the association between diet soft drink consumption and metabolic syndrome suggest that the association between diet soft drinks and vascular events may be largely mediated by adiposity and fasting glucose.[12] Although we controlled for BMI, blood sugar, blood pressure, and lipids, as well as intake of total calories, these variables may still be on the pathway linking diet soft drink consumption with vascular disease risk. We also found a lack of effect modification by BMI (data not shown); however, as the interaction test was limited by lack of power due to the small number of people (n=50) with normal BMI (<25 kg/m^2), larger studies are needed to determine the true impact of BMI on the association between diet soft drink and DR.

One strength of our study is that DR/DME and diabetes control parameters were objectively determined using fundus photography, ocular coherence tomography and standardized grading protocols, and fasting blood samples, respectively. Similarly, we used a well-validated FFQ to collect our soft drink data. However, the cross-sectional nature of our study means that we do not know if people who currently consume diet soft drink were former consumers of regular soft drink who may have modified their lifestyle upon diagnosis of DR.[44] Although we adjusted for change of dietary habit in the last five years, this would not capture change in diet soft drink consumption at diagnosis of diabetes or DR, which is when most patients are likely to implement dietary or lifestyle changes. Therefore, the relationship between diet soft drink consumption and DR may be overestimated in our study and our conclusions should be interpreted

taking this limitation into account. Longitudinal studies are required to overcome the limitations of our cross-sectional study design.

There is also potential for recall bias in our study as consumption of diet soft drink was self-reported. However, measurement validity[45] was increased by using a valid and reliable food frequency questionnaire[24] that provides a detailed assessment of beverage consumption. Future studies could consider using a 'soft drink consumption' diary or App with frequent reminders, as well as collecting data on previous dietary habits. Because the 145-item FFQ was usually conducted at the end of the 3-hour assessment procedure, it was often excluded if the patient was fatigued resulting in 21% missing data for diet and regular soft drink consumption. However, we were able to deal with the missing data using data imputation methods which increased the robustness of our results. Despite having imputed missing data, the small percentage of individuals who consumed diet and regular soft drinks at the intermediate levels of DR severity, particularly mild and severe DR, could still mean a lack of power to detect associations at these levels.

Finally, our analysis included people with both type 1 and type 2 diabetes mellitus. Given that the two conditions differ in their pathophysiology, etiology, epidemiology and management, it is also possible that people with type 1 and type 2 diabetes mellitus have different approaches to lifestyle and dietary behaviours. However, there were too few patients with type 1 diabetes mellitus (n=45) in our study to conduct a sub-analysis. Moreover, given the estimated effects remained in the same direction (albeit with a loss of statistical significance due to loss of power) when we explored the association between diet soft drink consumption and DR in patients with type 1 diabetes mellitus only and type 2 diabetes mellitus only, and that the results were unaffected when we adjusted for diabetes type in the multivariable analyses, it is unlikely that our combined analysis has produced misleading results.

In summary, our findings suggest that high consumption of diet soft drink is independently associated with increased risk of PDR in patients with diabetes. Our study adds to the growing body of evidence reporting on the harmful effect of artificially sweetened beverages on a range of health outcomes. However, given the limitations inherent in our study, longitudinal studies using prospectively collected dietary data are needed to determine whether diet soft drinks are indeed unhealthy substitutes for regular soft drinks in patients with diabetes and to inform clinical management guidelines for DR.

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