Dietary predictors of arterial stiffness in a cohort with type 1 and type 2 diabetes

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ABSTRACT

Objective: To determine the dietary predictors of central blood pressure, augmentation index and pulse wave velocity (PWV) in subjects with type 1 and type 2 diabetes.

Methods: Participants were diagnosed with type 1 or type 2 diabetes and had PWV and/or pulse wave analysis performed. Dietary intake was measured using the Dietary Questionnaire for Epidemiological Studies Version 2 Food Frequency Questionnaire. Serum lipid species and carotenoids were measured, using liquid chromatography electrospray ionization–tandem mass spectrometry and high performance liquid chromatography, as biomarkers of dairy and vegetable intake, respectively. Associations were determined using linear regression adjusted for potential confounders.

Results: PWV (n=95) was inversely associated with reduced fat dairy intake (β=-0.01; 95% CI -0.02, -0.01; p=0<0.05) in particular yoghurt consumption (β=-0.04; 95% CI -0.09, -0.01; p=0<0.05) after multivariate adjustment. Total vegetable consumption was negatively associated with PWV in the whole cohort after full adjustment (β = -0.04; 95% CI -0.07,-0.01; p<0.05). Individual lipid species, particularly those containing 14:0, 15:0, 16:0, 17:0 and 17:1 fatty acids, known to be of ruminant origin, in lysophosphatidylcholine, cholesterol ester, diacylglycerol, phosphatidylcholine, sphingomyelin and triacylglycerol classes were positively associated with intake of full fat dairy, after adjustment for multiple comparisons. However, there was no association between serum lipid species and PWV. There were no dietary predictors of central blood pressure or augmentation index after multivariate adjustment.

Conclusion: In this cohort of subjects with diabetes reduced fat dairy intake and vegetable consumption were inversely associated with PWV. The lack of a relationship between serum lipid species and PWV suggests that the fatty acid composition of dairy may not explain the beneficial effect.

Keywords: Diabetes, arterial stiffness, pulse wave velocity, dairy, lipidomics, carotenoids

INTRODUCTION

The number of people with diabetes is projected to be 7.7% worldwide by 2030, an increase of 54% since 2010 [1]. Individuals with type 1 and type 2 diabetes have approximately double the risk of cardiovascular disease (CVD) compared to the general population [2, 3]. Dietary intake is a modifiable risk factor for CVD with studies showing that better dietary quality reduces the risk of CVD [4, 5].

Arterial stiffness, which can be measured by carotid femoral pulse wave velocity (PWV) and augmentation index, is a predictor of CVD [6, 7]. A meta-analysis of individual participant data from 17 studies (17 635 subjects) showed that PWV independently predicted CVD and CVD mortality such that per 1 standard deviation increase in PWV the hazard ratio was 1.30 (95% CI 1.18, 1.43) and 1.28 (95% CI 1.15, 1.43), respectively, after adjustment for established risk factors. Furthermore, the addition of PWV to conventional Framingham risk factors improved 10 year CVD risk prediction by 13% in those at intermediate risk of CVD [7]. Elevated central blood pressure and a larger augmentation index have also been shown to increase the risk of a cardiovascular event [6].

The SEARCH for Diabetes in Youth Study, involving children aged ≥10 years with type 1 diabetes, showed that a dietary pattern, determined by reduced rank regression, high in sugar sweetened and diet beverages, eggs, potatoes, high fat meat and low in dairy was associated with higher augmentation index and PWV; however the relationship with PWV did not persist after adjustment for cofounders [8]. Epidemiological studies conducted in cohorts without diabetes suggest that fruit and vegetables [9-11] and dairy [12, 13] may be inversely associated with PWV. The aim of this study is to determine the dietary predictors of central blood pressure, augmentation index and PWV in subjects with type 1 and type 2 diabetes.

METHODS

Study methods

This is a cross-sectional study of the association between dietary intake and arterial stiffness in subjects with type 1 and type 2 diabetes. One hundred and fifty subjects were recruited by public advertisement between August 2012 and December 2013. Subjects eligible for inclusion were adults (age >18 years) with diagnosed type 1 or type 2 diabetes for any duration managed with diet, oral hypoglycaemic agents (OHA) and/or insulin. Subjects were excluded if they had cancer, unstable CVD requiring intervention, heart failure, significant renal impairment (eGFR <30ml/min) or liver disease. This study includes data from participants who had PWV (n=95) and/or pulse wave analysis measurements (n=111) performed. Thirty nine- participants did not have PWV or pulse wave analysis performed because the equipment was not available and PWV data were available for only 95 participants because of technical difficulty performing the measurement due to obesity. Ethics approval was obtained from the University of South Australia Human Research Ethics Committee and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612001052820).

Subjects attended the clinic after an overnight fast. Anthropometric measurements, pulse wave analysis, PWV and blood pressure were performed by one operator and a blood sample was taken. Participants completed the online version of the Dietary Questionnaire for Epidemiological Studies Version 2 Food Frequency Questionnaire (DQES v2 FFQ) to determine habitual dietary intake.

Anthropometric measurements

Height was measured using a stadiometer (SECA, Hamburg, Germany) to the nearest 0.1cm while barefoot or in flat footwear. Weight was measured to the nearest 0.05kg using calibrated electronic

scales (SECA, Hamburg, Germany) while the participants were barefoot or in light footwear and wearing light clothing.

Blood pressure

Clinic brachial blood pressure was measured using an automated sphygmomanometer (SureSigns VS3; Philips, North Ryde, Australia) once the participant had been seated for 5 minutes. A normal sleeve (16 x 52cm) was used for an arm circumference of 24-32cm and a large sleeve (16 x 70cm) for an arm circumference of 32-42cm. A minimum of four consecutive readings were taken at 1 minute intervals while subjects were sitting quietly and alone. The first reading was discarded and the following three consistent measurements i.e. systolic blood pressure within a range of 10mmHg, were used [14].

Pulse wave analysis

A SphygmoCor® XCEL (AtCor Medical, West Ryde, Australia) was used to perform pulse wave analysis as previously described [15]. Briefly, a cuff was placed over the brachial artery on the right arm to measure central blood pressure, augmentation index and augmented pressure. A normal sleeve (16 x 52cm) was used for an arm circumference of 23-33cm and a large sleeve (16 x 70cm) for an arm circumference of 31-40cm. After the participants had been quietly resting for 5 minutes, 3 consecutive measurements were taken. The coefficient of variation (CV) was 11% (n=5).

Pulse wave velocity

A SphygmoCor® XCEL (AtCor Medical, West Ryde, Australia) was used to measure right carotid femoral PWV as previously described [15]. Three measurements were performed and all of the measurements were taken by one operator with a CV of 4.2% (n=28).

Laboratory analysis

Serum total cholesterol, HDL cholesterol, triglycerides, C reactive protein (CRP) and glucose were measured using a Konelab 20XTi automatic analyser (Thermo Electron Corporation, Louisville, CO, USA) with reagents from Thermo Fisher Scientific (Melbourne, Australia). LDL cholesterol was calculated using the Friedewald formula ((total cholesterol - HDL cholesterol) – (triglycerides x 0.45)) [16]. Subjects with a serum CRP >10mg/L were excluded from the analysis. Serum carotenoids were measured by high performance liquid chromatography according to a previously published protocol [17]. Lipid analysis was performed by liquid chromatography, electrospray ionization–tandem mass spectrometry as previously published [18]. Briefly, 333 individual lipid species from 25 classes were measured and the median intra assay CV was 8%.

Dietary analysis

Habitual dietary intake was measured using the electronic version of the DQES v2 FFQ. This FFQ has been found to have relatively good agreement with a 3 day weight food record [19]. It classifies more than two thirds of subjects within one quintile, for all nutrients, compared with a 3 day weighed food record [20].

Statistical analysis

Data are presented as mean ± standard deviation or median (interquartile range) depending on the distribution. Data were checked for normality using Shapiro-Wilk and Kolmogorov-Smirnov values. Pearson's correlation was used to determine univariate relationships and stepwise multivariate linear regression was used to determine predictors of central blood pressure, augmentation index and PWV. Variables that were correlated ($p<0.1$) with the variable of interest in Pearson's correlation were entered into a stepwise linear regression to determine predictors. Linear regression analysis was adjusted for predictors identified in the stepwise linear regression and outliers (±2 standard deviations) were removed. To determine the level of under-reporting the Schofield equation was used to calculate Basal Metabolic Rate [21] and the Goldberg cut-off was applied [22]. Significant under-reporting was observed. Therefore intake is reported as a percentage of total energy intake or units per 1000kJ. Serum carotenoids and serum lipid concentrations were normalised to their respective interquartile ranges to account for the variation in relative abundance in serum. Therefore the β- coefficients obtained correspond to a change in the outcome measure associated with an increase in the serum carotenoid or lipid concentration relative to the interquartile range. The association between reported dairy intake from the FFQ and lipids was investigated using linear regression adjusted for age, sex, type of diabetes and BMI. P values were corrected for multiple comparisons using the Benjamini Hochberg approach [18]. Analysis was performed using SPSS (version 19, 2010, SPSS Inc, Chicago, IL). Statistical significance was set at $p<0.05$.

RESULTS

Subjects were 111 adults with diagnosed type 1 or type 2 diabetes, see Table 1. The subjects with type 2 diabetes were significantly older than those with type 1 diabetes (59 ± 11 vs. 42 ± 15 years; $p=0.0001$). There was no difference in weight, BMI or peripheral blood pressure by diabetes type. HDL cholesterol was higher in the subjects with type 1 diabetes (1.5 ± 0.4 vs. 1.1 ± 0.3mmol/L; $p=0.0001$), but there was no difference in total cholesterol, LDL cholesterol or triglycerides. Fasting

glucose was significantly higher in the subjects with type 1 diabetes (12.2 ± 3.8 vs. 7.8 ± 2.6 mmol/L; p= 0.0001), but there was no difference in HbA1c. Forty six (46%) of the subjects with type 2 diabetes were concurrently prescribed an anti-hypertensive and lipid lowering medication, and a further 18 subjects were prescribed either an anti-hypertensive or lipid lowering medication. Twenty two subjects with type 2 diabetes were not taking any medication to control their diabetes, 56 were taking an OHA and 3 were prescribed insulin, 20 subjects were taking an OHA and insulin. One subject with type 1 diabetes was prescribed anti-hypertensive and lipid lowering medication and 3 subjects were prescribed an antihypertensive medication. All the subjects with type 1 diabetes were prescribed insulin with no other concurrent hypoglycaemic agents.

Table 1: Subject characteristics

Characteristic	**Whole cohort (n=111)**	**Type 1 diabetes (n=10)**	**Type 2 diabetes (n=101)**	**P value^**
Age (years)	58 ± 12	42 ± 15	59 ± 11	0.0001
Weight (kg)	100.3 ± 21.6	98.8 ±29.5	100.4 ± 20.8	>0.05
Height (m)	1.7 ± 0.1	1.72 ± 0.1	1.72 ± 0.1	>0.05
BMI (kg/m^2)	34.0 ± 6.9	34.0 ± 11.1	34.0 ± 6.4	>0.05
Sex, male	66 (60)	4 (40)	62 (61)	>0.05
Diagnosed with diabetes (years)	9.3 ± 8.7	24.9 ± 9.2	7.7 ± 7.0	0.0001
Smoking (pack years)	9.8 ± 15.5	1.0 ± 2.0	10.7 ± 15.9	0.057
Peripheral systolic blood pressure (mmHg)	129 ± 14	130 ± 16	129 ± 14	>0.05
Peripheral diastolic blood pressure (mmHg)	73 ± 10	70 ± 13	73 ± 10	>0.05
Peripheral mean arterial	91 ± 9	90 ± 11	91 ± 9	>0.05

pressure (mmHg)				
Peripheral pulse pressure (mmHg)	56 ± 14	60 ± 19	56 ± 13	>0.05
Central systolic blood pressure (mmHg)	127 ± 15	122±17	127±15	>0.05
Central diastolic blood pressure (mmHg)	83 ± 10	81±12	84±10	>0.05
Central mean arterial pressure (mmHg)	101 ± 11	98±12	101±11	>0.05
Central pulse pressure (mmHg)	43 ± 12	41±18	44±12	>0.05
Central augmented pressure (mmHg)	9 ± 5	8±6	9±5	>0.05
Augmentation index (%)	20.4 ± 8.3	16.7±8.2	20.7±8.3	>0.05
Pulse wave velocity (m/s)	9.6 ± 1.8	8.2±1.5	9.7±1.8	<0.05
Total cholesterol (mmol/L)	4.2 ± 1.1	4.4 ± 1.4	4.1 ± 1.1	>0.05
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.5 ± 0.4	1.1 ± 0.3	0.001
LDL cholesterol (mmol/L)	2.4 ± 0.9	2.6 ± 1.2	2.4 ± 0.9	>0.05
Triglycerides (mmol/L)	1.4 ± 1.3	0.8 ± 0.3	1.5 ± 1.3	>0.05
Glucose (mmol/L)	8.2 ± 3.0	12.2 ± 3.8	7.8 ± 2.6	0.0001
HbA1c (mmol/mol)	57 ± 16	59 ± 13	57 ± 17	>0.05
CRP (mg/L)	2.6 ± 2.4	2.6 ± 2.4	2.6 ± 2.4	>0.05
Prescribed anti-hypertensive medication	68 (61)	4 (40)	64 (63)	>0.05
Prescribed lipid lowering medication	65 (59)	1 (10)	64 (63)	0.001

Diabetes treatment				0.0001
None	22 (20)	0 (0)	22 (22)	
OHA	56 (50)	0 (0)	56 (55)	
Insulin	13 (12)	10 (100)	3 (3)	
OHA + Insulin	20 (18)	0 (0)	20 (20)	

Data presented as mean ± standard deviation; ^type 1 vs. type 2

Table 1 shows central blood pressure, augmentation index and PWV data for the whole cohort. There was no difference in central blood pressure or augmentation index by diabetes type. PWV was significantly higher in subjects with type 2 diabetes compared to those with type 1 diabetes (9.7 ± 1.8 vs. 8.2 ± 1.5m/s; $p<0.05$), but this was no longer significant after age adjustment.

There was no statistically significant difference in dietary intake by diabetes type, therefore data is presented for the whole cohort, see Table 2. The median macronutrient composition was 21%, 36%, 14% and 40% of total energy from protein, fat, saturated fat and carbohydrate, respectively. Dietary intake of the major food groups is presented in Table 3. Serum α- carotene ($r= 0.31$; $p=0.001$) and β-carotene ($r=0.23$; $p<0.05$), expressed as interquartile normalised concentration were significantly associated with vegetable intake (g/1000kJ) derived from the FFQ and serum lycopene concentration approached statistical significance ($r= 0.19$; $p=0.053$). There were no correlations between total carotenoids, lutein/ zeaxanthin and β-cryptoxanthin and dietary intake.

Table 2: Nutrient intake as reported in the FFQ

Nutrient intake	**Value**
Energy	6837 (5601, 9074)
Protein (g/d)	87 (66,110)
% E protein	21 (18, 23)

Total fat (g/d)	65 (51, 91)
% E total fat	36 (33, 40)
Saturated fat (g/d)	24 (19, 34)
% E saturated fat	14 (12, 15)
Monounsaturated fat (g/d)	24 (18, 33)
% E monounsaturated fat	13 (11, 15)
Polyunsaturated fat (g/d)	11 (8, 14)
% E polyunsaturated fat	6 (5, 7)
Carbohydrate (g/d)	158 (135, 208)
% E carbohydrate	40 (37, 44)
Sugar (g/d)	75 (56, 94)
Fibre (g/d)	20 (16, 26)
Sodium (mg/d)	2199 (1693, 2953)
Potassium (mg/d)	2742 (2190, 3394)
Alcohol (g/d)	2 (0, 14)

166 Data presented as median (interquartile range)

167 **Table 3: Dietary intake by food group as reported in the FFQ**

Dietary intake	**g/d**	**g/1000kJ**
Total breads and cereals	188 (137, 281)	26 (21, 35)
Breakfast cereal	18 (4, 38)	2 (1, 6)
Bread	60 (53, 90)	9 (6, 13)
Pasta/rice	58 (32, 109)	8 (5, 14)
Total vegetables/ legumes	140 (108, 206)	22 (14, 30)

Vegetables	135 (103, 197)	20 (12, 28)
Legumes	7 (3, 13)	1 (0,2)
Total fruit	245 (124, 337)	33 (19, 46)
Fresh fruit	181 (97, 276)	25 (12, 39)
Canned fruit	5 (1, 14)	1 (0, 2)
Juice	8 (0, 53)	1 (0, 6)
Total dairy	387 (244, 477)	47 (31, 76)
Milk	375 (200, 377)	39 (25, 63)
Yoghurt	41 (10, 73)	5 (1, 12)
Cheese	8 (4, 15)	1 (1, 2)
Reduced fat dairy	299 (67, 448)	42 (10, 73)
Milk	200 (0, 375)	32 (0, 62)
Yoghurt	31 (0, 73)	4 (1, 11)
Cheese	0 (0, 5)	0 (0, 1)
Full fat dairy	9 (3, 20)	1 (0, 3)
Milk	0 (0, 4)	0 (0, 1)
Yoghurt	0 (0, 0)	0 (0, 0)
Cheese	8 (2, 14)	1 (0, 2)
Total meats and alternatives	190 (120, 242)	25 (20, 30)
Red meat	52 (27, 105)	8 (5, 13)
Processed meat	20 (10, 34)	3 (1, 4)
Chicken	31 (19, 51)	4 (3, 6)
Fish	27 (15, 47)	4 (2, 7)
Tofu	0 (0, 2)	0 (0, 1)
Nuts	7 (1, 13)	1 (0, 1)

Eggs	13 (13, 34)	2 (1, 4)
Total extra foods	140 (71, 307)	19 (12, 35)

168 Data presented as median (interquartile range)

169

170 Many lipid species, expressed as interquartile normalised concentration, were positively associated
171 with total full fat dairy intake (g/day), after adjustment for multiple comparisons (Table 4). Individual
172 lipids from the following classes were positively associated with intake of full fat dairy after
173 adjustment for multiple comparisons lysophosphatidylcholine (LPC14:0, LPC15:0, LPC17:1),
174 cholesterol ester (CE14:0, CE15:0), diacylglycerol (DG16:0/20:0), phosphatidylcholine (PC29:0,
175 PC30:0, PC31:0, PC31:1, PC33:0, PC33:1, PC33:3, PC35:0), sphingomyelin (SM31:1, SM32:0) and
176 triacylglycerol (TG14:0/17:0/18:1, TG15:0/16:0/18:1, TG16:0/16:0/16:0, TG16:0/16:1/17:0,
177 TG16:0/17:0/18:0). Many of these associations were also evident for full fat milk; very few
178 associations were observed with cheese consumption. There were no significant associations found
179 between the lipid species and intake of total or reduced fat dairy, after adjustment for multiple
180 comparisons. When dairy intake in grams per 1000kJ was examined many of the same associations
181 were evident (data not shown).

182 **Table 4: Linear regression of serum lipid species against dairy consumption reported in the FFQ,**
183 **adjusted for age, sex, BMI and type of diabetes.**

Dietary intake	**B- coefficient (95% CI)**	**P value**	**P value corrected^**
Total dairy			
Nil significant			
Total full fat dairy			
CE 14:0	50.31 (20.05, 80.58)	0.001	0.029

CE 15:0	77.15 (44.77, 109.52)	<0.001	<0.01
DG 16:0/20:0	33.75 (17.26, 50.25)	<0.001	0.006
LPC 14:0	66.97 (26.91, 107.04)	0.001	0.029
LPC 15:0	71.96 (32.84, 111.08)	<0.001	0.016
LPC 17:1	77.12 (29.84, 124.39)	0.002	0.03
PC 29:0	99.62 (64.31, 134.92)	<0.001	<0.01
PC 30:0	55.65 (24.71, 86.60)	0.0006	0.017
PC 31:0	108.09 (71.56, 144.63)	<0.001	<0.01
PC 31:1	78.41 (30.74, 126.07)	0.002	0.03
PC 33:0	109.33 (65.31, 153.35)	<0.001	0.0003
PC 33:1	75.81 (34.08, 117.55)	0.0005	0.017
PC 33:3	78.47 (37.56, 119.39)	0.0002	0.012
PC 35:0	85.94 (43.00, 128.87)	0.0001	0.008
SM 31:1	68.76 (25.29, 112.22)	0.002	0.039
SM 32:0	63.48 (29.09, 97.87)	0.0004	0.016
TG 14:0/17:0/18:1	33.15 (14.00, 52.30)	0.0009	0.025
TG 15:0/16:0/18:1	28.77 (10.25, 47.29)	0.003	0.044
TG 16:0/16:0/16:0	35.77 (13.17, 58.38)	0.002	0.039
TG 16:0/16:1/17:0	32.75 (13.15, 52.35)	0.001	0.029
TG 16:0/17:0/18:0	34.46 (14.00, 54.91)	0.001	0.029
Reduced fat dairy			
Nil significant			

184 ^corrected for multiple comparisons using the Benjamini Hochberg approach (all lipid species were
185 analysed). Only significant associations are shown. CE cholesterol ester; DG diacylglycerol; LPC
186 lysophosphatidylcholine; PC phosphatidylcholine; SM sphingomyelin; TG triacylglycerol

There was no association with any component of dietary intake and central systolic blood pressure. In univariate analysis inverse associations existed between central diastolic blood pressure and intake (per 1000kJ) of total dairy ($r=-0.21$; $p<0.05$), yoghurt ($r=-0.24$; $p=0.01$), vegetables ($r=-0.2$; $p<0.05$) and potassium ($r=-0.21$; $p<0.05$) and positive associations were observed for rice and pasta ($r=0.21$; $p<0.05$) and takeaway foods ($r=0.23$; $p<0.05$). However, after adjustment for other predictors of central diastolic blood pressure (age and weight) all of these univariate associations were attenuated to non-significance. After multivariate adjustment there were no dietary predictors of central pulse pressure (adjusted for age, sex, time since diabetes diagnosis and anti-hypertensive prescription) or augmentation index (adjusted for age, sex, BMI and total cholesterol). Total vegetable intake (g/1000kJ) was inversely associated with PWV ($\beta = -0.04$; 95% CI -0.07,-0.01; $p<0.05$) after multivariate adjustment (age, BMI, central mean arterial pressure, heart rate and antihypertensive medication prescription). There was no association between serum carotenoids and PWV.

After adjustment for predictors of PWV (age and central mean arterial pressure) there was a significant inverse relationship between dairy intake and PWV, such that per 100g/1000kJ increase in total dairy intake PWV was 1.1m/s lower (95% CI -2.2, -0.07; $p<0.05$), see Table 5. In addition, there was a significant association observed between total reduced fat dairy consumption and PWV, but no association was evident for full fat dairy. Total and reduced fat yoghurt consumption was inversely associated with PWV. After further adjustment for BMI, heart rate and antihypertensive medication prescription the association with total dairy intake was attenuated to non-significance, see Table 5. However, an inverse relationship remained between PWV and reduced fat dairy in particular yoghurt consumption. Calcium intake was also inversely associated with PWV ($\beta= -0.01$;

211 95% CI -0.02, -0.004; p=0.003). There was no association between any serum lipid species and PWV,

212 after multivariate adjustment for predictors and adjustment for multiple comparisons.

213 **Table 5: Linear regression of dairy consumption (g/1000kJ) against PWV**

Predictor	**Model 1[2]**		**Model 2[3]**	
	β- coefficient (95% CI)	**p value**	**β- coefficient (95% CI)**	**P value**
Pulse wave velocity				
Total dairy[1]	-0.01 (-0.02, -0.01)	0.038	-0.01 (-0.02, 0.01)	0.07
Milk	-0.01 (-0.02, 0.01)	0.13	-0.01 (-0.02, 0.01)	0.33
Cheese	-0.14 (-0.39, 0.11)	0.26	-0.11 (-0.35, 0.13)	0.37
Yoghurt	-0.04 (-0.08, -0.01)	0.046	-0.04 (-0.08, -0.01)	0.03
Reduced fat dairy	-0.01 (-0.02, -0.01)	0.031	-0.01 (-0.02, -0.01)	0.048
Milk	-0.01 (-0.02, 0.87)	0.12	-0.01 (-0.02, 0.01)	0.18
Cheese	-0.37 (-0.87, 0.13)	0.14	-0.34 (-0.82, 0.15)	0.18
Yoghurt	-0.05 (-0.09, -0.01)	0.026	-0.04 (-0.09, -0.01)	0.037
Full fat dairy	0.01 (-0.02, 0.03)	0.82	0.01 (-0.02, 0.03)	0.89
Milk	0.01 (-0.2, 0.04)	0.59	0.01 (-0.02, 0.04)	0.65

Cheese	-0.17 (-0.46, 0.12)	0.25	-0.18 (-0.47, 0.11)	0.21
Yoghurt	0.06 (-0.34, 0.22)	0.66	-0.01 (-0.28, 0.27)	0.99

[1]g/1000kJ; [2]Adjustment for age and central mean arterial pressure; [3]Model 1 + BMI, heart rate and antihypertensive medication prescription

DISCUSSION

In this cohort with type 1 and type 2 diabetes there was an inverse association between reduced fat dairy intake, in particular yoghurt consumption, and PWV, which persisted after multivariate adjustment. Serum lipid species, known to be of ruminant origin, were positively associated with full fat dairy consumption; however there was no association between these lipid species and PWV. In addition, higher vegetable intake was also associated with lower PWV. There were no dietary predictors of central blood pressure or augmentation index identified in this cohort.

Previous studies that have investigated the association between dairy intake and PWV have shown mixed results. In the Maine- Syracuse Longitudinal study frequency of overall dairy consumption was inversely associated with PWV [12]. Recio-Rodriguez et al [13] found in a study of healthy Spanish subjects that consumption of low fat dairy was associated with reduced PWV, such that per 100g/d increase PWV was 0.1m/s lower (95% CI -0.18, -0.02; p=0.011). In the study by Recio-Rodreiguez et al consuming whole fat dairy was associated with a 0.1m/s increase (95% CI 0.01, 0.21) in PWV per 100g/d [13]. In the Caerphilly Prospective study, involving healthy men, there was no relationship between PWV and dairy consumption [23]. With the exception of the Caerphilly Prospective study, these observational studies did not look at the association between types of dairy products and PWV. In the Caerphilly Prospective study, only cream consumption was associated with PWV, albeit inversely; intake of yoghurt was not included in the analysis. In our study there was an inverse

association between PWV and reduced fat dairy intake, but no association was observed with full fat dairy. After multivariate adjustment yoghurt consumption was inversely correlated with PWV, Yoghurt has a hypotensive effect [24] and has also been shown to lower cholesterol [25]. Ivey et al [26] showed that higher yoghurt consumption was associated with lower carotid intima media thickness, a measure of subclinical atherosclerosis. However, an association between yoghurt consumption and PWV has not been previously reported.

In this study full fat dairy intake was positively correlated with lipid species containing fatty acids 14:0, 15:0 and 17:1, in particular in the lysophosphatidylcholine lipid class. Previously, 15:0 and 17:0 fatty acids in lysophosphatidylcholine have been found to correlate with full fat dairy intake [27]. The finding that fatty acids from ruminant sources are not associated with PWV, in addition to the lack of an association with full fat dairy, suggests that fatty acids in dairy may not be responsible for the observed beneficial effect. A recent meta-analysis showed that 17:0 was protective against coronary outcomes, with a relative risk for the highest tertile of 17:0 compared with the lowest tertile of 0.77 (95% CI 0.63, 0.93) [28]. Fatty acids 14:0 (RR 0.96; 95% CI 0.83, 1.12) and 15:0 (RR 0.94; 95% CI 0.67, 1.32) were also non-significantly associated with a reduced risk of coronary events in this meta-analysis.

It is postulated that bioactive peptides in dairy products may account for some of the observed effect by inhibiting angiotensin-I-converting enzyme [29]. Both whey and casein milk proteins have been shown to improve arterial compliance [30, 31]. In addition, the calcium composition of dairy may also explain the effect. A four way cross-over study showed that a high intake of calcium from dairy products in the presence of a high fat intake attenuated the increase in total cholesterol and LDL cholesterol without changing the increase in HDL cholesterol, compared with a high fat, low calcium diet [32]. Similarly, in a study of a high fat diet (50% of total energy) with a milk protein enriched concentration containing 1990mg/10MJ of calcium compared with a control drink (470mg/10MJ of calcium), total cholesterol was 9% lower and LDL cholesterol was 6% lower after

the milk protein enriched drink compared with the control treatment, and there was no significant difference in HDL cholesterol [33]. In this analysis we observed an inverse association between calcium intake and PWV.

To the authors knowledge there have been no studies that have shown an association between vegetable consumption and PWV. In the Cardiovascular Risk in Young Finns Study total fruit and vegetable consumption, in both childhood and adulthood, was inversely associated with PWV in adulthood [9]. In addition, in a study of rheumatoid arthritis patients augmentation index was inversely associated with frequency of vegetable consumption (-4.2%: 95% CI -7.9, -0.5 with daily vegetable intake compared to less frequently) [34].

Previously, epidemiological studies have shown an inverse relationship between lycopene [35, 36], β- carotene [37], β- cryptoxanthin [37] and brachial ankle PWV. However, other studies have reported no effect of carotenoids on arterial stiffness [38]. In this analysis despite an inverse association existing between vegetable intake and PWV, serum carotenoids were not associated with PWV. Studies to date have shown that lycopene, either from supplements or consumption of tomato based products, up to 70mg/week for 2-3 months did not affect PWV [39, 40]. Similarly, a randomised controlled trial, of people at high risk of CVD with habitually low intake of fruit and vegetables, showed that greater intake of fruit and vegetables did not affect PWV after 18 weeks [41]. In this study subjects were randomised to consume +2, +4 and +6 portions/d (above habitual intake), each for 6 weeks, of high flavonoid fruit and vegetables or low flavonoid fruit and vegetables, compared to a control group continuing on their usual diet.

In this analysis we showed that reduced fat dairy and vegetable intake were inversely associated with PWV such that per 100g/1000kJ (~half a serve of dairy) increase in reduced fat dairy intake PWV was 0.9m/s lower and per 75g/1000kJ (1 serve of vegetables) increase in vegetable consumption PWV was 2.8m/s lower after full adjustment. A previous meta-analysis of longitudinal studies, with a

mean follow-up time of 7.7 years, showed that with a 1m/s lower carotid femoral PWV the risk of a cardiovascular event was reduced by 14% [42].

In this study there was no relationship between augmentation index and PWV, which has been previously reported [43]. Augmentation index is not a direct measure of arterial stiffness and is influenced by the timing and magnitude of the wave reflection[44]. In contrast, PWV is a robust measure of arterial stiffness as it is determined by measuring the velocity of the waveform between the carotid and femoral arteries [45]. Previously, it has been shown that in a population with diabetes PWV was elevated compared with healthy controls, however augmentation index was not different [46]. Lacy et al [46] concluded that augmentation index is not a reliable measure of arterial stiffness in people with diabetes. This may explain why we did not see an association between augmentation index and dietary intake, despite seeing correlations with PWV.

Limitations of this study include the inclusion of fewer individuals with type 1 diabetes than type 2 diabetes and the cross-sectional design. In addition, the use of a FFQ to capture dietary intake is a limitation, although serum carotenoids and lipid species were correlated with reported dietary intake. It is also possible that we did not see an association between serum lipid species and PWV because serum fatty acid composition is not a sensitive marker of dairy intake and the majority of our cohort was consuming reduced fat dairy. A randomised controlled trial showed that when full fat dairy intake was increased by 3 serves per day for 1 month only plasma pentadecanoic acid composition was significantly changed when compared to elimination all of dairy products [47]. The method used to measure augmentation index is based on the averaged transfer function which may blunt the sensitivity of this measure [48].

CONCLUSION

In this cohort of people with type 1 and type 2 diabetes greater consumption of reduced fat dairy and vegetables was associated with less arterial stiffening, as measured by PWV, the gold standard for non-invasively measuring arterial stiffness. However, these results need to be confirmed in a randomised controlled trial. In people with diabetes improving diet quality may reduce the burden of cardiovascular disease by virtue of reducing arterial stiffness.

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