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Plasma carotenoids and diabetic retinopathy

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Diabetic retinopathy increases with duration of diabetes and may be associated with carotenoid status. Carotenoids alter the pro-oxidation/anti-oxidation balance, and circulating levels depend largely on dietary intake. Lower levels have been reported in diabetes and age-related macular degeneration; however, little is known of the relationship between carotenoids and diabetic complications. Consequently, the purpose of the present study was to evaluate the relationship between plasma carotenoids and diabetic retinopathy. We assessed the carotenoid–retinopathy relationship in 111 individuals with type 2 diabetes in a community-based, cross-sectional study. We photodocumented retinal status and used HPLC to measure plasma carotenoid concentrations. Data for clinical and demographic variables and risk factors for diabetic retinopathy were obtained from 24 h urine and fasting blood samples, and an interviewer-assisted lifestyle questionnaire. We found that the combined lycopene and lutein/zeaxanthin (non-pro-vitamin A (non-PVA) carotenoid) concentration when compared with the pro-vitamin A (PVA) carotenoids (α-carotene, β-carotene and β-cryptoxanthin) was significantly lower in the retinopathy than non-retinopathy group (OR 1·2 (95 % CI 1·0, 1·4) *v.* 1·6 (95 % CI 1·4, 1·7), respectively; $P=0·009$). A higher non-PVA:PVA ratio also predicted a lower risk of diabetic retinopathy, after adjustment for potential confounders (OR 0·33 (95 % CI 0·12, 0·95); $P=0·039$). Finally, a higher concentration of PVA carotenoids was associated with greater odds of diabetic retinopathy, after adjustment for risk factors ($P=0·049$). We suggest synergies between carotenoids are implicated in diabetic retinopathy, independent of established risk factors. Importantly, our observations indicate dietary modulation of retinopathy risk may be possible by increasing intakes of lutein- and lycopene-rich foods.

Carotenoids: Diabetic retinopathy: Pro-vitamin A

Carotenoids demonstrate a vast array of biological activities, including vital roles in the eye, both functionally as precursors to retinol in the visual pathway (pro-vitamin A (PVA) carotenoids) and structurally as macular pigments. The major PVA carotenoids in plasma are α-carotene, β-carotene and β-cryptoxanthin. Of these, only β-carotene is found in ocular tissues[1].

In contrast, lutein/zeaxanthin and lycopene are the major non-PVA carotenoids, i.e. are not retinol precursors, and both are present in ocular tissues at high concentrations. Lutein and zeaxanthin comprise the macular pigments, essential for normal vision and for the protection of photoreceptors from phototoxic blue light, while lycopene is present in high concentrations in the human ciliary body and retinal pigment epithelium/choroid[2].

Plasma carotenoid concentrations have been linked to numerous conditions[3–6] including the major blinding conditions – age-related macular degeneration[7,8] and cataracts[9]. To date, the relationship between the major carotenoids and diabetic retinopathy has not been evaluated (Table 1). Consequently, we undertook to investigate the association between plasma carotenoids and diabetic retinopathy.

Subjects and methods

Diabetes status

Self-reported diabetes status was confirmed biochemically, according to the WHO diagnostic criteria for the classification of diabetes[10].

Subject selection

In order to broaden the range of dietary intakes and lifestyle exposures, we sourced subjects from the Melbourne Collaborative Cohort Study (MCCS), a community-based prospective cohort of 41 528 male and female volunteers, aged 40–69 years at baseline (1989–94), recruited from the electoral roll, and ethnic radio, clubs, and churches[11]. We invited 157 men with type 2 diabetes and not taking carotenoid supplements to participate in the present study. Of these, we excluded three (two men with type 1 diabetes and one man with ungradeable photographs). Of the eligible subjects (*n* 154), 72 % (*n* 111) participated in the study. Ethics approval was obtained from the MCCS scientific committee and Deakin and Monash Universities (Melbourne, Australia), and written informed consent was obtained from every participant.

Abbreviations: MCCS, Melbourne Collaborative Cohort Study; PVA, pro-vitamin A.

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Table 1. Diabetes-related studies of plasma concentrations of major carotenoids[5,6,24,25,35,37–54]

Authors	Year	Design	Country	Age and age range (years)	Number of subjects			Mean plasma carotenoid concentrations (μmol/l)					Subject characteristics and/or carotenoid-related findings
					ND	T1D	T2D	ACAR	BCAR	BCRY	LZ	LYC	
A Hodge (personal communication)	2008	P	Australia	55 (36–73)	2437			0·13	0·78	0·32	0·36	0·57	4-Year diabetes incidence associated with lower baseline PVA carotenoid levels despite similar carotenoid intakes to non-diabetic group ($P<0·0001$)
				58 (42–70)			255	0·06	0·41	0·24	0·35	0·52	
Neyestani *et al.*[24]*	2007	T	Italy	54			35		0·39			0·48	Lycopene at 10 mg/d for 2 months increased innate immunity and serum lycopene concentration (to 1·42 μmol/l)
Wang *et al.*[37]	2006	CC	USA	≥45 (women only)	470		470	0·16	0·34	0·47	0·20	0·37	10-Year diabetes incidence not associated with baseline plasma carotenoids
								0·17	0·35	0·48	0·21	0·39	
Hozawa *et al.*[38]*	2006	P	USA	18–30	4345		148	0·05	0·34	0·27	0·15	0·53	15-Year diabetes incidence associated with lower carotenoids in non-smokers only. Only grouped data presented
Granado-Lorencio *et al.*[35]*	2006	PH	Spain	21		145		0·08	0·39	0·42	0·28	0·56	Mean levels (over 2·5 years) higher than in Spanish population; seasonal differences only in cryptoxanthin and β-carotene
Quilliot *et al.*[39]	2005	CH	France	44	20			0·07		0·29		0·41	Lower lycopene in T1D and lower carotenes (α- and β-carotene and lycopene) in chronic pancreatitis
				55		25		0·06		0·19		0·22	
Coyne *et al.*[5]*	2005	Cc	Australia	≥25			132	0·10	0·42	0·19	0·35	0·35	All serum carotenoids lower in diabetes, after adjustment. All increased with age except lycopene, which decreased with age
			(AusDiab)		1145			0·13	0·59	0·22	0·42	0·44	
Sugiura *et al.*[40]*	2006	Cc	Japan	56	812			Ranges not means reported					Serum carotenoids inversely associated with insulin resistance (tertiles); sex differences in lycopene and α-carotene associations with diabetes
Granado *et al.*[41]*	2004	T	Spain		8			0·05	0·14	0·20	0·29	0·31	On a very low carotenoid intake, carotenoid depletion rates unaltered by T1D
						10		0·08	0·24	0·32	0·43	0·29	
Ylonen *et al.*[42]	2003	Cc	Sweden	53	81		Men	0·12	0·51			0·31	In non-diabetic relatives of individuals with T2D, β-carotene positively associated with fasting plasma glucose in women and inversely associated with insulin resistance in men
			Finland	53	101		Women	0·20	0·73			0·30	
Suzuki *et al.*[43]*	2002	Cc	Japan	64 (48–86)	302			0·13	1·24	0·26	1·17	0·46	Controls for non-diabetic high HbA1c (≥5·6 %) group
					151			0·10	0·87	0·20	0·97	0·38	Non-diabetic high HbA1c (≥5·6 %) group had lower levels of all carotenoids than controls
					266			0·12	1·21	0·25	1·11	0·45	Controls for diabetic group
						133		0·12	1·14	0·21	1·10	0·50	Self-reported diabetic group had lower cryptoxanthin level
Upritchard *et al.*[44]	2000	T	New Zealand	63			15					0·39	Tomato juice at 500 ml/d (4 weeks) raised lycopene to 1·08 μmol/l and increased lag time in LDL oxidation
Polidori *et al.*[45]	2000	CH	Germany/Italy	77	75			0·06	0·58	0·32	0·44	0·75	Mean diabetes duration 23 years, HbA1c 8·5 %
				76		72		0·03	0·18	0·04	0·14	0·11	All carotenoids lower in elderly T2D patients
Facchini *et al.*[46]	2000	T	USA	47 (19–32)	36			0·21	0·68	0·17	0·73	0·61	Baseline fasting levels
					12			0·25	0·95	0·19	0·40	0·62	Subset defined as insulin sensitive post-180 min infusion of octreotide, insulin, and glucose

Table 1. *Continued*

Authors	Year	Design	Country	Age and age range (years)	Number of subjects			Mean plasma carotenoid concentrations (μmol/l)					Subject characteristics and/or carotenoid-related findings
					ND	T1D	T2D	ACAR	BCAR	BCRY	LZ	LYC	
					12			0·08	0·34	0·15	0·30	0·55	Subset defined as insulin resistant after same infusion; lutein, α- and β-carotene lower in insulin-resistant subset
Levy *et al.* [47]	2000	T	Israel	52			20		0·52				β-Carotene at 60 mg/d (3 weeks) increased plasma levels 2·3-fold to 1·70 μmol/l and lag time in LDL oxidation; diabetic LDL had half the carotenoid concentration of control LDL
Ford *et al.* [6]*†	1999	Cc	USA	54 (47–74)	959			0·10	0·43	0·16	0·41	0·42	Normal glucose tolerance
				56 (47–74)	262			0·09	0·34	0·18	0·43	0·39	Impaired glucose tolerance; lower β-carotene levels than normal glucose tolerance group
				60 (47–74)			139	0·07	0·26	0·12	0·38	0·38	Newly diagnosed T2D; *P* (linear trend across first three groups) significant for β-carotene and lycopene
				61 (47–74)			203	0·09	0·41	0·18	0·57	0·45	Previously diagnosed T2D not associated with carotenoids
Abahusain *et al.* [48]*	1999	CC	Saudi Arabia	50 (28–74)			105	0·08	0·28				Serum β-carotene lower in T2D
				49 (27–75)	140			0·08	0·39				
Anderson *et al.* [49]*	1999	T	USA	47	21				0·05				Control group was not well matched for age and TAG
				63			16		0·10				β-Carotene at 24 mg/d (24 weeks) doubled serum β-carotene concentration
Reunanen *et al.* [50]*	1998	Cc	Finland	60 (15–99)			106		1·6				β-Carotene lower in diabetes, but lifestyle factors important confounders of β-carotene/diabetes link
					201				1·9				
Granado *et al.* [25]*	1998	CH	Spain			54	Men	0·06	0·31	0·55	0·25	0·42	T1D not associated with lower carotenoid levels; PVA carotenoids higher in T1D
							Women	0·09	0·44	0·56	0·28	0·40	
					214		Men	0·05	0·22	0·34	0·24	0·38	
					relatives		Women	0·07	0·32	0·47	0·26	0·39	
					236		Men	0·08	0·27	0·39	0·31	0·41	
					unrelated		Women	0·09	0·38	0·60	0·31	0·45	
Olmedilla *et al.* [51]*‡	1997	CC	Spain	32 (5–79)	210		Men	0·05	0·22	0·28	0·25	0·35	
					240		Women	0·06	0·28	0·39	0·24	0·36	
				27 (13–84)		Men	60	0·06	0·28	0·34	0·23	0·42	Dietary intake does not predict serum lycopene level in T2D
						Women	63	0·07	0·36	0·44	0·25	0·42	
Krill *et al.* [52]*	1997	Cc	USA	Not reported	27	13			Not reported				Total serum carotenoids in T1D *v.* non-diabetic relatives were 1·12 *v.* 1·28 μmol/l, respectively (NS)
Rock *et al.* [53]*	1997	CH	USA	57 (21–84)					Not reported				Plasma β-carotene lower in diabetic than non-diabetic ESRD (data not shown)
O'Brien *et al.* [54]*	1996	CH	Australia	40 (21–69)		121			0·48				No association between β-carotene and albuminuria (ACR > 3 mg/mmol)

ND, non-diabetic; T1D, type 1 diabetes; T2D, type 2 diabetes; ACAR, α-carotene; BCAR, β-carotene; BCRY, β-cryptoxanthin; LZ, lutein + zeaxanthin; LYC, lycopene; P, prospective; PVA, pro-vitamin A; T, controlled clinical trial; CC, case–control; H, hospital-based; C, cross-sectional; c, community-based; ESRD, end-stage renal disease; ACR, albumin:creatinine ratio (as indicator of glomerular dysfunction).

* Serum not plasma levels.

† Age-adjusted data.

‡ Median values.

Diabetic retinopathy

We used a mydriatic retinal fundus camera (Kowa FX-500S, Japan) to photodocument retinal status. Diabetic retinopathy grading was based on the EURODIAB protocol (validated against the Airlie House classification) in which the overall grading was that of the worse eye and diabetic retinopathy was defined as more than one microaneurysm and/or haemorrhage[12]. A medical retina specialist, masked to all other participant information, graded the slides on two separate occasions in order to assess internal validity. Agreement between gradings was excellent (κ value of 0·986).

Clinical measures and retinopathy risk factors

Systolic and diastolic blood pressure was recorded using a Dinamap XL portable automated adult vital signs monitor (model 9300; Critikon, FL, USA). Blood pressure was recorded as the average of the last two of three consecutive readings, obtained from the right arm of seated subjects at 1 min intervals after a 10 min rest period. Weight was measured to within 0·1 kg, using digital electronic scales (UC-300; A.N.D, Tokyo, Japan), before breakfast and following a 12 h fast, with subjects wearing light clothing and no shoes. Height was measured to within 0·1 cm using a wall-mounted stadiometer (Harpenden; Holtain Limited, Crymych, UK). BMI was calculated as weight (kg)/height $(m)^2$. A 'current smoker' was defined as a subject who smoked at least seven cigarettes per week at the time of completing the questionnaire.

Plasma biochemistry

A fasting blood sample was drawn between 08.00 and 10.00 hours on the morning of the clinical evaluation. Carotenoids were analysed in plasma separated from blood treated with EDTA as anticoagulant and stored at −80°C and protected from light until analysed. Extraction was as follows: 200 μl of plasma and 200 μl of 95 % ethanol (containing α-tocopheryl acetate (200 ng/ml) and retinyl acetate (750 ng/ml) as internal standards) were placed in 13 × 100 mm borosilicate tubes (Laboratory Supply, Melbourne, VIC, Australia). Hexane (1 ml) containing 0·01 % butylated hydroxytoluene was added. The different phases were then separated by centrifugation at 2000 ***g*** for 10 min. The organic phase was removed by evaporation under N_2. The residue was reconstituted in 30 μl chloroform before the addition of 70 μl acetonitrile–methanol (1:1, v/v) and transferred to light-protected vials maintained at 40°C.

A modified HPLC method, developed and reported elsewhere by Su *et al.*[13] was used to analyse the prepared samples. Calibration and peak identification for the carotenes were achieved by the use of pure standards obtained from Sigma (St Louis, MO, USA), while calibration and peak identification for lutein/zeaxanthin and β-cryptoxanthin were achieved by the use of pure standards obtained from Hoffman La Roche (Basel, Switzerland). The inter-batch CV was <10 % for all analytes except lycopene for which the CV was 12 %.

Plasma glucose concentrations were analysed using an automatic analyser (Hitachi model 705, Tokyo, Japan) and a commercial enzymic kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) by the glucose oxidase method. Plasma cholesterol and TAG concentrations were analysed with an automatic analyser (Hitachi model 705, Tokyo, Japan) using a commercial enzymic kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany).

Urinary biochemistry

Urinary albumin concentration was measured using immunonephelometry (Kallestadt QM300 or Beckman 360 Array nephelometers; inter-assay CV was 3–5 %). Urinary creatinine concentration was measured using an alkaline picrate method (Olympus AU800 autoanalyser; inter-assay CV was 2 %). The urinary albumin:creatinine ratio was then calculated as albumin (mg)/creatinine (mmol).

Statistical analyses

We used SPSS version 13 for Windows (SPSS Inc., Chicago, IL, USA) software to perform the statistical analyses. The data were cross-sectional observations. Descriptive statistics for the exposure and outcome variables were obtained, and variables with distributions that were not normally distributed were log-transformed before analysis. Associations between categorical variables were analysed using χ^2 tests.

Initially, variables assessed in univariate analyses, including known risk factors for diabetic retinopathy, were modelled using binomial logistic regression analysis to determine the best clinical predictors of diabetic retinopathy. Plasma carotenoid concentrations were then added to subsequent models that controlled for the major risk factors for diabetic retinopathy. The fit of each model was tested and the Nagelkerke R^2 approximation was compared. $P<0·05$ was considered statistically significant.

Results

The mean age of the participants was 64 (range 44–77) years. The clinical and demographic characteristics of the participants, according to retinopathy status, are shown in Table 2. Diabetic retinopathy was significantly associated with established risk factors, i.e. duration of diabetes, HbA1c, use of hypoglycaemic medication, and the albumin excretion rate. A longer duration of diabetes was, however, the only independent predictor of diabetic retinopathy, as demonstrated by multivariate modelling of these factors (data not shown). Mean plasma cholesterol and TAG concentrations were in the normal range and not significantly different between retinopathy and non-retinopathy cases. Consequently, subsequent analyses were not adjusted for cholesterol and TAG concentrations.

The observed mean concentration of each carotenoid for both retinopathy and non-retinopathy cases was within the range of means reported in other diabetic populations (Table 1). The observed α-carotene concentrations were at the higher end of values reported in other diabetic populations and were associated with diabetic retinopathy. Conversely, lycopene, a non-PVA carotenoid, demonstrated a trend to lower levels in the retinopathy group (Table 2). In addition, a higher plasma non-PVA:PVA carotenoid ratio was inversely

Table 2. Characteristics of diabetic subjects according to retinopathy status (Prevalence rates or mean values and 95 % confidence intervals)

	Retinopathy absent (*n* 78)		Retinopathy present (*n* 33)		
Characteristic	Mean	95 % CI	Mean	95 % CI	*P*
Age (years)	63	62, 65	65	61, 68	0·504
Fasting glucose (mmol/l)	10·1	9·6, 10·7	10·1	9·2, 11·0	0·895
HbA1c (%)	7·9	7·6, 8·3	8·8	8·2, 9·4	0·018
Diabetes duration (years)	9	7, 10	16	12, 19	<0·0001
Hypoglycaemic medication (%)	63		88		0·009*
BMI (kg/m^2)	29	28, 30	28	27, 30	0·228
Systolic blood pressure (mmHg)	142	137, 146	149	141, 156	0·114
Diastolic blood pressure (mmHg)	75	73, 77	74	71, 77	0·638
Current smoker (%)	13		6		0·311*
Urinary albumin excretion rate (μg/min)	47	22, 71	174	46, 302	0·008
Total cholesterol (mmol/l)	5·3	5·0, 5·5	5·0	4·7, 5·3	0·176
HDL-cholesterol (mmol/l)	1·1	1·0, 1·2	1·2	1·1, 1·3	0·375
TAG (mmol/l)	2·0	1·6, 2·3	1·7	1·4, 1·9	0·280
α-Carotene (μmol/l)	0·08	0·07, 0·10	0·12	0·09, 0·16	0·029
β-Carotene (μmol/l)	0·37	0·29, 0·44	0·40	0·29, 0·51	0·576
Cryptoxanthin (μmol/l)	0·21	0·16, 0·26	0·21	0·16, 0·26	0·940
Lutein + zeaxanthin (μmol/l)	0·36	0·30, 0·41	0·34	0·27, 0·42	0·736
Lycopene (μmol/l)	0·45	0·39, 0·51	0·36	0·30, 0·42	0·079

* Pearson χ^2 *P* values.

associated with diabetic retinopathy (1·6 (95 % CI 1·4, 1·7) *v.* 1·2 (95 % CI 1·0, 1·4), respectively; $P=0·009$).

In multivariate modelling of carotenoids as predictors of diabetic retinopathy (Table 3), the odds of diabetic retinopathy increased with higher plasma concentrations of the PVA carotenoids (model 1). The ratio of non-PVA:PVA carotenoid concentrations was the best carotenoid-related predictor of diabetic retinopathy. A higher combined plasma concentration of lycopene and lutein/zeaxanthin was associated with significantly lower odds of diabetic retinopathy, after adjusting for potential confounding by retinopathy risk factors (model 2).

Discussion

To date, little is known of the role of carotenoids in diabetes and its complications. Consequently, costly intervention studies of the potential impact of carotenoids on diabetic retinopathy are not yet justifiable, despite evidence of a biologically plausible mechanism, oxidative stress. The present cross-sectional study evaluated the association between diabetic retinopathy and the concentrations of major plasma carotenoids, which in turn are largely, but not exclusively, dependent on dietary intake[14]. Our key finding supports a protective role for a higher combined lutein/zeaxanthin and lycopene concentration against diabetic retinopathy, after adjustment for potential confounders. There are important distinctions between lutein/zeaxanthin and lycopene (non-PVA carotenoids) and the other three major (PVA) carotenoids in humans. Lutein/zeaxanthin and lycopene are absorbed intact, unlike α- and β-carotene and β-cryptoxanthin, which can be cleaved to form retinol and, before absorption, are partly metabolised to vitamin A in the intestinal mucosa[15]. Whether competitive inhibition influences PVA carotenoid cleavage at particular intake levels is not well understood. Furthermore, β-carotene is ubiquitous and food sources of various carotenoid combinations differ: lycopene and β-carotene, but not α-carotene, are present in tomatoes, while carrots and pumpkin are good sources of α-carotene and β-carotene, but not lycopene, and cryptoxanthin is primarily found in citrus fruits.

Apart from the key structural role of lutein and zeaxanthin as the macular pigments in the eye, evidence is emerging of a wider role for lutein in chronic disease prevention[16]. In particular,

Table 3. Adjusted regression models of plasma carotenoids (μmol/l) as predictors of diabetic retinopathy*

Model	R^2	Independent variable	B	SE	Exp(B)	95 % CI for Exp(B)	*P*
1	0·32	Duration of diabetes (years)	0·11	0·04	1·12	1·03, 1·20	0·004
		HbA1c (%)	0·17	0·16	1·19	0·87, 1·63	0·280
		Hypoglycaemic medication(s) (%)	0·37	0·76	1·45	0·33, 6·40	0·622
		Albumin excretion rate (μg/min)	0·00	0·00	1·00	1·00, 1·01	0·105
		PVA carotenoids (μmol/l)	1·09	0·55	2·97	1·00, 8·79	0·049
		Non-PVA carotenoids (μmol/l)	−1·16	0·66	0·31	0·09, 1·14	0·077
2	0·34	Duration of diabetes (years)	0·11	0·04	1·12	1·04, 1·20	0·004
		HbA1c (%)	0·17	0·16	1·19	0·87, 1·63	0·282
		Hypoglycaemic medication(s) (%)	0·40	0·74	1·49	0·35, 6·33	0·592
		Albumin excretion rate (μg/min)	0·00	0·00	1·00	1·00, 1·01	0·107
		Non-PVA:PVA ratio	−1·11	0·54	0·33	0·12, 0·95	0·039

PVA, pro-vitamin A carotenoids (α- + β-carotene + cryptoxanthin); non-PVA, non-pro-vitamin A carotenoids (lutein/zeaxanthin + lycopene).
* Carotenoid and albumin excretion rate data are log-transformed.

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lutein has been shown to attenuate oxidative stress in experimental models of early diabetic retinopathy[17,18]. A role for lycopene in ocular health is also plausible; lycopene is present in high concentrations in human ocular tissues[19], is the most potent carotenoid quencher of singlet oxygen[20], and has other important functions. *In vitro* studies have shown that lycopene can inhibit proliferation and induce differentiation of human blood cells, activate genes involved in cell-to-cell communication, and modulate lipoxygenase activity and therefore inflammation and immune function[21]. According to *in vitro* studies and animal research[9], lycopene can attenuate oxidative stress-induced experimental cataract in rat lenses via an antioxidant mechanism involving restoration of levels of endogenous antioxidant enzymes, such as superoxide dismutase and catalase. In one study, lycopene-treated diabetic rabbits demonstrated an increase in antioxidant activity in ocular capillaries and lacrimal fluid[22]. Low plasma lycopene concentrations have been associated with the very early stages of vascular disease in humans[23], indicating that lycopene contributes to the expression of the disease. Furthermore, lycopene bioavailability is impaired in older individuals[7]. In contrast, β-carotene bioavailability is maintained with age, as is the intestinal conversion of PVA carotenoids to vitamin A. Recently, a double-blind placebo-controlled clinical trial found a physiological dose of lycopene for 2 months suppressed oxidative stress and enhanced innate immunity in individuals with type 2 diabetes[24].

We also observed an association between retinopathy and plasma PVA carotenoid concentrations. This finding is consistent with one study in which individuals with type 1 diabetes had higher plasma concentrations of PVA (but not non-PVA) carotenoids and lower levels of retinol than their first-degree relatives[25], suggesting impaired bioconversion of carotenoids to retinol. Diabetes may also promote higher levels of PVA carotenoids *via* another mechanism, such as down-regulation of the bioconversion of PVA carotenoids to retinol, secondary to a nephropathy-induced accumulation of retinol, levels of which are homeostatically controlled[26,27] and can be higher in renal disease[28,29] and diabetes[30]. Importantly, our observation does not demonstrate a negative role of dietary PVA carotenoids. Prospective studies are needed to further evaluate the association between retinopathy and plasma PVA carotenoid concentrations.

Different food sources and intake levels may in part explain the large variation in the ratio of circulating α-carotene: β-carotene reported in previous studies (Table 1). In addition, previous studies point to a complex interplay between carotenoids: non-PVA carotenoids (lutein/zeaxanthin and lycopene) can diminish PVA carotenoid bioavailability. Moreover, synergies have been demonstrated between non-PVA carotenoids[31], and competitive inhibition has been demonstrated between carotenoids, for example, for incorporation into chylomicrons[32–34]. It is also noteworthy that while carotenoid levels reflect relatively recent intake, evidence suggests that most individuals maintain relatively consistent eating patterns over time, with seasonal fluctuations affecting only cryptoxanthin and β-carotene levels[35].

The main limitation of the present study is its observational nature. The lack of temporal direction prevents us from inferring causality. Consequently, prospective studies of diabetic retinopathy are needed to determine whether over time a higher combined plasma concentration of lutein/zeaxanthin and lycopene and/or a higher plasma non-PVA:PVA carotenoid ratio can reduce the risk of diabetic retinopathy. Residual confounding may have played a role in our observations, although sex, a key determinant of plasma carotenoid levels, was not a confounder in the present study as we selected only men with type 2 diabetes. However, the interaction between different food components may have confounded the present results. Furthermore, plasma carotenoids may be a marker for any of the many non-nutrient food components in vegetables and fruits[36]. Finally, although we used a validated protocol for retinal evaluation, our assessment of retinopathy may have underestimated diabetic maculopathy.

In conclusion, synergies between plasma carotenoids seem to be implicated in diabetic retinopathy, independent of established risk factors. In general, the present study provides additional data concerning the importance of carotenoid-rich foods for health maintenance and gives strength to the recommendation of increasing consumption of lutein- and lycopene-rich foods.

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