Plasma carotenoids and onset of dysglycemia in an elderly population: results of the Epidemiology of Vascular Ageing Study.

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Plasma carotenoid and onset of dysglycemia in an elderly population: results of the EVA study

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ABSTRACT

Objective: The hypothesis of carotenoid having a preventive role in diabetes is suggested by their antioxidant properties. In this report, we investigated the relationship between baseline total plasma carotenoid levels and 9-year onset of dysglycemia (Impaired Fasting Glucose or type 2 diabetes) in a healthy elderly population.

Research Design and Methods: The EVA ("Epidemiology of Vascular Ageing") study is a 9-year longitudinal study including 1389 volunteers aged 59-71. Fasting plasma glucose was measured at baseline, 2, 4 and 9 year after inclusion. The relations between plasma carotenoid at baseline and incidence of dysglycemia were determined by Cox proportional hazards regression analysis adjusting for potential confounders.

Results: At 9-year, 123 incident cases of dysglycemia had occurred. Risk of dysglycemia was significantly lower in participants with plasma carotenoid in highest quartile Q4 compared to participants with lowest quartile Q1 (Q4 vs. Q1: RR=0.26 [0.14; 0.49], $p<10^{-4}$; Q3 vs. Q1: RR=0.55 [0.34; 0.89], p=0.01; Q2 vs. Q1: RR=0.82 [0.51; 1.31], p=0.40). After controlling for socio-demographic variables, lifestyle habits, cardiovascular diseases, blood pressure, BMI and lipid profile, risk of dysglycemia remained significantly lower in participants in the highest quartile of total plasma carotenoid compared to participants in the lowest quartile (Q4 vs. Q1 : 0.42 [0.22;0.82], p=0.01; Q3 vs. Q1 : 0.69 [0.41;1.15], p=0.16; Q2 vs. Q1 : 0.80 [0.48;1.32], p=0.38).

Conclusions: This study confirms prospectively that plasma carotenoid levels have an independent relationship to onset of dysglycemia.

Keywords:

Total plasma carotenoid

Diabetes

Impaired Fasting Glucose

Elderly population

Prospective study

Carotenoids are natural pigments, synthesized by plants and micro-organisms, but not by animals nor by humans. These pigments are found in food, especially in fruits and vegetables. It is highly suggested that they play a protective role in chronic diseases (1) or cancers. Even if the biological mechanisms for such a protection are currently unclear (2), their protective effects could come from their antioxidant properties (3).

Several cross-sectional (4-6) and case-control studies (7-9) have shown an inverse relationship between serum carotenes and type 2 diabetes status. A longitudinal study on dietary intake of antioxidant found a significant relationship between β-cryptoxanthin intake and reduced risk of type 2 diabetes (10), however the association between serum carotenoid and diabetes was called into question by the results of two other longitudinal studies (11-12) and one randomized double-blind trial (13).

To investigate if carotenoids could have a role in diabetes incidence in the elderly, possibly through their antioxidant capacity, we explored the relationships between total plasma carotenoid at baseline and 9-year occurrence of type 2 diabetes or IFG in a healthy elderly population

RESEARCH DESIGN AND METHODS

The EVA study ("Epidemiology of Vascular Ageing") is a nine-year longitudinal study with 6 waves of follow-up (14) . At baseline, (EVA0, 1991-1993), 1389 volunteers (574 men and 815 women) born between 1922 and 1932 (mean age = 65) residing in the town of Nantes (Western France) were recruited from electoral rolls, and to a lesser extent, via information campaigns. The subsequent follow-up waves with biological measurements were EVA2 (2-year follow-up, n=1272), EVA3 (4-year follow-up, n=1188) and EVA6 (9-year follow-up, n=781). The study protocol was approved by the

Ethical Committee of the University Center Hospital of Kremlin-Bicêtre, Paris. Signed informed consent was obtained from all participants at enrolment. For the purpose of the present paper, analyses were carried on the 1165 participants who were normoglycemic at inclusion defined by a fasting blood glucose (FBG) ≤ 6.1 mmol/L and no anti-diabetic drugs, and for whom plasma carotenoid measurement was available.

Blood samples were drawn between 8.30 am and 9.30 am after a 12-hour fast. Biological procedures for the determination of glucose levels have been described elsewhere (15). According to WHO definition (16), participants with a FBG≥7.00 mmol/L or who used anti-diabetic drugs were defined as diabetic. Participants with impaired fasting glucose (IFG) were characterized by a 6.1 >FBG <7 mmol/L (16). Dysglycemia was defined by presence of IFG or diabetes status. During the 9-year follow-up, cumulative incidence of dysglycemia was considered. To account the reversibility of IFG, we defined "persistent IFG" as participants with dysglycemia at least 2 times on the 3 follow-up waves.

At baseline the general questionnaire allowed us to obtain information on socio-demographic factors (sex, age, education), lifestyle habits (smoking habits and alcohol intake). In addition, height and weight were measured. Two independent measures of systolic and diastolic blood pressure were taken with a digital electronic tensiometer after a 10-minute rest. Health characteristics considered in these analyses were BMI, total cholesterol/HDL cholesterol ratio, use of lipid lowering drugs, hypertension (systolic or diastolic blood pressure ≥ 140 or ≥ 90 mm Hg respectively or use of hypertensive drugs), history of vascular diseases (self-reported history of myocardial infarction, angina pectoris, stroke).

Total plasma carotenoid was determined only at baseline by using a spectrophotometric assay. Samples were immediately frozen at -20°C then stored at -80°C, and all the determinations were done at the same time, 2 years after the first inclusion. After precipitation of plasma proteins with ethanol, carotenoid were extracted with hexane and measurements of absorbance on the hexane phase at 350, 450 and 550nm were performed (spectrophotometer Uvikon 860, Kontron, Rotkreuz, Switzerland). Concentrations were calculated on the basis of a molecular extinction factor at 450 nm of 134,000 L/mol/cm. Absorbance values at 350 and 550 nm were used to correct the absorbance obtained at 450 nm by applying an adequate equation. Coefficients of intra- and inter-assay variation were 5.4% and 4.9%, respectively.

Statistical Methods

Sex, education (≤ primary school / ≥high school), smoking status (current, ex-smokers /non-smokers), alcohol intake (≥ 20ml / < 20ml per day), cardiovascular diseases antecedents (yes/no), and use of lipid lowering drugs (yes/no), were considered as categorical variables. Age, BMI, diastolic and systolic blood pressure and total cholesterol/HDL cholesterol ratio were considered as continuous variables. Total plasma carotenoid level was considered by quartile, the median and range values for each quartile were 1.42µmol/L, [0.21-1.82] for Q1 ($<25^{th}$), 2.16µmol/L, [1.83-2.53] for Q2 ($\geq 25^{th}$ $<50^{th}$), 2.90µmol/L, [2.55-3.43] for Q3 ($\geq 50^{th}$ $<75^{th}$) and 4.14 µmol/L, [3.44-10.1] for Q4 ($\geq 75^{th}$). By using the Student t-test (for continuous variables) and the chi-square test (for categorical variables), baseline characteristics were compared between participants who developed dysglycemia during the follow-up and those who did not, and between participants who completed the 9-year follow-up and those who did not. Survival analyses by actuarial methods were used to assess the probability to not develop dysglycemia

according to levels of plasma carotenoid. The effects of total plasma carotenoid levels on onset of dysglycemia were determined by Cox proportional hazards regression models in which years of age during the study was used as the time axis, with left truncation at age of entering the study. To take into account the reversibility of IFG, additional sensitivity analyses were performed after considering persistent IFG. To explore whether relationships with carotenoids could be confounded by their antioxidant capacity, similar analyses were performed on participants (n=875) for whom different markers of anti-oxidant status such as TBARS, vitamin E, activity of glutathione peroxydases and superoxyde dismutase were measured as baseline (method described in Berr et al. (15)). The proportional hazards assumption was verified by adding a time-dependent variable to the model. Results of Cox multivariate regressions were expressed by Hazard Ratio (HR) with their confidence interval (CI) at 95 %. All interactions between total plasma carotenoid and other variables were tested. Statistical analyses were performed using SAS software version 9.1 (SAS Institute, Inc. Cary, North Carolina).

RESULTS

During the 9-year follow-up, 127 new cases of dysglycemia (including 27 cases of type 2 diabetes) occurred. 67 (including 9 cases of diabetes) occurred during the first two year of follow-up (EVA2- baseline). 40 (including 9 cases of diabetes) occurred between EVA2 and EVA3, and 23 (including 9 of diabetes) occurred between EVA3 and EVA6. Characteristics of the 635 participants who completed the 9-year follow-up, were compared to the 530 who did not (including 101 deaths). Participants who did not complete the whole study were more likely to be current or former smokers (43.4% vs. 37.5% p=0.04), to have higher baseline diastolic blood pressure (79.5 ±11.0 vs. 78. 2±

10.7 mm Hg, p=0.03) and lower baseline total plasma carotenoid levels (2.71 ± 1.23 vs. 2.91±1.32 μmol/L, p=0.008).

Among the 127 participants who developed a dysglycemia during the 9-year follow-up, proportion of men, current or former smokers and regular consumer of alcohol was significantly higher compared to the 1042 participants who did not developed dysglycemia (Table1). A significantly higher BMI, diastolic and systolic blood pressure and lower HDL cholesterol concentrations at baseline, were also observed in the dysglycemic group. No significant difference was observed in baseline total cholesterol, lipid lowering drugs users cardiovascular diseases history and age between the two groups. Bivariate analyses also showed total baseline plasma carotenoid levels were significantly lower in participants who developed dysglycemia compared to those who did not (Table1).

Factors associated with total plasma carotenoid at baseline are described in Table 2. A higher proportion of women was found in higher quartiles of total plasma carotenoid, and higher percentage of smokers and alcohol consumers were observed in lower quartiles. BMI, diastolic and systolic blood pressure and cardiovascular disease history decreased with higher levels of plasma carotenoid. High levels of carotenoid were associated with an increase of total cholesterol and a decrease of HDL cholesterol, and there was a higher proportion of lipid lowering users in low quartiles of plasma carotenoid levels.

Comparisons of survival distributions between quartiles of plasma carotenoid showed that the lower the quartile, the greater the occurrence of dysglycemia (Figure 1). The analysis showed that risk of dysglycemia decreased significantly in participants with plasma carotenoid in Q4 and Q3 compared to participants in Q1 (Q4 vs. Q1: RR=0.26 [0.14; 0.49], p<10-4; Q3 vs. Q1: RR=0.55 [0.34; 0.89], p=0.01; Q2 vs. Q1: RR=0.82

[0.51; 1.31], p=0.40). Interactions tested between plasma carotenoid and different characteristics were not statistically significant. Results of multivariate Cox models (Table 3), after controlling for socio-demographic factors, smoking habits, alcohol intake, cardio-vascular disease history, blood pressure, BMI and lipid profile, showed that risk of dysglycemia during the 9 year follow-up remained significantly lower in participants in the highest quartile of total plasma carotenoid compared to participants in the lowest quartile (Q4 vs. Q1 : 0.42 [0.22;0.82], p=0.01). The other adjusted risks did not remained significant (Q3 vs. Q1 : 0.69 [0.41;1.15], p=0.16; Q2 vs. Q1 : 0.80 [0.48;1.32], p=0.38). To take into account reversibility of IFG, sensitivity analyses with persistent IFG as an end point were performed and showed similar graded association between quartiles of carotenoids and dysglycemia (data not shown).

To test if the relation was maintained after adjustement for antioxidant markers, similar analyses were performed after adjustment for markers of antioxidant status (TBARS, vitamin E, activity of glutathione peroxydases and superoxyde dismutase). Relative risks of dysglycemia associated to quartile of total plasma carotenoids levels were similar to those described in Table 3 (Q4 vs. Q1 : 0.38 [0.19;0.79], p=0.009, Q3 vs. Q1 : 0.66 [0.40;1.15], p=0.15; Q2 vs. Q1 : 0.74 [0.43;1.27], p=0.28). None of the markers of antioxidant stress measured at baseline was significantly associated with 9-year risk of dysglycemia.

CONCLUSIONS

Our results showed that high levels of plasma carotenoid at baseline were significantly associated with a lower 9-year risk of onset of dysglycemia, in a healthy elderly population, independently of factors classically associated with dysglycemia: socio-demographic factors, lifestyle habits, cardio-vascular disease history, blood

pressure, BMI and dyslipidemia. Furthermore, the relation persisted after adjustment for markers of antioxidant status (TBARS, vitamin E, activity of glutathione peroxydase and superoxyde dismutase). This suggests that plasma carotenoids could be in a direct relation with dysglycemia. To our knowledge, our study is one of the few that explore longitudinally the relationship between carotenoid and dysglycemia.

Many cross sectional studies have shown this association (4-9). In a population at high risk of type 2 diabetes, the Botnia Dietary Study showed that high dietary intake of α-carotene, β-carotene and lycopene, and high plasma β-carotene concentration were beneficially associated with glucose metabolism in participants (6). The Third National Health and Nutrition Examination Survey (5) and the Australian Diabetes, Obesity and Lifestyle Study (4) showed a significant association between low serum β-carotene levels and type 2 diabetes. In a previous publication, on baseline data of the EVA study, we also reported a significant correlation between total plasma carotenoid and glycemia (17). Finally, case-control studies also found lower plasma carotenoid levels in diabetic patients than in controls (8-9) and one of them was carried out on an elderly population (8). However in this cross sectional framework it is not possible to know if low levels of carotenoid in diabetic participants are a consequence-the results of increased utilization due to the oxidative stress effects of the diseases- or a cause : are the low concentrations involved in the pathogenesis of the diseases?

Our longitudinal findings confirm results from the prospective study led by Montonen et al. (10) (23-years of follow-up) which showed, in a cohort of 4303 participants free of diabetes at baseline, that β-cryptoxanthin intake was significantly associated with a reduced risk of diabetes of type 2 (RR=0.58 [0.44 ;0.78]). Other

longitudinal results are more conflicting. In a nested case-control study (11) (106 cases and 201 controls, mean age=59.9 years), risk of diabetes was found to be lower in the highest serum β-carotene tertile group compared to the lowest tertile, however the significant association did not remain after adjustment for cardiovascular risk factors (RR=0.94 [0.38;2.32]). More recently, results of the nested case-control study (470 cases, 470 controls) carried on middle-aged and older US women (age>45 years), led by Wang et al. (12), did not confirm the prospective association between different baseline plasma carotenoids compounds (lycopene, α-carotene, β-carotene, β-cryptoxanthin, lutein and/zeaxanthin) and risk of type 2 diabetes. The different methods and the biovariability of carotenoids, which is influenced by several factors such as characteristics of the food sources, interactions with other dietary factors and various participant characteristics, could explain the differences between studies. Finally a double blind randomised controlled trial (13) of 12-year supplementation of beta carotene in healthy US male cohort (n=22071, 40-84 years), did not show a significant benefit on risk of type 2 diabetes mellitus (396 incident cases in supplemented group vs. 402 cases in placebo group, RR=0.98 {0.85;1.12]). However, results of this trial should be interpreted with caution as type 2 diabetes was not the primary end-point and diagnosis of diabetes was self-reported without screening for glucose tolerance.

Our study has limitations. The EVA study included volunteers with higher educational status, higher incomes than the average elderly French population, however this should not have any effects on the relation between plasma carotenoid levels and dysglycemia. For reasons of statistical power (only 27 incidents cases of diabetes), we considered IFG in the dysglycemia definition. IFG is a risk situation for diabetes however we cannot exclude that some participants have reversed to normal. Thus, we cannot

exclude that we overdiagnosed dysglycemia. However, if this is the case, it is likely to have weakened the true association between carotenoid and dysglycemia. Moreover our sensitivity analyses using persistent IFG definition showed that the relation was maintained. Another limitation comes from our carotenoids measurement insofar as only one measurement was made using a spectrophotometry method while reversed-phase high performance liquid chromatography method provides more accurate measures with information on different carotenoids. Unfortunately, in 1991 when the EVA study started this assay was not available for epidemiological purpose. Furthermore, the blood samples were equally collected throughout the year in EVA study. Finally, another limitation is the high rate of attrition in this cohort. Participants lost to follow-up had lower baseline total plasma carotenoid. The most probable is that caused an underestimation of incidence of dysglycemia and consequently decreased the power of the study. Despite these limitations, the relationships we showed were strong, almost in a dose-dependent fashion and remained significant in the highest quartile after adjusting for various potential confounders.

Currently, the mechanism of this potential relationship is still under debate and, as it has been described by Paiva et al., several hypotheses can explain this observation (2). One of them involves the antioxidant properties. In our study, analyses were repeated after controlling on various antioxidative markers, and our results remained unchanged suggesting that the association between total plasma carotenoids and diabetes observed in our cohort is independent of the oxidative stress status of subjects. Other human studies have failed to show a clear benefit of antioxidants as it showed in the recent review of Yim et al.(18), whereas some studies have even suggested that they can be potentially harmful (19).

High plasma carotenoid is also a marker of fruits and vegetable consumption (20). A reduced risk of type 2 diabetes with vegetables consumption was suggested in several studies (21-22) but not all (23). This possible protective effect of vegetables and fruits consumption in diabetes described in these studies could result from the combined action of many protective compounds including antioxidants and could explain the controversial literature results between studies which were interested in blood measurement levels of carotenoids and those which were interested in carotenoids rich-vegetables and fruits consumption.

Finally we cannot exclude that carotenoids might have been serving as markers for other protective lifestyle habits and health behaviours but are not acting as effective agents themselves.

In conclusion, our results bring support to a possible role of carotenoids in onset of IFG and type 2 diabetes in elderly people. Further studies are necessary to explore the mechanism which could explain the relationship, and hopefully design original measures which could help preventing dysglycemia.

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TABLES AND FIGURES LEGENDS

Table 1: Comparison of baseline characteristics between participants who developed diabetes / IFG during the 9-year follow-up and those who did not. Results of bivariate analyses.

* analyses were performed on 1164 participants for education level, 1144 for alcohol consumption 1164 for blood pressure, 1162 for BMI, 1121 for LDL cholesterol , 1130 for HDL cholesterol, 1139 for total cholesterol.

Table 2: Factors associated to total plasma carotenoid at baseline

Table 3: Effects of total plasma carotenoid on onset of dysglycemia during the EVA follow-up: Results of multivariate Cox Proportional Hazards Regression Analyses.

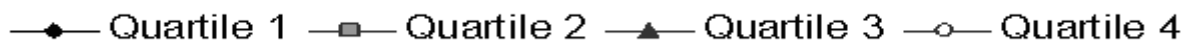
* adjustment for all variables listed in the table, model performed on 1035 participants

† p trend

Figure 1: Non occurrence distributions for each total plasma carotenoid quartile groups

Black diamond-shaped : Quartile 1; Grey rectangle : Quartile 2; black triangle : Quartile 3; White round : Quartile 4.

Quartile 1: < 1.82 µmol/L, Quartile 2 :1.82-2.55 µmol/L, Quartile 3 : 2.55-3.433 µmol/L, Quartile 4 : 3.43 µmol/L



TABLES

TABLES

Table 1: Comparison of baseline characteristics between participants who developed dysglycemia during the 9-year follow-up and those who did not. Results of bivariate analyses.

	Participants who		
	Did not developed dysglycemia	Developped Dysglycemia	
	N=1038	N=127	
	% or m ± SD	% or m ± SD	p-value
Socio-demographic factors			
Women	63.9	44.7	$<10^{-4}$
Age at baseline (years)	65.0 ±3.0	64.9±3.1	0.72
High Education*	48.5	47.1	0.78
Consumption factors			
Current/Former Smokers	38.7	52.8	0.002
Alcohol consumer > 20 ml / day*	26.0	39.2	0.002
Health factors			
BMI (Kg/ m^2)*	24.7±3.4	27.0±3.7	$<10^{-4}$
HDL cholesterol (mmol/L)*	1.69±0.43	1.50±0.42	$<10^{-4}$
Total cholesterol (mmol/L)*	6.43±1.01	6.30±0.98	0.17
total/HDL cholesterol ratio *	4.03±1.15	4.49±1.45	0.01
Lipid lowering drugs user	22.8	27.6	0.23
Diastolic blood pressure (mm Hg)*	130.0±17.6	136.4±16.0	0.0001
Systolic blood pressure (mm Hg)*	78.4±10.8	82.0±11.1	0.0006
Cardiovascular diseases antecedents	8.6	8.9	0.91
Total Plasma carotenoid (µmol/L)	2.87±1.29	2.37±1.10	$<10^{-4}$

Table 2: Factors associated to total plasma carotenoid at baseline

	Plasma carotenoid quartiles			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	<1.82 µmol/L	[1.82 -2.55[µmol/L	[2.55-3.43[µmol/L	≥3.43 µmol/L
	% or m± SD	% or m± SD	% or m± SD	% or m± SD
Socio-demographic factors				
Women	40.1	52.5	65.8	83.2
Age at baseline (years)	64.7±2.9	65.3±2.8	65.0±3.1	64.8±3.0
High Education*	43.0	49.3	50.8	49.4
Consumption factors				
Current/Former Smokers	56.2	48.2	37.9	22.5
Alcohol consumer(>20 ml/d)	41.2	33.2	24.7	14.1
Health factors				
BMI (Kg/ m^2)	26.5±3.7	25.4±3.4	24.6±3.3	23.6±3.0
HDL cholesterol (mmol/L)	1.55±0.41	1.56±0.35	1.71±0.42	1.82±0.46
Total cholesterol (mmol/L)	5.98±0.99	6.23±0.91	6.47±0.91	6.86±1.01
total/HDL cholesterol ratio	4.10±1.13	4.22±1.37	4.01±1.09	4.00±1.17
Lipid lowering drugs user	32.5	26.9	20.4	15.9
Diastolic blood pressure (mm Hg)	81.2±11.8	79.8±11.0	77.9±10.8	76.9±9.7
Systolic blood pressure (mm Hg)	133.4±18.1	134.2±18.0	130.0±18.0	126.2±15.1
Cardiovascular diseases antecedents	13.2	11.7	4.70	6.3

Table 3: Effects of total plasma carotenoid on onset of dysglycemia during the EVA follow-up: Results of multivariate Cox Proportional Hazards Regression Analyses.

		Multivariate models*			
Complete model		HR	CI at 95 %	p	
Total Plasma Carotenoid (by µmol/L)	Q4 vs. Q1	0.42	0.22 ; 0.82	0.01	
	Q3vs. Q1	0.69	0.41 ;1.15	0.16	0.08†
	Q2 vs. Q1	0.80	0.48 ;1.32	0.38	
Sex		0.90	0.53 ; 1.54	0.71	
Education (High vs Low level)		0.91	0.62 ; 1.34	0.63	
Smoking habits (Current/Former vs. Non smoker)		1.08	0.67 ; 1.75	0.74	
Alcohol intake (>20ml vs. ≤ 20ml)		1.18	0.75 ; 1.85	0.47	
Diastolic blood pressure (by mm Hg)*		0.99	0.96 ; 1.01	0.37	
Systolic blood pressure (by mm Hg)*		1.01	0.99 ; 1.03	0.11	
Cardiovascular diseases antecedents (Yes vs. No)		0.69	0.34 ; 1.40	0.30	
total/HDL cholesterol ratio		1.22	1.06 ; 1.40	0.005	
Lipid lowering drugs user (Yes vs. No)		1.30	0.85 ; 1.98	0.22	
BMI (by Kg/ m^2)		1.11	1.05 ; 1.17	0.0002	