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Plasma carotenoids and risk of acute myocardial infarction in The Singapore Chinese Health Study

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Abstract

Background—Modification of low density lipoprotein due to oxidative stress is essential in the development of coronary atherosclerosis. Data of specific carotenoids except β-carotene on cardioprotective effects in humans are limited.

Objective and methods—This study examined the associations between plasma concentrations of specific carotenoids and incidence of acute myocardial infarction. The study included 280 incident cases of acute myocardial infarction and 560 matched controls nested within the Singapore Chinese Health Study, a prospective cohort of 63,257 Chinese men and women aged 45 to 74 years old enrolled in 1993-1998 in Singapore. Retinol and carotenoids in prediagnostic plasma were quantified using high-performance liquid chromatography.

Results—High levels of plasma β-cryptoxanthin and lutein were associated with decreased risk of acute myocardial infarction after adjustment for multiple risk factors for coronary heart disease. For β-cryptoxanthin, the odds ratio (95% confidence interval) for the highest (Q5) versus the lowest (Q1) quintile was 0.67 (0.37-1.21) (*P* for trend = 0.03). For lutein, the odds ratios (95% confidence intervals) for Q2-Q3 and Q4-Q5 versus Q1 were 0.71 (0.45-1.12) and 0.58 (0.35-0.94) respectively (*P* for trend = 0.03). There was no statistically significant association between other carotenoids or retinol and risk of acute myocardial infarction.

Conclusions—High plasma levels of β-cryptoxanthin and lutein were associated with decreased risk of acute myocardial infarction. The findings of this study support a cardioprotective role of these two carotenoids in humans.

Keywords

antioxidants; carotenoids; coronary disease; nested case-control study; Chinese

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Introduction

Coronary heart disease (CHD) remains the top cause of death in the US and other developed countries, and has increasingly become a major health problem in developing countries as well [1]. Among the clinical spectrum of CHD, acute myocardial infarction (AMI) has the highest morbidity and mortality [2]. While smoking cessation and control of body weight, hypertension, diabetes mellitus and hyperlipidaemia remain the main options for primary prevention of CHD, identification of other modifiable risk/protective factors would lead to the development of more effective and efficient strategy for primary prevention of CHD.

The free radical theory of aging posits that oxidative stress is among the major mechanisms in aging and age-related disease, including CHD [3]. Studies on human tissues have supported the role of low-density lipoprotein (LDL) oxidation in atherosclerosis [4], thus leading to the hypothesis that antioxidants such as carotenoids could be used as an inexpensive means of prevention and possibly, treatment of CHD and other cardiovascular-related diseases. Although several large epidemiologic cohort studies found an inverse association between β-carotene and cardiovascular disease risk, randomized controlled trials failed to demonstrate a consistent benefit of supplementary β-carotene on CHD prevention [5]. While the reason for this apparent inconsistency between observational and interventional studies remains obscure, a likely explanation is that the β-carotene-CHD association noted in the former studies is merely marking the association between one or more unmeasured factor(s) that exert cardioprotective effect. Hence, there is a need for prospective studies that can provide data on multiple carotenoids and other antioxidants measured simultaneously in relation to CHD risk.

Utilizing the database of the Singapore Chinese Health Study, we conducted a case-control study nested within this established cohort of Chinese men and women in Singapore to simultaneously investigate the roles of antioxidants, including specific carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthins), retinol, vitamin E (tocopherols and tocotrienols) and coenzyme Q_{10} (ubiquinone and ubiquinol) in the etiology of AMI, the prototype of CHD. In the present report, we focused on the role of individual carotenoids and retinol in the initial incidence of AMI.

Materials and methods

Study population

The subjects were participants of the Singapore Chinese Health Study, a population-based, prospective cohort of 63,257 Chinese women and men who were aged 45-74 years at recruitment between April 1993 and December 1998 [6]. They were recruited from among the 86% of the Singapore population who resided in government housing estates. We restricted study subjects to the two major dialect groups of Chinese in Singapore, the Hokkiens and the Cantonese. The Institutional Review Boards at the National University of Singapore and the University of Minnesota had approved this study.

At recruitment, a face-to-face interview was conducted in the subject's home by a trained interviewer using a structured questionnaire for the collection of information on demographics, smoking, physical activity, menstrual and reproductive history (women only), medical history, and family history of cancer. The validated semi-quantitative food frequency questionnaire was used for the assessment of current dietary intake [6]. For the assessment of physical activity, subjects were asked to estimate the number of hours spent watching television per day, and the numbers of hours per week spent on moderate activities such as brisk walking, bowling, bicycling on level ground, tai chi or chi kung, and on

strenuous sports such as jogging, bicycling on hills, tennis, squash, swimming laps or aerobics.

Between April 1994 and July 1999, we attempted to collect blood and single-void urine specimens from a random 3% sample of study enrollees. Details of the biospecimen collection, processing and storage procedures have been described previously [7]. Between January 2000 and April 2005, we extended our biospecimen collection to all surviving cohort members and collected biospecimens from 32,543 subjects, representing a consent rate of about 60% of surviving cohort participants. During the visits at the subjects' homes for the collection of biospecimens, systolic (SBP) and diastolic (DBP) blood pressures were also measured for each subject using a standard protocol. The means of the three readings of the SBP and DBP, respectively, were used in the statistical analysis.

Case ascertainment

Participants of the Singapore Chinese Health Study who were free of coronary heart disease or stroke at baseline and who donated blood samples prior to the initial occurrence of AMI were eligible for the present study. We identified incident AMI cases through 2 independent courses. 1) A computer linkage was carried out between the cohort database and the Singapore Myocardial Infarction Registry (SMIR). This registry, established in 1986, is a centralized population-based AMI registry that captures records of AMI patients in Singapore who were either discharged from hospitals with an AMI diagnosis, who died from AMI, who had evidence of AMI in postmortem autopsy, or who had plasma creatinine phosphokinase greater than 400 IU in a clinical laboratory test. All registered AMI cases are classified, according to WHO's diagnostic categories [8] into definite AMI, probable AMI, ischemic cardiac arrest, clinical AMI, or death with or without necropsy. Only definite or probable AMI cases were eligible for the present study. Up to 31 December 2002 when the registry had complete datasets available to us, we identified 78 incident cases of definite or probable AMI. 2) We also carried out linkage analysis of our cohort database with the nationwide hospital discharge database. This database was set up in 1990 by the Singapore government to capture all inpatient discharge information (including diagnosis) nationwide [9]. As of 31 December 2004, we identified 248 cohort patients with an ICD-9 code 410 (AMI) for reason of hospital admission. After medical record reviews, 202 cases were confirmed by a cardiologist (Y-P.L.) according to the criteria of the Multi-Ethnic Study of Atherosclerosis (MESA) in the US [10] and included in our study. Among them, 189 were definite AMI and 13 probable AMI cases.

Control selection

For each of the 280 eligible AMI patients, 2 control subjects were randomly selected among all cohort participants who donated blood samples at baseline, and who were alive and free of cardiovascular disease at the time of AMI diagnosis of the index cases. The chosen controls were matched to the index case on gender, dialect group, year of birth (±2 years), year of recruitment (±1 year) and date of blood collection (±6 months).

Laboratory Measurements

Plasma samples of a given matched set (containing the samples from the case and 2 matched controls) were arranged in random order, identified only by unique codes, and tested in the same laboratory batch for all measurements. In the present study, plasma concentrations of lipophilic antioxidants were determined by high-performance liquid chromatography [11]. The plasma levels of retinol and individual carotenoids (including α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin) were quantified using Photo-Diode Array detection. The vitamin E (α-, γ- and δ-tocopherols, and α-, γ- and δ-tocotrienols) were determined using fluorescence detection, and the coenzyme Q_{10} (ubiquinol and ubibuinone)

were determined using electrochemical method. The within-batch variations of coefficients (CVs) were 2-4% for carotenoids, 0.8% for retinol, and 4-5.7% for other fat-soluble micronutrients measured. The between-batch CVs were approximately 7% for carotenoids, 3.9% for retinol, 4.4% for vitamin E and 10% for Coenzyme Q_{10}.

Total and HDL cholesterol concentrations were measured with the enzymatic, colorimetric method with sterol esterase, cholesterol oxidase, and 4-aminoantipyrine. HDL cholesterol concentrations were measured in the supernate after centrifugation at 1500 × g for 10 min at room temperature to precipitate the chylomicrons and LDL and VLDL cholesterol by using phosphotungstic acid and magnesium ions. Triglycerides concentrations were measured by using the enzymatic, colorimetric method with glycerol-3-phosphate oxidase and 4-aminoantipyrine. LDL cholesterol concentrations were calculated with the Friedewald formula:

LDL cholesterol = total cholesterol − HDL cholesterol − triglycerides/5, where all the variables are expressed in mg/dL (To convert to mmol/L, multiply the product by 0.02586).

Statistical analysis

The chi-square test and the *t* test were used to compare the distributions of selected demographic and lifestyle factors, and measurements of plasma lipids between cases and controls. The distributions of all plasma antioxidants measured were markedly skewed with a long tail toward high values, which were corrected, to a large extent, by transforming the original values to logarithmic values. Therefore, formal statistical testings were performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented. The analysis of covariance (ANCOVA) method was used to examine the differences in the concentrations of plasma antioxidants between AMI cases and control subjects.

The conditional logistic regression method was used to examine the associations between plasma carotenoids and retinol and risk of AMI. Study subjects were grouped into quintiles of individual plasma antioxidants based on the distributions in all control subjects (see the quintile cut-off values in Appendix 1). The magnitude of the association was assessed by the odds ratio (OR) and its corresponding 95% confidence interval (CI) and *P* value. The following covariates (potential confounders) were included in all regression models: level of education (no formal schooling, primary school, or secondary school or higher), body mass index (BMI) (<20, 20-<24, 24-<28, 28+ kg/m^2), smoking status (never, former, and current smokers), number of cigarettes per day, histories of diabetes (no, yes) and hypertension (no, yes), SBP (mmHg), DBP (mmHg), ratio of plasma concentrations of total cholesterol to HDL, triglyceride (μmol/L), α-tocophenol (mg/L), α-tocotrienol (mg/L) and ubiquinone (mg/L) in logarithmic values.

Statistical computing was carried out using the SAS version 9.1 software (SAS Institute, Cary NC). All *P* values quoted are two-sided. Two-sided *P* values less than 0.05 were considered statistically significant.

Results

The present study included 280 patients (188 men and 92 women) with incident AMI and 560 matched control subjects. The mean ages [standard deviations (SD)] of cases and controls at the time of AMI diagnosis were identical (69.2, SD=7.5) years. The time interval between the collection of blood at baseline and the diagnosis of AMI was 1.85 (SD=1.60) years (ranged from 0.1 months to 9.5 years). Compared with control subjects, patients who developed AMI had lower level of education, were more likely to be obese, consumed

cigarettes but less alcoholic beverages, and more likely to have a history of diabetes or hypertension. AMI cases also had higher levels of total cholesterol and triglycerides than controls (Table 1). In addition, they also had lower plasma levels of high density lipoproteins (HDL) cholesterol but higher ratio of total cholesterol to HDL cholesterol (data not shown).

Patients with AMI had statistically significantly lower levels of β-cryptoxanthin in plasma at baseline but higher levels of retinol than control subjects. The levels of plasma α-carotene, β-carotene, lycopene and lutein also were lower for AMI cases than controls, but their differences were not statistically significant (Table 2). The association between quintile levels of plasma antioxidants and AMI risk was presented in Table 3. After adjustment for risk factors for CHD, increasing levels of β-cryptoxanthin were associated with decreased risk of AMI (*p* for trend = 0.03). A similar inverse association for AMI risk was present for plasma lutein. Relative to Q1, the odds ratios (95% CI) for the combined Q2-Q3 and the combined Q4-Q5 were 0.71 (0.45-1.12) and 0.58 (0.35-0.94), respectively (*P* for trend = 0.03).

Discussion

This study demonstrated for the first time a statistically significant, inverse association between prediagnostic plasma levels of β-cryptoxanthin and lutein, and risk of developing AMI. These inverse associations were independent of cigarette smoking, obesity, hypertension, hyperlipidaemia, diabetes and other risk factors for CHD. Further adjustment for other antioxidants including tocopherols, tocotrienols, and coenzyme Q did not materially alter the association between these two carotenoids and AMI risk. Conversely, there was no statistically significant association between plasma levels of other individual carotenoids and risk of developing AMI.

The strength of this study is the use of a biomarker approach to examine the effect of carotenoids on risk of AMI, which overcomes the inherent limitation of using a food frequency questionnaire as an assessment tool. All diagnoses of AMI were verified through review of patients' medical records by a cardiologist according to the pre-set criteria. Given a prospective study design with blood samples collected prior to the onset of disease, the study ruled out the possibility that disease symptoms and/or treatment had any impact on the plasma levels of antioxidants under study. Simultaneous measurements of multiple risk factors for CHD and other antioxidants including tocopherols, tocotrienols, and coenzyme Q_{10} on all study subjects allowed for the adjustment for their potential confounding effect on the associations between individual carotenoids and AMI risk. Given that plasma levels of carotenoids could reflect the recent dietary intake, the present study using single nonfasting plasma for assessing an individual's long-term exposure to carotenoids could suffer from a large measurement error that would lead to an exposure-disease association biased towards null result. Another limitation of the present study was its relatively small sample size, resulting in relatively low statistical power to detect a moderate effect of antioxidants on risk of AMI. Since the study included cases diagnosed before the inclusion of troponin became a criterion in the diagnosis of acute myocardial infarction, the findings of the present study could be applicable to AMI cases with more severe symptoms. On the other hand, the potential inclusion of silent myocardial infarction cases in the control group could lead to the underestimation of true effect of β-cryptoxanthin/lutein on the risk of developing AMI.

There is increasing epidemiologic data to suggest that β-cryptoxanthin and lutein reduce CHD risk. The Atherosclerosis Risk in Community (ARIC) study in adult Americans reported a strong inverse relationship between serum levels of β-cryptoxanthin and carotid intima-media thickness, an intermediate marker for CHD [12], suggesting the potential early

cardioprotective effect. A study among a Dutch population aged 65-85 years also reported a statistically significant, inverse relationship between all-cause mortality (largely attributable to CHD) and serum levels of β-cryptoxanthin [13]. Higher serum β-crypoxanthin levels were also associated with lower pulse wave velocity, a marker of atherosclerosis [14]. Plasma concentrations of lutein in baseline were associated with significantly slowed progression of carotid intima-media thickness over 18 months in 480 middle-aged men and women [15]. A nested case-control study of AMI within a prospective cohort of Americans also reported a statistically borderline significant inverse association between serum lutein levels and risk of myocardial infarction (*p* for trend=0.09) [16].

Cryptoxanthin and lutein belong to the xanthophyll subfamily of carotenoids, which also include zeaxanthin, neoxanthin and violaxanthin. Xanthophyll carotenoids are distinguished from carotene carotenoids by the conjugation of oxygen-containing cyclic end groups to the polyene backbone, and are therefore more polar and hydrophilic in their physicochemical properties [17]. The main dietary sources of lutein are leafy green vegetables, including broccoli, kale and spinach while β-cryptoxanthin is mainly derived from papaya, tangerine, orange, mango, and their juices. Recently, *in-vitro* experiments have demonstrated that the polar xanthophyll carotenoids could preserve cell membrane structure and decrease lipid hydroperoxide levels, and display a stronger antioxidant capacity than the nonpolar carotene carotenoids, which disordered membrane bilayer and stimulated membrane lipid peroxidation instead [17]. Other experimental evidence also suggests that the unique physicochemical properties of the xanthophyll carotenoids enable them to modulate the production of excessive reactive oxygen and nitrogen species, and hence may play an important role in the regulation of the inflammatory process driven by oxidative stress in cardiovascular pathologies [18].

Two recent case-control studies of coronary artery disease that investigated plasma levels of carotenoids have found that the levels of the xanthophyll carotenoids (lutein/zeaxanthin and β-cryptoxanthin) were lower in cases than in controls [19-20]. Both studies did not discriminate between lutein and zeaxanthin, and the sum of these two carotenoids was presented as a single value. In our study, plasma level of zeaxanthin, a member of the xanthophyll carotenoid subfamily, did not display any association with AMI risk. However, the plasma level of zeaxanthin was only 17% of lutein and 34% of β-crytoxanthin, which may explain the lack of biological effect of zeaxanthin in this study.

Although observational studies generally indicated that serum or adipose β-carotene levels are inversely associated with CHD risk [16,21-22], our results on the null association between β-carotene and AMI risk concurred with intervention trials on β-carotene and CHD prevention [23-25]. In fact, human studies have showed that dietary supplementation of β-carotene alone is ineffective at preventing LDL oxidation *in vitro* [26-27]. Epidemiologic studies of diet and CHD have shown a stronger, inverse association of CHD risk with consumption of dark green or orange vegetables than with intake of carotenes [28-29], suggesting that constituents other than carotenes in those vegetables, such as β-cryptoxanthin and lutein as discussed above, may exert cardioprotective effect against CHD development. Finally, although a prospective, nested case-control analysis in the Physicians' Health Study did not show any overall protective relation between plasma carotenoids, including the xanthophyll carotenoids, and myocardial infarction, this study was nested in an interventional trial with exceptionally low cardiovascular mortality rates compared to the general population [30], and may not reflect the benefit of these carotenoids on CHD risk in the general population.

In conclusion, this study demonstrated a statistically significant, independent inverse association between plasma levels of β-cryptoxanthin and lutein and the risk of developing

AMI. The findings of this study support that the intake of foods rich in β-cryptoxanthin and lutein may possibly be considered useful to decrease the risk of CHD. It is warranted that future clinical trials demonstrate the effectiveness of the supplementation with the pure compounds of these carotenoids on AMI protection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix 1

Quintile cut-points of plasma concentrations of retinol and carotenoids among all control subjects (n = 560), Singapore Chinese Health Study

Antioxidant	Range of plasma concentrations of each antioxidant by quintile				
	1st (low)	2nd	3rd	4th	5th (high)
α-carotene (μmol/L)	<0.012(63/113)	0.012-0.025(58/112)	0.025-0.036(70/111)	0.036-0.055(52/112)	>0.055(37/112)
β-carotene (μmol/L)	<0.171 (62/112)	0.171-0.286(68/113)	0.286-0.411 (60/111)	0.411-0.610(43/112)	>0.610(47/112)
Lycopene (μmol/L)	<0.055(63/112)	0.055-0.083(53/112)	0.083-0.117(56/112)	0.117-0.184(59/112)	>0.184(49/112)
β-cryptoxanthin (μmol/L)	<0.101 (67/112)	0.101-0.167 (77/112)	0.167-0.239 (54/112)	0.239-0.392 (39/112)	>0.392 (43/112)
Lutein(μmol/L)	<0.283 (67/112)	0.283-0.383 (54/112)	0.383-0.500 (60/113)	0.500-0.664 (42/111)	>0.664 (57/112)
Zeaxanthin (μmol/L)	<0.040 (46/112)	0.040-0.066 (56/114)	0.066-0.092 (60/110)	0.092-0.141 (65/112)	>0.141 (53/112)
Retinol (μmol/L)	<1.832 (53/112)	1.832-2.111 (50/112)	2.111-2.423 (58/112)	2.423-2.761 (53/112)	>2.761 (66/112)

Number of acute myocardial infarction cases/number of control subjects in parenthesis.

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Table 1

Distribution of demographic characteristics and selected risk factors for AMI in cases and control subjects, The Singapore Chinese Health Study

Characteristic or risk factor	Cases (n=280)	Controls (n=560)	2-sided *P*
Level of education			
No formal school	24.6%	22.0%	0.006
Primary school	54.3%	46.4%	
Secondary or higher	21.1%	31.6%	
Cigarette smoking			
Never	45.7%	57.3%	0.001
Former smokers	18.2%	18.8%	
Current smokers	36.1%	23.9%	
Alcohol consumption			
Non/occasionally	90.4%	84.8%	0.08
Weekly	6.1%	9.8%	
Daily	3.6%	5.4%	
Weekly moderate or strenuous physical activity	34.6%	37.0%	0.51
History of diabetes	23.2%	8.2%	<0.0001
History of hypertension	55.4%	39.1%	<0.0001
Obesity (BMI >=30 kg/m2)	3.6%	2.9%	0.57
Hypercholesterolemia (>=6.2 mmol/L)	20.6%	13.7%	0.01
Hypertriglyceridemia (>=2.3 mmol/L)	24.2%	18.9%	0.08

AMI, acute myocardial infarction; SD, standard deviation.

P-values are based on chi-square test or *t* test.

Moderate activities include brisk walking, bowling, bicycling on level ground, tai chi or chi kung; strenuous activities include sports such as jogging, bicycling on hills, tennis, squash, swimming laps or aerobics and vigorous work such as moving heavy furniture, loading or unloading trucks, shoveling, or equivalent manual labor.

Table 2

Geometric means of carotenoids and retinol in patients with acute myocardial infarction (cases) and control subjects, The Singapore Chinese Health Study

	Cases (n=280)	Controls (n=560)	2-sided *P**
α-carotene (μmol/L)	0.026	0.028	0.077
β-carotene (μmol/L)	0.290	0.314	0.162
Lycopene (μmol/L)	0.091	0.099	0.155
β-cryptoxanthin (μmol/L)	0.171	0.196	0.027
Lutein (μmol/L)	0.409	0.429	0.215
Zeaxanthin (μmol/L)	0.080	0.076	0.314
Retinol (μmol/L)	2.343	2.251	0.035

* Based on the analysis of covariance (ANCOVA) models that also included age, sex, dialect group, year of interview and year of blood drawn.

Table 3

Levels of prediagnostic plasma carotenoids and retinol in relation to risk of AMI, The Singapore Chinese Health Study

	Odds Ratio (95% confidence interval)					
Antioxidant	**Q1 (low)**	**Q2**	**Q3**	**Q4**	**Q5 (high)**	***P* for trend**
α-carotene	1.00	0.96(0.55-1.66)	1.18(0.67-2.06)	0.70(0.38-1.30)	0.65(0.35-1.20)	0.10
β-carotene	1.00	1.11 (0.65-1.89)	0.89(0.51-1.55)	0.85(0.48-1.53)	1.03(0.56-1.89)	0.75
Lycopene	1.00	0.89(0.52-1.52)	1.11 (0.63-1.95)	1.08(0.60-1.93)	0.97(0.53-1.77)	0.84
β-cryptoxanthin	1.00	1.16(0.69-1.92)	0.69(0.39-1.21)	0.57(0.32-1.02)	0.67(0.37-1.21)	0.03
Lutein	1.00	0.63 (0.37-1.06)	0.80(0.48-1.35)	0.54(0.30-0.95)	0.62(0.35-1.10)	0.09
Zeaxanthin	1.00	1.18(0.66-2.12)	1.11 (0.63-1.99)	0.98(0.55-1.77)	0.96(0.50-1.83)	0.58
Retinol	1.00	1.14(0.66-1.98)	1.12(0.66-1.92)	0.80(0.46-1.42)	0.99(0.55-1.77)	0.55

Adjusted for age, sex, dialect group, year of interview, year of blood drawn, level of education, body mass index, smoking status (never, former and current smokers), number of cigarettes per day, histories of diabetes and hypertension, systolic and diastolic blood pressures, ratio of plasma concentrations of total to HDL cholesterol, triglyceride, and α-tocopherol, α-tocotrienol and ubiquinone in logarithmic values. AMI, acute myocardial infarction.