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□ **Original Article** □

Plasma Carotenoid Levels in Healthy Men and Acute Cardiovascular Disease Patients in Taegu*

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ABSTRACT

Plasma carotenoid levels were compared among 64 healthy male subjects(control) and 38 patients of ischemic heart disease(IHD) and 20 ones of cerebral infarction(CI) all of whom were over 50 years of age. Another 98 healthy male subjects aged 23 to 58 were selected to compare their plasma carotenoid levels by age groups. Levels of lutein, zeaxanthin and crpytoxanthin were lower in IHD(34 ± 2, 13 ± 1 and 62 ± 7μg/dl) and CI(36 ± 3, 12 ± 2 and 41 ± 6μg/dl) patient groups than in control group(84 ± 5, 16 ± 2 and 69 ± 3μg/dl) while those of lycopene, α-and β-carotene varied little among the three groups. The sum of the six carotenoid levels were, therfore, highest(205 ± 14μg/dl) in the control group followed by IHD(155 ± 15μg/dl) and CI(128 ± 17μg/dl) patient groups. Among the 98 healthy male subjects for the age group study, levels of the three major carotenoids increased with age from the twenties to the fifities ; lutein, from 64 ± 6 to 89 ± 8μg/dl, cryptoxanthin, 57 ± 8 to 73 ± 4μg/dl and β-carotene, 29 ± 6 to 47 ± 4μg/dl, respectively, but those of minor ones such as zeaxanthin, lycopene and α-carotene did not differ. Also the levels of lutein, cryptoxanthin and β-carotene were more significantly correlated($r=0.30$ to 0.61, $p<0.01$), whereas levels of lycopene and α-carotene were less significantly($r=0.21-0.23$, $p<0.05$) correlated. (*Korean J Community Nutrition* 2(5) : 728~734, 1997)

KEY WORDS : plasma carotenoids · healthy men · ischemic heart disease · cerebral infarction · age.

Introduction

Carotenoids are natural compounds with lipophilic properties. More than 500 different compounds have been identified and β-carotene is the most prominent. Most carotenoids contain an extended system of conjugated double bonds, which is responsible for their antioxidant activity. Numerous epidemiologic studies have suggested that dietary carotenoids aid in prevention of cancer(Ziegler 1991), cardiovascular disease(CVD, Gaziano & Hennekens 1993 ; Kohlmeier & Hastings 1995) and cataract(Jacques & Chylack 1991), although posssible adverse effects on the incidence of lung cancer have been reported in β-carotene treatment of smokers(The ATBC Cancer Prevention Study Group 1994).

Plasma or serum concentrations of carotenoids have

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been correlated with dietary intakes(Ahn & Paik 1997 ; Forman et al. 1993 ; Granado et al. 1996 ; Scott et al. 1996 ; Yeum et al. 1996), supplementation of β-carotene(Fotouchi et al. 1996 ; Kostic et al. 1995 ; Wahlqvist et al. 1994) and life style(Brady et al. 1996 ; Forman et al. 1995 ; Handelman et al. 1996). Carotenoids appear to have specific physiological roles depending on their chemical characteristics. Hydrocarbon carotenoids such as β-carotene may play a role on reducing risk of cancer(Ziegler 1991) and heart disease(Gaziano & Hennekens 1993 ; Kohlmeier & Hastings 1995), whereas oxygenated carotenoids such as lutein and zeaxanthin may have a protective role in the eye, that is presumably not related to antioxidant activity(Snodderly 1995). However, guidelines for the optimal nutritional status of carotenoids have been suggested only for β-carotene (Fernandez-Banares et al. 1993), which is not agreed upon among researchers.

During the last decade, incidences of chronic diseases including CVD have been notably increased in Korea. Changes in diet and life style have been repeatedly suggested to be the important causes for the CVD increase in Korea. Energy balance and fat intake have been major dietary factors studied to elucidate the correlation with CVD. However, only in recent years status of antioxidant nutrients including vitamins E and A and β-carotene(Cho et al. 1995 ; Choi et al. 1996 ; Kim et al. 1996 ; Lee & Paik 1995 ; Park et al. 1997 ; Suh et al. 1997 ; Yeum et al. 1992 ; Yu et al. 1996) have been actively reported in studies for case-control comparison, general population and the other types as their roles are increasingly recognized on prevention of CVD. But studies on the levels of many other different carotenoids are very limited(Ahn & Paik 1997 ; Kim et al. 1996), probably due to difficulties obtaining standard materials for various carotenoids as well as the low sensitivity of analytic methods.

In the present study, we report the plasma levels of six carotenoids in normal subjects and patients with acute ischemic heart disease(IHD) and cerebral infarction(CI). These two types of patients have been rarely studied in Korea(Park et al. 1997). For IHD pateints, acute type has not been studied whereas chronic type has(Kim et al. 1996 ; Suh et al. 1997) And CI patients are important group because of their high prevalence. We also studied another set of healthy subjects to see changes in the carotenoid status by age, since CVD are closely related to age.

Subjects and Methods

1. Subjects

All healthy subjects for case-control and age group comparisons were male and randomly chosen from the annual health examination for educational public service employees in May, 1994. Patients of ischemic heart disease(IHD) and cerebral infarction(CI) were those who were hospitalized due to acute myocardiac infarctions during the period from June 1st through December 31st in 1994 and cerbral infarctions during the period from May 1st through August 31st in 1995, respectively. Even though many patients were older than controls as shown in Table 1, body mass indices were not different. For case-control comparisons, subjects over 50 years of age were selected using 64 control subjects, 38 IHD and 20 CI patients. For the comparison of different age groups, a total of another 98 healthy male subjects were grouped into 13 men of 20–29 years, 29 of 30–39 years, 30 of 40–49 years and 26 of 50–59 years.

2. Plasma Preparation

Five to ten ml of fasting blood was collected into 15ml cornical plastic tubes containing 100μl of 31% trisodium citrate and mixed gently. Plasma samples were obtained by centrifugation at 800rpm for 15

Table 1. Age and anthropometric indices of the subjects for case-control study

	Control(64)	IHD(38)	CVD(20)
Age	55.6 ± 3.5^{a}	60.8 ± 9.1^{b}	65.2 ± 9.6^{c}
Weight	67.3 ± 8.4^{a}	63.1 ± 10.8^{b}	64.5 ± 5.8^{ab}
Height	169.1 ± 4.8^{a}	164.2 ± 8.1^{b}	164.4 ± 4.6^{b}
BMI	23.1 ± 2.7	23.6 ± 2.9	24.0 ± 2.7

Mean ± S.D
The number of subjects is in parenthesis
Values with different superscript letters in the same row are significantly different at $p < 0.05$

minutes at room temperature.

3. Analysis of Plasma Carotenoids

HPLC analysis of carotenoid was modified from the method of Kim(1989). Hundred μl of plasma was mixed with the same volume of 0.5μg/ml echinonene(La Roche Co. Switzerland) as an internal standard. To this was added 0.4ml of 1M methanolic KOH. The mixture was shaken and placed in a 60℃ water bath for 30min. Then the tube was allowed to cool in ice water for 1 – 2min. The extraction was made by adding 2ml of petroleum ether (PE), vortexing for 1min and centrifuging at 2000 rpm for 5min. The upper PE layer was filtered through 0.45μ nylon membrane into a small amber vial. The extraction procedure was repeated and the first and second PE layer were combined. Just prior to HPLC, the extract was dried with nitrogen gas and dissolved in 40 – 60μl of chromatographic solvent (acetonitrile/methanol/acetone : 40/40/20). Ten to 20μl of the reconsituted sample was injected into HPLC(Youngin model 910) equipped with C_{18} Novapak cartridge column(8×100mm, 4μ). Chromatography was carried out isocratically at the flow rate of 1.3ml/min and monitored at 450nm. Standard materials for α- and β-carotene and lycopene were obtained from Sigma. Echinenone was a gift from Roche Korea and lutein and cryptoxanthin produced by La Roche Co. were kindly donated by Dr. Paik of Seoul National University. The purity of all standards were determined spectrophotometrically. Extinction coefficients for($E^{1\%}_{1cm}$) used were 2,800 at 444nm and 2,592 at 453 nm for α- and β-carotene, for lycopene, 2,370 at 452nm for echinenone, 2,236 at 458nm for lutein, 2,350 at 451nm for zeaxanthin, and 2,380 at 452nm for cryptoxanthin.

4. Statistical Analysis

Statistical analysis was done with SPSS package and the reults are expressed as mean±SEM. Comparisons between control and patient groups and among age groups were made using one way analysis of variance and significance by the Tukey test. Correlations among plasma carotenoids were examined by the Pearson Correlation.

Results and Discussion

1. Plasma Carotenoid Levels of Control and Cardiovascular Patients

Fig. 1 shows a typical HPLC chromatogram of plasma six carotenoids and internal standard, echinenone which were clearly separated by our analytical procedure. Considering that in most of the studies using a more complicated system zeaxanthin was not separable from lutein
(Ahn and Paik 1997 ; Handelman et al. 1996 ; Kim et al. 1996 ; Zaman et al. 1993), it is significant in the present study that zeaxanthin, albeit in small amount, was well separated from lutein.

Fig. 2 shows the plasma levels of six carotenoids from control subjects and IHD and CI patients. Levels of lutein were significantly higher in control subjects than in IHD and CI patients and those of zeaxanthin and cryptoxanthin were in a similar pattern. On the other hand, levels of α- and β-carotene did

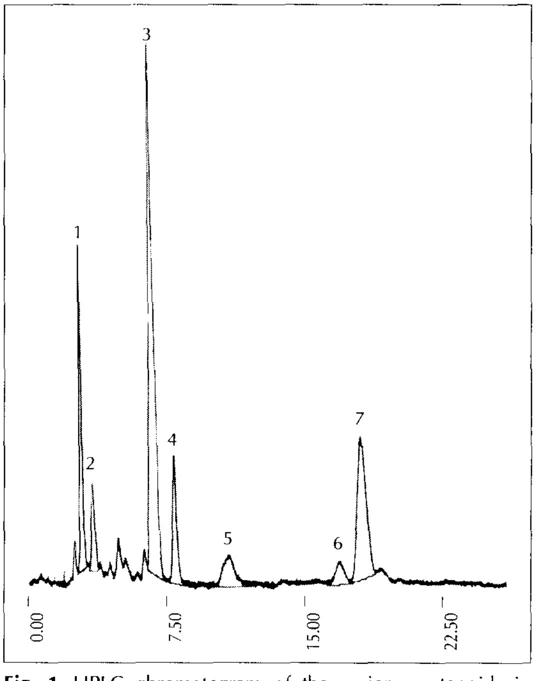

Fig. 1. HPLC chromatogram of the major carotenoids in subject plasma of the present study. The numbered peaks are 1, lutein ; 2, zeaxanthin ; 3, cryptoxanthin ; 4, echinenone(internal standard) ; 5, lycopene ; 6, α-carotene ; 7, β-carotene. Numbers in x-axis are time as minutes.

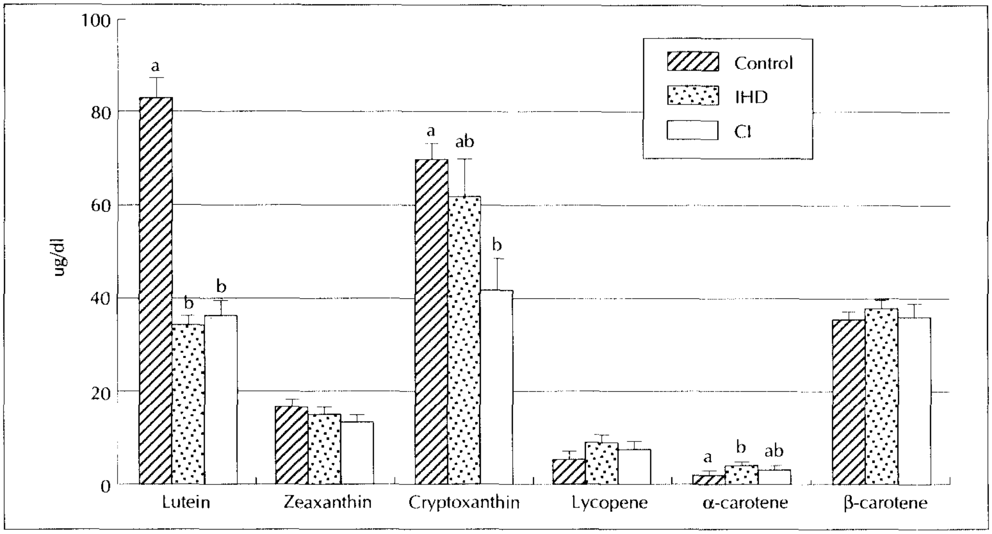

Fig. 2. Plasma carotenoid levels in control and IHD and CI patients. Values with different alphabet letters in the same type of carotenoid are significantly different from each other at $p<0.05$.

not differ significantly among the three groups. The levels of lycopene and α-carotene which were much lower than those of other carotenoids appeared to be a little higher in IHD patients. Therefore, differences in total carotenoids(Table 2) resulted mostly from differences of lutein and cryptoxanthin. Circulating concentrations of β-carotene have been shown to be lower in patients with cardiovascular disease than in control subjects in case-control studies and cohort and supplemetation studies have also supported the importance of β-carotene in the body as one of the protective factors for the disease(Ko-hlmeier & Hastings 1995). But the levels of whole carotenoids in plasma and tissues have been sparingly reported. Kim et al.(1996) have reported that serum levels of lycopene, cryptozanthin and zeaxanthin as well as β-carotene were reduced in both male and female patients with angiographically confirmed coronary artery disease. The present results were similar to theirs except for the β-carotene data. Previously we have reported that IHD patients consumed kimchi more frequently and other vegetable dishes less frequently than control subjects(Choi et al. 1996). Preference for hot taste has been shown to be positively related to intake of red pepper(Lee & Paik, 1995) and serum β-carotene levels(Yu et al. 1996). Therefore, plasma β-carotene levels in the patients that were

Table 2. Levels of total plasma carotenoids in control subjects and IHD and CI patients

Control(64)	IHD(38)	CI(20)
	μg/dl	
205±14[1)a]	155±15[b]	128±17[b]

1) Values are mean SE and those with different suprescript letters are significantly different at $p<0.05$

not lower than control subjects may be partly due to their higher intake of hot kimchi which is very common in the Taegu area. But this explanation has to be confirmed along with the underlying causes for lower levels of lutein and cryptoxanthin in patient groups in relation to their low intakes of other vegetable foods and disease status.

2. Plasma Carotenoid Levels of Healthy Men with Different Ages

Fig. 3 shows the plasma levels of six carotenoids of different age groups of healthy men. The levels of most carotenoids increased with age and this finding concurs with some other reports(Ahn & Paik 1997 ; Brady et al. 1996 ; Yeum et al. 1996 ; Zaman et al. 1993). But the values of each carotenoid in the present study, notably those of lutein and cryptoxanthin were relatively higher than those of other reports both from home(Ahn & Paik 1997 ; Kim et al. 1996) and abroad(Brady et al. 1996 ; Cantilena et

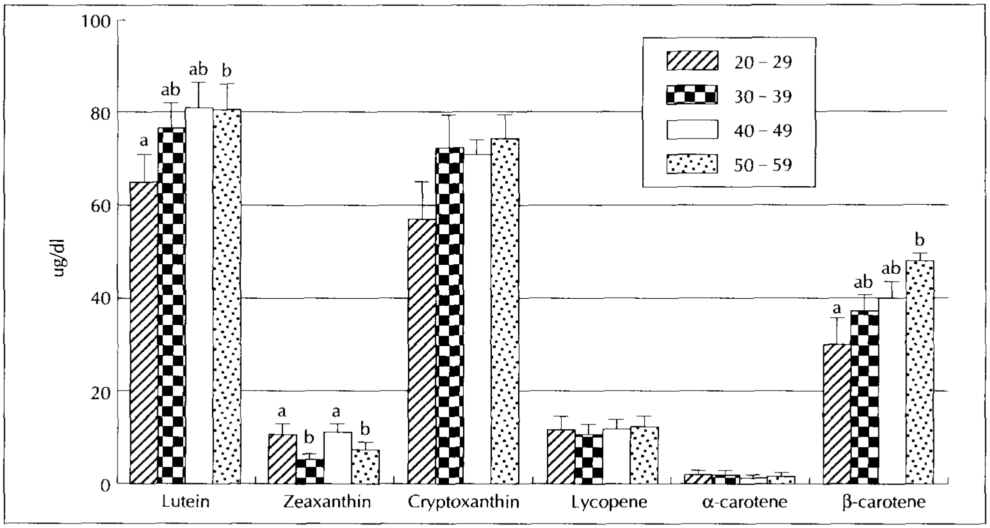

Fig. 3. Plasma carotenoid levels in healthy Korean men of different age groups. Values with different alphabet letters in the same type of carotenoid are significantly different from each other at p<0.05.

Table 3. Correlation between the six plasma carotenoids levels in 98 healthy subjects

	Lutein	Zeaxanthin	Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Lutein	1.00	0.28**	0.45***	0.17*	0.08	0.30**
Zeaxanthin		1.00	0.02	0.04	0.01	0.00
Cryptoxanthin			1.00	0.21*	0.20*	0.61***
Lycopene				1.00	0.21*	0.21*
α-Carotene					1.00	0.23**
β-Carotene						1.00

*p<0.05, **p<0.01, ***p<0.001

al. 1992 ; Scott et al. 1996). High levels of lutein and cryptoxanthin appear to be distinctive in Koreans but the level of lycopene in the present study was lower when compared with most results reported from western countries. This discrepancy seemed to be attributable to seasonal differences in food intakes, aside from variations in analytical procedure. Our plasma samples were collected during spring when both intakes and plasma levels of carotenoids have been reported to be high, while Ahn & Paik (1997) collected their samples in winter. Carotenoids do not exist evenly in all vegetables and fruits but each one has its own main sources. Oxygenated carotenoids such as lutein, zeaxanthin and cryptoxanthin are found mostly in green leafy vegetables and beans and peas, while α- and β-carotene are in yellow to orange vegetables as well as green ones and lycopene is exclusively in tomatoes and watermelons(Grando et al. 1996 ; Scott et al. 1996). Therefore, the intake of each carotenoid varies with the season. The high plasma level of lutein in the present study is in good agreement with the report that its intake was highest in the spring(Scott et al. 1996). Brady et al.(1996) have reported that lower serum concentrations of carotenoids were associated with younger age, male gender and lower non-HDL cholesterol. Increases in plasma lipid levels with age that were found in the same population (Shin 1996) seem to be associated with higher levels of carotenoids in older ages in the present study.

3. Relations between Plasma Carotenoid Levels of Healthy Men

Table 3 shows correlations between values of six plasma carotenoids measured in 98 healthy men of 23 to 60 years of age. All of the three major plasma carotenoids – lutein, cryptoxanthin and β-carotene were positively correlated each other. The same trend was seen when correlations analysis was done with case-control subjects(data not shown). It is noteworthy that most significant correlations were between cryptoxanthin and either lutein($r=o{,}45$, $p<0.001$) or β-carotene($r=o{,}61$, $p<0.001$). Similar results have been reported by Cantilena et al.(1992) and Ahn & Paik(1997). Less significant correlations found with zeaxanthin, lycopene and α-carotene may be partly due to their low values that were likely to be less accurate. Several investigators reported that plasma or serum concentrations of various carotenoids were correlated with their intakes but with varying degrees. Correlations between intakes and plasma levels of lutein, cryptoxanthin and lycopene have been shown to be higher than those of α- and β-carotenes(Forman MR et al. 1993 ; Scott et al. 1996). These are in accordance with the report from Ahn & Paik (1997), in which a significance correlation between vegetable intakes and serum concentration of lutein/zeaxanthin only was shown. On the other hand, Kim et al.(1996) have reported that the ratio of n-6/n-3 fatty acid in diet were positively correlated with serum lycopene and β-carotene, but not with other types of carotenoids. We also found that plasma β-carotene concentration was strongly correlated($r=0.53$, $p<0.001$) with the linoleic acid level in red blood cells(unpublished observations). Since RBC fatty acid composition is regarded to reflect diet for a relatively long period(Sarkkinen et al. 1994), this observation provides an indirect evidence of association between intakes and plasma levels of carotenoids. To clarify and confirm these correlations, more work is needed in relation to other factors influencing carotenoid status in the body.

Summary and Conclusion

Plasma levels of six cartenoids were measured in 162 healthy male subjects and 38 ischemic heart disease(IHD) and 20 cerebral infarction(CI) patients, to compare between age-macthed healthy controls and both patient groups and also among different age groups in healthy subjects.

Results are summarized as follows.

1) Levels of lutein, zeaxanthin, crpytoxanthin were lower in IHD and CI patient groups than those of control group while those of lycopene, α- and β-carotene were not very different among three groups.

2) Sum of the six carotenoid levels were highest in control group followed by IHD and CI patient groups.

3) Among healthy subjects, levels of lutein, cryptoxanthin, and β-carotene increased with age from twenties up to fifities, but those of zeaxanthin, lycopene and α-carothene did not differ.

4) The plasma levels of lutein, cryptoxanthin and β-carotene were significantly correlated($r=0.30$ to 0.61, $p<0.01$) in 98 healthy subjects.

In conclusion, low body status of carotenoids appears to be related to cardiovascular disease in Korean men and their higher status in old ages to plasma lipid levels. Although it remains to be elucidated in detail whether the carotenoid status is viewed in terms of total antioxidant and lipid nutrition, the present results suggest that sufficient intakes of various carotenoids are advisable to Korean adults both in health and cardiovascular diseases.

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