Original Article

Serum β-carotene, lycopene and α-tocopherol levels of healthy people in northeast Thailand

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Human serum contains many different antioxidants which may be important in the maintenance of antioxidant status. β-carotene and lycopene are carotenoids with potent antioxidant activity. Carotenoids intake probably protects against cancers and may affect the risk of several chronic conditions. α-tocopherol is well known for its function as antioxidant and in reduction of heart disease and cancer risk. We aimed to establish baseline values for serum β-carotene, lycopene and α-tocopherol concentrations in healthy northeast Thais. Fasting serum β-carotene, lycopene and α-tocopherol levels from 294 subjects aged 23-75 years old in northeast Thailand were determined by high performance liquid chromatography (HPLC). The mean serum β-carotene, lycopene and α-tocopherol levels were 0.53 ± 0.32 μmol/L, 0.57 ± 0.37 μmol/L, and 26.64 ± 14.85 μmol/L respectively. Serum β-carotene and lycopene levels in females (N = 118) were significantly higher than the value for males (N = 176), ie 0.60 ± 0.31 μmol/L versus 0.48 ± 0.32 μmol/L (p = 0.002) for β-carotene and 0.74 ± 0.38 μmol/L versus 0.46 ± 0.33 μmol/L (p<0.001) for lycopene whereas α-tocopherol level in males (28.60 ± 14.34 μmol/L) was significantly higher than in females (23.72 ± 15.16 μmol/L) (p = 0.006). β-carotene level was positively correlated with α-tocopherol (r = 0.22, p<0.001) and lycopene levels (r = 0.63, p <0.001). The results from this study give the baseline data of serum β-carotene, lycopene and α-tocopherol levels in healthy northeast Thai population and also suggest future study on the relationship of dietary intake.

Key Words: β-carotene, lycopene, α-tocopherol, northeast Thais, HPLC

Introduction

β-carotene, lycopene and α-tocopherol are micronutrient antioxidants that play important role in regulation the metabolic reactions in the body. Optimal status of these micronutrients is an essential requirement in any population because of their inverse relationship with the development of numerous types of cancers and cardiovascular disease.[1] β-carotene and lycopene intake probably protects against cancers and may affect the risk of several chronic conditions. Higher blood lycopene level is associated with lower prostate cancer risk.[2] α-tocopherol level is inversely related with mortality from ischaemic heart disease.[3, 4] β-carotene and α-tocopherol exhibited synergistic cooperative effects scavenging reactive nitrogen species.[5] The cooperative interaction between β-carotene and α-tocopherol was also examined in a membrane.[6] Micronutrient antioxidant status may vary among populations.[7] Dietary habits may be one of factors affecting these micronutrient status of different populations.[7] It was reported that dietary patterns in northeast Thailand are characterized by high grain and vegetable but low fat intake.[8] Endemic illnesses in northeast Thailand include renal stones, liver fluke *Opisthorchis viverrini* infection, cholangiocarcinoma and liver cancer may be from the characteristic eating habits of the northeast Thais.[9] Impaired micronutrient status has been observed in some diseases including protein calorie malnutrition.[10] For correction these deficiency, reference ranges of these micronutrients obtained from the healthy population living in the same area should be used to define these thresholds. The aim of this study, therefore, was to establish baseline data for serum concentrations of β-carotene, lycopene and α-tocopherol in healthy northeast Thais for use in comparisons with other control populations, for interpretating values in different clinical conditions, and for application in diet and in health and disease epidemiological studies.

Materials and methods

Subjects

Two hundred and ninety four apparently healthy northeast Thais, with age range between 23 and 75 years, whom yearly checked up their health at Faculty of Associated Medical Sciences, Khon Kaen University were enrolled in this study.

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Table 1. Background information of the studied population.

Characteristics	Male (N = 176)	Female (N = 118)	*p*-value
	Median (95% CI)	Median (95% CI)	
Age (year)	46 (44.77 -47.03)	44 (42.41-44.64)	0.003
Weight (kg)	64 (62.32-65.90)	53 (54.61-59.20)	<0.001
Glucose (mg/dL)	91 (89.38-92.27)	86 (85.36-88.55)	0.001
Blood urea nitrogen (mg/dL)	12 (11.49-12.35)	10 (10.22-11.27)	0.001
Creatinine (mg/dL)	1.10 (1.03-1.09)	0.80 (0.79-0.85)	<0.001
Uric acid (mg/dL)	5.80 (5.48-5.89)	4.00 (3.99-4.46)	<0.001
Total cholesterol (mg/dL)	117 (169-175)	172 (68-175)	0.755
High-density lipoprotein-cholesterol (mg/dL)	42 (43.35-46.69)	45 (46.62-51.28)	0.002
Low-density lipoprotein-cholesterol (mg/dL)	108 (98.80-106.17)	105 (100.01-107.12)	0.662
Triglycerides (mg/dL)	107 (104.73-118.87)	81 (78.11-93.12)	<0.001
Alanine aminotransferase (unit/L)	23 (21.16-23.77)	17 (16.37-18.99)	<0.001
Aspartate aminotransferase (unit/L)	24 (23.67-25.26)	22 (21.31-22.94)	<0.001
Alkaline phosphatase (unit/L)	73 (72.75-79.25)	65 (64.85-73.53)	0.014

They were members of an urban population with no disease history, mostly residing in Khon Kaen, with only a few residing in Mahasarakham, Loei and Nakhonratchasima. Fasting blood samples were collected during the year 2002 and 2003. Their plasma glucose, serum uric acid, lipid profile (triglyceride, total cholesterol, low-density lipoprotein-cholesterol; LDL-C, high-density lipoprotein-cholesterol; HDL-C), liver function enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase), kidney function parameters (blood urea nitrogen and creatinine) (Table 1) and urinary analysis were within the normal ranges. The stool examination revealed no parasites found.

Blood specimen collections

Two-milliliter blood samples were collected in the morning by venipuncture from fasting subjects for β-carotene, lycopene and α-tocopherol analysis. Serum samples were obtained from the spontaneous coagulation of blood. The blood was then centrifuged at 2,500 rpm at 4°C for 10 min to obtain the serum. Hemolyzed samples were excluded. The serum was stored at -20°C until analyzed.

Ethics

The Ethics Committee, Faculty of Medicine, Khon Kaen University, approved the study protocols and informed consent was given by the participants (HE470904).

β-carotene, lycopene and α-tocopherol determination

β-carotene, lycopene and α-tocopherol are fat-soluble compounds. They were extracted from serum samples and measured by reverse-phase high performance liquid chromatography (HPLC) and a spectrophotometric detector using a modification of the Thurnham method.[11] Samples were protected from photodegradation by extraction under dimmed natural lighting, excluding direct sun and fluorescent light all times. One hundred microliters of serum sample was mixed with 200 µL of 0.1 µmol/L sodium dodesyl sulfate (SDS) reagent for 1 min in a glass tube (10 x 0.75 cm). Next 200 µL of ethanol contained 42 µmol/L of tocopherol acetate (as internal standard) was added to the sample. One milliliter of n-heptane contained 0.5 g of butylated hydroxytoluene (BHT) per liter was then added, mixed vigorously for 2.5 min and centrifuged at 5,000 rpm for 10 min at 20°C to separate the organic from the aqueous phase. A 700-µL layer of heptane was transferred to a glass tube and evaporated under nitrogen at 40°C. The residue was reconstituted with 250 µL of freshly prepared mobile phase. The mobile phase consisted of 4:4:1 of acetonitrile, methanol and dichloromethane. This was filtered under a vacuum pump using 2-µm filter paper (Cat. No. 3400, Sartorius, Germany). BHT (0.01 g) was added to the mobile phase and degassed by sonication for 30 min. The HPLC column (C18 ODS-2 Spherisorb column, diameter 5 µm, 100 x 4.6 mm, Waters) was equilibrated with the mobile phase for 5 column volume. Then a 20 µL of sample was injected into the column using a Rheodyne injector (model 7125, Rheodyne) at a flow rate of 1 mL/min by an isocratic pump (model 501, Waters). Standard β-carotene, (Cat. No. C-4582), lycopene (Cat. No. L-9879) and α-tocopherol (Cat. No. T-3251) were purchased from Sigma Chem. Co. (St. Louis, MO). UV detector (model Lambda-Max 481, Waters) was set at wavelength 450 nm and 292 nm for detecting carotenoids (β-carotene and lycopene) and α-tocopherol respectively. All samples were analyzed in duplicate. The area under the main peaks was calculated quantitatively using an integrator (model 746 Data Module, Waters).

Statistical analysis

The data was analyzed with SPSS Version 10.0 (SPSS Inc., Chicago, IL). Data were presented as means ± SD. Differences in mean values between two groups were evaluated using Student's *t* test. Statistical significance was considered at $p < 0.05$.

Results

The total number of samples collected was 294, from apparently healthy persons between 23 and 75 years of age. This study group comprised 176 (59.9%) males and 118 (40.1%) females, living in 4 provinces of northeast Thailand (viz. Khon Kaen, Mahasarakham, Loei and Nakhonratchasima). Weight was normally distributed in these study groups. The background information of the studied population are presented in Table 1.

Table 2. β-carotene, lycopene and α-tocopherol levels in males and females at various age ranges.

Group[a]	Mean±SD (95% CI) µmol/L			
	Total (N=294)	Male (N=176)	Female (N=118)	*p*-value[*]
β-carotene				
Group 1	0.54±0.30 (0.50-0.59)	0.51±0.30 (0.44-0.57)	0.58±0.29 (0.52-0.65)	0.108
Group 2	0.51±0.34 (0.45-0.57)	0.46±0.34 (0.39-0.53)	0.63±0.33 (0.52-0.74)	0.100
Both groups	0.53±0.32 (0.49-0.56)	0.48±0.32 (0.44-0.53)	0.60±0.31 (0.54-0.65)	0.002
p-value[**]	0.367	0.303	0.425	
Lycopene				
Group 1	0.59±0.35 (0.54-0.64)	0.47±0.27 (0.41-0.52)	0.73±0.37 (0.64-0.81)	<0.001
Group 2	0.55±0.40 (0.48-0.62)	0.46±0.35 (0.38-0.54)	0.77±0.39 (0.64-0.90)	<0.001
Both groups	0.57±0.37 (0.53-0.62)	0.46±0.33 (0.42-0.51)	0.74±0.38 (0.67-0.81)	<0.001
p -value[**]	0.368	0.941	0.569	
α-tocopherol				
Group 1	26.79±14.84 (24.53-29.05)	28.97±13.92 (26.01-31.94)	24.45±15.51 (21.02-27.89)	0.048
Group 2	26.44±14.91 (23.82-29.07)	28.24±14.81 (25.12-31.36)	22.13±14.44 (17.32-26.94)	0.035
Both groups	26.64±14.85 (24.94-28.35)	28.60±14.34 (26.47-30.74)	23.72±15.16 (20.96-26.49)	0.006
p -value[**]	0.842	0.736	0.441	

M = males, F = females, y = years. [*] = *p* value between males and females; [**] = *p* value between group 1 and group 2. [a]Group 1 consisted of 87M/81F with age range 20 – 45 y and group 2 consisted of 89M/37F with age range 46 – 75 y

Analysis of quality control materials for the concentrations of β-carotene, lycopene and α-tocopherol were performed repeatedly to obtain between run and within run coefficients of variation (CVs). The between run CVs were 5.5, 4.6, 6.2% and within run CVs were 2.7, 3.3, 3.2% for β-carotene, lycopene and α-tocopherol respectively. Percent recovery was performed on serum samples spiked with known concentrations of each micronutrient. The mean percent recoveries of β-carotene, lycopene and α-tocopherol were 95, 90, 96% respectively.

In this study, the subjects were divided into 2 groups according to their age. Group 1 was 20 - 45 and group 2 was 46 – 75 years of age. The concentration of serum β-carotene, lycopene and α-tocopherol among these northeast Thais at various age ranges were summarized in Table 2. The mean levels of serum β-carotene, lycopene and α-tocopherol of the total population were 0.53 ± 0.32 µmol/L (95% CI = 0.49 – 0.56 µmol/L), 0.57 ± 0.37 µmol/L (95% CI = 0.53 – 0.62 µmol/L) and 26.64 ± 14.85 µmol/L (95% CI = 24.94 – 28.35 µmol/L) respectively. β-carotene and lycopene concentrations of serum in males were significantly lower than those in females ($p = 0.002$ and $p<0.001$ respectively), whereas serum α-tocopherol concentrations in males were significantly higher than those in females ($p = 0.006$).

In both age groups, β-carotene concentration in males were similar to females ($p = 0.108$ and $p = 0.100$ for group 1 and 2 respectively) whereas serum lycopene concentration in females were significantly higher than males for all age range ($p<0.001$). α-tocopherol level in males were higher than in females in both groups ($p = 0.048$ and $p = 0.035$ in group 1 and 2 respectively). No statistically difference for the effect of age on these serum micronutrient antioxidants was found between group 1 and 2. Regardless of gender of the subjects, we obtained high correlation coefficient between β-carotene and lycopene (r = 0.63, $p<0.001$). β-carotene and α-tocopherol was also significantly positively correlated with each other (r = 0.22, $p<0.001$).

Discussion

Carotenoids and α-tocopherol are among the most widely studied compounds in various populations, for both serum concentrations and dietary intake. The present study is a biochemical study and did not include dietary surveys. The free-living, apparently healthy subjects in this study were presumed to have ordinary eating habits. The levels of the compounds determined (Table 1 and 2) reflect serum concentrations before the enrolment of subjects under habitual dietary intakes in each province. Serum carotenoids may be considered as biomarkers of fruit and vegetable intake.[12] Local fruits and vegetables, such as papaya, mango, pumpkin and tomato are the main source of carotenoids in presenting specific carotenoid profiles. Reference ranges for β-carotene, lycopene and α-tocopherol have been obtained for different apparently healthy populations including American[13], European[14], Arabian[15], Chinese[16], Japanese[17], and Vietnamese[18] (Table 3) using reverse phase HPLC technique. A great variability is evident in these serum micronutrient antioxidants from various populations. However, serum β-carotene, lycopene and α-tocopherol of the healthy northeast Thais fall within the ranges of these countries.

In many studies of serum carotenoids and fat-soluble vitamin levels, subjects were established according to sex. The present study demonstrates significant higher serum

Table 3. Mean or median β-carotene, lycopene and α-tocopherol concentrations (μmol/L) in plasma[13] or serum[14-19, 27] in different populations

Country [(Ref)] [a]	β-carotene		lycopene		α-tocopherol	
	M	F	M	F	M	F
USA[13] 121M/186F (45 – 65 y)	0.46	0.58	0.82	0.76	27.1	26.2
European countries[b] [14] 175M/174F (25 – 45 y)	0.40	0.47	0.30	0.29	26.10	26.75
Arab [15] 159M/101F (18 - 63 y)	0.29	0.88	0.71	1.31	21.30	17.30
Japan[c] [17] 618M/1196F (7 - 86 y)	0.35	0.64	0.68	0.50	23.00	24.10
China[16] 570 M (45 – 64 y)	0.17	-	-	-	19.97	-
Vietnam[d] [18] 111M/185F (40 – 59 y)	0.19	0.28	-	-	13.00	12.54
Thailand[c] (Bangkok) [27] 14M/58F (20 – 60 y)	-	-	-	-	18.73	18.53
Thailand (Khon Kaen)[e] [19] 254M/296F (30 – 93 y)	0.61	0.70	-	-	24.84	25.07
This study (northeast Thailand) 176M/118F (23 – 75 y)	0.48	0.60	0.46	0.74	28.60	23.72

[a] = Population studied is described by sex (M = male, F = female) and age range (y = years). [b] = Population studied: France (38M/37F), Northern Ireland (32M/33F), Republic of Ireland (40M/33F), The Netherlands (33M/39F) and Spain (32M/32F). [c] = values reported in median. [d] = Vietnamese populations with medium income. [e] = Population studied: Ban-Fang and Chonnabot districts in Khon Kaen province

levels of both β-carotene and lycopene in females than in males. This is in agreement with data reported earlier[13-15, 17, 19] that females generally have higher concentrations of many carotenoids, including α-carotene and β-carotene but this relationship was also not observed consistently with lycopene.[13] The difference between the gender may be due to quantitative and qualitative differences in their intake which may be related to energy intake, absorption and metabolism. Hormonal changes during the menstrual cycle have been shown to affect carotenoid serum levels in females.[20] It was reported that consumption of alcoholic beverages in males was inversely associated with blood concentration of β-carotene[21, 22]; an inverse association with alcohol was also observed in some studies of lycopene.[23]

With regard to α-tocopherol, the mean values observed in all populations fall within a narrower range than for β-carotene and lycopene. Significant difference in serum α-tocopherol levels was observed between gender (p = 0.006). Males seemed to retain a higher level of α-tocopherol compared to females in all age ranges (group 1 p = 0.048, group 2 p = 0.035) which is agreed with Abiaka *et al*[15] but contrasts with some studies.[14, 17, 19] The possible explanation may be due to the amount of α-tocopherol consumed which is positively associated with plasma concentration of α-tocopherol[13] and low fat-intake in northeast Thais[8] may limit the absorption of fat-soluble vitamins.

Serum β-carotene and lycopene concentrations were not different between age groups suggesting that carotenoid isomer distribution in human serum is peculiar to each carotenoid as described in other population with different dietary patterns.[24, 25] However, several studies suggest that lycopene levels are inversely associated with age.[13, 26] The high correlation coefficients between β-carotene and lycopene (r = 0.63, p<0.001) was obtained. A similar correlation between β-carotene and lycopene has been described by Ascherio *et al*.[13] β-carotene and α-tocopherol also showed significantly positively correlated with each other (r = 0.22, p<0.001). This may be due to their simultaneous occurence in several vegetables and fruits.

Consideration variations in serum β-carotene and α-tocopherol levels were found among 3 regions in Thailand: Bangkok[27], Ban-Fang and Chonnabot districts in Khon Kaen province[19] and the 4 provinces in northeast Thailand (this study). Similar to our study, Olmedilla *et al*[14] reported wide variability of serum carotenoids between northern and southern Europe. This finding suggests the northeast Thais consumed qualitatively and quantitatively more fruit and vegetables than those in Bangkok.

Conclusion

The baseline data of serum β-carotene, lycopene and α-tocopherol obtained in this study is the informative on the physiological ranges achievable under habitual dietary conditions in healthy northeast Thais, aged 23 - 75 years, because the subjects were assessed as consuming diets typical of northeast Thailand. The data indicate the gender difference in these micronutrients. No concentration difference of these micronutrients between the age groups (20 - 45, 46 – 75 years) was found. As the northeast Thais rely on their characteristic food, further research is needed to determine the extent to which other environmental, dietary factors and pathological factors may play a role in serum levels of these micronutrients in northeast Thais.

Acknowledgements

This research was supported by a Research Grant from Khon Kaen University. The authors wish to thank the Faculty of As-

sociated Medical Sciences, Khon Kaen University, for providing serum samples.

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