

Seasonal variation of serum concentrations of β -carotene and α -tocopherol¹⁻³

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ABSTRACT We studied the seasonal variation in serum concentrations of β -carotene and α -tocopherol (HPLC) in 17 247 Finnish men who smoked. Month of blood sampling was a statistically significant determinant of serum concentration of β -carotene in a regression model including age, body mass index, alcohol and fat intakes, total serum cholesterol, and daily cigarettes as covariates. The serum concentrations were lowest in April–June and highest in October–November. The 1.5-fold increase in the serum concentration of β -carotene during the fall reflects the seasonality of dietary sources of carotenoids in Finland. The serum concentrations of α -tocopherol demonstrated no seasonal variation but remained close to 27.6 $\mu\text{mol/L}$ throughout the year. The results indicate that the seasonal variation of serum concentrations of β -carotene should be taken into account in long-term studies in which comparison of groups or individuals is based on serum concentrations. *Am J Clin Nutr* 1993;57:551–6.

KEY WORDS Seasonal variation, serum vitamins, β -carotene, α -tocopherol

Introduction

Assuming that the physiological functions of antioxidants are dependent on their concentrations in body fluids and tissues, knowledge of factors affecting those concentrations is essential in attempting to use antioxidants as preventive or therapeutic agents. The serum concentration of certain antioxidants, such as β -carotene, reflects among other things dietary intake (1). The variation in serum concentration of β -carotene can be related to differences in the amount of substrate in stable diet or to the seasonality of dietary sources. On the other hand, serum concentrations of α -tocopherol, another antioxidant, appear to be much less sensitive to changes in dietary intake, though diet is an important determinant of serum concentration (2). The purpose of this study was to investigate the degree of seasonal variation in the serum concentrations of β -carotene and α -tocopherol in Finnish men.

Subjects and methods

The subjects were participants of the Alpha-tocopherol, Beta-carotene Lung Cancer Prevention Study (ATBC study) (3), who were randomly recruited during a 2-y period from 1985 to 1987. The study was approved by the ethical committees of the National Public Health Institute of Finland and the National Cancer

Institute of the United States. The vitamin concentrations of 17 247 men were available for this study. At the beginning of the ATBC study the members of the all-male study population were Finns aged 50–69 y, smoked at least five cigarettes per day and were free of cancer. Background information, medical and dietary histories, and a blood sample were collected at baseline of the study. Serum from all men whose blood was drawn during a given month contributed to the mean serum concentration of that month. No men were enrolled, and thus no samples were collected, in July.

Before the blood sampling the men were asked to fast for 12 h. Blood was collected into 10-mL heparinized vacuum tubes between 0800 and 1100 each day. After centrifugation at 1100 $\times g$ for 10 min at room temperature, serum was transferred to glass freezer tubes and stored at -70°C until analyzed. Total serum cholesterol was determined enzymatically (cholesterol CHOD-PAP method, Monotest Cholesterol Kit from Boehringer-Mannheim, Germany).

Alpha-tocopherol and β -carotene were determined by using an HPLC assay (4). A reversed-phase column (octadecylsilica) was used for the simultaneous determination of the vitamins by using isocratic elution with methanol as the single eluant. The vitamins were extracted with *n*-hexane from the aqueous serum solution in which the proteins had been precipitated with ethanol. The *n*-hexane phase was evaporated in vacuum and the vitamins were redissolved in ethanol and a portion was injected in the liquid chromatograph. The light absorption of the compounds was measured with a diode-array detector at wavelengths 450 nm for carotenoids and 292 nm for α -tocopherol and tocol. The amounts were calculated by using echinenone at 450 nm and tocol at 292 nm as internal standards. The heights of the chromatographic peaks were used in the calculations. All manipulations were carried out in yellow light to avoid photoisomerization of the compounds. The between-runs CV was 0.036 for β -carotene and 0.022 for α -tocopherol.

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Dietary intakes of β -carotene and α -tocopherol were calculated, by using recently analyzed food-composition data (5, 6), from a food-use questionnaire covering the habitual consumption of > 300 food items for the past 12 mo. This method, modified from dietary history, facilitates a qualitative assessment of individual diet over a period of 1 y before entry into the study (7).

Statistical analyses

Analysis of covariance was used to study dependence between serum β -carotene and α -tocopherol concentrations and month of blood sampling (8). Age, body mass index (BMI; in kg/m²), serum cholesterol (mmol/L), number of daily cigarettes, and daily intakes of fat (g) and alcohol (g) were used as confounding variables in every model. These variables were chosen on the basis of prior studies (2, 9–12). Also, daily vitamin E intake for serum α -tocopherol and daily β -carotene intake for serum β -carotene were used as explanatory variables.

Adjusted means for serum vitamins were calculated according to Willett's (13) energy-adjustment approach. First, a regression model with confounders and vitamin intake as the independent variables and serum vitamin concentration as the dependent variable was calculated. Population means of serum vitamin values were added to residuals of this model to get adjusted values.

Results

The characteristics of the study population are presented in **Table 1**. Beta-carotene supplements were used by 169 (1%) and vitamin E supplements by 1460 (8.6%) participants. Doses of supplemental β -carotene ranged from 0.2 to 18 mg/d (5.8 ± 2.1 mg/d, $\bar{x} \pm$ SD) and those of vitamin E (as *d*- α -tocopherol equivalents) from 0.4 to 471 mg/d (25.3 ± 41.4 mg/d).

Month of blood sampling was a statistically significant determinant of serum concentration of β -carotene in a regression model including age, BMI, alcohol and fat intakes, total serum cholesterol, and number of daily cigarettes as covariates (**Table 2**). In this model, June was chosen as the reference (fixed) month because the serum concentrations were the lowest in June. The

TABLE 1
Characteristics of the study population*

	$\bar{x} \pm$ SD
Age (y)	57.6 \pm 5.0
BMI†	26.1 \pm 3.7
Smoking (cigarettes/d)	20.8 \pm 9.0
Diet	
β -carotene (mg/d)	2.1 \pm 1.5
Vitamin E (mg/d)	11.9 \pm 5.9
Alcohol (g/d)	18.8 \pm 22.0
Fat (g/d)	122.8 \pm 40.6
Serum	
β -carotene (μ mol/L)	0.40 \pm 0.35
α -tocopherol (μ mol/L)	27.9 \pm 7.9
Cholesterol (mmol/L)	6.3 \pm 1.2

* $n = 17\ 247$.

† BMI, body mass index; weight (kg)/height (m)².

TABLE 2
Regression model of serum β -carotene (μ mol/L) and certain factors*

	Beta \times 1000†	SE‡	Partial r^2
Age (y)	-0.49	0.52	0.0001
BMI§	-10.3	0.69	0.013
Smoking (cigarettes/d)	-2.16	0.30	0.0031
Serum cholesterol (mmol/L)	49.5	2.19	0.029
Dietary alcohol (g/d)	-3.04	0.12	0.036
Dietary fat (g/d)	0.34	0.06	0.0017
Month of blood sampling	—	—	0.015
January	47.2	19.1	
February	49.4	19.3	
March	46.3	18.9	
April	22.9	18.9	
May	14.7	19.0	
June¶	0.0	0.0	
July**	—	—	
August	82.5	20.0	
September	113.6	18.6	
October	129.2	18.7	
November	116.3	19.0	
December	78.2	19.7	

* $n = 17\ 247$.

† The actual beta's have been multiplied by 1000 to make the numerical values easier to compare.

‡ SE, standard error of beta (multiplied by 1000).

§ Weight (kg)/height (m)².

|| In the model as a categorical variable; its β value was fixed to 0.0.

¶ Reference variable in the model.

** No sampling in July.

other 10 mo of blood sampling were included into the model as independent indicator variables.

Adding daily intake of β -carotene as calculated from the dietary-history questionnaire into the regression model did not decrease the statistical significance of the month of blood sampling nor did it increase the proportion of variance explained by the model. Similarly, removing all participants consuming β -carotene supplements or adding into the model any supplemental doses of β -carotene the men reported using did not change the results.

Mean monthly serum β -carotene concentrations showed seasonal variation with lowest (0.33–0.36 μ mol/L) values in April–June and highest values (0.45–0.47 μ mol/L) in September–November (**Table 3**). BMI, total serum cholesterol, number of daily cigarettes, and daily intakes of alcohol and fat did not change the seasonal pattern when the means for age were adjusted (**Table 3**). The seasonality pattern was very similar for 2 consecutive years when the year and month were used as determinants of the sampling time (**Fig 1**). Variance of the serum concentrations of β -carotene became noticeably large during June 1987 because of the small number of men ($n = 42$) and few individuals with high serum concentration.

The main data available for this study allowed evaluation of seasonal changes between but not within individuals. The latter approach was used in a small subgroup of participants ($n = 66$) whose baseline sample had been taken in May and follow-up sample during the months of October, November, or February and who received no study supplementation with β -carotene. The differences between the monthly means of serum concen-

TABLE 3
Unadjusted and adjusted serum β -carotene concentrations by month of blood drawing

Month	Unadjusted	Adjusted*
	$\bar{x} \pm SD$	$\bar{x} \pm SD$
	$\mu\text{mol/L}$	
January (n = 1666)	0.38 \pm 0.31	0.38 \pm 0.30
February (n = 1449)	0.38 \pm 0.33	0.38 \pm 0.31
March (n = 1845)	0.38 \pm 0.36	0.38 \pm 0.34
April (n = 1818)	0.36 \pm 0.31	0.35 \pm 0.30
May (n = 1748)	0.35 \pm 0.34	0.35 \pm 0.33
June (n = 376)	0.33 \pm 0.31	0.33 \pm 0.30
July† ---	---	---
August (n = 1090)	0.42 \pm 0.30	0.41 \pm 0.28
September (n = 2212)	0.45 \pm 0.29	0.45 \pm 0.27
October (n = 2120)	0.47 \pm 0.36	0.46 \pm 0.34
November (n = 1712)	0.45 \pm 0.47	0.45 \pm 0.45
December (n = 1211)	0.42 \pm 0.42	0.41 \pm 0.33

* Adjusted for age, BMI, serum cholesterol, number of daily cigarettes, and daily intake of alcohol and fat.

† No blood sampling was done because July is a popular vacation month in Finland.

trations within individuals support the results obtained from the main data of this study (Table 4). Serum concentrations of β -carotene rose from May through November and remained elevated in February.

The monthly means of serum β -carotene in the tertiles of β -carotene intake demonstrated similar seasonal variation as in the combined group (Fig 2), though the decrease in spring and

TABLE 4
Serum β -carotene concentrations at baseline and during follow-up among men whose baseline sample was taken in May and follow-up sample in October, November, or February

Baseline $\bar{x} \pm SE$	Follow-up		Difference*	
	Month	$\bar{x} \pm SE$	Observed	Expected†
	$\mu\text{mol/L}$			
0.28 \pm 0.03 (n = 21)	Oct	0.48 \pm 0.09	0.20	0.12
0.35 \pm 0.06 (n = 14)	Nov	0.54 \pm 0.09	0.19	0.11
0.28 \pm 0.02 (n = 31)	Feb	0.37 \pm 0.03	0.09	0.03

* Follow-up value minus baseline value.

† Based on unadjusted monthly means between individuals (Table 3).

increase in autumn were not as clear in the lowest intake tertile. Serum concentrations went down in April–June and increased during autumn. The monthly means of serum concentrations in each intake tertile retained their position in respect to the other tertiles. Thus, for each month, the men in the lowest intake tertile had the lowest serum concentrations, the men in the middle tertile had intermediate concentrations, and the men in the highest tertile had the highest serum concentrations of β -carotene compared with the men in the other intake groups. In similar regression models as described previously, the month of blood sampling was a statistically significant determinant of serum β -carotene concentration in each of the intake tertiles. The interaction term of dietary β -carotene and month of blood sampling was not statistically significant in these models.

The serum concentrations of α -tocopherol demonstrated no seasonal variation whatsoever but remained close to 27.6 $\mu\text{mol/}$

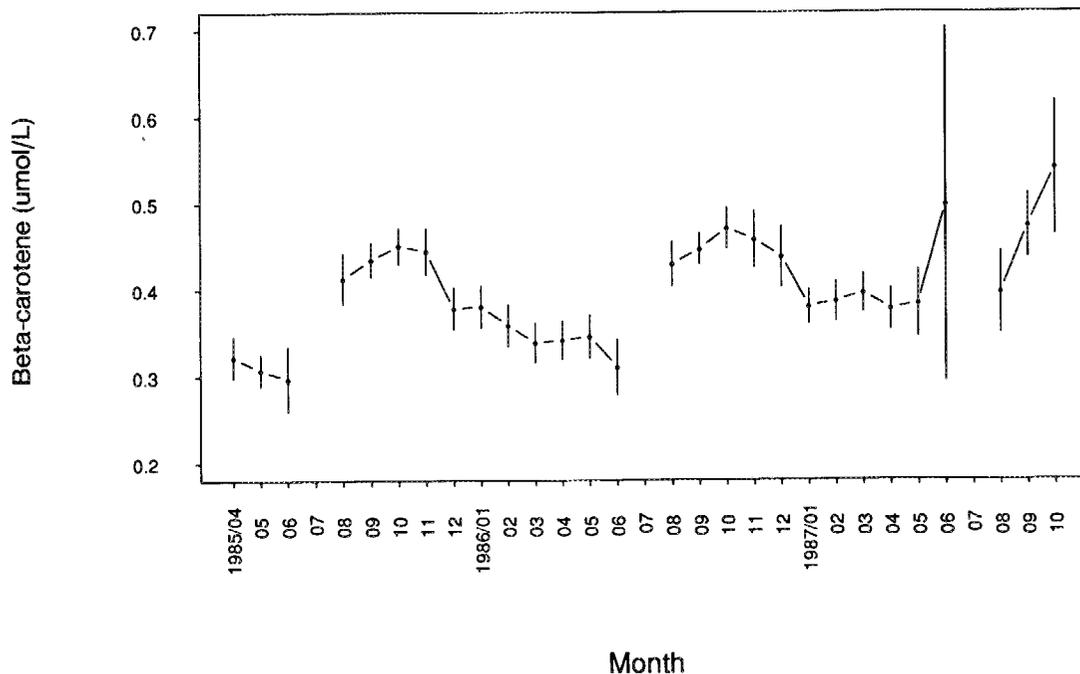


FIG 1. Mean serum β -carotene concentrations by year and month of blood sampling. The bars around the means denote 95% confidence interval of the mean ($2 \times SE$). No blood was collected in July. The number of men per month ranged from 42 in June 1987 to 1260 in March 1987.

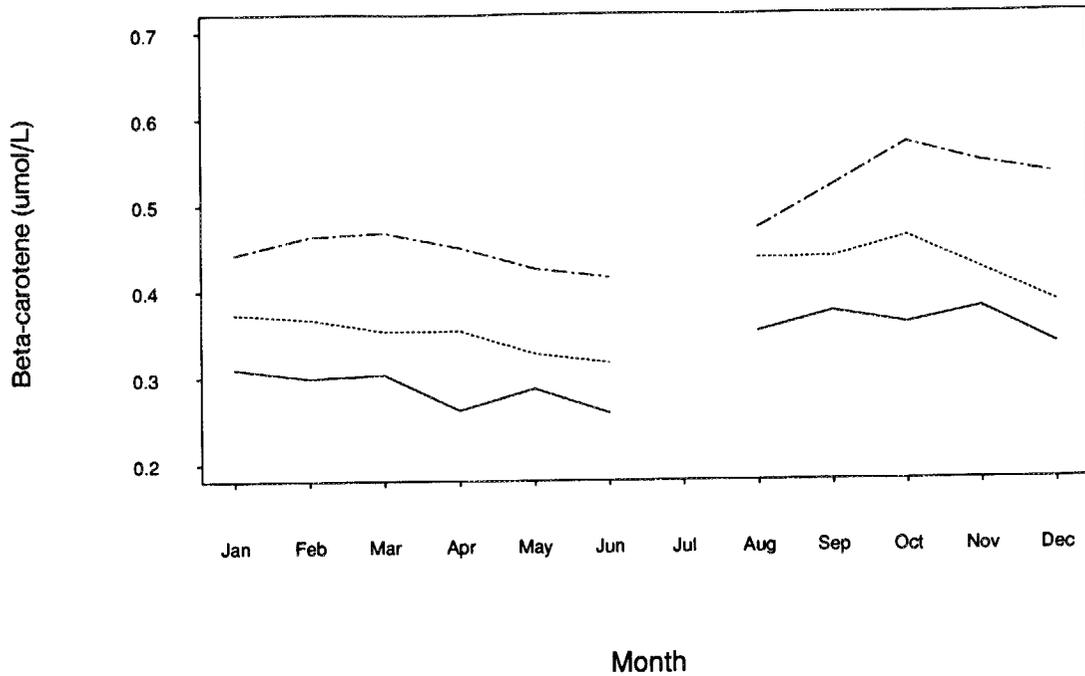


FIG 2. Mean serum β -carotene concentrations by month of blood sampling in tertiles of β -carotene intake. No blood was collected in July. (—), lowest intake tertile; (· · · · ·), middle intake tertile; and (- - - - -), highest intake tertile.

L throughout the year (Fig 3). The serum concentrations in the tertiles of vitamin E intake also manifested no clear seasonal patterns, based on the month of blood sampling (Fig 4). Similar to β -carotene, the serum concentrations of α -tocopherol correlated with vitamin E intake ($r = 0.21$ for both). Thus the men

in the lowest intake group had the lowest serum concentrations, the men in the medium intake group had intermediate serum concentrations, and the men in the highest intake tertile had the highest serum concentrations of α -tocopherol when compared with the other intake groups.

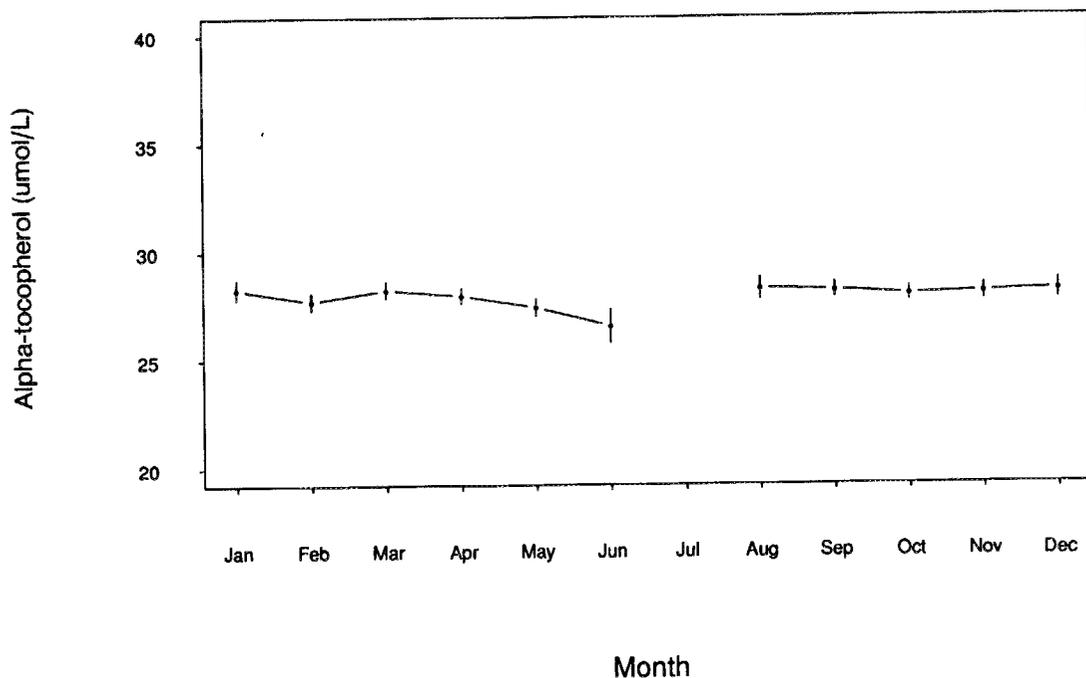


FIG 3. Mean serum α -tocopherol concentrations by month of blood sampling. The bars around the means denote 95% confidence interval of the mean ($2 \times SE$). No blood was collected in July.

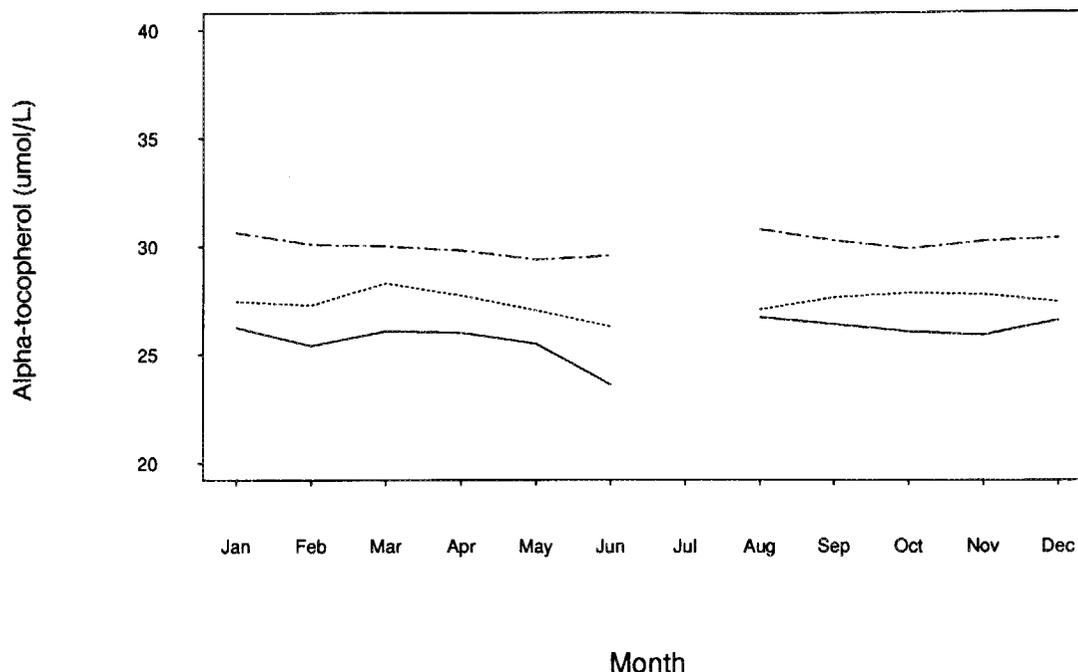


FIG 4. Mean serum α -tocopherol concentrations by month of blood sampling in tertiles of α -tocopherol intake. No blood was collected in July. (—), lowest intake tertile; (· · · · ·), middle intake tertile; (- - - -), highest intake tertile.

Discussion

Dietary intake has been shown to be one of the major determinants of serum β -carotene (1, 9). Changes in dietary intake of green-yellow vegetables have been shown to be positively associated with changes in serum concentrations of β -carotene (14). The increase in the serum concentration of β -carotene during autumn, in this study, reflect the seasonality of dietary sources of carotenoids in Finland. Other studies addressing this issue are few and from various parts of the world (11, 15–18). Although comparisons with these earlier works are difficult because of major differences in food supply, culture, and seasonal changes, they too attribute the fluctuations in serum concentrations to variations in food consumption.

The increase in serum concentrations of β -carotene during the months of August through November most probably reflects the fact that at least a proportion of this study population consisted of opportunistic vegetable consumers. These are men who normally eat relatively small amounts of vegetables such as carrots, spinach, sweet pepper, and lettuce, but who increase their intake as supplies increase and prices decrease during the fall. Many of the men normally consuming low or moderate amounts of vegetables are more prone to take advantage of better availability of these foodstuffs. Also, those men who grow their own vegetables have an ample supply during the months after harvest.

Changes in the serum concentration of β -carotene within individuals have to be interpreted cautiously because of the small number of men in this subsample. The results, however, lend further support to the seasonal pattern of serum concentrations and the possible seasonality of intake. Cantilena et al (18) recently reported that they observed no within-person seasonal variation among the 29 subjects in their study. One reason for this could be lack of variability in dietary intake, which they were unable to report, during the different seasons. The fact that men in each

of the intake tertiles demonstrated a similar monthly fluctuation in serum concentrations supports the possibility of similar seasonal consumption pattern regardless of the intake amount.

Dietary intake of β -carotene and other nutrients, as measured by the method used in our study, did not explain the fluctuation in the serum concentrations. Even though the dietary information was collected on the same day as the blood sample, the calculated measures of intake should not reflect the diet of that particular season because the dietary method was designed to provide a quantitative assessment of individual diet over a period of 1 y. The results of this study implicate that it indeed meets this objective.

However, other sources of information on the Finnish diet (19, 20) indicate that the variation in serum concentrations of β -carotene coincides with that of β -carotene intake. It is of interest that Ziegler et al (21) found carotenoid intake in winter and fall to be $\approx 66\%$ of that in summer and spring in the North-eastern parts of the United States. The pattern thus appears to be different in Finland, where vegetables contribute $\approx 85\%$ of the total dietary β -carotene and carrot alone some 70% (20). Reasons for these differences between various parts of the world most probably include, in addition to diverse dietary habits, contrasts in climatic conditions and food-distribution systems.

Another quite interesting explanation for the monthly fluctuations observed in the serum concentration of β -carotene is the seasonal changes in the amount of ambient daylight. Light has been shown to reduce plasma carotenoid concentrations in human subjects (22). In this experimental study, photodegradation was induced with controlled light exposure of mainly ultraviolet-A irradiation and mean plasma carotenoids were reduced by 0.16 $\mu\text{mol/L}$. The seasonal pattern of ambient light is very pronounced in Finland. The amounts of both global radiation (MJ/m^2) and sunshine duration (h) are lowest in De-

ember–January, increase almost linearly during spring, reach maximum amounts in May–July, and decline again linearly during the fall (23). These changes, together with the variation in intake, could very well be reasons for the seasonal pattern of serum β -carotene concentrations in this study.

Dietary intake has also been shown to be a significant predictor of serum α -tocopherol concentrations (2, 24). Serum concentrations of α -tocopherol do not, however, respond to changes in intake as readily and strongly as those in β -carotene (25, 26). Data on seasonal variation of serum concentrations for this nutrient are scant. Contrary to our results, one study did report seasonal variation with higher serum concentrations of α -tocopherol in the autumn than during other seasons (10). This study, which was also conducted in Finland, attributed the autumnal rise with the increased supply of fresh vegetables and high α -tocopherol content of certain foodstuffs (6). Our dietary data indicated no seasonal changes in the intake of vitamin E. Other reports on the determinants of serum α -tocopherol concentrations do not address the issue of seasonal differences (27, 28). Comstock et al (29) observed no association between serum concentrations and month of blood sampling, but their observation period was limited to only 3 consecutive months.

In conclusion, the results of this study indicate that the serum concentrations of β -carotene, but not α -tocopherol, can be dependent on the month of blood sampling. The possibility of seasonal variation should be taken into account when conducting long-term studies in which comparison of groups or individuals is based on serum concentrations. \square

References

- Shibata A, Sasaki R, Ito Y, et al. Serum concentration of beta-carotene and intake frequency of green-yellow vegetables among healthy inhabitants of Japan. *Int J Cancer* 1989;44:48–52.
- Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* 1988;127:283–96.
- Albanes D, Virtamo J, Rautalahti M, et al. Pilot study: the US-Finland lung cancer prevention trial. *J Nutr Growth Cancer* 1986;3:207–14.
- Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene, and alpha and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin Chem* 1986;32:874–6.
- Heinonen M. Carotenoids and retinoids in Finnish foods and the average diet. EKT-series 811. Helsinki: University of Helsinki, 1990.
- Piironen V. Tocopherols and tocotrienols in foods and in the average Finnish diet. EKT-series 726. Helsinki: University of Helsinki, 1986 (in Finnish with an English abstract).
- Pietinen P, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments. *Am J Epidemiol* 1988;128:655–66.
- Snedecor GW, Cochran WG. *Statistical methods*. 7th ed. Ames, IA: Iowa State University Press, 1980.
- Russell-Briefel R, Bates MW, Kuller LH. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am J Epidemiol* 1985;122:741–9.
- Knekt P, Seppanen R, Aaran R. Determinants of serum alpha-tocopherol in Finnish adults. *Prev Med* 1988;17:725–35.
- Nierenberg DW, Stukel TA, Baron JA, Dain BJ, Greenberg ER, The Skin Cancer Prevention Study Group. Determinants of plasma levels of beta-carotene and retinol. *Am J Epidemiol* 1989;130:511–21.
- Albanes D, Virtamo J, Rautalahti M, et al. Serum beta-carotene before and after beta-carotene supplementation. *Eur J Clin Nutr* 1992;46:15–24.
- Willett W. *Nutritional Epidemiology*. London: Oxford University Press, 1990.
- Suzuki S, Sasaki R, Ito Y, et al. Changes in serum concentrations of beta-carotene and changes in the dietary intake frequency of green-yellow vegetables among healthy male inhabitants of Japan. *Jpn J Cancer Res* 1990;81:463–9.
- Le Francois P, Chevassus-Agnes S, Benefice E, et al. Vitamin A status of populations in three West African countries. *Int J Vitam Nutr Res* 1980;50:352–63.
- Bates C, Villard L, Prentice A, Paul A, Whitehead R. Seasonal variations in plasma retinol and carotenoid levels in rural Gambian women. *Trans R Soc Trop Med Hyg* 1984;78:814–7.
- Zheng S, Ershow A, Yang C, et al. Nutritional status in Linxian, China: Effects of season and supplementation. *Int J Vitam Nutr Res* 1988;59:190–9.
- Cantilena LR, Stukel TA, Greenberg ER, Nann S, Nierenberg DW. Diurnal and seasonal variation of five carotenoids measured in human serum. *Am J Clin Nutr* 1992;55:659–63.
- Heinonen M, Ollilainen V, Linkola E, Varo P, Koivistoinen P. Carotenoids in Finnish foods: vegetables, fruits, and berries. *J Agric Food Chem* 1989;37:655–9.
- Heinonen M. Food groups as the source of vitamin A and beta-carotene in Finland. *Int J Vitam Nutr Res* 1990;61:3–9.
- Ziegler RG, Wilcox HB III, Mason TJ, Bill JS, Virgo PW. Seasonal variation in intake of carotenoids and vegetables and fruits among white men in New Jersey. *Am J Clin Nutr* 1987;45:107–14.
- Roe D. Photodegradation of carotenoids in human subjects. *Fed Proc* 1987;46:1886–9.
- Finnish Meteorological Institute. *Global radiation and duration of sunshine. Monthly reports*. Helsinki: Finnish Meteorological Institute, 1991 (in Finnish).
- Herbeth B, Chavance M, Musse N, Mejean L, Vernhes G. Dietary intake and other determinants of blood vitamins in an elderly population. *Eur J Clin Nutr* 1989;43:175–86.
- Willett W, Stampfer M, Underwood B, Taylor J, Hennekens C. Vitamins A, E, and carotene: effect of supplementation on their plasma levels. *Am J Clin Nutr* 1983;38:559–66.
- Lehmann J, Rao D, Canary J, Judd J. Vitamin E and relationships among tocopherols in human plasma, platelets, lymphocytes, and red blood cells. *Am J Clin Nutr* 1988;47:470–4.
- Kivela S, Maenpaa P, Nissinen A, et al. Vitamin A, vitamin E and selenium status in an aged Finnish male population. *Int J Vitam Nutr Res* 1989;59:373–80.
- Bjorneboe A, Bjorneboe GA, Drevon CA. Absorption, transport and distribution of vitamin E. *J Nutr* 1990;120:233–42.
- Comstock GW, Menkes MS, Schober SE, Vuilleumier J, Helsing KJ. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. *Am J Epidemiol* 1987;127:114–23.