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Relations between Antioxidant Vitamins in Adipose Tissue, Plasma, and Diet

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For an evaluation of fat-soluble vitamin concentrations in adipose tissue as biomarkers of intake, estimates of usual intake of β-carotene, total vitamin A, and vitamin E (assessed by food frequency questionnaire) were compared with plasma and adipose tissue concentrations of β-carotene, retinol, and α-tocopherol, respectively. Data were collected in 1992 in the Netherlands for 85 healthy, nonsmoking volunteers aged 50–70 years (38 males and 47 females). For α-tocopherol, a significant age- and sex-adjusted partial correlation ($r = 0.24$, $p < 0.05$) was observed between adipose tissue levels and intake. For β-carotene, the partial r was 0.20. Adipose tissue retinol did not reflect intake (partial $r = 0.08$). Correlations of adipose tissue vitamin levels with plasma vitamin levels were higher overall ($r = 0.34$ for α-tocopherol, $r = 0.56$ for β-carotene, and $r = 0.17$ for retinol) than correlations with intake. Plasma concentrations of α-tocopherol, β-carotene, and retinol were not associated with dietary intake (partial r's were 0.05, 0.17, and −0.12, respectively). Pearson correlations of repeated measurements in adipose tissue (after 4 months) were 0.24 for retinol, 0.50 for β-carotene, and 0.78 for α-tocopherol. Adipose tissue β-carotene was shown to increase sixfold after 6 months' supplementation with 30 mg of β-carotene daily. It is concluded that adipose tissue vitamin concentrations are an acceptable alternative to plasma levels as relatively stable indicators of dietary intake. However, both plasma and adipose tissue levels are more useful as markers of internal dose, taking into account variations in absorption and metabolism, than of dietary intake. *Am J Epidemiol* 1995;141:440–50.

adipose tissue; biological markers; carotene; diet; vitamin A; vitamin E

Antioxidant vitamins may have an important role in the prevention of certain types of cancer and cardiovascular disease (1, 2). In epidemiologic studies, antioxidant intake is often assessed by means of a food frequency questionnaire. In case-control studies, however, this type of assessment is susceptible to differential recall by cases and controls and therefore may bias the outcome of the study; in multicountry studies, important in achieving larger ranges in intake, diet questionnaires are difficult to standardize. The reliability of intake estimates also depends on the quality of the food composition tables, which can only give average values for vitamin content in specific foods. To overcome these problems, biomarkers of intake, e.g., plasma β-carotene, may be used as an alternative to diet assessment. However, dietary intake is not the only determinant of biomarker status. Antioxidant levels in blood or tissue are also affected by genetic and lifestyle factors, as well as intake of other nutrients. In case-control studies, a useful biomarker of intake should be related to intake over a relatively long period of time, because the onset of chronic diseases is usually related to long-term exposures. Levels of vitamin E and β-carotene in plasma are related to intake but represent a relatively short time period. In assessing the fatty acid composition of the diet, especially for polyunsaturated fats, the composition of adipose tissue has been shown to be a reliable indicator (3, 4). Since vitamin E, β-carotene, and retinol are fat-soluble compounds that are stored in subcutaneous adipose tissue, concentrations in adipose tissue may be a better indicator of intake than are plasma levels. Information on the relation between intake and adipose tissue levels of these vitamins in free-living human populations is very limited (5, 6).

Therefore, we studied the relation between dietary intake and plasma and adipose tissue levels of vitamin E, β-carotene, and retinol; linoleic acid content was included as well, to provide an independent means of comparison with other studies. The interrelation of these parameters was studied in a cross-sectional design. Moreover, reproducibility was assessed by repeated measurement, and for β-carotene the tissue response to supplementation was examined.

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MATERIALS AND METHODS

Study population

Subjects in this study were men and women aged 50–70 years who were recruited in the municipalities of Utrecht and Zeist, The Netherlands, by an advertisement in a local newspaper. All 85 volunteers (38 men and 47 women) were apparently healthy nonsmokers, did not use vitamin supplements or prescribed medication, had not lost weight in excess of 5 kg, and had had stable food consumption habits during the previous year. The protocol was approved by an independent medical-ethical review board.

Design

A group of 30 men and 26 women (group 1) was invited to the TNO Nutrition and Food Research Institute for two visits held 4 months apart (in May and September 1992). Both times, a fat aspirate from the gluteal region and a blood sample were obtained, and a food frequency questionnaire was distributed to the participant and returned by mail. Alcohol intake was assessed with a separate questionnaire during the first visit, and anthropometric measurements were taken. A second group (8 men and 21 women) was asked to take two capsules containing β-carotene (15 mg each) daily for a period of 6 months (from April to October of 1992) before the second visit. The same data were collected as in the first group. The groups were not randomized. Data for group 1 were used to assess the reproducibility of measurements. Results from group 2 provided information on the responsiveness of adipose tissue concentrations to dietary intake of β-carotene. Data from the first visit of both groups were used to assess correlations between dietary intake and plasma and adipose tissue concentrations.

β-Carotene supplementation

At their first visit, subjects in group 2 were given written instructions to take two β-carotene capsules daily with their main meal. The capsules used were commercially available (Lamberts Natural Beta Carotene; Lamberts, Tunbridge Wells, England) and contained 15 mg of β-carotene encapsulated with gelatin, glycerine, soybean oil, and water. Analysis in our laboratory indicated a mean value of 17.8 mg of β-carotene per capsule. Participants received capsules in 2-month batches and were instructed to store them in a cool, dry place and protect them from light. Compliance was monitored by a repeated questionnaire in combination with a calendar on which intake was to be recorded. Two subjects stopped taking the capsules prematurely; among the other subjects, 98 percent of the capsules were said to have been taken. group 1 did not receive β-carotene capsules was no formal placebo group.

Fat aspiration and blood collection

Subcutaneous fat aspirates were obtained b biopsy from the lateral buttock as described by and Katan (7), using a 16-gauge needle attached to a plastic container in which the tissue (mean amount = 39 mg (standard deviation, 15 mg)) was collected by connecting a vacuum tube. The plastic container was immediately placed on dry ice and stored at −80°C until analysis. A venous blood sample was drawn in a 10-ml heparin tube from nonfasting participants and was refrigerated until plasma was separated. The blood was centrifuged (10 minutes at 1,500 *g*) within 2 hours, and plasma was stored at −80°C.

Biochemical analyses

Samples taken at the first and second visit for each subject were analyzed in the same run. Concentrations of retinol, β-carotene, and α-tocopherol in adipose tissue and plasma were determined by reverse-phase high-performance liquid chromatography (8, 9) and spectrophotometric detection. Samples were protected from light during the analysis. The adipose tissue sample was saponified and quantitatively split for vitamin and fatty acid determination. The coefficients of variation for the analysis of retinol, β-carotene, and α-tocopherol in adipose tissue were 4.3 percent, 7.1 percent, and 4.9 percent, respectively (at mean values of 4.66 μg/g, 2.36 μg/g, and 85.3 μg/g, respectively, in the quality control samples). For determination in plasma, coefficients of variation were 4.3 percent for retinol (mean = 1.78 μmol/liter), 7.3 percent for β-carotene (mean = 0.26 μmol/liter), and 3.2 percent for α-tocopherol (mean = 25.4 μmol/liter). Vitamin concentrations in adipose tissue were expressed in μg/g of total fatty acids (the mean fatty acid content of aspirates was 14.5 mg (standard deviation, 9.1 mg)). Concentrations were unaffected by storage.

Fatty acid content was assessed by gas liquid chromatography in an aliquot of the same extract as that of the vitamins, adding heptadecanoic acid (C17 : 0) as an internal standard to the sample before saponification. Linoleic acid (C18 : 2) was quantitated as the percentage of the peak area of total fatty acids. The coefficient of variation for linoleic acid was 3.4 percent. Total plasma cholesterol was determined enzymatically (CHOD-PAP method; Boehringer, Mannheim, Germany).

Food frequency questionnaire

Usual dietary intake of retinol, β-carotene, and vitamin E during the previous year was assessed by a 95-item self-completed semiquantitative food frequency questionnaire that was adapted from a questionnaire on retinol and β-carotene intake developed and validated by Stiggelbout et al. (10). Additional items representing vitamin E intake were selected on the basis of their high vitamin E content and regular use according to the 1987 Dutch National Food Consumption Survey (11). Frequency of consumption was reported in eight categories: never, seldom, 1 day per month, 1 day per 2–3 weeks, 1–2 days per week, 3–4 days per week, 5–6 days per week, and 7 days per week. The number of servings per consumption day was quantified by respondents in terms of natural or household measures (slices, spoons, cups) to which standard weights were assigned. For some foods (e.g., milk and dairy products), individual portion sizes were calculated from the number of glasses obtained from 1 liter. Participants were asked to report consumption of a number of vegetables and fruits separately by season (summer or winter). The use of fats and oils was specified according to type and brand.

Questionnaire responses were entered and processed using an automated processing system. Completeness, credibility of the reported number of servings, and consistency in reported consumption frequencies were checked. If necessary, participants were contacted for further information. Data on mean daily consumption of food products in grams were converted to nutrient intakes using the computerized Dutch Food Composition Table (12) for retinol equivalents and linoleic acid. Information on vitamin E and β-carotene content was derived primarily from recent analyses of Dutch food products (13). Since these analyses did not provide complete coverage of all foods in the questionnaire (75 percent for vitamin E, 97 percent for β-carotene), additional information was obtained from calculations and from the British food composition table (14). The values for vitamin E take into account the contribution of the different tocopherols and tocotrienols. Intake of linoleic acid was calculated, although the questionnaire was not specifically designed to measure this nutrient. However, since the important sources of linoleic acid and vitamin E are largely the same foods, the questionnaire was considered to measure linoleic acid well enough to obtain an adequate ranking of subjects according to intake.

Statistical analysis

In the group of 56 subjects who did not take supplements, the fat aspirate was missing for three persons at the first measurement. For two others, the vitamin values were outside three standard deviations of the mean; these values were considered unreliable because of very little fat tissue in the fat aspirate, and therefore were excluded. For three subjects, the food frequency questionnaire data were excluded from the analysis, because the food intake pattern of these people differed substantially from the usual Dutch diet, for which the questionnaire was designed. Three other subjects had missing plasma data for the second measurement. In the group of 29 subjects who took supplements, three subjects had missing fat aspirate data for the first measurement. For the second measurement, aspirate data were missing for two subjects, plasma data for two other subjects, and food frequency questionnaire data for one person.

Skewed distributions of all dietary, plasma, and adipose tissue nutrients, except for dietary retinol equivalents and relative intake of linoleic acid (percentage of total fat intake), were normalized by natural logarithm. All correlation analyses were performed on the $\log_e$-transformed data. In tables 1 and 3, untransformed mean levels are presented; in table 5, values were retransformed from the $\log_e$ value for ease of interpretation. Plasma α-tocopherol values were strongly correlated with total plasma cholesterol ($r = 0.64$, $p < 0.001$), but plasma β-carotene was not ($r = 0.15$, $p = 0.16$). Therefore, absolute plasma α-tocopherol values as well as the ratio of α-tocopherol to total plasma cholesterol are presented where necessary.

Mean vitamin levels and their standard deviations were calculated. The reproducibility of all three independent measurements (in diet, plasma, and adipose tissue) was assessed by calculating Pearson correlation coefficients. The associations between adipose tissue vitamin levels and age, sex, body mass index (weight (kg)/height $(m)^2$), waist : hip ratio, alcohol intake, and socioeconomic status were tested by linear regression analysis. The change in adipose tissue and plasma antioxidant concentrations in the supplemented group was adjusted for changes in the nonsupplemented group through the use of linear regression of the second measurement on group and first measurement. Simple Pearson and age- and sex-adjusted correlations between adipose tissue and plasma levels and dietary intake (as assessed by food frequency questionnaire) were calculated. To account for variability in the plasma and adipose tissue measurements, correlation coefficients were deattenuated using the within-person : between-person variance ratios (15), calculated with analysis of variance from the repeated measurements in the nonsupplemented group. The

deattenuated correlation (r_t) was calculated from the observed correlation (r_o) as follows:

$$r_t = r_o \sqrt{[(1 + \lambda_x/2)(1 + \lambda_y/2)]},$$

where λ is the variance ratio, x's are plasma measurements, and y's are adipose tissue measurements.

Finally, age- and sex-adjusted mean dietary intake and plasma levels are presented across quartiles of adipose tissue concentrations. Tests for trends over quartiles were performed by assigning the median value of the quartile to each category. Analyses were performed using the BMDP software package (BMDP Statistical Software, Inc., Los Angeles, California).

RESULTS

Characteristics of the study population at the start of the study are presented in table 1. Men and women differed with respect to socioeconomic status and waist : hip ratio. Usual intake of vitamin E ranged from 5.5 mg/day to 37.3 mg/day; intake of β-carotene varied from 0.64 mg to 4.05 mg. Plasma levels of α-tocopherol ranged from 20.1 μmol/liter to 55.6 μmol/liter (the α-tocopherol : cholesterol ratio ranged from 2.8 to 8.0), and plasma β-carotene ranged from 0.09 μmol/liter to 4.7 μmol/liter. Adipose tissue concentrations of α-tocopherol varied 11-fold, between 87 μg/g and 950 μg/g; the range for β-carotene was about 20-fold, from 0.33 μg/g to 6.9 μg/g.

TABLE 1. Characteristics of the study population and mean dietary intakes, plasma levels, and adipose tissue levels of fat-soluble vitamins and linoleic acid, The Netherlands, 1992

	Men (n = 38)		Women (n = 47)	
	Mean	SD*	Mean	SD
Age (years)	59.5	6.3	58.3	5.9
BMI* (weight (kg)/height (m)^2)	24.4	2.7	24.7	3.3
Waist:hip ratio	0.90	0.06	0.83	0.10
Serum cholesterol (mmol/liter)	5.9	1.2	5.7	0.9
Alcohol use (%)	95		94	
Socioeconomic status (%)				
Low	32		52	
High	68		48	
Dietary intake				
β-carotene (mg/day)	1.73	0.66	1.75	0.72
Retinol (RE*/day)	0.83	0.23	0.79	0.24
Vitamin E (mg/day)	17.3	6.5	13.1	5.9
Linoleic acid (% of total fat/day)	19.0	7.0	13.8	7.2
Plasma				
β-carotene (μmol/liter)	0.41	0.26	0.63	0.67
Retinol (μmol/liter)	2.47	0.59	2.11	0.68
α-tocopherol (μmol/liter)	35.5	8.6	32.6	7.4
α-tocopherol: cholesterol ratio	6.0	1.0	5.7	1.0
Adipose tissue				
β-carotene (μg/g)	1.08	0.66	1.81	1.20
Retinol (μg/g)	2.99	3.08	2.35	1.30
α-tocopherol (μg/g)	240	106	281	152
Linoleic acid (%)	15.9	4.8	13.0	4.4

* SD, standard deviation; BMI, body mass index; RE, retinol equivalents.

Pearson correlation coefficients were calculated for repeated measurements of vitamin and linoleic acid content in adipose tissue and plasma and dietary intake in the nonsupplemented group (table 2). The highest reproducibility was found for plasma values. (The correlation for cholesterol-standardized α-tocopherol was lower than that for absolute levels of α-tocopherol.) For adipose tissue, the reproducibility of retinol levels was low (r = 0.24). The highest correlation in adipose tissue was observed for α-tocopherol (r = 0.78, $p < 0.001$). Reproducibility of adipose tissue β-carotene values was considerably better for women than for men (r = 0.62 and r = 0.39 for women and men, respectively). The same could be said for linoleic acid content (r = 0.91 for women, r = 0.36 for men). In the smaller samples containing less than 10 mg of fatty acids, the correlation for α-tocopherol was 0.69, that for β-carotene was only 0.21 (p = 0.3), and that for linoleic acid was 0.44 (p = 0.02); corresponding correlation coefficients in samples containing more than 10 mg of fatty acids were 0.90, 0.90, and 0.89, respectively, over the same range. These data point to larger analytical variation in smaller samples. Repeated assessment of dietary intake by food frequency questionnaire showed somewhat higher correlation coefficients for linoleic acid (r = 0.80) than for the vitamins.

To evaluate whether an increased β-carotene intake resulted in enhanced β-carotene levels in adipose tissue, we compared concentrations of the vitamins in adipose tissue and plasma before and after the 6-month supplementation period. The difference was corrected for any changes observed in the nonsupplemented group (table 3). In adipose tissue, a sixfold increase in mean β-carotene levels was measured. The difference in the supplemented group, after correction, was 7.56 μg/g (95 percent confidence interval 4.81–10.3). Adipose tissue α-tocopherol and retinol did not change significantly. In plasma, we observed an (eightfold) increase in β-carotene as well, on average with 3.28 μmol/liter (95 percent confidence interval 2.75–3.81). For α-tocopherol and retinol, no change was seen. There appeared to be no correlation between the increase in plasma β-carotene and the increase in adipose tissue β-carotene (r = 0.09); however, when two

TABLE 2. Pearson correlations† between repeated measurements of vitamin and linoleic acid content in adipose tissue and plasma and dietary intake as measured by food frequency questionnaire, The Netherlands, 1992

	Adipose tissue			Plasma			Dietary intake		
	Men (n = 26)	Women (n = 25)	Overall (n = 51)	Men (n = 28)	Women (n = 25)	Overall (n = 53)	Men (n = 29)	Women (n = 24)	Overall (n = 53)
β-carotene	0.39*	0.62***	0.50***	0.90***	0.95***	0.93***	0.60***	0.74***	0.67***
Retinol‡	0.24	0.27	0.24	0.53**	0.79***	0.74***	0.38*	0.47*	0.43
α-tocopherol‡	0.77***	0.86***	0.78***	0.82***	0.89***	0.86***	0.68***	0.55**	0.63***
α-tocopherol:cholesterol ratio				0.72***	0.67***	0.69***			
Linoleic acid (%)	0.36	0.91***	0.58***				0.81***	0.76***	0.80***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
† All data were log-transformed, except retinol equivalents and linoleic acid from the food frequency questionnaire.
‡ The food frequency questionnaire measured vitamin E and retinol equivalents.

extreme values were excluded, a significant correlation was observed ($r = 0.56$, $p < 0.005$).

The associations of adipose tissue α-tocopherol and β-carotene with sex, age, socioeconomic class, alcohol intake, body mass index, and waist : hip ratio were tested by linear regression analysis, using the first measurement for all subjects. None of these factors were associated with adipose tissue α-tocopherol; β-carotene levels were significantly higher in women than in men, and were inversely associated with waist : hip ratio ($p = 0.01$) but not with body mass index ($p = 0.25$). When sex was taken into account, waist : hip ratio was no longer associated with adipose tissue β-carotene.

Intake of vitamin E was significantly associated with intake of β-carotene ($r = 0.34$, $p < 0.05$); since this may be caused by the association of both nutrients with energy intake, we also examined the correlation of vitamin intake relative to total fat intake. The significant association remained ($r = 0.45$, $p < 0.001$). Plasma β-carotene and cholesterol-standardized plasma α-tocopherol were not significantly associated ($r = 0.06$), but adipose tissue β-carotene and α-tocopherol were ($r = 0.29$, $p = 0.01$).

Correlations between adipose tissue and plasma measurements and diet are displayed in table 4. Plasma and dietary measurements of retinol were not correlated ($r = 0.06$). Intake of vitamin E was weakly correlated with cholesterol-standardized plasma α-tocopherol ($r = 0.22$), but not with absolute plasma levels. Overall, adipose tissue levels were correlated more strongly with intake than were plasma levels; for β-carotene, the crude correlation was 0.16 (not significant), and the age- and sex-adjusted partial correlation was 0.20 ($p = 0.09$). For α-tocopherol, similar correlations were calculated (crude $r = 0.19$, partial $r = 0.24$, $p = 0.05$). The linoleic acid content of adipose tissue was significantly related to intake ($r = 0.44$,

TABLE 3. Effect of β-carotene supplementation on β-carotene, α-tocopherol, and retinol levels in adipose tissue and plasma, The Netherlands, 1992

Vitamin	Group	Baseline		End		Difference		Corrected difference	95% CI*
		Mean	SD*	Mean	SD	Mean	SD		
				Adipose tissue (μg/g)†					
β-carotene	Supplemented	1.47	0.83	9.31	9.74	7.84	9.42	7.56	4.81–10.3
	Reference	1.54	1.18	1.84	2.45	0.31	2.43		
α-tocopherol	Supplemented	278	134	327	155	49	135	22	−35 to 80
	Reference	258	138	286	184	28	114		
Retinol	Supplemented	2.21	1.25	3.07	2.12	0.86	2.27	−0.00	−1.76 to 1.76
	Reference	2.81	2.60	3.07	4.23	0.26	5.02		
				Plasma (μmol/liter)‡					
β-carotene	Supplemented	0.46	0.28	3.77	2.01	3.31	1.95	3.28	2.75–3.81
	Reference	0.58	0.65	0.63	0.67	0.04	0.14		
α-tocopherol	Supplemented	34.2	8.6	35.0	9.6	0.8	6.2	−0.4	−2.7 to 1.9
	Reference	33.1	7.3	34.3	8.3	1.2	4.2		
Retinol	Supplemented	2.32	0.79	2.43	0.98	0.11	0.70	0.10	−0.14 to 0.34
	Reference	2.21	0.59	2.26	0.50	0.04	0.46		

* SD, standard deviation; CI, confidence interval.
† Supplemented group, n = 25; reference group, n = 51.
‡ Supplemented group, n = 27; reference group, n = 53.

TABLE 4. Pearson correlations† between vitamin and linoleic acid levels in adipose tissue, plasma, and diet (assessed by food frequency questionnaire), The Netherlands, 1992

Overall	Adipose tissue vs. plasma (n = 77)			Adipose tissue vs. diet (n = 74)			Plasma vs. diet (n = 82)		
	Crude	Age- and sex-adjusted	Deattenuated‡	Crude	Age- and sex-adjusted	Deattenuated‡	Crude	Age- and sex-adjusted	Deattenuated‡
α-tocopherol§	0.31**	0.34**	0.34**	0.19	0.24*	0.20	0.11	0.05	0.11
Men	0.53**			0.16			0.18		
Women	0.21			0.30*			−0.06		
β-carotene	0.62***	0.56***	0.77***	0.16	0.20	0.20	0.15	0.17	0.15
Men	0.53**			0.30			−0.07		
Women	0.59***			0.12			0.33*		
Retinol‖	0.21	0.17	0.31**	0.12	0.08	0.14	0.06	−0.12	0.07
Men	0.25			0.14			−0.02		
Women	0.16			0.08			−0.12		
Linoleic acid				0.44***	0.42***	0.51***			
Men				0.40*					
Women				0.40*					
α-tocopherol:cholesterol ratio	0.30**	0.34***	0.35**				0.22*	0.19	0.24*
Men	0.60***						0.33*		
Women	0.18						0.09		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
† Correlations for $\log_e$-transformed values, except for retinol equivalents and linoleic acid from the food frequency questionnaire.
‡ Deattenuation of the crude correlation for within-person:between-person variance ratio.
§ α-tocopherol in adipose tissue and plasma; vitamin E in the diet.
‖ Retinol in adipose tissue and plasma; retinol equivalents in the diet.

$p < 0.001$). Figures 1–3 graphically depict the relations between dietary intakes and adipose tissue levels of α-tocopherol, β-carotene, and linoleic acid, respectively.

Mutual correlations were higher for plasma and adipose tissue levels than for dietary intake. Correlation was especially high for β-carotene (partial $r = 0.56$, $p < 0.001$). Standardization of plasma α-tocopherol to cholesterol did not affect the correlation with adipose tissue α-tocopherol. A higher correlation between plasma and adipose tissue α-tocopherol was observed for men than for women (crude r's = 0.53 and 0.21 for men and women, respectively).

From the repeated measurements in the nonsupplemented group, the within-person : between-person variance ratio was calculated. The ratio varied between 0.08 for β-carotene and 0.45 for cholesterol-standardized α-tocopherol in plasma, and between 0.28 for α-tocopherol and 1.7 for retinol in adipose tissue. Crude correlation coefficients for the interrelations between dietary intake and plasma and adipose tissue levels were deattenuated for this variance ratio (table 4), which did not change the results substantially.

Table 5 shows the age- and sex-adjusted mean plasma values and intakes for β-carotene, vitamin E, and linoleic acid, by quartile of adipose tissue concentration. Mean intake of β-carotene changed little across adipose tissue quartiles. Intake of vitamin E was 27 percent higher in the highest adipose tissue quartile than in the lowest quartile. For relative intake of linoleic acid, the difference was 42 percent. Mean plasma levels of β-carotene in the highest adipose tissue quartile (0.63 μmol/liter) were more than twice as high as those in the lowest quartile (0.30 μmol/liter). For absolute plasma α-tocopherol, the difference

TABLE 5. Age- and sex-adjusted mean vitamin and linoleic acid concentrations* in plasma and dietary intakes (assessed by food frequency questionnaire), by quartile of adipose tissue concentration, The Netherlands, 1992

	Adipose tissue quartile†				p-trend
	1	2	3	4	
Plasma					
β-carotene (μ-mmol/liter)	0.30	0.32	0.51	0.63	<0.01
α-tocopherol (μmol/liter)	30.3	33.5	33.6	34.6	0.11
α-tocopherol: cholesterol ratio	5.27	5.89	5.90	6.08	<0.05
Dietary intake					
β-carotene (mg/day)	0.90	0.89	0.98	1.00	0.35
Vitamin E (mg/day)	13.1	12.5	12.7	16.6	<0.05
Linoleic acid (g/day)	13.8	15.0	16.4	19.6	0.01

* Retransformed from the $\log_e$-transformed values.
† Quartile cutpoints: β-carotene, 0.83, 1.14, and 2.01 μg/g; α-tocopherol, 180, 238, and 309 μg/g; linoleic acid, 11.7%, 13.1%, and 16.4%.

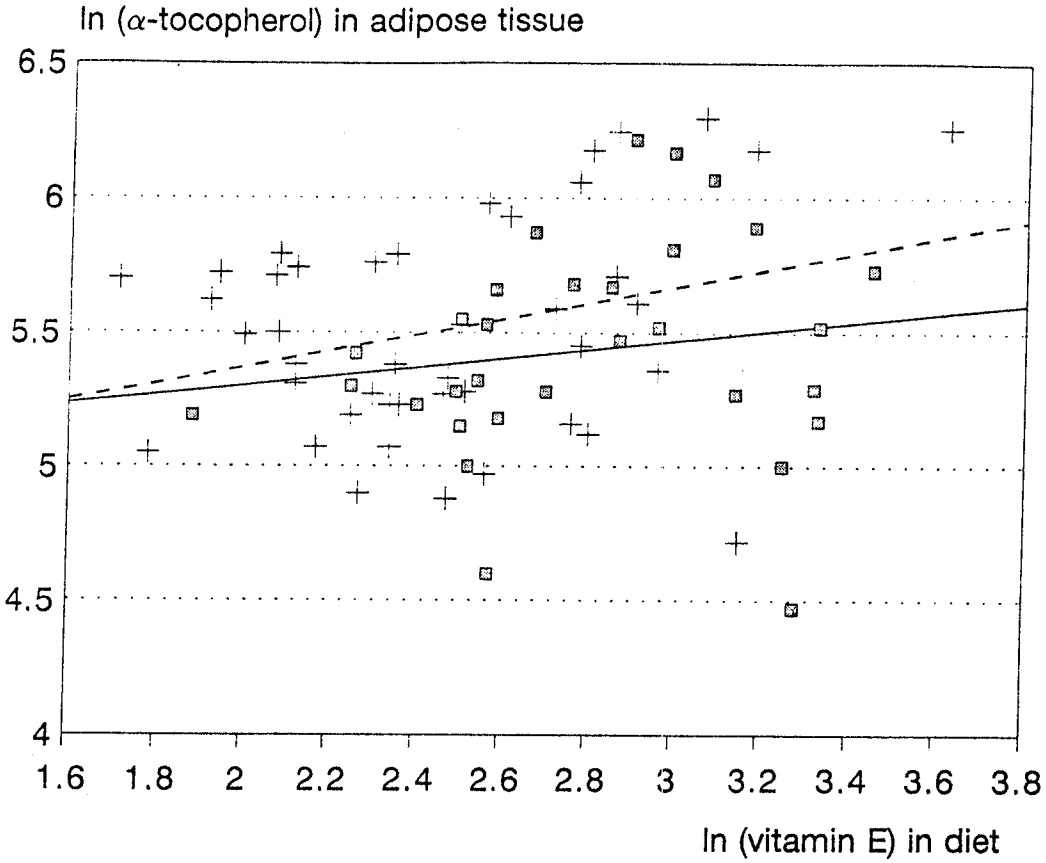

FIGURE 1. Correlation between dietary intake and the adipose tissue level of α-tocopherol among men and women in the Netherlands, 1992. □, men; ·, women; —, men; - - -, women.

between quartiles was not significant ($p = 0.11$); for the α-tocopherol : cholesterol ratio, there was a 15 percent increase over adipose tissue quartiles and a significant trend ($p < 0.05$).

DISCUSSION

The aim of the present study was to evaluate the use of fat-soluble vitamins in adipose tissue as biomarkers of dietary intake in epidemiologic studies. Comparison of dietary intakes of β-carotene and α-tocopherol with plasma and adipose tissue levels among 85 healthy men and women showed a moderate correlation between adipose tissue levels and dietary intake assessed by food frequency questionnaire, somewhat higher than the correlation observed between plasma levels and intake. This is consistent with the notion that adipose tissue better reflects long-term intake and is less subject to short-term fluctuations. However, the within-person variability of adipose tissue levels was higher than that of plasma levels. Adipose tissue β-carotene was shown to respond to prolonged oral supplementation.

The strength of the correlations depended partly on the analytical and intraindividual variation of measurements. We were able to adjust for those, because data from repeated measurements were available. Repeated measures of adipose tissue levels showed relatively larger differences than plasma levels, although less fluctuation was expected. A likely explanation is the larger analytical variation observed in smaller samples; optimization of the aspiration procedure, yielding somewhat larger samples, might improve the reproducibility. Perhaps the less homogeneous texture of adipose tissue in comparison with plasma can cause an uneven distribution of the vitamins in the tissue. Overall analytical variation was within acceptable ranges. However, unlike plasma levels, adipose tissue values were expressed as μg per gram of fatty acids. This means that the analytical variation of both the vitamin and the fatty acid analyses contributed to the error in the vitamin concentration.

Food frequency questionnaires have become the primary method of measuring dietary intake in epidemiologic studies (15). Generally, short-term recall and

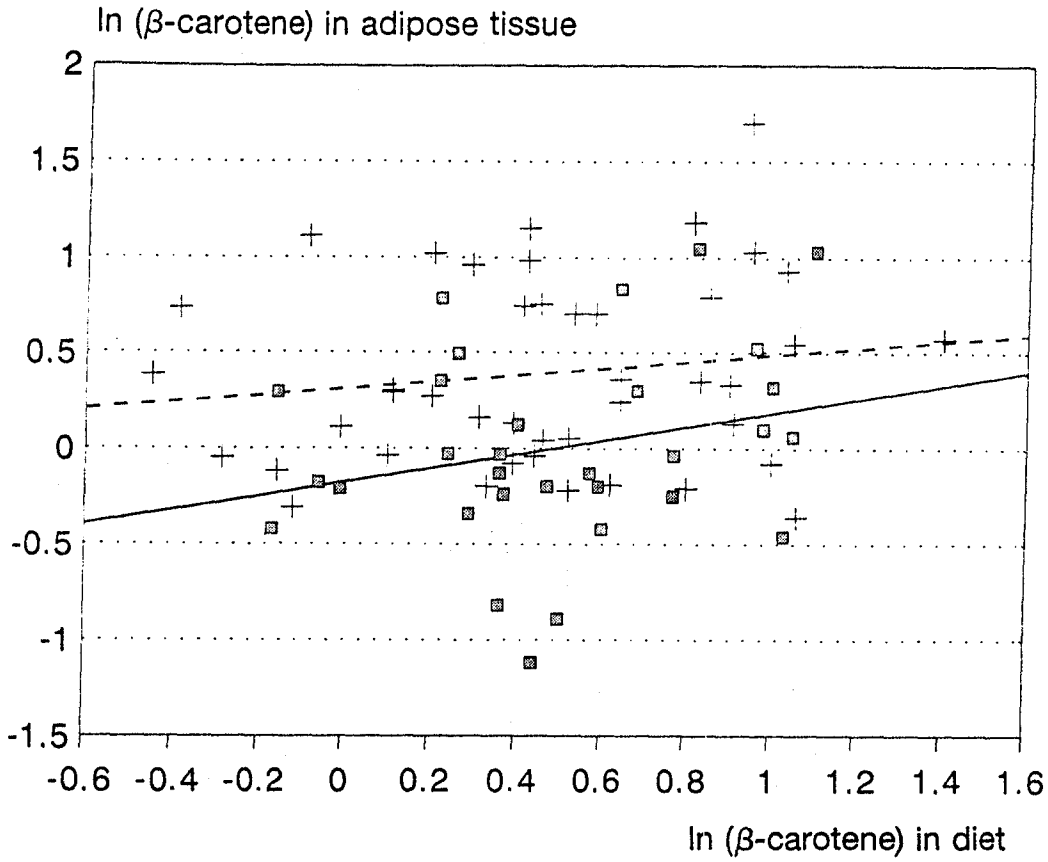

FIGURE 2. Correlation between dietary intake and the adipose tissue level of β-carotene among men and women in the Netherlands, 1992. □, men; +, women; —, men; - - -, women.

diet record methods are unrepresentative of usual intake and are inappropriate for assessment of past diet. An important characteristic of food frequency questionnaires is their ability to rank individuals according to intake of specific nutrients. Therefore, we chose a food frequency questionnaire to compare dietary intake with vitamin levels in plasma and adipose tissue. Intake of vitamin E in the present study was similar to intake assessed in other studies using different food consumption methods (5, 16–18); several other studies have reported lower intakes (19–22). Mean intake of α-tocopherol in a Dutch total diet study among 18-year-old men was 13.9 mg (23). However, β-carotene intake in this study was considerably lower than that in other studies (18, 20, 24, 25). This may be due to the method of calculation of intake: In our study, as well as in that of Albanes et al. (24) (with a mean intake of 2.1 mg/day, closest to our results), recently analyzed food composition data were used. Other studies determined β-carotene values by partitioning total vitamin A activity into β-carotene, retinol, and other carotenoids. Perhaps this latter method leads to overestimation of β-carotene intake. Our results agree with those of a total diet study conducted in the Netherlands (23) in which a mean β-carotene intake of 1.3 mg/day was found among male adolescents. Relative intake of linoleic acid was similar to that found in the Dutch Food Consumption Survey (11).

The α-tocopherol content of adipose tissue has been reported by few authors (5, 8, 26–29); mean α-tocopherol levels have varied from 141 μg/g of triglycerides to 402 μg/g of triglycerides. Parker (8) reported a range of 61–811 μg/g of adipose tissue. Concentrations cannot simply be compared between studies, because these concentrations can be expressed in different ways. In all studies, relatively large between-person variation has been observed; in the present study, levels varied 11-fold for α-tocopherol and 20-fold for β-carotene. The mean β-carotene content of adipose tissue in our study was of the same order as reported by Parker (8).

The correlation of β-carotene levels in adipose tissue and plasma was much higher than the correlation of α-tocopherol levels. This may be explained in part by the larger range of adipose tissue β-carotene levels

FIGURE 3. Correlation between dietary intake and the adipose tissue level of linoleic acid among men and women in the Netherlands, 1992. □, men; +, women; —, men; - - -, women.

as compared with α-tocopherol. It might indicate a quicker response of adipose tissue β-carotene to fluctuations in plasma levels; if so, this would contradict the notion that adipose tissue β-carotene levels reflect long-term intake. However, a more likely explanation is that plasma α-tocopherol levels are regulated by some mechanism, thus being less susceptible to variations in intake, as has been suggested recently (30).

Correlation between intake and biomarkers is always weakened by interindividual differences in absorption and metabolism and by the effect of other, mostly unknown determinants of the biomarker levels. For β-carotene, the sensitivity of plasma concentrations to intake has been shown previously (24, 31). Plasma vitamin E concentration as such is a questionable index of intake, since it is strongly correlated with plasma lipid concentration. Therefore, the use of the α-tocopherol : lipid ratio is recommended (32) as an indicator of nutritional status. In our study, plasma α-tocopherol (relative to plasma cholesterol) was weakly correlated with intake. This may be explained by the proposed regulatory mechanism for plasma α-tocopherol (30). Moreover, vitamin E in our food composition table included other tocopherols and tocotrienols, which may have weakened the correlation with α-tocopherol. Of the other plasma parameters that were assessed, only β-carotene concentration was significantly associated with intake, among women. Others have found similar low correlations between intake of vitamin E and plasma α-tocopherol, among nonusers of vitamin supplements (19, 21). Some other studies (17, 20, 22) have observed significant correlations slightly over 0.3. Correlation between intake of carotenoids and plasma or serum β-carotene varies between 0.21 (16, 25) and 0.49 (21) among nonsupplement-users. In the present study, we observed a similar correlation for women (0.33), but for men there was no correlation between intake and plasma levels at all. To some extent, these findings may be due to the larger range of intake among women, but they may also be due to chance.

As yet, little information is available on the relation between intake and adipose tissue levels. Kayden et al. (27) reported increased adipose tissue α-tocopherol

levels in two subjects who took supplemental vitamin E for more than a year; in one of them, who discontinued the supplement 20 months prior to the study, plasma α-tocopherol levels had returned to normal but the adipose tissue level remained elevated. The only human study, to our knowledge, in which the effect of vitamin E supplementation on adipose tissue levels has been studied systematically dates from the mid-1960s (33). Two-week supplementation with 1 g of vitamin E daily resulted in a doubling of adipose tissue levels. Experimental studies carried out in animals have shown that concentrations in adipose tissue reflect intake over a period of time (34–36).

Accumulation of β-carotene in adipose tissue was observed 5 days after a single dose of 120 mg of β-carotene (37). In the present study, we observed an increased level of adipose tissue β-carotene in response to long-term supplementation. Our results can only be interpreted qualitatively, since we did not use a controlled design. The increase in adipose tissue appeared to be correlated with the increase in plasma levels, suggesting that an equilibrium had been reached; both showed large differences between individuals. We may conclude that adipose tissue levels of β-carotene respond to oral intake. A controlled trial is needed to provide information on how much time it takes for adipose tissue levels to start to increase, whether a steady state has been reached after 6 months, and how much time is required for β-carotene to return to normal levels after the end of supplementation.

For linoleic acid, the correlation between intake and adipose tissue levels was in the same range as reported by others (3, 38). Aro et al. (6) recently reported a correlation of 0.46 ($p = 0.015$) between β-carotene intake assessed by 5-day food records and β-carotene levels in adipose tissue sampled immediately thereafter. A lower correlation ($r = 0.33$) was found between intake assessed by analysis of duplicate portions collected over 3 days and a biopsy done 6 months later. The conclusion that adipose tissue levels reflect recent dietary intake may be valid, but it could also be attributed to this particular population, which may have had relatively stable dietary habits. In our study, adipose tissue α-tocopherol correlated better with intake than did plasma levels and intake, among women; for β-carotene, adipose tissue concentrations correlated well with intake among men, but not among women. Considering the small number of subjects, this may well be a chance finding. However, it seems possible that metabolism and the distribution of fat-soluble vitamins over different body compartments may differ between men and women. This suggestion is supported by the finding that concentrations of β-carotene in plasma and adipose tissue were substantially higher in women than in men, although intake was similar.

Overall, adipose tissue levels of linoleic acid enable us to rank subjects adequately according to intake, and high adipose tissue α-tocopherol was related to relatively high dietary intake. Adipose tissue β-carotene seemed to provide a less direct indication of intake as assessed by food frequency questionnaire. Whether this was caused by a larger dependence on plasma concentrations, and thus recent dietary intake, or perhaps an insufficient ability of our food frequency questionnaire to adequately rank subjects according to intake for the limited range observed in this population remains to be clarified.

In conclusion, adipose tissue concentrations of β-carotene and α-tocopherol seem to be biomarkers of usual dietary intake, in a manner similar to plasma concentrations. Overall, the association with intake is moderate and only slightly better than the association of plasma levels with intake. The use of biomarkers in general is less appropriate in retrospective studies of diseases that may have affected the biomarker levels. Concentrations in adipose tissue may, for example, be influenced by weight loss. The use of biomarkers in adipose tissue may be preferred to plasma in studies of persons who may have changed their diet recently or endured the effects of an acute event, such as a myocardial infarction, that affected plasma levels (adipose tissue levels will most likely be constant for a longer period of time). In prospective studies, the choice of biomarker may be determined by factors such as feasibility and cost; in most cases, plasma levels will then be preferable. The use of fat aspirates in large epidemiologic studies has been proven feasible (39). However, about half of the subjects in the present study found fat aspiration more unpleasant than having a blood sample drawn.

Finally, even if these biomarkers are not perfect indicators of dietary intake, they may still be relevant predictors of disease risk, since they account for the individual differences of absorption and metabolism that affect nutritional status.

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