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Plasma levels of antioxidant vitamins and cholesterol in a large population sample in Central-Northern Italy

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Abstract *Background:* In a population-based multicenter case-control study of diet, life-style, and gastric cancer a large series of adults, aged 30–75 years (mean 58.9 years), were randomly sampled from the general population in 3 areas of Central-Northern Italy.
Aim of the study: To evaluate the relationship between plasma levels of antioxidant vitamins and cholesterol, and sociodemographic characteristics, life-style factors, and dietary intake of selected nutrients in a sample of the Italian population.
Methods: A fasting blood sample was available for 945 subjects (553 men, 392 women). The plasma concentrations of ascorbic acid, carotene, retinol, alpha-tocopherol, and cholesterol were determined by a centralized laboratory. All participants answered to a detailed questionnaire collecting information on sociodemographic and anthropometric characteristics, smoking, alcohol drinking, and dietary habits. Covariance analysis models, with post hoc Dunnett tests, including terms for age, sex, study center, and period of blood drawing, were used for selected multiple-way comparisons of mean values of plasma nutrients.
Results: Mean plasma values of retinol were higher among men while women had higher levels of plasma carotene, ascorbic acid, alpha-tocopherol, and cholesterol. Plasma carotene levels showed an inverse association with body mass index, alcohol consumption, and smoking and a positive association with social class. Carotene concentrations were higher in plasma samples obtained in spring/summer, while ascorbic acid levels were higher in autumn/winter. Partial correlation coefficients between plasma vitamin levels showed a strong correlation between carotene and ascorbic acid (0.69 in men; 0.74 in women), between carotene and alpha-tocopherol (0.44; 0.37), and between alpha-tocopherol and ascorbic acid (0.45; 0.41). Plasma alpha-tocopherol and retinol correlated with plasma cholesterol. On the other hand, plasma carotene and ascorbic acid were correlated with their estimated dietary intakes, while the intakes of other nutrients, as expected, correlated rather poorly with the respective plasma concentrations.
Conclusions: Socio-economic factors, life-style, and specific nutrient intake, in addition to gender, are related to nutrient plasma levels in Italian adults and may provide specific suggestions for the prevention of chronic diseases.

Key words Diet – carotene – retinol – alpha-tocopherol – ascorbic acid

Introduction

The increasing evidence of the potential role of antioxidant vitamins in the prevention of cancer and coronary heart disease has multiplied epidemiologic studies focusing on blood levels of these micronutrients. Exogenous antioxidants have been shown to remove free radicals produced by inner metabolism (17). In 1981 Peto et al. (35) hypothezised a reduction in human cancer risk by dietary intake of beta-carotene, particularly for smoking related cancers. The debate during the last decade has often focused on this topic (50). A case-control study in the US and a large trial in China have indicated that dietary beta-carotene and alpha-tocopherol supplements decreased the risk of lung cancer mortality (6, 30), but more recently the Finnish Trial reported opposite results among heavy smokers (1).

Several studies have specifically examined the relationship between dietary intake of nutrients estimated by questionnaire and blood levels of the same nutrient (5, 7, 13, 25, 26, 44, 49). Overall, these studies have found low to moderate correlations for most of the antioxidant vitamins, with Pearson correlation coefficients in the range of 0.2 to 0.6. Other determinants and complex homeostatic mechanisms probably modify the relationship between the intake of a nutrient and its levels in biological fluids. A few reports have investigated the relationship between plasma vitamin levels and individual factors, including dietary habits, among healthy subjects (8, 14, 36, 39).

The availability of measurements of plasma concentrations of antioxidant vitamins and cholesterol carried out in a centralized laboratory together with data on sociodemographic characteristics, life-style factors, and dietary habits for a large sample of the Central-Northern Italian population, gave us the unique opportunity to explore the relationship between these variables in this representative group of Italian adults.

Materials and methods

Study population

During 1985–1988, 1.423 adults aged 30–75 years were randomly selected from the general population of several geographic areas in Italy and invited to participate as controls in a multicenter epidemiological study to evaluate dietary risk factors for gastric cancer (9). Computerized lists of municipalities and of the National Health Service were used to identify an age- and sex-stratified random sample of eligible controls. Eighty-one percent (1,159) of those contacted agreed to participate and were interviewed using a structured questionnaire seeking dietary and other information (10). Ninety-three percent (1.078) of those interviewed also donated a blood sample (33). Plasma samples of 133 (12 %) subjects resulted insufficient for all laboratory assays and were excluded from analyses. Results are therefore presented for 945 subjects from three areas: Florence in Tuscany, Central Italy; Genoa and Forlì/Imola in Liguria and Emilia-Romagna, two regions of Northern Italy. Results for ascorbic acid were available only for a subgroup of 745 subjects, due to a more complex laboratory protocol for sample processing and storage. The mean age of study subjects was 58.9 years, and 59 % were males.

Dietary and other interview data

A detailed description of the questionnaire has been published elsewhere (11). Briefly, the questionnaire recorded demographic, anthropometric, socio-economic, residential, occupational, smoking, medical, family and dietary information. Diet was assessed by asking the usual frequency of consumption of 181 food items and beverages. With the aid of an instruction manual and an atlas containing pictures of the most frequently consumed food items, the usual portion size (small, medium, and large) in a 12-month period before the interview was assessed for 146 food and beverage items. A standard portion size was assumed for the remaining items. All interviews were carried out face to face in the period 1985–88. Amounts of nutrients and energy provided by each food were estimated using newly updated Italian food composition tables (38). Thermolability was taken into account by reducing estimated contents of ascorbic acid by 50 % and carotene by 15 % in cooked foods. For all subjects, a cumulative average intake for each nutrient per day was computed by summing values for each food. Average daily alcohol consumption was computed by multiplying the amount of alcohol present in each alcoholic beverage reported at interview by the reported frequency of consumption; drinkers were grouped into 4 categories according to daily consumption (<20, 20–40, 41–60, and 60+ g/d).

Use of vitamin supplements was shown to be uncommon during a pilot phase carried out in 1985 and was not recorded in our study questionnaire.

The body mass index (BMI), calculated as weight in kilograms divided by height in squared meters, was used as a measure of overall obesity. Tertiles of BMI were based on the joint distribution of males and females.

Smokers were defined as individuals who had smoked at least one cigarette, pipe or cigar per day for at least one year. Ex-smokers were defined as those who had stopped smoking at least one year before study entry.

Blood collection and analysis

In each center, a physician, usually at the subject's home or at the local health department, collected a sample of about 20 ml of whole blood by venipuncture from fasting participants. Blood was placed in heparinized tubes, cov-

ered with aluminium foil, kept cool (4–8 °C) in a cold bag, delivered to the local study laboratory within 2 h of collection, and processed according to a rigid protocol. Plasma obtained by centrifugation was subdivided into several aliquots for subsequent analysis and immediately frozen at -30 °C. A 1-ml plasma aliquot was mixed with a solution of metaphosphoric acid for ascorbic acid assays. One set of the frozen plasma aliquots was air shipped on dry ice for nutrient assays to Hoffman-La Roche (Basel, Switzerland) every other month during the study period. Plasma concentrations of carotene, retinol and alpha-tocopherol were all determined at the end of the study using a high-pressure liquid chromatography method (HPLC). The method used three separate HPLC lines, one for each of the analysis parameters, each conducted under optimal conditions and consisting of an isocratic system using silica gel (adsorption) as the stationary phase (46). The carotene value represents mainly beta-, alpha-, and gamma-carotene, where beta-carotene is the most abundant form of carotene in human plasma. Lycopene or lutein are not included. Total plasma ascorbic acid was assessed by using a standard method (45). Briefly, thawed samples were centrifuged at 4 °C to yield a clear protein-free supernate, which was analyzed on a Roche Cobas Bio (Basel, Switzerland) centrifugal analyzer, with fluorescent attachment. Pre-incubation with ascorbate oxidase enzyme in buffer, pH 6.2, was followed by the addition of o-phenylene diamine to produce a fluorescent quinoxaline derivative from the dehydroascorbate formed from ascorbate by the preliminary oxidation step. Fluorescence yield is linearly proportional to the sum of ascorbate plus dehydroascorbate over the concentration range 0–57 μmol/l. A calibration curve of freshly prepared ascorbate standard and quality assurance samples of metaphosphoric acid-treated heparinized plasma with 3 concentrations of ascorbate, subdivided and stored at -85 °C, were used in each run. Each unknown sample was measured in duplicate, with additional repeats if duplicate agreement was poor (>20 % discordance).

Data analysis

Analysis of covariance, including in the model terms for age, sex, study center, and period of blood drawing (to control for seasonal variation), was used to estimate the multivariate-adjusted means. The association between mean plasma values of vitamin concentration and socio-demographic characteristics and life-style was evaluated by comparing adjusted means across each subgroup. Post hoc Dunnett tests were carried out for multiple-way comparisons among subgroups using the first level of each variable as the reference category. Differences in mean nutrient levels across study centers were assessed by means of post hoc Scheffè test for all multiple-way comparisons. Partial correlation coefficients, to control for age, period of blood drawing, social class, smoking habits, alcohol intake, and total caloric intake, were also computed. Statistical analysis was performed using the Statistical Analysis System.

Results

The main characteristics and estimated dietary intake of nutrients in the study population are shown by sex in Table 1. The 945 subjects included 553 men (59 %) and 392 women (41 %). The mean age at interview was 59.5 years for males and 57.8 for females. Samples were collected in the centers of Florence (40 %), Forlì/Imola (44 %) and Genoa (16 %). Overall, study subjects were mostly resident in urban areas and of lower social class. Blood samples were more frequently obtained during winter and autumn (59 %). Females were mostly classified as never smokers (64 % vs. 20 % in males), while there were more current smokers among males (36 %) than females (21 %). Male smokers also tended to smoke more heavily than female smokers (data not shown). Teetotallers were more represented among women (22 % vs. 6 %); female drinkers in general also consumed less alcohol than males (only 19 % of women consumed more than 40 grams of alcohol per day, compared to 62 % of men). Interviewed subjects who did not provide a blood sample did not show any significant difference in comparison to responders when their individual characteristics were taken into account (data not shown).

Mean plasma levels adjusted according to selected characteristics are shown in Table 2, separately for males and females. Plasma retinol concentration was higher among men while women had higher levels of plasma carotene, alpha-tocopherol, ascorbic acid, and plasma cholesterol. Carotene levels did not vary with age in both sexes. In contrast, retinol presented an inverse association with age in males, and a positive one in females. Also alpha-tocopherol showed a positive relationship with age in females, but not in males. Ascorbic acid declined with age in both sexes, but only in males was the difference between the oldest and the youngest age group significant. Mean levels of cholesterol increased with age in both sexes; the increasing trend was particularly evident among females.

Seasonal variation of plasma levels was observed for carotene and ascorbic acid: carotene levels were significantly higher, in both men and women subjects, during spring and summer, while, in contrast, ascorbic acid was higher in autumn and winter in both sexes, although the difference reached significance only in females.

Female subjects recruited in Forlì/Imola tended to have lower mean plasma levels of retinol and alpha-tocopherol than subjects recruited in Florence, while cholesterol levels were also lower than those in Genoa. Male subjects recruited in Forlì/Imola had alpha-tocopherol and ascorbic acid levels lower than in Florence. Ascorbic acid

Table 1 Main individual characteristics, mean dietary intakes and corresponding standard errors (SE) of the study population, by sex (Central-Northern Italy, 1985–88)

Characteristics	Males (553)		Females (392)		Total (945)	
	N	%	N	%	N	%
Age (years)						
< 50	129	23	114	29	243	26
50–64	202	37	131	33	333	35
> 64	222	40	147	38	369	39
Center						
Florence	230	42	151	38	381	40
Forlì/Imola	240	43	171	44	411	44
Genoa	83	15	70	18	153	16
Period of blood drawing						
Autumn/winter	333	60	223	57	556	59
Spring/summer	220	40	169	43	389	41
Residence						
Urban	423	76	320	82	743	79
Rural	130	24	72	18	202	21
Social class						
Low	358	65	211	54	569	60
Medium	120	22	117	30	237	25
High	75	13	64	16	139	15
Body Mass Index (tertiles)						
< 23.4	139	25	178	45	317	34
23.4–26.3	217	39	105	27	322	34
> 26.3	197	36	109	28	306	32
Smoking habits						
Never	109	20	250	64	359	38
Ex-smoker	245	44	58	15	303	32
Current	199	36	84	21	283	30
Alcohol (grams/day)						
Never	31	6	88	22	119	13
1–20	65	12	91	23	156	16
21–40	113	20	140	36	253	27
41–60	168	30	61	16	229	24
> 60	176	32	12	3	188	20
Dietary intakes*	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Carotene (µg/day)	2690.8	47.9	2786.6	53.6	2732.6	38.0
Retinol (µg/day)	553.6	23.5	541.7	26.3	548.4	18.6
Alpha-tocopherol (mg/d)	7.9	0.1	7.9	0.1	7.9	0.1
Ascorbic acid (mg/day)	92.7	2.0	95.4	2.2	93.9	1.6
Cholesterol (mg/day)	321.2	5.4	283.8	6.0	304.9	4.3
Energy intake (Kcal/d)	2689.8	25.2	2285.3	28.1	2513.3	21.3
Alcohol (g/day)	49.0	1.3	24.0	1.5	38.1	1.1

***** From analysis of covariance including terms for age, center, and period of blood drawing

levels among Florence males were also higher than those in Genoa.

Subjects living in rural areas tended to have lower levels of all nutrients compared to subjects living in urban areas, but differences were significant only for alpha-tocopherol in males.

With respect to social class, a positive association with levels of carotene was found in men. Also retinol, alpha-tocopherol, and ascorbic acid tended to be higher among upper class subjects, although the association was not statistically significant. On the other hand, cholesterol levels were lower in upper class women.

Body mass index showed in both sexes an inverse association with plasma carotene (significant in females) and a positive association with plasma retinol (not significant). Ascorbic acid and cholesterol tended to be stable across BMI tertiles. In contrast, alpha-tocopherol plasma levels were significantly higher in overweight males.

When smoking history was considered, male never smokers had significantly higher concentrations of plasma carotene. Among women, beta-carotene concentrations were highest among ex-smokers, but differences were not statistically significant. Also ascorbic acid showed a tendency to the same inverse relationship with

European Journal of Nutrition, Vol. 38, Number 2 (1999)

Table 2 Crude and adjusted mean (§) plasma levels of carotene, retinol, alpha-tocopherol, ascorbic acid and cholesterol and corresponding standard errors (SE) by sex and according to several sociodemographic characteristics and life-style factors (Central-Northern Italy, 1985-88)

	Carotene µg/dl		Retinol mg/dl		Alpha-tocopherol mg/L		Asorbic acid# mg/L		Cholesterol mg/dl	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Age										
< 50	34.0-2.1	44.3-2.9	63.9-1.4	50.2-1.4	12.0-0.3	10.8-0.3	7.9-0.4	8.6-0.5	202.6-4.0	196.8-4.2
50–64	32.5-1.6	46.9-2.7	60.1-1.1*	53.3-1.3	11.9-0.2	12.4-0.2*	7.0-0.3	7.9-0.5	205.3-3.2	224.9-3.9*
> 64	32.5-1.5	44.7-2.4	60.2-1.1*	56.3-1.2*	12.0-0.2	13.3-0.2*	5.9-0.4*	7.4-0.4	209.8-3.0	236.2-3.5*
Center										
Florence	33.4-1.5	46.2-2.4	61.8-1.0	55.0-1.1	12.2-0.2	12.6-0.2	7.9-0.3	8.2-0.4	209.2-3.0	220.0-3.5
Forlì/Imola	30.2-1.4	44.6-2.2	59.6-1.0	52.3-1.1**a**	11.5-0.2**a**	12.1-0.2**a**	6.6-0.3**a**	8.0-0.4	201.0-2.8	215.2-3.3**c**
Genoa	35.4-2.4	45.1-3.6	62.8-1.6	52.6-1.7	12.2-0.3	11.8-0.3	6.2-0.5**b**	7.7-0.6	207.6-4.8	222.7-5.2
Period of blood drawing										
Autumn/winter	30.1-1.3	41.5-2.0	62.3-0.9	54.2-0.9	12.0-0.2	12.2-0.2	7.2-0.3	8.7-0.3	207.2-2.4	223.5-2.8
Spring/summer	35.7-1.6*	50.3-2.3*	60.9-1.1	52.3-1.1	12.0-0.2	12.2-0.2	6.9-0.4	7.5-0.4*	205.5-3.1	215.8-3.3
Residence										
Urban	33.2-1.2	46.0-1.7	61.3-0.8	53.6-0.8	12.0-0.2	12.3-0.2	6.9-0.3	8.0-0.3	206.7-2.3	221.1-2.5
Rural	31.3-2.1	40.5-3.7	60.1-1.4	53.8-1.8	11.6-0.3*	12.0-0.3	6.7-0.5	7.6-0.6	205.3-4.1	221.4-5.5
Social class										
Low	31.2-1.3	43.9-2.1	60.4-0.9	52.7-1.0	11.9-0.2	12.1-0.2	6.9-0.3	7.6-0.4	205.8-2.6	219.7-3.1
Medium	35.2-2.0	43.2-2.7	62.0-1.4	55.2-1.3	11.8-0.3	12.6-0.3	6.1-0.4	8.0-0.5	205.2-4.0	226.6-4.0
High	36.8-2.5*	52.4-3.7	62.6-1.7	53.7-1.8	12.4-0.4	12.3-0.3	7.4-0.5	8.6-0.6	211.0-5.0	216.4-5.4*
BMI (tertiles)										
< 23.4	32.2-1.9	51.1-2.2	59.5-1.3	53.0-1.1	11.5-0.3	12.3-0.2	7.2-0.4	8.2-0.4	205.4-3.8	220.1-3.4
23.4–26.3	35.3-1.5	43.9-2.9	61.0-1.0	53.8-1.4	11.9-0.2	12.3-0.3	6.6-0.3	7.7-0.5	206.9-3.0	223.3-4.3
> 26.3	30.3-1.7	37.2-2.8*	62.2-1.1	54.4-1.3	12.4-0.2*	12.1-0.3	6.9-0.4	7.6-0.5	206.6-3.3	220.7-4.1
Smoking habit										
Never	37.4-2.1	46.4-2.0	59.9-1.5	52.9-1.0	11.8-0.3	12.2-0.2	7.2-0.5	8.2-0.3	203.9-4.2	219.2-3.0
Ex-smoker	35.3-1.5	47.7-3.8	63.2-1.0	54.5-1.8	12.2-0.2	12.4-0.3	6.9-0.3	8.0-0.6	205.3-2.9	219.5-5.5
Current	27.5-1.6*	39.8-3.3	58.9-1.1	54.8-1.6	11.8-0.2	12.4-0.3	6.5-0.4	7.0-0.6	209.2-3.2	228.0-4.9
Alcohol										
Never	32.8-3.7	48.0-3.1	57.5-2.6	50.6-1.5	10.8-0.5	12.0-0.3	6.5-0.8	7.7-0.5	190.8-7.6	217.6-4.6
1–20	41.6-2.6	46.8-3.1	56.3-1.8	50.9-1.4	11.8-0.4	12.3-0.3	7.3-0.6	9.1-0.5	208.1-5.3	222.5-4.5
21–40	38.0-2.0	46.0-2.5	60.4-1.4	53.9-1.2	12.3-0.3*	12.2-0.2	7.3-0.4	7.8-0.4	205.0-4.0	220.2-3.7
41–60	33.0-1.8	38.5-3.8	63.0-1.2	59.8-1.7*	12.3-0.3*	12.7-0.3	6.9-0.4	6.8-0.6	211.4-3.5*	226.5-5.5
> 60	24.9-1.7	40.1-8.3	62.5-1.2	59.4-3.8	11.7-0.2	11.5-0.8	6.1-0.4	6.9-1.6	205.5-3.4	216.9-12.2
Overall°	32.7-1.2	45.7-1.3	60.7-0.7	54.3-0.8	11.9-0.1	12.4-0.2	6.8-0.2	8.0-0.2	205.9-2.0	222.2-2.2

§ From analysis of covariance including terms, as appropriate, for age, center and period of blood drawing.
* Dunnett test for multiple comparisons significant at the 0.05 level (first level is the reference category)
a Plasma level of Forlì/Imola was different from Florence
b Plasma level of Genoa was different from Florence
c Plasma level of Forlì/Imola was different from Genoa
} Scheffè test for all possible multiple comparisons significant at the 0.05 level
Analyses for ascorbic acid are based on 745 subjects
° All comparisons between nutrient levels in men and women are significant ($p < 0.01$)

smoking habits, while cholesterol tended to be lower among never smokers. An inverse dose-response relationship existed between the cumulative number of cigarettes smoked (pack-years) and plasma carotene and ascorbic acid levels: only in males such a difference was significant (data not shown). Although the size of the study was quite large, stratified analyses by sex and smoking history specific subsets were impaired by small numbers in most strata.

A non-significant inverse association was observed in women between plasma carotene concentrations and alcohol consumption, while retinol concentration was positively associated with alcohol intake in women.

Table 3 shows partial correlation coefficients between plasma vitamin levels and intake of nutrients estimated from the study food-frequency questionnaire, for each sex separately. A strong correlation was observed between plasma carotene and ascorbic acid (0.69 in males and 0.74 in females) and between plasma carotene and alpha-tocopherol (0.44 in males and 0.37 in females). Alpha-tocopherol was also correlated with plasma ascorbic acid (0.45 in males and 0.41 in females) and with plasma levels of cholesterol (adjusted correlation coefficient was 0.27 in males and 0.20 in females). Also plasma retinol levels were correlated with plasma cholesterol (0.37 in males and 0.40 in females). The adjusted correlation between estimated dietary intake of carotene and its plasma

Table 3 Partial correlation coefficients of selected nutrients (§) between dietary intakes estimated from food-frequency questionnaire and plasma levels for each sex separately: A) 553 males and B) 392 females (Central-Northern Italy, 1985-88)

A)

Males (n = 553)		Dietary intake					Plasma levels			
		Carotene	Retinol	Ascorbic acid	Alpha-tocopherol	Cholesterol	Cholesterol	Alpha-tocopherol	Ascorbic acid	Retinol
Plasma	Carotene	0.27	-0.05	0.20	0.06	-0.02	0.02	0.44	0.69	0.05
	Retinol	0.08	0.06	-0.04	0.07	0.07	0.37	0.11	0.01	
	Ascorbic acid	0.13	-0.10	0.27	0.11	-0.01	-0.01	0.45		
	Alpha-tocopherol	0.15	-0.11	0.11	0.11	0.04	0.27			
	Cholesterol	0.08	-0.04	0.02	0.04	0.06				
Dietary	Cholesterol	0.21	0.32	0.12	0.68					
	Alpha-tocopherol	0.21	0.37	0.17						
	Ascorbic acid	0.23	0.02							
	Retinol	0.04								

B)

Females (n = 392)		Dietary intake					Plasma levels			
		Carotene	Retinol	Ascorbic acid	Alpha-tocopherol	Cholesterol	Cholesterol	Alpha-tocopherol	Ascorbic acid	Retinol
Plasma	Carotene	0.23	-0.03	0.11	-0.07	-0.10	0.12	0.37	0.74	0.23
	Retinol	-0.04	0.08	-0.01	0.00	0.02	0.40	0.12	0.18	
	Ascorbic acid	0.19	0.04	0.23	0.06	0.00	0.03	0.41		
	Alpha-tocopherol	0.14	-0.07	0.07	-0.02	-0.05	0.20			
	Cholesterol	0.04	-0.08	-0.02	-0.01	-0.05				
Dietary	Cholesterol	0.08	0.21	-0.03	0.70					
	Alpha-tocopherol	0.24	0.30	0.07						
	Ascorbic acid	0.15	0.00							
	Retinol	-0.03								

§ adjusted for age, period of blood drawing, social class, smoking habits, alcohol, and energy intake.
Note: correlations between plasma levels and dietary intakes are reported in the gray area, correlations between plasma levels are reported in the upper right corner of the tables and correlations between dietary intakes are reported in the lower left corners.

levels was 0.27 for males and 0.23 for females. Correlation between ascorbic acid dietary intake and plasma levels was 0.27 for males and 0.23 for females. Other nutrient intakes estimated from our food-frequency questionnaire correlated rather poorly with the respective plasma concentrations. In general, correlations between dietary intakes of nutrients were stronger in males than in females; moreover, as expected, retinol and carotene intakes were not correlated among each other.

Discussion

This study represents a first survey of plasma antioxidants in a large sample of the adult population in Central-Northern Italy. Participants were originally enrolled as population controls in a case-control study of diet and gastric cancer; thus, men and older subjects were more represented. However, considering the high compliance rate and that non-responders were very similar to those who agreed to provide a blood sample, this large cross-sectional study of nearly 1,000 subjects may be considered representative of the adult population of Central-Northern Italy. The distribution of study subjects according to other demographic and individual characteristics (residence, migration from Southern Italy, social class, BMI, smoking history, alcohol consumption, and medical history) was consistent with *a priori* expectations.

In our study, plasma levels of vitamins were comparable to those found in studies conducted in the US (14, 24, 29, 42), in South America (8), in other European countries (26, 41), and in Japan (2, 23, 47). Carotene and alpha-tocopherol levels, however, are slightly higher in our population, possibly reflecting a more frequent consumption of carotene-rich food items and olive oil.

Higher levels of plasma retinol among males than among females and higher levels of plasma carotene in females have been consistently reported in other investigations (2, 14, 18, 19, 21, 23, 28, 31, 32, 39, 42). In this study, women had also higher levels of alpha-tocopherol in comparison to men, in agreement with some other reports (19, 26), although most studies did not find such a difference (4, 14, 16, 18, 21, 28, 32, 42, 48, 49). The biological explanation for differences between genders is not yet clear. Overall, sex differences in plasma vitamin levels could be due to differences in life-style, but stratification by smoking and drinking habits confirmed these results also in non-smokers and non-drinkers. Since plasma carotene levels reflect dietary intake and women tend to have a higher dietary intake of carotene, we speculated that the difference could be simply due to this differential consumption: adjustment by carotene intake, actually, reduced this gender difference, although not completely. Also genetic and hormonal factors have been invoked to explain these findings, but the sex difference is still evident in this population even considering only postmenopausal females.

Published data on the association of plasma vitamin levels with age, adjusted for the effects of sex, are less consistent. Studies from other countries indicate a wide range of results, mostly showing no association for carotene and retinol levels (14, 19–21, 23, 28, 31, 37, 42), and a somewhat positive one with alpha-tocopherol (4, 16, 18, 20, 21, 28, 42, 48). For carotene, retinol, and ascorbic acid we found a negative association with age in males, while retinol was higher in elderly women (above 64 years of age). Also cholesterol and alpha-tocopherol increased significantly with age only in women.

Considering the geographical distribution, significant differences between the three study areas were found. Subjects recruited from Genoa were more similar to those from Florence. The mean plasma levels of carotene and alpha-tocopherol in males, and cholesterol in both sexes were lower among the subjects recruited in Forlì/Imola where subjects residing in rural areas are more represented. Overall, residents of rural areas showed slightly lower plasma levels for all micronutrients, in agreement with findings from Japan and Finland (26, 39); this could possibly explain the lower levels of nutrients in subjects recruited in Forlì/Imola. Dietary habits in rural areas tend to be more traditional and dependent on seasonal availability of food.

As reported by Olmedilla et al. (32), we found a seasonal difference in some antioxidants. Carotene plasma levels were higher in spring and summer reflecting higher consumption of vegetables and fruits, while ascorbic acid concentration was higher in winter, possibly reflecting frequent intake of citrus fruits.

According to BMI, leaner females had higher levels of carotene and lower levels of retinol, in comparison to overweight females. In overweight men, the concentration of alpha-tocopherol was significantly higher than in leaner subjects. Similar results for carotene and alpha-tocopherol, but not for retinol, have been reported in other studies (20, 21, 26, 31, 39, 42, 49).

Our results have shown that socio-economic status tended to be positively associated with carotene (and possibly retinol and alpha-tocopherol) levels in males, while in females this pattern was less evident. Social class is strongly related to life-style factors in this Italian population; differences in dietary habits could explain these findings. In fact, a significantly higher intake of carotene was observed in men of higher social class. A positive relation between alpha-tocopherol and social class in males had been previously reported (26).

Recently, cigarette smoking and alcohol consumption have been reported, in several different populations, to affect plasma levels of vitamins, particularly beta-carotene (2, 12, 14, 15, 19, 21, 23, 31, 34, 37, 39, 42, 43). In this study, an inverse relation between smoking and carotene and ascorbic acid was evident in both sexes, but male

current-smokers had markedly lower levels of carotene, possibly due to the higher number of cigarettes smoked. In contrast, no similar trend was shown for retinol and alpha-tocopherol. Smoking alters metabolism in a number of ways and induces various hepatic enzymes, which may increase clearance.

Our findings among alcohol consumers are more complex. Decreasing levels of carotene with increasing consumption of alcohol were observed in females, while in males light drinkers (1–20 g/d) had higher carotene levels than both abstainers and heavier drinkers. The role of smoking and alcohol drinking in reducing plasma levels of micronutrients has been discussed in several recent papers, recommending vitamin supplementation in smokers and/or drinkers for cancer prevention (2, 12, 14, 15, 18, 19, 21, 23, 27, 31, 34, 37, 39, 42, 43). However, the results of a large human trial in male smokers in Finland have shown that beta-carotene and alpha-tocopherol supplements have no beneficial effects and actually may increase the risk of lung cancer (1).

Several studies have examined the role of vitamin supplements (14, 31, 37): information on their use was not collected in our study according to the results of a pilot study, indicating no relevant consumption of vitamin pills in this Italian population in the previous decade. The current pattern has probably changed, also according to preliminary results of EPIC-Italy, a large prospective study with over 42,000 volunteers enrolled in four Italian centers (V. Krogh, personal communication).

Apart from retinol, plasma levels of antioxidants were positively, but not strongly, correlated to each other. Plasma alpha-tocopherol and retinol also correlated with plasma cholesterol. This correlation was particularly strong before adjustment for smoking and alcohol (0.68 in males and 0.70 in females). It is well known that studies exploring the role of alpha-tocopherol in the occurrence of cancer or cardiovascular diseases should carefully be controlled for total plasma lipids (22). Other plasma nutrients were weakly correlated with each other.

As expected, retinol levels did not reflect intake, while carotene and ascorbic acid dietary intakes were related to blood. On the other hand, alpha-tocopherol, which *a priori* we expected to be correlated with intake, was only poorly correlated. Ascherio et al. (3) found a strong positive correlation between total vitamin E intake and plasma alpha-tocopherol ($r = 0.51$ in men and $r = 0.41$ in women), but this was primarily due to vitamin E supplements. Further analyses should be devoted to explore and better understand these relationships, concentrating on details of dietary intake.

In conclusion, our results indicated that socioeconomic factors, life style, and dietary habits are relevant determinants of plasma concentration of antioxidants and cholesterol in the Italian population. These factors might play a role in explaining differences in cancer incidence and overall mortality across different areas in Italy.

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