Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake[1–3]

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ABSTRACT

Background: Plasma and adipose tissue concentrations of carotenoids are thought to reflect short- and long-term intakes of carotenoids, respectively. The ability of adipose tissue carotenoid concentrations to reflect dietary intake in population studies is unknown.

Objective: We examined the relation between intakes of the major dietary carotenoids and their concentrations in plasma and adipose tissue.

Design: A blood sample and an adipose tissue biopsy sample were collected from 115 women and 344 men in Costa Rica after they had fasted overnight, and a dietary interview based on a 135-item food-frequency questionnaire was administered. After carotenoid intake was adjusted for total energy intake and plasma concentrations were adjusted for HDL-, LDL-, and VLDL-cholesterol concentrations, we calculated partial Spearman correlation coefficients that were adjusted for age, sex, smoking, and body mass index.

Results: In women, the correlations (r) between intakes and concentrations of α-carotene, β-carotene, β-cryptoxanthin, and lutein+zeaxanthin were 0.25, 0.29, 0.44, and 0.17, respectively ($P < 0.05$ for $r \geq 0.19$), in adipose tissue and 0.26, 0.13, 0.55, and 0.22 in plasma. In men, these values were 0.04, 0.07, 0.23, and 0.06 in adipose tissue and 0.24, 0.22, 0.44, and 0.20 in plasma. In women and men, correlations for lycopene were higher in plasma (r = 0.19 and 0.35, respectively) than in adipose tissue (r = 0.14 and 0.26). The relative abundance of each carotenoid in the diet was similar to its distribution in plasma but not in adipose tissue.

Conclusion: The usefulness of adipose tissue and plasma carotenoids as biomarkers of intake is similar, although correlations for individual carotenoids vary substantially. *Am J Clin Nutr* 2002;76:172–9.

KEY WORDS Carotenoids, biomarkers, adipose tissue, dietary questionnaire, Costa Rica, Hispanics, population study

INTRODUCTION

Consumption of fruit and vegetables rich in carotenoids is associated with a reduced risk of several chronic diseases (1–3). Although β-carotene has been studied the most extensively, no benefits have been found for it in randomized trials (4, 5). Thus, emphasis has shifted toward other carotenoids that are abundant in a variety of foods and that, like β-carotene, are found at appreciable concentrations in human plasma and tissues (6). Investigating the role of carotenoids in human nutrition has been facilitated by updated food-composition tables, which permit more precise estimates of the intake of individual carotenoids (7, 8). Nonetheless, measurement errors inherent in estimating intake by dietary questionnaire, differential recall in case-control studies, the bioavailability of carotenoids from different foods, and individual differences in absorption and metabolism suggest that biomarkers of intake may provide a more accurate assessment of underlying carotenoid exposure.

Several studies have compared dietary intakes of the major carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin) with their concentrations in plasma or serum (9–17). Although plasma and serum concentrations correlate reasonably well with intakes, they may not reflect long-term intake (18), and they cannot be used in case-control studies if the disease under study itself affects plasma concentrations (19). Furthermore, the concentrations of carotenoids in plasma may be altered by a variety of factors independent of intake (9, 20). Thus, there is a need to evaluate other biomarkers of carotenoids that may better reflect habitual intake and that are less prone to distortion from occasional fluctuations in intake.

Because adipose tissue is the major storage site for carotenoids, carotenoid concentrations in this tissue have been proposed as a better predictor of long-term intake than concentrations in plasma (6). However, the results of the few studies that examined the relation between carotenoid intake and adipose tissue concentrations are inconclusive (21–24). The purpose of the present study was to determine whether adipose tissue concentrations of the major carotenoids are better biomarkers of long-term dietary intake than plasma concentrations.

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SUBJECTS AND METHODS

Study population

The participants in this study were the 531 control subjects of a case-control study of diet and heart disease in Costa Rica. The control subjects were randomly selected from the greater metropolitan area of San José, the capital city, and the surrounding 18 counties by using the national census information available at the National Census and Statistics Bureau of Costa Rica. Subjects were visited at their homes for recruitment and data collection, and 90% of those selected participated. Subjects were ineligible if they reported a past diagnosis of myocardial infarction or if they were physically or mentally unable to answer the questionnaire because of a stroke or other serious illness. The total study area comprised 2225 km^2 and 1 092 000 persons who were ethnically Mestizo as a result of 4 centuries of racial tripartite mixing of peoples of European, African, and Native American ancestry (25). All subjects gave their informed consent, and the study was approved by the Ethics Committee of the Harvard School of Public Health and the National Institute of Health Research at the University of Costa Rica.

Data collection

Data collection included a general questionnaire consisting of closed-ended questions regarding sociodemographic characteristics, smoking, socioeconomic status, and medical history, including personal history of diabetes and hypertension. Self-reported diabetes and hypertension were validated by using the definitions recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (26) and the Third Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (27).

Anthropometric measurements were collected by trained fieldworkers while the subjects wore light clothing and no shoes. Measurements were performed in duplicate and the average was used for analyses. Height was measured with steel anthropometers (GPM Anthropological Instruments, distributed by Siber Precision Inc, Carlstadt, NJ). To ensure correct readings (to the nearest 0.1 cm) for height, subjects always stood on a flat surface against a wall. Weight was measured with mechanical portable scales to the nearest 0.25 kg (model 761; Seca, Hamburg, Germany). Scales were calibrated biweekly. Body mass index (BMI) was calculated as weight (in kg) divided by height squared (in m).

Dietary assessment

Dietary information was obtained by using a 135-item semiquantitative food-frequency questionnaire (FFQ) that is a modification of the Willett questionnaire (28) and that was developed specifically for use among the Costa Rican population (29). This FFQ inquires about the intake of 135 food items; 20 vitamin, mineral, and food supplements; types of fat used for cooking and frying; consumption of fried foods in and away from home; and food habits related to meat consumption during the past year. Energy and nutrient intakes were computed by multiplying the consumption frequency of each food by the nutrient content of the specific portion, by using composition values from the US Department of Agriculture (USDA; 30) supplemented with other data from manufacturers and published reports. Carotenoid intake, including intakes of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin, was calculated by using the new carotenoid database of >2400 fruit, vegetables, and selected multicomponent foods (7, 8). The carotenoid content of tomato-based products was updated with values from the USDA, which were recently derived by the use of reversed-phase HPLC (31). Trained interviewers administered the questionnaire.

We carried out a validation study in which we compared the FFQ with 7-d, 24-h recalls in a subset of 120 subjects. For reproducibility, a second FFQ was administered 1 y after the first interview. The Spearman correlation coefficients for total carotenoids, α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin between the average of the 2 FFQs and the 7-d, 24-h recalls were 0.85, 0.40, 0.58, 0.46, 0.37, and 0.53 and were 0.57, 0.63, 0.56, 0.64, 0.37, and 0.43 for the first compared with the second FFQ ($P < 0.008$ for all) (29). The average macronutrient intakes assessed by this FFQ were also similar to the average intakes assessed by multiple 24-h recalls in the National Nutrition Survey in Costa Rica (29).

Biochemical analyses

Biological specimens were collected in the morning at each subject's home after he or she had fasted overnight. A subcutaneous adipose tissue biopsy sample was collected from the upper buttock with a 16-gauge needle and disposable syringe according to procedures previously described (32). Blood samples were collected in tubes containing 0.1% EDTA. Both samples were stored in a cooler with ice packs at 4°C and were transported to the fieldwork station within 4 h. Blood was centrifuged for 20 min at 4°C and 1430 × *g* to separate plasma. Adipose tissue and plasma were stored at −80°C and were transported on dry ice to the Harvard School of Public Health within 6 mo for analysis. The average ($\bar{x} \pm$ SD) yield of adipose tissue was 38 ± 33 mg.

Concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin in adipose tissue and plasma samples were measured by using the method described by Hess et al (33) with modifications. Plasma samples (250 μL) were mixed with 250 mL ethanol containing 10 μg *rac*-tocopherol/mL (Tocol; Matreya Inc, Pleasant Gap, PA) as an internal standard, extracted with 4 mL hexane, evaporated to dryness under nitrogen, and reconstituted in 100 mL ethanol:dioxane (1:1, by vol) and 150 mL acetonitrile. Carotenoids from adipose tissue biopsy samples that weighed >2 mg were extracted as described above, but samples were first weighed and homogenized in an aqueous solution containing 20% ascorbic acid. The extracted samples were saponified with potassium hydroxide for 15 min at 50°C. Before saponification, 500 μL of 20% (wt:vol) ascorbic acid in aqueous solution and 1000 μL of 0.167 μg *trans*-β-apo-8′-carotenal/mL in ethanolic solution were added to all samples. Samples were reconstituted with 100 μL of a dioxane:ethanol solution (1:1, by vol) and 150 μL acetonitrile. Recovery for *trans*-β-apo-8′-carotenal after saponification was >96% and that for plasma carotenoids after saponification of plasma samples that were also run without saponification was >90%.

Samples were quantitated by HPLC on a Restek Ultra C_{18} 150 mm × 4.6 mm column with a 3-μm particle size (Restek Corp, Bellefonte, PA). The column was encased in a water bath to prevent temperature fluctuations and was equipped with a trident guard cartridge system. A mixture of acetonitrile, tetrahydrofuran, methanol, and a 1% ammonium acetate solution (68:22:7:3) was used as the mobile phase with a flow rate of 1.1 mL/min.

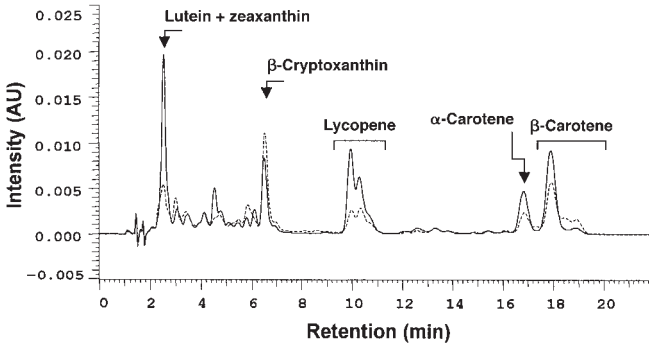

FIGURE 1. Chromatogram of the major carotenoids in human plasma and subcutaneous adipose tissue. The solid line indicates the plasma control pool and the dashed line the adipose tissue control pool. AU, arbitrary units.

The system included a Hitachi L-7100 pump in isocratic mode, an L-4250 ultraviolet-visible light (445 nm) detector, and a programmable AS-4000 autosampler with water-chilled tray interfaced with a D-6000 interface module (Hitachi, San Jose, CA). The system manager software (D-7000, version 3.0; Hitachi) was used for peak integration and data acquisition.

The minimum detection limits in plasma were 7.74 μg/L for α-carotene, 7.31 μg/L for β-carotene, 5.51 μg/L for β-cryptoxanthin, 8.49 μg/L for lycopene, and 6.31 μg/L for lutein+zeaxanthin. The minimum detection limits for a 10-mg adipose tissue sample were 0.063, 0.069, 0.052, 0.079, and 0.058 μg, respectively. A single large sample of abdominal adipose tissue was used to calculate between-run analytic variations. One adipose tissue portion from the core of this large sample with similar weight to that of most (75%) of the unknown samples (15–70 mg) was included in every run over the period of the study. Every run also included one plasma pool sample.

Typical HPLC chromatograms for the adipose tissue and plasma quality-control samples included in every run are shown in **Figure 1**. Because lutein and zeaxanthin co-elute on the chromatogram, the 2 were grouped and are presented as lutein+zeaxanthin. The between-run CVs for α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin were 7.2%, 7.5%, 12.6%, 8.1%, and 8.6%, respectively, in the plasma control pool and 18.6%, 21.8%, 26.4%, 16.7%, and 19.6%, respectively, in the adipose tissue control pool. The CVs for α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein+zeaxanthin in 8 blinded buttock adipose tissue duplicate samples were 19.8%, 15.8%, 16.6%, 12.1%, and 18.3%, respectively. To estimate the method variation independent of the heterogeneity of the adipose tissue itself, we also analyzed 82 samples that were split after homogenization and measured in the same run. The CVs for these duplicates were 6.1%, 7.4%, 6.3%, 7.4%, and 6.4%, respectively. For external quality control, our laboratory participates in the standardization program for carotenoid analysis from the National Institute of Standards and Technology.

Plasma triacylglycerol, cholesterol, and HDL-cholesterol concentrations were measured by using enzymatic reagents (Boehringer-Mannheim, Indianapolis) in a Roche Cobas Mira Plus autoanalyzer (Roche Diagnostic Systems, Somerville, NJ). LDL-cholesterol concentrations were calculated by using the Friedewald equation (34). Cholesterol determinations in our laboratory are standardized according to the program for research laboratories specified by the Centers for Disease Control and Prevention and the National Heart, Lung, and Blood Institute.

Statistical analyses

All data were analyzed with the SAS software package (version 8; SAS Institute Inc, Cary, NC). Of the 531 subjects recruited, those with missing or insufficient (≤2 mg) adipose tissue samples or those with missing plasma samples were excluded (n = 72). Among those analyzed, 5% had adipose tissue biopsy samples with <10 mg available. Analysis with and without these samples did not differ, so we included them in this report. The final sample of 459 subjects consisted of 344 men and 115 women. Carotenoid intake was adjusted for total energy intake by calculating the residuals from a regression of $\log_e$ dietary carotenoid concentrations on total energy intake (19). Log_e plasma carotenoid concentrations were regressed on plasma HDL, LDL, and VLDL cholesterol because most carotenoids are transported in plasma lipoproteins, which contributes to extraneous variation in the plasma carotenoids. Differences between men and women were compared by using Student's t test for continuous variables and the chi-square test for categorical variables. We used Spearman's correlations and Wilcoxon's rank-sum test to identify associations between dietary, plasma, and adipose tissue carotenoid concentrations with age, sex, smoking status, BMI, and other potential confounders such as alcohol intake. The associations between dietary, plasma, and adipose tissue carotenoids were determined by calculating partial Spearman's correlation coefficients adjusted for age, sex, smoking status, and BMI. Tests for trends were computed by assigning the median value of the corresponding quintile to each subject and entering that variable as a continuous one in the model. Robust estimators of the variance were used in regression models (35), thus eliminating the need for normalizing the dependent variable in the models described above.

RESULTS

The general characteristics and macronutrient intakes of the study participants are shown in **Table 1**. Intakes of most carotenoids were higher in the women than in the men (from 20% higher for lutein+zeaxanthin to 63% higher for α-carotene), although the range of intake observed was wide for both sexes (**Table 2**). Intakes of α-carotene and β-cryptoxanthin were comparable and were lower than the intakes of β-carotene, lycopene, and lutein+zeaxanthin by a factor of 10. As with dietary intake, the plasma concentrations of most carotenoids were higher in the women than in the men (from 17% higher for lycopene to 70% higher for β-carotene). Additionally, in both groups, the plasma concentrations of α-carotene and β-cryptoxanthin were ≈3-fold lower than those of β-carotene, lycopene, and lutein+zeaxanthin. Thus, the relative abundance of each carotenoid in plasma mirrored its level in the diet.

As expected from the intakes and plasma concentrations, concentrations of carotenoids in adipose tissue were also higher in the women than in the men (2-fold higher for β-carotene, 70% higher for β-cryptoxanthin, 65% higher for lycopene, and 32% higher for lutein+zeaxanthin). However, the distribution of carotenoids in adipose tissue did not reflect intake. For example, lycopene was the least abundant carotenoid in adipose tissue despite being one of the most abundant in the diet and plasma. The adipose tissue concentration of lutein+zeaxanthin was

TABLE 1
General characteristics and dietary profile of the study population

	Men (n = 344)	Women (n = 115)
Age (y)	56 ± 11[1]	59 ± 10[2]
Weight (kg)	71.2 ± 13.2	61.9 ± 10.9[3]
BMI (kg/m^2)	25.5 ± 4.0	26.2 ± 4.5
Waist-to-hip ratio	0.96 ± 0.06	0.86 ± 0.07[3]
Plasma triacylglycerol (mmol/L)	2.37 ± 1.37	2.24 ± 1.08
Plasma cholesterol (mmol/L)	5.10 ± 1.01	5.34 ± 1.01
HDL cholesterol (mmol/L)	1.04 ± 0.28	1.22 ± 0.34[3]
Diabetes (%)	8	21[4]
Hypertension (%)	23	37[2]
Angina (%)	4	9[5]
Smoking status (%)[6]		
Never smoked	22	74
Past smoker	45	16
Current smoker (<10 cigarettes/d)	15	5
Current smoker (≥10 cigarettes/d)	18	5
Drinkers (%)	42	7[3]
Alcohol intake among drinkers (g/d)	21.0 ± 22.8	7.2 ± 3.4[3]
Postmenopausal women (%)	—	80
Energy (MJ/d)	10.1 ± 3.1	9.0 ± 2.4[4]
Total fat (% of energy)	32.4 ± 5.9	34.1 ± 6.2[5]
Saturated	11.4 ± 2.7	12.3 ± 2.9[2]
Monounsaturated	12.1 ± 3.2	12.6 ± 3.8
Polyunsaturated	5.5 ± 1.5	5.5 ± 1.4
Protein (% of energy)	13.4 ± 2.4	12.9 ± 2.6
Carbohydrate (% of energy)	54.3 ± 8.3	53.0 ± 7.7
Dietary fiber (g/d)	24.5 ± 9.4	25.0 ± 9.9[4]
Cholesterol (mg/d)	301 ± 172	253 ± 143

[1] $\bar{x}$ ± SD.
[2–5] Significantly different from men: [2] $P < 0.01$, [3] $P < 0.0001$, [4] $P < 0.001$, [5] $P < 0.05$.
[6] Significantly different between men and women, $P < 0.0001$.

TABLE 2
Intake of carotenoids and concentrations in plasma and adipose tissue[1]

	Men (n = 344)	Women (n = 115)
Diet (μg/d)		
α-Carotene	447 ± 449	727 ± 849[2]
β-Carotene	3407 ± 2407	4668 ± 3564[3]
β-Cryptoxanthin	383 ± 446	552 ± 523[4]
Lycopene	5451 ± 5274	5772 ± 4789
Lutein+zeaxanthin	2412 ± 2857	2893 ± 2487[2]
Plasma (nmol/L)		
α-Carotene	135 ± 92	180 ± 151[3]
β-Carotene	484 ± 407	821 ± 725[3]
β-Cryptoxanthin	181 ± 202	275 ± 295[4]
Lycopene	501 ± 338	585 ± 338[2]
Lutein+zeaxanthin	316 ± 143	328 ± 165
Adipose tissue (μg/g)		
α-Carotene	0.20 ± 0.15	0.23 ± 0.17
β-Carotene	0.20 ± 0.18	0.41 ± 0.30[3]
β-Cryptoxanthin	0.12 ± 0.09	0.20 ± 0.13[3]
Lycopene	0.17 ± 0.19	0.28 ± 0.21[3]
Lutein+zeaxanthin	0.92 ± 0.53	1.21 ± 0.72[3]

[1] $\bar{x}$ ± SD.
[2–4] Significantly different from men: [2] $P < 0.01$, [3] $P < 0.0001$, [4] $P < 0.001$.

greater than that of any other carotenoid, yet β-carotene and lycopene were much more abundant than lutein+zeaxanthin in the diet and plasma.

Overall, intakes of each carotenoid were better correlated with plasma concentrations than with adipose tissue concentrations. In both the men and the women, dietary intake was significantly ($P < 0.05$ for all) correlated with plasma concentrations of all carotenoids except β-carotene in women ($r = 0.13$). Carotenoid intake was significantly correlated with adipose tissue concentrations of β-cryptoxanthin in men ($r = 0.23$) and women ($r = 0.44$), but the correlations for α-carotene and β-carotene were significant only in the women and that for lycopene was significant only in the men (**Table 3**). In the men, intakes of all carotenoids correlated better with concentrations in plasma than in adipose tissue. In the women, intakes of β-cryptoxanthin, lycopene, and lutein+zeaxanthin correlated better with plasma concentrations, whereas intake of β-carotene correlated better with adipose tissue concentrations.

We next determined whether the concentrations of each carotenoid in plasma or adipose tissue could discriminate between individuals with different intakes. We limited this analysis to β-carotene, β-cryptoxanthin, and lycopene because intakes of these 3 carotenoids were correlated with adipose tissue concentrations. The average concentrations and 95% CIs of plasma and adipose tissue carotenoids within each quintile of dietary carotenoid intake are shown in **Figures 2–4**. Despite relatively low correlations between carotenoid intake and plasma concentrations, mean plasma concentrations of each carotenoid increased substantially across the quintiles of intake. For example, plasma concentrations of lycopene were almost 2-fold greater in the highest compared with the lowest quintile of dietary lycopene. The corresponding difference for β-cryptoxanthin was 5-fold. For the adipose tissue concentrations of carotenoids within each quintile of intake, a significant trend was detected

TABLE 3
Spearman correlation coefficients for dietary, plasma, and adipose tissue carotenoids[1]

	Plasma versus diet	Adipose tissue versus diet	Adipose tissue versus plasma
α-Carotene			
Men	0.24[2]	0.04	0.13[3]
Women	0.26[4]	0.25[4]	0.09
β-Carotene			
Men	0.22[2]	0.07	0.33[2]
Women	0.13	0.29[4]	0.30[4]
β-Cryptoxanthin			
Men	0.44[2]	0.23[2]	0.40[2]
Women	0.55[2]	0.44[2]	0.54[2]
Lycopene			
Men	0.35[2]	0.26[2]	0.40[2]
Women	0.19[3]	0.14	0.23[3]
Lutein+zeaxanthin			
Men	0.20[4]	0.06	0.31[2]
Women	0.22[3]	0.17	0.38[2]

[1] Partial Spearman's correlations were adjusted for BMI, age, and smoking status. Variables are the residuals obtained from regression analyses adjusted for total energy in the diet; HDL cholesterol, LDL cholesterol, and VLDL cholesterol in plasma; and grams of adipose tissue collected (except in the analyses of β-cryptoxanthin and lutein+zeaxanthin). n = 344 M, 115 F.
[2] $P < 0.001$.
[3] $P < 0.05$.
[4] $P < 0.01$.

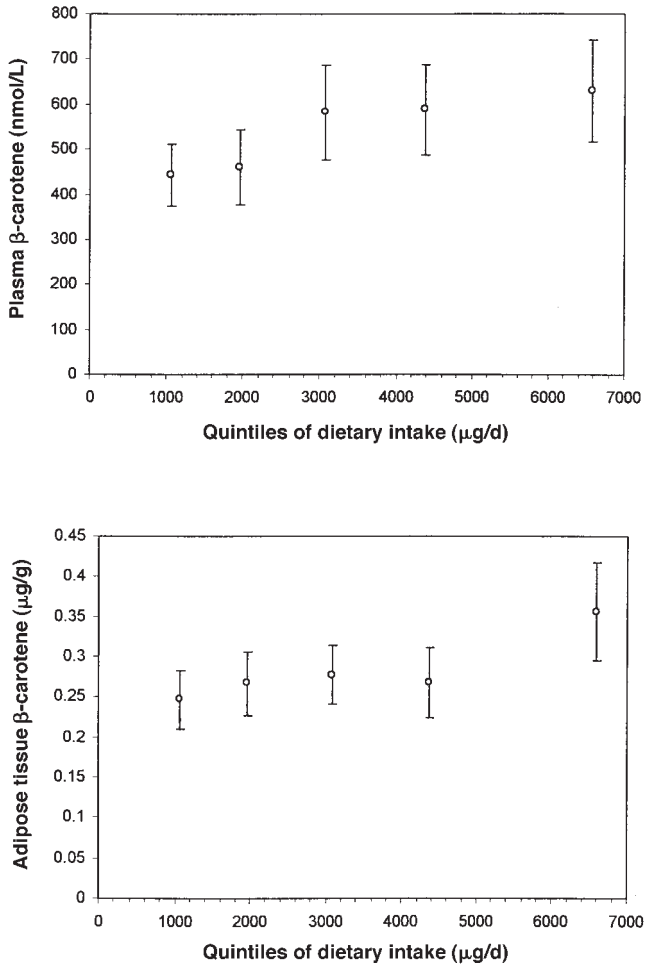

FIGURE 2. Plasma and adipose tissue concentrations of β-carotene plotted against median quintiles of intake of β-carotene. *P* for trend < 0.001 for plasma and *P* for trend = 0.002 for adipose tissue.

for β-carotene, β-cryptoxanthin, and lycopene. No significant trends were observed for plasma or adipose tissue α-carotene or lutein+zeaxanthin (data not shown).

DISCUSSION

Adipose tissue concentrations of fat-soluble nutrients are thought to represent a time-integrated measure of dietary intake, whereas blood concentrations are thought to change more rapidly with short-term fluctuations in intake (36, 37). This view is supported by the slow turnover rates for adipose tissue vitamin E (38) and fatty acids (39). However, few studies have assessed the ability of adipose tissue carotenoids to reflect long-term dietary intakes, and the findings of these studies have not been clear (21, 23).

Our results show that average long-term intakes of each carotenoid assessed by the FFQ correlated somewhat better with concentrations in plasma than with those in adipose tissue, and the difference between these 2 biomarkers was greater among the men than the women. Although this difference between men and women might be due to differential reporting in the FFQ, the correlations between intake and plasma carotenoids did not differ significantly between men and women, suggesting that adipose tissue carotenoids are affected by factors other than intake (40). Indeed, the relative abundance of each carotenoid in the diets of either the men or the women differed considerably from their distribution in adipose tissue, yet were similar to their distribution in plasma. For example, β-carotene and lycopene were the most abundant carotenoids in the diet and plasma, but lutein+zeaxanthin was more abundant in adipose tissue than β-carotene and lycopene combined. Although it could also be argued that the FFQ is not a good measure of long-term intake, the correlations between plasma and adipose tissue carotenoids, which are independent of any measurement errors associated with the FFQ, were low (r = 0.11–0.43) and were substantially different for each carotenoid. Similar correlations between plasma and adipose tissue for β-carotene (r = 0.39) and lycopene (r = 0.24) were previously reported by others (41).

The first study to suggest that adipose tissue carotenoids may be useful biomarkers of long-term intake showed a significant increase in adipose tissue β-carotene after 6 mo of daily supplementation with 30 mg β-carotene (21). However, this level of supplementation is >10-fold higher than typical dietary intakes, and there was no association between dietary β-carotene assessed by an FFQ and adipose tissue β-carotene among individuals not taking supplements (21). Zhang et al (23) also found

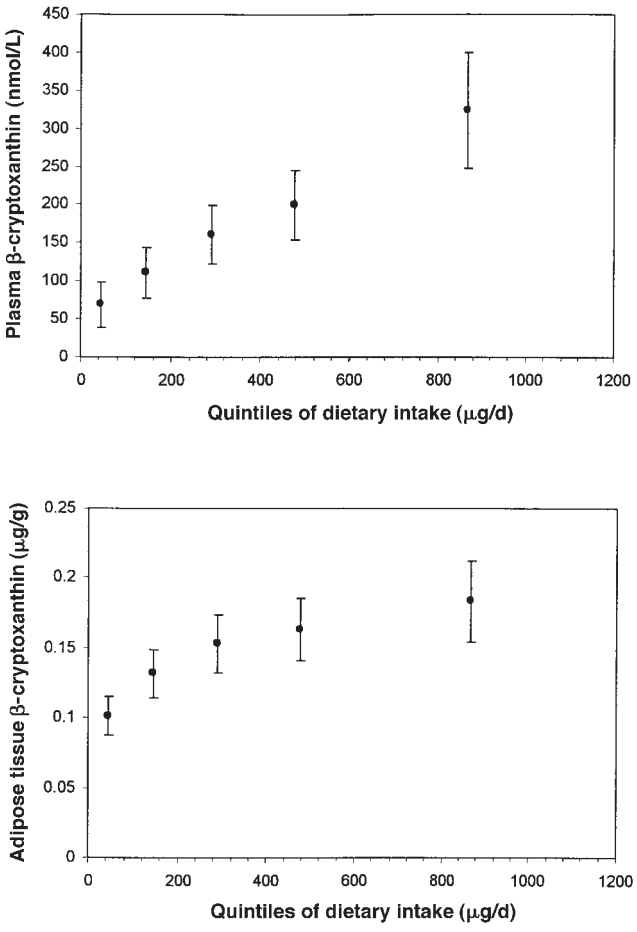

FIGURE 3. Plasma and adipose tissue concentrations of β-cryptoxanthin plotted against median quintiles of intake of β-cryptoxanthin. *P* for trend < 0.0001 for both plasma and adipose tissue.

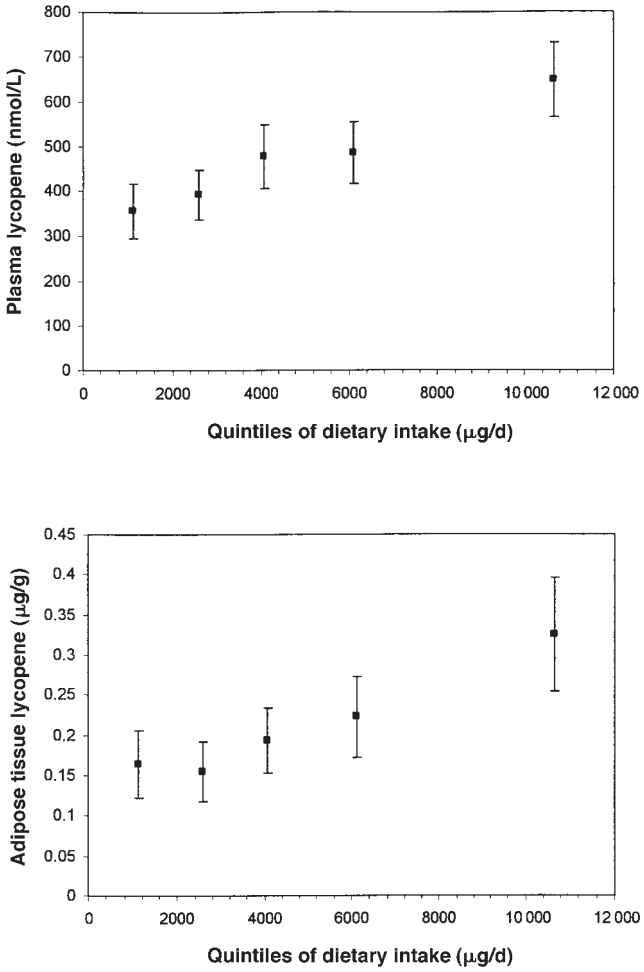

FIGURE 4. Plasma and adipose tissue concentrations of lycopene plotted against median quintiles of intake of lycopene. *P* for trend < 0.0001 for both plasma and adipose tissue.

no association between intakes of lycopene, β-carotene, and lutein+zeaxanthin as assessed by an FFQ and their concentrations in breast adipose tissue. In other studies, a single 120-mg dose of β-carotene increased adipose tissue β-carotene concentrations by 2-fold in only 5 d (22), and eating a lutein-rich diet for 8 wk increased adipose tissue concentrations of lutein by almost 2-fold (24). In the latter study, women had much higher lutein concentrations in adipose tissue than did men despite having similar serum concentrations and significantly lower intakes. Thus, the notion that adipose tissue carotenoids reflect long-term dietary intake and are stable against occasional fluctuations in intake is not supported by any of the studies conducted to date.

One of the major concerns with using adipose tissue carotenoids is the poor reproducibility of measurement of concentrations (21, 23). In the present study, the CVs for adipose tissue were 2-fold greater than those for plasma. Similarly, Kardinaal et al (21) observed that the reproducibility of measures of adipose tissue β-carotene repeated over 4 mo was much lower ($r = 0.50$) than that of plasma β-carotene measurements ($r = 0.93$) or intake of β-carotene as assessed by an FFQ ($r = 0.67$). Zhang et al (23) also reported poor reproducibility in their measurements of adipose tissue carotenoids.

Another issue is the lack of a uniform unit of measurement for adipose tissue carotenoid concentrations and the difficulty in converting from one unit to another. In previous studies, the concentration of adipose tissue carotenoids was expressed as μg/g (wet or dry) adipose tissue or as μg/g fatty acid. Regardless, the relative concentrations of each carotenoid in adipose tissue, which are independent of the unit of measurement, are similar in the present study to values previously reported (23, 40–43). More importantly, the distribution of carotenoids in adipose tissue is different from the distribution in the diet and plasma found in this and other studies (23, 40–43). However, an earlier study involving 19 subjects found the relative abundance of α-carotene, β-carotene, and lycopene to be similar in adipose tissue and plasma (44), and a more recent study found that the relative concentrations of the major carotenoids are similar in plasma and prostate tissue (45). These observations suggest that carotenoid uptake or retention may differ depending on the tissue type. Preliminary data from an unpublished study reported by Johnson et al (24) show that lutein concentrations in adipose tissue differ among body fat sites (abdomen, buttocks, and thigh). Thus, it appears that the distribution of carotenoids within adipose tissue is not homogeneous between sites, and this could explain the poor reproducibility in measuring adipose tissue carotenoids.

It has been suggested that plasma and adipose tissue carotenoids represent different aspects of carotenoid status and should not be used interchangeably as markers of carotenoid intake (41). This assertion was based primarily on a case-control study showing that plasma lycopene was positively associated with heart disease, whereas adipose tissue lycopene was inversely associated with it (41). In the present study, we provide further evidence that these 2 pools represent distinct markers of carotenoid status by showing that the relative concentrations of the major carotenoids differed between plasma and adipose tissue. Differences between plasma and adipose tissue may be due to selective uptake of certain carotenoids by adipose or other tissues; alternatively, the turnover rates of individual carotenoids in various tissues may differ. The partitioning of carotenoids into adipose tissue, however, does not appear to be related to their lipophilicity because the more polar carotenoids lutein and zeaxanthin were the most abundant in adipose tissue.

The correlation coefficients we observed between intakes of individual carotenoids and their concentrations in plasma, although similar to those observed by others (9–17), tended to be near the lower end for α-carotene and β-carotene, intermediate for lutein+zeaxanthin, and at the upper end for β-cryptoxanthin and lycopene. These variations among reported correlations may be due to different ranges of intake and measurement error between populations, different methods of dietary assessment, or different methods of measuring carotenoids. Differences in dietary patterns between populations can also enhance or inhibit the absorption of specific carotenoids, which could also explain the variation in reported values. For example, intake of lycopene was shown to reduce the absorption of β-carotene (46). Therefore, populations who consume their main sources of carotenoids together may have lower correlations for β-carotene between the diet and plasma. Although we used the updated carotenoid content of foods listed in the USDA–National Cancer Institute carotenoid database, this database was derived from measurements determined by a variety of methods at different points in time and in different laboratories. Measuring the carotenoid content of foods by the same analytic methods should improve the

accuracy of associations between diet and biomarkers of intake found in studies such as this. In this Costa Rican population, intakes and plasma concentrations of each carotenoid were similar to those reported for other populations (9–13, 41, 47). Intakes of β-cryptoxanthin, however, were 2-fold greater in the present study, probably because of more frequent consumption of papayas, which are rich in β-cryptoxanthin (761 μg/100 g edible portion; 8) and which were consumed 2–4 times/wk by one-half the population of this study.

In summary, we found no evidence that adipose tissue carotenoid concentrations are better biomarkers of intake than are plasma concentrations. Differences in the relative abundance of each carotenoid in adipose tissue compared with that in plasma and the diet suggest that the uptake, storage, or turnover rates of each carotenoid differ and depend on factors other than intake. The reproducibility of measurements of adipose tissue carotenoids has been poor in the studies carried out to date, indicating that refinements to the methods used are required to improve the accuracy of the associations. Studies that examine the kinetics of individual carotenoids would greatly improve our understanding of the role these compounds may play in human health and disease.

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