This study evaluated how prostatic levels of antioxidants relate to plasma levels and self-reported usual dietary intake. Definition of these relations may aid in interpreting studies of antioxidant exposure and prostate cancer risk. Between July 1996 and April 1997, plasma and prostatic tissue levels of tocopherols, carotenoids, and retinol were measured in 47 men undergoing radical prostatectomy or transurethral prostatectomy at Loyola University Medical Center, Maywood, Illinois, and an affiliate hospital. Dietary intake was measured by using a 122-item version of the Block Health Habits and History Questionnaire, and correlations were assessed with Pearson's coefficients. Prostatic levels of tocopherols and carotenoids (but not retinol) were significantly correlated with plasma levels ($r = 0.31-0.56$, $p < 0.05-0.0001$); the strongest correlations were associated with lycopene, beta-carotene, and gamma-tocopherol (0.56, 0.54, and 0.52, respectively; $p < 0.0001$). Relative concentrations of tocopherols and carotenoids in prostate tissue were proportionate to those in plasma. No correlation between prostatic levels and reported dietary intake was observed ($r = -0.09$ to $0.16$, $p <$ not significant). Adjustment for energy intake, body mass index, and serum lipids did not impact these relations. These results suggest that plasma levels of tocopherols and carotenoids better reflect prostatic exposure than self-reported usual dietary intake. Am J Epidemiol 2000;151:109-18.
dietary intake is unknown. Furthermore, definition of the relations between antioxidant levels in plasma, which are believed to reflect short-term intake, and those in prostate tissue may aid in interpreting studies involving circulating antioxidants and prostate carcinogenesis. To evaluate these questions, we compared prostatic tissue levels of major dietary antioxidants (alpha- and gamma-tocopherol, lutein, cryptoxanthin, lycopene, alpha- and beta-carotene, and retinol) with corresponding levels in plasma and in the diet of 47 men undergoing total or subtotal surgical removal of the prostate gland.

MATERIALS AND METHODS

Identification and recruitment of subjects

Subjects were men awaiting radical prostatectomy for localized adenocarcinoma of the prostate gland or transurethral prostatectomy for benign prostatic hyperplasia at Loyola University Medical Center (Maywood, Illinois) and its Veterans Administration affiliate, Hines Hospital (Hines, Illinois). They were identified through periodic review of operating room schedules between July 15, 1996, and April 30, 1997 (n = 81). After receiving a letter about the study from their attending physicians, potential subjects were contacted by telephone to solicit their participation. Men who during the last 12 months either changed their diet significantly (n = 5) or lost >15 pounds (6.81 kg) unintentionally (n = 2) were excluded, as were those who received presurgical hormonal therapy with leuprolide acetate (n = 8). Of the remaining 66 eligible potential subjects, 59 agreed to participate (response rate, 89 percent). After providing their informed consent, subjects were scheduled for an outpatient basis prior to surgery. Family history of prostate cancer, smoking exposure, medical history, and medication were also ascertained by using a separate questionnaire. The average time between diet interview and subsequent tissue procurement (blood and prostate) was 16 days; the maximum was 6 weeks.

Semiquantitative food frequency questionnaire and administration

Usual dietary intake of antioxidants and various other nutrients during the last 12 months was measured by using a modified version of the Health Habits and History Questionnaire (HHHQ) developed by Gladys Block and administered by a trained interviewer (11). This instrument, referred to as the "Block questionnaire," provides intake estimates for 33 nutrients: total calories; protein; total fat; total saturated fat; oleic and linoleic acids; carbohydrates; calcium; phosphorus; iron; sodium; potassium; vitamins A (International Units and retinol equivalents), B1, B2, B6, C, and E; niacin; total cholesterol; dietary fiber; folate; zinc; zinc from animals; magnesium; alpha- and beta-carotene; beta-cryptoxanthin; lutein; lycopene; retinol; and provitamin A carotenoids. We included 22 additional items, mainly ethnic (n = 9), reduced fat (n = 7), and deep fried and creamy (n = 3) foods likely to be consumed in our patient catchment area. Subjects were asked to report their frequency of consumption of 122 food items by selecting one of nine categories ranging from "never or less than once per month" to "six or more times per day" in terms of a standard portion size. Food models (Nasco, Fort Atkinson, Wisconsin) were used to assist subjects in their reporting of portions. The questionnaire also included a write-in section for frequencies not listed, foods not listed, and the exact brand of margarine and type of fat used in frying, cooking, and baking.

Responses were entered by hand first by one data entry clerk and again by a second (the "double-keying" method) and were then passed through various audit checks before nutrient intakes were calculated. Mean daily consumption of each food item in grams was calculated and was converted into daily nutrient intake by using HHHQ-DIETSYS analysis software, version 3.0, from the National Cancer Institute (NCI) (12). The basic form of the algorithm used by this software is as follows: (gram portion size × nutrient content in 100 g × reported food frequency)/100. The "OnQuest" option was selected for these conversions. Nutrient estimates for food items listed in the Block questionnaire are based on findings from the Second National Health and Nutrition Examination Survey and its nutrient content database (13, 14). Nutrients were summed for all foods and were expressed as average intake per day in mg/day of each antioxidant except retinol, which was expressed as retinol equivalents/day.

Tissue procurement

Blood. On the morning of a subject's surgery, 10 ml of fasting blood for plasma tocopherols, carotenoids, and retinol were collected into an aluminum-foil-wrapped heparinized blood collection tube and immediately placed on ice. A second 10 ml sample was collected into a "red-top" tube for serum lipids. Within 30 minutes of collection, the heparinized tube was refrigerated while the red-top tube remained at room temperature. After 1 hour, the tubes were inverted once and were centrifuged at 1,500 g for 12 minutes at 4°C. One milliliter aliquots of plasma and serum were then placed into separate 2 ml cryovials (Nalgene, Rochester, New York) and were stored at −70°C until analysis.

**Prostate tissue.** Fresh prostate tissue was obtained from radical prostatectomies performed for localized-stage prostate cancer (n = 41) and transurethral prostatectomies performed for benign prostatic hyperplasia (n = 6); the former made available whole prostate specimens and the latter yielded “prostate chips.” Immediately after surgical removal, the prostate tissue was placed into sterile saline and was delivered to the medical facility’s department of pathology. Whole prostate specimens were assessed for the presence of palpable tumor by using sterile instruments and gloves. Then, 2–3 g of fresh, grossly normal prostate tissue were resected from the peripheral zone prior to fixation according to the method described by Bova et al. (15). Normal tissue was used to estimate nutrient levels, since levels in tumor likely reflect the effects of disease rather than internal exposure (16). The peripheral zone was selected because the vast majority of prostatic carcinomas originate here (17). In addition, frozen-section histologic controls were obtained from the same areas to ensure that the apparently “normal” tissue did not contain cancer. Prostate chips (average aggregate weight, 2.3 g) were inspected for areas suspicious for tumor. Grossly yellow tissue (a marker for the presence of tumor) or tissue of <4 mm (increased probability of microscopic tumor) was not used for the study. Tissue samples were placed into a 1 inch (2.54 cm) resealable plastic bag, were wrapped in foil, and were then stored frozen in plastic vials at -70°C until analysis.

Prostate tissue samples and the corresponding plasma were transferred on dry ice to the US Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University in Boston, Massachusetts, for antioxidant analyses. Serum samples for total cholesterol, triglycerides, high density lipoproteins, and low density lipoproteins were similarly transferred to the Northwest Lipid Research Laboratories, University of Washington, Seattle (Dr. Santica Marcovina, Core Laboratory Director). Low density lipoprotein fractions were estimated by using the Friedewald et al. equation (low density lipoproteins = total cholesterol – high density lipoproteins – (triglycerides/5)) (20).

**Biochemical assays**

All carotenoid, tocopherol, and retinol analyses were performed under red light to prevent sample degradation by photooxidation. Echinonone, alpha-tocopheryl acetate, and retinyl acetate were used as internal standards for carotenoid, tocopherol, and retinol analyses, respectively, by high-performance liquid chromatography (HPLC), helping to account for recovery of associated analytes. After homogenization (PowerGen 125; Fisher Scientific, Pittsburgh, Pennsylvania) in a chloroform-methanol (2:1) solution containing internal standards, prostate tissue was extracted by hexane for antioxidant analyses. Plasma was extracted similarly. Organic phases were dried under nitrogen and were reconstituted in ethanol. Concentrations of carotenoids, tocopherols, and retinol were then analyzed simultaneously by HPLC. The chromatographic separation and HPLC column were performed according to the method of Tang et al. (18, 19). This method measures alpha- and gamma-tocopherol, lutein, beta-cryptoxanthin, lycopene, alpha- and beta-carotene, and retinol. Concentrations were expressed as μmol/liter in plasma and as nmol/g in prostate tissue. The coefficients of variation for each analyte in plasma were as follows: alpha-tocopherol, 1.5 percent (mean, 32.15 μmol/liter); gamma-tocopherol, 1.8 percent (mean, 3.42 μmol/liter); lutein, 7.6 percent (mean, 0.68 μmol/liter); beta-cryptoxanthin, 21.8 percent (mean, 0.24 μmol/liter); lycopene, 10.5 percent (mean, 0.62 μmol/liter); alpha-carotene, 12 percent (mean, 0.11 μmol/liter); beta-carotene; 10.3 percent (mean, 0.42 μmol/liter); and retinol, 4.5 percent (mean, 2.83 μmol/liter). The corresponding coefficients of variation for each analyte in prostate tissue were similar.

Total cholesterol, high density lipoproteins, and triglycerides were measured enzymically on the Abbott Spectrum Multichromatic Analyzer (Abbott Laboratories, North Chicago, Illinois) at Northwest Lipid Research Laboratories, University of Washington, Seattle (Dr. Santica Marcovina, Core Laboratory Director). Low density lipoprotein fractions were estimated by using the Friedewald et al. equation (low density lipoproteins = total cholesterol – high density lipoproteins – (triglycerides/5)) (20).

**Statistical analysis**

All 59 eligible subjects completed the dietary questionnaire and blood collection, both of which were repeated for a random 25 percent sample of subjects (n = 15) after 2–6 weeks (mean, 3.8 weeks) to assess reproducibility of nutrient consumption and blood level estimates. However, prostate tissue from 12 subjects could not be analyzed for the following reasons: radical prostatectomy abandoned because of inoperable cancer detected during surgery (n = 3), radical prostatectomy completed but normal tissue technically unresectable because of diffuse involvement of the gland with tumor cells (n = 1), tissue embedded in paraffin before storage (n = 5), and sample quantities insufficient for biochemical analyses (n = 3). Thus, the results from 47 subjects (12 with repeat dietary intake and blood nutrient measurements) were available for statistical analysis.

Mean dietary intake, plasma, and prostatic tissue levels of each antioxidant, and their standard errors, were calculated. Since the distributions of dietary intake were skewed, values were also transformed by using the natural logarithm (21). Linear regression analysis revealed that daily intake of alpha-tocopherol, lutein, cryptoxanthin, lycopene, and beta-carotene (but
not alpha-carotene and retinol) was significantly correlated with daily total caloric intake ($p = 0.0001-0.036$) in our sample. Therefore, crude intakes were also adjusted for total caloric intake by using the method described by Willett and Stampfer (22).

For each antioxidant, correlations between levels observed in prostate tissue and plasma, prostate tissue and the diet, and plasma and the diet were assessed by using Pearson's correlation coefficients. Correlations with crude, calorie-adjusted, and log$_e$-transformed intake values were evaluated separately. Blood levels of some antioxidants may also be associated with lipid levels (23–25). In our sample, linear regression analysis revealed that plasma gamma-tocopherol and lycopene levels were significantly associated with serum total cholesterol, while plasma alpha- and gamma-tocopherol, lycopene, and retinol levels were significantly associated with serum triglycerides. Therefore, partial Pearson's correlation coefficients were calculated to adjust for the effect of serum cholesterol and triglycerides on the corresponding plasma-diet and plasma-prostate-tissue tocopherol, lycopene, and retinol correlations. Linear regression was also used to evaluate the association of plasma and prostate tissue antioxidant levels with age, body mass index, and alcohol intake, but no significant relations were observed. For the subsample of subjects who repeated the questionnaire and blood draw, nutrient intake and blood-level estimates obtained at the first and second visits were compared by using the Wilcoxon signed-rank test. All measurements were reproducible ($p = 0.15–1.00$) except plasma alpha-tocopherol (mean difference, 5.25 μmol/liter; $p = 0.004$).

**RESULTS**

The mean (standard deviation) age of subjects was 65.9 (9.2) years. Mean (standard deviation) body mass index, total caloric intake, and serum cholesterol were 24.0 (3.4) kg/m$^2$, 2,071 (818) kcal/day, and 200.6 (40.5) mg/100 ml, respectively. These values closely resembled those reported for similarly aged men in the general population: 26 kg/m$^2$, 2,109 kcal/day, and 200 mg/100 ml, respectively (26–28). Estimates of total caloric intake and serum cholesterol appeared reproducible in the subset of 12 subjects with repeat measurements (mean difference, 274 kcal ($p = 0.38$) and 4.9 mg/100 ml ($p = 0.86$), respectively). All 47 subjects were nonsmokers; nine were African American. Annual income ranged from approximately $11,000 to $170,000, and 82 percent of the subjects had completed high school whereas 67 percent were college graduates.

Levels of tocopherols, carotenoids, and retinol in the diet, plasma, and prostate tissue are presented in table 1. Alpha-tocopherol was the most abundant nutrient measured at each level (mean (standard error): 10.11 (0.70) mg/day in the diet, 32.15 (2.25) μmol/liter in plasma, and 38.95 (4.28) nmol/g in prostate tissue). In the following two ways, concentrations of tocopherols and carotenoids in prostate tissue closely resembled the pattern observed in plasma but not that reported in the diet: 1) concentrations followed exactly the rank order observed in plasma, and 2) relative concentrations were comparable in magnitude to those measured in plasma (relative to alpha-carotene, mean concentrations of alpha-tocopherol, gamma-tocopherol, lutein, lycopene, beta-carotene, and beta-cryptoxanthin were 292.3, 31.1, 6.2, 5.6, 3.8, and 2.2 times higher, respectively, in plasma and 486.9, 51.4, 4.6, 3.0, 3.0, and 1.8 times higher, respectively, in prostate tissue).

Pearson's correlations between tocopherols, carotenoids, and retinol levels in prostate tissue, plasma, and the diet are presented in table 2. Prostatic levels of tocopherols and carotenoids (but not retinol) were significantly correlated with plasma levels. Lycopene, beta-carotene, and gamma-tocopherol exhibited the strongest

| TABLE 1. Mean dietary intake, plasma, and prostatic tissue levels of carotenoids, tocopherols, and retinol in 47 men aged 50–75 years undergoing prostate surgery, Maywood, Illinois, 1996–1997 |
|------------------------------------------|-----------------------------|
| **Dietary intake (mg/day)** | **Mean (standard error)** |
| α-Tocopherol | 10.11 (0.70) |
| Lutein | 2.30 (0.37) |
| β-Cryptoxanthin | 0.12 (0.01) |
| Lycopene | 2.27 (0.37) |
| α-Carotene | 0.38 (0.05) |
| β-Carotene | 3.65 (0.61) |
| Retinol | 0.97 (0.16) |
| **Plasma (μmol/liter)** |  |
| γ-Tocopherol | 3.42 (0.42) |
| α-Tocopherol | 32.15 (2.25) |
| Lutein | 0.68 (0.08) |
| β-Cryptoxanthin | 0.24 (0.03) |
| Lycopene | 0.62 (0.05) |
| α-Carotene | 0.11 (0.01) |
| β-Carotene | 0.42 (0.06) |
| Retinol | 2.83 (0.16) |
| **Prostate tissue (nmol/g)** |  |
| γ-Tocopherol | 4.11 (0.47) |
| α-Tocopherol | 38.95 (4.28) |
| Lutein | 0.37 (0.04) |
| β-Cryptoxanthin | 0.14 (0.03) |
| Lycopene | 0.24 (0.03) |
| α-Carotene | 0.08 (0.01) |
| β-Carotene | 0.24 (0.05) |
| Retinol | 0.002 (0.0003) |

* α-Tocopherol in prostate tissue and plasma, vitamin E in the diet.
† Measured in retinol equivalents/day.
between-tissue correlations, with respective coefficients of 0.56, 0.54, and 0.52; \( p < 0.0001 \). However, no correlation between prostatic tissue levels of antioxidants and reported dietary intake was observed. The results of the comparison between plasma and reported dietary intake levels were less consistent. The highly significant correlations observed between prostatic and plasma levels of lycopene and beta-carotene disappeared when levels in plasma were compared with the corresponding reported intake. On the other hand, the correlations observed between prostatic tissue and plasma levels of lutein and beta-cryptoxanthin, both lower than the correlations observed for lycopene and beta-carotene, remained significant when levels in plasma were compared with reported intake (\( r = 0.33 \) and 0.31, respectively; \( p < 0.05 \)). In fact, the strength of the between-tissue correlations for lutein remained fairly constant (\( r = 0.30 \) and 0.33 for prostatic tissue vs. plasma and plasma vs. diet, respectively; \( p < 0.05 \)). Neither log-transformed nor calorie-adjusted intake values materially altered any of the relations described.

Scatter plots of the plasma-prostate relations, subject by subject for each nutrient, are displayed in figure 1. The plots for beta-cryptoxanthin and beta-carotene suggest that the observed prostate-plasma correlations may be driven by one or two subjects in the upper range of prostatic values. Levels of the other nutrients also tended to be higher in these subjects and were confirmed with biochemical analyses of tissue adjacent to the original sample. Therefore, these observations were retained in the statistical analysis.

Plasma levels of alpha- and gamma-tocopherol, lycopene, and retinol were variably associated with serum total cholesterol and triglycerides in our sample. Since these associations could have affected our estimates of the prostate tissue versus plasma antioxidant correlations, partial Pearson’s correlations “adjusting” for their effects, if any, were calculated and are presented in table 3. The most substantive change was in the correlation between prostatic and plasma gamma-tocopherol levels. After adjustment for serum total cholesterol levels, the significance of the correlation was lowered from \( p < 0.0001 \) to \( p < 0.001 \). Thus, adjustment for lipid levels in this sample had little impact on the observed crude associations.

Table 4 compares our correlations between diet and plasma carotenoids with estimates reported elsewhere (29, 30). Both studies cited used the semi-quantitative food frequency questionnaire developed by Block, with minor modifications. However, unlike our analysis using the literature-based Block carotenoid composition database (11), Forman et al. (29) used the USDA-NCI laboratory-based composition database recently developed by Chug-Ahuja et al. and Mangels et al. (31, 32), while Ritenbaugh et al. (30) compared the results obtained with both. Generally, our correlations were comparable to those reported in these two samples. From a qualitative standpoint, the most substantive differences were observed with the estimates for lutein by Forman et al. (0.33 vs. 0.11) and for alpha-carotene by Ritenbaugh et al. (0.23 vs. 0.49 and 0.51 for Block and USDA-NCI carotenoid composition databases, respectively).

Finally, table 5 compares prostatic levels of carotenoids in our sample with those measured in a study by Clinton et al. of 25 radical prostatectomy specimens from the Mayo Clinic (33). In contrast to our results, lycopene was the most abundant carotenoid measured in prostate tissue.
TABLE 3. Partial Pearson’s correlations for plasma vs. prostate concentrations of γ-tocopherol, α-tocopherol, lycopene, and retinol, after controlling for serum total cholesterol and triglyceride levels, for 47 men aged 50–75 years undergoing prostate surgery, Maywood, Illinois, 1996–1997

<table>
<thead>
<tr>
<th></th>
<th>Crude Pearson’s correlation</th>
<th>Lipid</th>
<th>Partial Pearson’s correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma vs. diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.06</td>
<td>Triglycerides</td>
<td>0.09</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.16</td>
<td>Total cholesterol</td>
<td>0.20</td>
</tr>
<tr>
<td>Retinol</td>
<td>-0.01</td>
<td>Triglycerides</td>
<td>-0.04</td>
</tr>
<tr>
<td><strong>Plasma vs. prostate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.52***</td>
<td>Total cholesterol</td>
<td>0.49**</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.31</td>
<td>Triglycerides</td>
<td>0.34*</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.56***</td>
<td>Total cholesterol</td>
<td>0.56**</td>
</tr>
<tr>
<td>Retinol</td>
<td>-0.16</td>
<td>Triglycerides</td>
<td>-0.20</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.0001.

TABLE 4. Correlations between diet and plasma carotenoids in men aged 50–75 years, Maywood, Illinois, 1996–1997, compared with estimates from other samples of US men†

<table>
<thead>
<tr>
<th></th>
<th>Present study (n = 47)</th>
<th>Forman et al. study (n = 57)</th>
<th>Rittenbaugh et al. study (30)††</th>
<th>USDA-NCI# Block§</th>
<th>USDA-NCI§ Block</th>
<th>Clinton et al. study (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>0.33*</td>
<td>0.11</td>
<td>0.33</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.31*</td>
<td>0.33**</td>
<td>0.26</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.16</td>
<td>0.24</td>
<td>0.25</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.23</td>
<td>0.25</td>
<td>0.49***</td>
<td>0.51***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.21</td>
<td>0.36**</td>
<td>0.25</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001.
† All studies used a version of the Block food frequency questionnaire; results are presented for nonsmokers only, and correlations are unadjusted for plasma lipids.
†† Age group: 20–40 years.
§ Block carotenoid composition database, which estimates carotenoid content of food items by using literature-derived algorithms.
|| Age group: 50–75 years.
# US Department of Agriculture-National Cancer Institute (USDA-NCI) carotenoid composition database, which estimates carotenoid content of food items by using standardized laboratory determinations.
†† Age group: 50–65 years. Correlations have been adjusted for body mass index and energy intake; results for β-cryptoxanthin not reported by the authors.
‡‡ Sample sizes for lutein, lycopene, α-carotene, and β-carotene: 29, 58, 67, and 58, respectively.

**DISCUSSION**

Our understanding of how nutrition relates to prostate cancer disease in humans is based almost exclusively on historical measures of exposure (34).

Furthermore, detection of an effect tempts us to believe that the measure used actually discriminates between persons with different degrees of nutrient exposures at the level of the target organ, that is, high reported nutrient intake corresponds to high tissue nutrient levels, and so forth. Our data, to our knowledge the first reported estimates of the correlations between dietary, plasma, and prostatic tissue levels of individual antioxidants, indicate that this may not be the case. In our sample, self-reported habitual dietary intake could not classify exposure to major antioxidants at the level of the prostate gland. Failure to observe significant correlations in this context may be due to physiologic factors that can obscure diet-plasma-tissue nutrient relations between persons, including interindividual differences in intestinal absorption, metabolism, and uptake and turnover at the tissue level, which, in turn, may be organ specific (35). These are fundamental variations. Unfortunately, quantifying them in a manner useful for routine epidemiologic investigation is beyond current technical expertise.

Methodological error could have also concealed an association between reported intake and prostatic antioxidant levels. Possible sources include variation in interviewer technique, an inadequate antioxidant composition database, and tissue sampling errors. The Block semiquantitative food frequency questionnaire is designed to be self-administered (36). Therefore, the role of the interviewer in our study was to help respondents report portion sizes by using food models, to pace completion of the questionnaire, and to answer questions about its content. The impact of interviewer technique on the accuracy of self-report in this setting is probably very limited. Furthermore, our use of a single interviewer should have minimized variation in questionnaire administration.

The Block database of carotenoid concentrations for specific foods relies on estimates from the literature (13, 14). Since the measurement techniques used in the studies varied, the ability of the Block database to accu-
rately convert food frequencies and portion sizes into specific carotenoid intakes may have been affected. The recently developed USDA-NCI database should overcome this potential limitation since it is based mainly on standardized laboratory assessment (31, 32). However, if the results presented in table 4 are any indication, obtaining qualitatively superior associations for diet-tissue carotenoid relations by using laboratory-based instead of literature-based estimates alone should not be taken for granted. For example, in a sample of 110 male nonsmokers in the Physicians Health Follow-up Study, Michaud et al. recently reported diet-plasma correlations for carotenoids ranging from 0.35 to 0.47 (adjusted for age, energy intake, body mass index, and plasma lipids) using the USDA-NCI carotenoid database (37). However, their results are also based on the average of two food frequency questionnaires completed 1 year apart, with responses to the second one preceded by 2 weeks of diet records completed approximately 6 months apart and blood collected just prior to the second questionnaire. While this strategy clearly resulted in improved diet-plasma correlations, it may be of limited applicability to the study of cancer outcomes, since it may be restricted to relatively small and highly motivated patient samples.

Finally, 75 percent of prostatic glandular tissue is localized to the peripheral zone, the largest of its three concentric anatomic zones (17). Variation could exist in antioxidant concentrations between zones, but this is only speculative. Nevertheless, we attempted to address this issue by limiting our analysis to tissue collected from the same anatomic zone in subjects, when possible. This procedure resulted in over 80 percent of the tissue analyzed originating from the peripheral zone. Within-zone variability was not assessed directly.

Perhaps the methodological error most likely culpable is inaccurate self-report of usual dietary intake. The evidence is circumstantial. Studies of the accuracy of questionnaire measurements of habitual intake are typically based on a comparison with repeated weighed-food consumption records (4- to 7-day food records) or 24-hour recalls used as reference measurements (38). When compared with biochemical markers, self-reported usual intake tends to be less correlated than reference measurements (39-41). Participants in validation studies may also tend to report intake more accurately than nonparticipants (42). Most importantly, the semiquantitative questionnaire is a 365-day recall instrument, and the study's cross-sectional design, necessitated by the research question at hand, precluded verification of subjects' responses. These responses appeared to be reproducible, and the use of food models probably improved estimates of portion sizes. However, the extent to which they agreed with actual intake could not be assessed.

Plasma levels of tocopherols and carotenoids were correlated with prostatic tissue levels. This finding supports their potential to provide better estimates of internal dose, and thus target organ exposure, than reported intake. However, use of plasma measurements to test diet-prostate cancer hypotheses must be considered carefully, since little is known about the natural history of prostate cancer and antioxidant levels in plasma can vary substantially within persons over time (43, 44). An alternative explanation for the significant correlations observed between plasma and prostate tissue is that both may actually reflect short-term intake, while the food frequency questionnaire reflects long-term intake. A setting in which highly correlated plasma-prostate nutrient relations might be exploited is a clinical trial, for example, one relating antioxidant intake (e.g., structured dietary modification or supplementation) to prevention of various prostate cancer outcomes (progression, mortality). However, our fragmentary understanding of prostate tissue responsiveness to changes in plasma levels limits this application as well. No correlation was found between plasma and prostatic tissue levels of retinol, which might have been due to potential metabolism of retinol to retinoic acid that we could not detect with the HPLC setup for these analyses.

Information on the antioxidant composition of prostate tissue could provide clues to how these nutrients are absorbed from the bloodstream, helping to illuminate additional mechanisms underlying their potential to protect against prostate cancer. To date, the study by Clinton et al. is probably the only one that makes these data available (33). In both samples, lycopene is a major carotenoid in prostate tissue. Lycopene has also been associated with a lower risk of prostate cancer, including advanced cases (5). Whether such an effect is due to properties unique to lycopene or is a function of the probability of exposure is not yet clear. For example, while lycopene possesses exceptionally high antioxidant activity in vitro compared with other carotenoids (45), it is also a predominant carotenoid in blood and many other tissues (46, 47). In our sample, the close similarity between relative mean concentrations of tocopherols and carotenoids in prostate tissue and those in plasma further suggests that prostatic absorption is not selective but proportionate to concentrations in plasma.

The most abundant antioxidant measured in the prostate glands and plasma of our sample was alphatocopherol followed by lutein, with lycopene and beta-
carotene closely behind. Perhaps it is no coincidence then that protective effects have also been observed with alpha-tocopherol and, less consistently, beta-carotene (4, 6). The reasons for the generally lower carotenoid concentrations observed in our sample compared with the series analyzed by Clinton et al. (33) are not immediately clear. They may reflect differences in the "true" carotenoid intakes between the two groups, unmeasured lifestyle factors, geography, extraction methodology, or some combination thereof.

In conclusion, we found that self-reported intake of major dietary antioxidants and retinol could not predict their levels in prostate tissue. This finding may be due to interindividual differences in absorption and metabolism in combination with reporting errors. Since our dietary intake assessment was conducted cross-sectionally and was based on only the Block questionnaire and the accompanying literature-based carotenoid composition database, our results may not necessarily be generalizable to other questionnaires and other strategies of application. Plasma levels of tocopherols and carotenoids were correlated with their levels in prostate tissue, supporting their potential to assess and monitor exposure at the level of the prostate gland. The most abundant antioxidant in the diet and in plasma was alpha-tocopherol. Concentrations of tocopherols and carotenoids in prostate tissue closely resembled the pattern observed in plasma in terms of their rank order and ratios, suggesting that prostatic absorption is simply proportionate to plasma concentrations. The most abundant tocopherols and carotenoids in prostate tissue included alpha-tocopherol, lycopene, and beta-carotene, antioxidants variously associated with a lower risk of prostate cancer. It may be that if an association exists between antioxidants and lowered prostate cancer risk, the association will most likely be evident with the antioxidant exposures most likely encountered at the prostate level.

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