Assessing the effect of fatty acids on prostate carcinogenesis in humans: does self-reported dietary intake rank prostatic exposure correctly?[1–3]

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ABSTRACT

Background: Dietary fatty acids may influence prostate carcinogenesis. Although the standard for assessing dietary effects in humans is the semiquantitative food-frequency questionnaire, the extent to which self-reported intake correctly ranks prostatic exposure is unknown.

Objective: The objective was to examine the correlation between reported intakes of different fatty acids and their concentrations in prostate tissue.

Design: This was a cross-sectional study of 52 men undergoing surgical resection of the prostate gland. Usual dietary intake of saturated, total unsaturated, oleic, and linoleic fatty acids over the previous year was estimated with use of a 122-item version of the Health Habits and History Questionnaire. Concentrations in prostate tissue were measured directly by use of gas chromatography in healthy tissue collected at the time of surgery and were expressed as a percentage of total fatty acids. Correlations with 4 measures of dietary intake [g/d, g/d adjusted for total daily energy intake, % of total fat (as g/d), and % of total energy] were evaluated by Spearman's rank-order correlation coefficients.

Results: Linoleic acid concentrations in prostate tissue were significantly correlated with dietary intake expressed as g/d adjusted for total energy [$r = 0.29$ (95% CI: 0.03, 0.49), $P = 0.04$], % of total fat [$r = 0.36$ (0.14, 0.550), $P = 0.008$], and % of total energy [$r = 0.28$ (0.04, 0.49), $P = 0.042$], but not as g/d. Although mean concentrations of saturated, total unsaturated, and oleic fatty acids in prostate tissue resembled mean intakes for the group, prostatic concentrations did not correlate with individual intakes.

Conclusion: Self-reported intake of fatty acids is a satisfactory marker of prostatic exposure at the group level, but, with the exception of linoleic acid, does not correctly rank individuals with respect to intensity of exposure. *Am J Clin Nutr* 2001;73:815–20.

KEY WORDS Fatty acids, dietary intake, prostate tissue, correlation, prostatic neoplasms, risk factors, men, prostate cancer, food-frequency questionnaire

INTRODUCTION

Nutrition, hormones, and genes likely influence the natural history of prostate cancer (1–8). Of the possible nutritional determinants evaluated to date, fatty acids have been studied the most extensively (9). Self-reported dietary intake as measured with use of a semiquantitative food-frequency questionnaire (FFQ) is the standard for assessing long-term exposure. However, whether self-reported habitual dietary intakes of fatty acids accurately rank prostatic exposure is unknown. Clarifying this relation would help in planning studies that either attempt to relate individual-level measures of exposure with clinical outcomes or examine nutrient-gene interactions affecting prostate cancer risk and prognosis. Using a cross-sectional study design, we compared reported intakes of saturated, total unsaturated, oleic, and linoleic fatty acids with corresponding concentrations in prostate tissue in 52 men undergoing prostate surgery.

SUBJECTS AND METHODS

Identification and recruitment of subjects

By periodically reviewing operating room schedules between 1 July 1996 and 15 May 1997, we identified 89 men awaiting radical prostatectomy for clinically localized adenocarcinoma of the prostate or transurethral prostatectomy for benign prostatic hyperplasia at Loyola University Medical Center (Maywood, IL) and its affiliate, Hines VA Hospital (Hines, IL). Potential subjects were contacted by letter and then by telephone. Men who during the past 12 mo either changed their diet significantly ($n = 5$) or lost >7 kg unintentionally ($n = 2$) and those who received presurgical hor-

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monal therapy with leuprolide acetate (n = 8) were excluded. Of the remaining 74 eligible subjects, 65 (88%) agreed to participate. After providing informed consent, the subjects were scheduled for an ≈1-h diet interview conducted on an outpatient basis before surgery. Family history of prostate cancer, smoking exposure, medical history, and medication use were ascertained with a separate questionnaire. The average time between the diet interview and prostate tissue collection was ≈2 wk, with a maximum of 6 wk. The study was approved by the institutional review boards of Loyola University Medical Center and Hines VA Hospital. All patients completed an informed consent form and were advised of the risks associated with participation.

Semiquantitative food-frequency questionnaire and administration

Usual dietary intakes of antioxidants and various other nutrients during the past 12 mo were measured with a modified version of the Health Habits and History Questionnaire [HHHQ (10)] administered by a trained interviewer. This instrument provides intake estimates for 33 nutrients: total energy, protein, total fat, total saturated fat, oleic and linoleic acids, carbohydrate, calcium, phosphorus, iron, sodium, potassium, vitamin A (IU and retinol equivalents), thiamine, riboflavin, vitamin B-6, vitamin C, vitamin E, niacin, total cholesterol, dietary fiber, folate, zinc, zinc from animal sources, magnesium, α-carotene, β-carotene, β-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 consumed in our catchment area. The subjects were asked to report their frequency of consumption of 122 food items by selecting 1 of 9 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, WI) 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 by the double-keying method and then passed through various coding, frequency, and portion size audit checks with the DIETSYS edit checking feature (11) before nutrient intakes were calculated. Questionnaires with potential errors or extreme values were checked by hand; none were excluded. Mean daily consumption of each food item in grams was calculated and converted into daily nutrient intakes by using HHHQ-DIETSYS (11). 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 HHHQ are based on findings from the second National Health and Nutrition Examination Survey and its nutrient database (12, 13). Nutrients were summed over all foods and expressed as average intake in mg/d. Estimated total daily intakes of oleic, linoleic, and total saturated fatty acids were expressed as g/d, % of total fat (as g/d), and % of total energy. Total unsaturated fatty acid intakes were derived by subtracting total saturated fatty acid intake from total fatty acid intake.

Prostate tissue collection

Fresh prostate tissue was obtained from radical prostatectomies performed for clinically localized prostate cancer and from transurethral prostatectomies performed for benign prostatic hyperplasia. The former made available whole prostate gland specimens whereas the latter yielded "prostate chips." Immediately after surgical removal, the prostate gland was placed into sterile saline and delivered to the Department of Pathology. After the gland was assessed for the presence of palpable tumors with use of sterile techniques, ≈2 g fresh, grossly noncancerous prostate tissue was resected from the peripheral zone and fixed according to the method described by Bova et al (14). Nonmalignant tissue was used to estimate nutrient concentrations because concentrations in tumors likely reflect the effects of disease rather than internal exposure (15). The peripheral zone was selected because most prostatic carcinomas originate from this zone (16). In addition, frozen, sectioned histologic controls were obtained from the same areas to ensure that the apparently healthy tissue did not contain cancer. Prostate chips (average aggregate weight: 2 g) were inspected for areas suspicious of containing tumors. Grossly yellow tissue (a marker of the presence of cancerous tissue) or tissue <4 mm in length (increased probability of a microscopic tumor) was not used for the study. Tissue samples were placed into a 2.54-cm (1-in) resealable plastic bag, wrapped in foil, and stored frozen in plastic vials at −70°C until analyzed. Measurements of fatty acids in prostate tissue were made at the US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston.

Biochemical analyses

After the prostate tissue samples were placed into separate test tubes, 2 mL of a chloroform:methanol (1:1, by vol) mixture and 100 μL heptadecanoic acid (400 mg/L) were added to each and the samples were homogenized for 30 s (PowerGen Homogenizer 125; Fisher Scientific, Fair Lawn, NJ). Next, 3 mL of the chloroform: methanol mixture was added and the samples were mixed by vortex for 1 h and then centrifuged for 5 min at 2000 × g at room temperature. After the addition of 2.5 mL chloroform and 1.5 mL water to each, supernates were mixed by vortex for 30 s and centrifuged at 2000 × g for 5 min at room temperature. The lower phase of each was removed and dried under nitrogen and the residue was resuspended in 1 mL benzene and 3 mL cold methanolic hydrogen chloride. Samples were then mixed by vortex for 30 s and incubated for 2 h in a water bath at 70°C. Next, 5 mL 7% NaCl and 0.5 mL hexane were added to the samples, which were mixed by vortex for 30 s. Top layers were removed and dried under nitrogen and the residues were resuspended in 75 μL hexane and injected into a gas chromatograph (model 5890; Hewlett-Packard, Avondale, PA) fitted with a 30-m silica capillary column (AT-WAX; Alltech, State College, PA) with an internal diameter of 0.25 mm (17, 18).

Fatty acid methyl esters of the eluted peaks were identified by using a chromatogram of an authentic mixture of fatty acid methyl esters (NuChek Prep Inc, Elysian, MN) generated under the same conditions of the gas chromatograph. Peaks were integrated with a computer program (CHEMSTATION; Hewlett-Packard) that calculated fatty acid concentrations as a percentage of the total area of the identified fatty acid peaks. This process measured the following fatty acids: 14:0, 16:0, 16:1, 18:1, 18:2, 18:3n−3, 18:3n−6, 20:1, 20:2, 20:3, 20:4, 20:5, 22:3, 22:4, 22:5, and 22:6. Concentrations were totaled for each subject to check whether the combination summed to 100%. CVs ranged from 2% to 22% but generally were <4%.

Statistical analysis

All 65 eligible subjects completed the dietary questionnaire. The questionnaire was repeated in a random 25% sample of the

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TABLE 1
Percentages of fatty acids in the diet and in prostate tissue of US men undergoing prostate surgery[1]

	Total fatty acids		Unsaturated fatty acids[2]	
	Saturated	Unsaturated	Oleic acid	Linoleic acid
		% of total fat		
Diet	35.10 ± 0.66	64.90 ± 0.66	37.11 ± 0.44	16.37 ± 0.64
Prostate tissue	38.94 ± 0.82	61.08 ± 0.82	24.53 ± 0.74	9.16 ± 0.38

[1] $\bar{x}$ ± SE; n = 52. Surgery entailed radical prostatectomy for localized prostate cancer (n = 41) or transurethral prostatectomy for benign prostatic hyperplasia (n = 11); 80% of tissue analyzed was collected from the peripheral zone of the prostate gland.

[2] Unsaturated fatty acids other than oleic and linoleic acids are not measured by the version of the Health Habits and History Questionnaire used in this study (10). Therefore, the percentages shown in columns 3 and 4 do not add up to the total percentage of unsaturated fat reported in column 2.

subjects (n = 15) between 2 and 6 wk later ($\bar{x}$: 3.8 wk) to assess the reproducibility of the nutrient intake estimates. Prostate tissue from 12 subjects could not be analyzed for the following reasons: inoperable cancer detected at the time of surgery (n = 3), diffuse involvement of the gland with tumor cells (n = 1), tissue embedded in paraffin before storage (n = 5), and sample amounts insufficient for biochemical analyses (n = 3). Another subject was found to have fatty acid concentrations in prostate tissue consistently <2.5 SDs from the mean and thus was not included with the rest of the sample. This left the results from 52 subjects available for statistical analysis.

Average daily dietary intakes and concentrations in prostate tissue of total saturated, total unsaturated, oleic, and linoleic fatty acids and their SEs were calculated. Crude intakes were also adjusted for total energy intake by using the method described by Willett and Stampfer (19). Correlations between average daily dietary intake and concentrations in prostate tissue were assessed by using Spearman's rank-order correlation coefficients. CIs were calculated by using the bootstrap methods described by Efron and Tibshirani (20). Four measures of average intake were evaluated separately: g/d, g/d adjusted for total daily energy intake, % of total fatty acids (as g/d), and % of total energy intake. Linear regression was also used to evaluate the association of prostate tissue fatty acid concentrations with age, body mass index (BMI; in kg/m^2), and alcohol intake, but no significant relations were observed.

In the subsample of subjects who repeated the questionnaire, fatty acid (as g/d) and total energy intake estimates obtained at the first visit were plotted against those obtained at the second visit and then compared by using Spearman's rank-order correlation coefficients. Estimates were reproducible: for saturated, unsaturated, oleic, and linoleic fatty acids and total energy, r = 0.86 (P = 0.0065), 0.76 (P = 0.0280), 0.79 (P = 0.0208), 0.91 (P = 0.0020), and 0.86 (P = 0065), respectively; the mean difference in total energy intake was 1146 kJ (P = 0.38).

Bootstrap CI calculations were performed with STATA (version 5.0; Stata Corp, College Station, TX). All other statistical analyses were performed with SAS (version 8; SAS Institute Inc, Cary, NC). P values <0.05 were taken to indicate significance.

RESULTS

The mean (±SD) age, BMI, and total energy intake of the subjects were 66.2 ± 8.9 y, 24.4 ± 3.1, and 8602 ± 3343 kJ/d, respectively. BMI and energy intake closely resembled values reported for similarly aged men in the general US population: 26 and 8824 kJ/d, respectively (21, 22). Of the 52 subjects evaluated, 2 were current smokers and 9 were African American.

Overall, the fatty acid composition of prostate tissue resembled that of the diet (**Table 1**). Expressed as a percentage of total fat, both the rank order and the absolute mean concentrations of saturated, unsaturated, oleic, and linoleic fatty acids measured in the diet and in prostate tissue appeared to be similar. The ratios of total saturated to unsaturated fatty acids were 0.64 and 0.54 in the diet and prostate tissue, respectively. Although oleic and linoleic acids combined represented 82% of the unsaturated fatty acid reportedly consumed, they accounted for only 55% of the unsaturated fatty acids measured in prostate tissue. This result was due mainly to the larger difference in linoleic acid concentrations (16.41% in the diet compared with 9.05% in prostate tissue).

Spearman's rank-order correlations between fatty acid concentrations in prostate tissue and the 4 measures of self-reported habitual dietary intake are presented in **Table 2**. Concentrations of linoleic acid in prostate tissue were significantly correlated with reported intake expressed as g/d adjusted for total energy, % of total fat (as g/d), and % of total energy. Scatter plots of linoleic acid concentrations in prostate tissue versus each measure of dietary intake (**Figure 1**) did not suggest that the correlations were unduly influenced by any one observation.

Saturated, unsaturated, and oleic fatty acids in prostate tissue did not correlate with self-reported usual dietary intake, irrespective of the intake measure. Furthermore, none of the prostate tissue concentrations were predicted by the intake frequencies of any of the food groups included in the questionnaire.

DISCUSSION

The evidence linking fatty acids to risk of prostate cancer is largely based on ecologic measures of exposure obtained at the population level and historical measures in individuals evaluated in relation to patterns of prostate cancer incidence and mortality (1, 23–25). Physiologic markers of long-term fatty acid exposure that are also suitable for routine epidemiologic investigation have yet to be fully developed. Current methods, such as tissue aspiration or biopsy, are cumbersome for both subjects and investigators and are susceptible to sampling errors (26). Consequently, indirect measurements based on reported consumption frequencies remain the standard for assessing the effect of fatty acids on human prostate carcinogenesis.

Our data suggest what at first appears to be a paradox: although the fatty acid composition of the diet closely resembled that of prostate tissue at the group level, the ability of self-reported usual dietary intake to correctly rank prostate tissue exposure at the level of the individual was generally poor. This is an important technical consideration in terms of study design. Our findings suggest that historical measurements based on semiquantitative food-frequency

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TABLE 2
Spearman's rank-order correlations between concentrations of fatty acids in prostate tissue as a percentage of total fat and 4 measures of daily dietary intake in US men undergoing prostate surgery[1]

Fatty acids in prostate tissue	Daily dietary intake			
	(g/d)	(g/d adjusted for total energy)[2]	[% of total fat (as g/d)]	(% of total energy)
Saturated	0.19 (−0.09, 0.47)	0.21 (−0.06, 0.45)	0.20 (−0.05, 0.48)	0.26 (−0.02, 0.50)
Unsaturated	−0.15 (−0.42, 0.12)	−0.13 (−0.37, 0.17)	0.21 (−0.05, 0.48)	−0.07 (−0.34, 0.22)
Oleic acid	0.01 (−0.26, 0.30)	0.05 (−0.26, 0.31)	−0.02 (−0.31, 0.27)	0.04 (−0.26, 0.29)
Linoleic acid	0.20 (−0.06, 0.43)	0.29 (0.03, 0.49)[3]	0.36 (0.14, 0.55)[4]	0.28 (0.04, 0.49)[5]

[1] 95% CIs in parentheses; $n = 52$. Surgery entailed radical prostatectomy for localized prostate cancer ($n = 41$) or transurethral prostatectomy for benign prostatic hyperplasia ($n = 11$); 80% of tissue analyzed was collected from the peripheral zone of the prostate.
[2] According to the method of Willett and Stampfer (19).
[3] $P = 0.034$.
[4] $P = 0.008$.
[5] $P = 0.042$.

data would be satisfactory ecologic indicators of exposure. However, if the goal is to relate individual-level fatty acid exposure to individual-level characteristics such as prostate cancer grade, stage, or risk of cancer progression, historical measurements pose formidable methodologic challenges. With use of current statistical approaches, repeated measures conducted in very large samples would be necessary to assess these effects, especially interactions with genetic factors (27).

Measurements of fatty acid concentrations in prostate tissue could provide a physiologic alternative to assessment of long-term exposure. The underlying assumption that concentrations in prostate tissue reflect long-term exposure is reasonable because the half-life of linoleic acid is ≈680 d in subcutaneous adipose tissue (28). Conceptually, it is important to distinguish 2 overlapping concerns when interpreting data such as those presented here: how well diet is measured and how well dietary fatty acids correlate with tissue fatty acid concentrations. This study is concerned primarily with the latter. Actual dietary intake may indeed predict subject-level exposure, but self-reported dietary intake as measured with the semiquantitative instrument used in this study did not.

Although fatty acid concentrations in prostate tissue tended not to correlate with reported intake, linoleic acid appeared to be an exception. Concentrations of linoleic acid in prostate tissue

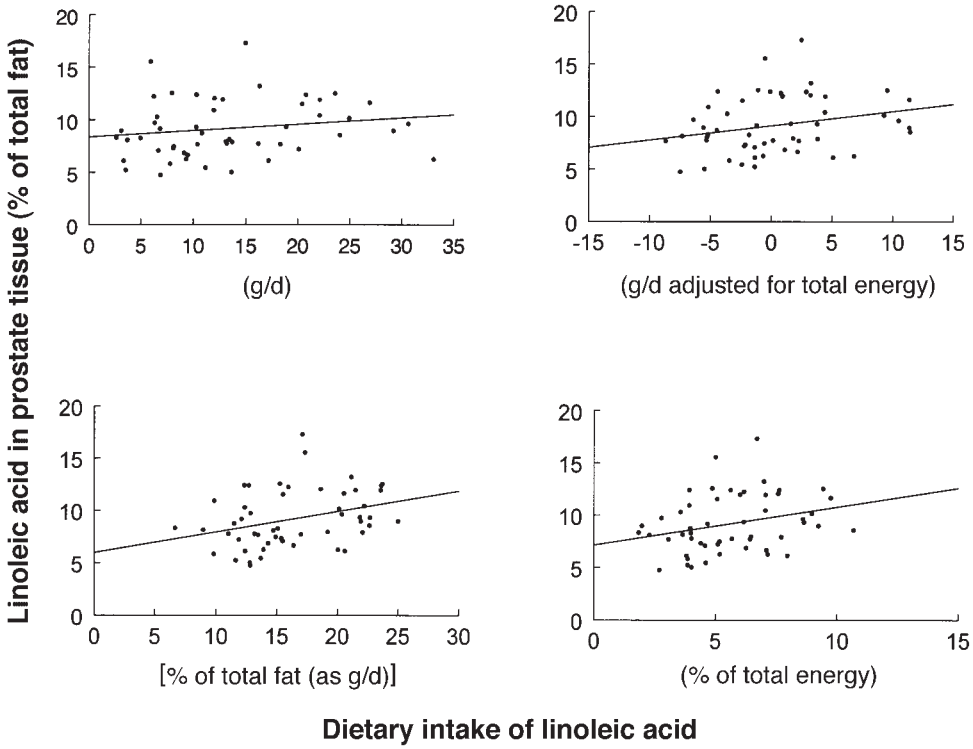

FIGURE 1. Linoleic acid concentrations in prostate tissue (as a percentage of total fat) versus 4 measures of corresponding dietary intakes as assessed with a 122-item version of the Health Habits and History Questionnaire (10). Energy-adjusted intakes were calculated according to the method described by Willett and Stampfer (19). This method involves comparing observed values with expected values predicted by using a linear regression model and results in negative values for some energy-adjusted intakes. Spearman's rank-order correlation coefficients were significant ($r = 0.28$–0.36, $P = 0.042$–0.008).

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were significantly correlated for 3 of the 4 intake measures evaluated, suggesting that self-reported usual dietary intake correctly classified prostatic exposure. This finding may be a consequence of linoleic acid's being an essential fatty acid. Hence, exposure could be due only to exogenous sources measured by the questionnaire rather than to endogenous sources that the questionnaire cannot assess. Linoleic acid may be a particularly relevant fatty acid to assess because data now suggest that linoleic acid may influence the development and course of prostate cancer through the immune system (29–30).

To our knowledge, other estimates of the correlation between reported intake and fatty acid concentrations in prostate tissue with which our data can be compared are not available. However, the relation between the fatty acid composition of subcutaneous adipose tissue and that of the diet has been examined and may provide an informative comparison (28, 31–36). Polyunsaturated fatty acids often showed the best correlations, presumably because many are derived primarily from exogenous sources, whereas saturated and monounsaturated fats can be synthesized endogenously (37). In a study of 118 healthy US male health professionals that used a 131-item FFQ developed by Willett (38), Hunter et al (36) reported Spearman's rank-order correlations between polyunsaturated fat intake (as a percentage of total fat intake) and subcutaneous adipose tissue concentrations of 0.50 ($P = 0.0001$). Linoleic acid was also significantly correlated ($r = 0.48$, $P = 0.0001$), whereas the correlation for saturated fat was at the threshold of statistical significance ($r = 0.18$, $P = 0.05$). Our findings for prostate tissue are generally consistent with these observations.

This study has several limitations. Interindividual differences in intestinal absorption, metabolism, uptake, and turnover at the tissue level and other physiologic factors may impede greater correlation between prostate tissue and self-reported dietary intake. Methodologic error could have also obscured an association between reported intake and fatty acid concentrations in prostate tissue. In this category, variations in interviewer technique are a potential source of error. The HHHQ is designed to be self-administered (21). The role of the interviewer in our study was to assist the subjects in reporting portion sizes through the use of food models, to pace completion of the questionnaire, and to answer questions about the questionnaire's content. In this context, the effect of interviewer technique on the accuracy of self-report is probably limited. Furthermore, use of a single interviewer likely minimized variation in administration.

Another potential source of methodologic error is variation in tissue sampling. Seventy-five percent of glandular tissue in the prostrate is localized in the peripheral zone, the largest of 3 concentric zones (16). Variation in fatty acid composition between zones is plausible. We addressed this by limiting our analysis to tissue collected from the peripheral zone when possible. This resulted in ≈80% of the tissue analyzed originating from a single anatomical zone. Within-zone variability was not directly assessed.

Inaccurate self-report of usual dietary intake is, perhaps, a more likely source of error, but the evidence of this is circumstantial. Studies of the accuracy of questionnaire measurements of habitual intake are typically based on a comparison with repeated daily weighed-food consumption records or 24-h recalls as reference measurements (39). When compared with biochemical markers, self-reported usual intake tends to be less correlated than the reference measurements (40–42). Participants of validation studies may also tend to report intake more accurately than do nonparticipants (43). Most importantly, the cross-sectional design of this study did not permit verification of the 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 these estimates agreed with actual intake was not assessed.

In conclusion, our data suggest that self-reported usual dietary intake of fatty acids is a marker of exposure at the group level but does not correctly rank individuals with respect to the intensity of prostatic exposure. Linoleic acid, an essential fatty acid, appears to be an exception: in this study, reported intake was significantly correlated with concentrations in prostate tissue. These data are unique in that they relate reported amounts of fatty acids in the diet to the fatty acid composition of prostatic epithelium and illustrate the feasibility of conducting fatty acid exposure assessment at the level of the prostate gland. Physiologic measures like these may be necessary to more adequately assess the effect of various fatty acids, especially the endogenously synthesized (nonessential) fatty acids, on the risk and prognosis of prostate cancer in individuals.

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