Fatty Acid Composition of Red Blood Cell Membranes and Risk of Squamous Cell Carcinoma of the Skin

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Abstract

Differential effects of fatty acids on carcinogenesis suggest that fatty acid composition is important in tumor development. Arachidonic acid and its metabolites elicit inflammation and promote tumor formation in mouse skin. Inhibitors of the arachidonic cascade inhibit tumor incidence. A population-based case control study in Southeastern Arizona tested the hypothesis that lower levels of arachidonic acid in RBC membranes were associated with decreased risk of skin squamous cell carcinoma (SCC; n = 335 SCC cases and 321 controls). Extracted and esterified RBC fatty acids were analyzed using capillary gas chromatography. Individual peaks for 14 fatty acids were measured as a percentage of total fatty acids. Logistic regression was used to estimate odds ratios (OR), adjusting for SCC risk factors (age, gender, actinic keratosis history, freckling, and tanning ability). Increased levels of arachidonic acid in RBC membranes were associated with increased risk of SCC [odds ratio (OR), 1.08 per mg/100 mL change; 95% confidence interval (95% CI), 1.02-1.15] and this association remained when controls with actinic keratosis precursor lesions were excluded. SCC risk was highest among the upper quartile of arachidonic acid (OR, 2.38; 95% CI, 1.37-4.12). In contrast, increasing proportions of palmitic acid (OR, 0.94; 95% CI, 0.89-1.00) and palmitoleicacid (OR, 0.49; 95% CI, 0.30-0.81) were associated with reduced SCC risk. More studies are needed to elucidate the function of RBC fatty acids so that recommendations can be made to alter the human diet for cancer prevention. (Cancer Epidemiol Biomarkers Prev 2005;14(4):906–12)

Introduction

Skin cancers are the most prevalent malignancy, with nonmelanoma skin cancers accounting for more than one third of all cancers in the United States. Basal cell and squamous cell carcinomas (SCC) comprise the major histologic types of nonmelanoma skin cancers. The incidence of these skin cancers has increased (1, 2), with the rates in Arizona among the highest in the world (3). Whereas SCC is not usually associated with mortality, these cancers are associated with increased health care burden and morbidity (4, 5). Furthermore, increases in cancer mortality and incidence of other invasive cancers following SCC have been reported (6-8).

The major constitutional risk factors for all skin cancers seem to be skin color and the skin response to strong sunlight (9, 10). Sunlight is considered the main etiologic factor for SCC of the skin with wavelengths in the UV light B spectrum (UV-B, wavelengths between 280 and 320 nm) most associated with SCC risk (11). Although geographic comparisons of SCC data suggest that differences in UV-B exposure may account for some of the differences in SCC incidence, preventive agents, or enhancing factors may also be important (12).

Whereas numerous animal studies indicate that high levels of dietary fat reduce the time between UV exposure and tumor appearance, increase the number of tumors, and affect the promotional stage of UV carcinogenesis (13-16), there have been few human studies. An intervention study by Black et al. showed a reduction in the occurrence of actinic keratoses and nonmelanoma skin cancers in participants randomized to an isocaloric low-fat diet (17-19). We previously noted that participants consuming higher percentages of dietary energy from fat seemed at increased risk for skin SCC (20). In this same study, reduced risk of cutaneous SCC was associated with higher dietary intakes of $n-3$ fatty acids and with higher ratios of n-3 to n-6 fatty acids (20).

The fatty acid profile of the erythrocyte membrane reflects dietary macronutrient intake and the interactions between dietary intake and endocrine changes (21, 22). Furthermore, in part due to the long half-life (120 days) of erythrocytes, these fatty acids may be more than mere biomarkers of dietary fat intake and are appropriate for investigating the relations of the patterns of fatty acid metabolism to skin cancer risk.

A population-based case control study in Southeastern Arizona was designed to test the hypothesis that lower levels of arachidonic acid in erythrocyte membranes were associated with reduced risk of SCC of the skin. In the present study, we also investigated the associations between various other erythrocyte membrane fatty acids and risk of skin SCC.

Materials and Methods

Eligibility and Recruitment. Cases were eligible if they were ≥30 years of age, no prior history of a skin cancer, and had a histopathologically confirmed, nonmetastatic squamous cell cancer (SCC) of the skin diagnosed within 4 months of contact. All cases were randomly selected from the Southeastern Arizona Skin Cancer Registry to reflect the age and gender distribution of SCC within the registry (3). Only non-Hispanic and Hispanic White cases were eligible. Upon identification of a potential case, a letter was sent to the case's personal physician for permission to contact the person regarding study participation. Once the physician granted permission, a letter describing the study was sent to the case. Approximately 2 weeks after mailing of the letter, the study interviewer called the potential case to determine eligibility, invite participation, and schedule the interview.

Controls were selected using a random digit dialing technique, basing the randomly generated numbers from the

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first four digits of the cases' residential phone numbers. Phone numbers were dialed until resolved as nonworking, nonresidential, or residential. Nonresidential and nonworking numbers were excluded. At least six attempts were made to interview a household member; if no eligible person was identified, a new phone number was selected. Controls were frequency matched to the cases by age category and gender with one control per household invited to participate using modified Waksberg criteria (23). Control subjects were eligible if they had no prior history of skin cancer, lived within southeastern Arizona, and were within the age, gender, and ethnicity grouping.

A total of 731 cases were interviewed for eligibility and interest. Of the 531 eligible cases, 404 (76%) completed the baseline interview and donated a blood sample. To identify the controls, over 3,384 telephone numbers were called and 1,641 individuals interviewed for interest and eligibility. Only 48% were deemed eligible and 391 of those persons participated. The institutional review board of the University of Arizona reviewed and approved all study procedures.

Assessment of Exposures. All subjects completed an extensive interview for behavioral and past UV exposure information and donated a blood sample. The given questionnaire sought information on personal characteristics, past and present residential history, occupational history, history of sunburns, use of sunscreens, medical history, use of medications, and behavioral information (smoking and alcohol use and physical activity).

Skin characteristics were measured through questions asking about reaction to sun exposure. Two questions evaluated ability of the skin to tan (coded as never tan, mildly tan, moderately tan, tan deeply, or very brown) and initial reaction of unexposed skin to the sun (coded as always burn, usually burn, burn moderately, burn minimally, and rarely or never burn). Past skin reaction to the sun was assessed by asking about number of blistering and peeling sunburns at various ages. Mole and freckling patterns were sought for various time periods. The study interviewer counted the current numbers of large (defined as greater than the diameter of a pencil eraser or 5 mm) freckles and moles on the arms of the subject.

Trained interviewers used a standard questionnaire to conduct the interviews at either the home or the clinic. One interviewer conducted over 90% of the personal interviews. All questionnaires were reviewed for completeness and coding. Data were compiled using screen-based entry programs that included range checks. Duplicate entry of a random 10% sample of the information indicated <1% transcription error. In addition, quality checks were done to assess missing values, extreme values, and incorrect coding.

RBC Fatty Acids. The method of Ferrante and Thong was used to separate WBC and RBC (24). Briefly, 7 mL of fresh human blood were layered on Hypaque-Ficoll solution (density = 1.114 g/mL) in 14-mL polypropylene conical tubes and then centrifuged at 200 × *g* for 30 minutes at room temperature. RBC samples were washed with isotonic saline solution and frozen at −80°C until the time of extraction. Fatty acid composition of RBC membranes was assessed using capillary gas chromatography. Fatty acids were extracted and esterified using methods of Rose and Oklander (25). The methylesters were quantified in mg/100 mL by capillary gas chromatography using methods of Alexander and Justice (26). Each injection fraction was adjusted with hexane to be between 1 and 0.50 mg/mL depending upon the quantity of fatty acid in the sample using a 100-m column (Supelco Sp-2380). Both flame and ionization detector and injection port temperatures were set at 250°F and the flow rate of 0.463 mL/min. A complete run cycle was 93 minutes and the methylesters quantified in mg/100 mL by capillary gas chromatography using a 100-m column. Peaks were integrated by computer and identified by comparison of the absolute and relative retention times of 20 fatty acids in an authentic standard (NuCheck Prep, Inc., Elysian, MN). The standard was changed daily and palmitic and arachidonic acids were used as retention time reference points in each run. Duplicate samples collected from 30 subjects indicated no differences in mean levels of any of the fatty acids assessed. Manual reinjection, which optimized recovery, was completed for a small proportion of samples in which the total recovery area was less than the established minimum.

Fatty acid analyses were done by two laboratories during the study, using the same procedures, chromatographic conditions, and internal standards. Comparisons between laboratories showed both laboratories consistently analyzed for 14 fatty acids, with the 14 fatty acids accounting for 86% to 90% of the total fatty acids recovered. Individual fatty acid values were standardized by the total recovery of the 14 fatty acids for that individual and these standardized values used in the subsequent comparisons between cases and controls.

Statistical Analyses. Means or proportions of various exposures were calculated for cases and controls. Statistical significance tests included the χ^2 statistic for categorical variables, the Student's t test for normally distributed continuous variables, and the Wilcoxon rank sum test for those values that were not normally distributed. All statistical tests were two sided.

The mean relative percent was calculated for each of the 14 fatty acids for case and control subjects. In addition to the individual 14 fatty acids, groupings of fatty acids were calculated, specifically the ratio of $n3$ fatty acids to $n6$ fatty acids ($n3/n6$) and the polyunsaturated fatty acids to saturated fatty acids ratio (P/S ratio). Calculations included both the unadjusted means and mean levels adjusted for age, gender, and laboratory using the method of least squares.

Logistic regression was used to calculate the odds ratio (OR) and 95% confidence intervals (95% CI) as an estimate of the strength of the association between the fatty acid and SCC risk. In analyses, fatty acid levels were treated as continuous variables and as categorical variables, with the quartile distribution of each fatty acid in the control group used to categorize the population. For those fatty acids that had very small values (e.g., *trans*-linoleic fatty acid), the population was dichotomized by whether the sample contained a detectable amount of the specific fatty acid or not. The referent category for the categorical variables was the lowest quartile. Trends were tested by inclusion of the continuous variable in the logistic regression models.

Independence of the associations was assessed with inclusion of potential confounding variables. Age, gender, and lab were considered probable confounders because of the study design. Tanning ability with chronic exposure to the sun, current freckles on the arm, and history of actinic keratoses were also included because prior analyses indicated these variables were strong, independent risk factors for SCC of the skin. Because actinic keratoses are probable precursors for SCC of the skin (27) and controls with an actinic keratosis might be considered as potentially misclassified as not having disease, separate analyses excluded controls with an actinic keratosis history. Use of nonsteroidal anti-inflammatory drugs (NSAIDS) was also evaluated as a potential confounder and effect modifier, by inclusion of interaction variables in the models and by stratifying the population by NSAID use and nonuse.

Results

From January 1993 through November 1996, 404 cases with a recent and first diagnosis of SCC and 391 control subjects with no history of skin cancer were interviewed. Adequate fatty acid analyses were available for 321 control subjects (82%) and

336 (83%) cases. There were no statistically significant differences for any of the demographic or skin characteristics between those subjects for whom fatty acid analyses were available (n = 657) and those not available (n = 138).

Table 1 presents selected characteristics of the sampled SCC cases and controls. Despite use of frequency matching, controls were somewhat younger than cases. Over 97% of both cases and controls were non-Hispanic White of European descent (data not shown) and 66% of the population had some college education. Cases were more likely, however, to report more freckling, inability to tan, usually burning when exposed to the sun, and history of a prior actinic keratosis.

The mean values for the individual RBC fatty acids and fatty acid groups for cases and controls are presented in Table 2. Mean levels are the mg/100 mL per total recovery of the 14 fatty acids assessed and are adjusted for age, gender, and lab. Cases had marginally lower RBC levels of palmitic acid (16:0) compared with controls but did not differ in the level of the other primary saturated fatty acids, stearic acid (18:0). Of the four monounsaturated fatty acids, only palmitoleic acid (16:1n-7) was lower among cases compared with controls (0.646 versus 0.725, $P < 0.011$). The mean mg/100 mL of arachidonic acid (20:4n6) was higher for cases than controls (15.30 mg/100 mL for cases compared with 14.60 mg/100 mL for controls, P = 0.005). In addition, SCC cases had marginally lower RBC levels of *trans*-linoleic acid (18:2n6t) and a greater polyunsaturated to saturated fatty acid ratio.

The adjusted odds ratios for the relationship between RBC fatty acids and SCC are also shown in Table 2 with the fatty acids modeled as continuous variables. The saturated RBC fatty acid, palmitic acid (16:0), is shown to be significantly associated with a decreased risk of SCC (OR, 0.91 per 1 mg unit change; 95% CI, 0.85-0.97). SCC risk is also reduced with higher levels of monounsaturated fatty acids, palmitoleic (16:1n-7) and vacceinic acid (18:1n7c). In contrast to the previous fatty acids, higher RBC membrane arachidonic acid levels are significantly associated with an increased risk of SCC of the skin (OR, 1.11 per 1 mg unit change; 95% CI, 1.04-1.18).

Table 3 presents a second approach to analyzing the association of RBC membrane fatty acid profile and risk of skin SCC. The quartile distribution of each fatty acids in the control group was used to categorize the fatty acid levels for all study subjects. The relationship between the membrane fatty acid levels and SCC was then evaluated without assuming a linear relationship across the distribution of the fatty acid (OR1). For those fatty acids in which levels of >25% of the control group fell below the detectable level, the distribution of the fatty acids was dichotomized as whether there was a reportable or detectable amount. For each fatty acid and fatty acid group, analyses were also completed excluding the 94 controls with an actinic keratosis history, a probable confounder in SCC risk (OR2). As seen in the table, arachidonic acid was associated with increased risk of SCC of the skin; however, the risk was most pronounced, at the extreme end of the distribution. It was also more evident when those controls with an actinic keratosis history were excluded. Cases were 2.38 times (95% CI, 1.37-4.12) more likely to have arachidonic acid levels above 16.60 mg/100 mL than were controls without a history of actinic keratosis. A higher P/S ratio was also associated with increased ORs, although the trend was not linear. In contrast to arachidonic acid and the P/S ratio, palmitic and palmitoleic fatty acids seemed protective. The OR for palmitic acid levels above the 75th percentile compared with the lowest quartile was 0.34 (95% CI, 0.18-0.64), with a statistically significant trend (P = 0.003). Higher levels of vacceinic acid was also associated with reduced risk of skin SCC, although the increased levels in the highest quartile are not associated with further reduction in risk. A detectable level of *trans*-linoleic acid was associated with a decreased risk of SCC, but this relationship was no longer evident once control subjects with an actinic keratosis history were excluded from the analyses.

Current use of NSAIDs, known inhibitors of prostaglandins and arachidonic acid metabolism (28), was evaluated as a potential effect modifier of the relationship between membrane fatty acid profile and skin SCC risk. Analyses were completed for all cases and all controls (OR1) and for all cases and those controls without a history of the lesions (actinic keratosis) potentially representing early disease (OR2). As evident in Table 4, the association between arachidonic acid levels and risk of SCC are most evident and consistent among those not using NSAIDs. Furthermore, the relationship is stronger when controls with potential precursor lesions are excluded.

Discussion

In the present case control study of incident SCC of the skin, alterations in the erythrocyte membrane fatty acid profile were associated with skin cancer risk. Reduction in skin cancer risk was associated with higher levels of one of the primary saturated fatty acids, palmitic acid. Levels of arachidonic acid, a polyunsaturated fatty acid, were significantly greater among SCC cases compared with controls. Consistent with findings of the differential effects of these two types of fatty acids, individuals with a first skin SCC had an increased ratio of polyunsaturated to saturated fatty acids in erythrocyte membranes compared with noncancer-affected controls.

The associations of RBC membrane fatty acid profile with SCC of the skin are consistent with findings using animal

Table 1. Selected characteristics of skin SCC cases and controls

Variable	Controls, n (%)	Cases, n (%)	P*
Gender			
Females	132 (41)	129 (38)	0.475
Males	189 (59)	207 (62)	
Age group (y)			
<49	50 (16)	30 (9)	0.035
50-59	38 (12)	38 (11)	
60-69	113 (35)	110 (33)	
70-79	94 (29)	129 (38)	
>79	26 (8)	29 (9)	
Education			
<High school	27 (8)	30 (9)	0.965
High school	83 (26)	85 (25)	
Some college or higher	210 (65)	221 (66)	
Smoking			
Never	116 (36)	112 (33)	0.050
Former	146 (45)	181 (54)	
Current	59 (18)	43 (13)	
Actinic keratosis history			
No	227 (71)	85 (25)	<0.001
Yes	94 (29)	251 (75)	
Freckles number on arm			
None	24 (7)	5 (1)	<0.001
<50	270 (84)	263 (78)	
≥50	27 (8)	68 (20)	
Tanning ability			
Never	2 (1)	14 (4)	<0.001
Mild	50 (16)	86 (26)	
Moderate	173 (54)	188 (56)	
Deep	94 (29)	48 (14)	
Reaction of skin to first sun exposure			
Always burn	18 (5.6)	31 (9.2)	<0.001
Usually burn	46 (14.4)	67 (19.9)	
Moderate burn	101 (31.7)	137 (40.8)	
Minimal burn	105 (32.9)	75 (22.3)	
Rarely/never burn	49 (15.4)	26 (7.7)	
Current use of NSAIDs	31 (9.2)	35 (10.9)	0.746

*P of comparison in factors between cases and controls.

Table 2. Relative percent content of various fatty acids in RBC membranes of skin SCC cases and controls

Fatty acid	Cases, n = 336, mean (SE)*	Controls, n = 321, mean (SE)*	$P^{\dagger}$	OR‡ (95% CI)
Saturated fatty acids				
16:0 (palmitic)	26.799 (.172)	27.243 (.172)	0.063	0.94 (0.89-1.00)
18:0 (stearic)	17.453 (.147)	17.410 (.147)	0.830	1.02 (0.95-1.09)
Monounsaturated fatty acids				
16:1*n*-7 (palmitoleic)	0.646 (0.022)	0.725 (0.023)	0.011	0.49 (0.30-0.81)
18:1*n*-7c (vacceinic)	0.715 (0.021)	0.750 (0.021)	0.217	0.67 (0.41-1.10)
18:1*n*-9c (oleic-*cis*)	17.798 (0.105)	18.001 (0.106)	0.160	0.96 (0.87-1.06)
18:1*n*-9t (oleic-*trans*)	1.326 (0.039)	1.375 (0.039)	0.361	0.86 (0.67-1.12)
Polyunsaturated fatty acids				
18:2*n*-6c (linoleic-*cis*)	13.073 (0.106)	13.034 (0.106)	0.791	1.01 (0.92-1.12)
18:2*n*-6t (linoleic-*trans*)	0.293 (0.017)	0.336 (0.017)	0.063	0.44 (0.24-0.82)
18:3*n*-3 (linolenic)	0.289 (0.020)	0.304 (0.020)	0.591	0.79 (0.46-1.36)
20:2*n*-9 (eicosadenoic)	0.479 (0.027)	0.476 (0.028)	0.941	1.02 (0.70-1.49)
20:3*n*-9 (eicosatrienoic)	1.786 (0.044)	1.742 (0.044)	0.465	1.04 (0.83-1.31)
20:4*n*-6 (arachidonic)	15.301 (0.171)	14.644 (0.171)	0.005	1.08 (1.02-1.15)
Long-chain *N*-3 polyunsaturated fatty acids				
20:5*n*-3 (eicosapentaenoic)	0.601 (0.030)	0.606 (0.030)	0.904	0.97 (0.68-1.37)
22:6*n*-3 (docosahexaenoic)	3.441 (0.060)	3.355 (0.060)	0.301	1.12 (0.95-1.33)
N3/N6 ratio	0.1511 (0.002)	0.1518 (0.002)	0.840	4.57 (1.27-16.49)
P/S ratio	0.8076 (0.008)	0.7846 (0.008)	0.046	3.00 (0.88-10.18)

*Relative % mean and SE, standardized as weight percentages of the total 14 fatty acids extracted; adjusted for age, sex, lab.
†P for t test of difference between adjusted means for cases and controls.
‡OR and 95% CI adjusted for age, sex, lab, freckles on arm, ability to tan, and history of actinic keratosis.

models of photocarcinogenesis. Rodent models of skin cancer provide some of the earliest evidence that dietary fat acts as a tumor promoter (13, 29) with higher levels leading to increases in tumor incidence (14, 29, 30). Specific types of fat exert differential carcinogenic effects in rodent models (15, 31-33), with the greatest response, including accelerated time to tumor appearance and increases in tumor burden, associated with higher dietary intakes of polyunsaturated fatty acids (32, 34, 35).

With the ability to act as both an initiator and as a promoter, UV radiation is a complete carcinogen. UV radiation lowers cell-mediated immunity by increasing suppressor T cells. Sunburn is the inflammatory response of cells to UV-B-induced damage. Dietary fat composition modifies cellular immunity affecting cell membrane fluidity and integrity, which in turn alters inflammatory response (16). Arachidonic acid, necessary for eicosanoid production providing prostaglandins, thromboxanes, and leukotrienes, mediates the inflammatory response (36). These eicosanoids act locally as hormones, including responding to damage such as that caused by UV radiation (37).

Although studies in rodent models of skin carcinogenesis have considered potential effects of dietary fat and other nutritional factors, data of trials among humans are limited. Several observational studies indicate no effect of dietary fat (38, 39), but these studies may not be ideally designed to discriminate expected effects. Nonmelanoma skin cancer (NMSC) is not generally included in cancer registries in the United States; hence, studies rely on validity of self-report including the distinction between preoperative diagnoses and pathologic verification of the various types of nonmelanoma lesions. Additionally, dietary assessments vary in the ability to distinguish types of dietary fat and in applicability to provide more than generalized ranges of intake. In a previous study, we reported an increased risk of cutaneous SCC with the percent energy received from dietary fat assessed using recall methodology (20). No observational studies employing diet history assessments report significant associations of dietary fat intake and SCC skin cancer.

One human intervention study, however, showed a decreasing incidence of NMSC and actinic keratosis with decreasing fat intake. In this study, the effect of an isocalorically reduced-fat diet on occurrence of actinic keratosis and NMSC was studied in a 2-year clinical intervention trial (17). The cumulative numbers of new actinic keratosis per patient as well as the incidence of NMSC in the dietary intervention group were significantly lower than those in the control group. Because animal evidence indicated lower total energy intake was associated with reductions in carcinogenesis (40), the study sought to ensure energy intake did not differ between the control and intervention groups by balancing the 47% reduction in percent of calories from fat with a 36% increase in carbohydrate intake among the intervention group. The successful intervention to reduce dietary fat from 39% to 21% also resulted in a small but significant change in the ratio of polyunsaturated to saturated fat intake. The reduction in saturated fat intake was greater than the reduction in polyunsaturated fatty acid intake, thereby increasing the P/S ratio by the end of the 2-year study. Both the animal data and the present study indicate that a greater proportion of fat calories derived from polyunsaturated fatty acid sources increases actinic keratosis and NMSC risk. In addition to the carcinogenic effect of higher levels of arachidonic acid, saturated fatty acids independently alter actinic keratosis and NMSC risk.

Most human diets contain a variety of saturated fatty acids of different chain lengths. The major saturated fatty acid in the diet is palmitic acid (C16:0), followed by stearic acid (C18:0). Palmitic acid is found in all edible fats and oils and is particularly abundant in palm oil and in butter, milk, cheese, and meats (41). In the current study, a significant reduction in skin cancer risk was associated with increasing levels of palmitic acid in erythrocyte membranes. A similar reduction in risk was also noted for palmitoleic acid. Palmitoleic acid (16:1) is a minor monounsaturated fatty acid in the diet and is principally derived from the desaturation of palmitic acid (16:0). The relation between palmitoleic acid and skin SCC that we have observed may, in part, reflect the absorption, synthesis, and metabolism of palmitic acid (42).

As suggested by the palmitic and palmitoleic relationship, the lipid composition of erythrocyte membranes is more than a direct reflection of dietary fatty acid intake. Membrane lipids reflect the balance of dietary carbohydrates and fats. This membrane property is well known and shown in studies like the one by Farquhar and Ahrens (22). A fat-free, carbohydrate-rich diet fed to adult subjects for 4 to 11 weeks resulted in a significant increase in C16:0, C16:1, C18:0, and C18:1, the fatty

acid products of lipogenesis (22). Because the *n*-7 and *n*-9 unsaturated fatty acids can be synthesized from endogenous precursors, the concentrations maintained in tissues and RBC may have little use for interpreting dietary exposure (21, 43). If intake of carbohydrate is very high and the intake of fat is very low, however, little new unsaturated fatty acid is provided by the carbohydrate-rich diet, and elevated insulin concentrations suppress movement of fatty acids from adipose tissue to liver. In the absence of an influx of unsaturated fatty acids, the liver rapidly converts carbohydrate into fatty acids, introducing unusually high transient concentrations of palmitoleic (16:1n-7) and its elongation product vacceinic acid (18:1n-7) into glycerolipids (44).

In the current study, the association of RBC lipid composition with skin cancer risk significantly increases once volunteers with actinic keratoses or the precursor lesions of the cancer of interest, are excluded in the analyses. Almost 30% of the selected ''controls'' had a history of these precursor lesions. As actinic keratoses are in the continuum of skin SCC, exclusion of individuals with these lesions represents a more appropriate control selection, reflecting a more ''true'' comparison of cases with SCC compared with controls with no evidence of skin carcinoma (i.e., no precursor lesion or likely misclassification). The reduction of SCC risk with increasing levels of unsaturated fatty acids (palmitic, palmitoleic, and vacceinic) and increased SCC risk with greater arachidonic and P/S ratio were substantially enhanced in analyses in which control subjects with actinic keratosis lesions were excluded.

NSAID, including aspirin, have long been used to control sunburn pain and inflammation. Inflammation is a characteristic response to exposure to UV-B and mediators of this response are the arachidonic acid metabolites (37, 45). Laboratory and animal studies provide evidence that NSAIDs inhibit prostaglandin biosynthesis and, in particular, inhibit the enzymes cyclooxygenase-1 (COX-1) and COX-2 associated with inflammatory responses (28, 46-49). Approximately 10% of the current study population reported regular use of NSAIDs. Although users did not compose a large proportion of the study population, we were presented with an opportunity to further investigate the hypothesis that the increased risk of SCC associated with higher RBC membrane concentrations of arachidonic acid was due to the role of arachidonic acid in inflammation. Overall, increasing levels of arachidonic acid augmented SCC risk. The association of arachidonic acid levels with SCC risk was enhanced among subjects not taking NSAIDs. Additionally, increasing levels of RBC membrane arachidonic acid was not associated with SCC risk among non-NSAID users providing evidence that the mechanism by which arachidonic acid increases SCC development is through the inflammatory response to UV radiation.

Some limitations and strengths of the study deserve consideration. As in all case control studies, there remain issues of bias and its effect on study findings. Differential participation or recall of history between cases and controls could lead to biased estimates of effect. Two areas of concern

Table 3. Comparison between ORs of skin SCC and quartiles of RBC fatty acids among participants in the Southeastern Arizona Health Study by inclusion of all controls (OR1) and only those without history of prior actinic keratoses (OR2)

		Level of fatty acid*				*P* for trend
		1st quartile	2nd quartile	3rd quartile	4th quartile	
Saturated fatty acids						
16:00	OR1	1.00	0.59 (0.36-0.96)	0.68 (0.42-1.10)	0.46 (0.26-0.82)	0.003
	OR2	1.00	0.92 (0.56-1.51)	0.61 (0.36-1.04)	0.34 (0.18-0.64)	
18:00	OR1	1.00	1.01 (0.51-1.99)	1.04 (0.51-2.12)	0.96 (0.45-2.03)	0.311
	OR2	1.00	1.05 (0.52-2.14)	1.13 (0.54-2.38)	1.20 (0.55-2.65)	
Monounsaturated fatty acids						
16:1*n*-7	OR1	1.00	0.82 (0.49-1.36)	0.70 (0.42-1.18)	0.51 (0.30-0.89)	0.066
	OR2	1.00	1.03(0.60-1.76)	0.84 (0.48-1.46)	0.78 (0.44-1.39)	
18:1*n*-7c	OR1	1.00	0.67 (0.33-1.35)	0.34 (0.15-0.79)	0.34 (0.14-0.78)	0.042
	OR2	1.00	0.67 (0.33-1.36)	0.32 (0.14-0.76)	0.24 (0.10-0.57)	
18:1*n*-9c	OR1	1.00	1.42 (0.88-2.27)	0.73 (0.41-1.30)	0.91 (0.45-1.85)	0.278
	OR2	1.00	1.25 (0.73-2.12)	0.95 (0.53-1.70)	0.92 (0.44-1.91)	
18:1*n*-9t	OR1	1.00	1.19 (0.70-2.03)	0.91 (0.55-1.49)	0.87 (0.53-1.44)	0.286
	OR2	1.00	1.05 (0.60-1.85)	0.65 (0.36-1.17)	0.64 (0.36-1.12)	
Polyunsaturated fatty acids						
18:2*n*-6c	OR1	1.00	1.07 (0.65-1.76)	1.36 (0.83-2.25)	0.96 (0.55-1.66)	0.735
	OR2	1.00	1.00 (0.59-1.69)	1.39 (0.82-2.38)	0.99 (0.56-1.75)	
18:2*n*-6t	OR1	1.00	0.45 (0.27-0.76)	—	—	0.003
	OR2	1.00	0.71 (0.42-1.19)	—	—	0.780
18:3*n*-3	OR1					0.753
	OR2	1.00	1.28 (0.87-1.89)	—	—	
20:2*n*-9	OR1	1.00	1.47 (0.87-2.49)	1.30 (0.77-2.20)	1.26 (0.74-2.13)	0.560
	OR2	1.00	1.64 (0.95-2.83)	1.43 (0.84-2.45)	1.03 (0.61-1.74)	
20:3*n*-9	OR1	1.00	1.31 (0.78-2.21)	0.88 (0.51-1.50)	1.45 (0.88-2.39)	0.450
	OR2	1.00	1.24 (0.72-2.13)	0.83 (0.48-1.43)	1.96 (1.12-3.42)	
20:4*n*-6	OR1	1.00	0.98 (0.58-1.64)	1.09 (0.65-1.85)	1.52 (0.91-2.52)	0.001
	OR2	1.00	1.61 (0.92-2.80)	1.40 (0.79-2.49)	2.38 (1.37-4.12)	
Long-chain N-3 polyunsaturated fatty acids						
20:5*n*-3	OR1	1.00	1.60 (0.94-2.73)	1.60 (0.95-2.70)	1.22 (0.72-2.08)	0.448
	OR2	1.00	1.87 (1.07-3.24)	2.28 (1.31-3.95)	1.54 (0.91-2.60)	
22:6*n*-3	OR1	1.00	1.61 (0.96-2.72)	1.66 (0.97-2.86)	1.71 (0.99-2.94)	0.726
	OR2	1.00	1.37 (0.80-2.33)	1.61 (0.92-2.81)	1.17(0.67-2.05)	
P/S ratio	OR1	1.00	1.55 (1.02-2.36)	1.19 (0.71-1.99)	1.94 (1.02-3.66)	0.020
	OR2	1.00	1.56 (0.92-2.63)	1.89 (1.11-3.21)	1.28 (0.76-2.17)	
N3/N6 ratio	OR1	1.00	1.45 (0.85-2.47)	1.38 (0.80-2.39)	1.28 (0.73-2.25)	0.731
	OR2	1.00	1.53 (0.88-2.64)	1.45 (0.82-2.56)	1.32 (0.74-2.35)	

NOTE: OR1 is OR adjusted for age, sex, lab, tanning ability, freckles, history of actinic keratosis and includes all cases and controls (n = 657). OR2 is OR adjusted for age, sex, lab, tanning ability, freckles and excludes 94 controls with history of prior actinic keratosis (n = 563).
*Quartiles defined by control group (all controls for OR1 and controls without AK for OR2); for 18:2n6t and 18:3n3 groups were categorized as detectable or not.

Table 4. Relationship between RBC arachidonic acid levels and SCC risk, stratified by current use of NSAIDs

		Red blood cell arachidonic acid				*P* for trend
		1st quartile	2nd quartile	3rd quartile	4th quartile	
All						
n = 657	OR1	1.00	1.20 (0.74-1.95)	1.23 (0.76-2.00)	1.86 (1.16-2.99)	0.04
n = 563	OR2	1.00	1.59 (0.93-2.72)	1.45 (0.85-2.40)	2.36 (1.38-4.02)	0.001
NSAID nonuser						
n = 589	OR1	1.00	1.32 (0.79-2.19)	1.43 (0.85-2.38)	2.01 (1.21-3.34)	0.03
n = 508	OR2	1.00	1.82 (1.03-3.21)	1.59 (0.99-2.78)	2.59 (1.47-4.58)	0.003
NSAID user						
n = 66	OR1	1.00	0.61 (0.11-3.32)	0.15 (0.02-0.98)	0.88 (0.18-4.30)	0.77
n = 55	OR2	1.00	0.43 (0.07-2.80)	0.49 (0.06-4.29)	1.14 (0.20-6.58)	0.85

NOTE: Quartiles defined by control group (all controls for OR1 and controls without actinic keratosis for OR2); for 18:2*n*6t and 18:3*n*3 groups were categorized as detectable or not.
OR1 is OR adjusted for age, sex, lab, tanning ability, freckles, history of actinic keratosis and includes all cases and controls. OR2 is OR adjusted for age, sex, lab, tanning ability, freckles and excludes controls with history of prior actinic keratosis. Model with all subjects also adjusts for NSAID use.

are that the response rates were less than desirable and cases might have altered their behavior between diagnosis and interview. The primary issue with low response is that subjects who did not participate would have responded differently than did the actual participants. Whereas it is not possible to completely rule this problem out, our primary outcome is erythrocyte membrane composition. It is not likely that subjects preferentially refused based on their RBC lipid levels. Several strategies were used to reduce potential bias. Standard questionnaires were given to all subjects and the interviewer was not aware of the case status of the subjects at the time of interview or the specific hypothesis under consideration.

Because the cases were interviewed within 1-year of their diagnosis (average of 4 months), it is possible that cases might have altered their behavior because diagnosis and reported more recent behaviors. However, given the general good health of SCC patients, these behavior changes would most likely be in those areas that the case thought to be related to their diagnosis (i.e., sun behavior and exposures) and not in areas not commonly considered to be associated with skin cancer (i.e., dietary factors). There is indeed some evidence that skin cancer cases did recently alter their behavior for risk factors they thought were related to skin cancer. For instance, skin cancer cases and controls reported similar recent (past year) exposure to the sun. Cases reported more current use of sunscreens. There was no difference, however, between the cases and controls in past use of sunscreens.

Most work examining fatty acid profile and disease risk have used adipose tissue, which reflects long-term integrated dietary intake, individual metabolism, and physical activity. Turnover of blood cells is more rapid in comparison with adipose tissue, reflecting a smaller window of exposure. Despite RBCs potentially reflecting a relatively short-term response to diet, substantial information indicates that population groups adopt consistent patterns of energy intake and expenditure. Furthermore, focused, intense interventions are needed to effect dietary changes like reducing fat intake or altering the proportions of the various types of dietary fat. Because erythrocyte membrane composition reflects dietary lipid intake along with endogenous lipid biosynthesis, it can be argued that these membranes should be better indicator of nutritional status and functional energy requirement compared with adipose tissue.

A final limitation is in the actual quantitation of fatty acids, especially when assessing specific fatty acids that are normally of small quantity (e.g., the *trans*-fatty acids). The quantification techniques were selected and standardized to be able to measure these fatty acids; however, we observed that less than half of the population had levels above the detectable level of the assay. This low level of response makes the associations less interpretable.

Conclusions

With the high prevalence of skin cancer in the United States and increasing health care costs, there is a need to understand the etiology of the various skin cancers, to define modifying risks, and to develop effective intervention strategies. Primary prevention strategies for skin cancer have focused on behavior modifications for minimizing UV exposure; however, other strategies are needed.

The current study provides further evidence that dietary factors and lipids, in particular, are important in SCC development in human skin, confirming numerous findings in rodent models of photocarcinogenesis. This case control study lends support to the hypothesis that modifications of arachidonic acid metabolism are involved in the promotion and/or progression to SCC of the skin in humans. It is not clear, however, whether the associations are solely due to alterations in arachidonic acid metabolism or whether palmitic and its related saturated fatty acids alter SCC risk through other mechanisms. The lack of apparent dietary fat and skin cancer associations in observational studies, yet significant findings in a randomized intervention study and this current study highlight the need for biological markers and pathologic validation to minimize potential confounding in human studies. Despite the challenge of completing well-designed studies in population groups, technological science is continually improving. Dietary and other lifestyle modifications that can significantly reduce cancer morbidity or mortality deserve attention and clarification.

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