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Toenail Trace Element Levels and Breast Cancer: A Prospective Study

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The associations between toenail levels of five trace elements and breast cancer risk were studied among a cohort of 62,641 US women who provided toenail clippings and were free from diagnosed breast cancer in 1982. Among 433 cases of breast cancer identified during 4 years of follow-up and their matched controls, the odds ratios comparing the highest with the lowest quintiles and adjusted for established breast cancer risk factors were as follows: for arsenic, 1.12 (95% confidence interval (CI) 0.66–1.91); for copper, 0.91 (95% CI 0.59–1.42); for chromium, 0.96 (95% CI 0.61–1.52); for iron, 0.89 (95% CI 0.56–1.40); and for zinc, 1.09 (95% CI 0.70–1.70). Among postmenopausal women, a marginally significant positive association was observed between toenail chromium levels and breast cancer risk (odds ratio = 1.71, 95% CI 0.87–3.35) (p for trend = 0.07). However, the association between chromium and breast cancer risk was inverse among premenopausal women. Although data on the validity of toenail levels of certain of these elements are limited, these results do not provide evidence for an important effect of arsenic, copper, chromium, iron, or zinc on breast cancer risk. *Am J Epidemiol* 1996;144:653–60.

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The importance of environmental factors in the epidemiology of breast cancer is supported by the fivefold variation in national breast cancer incidence rates and by the observation that offspring of migrants to areas of high breast cancer incidence from areas of low incidence acquire the rates of the new location (1). However, to date, few environmental risk factors for breast cancer have been confirmed (1). Environmental exposure to certain trace elements has been shown to modify cancer risk, and the relation of dietary intake of these elements to breast cancer risk merits further study.

Arsenic is an established human carcinogen. Inhalation exposure has been shown to lead to an increased risk of lung cancer (2), and chronic oral exposure to arsenic has been shown to increase the risk of skin cancer (3). Recent evidence indicates that oral arsenic exposure may increase the risk of cancers of the bladder, lung, liver, and kidney (4). Excess iron intake has been shown to increase the incidence of mammary tumors in rats (5), and high iron status has been linked to an increased risk of cancer at all sites combined in several epidemiologic studies (6–9). Considerable epidemiologic data support an increased risk of lung cancer with occupational exposure to chromium (10), although there are few data on the relation between dietary intake of chromium in relation to cancer risk. Low copper levels may enhance cancer risk due to inhibition of the antioxidant enzyme superoxide dismutase, while high copper levels may enhance cancer risk by catalyzing free radical formation (11). In prospective studies of risk of death from cancer (11) and breast cancer incidence (12), elevated risks were found to be associated with either high or low (as compared with intermediate) blood copper levels. In a third prospective study, a significantly increased risk of cancer associated with high blood copper levels disappeared upon exclusion of cases diagnosed early in the follow-up period (13). For zinc, as with copper, hypotheses have been suggested for both a carcinogenic and anticarcinogenic role. Zinc may inhibit carcinogenesis by protecting against free radical damage (11) but may also be necessary for tumor growth due to its role in gene transcription and cell proliferation (14). The epidemiologic data on the relation between zinc and cancer are limited and inconsistent.

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Abbreviations: CI, confidence interval; OR, odds ratio.

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We examined the relation between breast cancer risk and toenail levels of arsenic, copper, chromium, iron, and zinc in a large prospective study. Nail levels of arsenic have been shown to reflect arsenic intake (15, 16). The validity of toenail levels to reflect trace element status is less well established for copper, chromium, iron, and zinc. Levels of chromium and zinc in hair may reflect chromium and zinc status, respectively (17), and because of the biologic similarity between hair and nails, it seems likely that nail levels of these elements are useful measures of exposure. Nail iron levels are elevated in patients with hemochromatosis (David Hunter et al., Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, unpublished manuscript), and nail copper levels are elevated among patients with Wilson's disease (18). The reproducibility of toenail trace element levels over a 6-year period was assessed by comparing levels in paired specimens collected in 1982–1983 and in 1988 from 127 women in this cohort. The correlation coefficient for the reproducibility of these trace elements was 0.26 for copper, 0.33 for chromium, 0.43 for iron, 0.54 for arsenic, and 0.58 for zinc (19). Thus, a single measure of toenail levels of these elements may provide a reasonable measure of long-term intake, particularly for iron, arsenic, and zinc.

MATERIALS AND METHODS

Population

The population for this study has been described previously (20). The Nurses' Health Study Cohort began in 1976 when 121,700 nurses aged 30–55 years living in 11 US states completed a questionnaire on risk factors for cancer (21) and coronary heart disease (22). Follow-up questionnaires are mailed to participants every 2 years to update information on exposures and to identify new cases of disease (including cancers). In 1980, a food frequency questionnaire was added. In 1982, 94,115 cohort members were requested to provide toenail clippings from all 10 toes, and 68,213 complied. Of these women, 62,641 had no prior history of cancer (other than nonmelanoma skin cancer) and constitute the baseline cohort for this analysis. We conducted a case-control study nested within this baseline cohort.

Identification of cases and controls

Cases were identified from the 1984 and 1986 follow-up questionnaires. As many as five mailings were sent to women who did not respond. The response rate among women who provided nail specimens exceeded 95 percent in both 1984 and 1986. Information on deaths was obtained from family members, the postal system, and the National Death Index. Fewer than 1 percent of the participants died during the follow-up period. When an incident case of breast cancer was identified from the questionnaires, permission was sought to obtain the medical records. There were 437 diagnoses of breast cancer that were reconfirmed by the participant and for which the date of diagnosis was after the date of nail return. Pathology reports were obtained for 96 percent of these women, and the diagnosis was rejected on the basis of the pathology report in only two instances. Because of the high rate of confirmation, we included cases who had self-reported the first diagnosis of breast cancer but for whom we were unable to obtain the pathology report, leaving 435 cases. We later identified an additional 86 eligible cases but were unable to analyze their toenail specimens because of time and budget constraints. We compared the breast cancer risk profile of these missing cases with that of the cases included in the analysis. Controls were chosen at random from women in the baseline cohort who were free of cancer, matched on a one-to-one basis to the cases by year of birth and month of nail return (the nails were returned over a 15-month period). There were 459 controls, the excess of controls relative to cases being due to the exclusion of cases who had originally been chosen and subsequently determined to be ineligible (if their date of nail return was after their date of diagnosis or if their diagnosis was rejected based on the medical record review). Samples from two cases were lost, leaving 433 cases and 459 controls for analysis.

Laboratory analyses

Concentrations of toenail trace element levels were determined by instrumental neutron activation analysis, as described previously (19), at the University of Missouri Research Reactor Facility, Columbia, Missouri. Each case-control pair was analyzed together, with the within-pair order randomized. Laboratory personnel were blinded to case-control status. The analyses were conducted in four batches: batch 1 consisting of 128 matched sets, batch 2 consisting of 67 matched sets, batch 3 consisting of 114 matched sets, and batch 4 consisting of 150 matched sets. (For copper, batches 2 and 3 were run together as one batch). For technical reasons, arsenic levels were not determined for batch 1.

Data analyses

In the final data set of 892 women (n = 643 for arsenic analyses), the numbers of values that were undetectable (below the sensitivity of the laboratory technique) were 47 for arsenic, 23 for iron, and seven for copper. These observations were set to the value of the limit of detection. The toenail trace element levels were $\log_e$ transformed to improve normality. To adjust for nail weight and laboratory batch, we regressed each toenail trace element (the dependent variable) on the weight of the specimen, the laboratory batch, and interaction terms for weight by laboratory batch (the independent variables). We obtained the residuals from this regression and added back the mean trace element value. Analyses conducted on trace element levels unadjusted for these factors gave very similar results (data not shown). We compared conditional and unconditional logistic regression models; as the two methods gave very similar results, we used unconditional logistic regression models that allowed the inclusion of all controls. Quintiles of the toenail trace element levels were derived based on the distribution among the controls. These quintiles were entered into logistic models as indicator variables or as ordinal variables (to test for trend). The odds ratio was used as an approximation to the relative risk (23). Information on covariates was derived from the biennial questionnaires from 1976 to 1982. Menopausal status was assessed in 1982. Women reporting a hysterectomy without bilateral oophorectomy were considered to be of uncertain menopausal status unless they were above the age at which 90 percent of participants had experienced natural menopause or below the age at which only 10 percent of participants had experienced natural menopause (in which cases they were considered postmenopausal or premenopausal, respectively). Women of uncertain menopausal status were excluded from analyses conducted separately by menopausal status. To test for interaction with menopausal status, menopausal status coded as a dichotomous variable (pre/post), the toenail trace element quintile coded as an ordinal variable, and the product of these two terms were entered in logistic regression models.

RESULTS

The distributions of potential breast cancer risk factors (age, menopausal status, smoking, age at first birth, parity, age at menarche, history of benign breast disease, history of breast cancer in the mother, history of breast cancer in a sister, body mass index, and alcohol intake) were similar between the 433 included cases and the 86 potential cases who were not included (data not shown). In most instances, traditional breast cancer risk factors had the expected associations with breast cancer risk in this population. For women whose age at first birth was 30 years or older, the odds ratio was 2.5 compared with those whose age at first birth was 24 years or younger. The odds ratio associated with a history of benign breast disease was 1.65, and the odds ratios associated with a history of breast cancer in a sister or mother were 1.3 and 2.0, respectively. The odds ratio of 1.07 for an age at menarche of 14 years or older compared with 12 years or younger was not in the expected direction, but this is among the weakest of the traditional breast cancer risk factors and thus more easily obscured by random fluctuation.

The associations among the 892 study participants between potentially confounding variables and toenail levels of arsenic, copper, chromium, iron, and zinc and several potential breast cancer risk factors were examined (data not shown). Arsenic and chromium levels were significantly inversely associated with age, while zinc levels were significantly positively related. Current smokers had higher levels of arsenic than had never or former smokers. Iron levels were significantly lower in women with a later age of first birth. Zinc levels were significantly elevated in women whose mother had had breast cancer. Arsenic levels were significantly inversely, and iron levels significantly positively, associated with body mass index. Arsenic, copper, and chromium were each significantly positively associated with alcohol consumption. No notable associations were observed between trace element levels and parity, age at menarche, history of benign breast disease, or a history of breast disease in a sister.

For all five elements, the mean and median toenail trace element levels were similar in cases and controls, and no significant differences were observed (table 1). In the overall analysis, the results from conditional and unconditional logistic regression models gave very similar results. Thus, the results from unconditional models are presented. The age-adjusted and multivariate odds ratios of breast cancer by quintile of the toenail trace element levels were similar; thus, only the multivariate results are presented in table 2. No notable associations were observed between any of the toenail trace elements and breast cancer risk. For cases, the follow-up period was assumed to be the time interval between the return of the nail clippings and diagnosis. Shown in table 3 are the multivariate relative risk estimates after exclusion of cases diagnosed during the first 2 years of the follow-up period. These results were similar to the results of analyses in which the cases diagnosed during the first 2 years of follow-up were included (table 2).

The trace element levels also were analyzed as deciles in multivariate models. Among all women, the odds ratios comparing the highest with the lowest deciles were as follows: for arsenic, 0.59 (95 percent CI 0.28–1.26); for copper, 0.83 (95 percent CI 0.43–

TABLE 1. Mean and median toenail trace element levels (µg/g) among 433* matched breast cancer case-control pairs from the Nurses' Health Study at baseline (December 1982 to February 1984)

	Mean	Median	p value†
Arsenic			
Cases	0.12 (0.14)‡	0.09	0.42
Controls	0.12 (0.16)	0.09	
Copper			
Cases	5.61 (3.88)	4.72	0.81
Controls	5.68 (4.47)	4.76	
Chromium			
Cases	1.72 (2.10)	1.16	0.53
Controls	1.69 (1.98)	1.13	
Iron			
Cases	40.2 (48.9)	32.0	0.38
Controls	41.4 (43.6)	33.0	
Zinc			
Cases	119 (47.9)	110	0.68
Controls	120 (51.5)	110	

* The analysis of arsenic is based on 312 matched sets.
† From a paired t test on $\log_e$-transformed values.
‡ Numbers in parentheses, standard deviation.

1.58); for chromium, 1.41 (95 percent CI 0.73–2.75); for iron, 0.70 (95 percent CI 0.36–1.35); and for zinc, 0.98 (95 percent CI 0.52–1.82).

The results of multivariate unconditional logistic regression analyses conducted among strata of menopausal status are shown in table 4. Tests for interaction with menopausal status gave statistically significant results only for chromium ($p = 0.02$). For the analysis of chromium among premenopausal women, the odds ratio (OR) was decreased in the highest quintile compared with the lowest (OR = 0.47), and this result was statistically significant. For postmenopausal women, a positive association with chromium was observed. The odds ratio in the fourth quintile relative to the lowest was significantly elevated (OR = 1.91), and the test for trend was marginally significant ($p = 0.07$). Among premenopausal women, the odds ratio associated with being in the highest quintile of toenail iron relative to the lowest was 0.45, which was statistically significant. Among postmenopausal women, an elevated odds ratio was observed, comparing the highest quintile of toenail iron with the lowest (OR = 1.56), but this was not significant, and the test for trend did not reach statistical significance. For zinc, the results among premenopausal women were null, while among postmenopausal women, a positive association was observed, with the odds ratio in the fourth quintile compared with the lowest quintile (OR = 2.58) reaching statistical significance; however, the trend was nonsignificant.

Because menopausal status was not one of the matching factors in this study, we examined the relations between toenail trace element levels and breast cancer using conditional logistic regression models based on matched pairs (data not shown). Results from these analyses were similar to the results based on unconditional models shown in table 4.

Among the 892 subjects in the study population, the toenail trace element levels were positively correlated with each other for all pairs of elements. The strongest correlation was between chromium and iron (Spearman's $r = 0.39$; $p = 0.0001$). To address the possibility of confounding, we conducted, separately by menopausal status, multivariate analyses with pairs of elements entered in the model simultaneously. All possible combinations of two elements were considered; each element was entered into an unconditional logistic regression model with indicator variables for quintiles. When iron and chromium were considered simultaneously among postmenopausal women, the estimates for iron shifted toward the null; the odds ratio for the highest quintile of toenail iron compared with the lowest was 1.19 (95 percent CI 0.57–2.48). In this same model, the estimates for chromium were similar to those unadjusted for iron (data not shown). There was little evidence of confounding between any other pair of trace elements (data not shown).

DISCUSSION

In this large prospective study, we found little evidence for associations between arsenic, copper, iron, and zinc and the risk of breast cancer. Because of the prospective design of this study, disease is not likely to have affected trace element intake or levels. Moreover, our results are unlikely to be biased by an effect of preclinical disease, as results were similar upon excluding cases diagnosed in the first 2 years of follow-up. Although several of the toenail element levels were associated with one or more known risk factors for breast cancer, our findings are unlikely to be appreciably confounded, as we were able to adjust for these breast cancer risk factors in multivariate analyses. Eighty-six eligible cases (representing 16.5 percent of the total) were not included. However, the similar distribution of breast cancer risk factors between these potential cases and the cases who were included suggests that our results are unlikely to be biased by underascertainment.

Arsenic levels in nails have been shown to reflect arsenic intake from drinking water (15; Margaret Karagas et al., Department of Community and Family Medicine, Dartmouth Medical School, unpublished manuscript) and in a case of arsenic poisoning (16). In addition, among members of this cohort, the reproduc-

TABLE 2. Multivariate* odds ratios of breast cancer, according to quintile of toenail trace element levels, among 877† participants in the Nurses' Health Study, 1982–1987

	Cutpoints (μg/g)	Cases/ controls	Multivariate OR	*p* for trend
		Arsenic		
Quintile 1	<0.059	54/66	1.00	0.78
Quintile 2	0.059–0.078	67/65	1.19 (0.71–1.98)‡	
Quintile 3	0.079–0.103	56/64	1.01 (0.59–1.73)	
Quintile 4	0.104–0.138	62/65	1.12 (0.67–1.90)	
Quintile 5	>0.138	69/65	1.12 (0.66–1.91)	
		Copper		
Quintile 1	<3.61	87/90	1.00	0.65
Quintile 2	3.61–4.33	85/90	1.01 (0.65–1.56)	
Quintile 3	4.34–5.26	88/90	1.03 (0.67–1.60)	
Quintile 4	5.27–6.77	87/90	0.96 (0.62–1.50)	
Quintile 5	>6.77	80/90	0.91 (0.59–1.42)	
		Chromium		
Quintile 1	<0.52	75/91	1.00	0.64
Quintile 2	0.52–0.88	81/89	1.12 (0.72–1.75)	
Quintile 3	0.89–1.37	97/91	1.22 (0.79–1.89)	
Quintile 4	1.38–2.37	101/89	1.47 (0.95–2.28)	
Quintile 5	>2.37	73/90	0.96 (0.61–1.52)	
		Iron		
Quintile 1	<20.3	84/90	1.00	0.36
Quintile 2	20.3–28.8	102/90	1.15 (0.75–1.77)	
Quintile 3	28.9–38.0	94/90	1.20 (0.78–1.86)	
Quintile 4	38.1–50.8	73/90	0.90 (0.57–1.41)	
Quintile 5	>50.8	74/90	0.89 (0.56–1.40)	
		Zinc		
Quintile 1	<96.3	76/91	1.00	0.35
Quintile 2	96.3–104	70/90	0.92 (0.58–1.46)	
Quintile 3	105–114	100/89	1.23 (0.79–1.91)	
Quintile 4	115–128	97/91	1.26 (0.81–1.96)	
Quintile 5	>128	84/89	1.09 (0.70–1.70)	

* Unconditional logistic regression models were used because conditional and unconditional logistic regression models gave very similar results. Multivariate logistic regression models contained terms for age, date of nail return, smoking, age at first birth, parity, history of benign breast disease, history of breast cancer in mother, history of breast cancer in a sister, age at menarche, menopausal status, body mass index, and alcohol consumption.

† Because of missing information on smoking, parity, or menopausal status, six cases and nine controls were deleted from this analysis (for the analysis of arsenic, 308 cases and 325 controls were included).

‡ Numbers in parentheses, 95% confidence interval.

ibility of toenail arsenic levels over a 6-year period is moderately high ($r = 0.54$), indicating that a single measure of toenail arsenic levels provides a reasonable measure of long-term intake (19).

In addition to the well-established associations between inhalation exposure to arsenic and lung cancer (2) and between oral exposure to arsenic and skin cancer (3), other data suggest that oral arsenic exposure may increase the risk of internal cancers. Several case reports have linked arsenic exposure to hepatic angiosarcoma, an extremely rare cancer (24, 25). Investigators studying populations exposed to high levels of arsenic in drinking water in Taiwan and Japan have demonstrated dose-response relations between arsenic exposure and cancers of the bladder, lung, liver, and kidney (26–31). Our findings do not provide evidence for an increased risk of breast cancer with increased arsenic exposure. However, the level of arsenic consumed daily by exposed persons in the studies from Taiwan has been estimated to be in excess of 1 mg/day (4) (and the range of arsenic levels in drinking water in the study conducted in Japan (31) was similar to the range of arsenic levels in drinking water in the studies from Taiwan (28, 30)), whereas arsenic intakes in the United States, even among persons consuming relatively high amounts of arsenic from drink-

TABLE 3. Multivariate odds ratios* of breast cancer, according to quintile of toenail trace element levels, among participants in the Nurses' Health Study after exclusion of cases diagnosed in the first 2 years of follow-up (diagnosis dates of cases 1984–1987)

	Cases/ controls†	Quintile‡ 1	2	3	4	5	p for trend
Arsenic	230§/325	1.00	1.22 (0.69–2.15)‖	1.07 (0.59–1.92)	1.10 (0.61–1.97)	1.16 (0.65–2.07)	0.79
Copper	230/450	1.00	1.06 (0.63–1.80)	1.22 (0.72–2.06)	0.86 (0.50–1.49)	1.13 (0.67–1.91)	0.95
Chromium	230/450	1.00	1.33 (0.77–2.28)	1.44 (0.84–2.47)	1.61 (0.94–2.75)	0.99 (0.56–1.76)	0.73
Iron	230/450	1.00	0.96 (0.58–1.58)	1.00 (0.60–1.66)	0.69 (0.40–1.20)	0.72 (0.42–1.24)	0.13
Zinc	230/450	1.00	1.00 (0.59–1.70)	0.95 (0.56–1.60)	0.94 (0.56–1.61)	0.92 (0.54–1.57)	0.72

* From unconditional logistic regression models containing terms for age, date of nail return, smoking, age at first birth, parity, history of benign breast disease, history of breast cancer in mother, history of breast cancer in a sister, age at menarche, menopausal status, body mass index, and alcohol consumption.

† Subjects missing information on smoking, parity, or menopausal status were excluded from this analysis, as described in table 2.

‡ Quintile cutpoints are defined as in table 2.

§ The number of cases for the arsenic analyses was the same as for the other analyses after exclusion of cases diagnosed in the first 2 years of follow-up, because the 128 cases for whom arsenic determinations were not done (see Materials and Methods) were all diagnosed in the first 2 years of follow-up.

‖ Numbers in parentheses, 95% confidence interval.

ing water, are estimated to be from one to two orders of magnitude lower (2). Thus, while our data provide reassurance that arsenic consumed within the range of the general US population does not substantially increase the risk of breast cancer, we cannot exclude the possibility that highly exposed individuals are at increased risk.

Nail iron levels have been shown to be lower in some (32) but not all (33) studies of persons who were iron deficient. Eighteen patients with hemochromatosis, a disorder of iron metabolism characterized by an inappropriate increase in absorption of ingested iron, were found to have significantly higher toenail iron levels as compared with those of controls; for great toenails, the mean iron concentration among the hemochromatosis patients was 105 μg/g as compared with 58 μg/g among controls (David Hunter et al., Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, unpublished manuscript). Several prospective studies have shown a positive association between cancer at all sites combined and iron levels, as assessed by serum ferritin (6), total iron binding capacity (7, 9), or transferrin saturation (8, 9). However, in the one prospective study in which results were presented for breast cancer specifically (8), none of the indica-

TABLE 4. Multivariate odds ratios* of breast cancer, according to quintile of toenail trace element levels, among premenopausal (pre) and postmenopausal (post)† participants in the Nurses' Health Study, 1982–1987

	Cases/ controls‡	Quintile§ 1	2	3	4	5	p for trend	p for interaction
Arsenic								
Pre	137/127	1.00	0.77 (0.32–1.85)‖	0.98 (0.40–2.42)	0.93 (0.37–2.32)	0.74 (0.31–1.77)	0.68	0.45
Post	152/184	1.00	1.56 (0.75–3.25)	1.42 (0.65–3.14)	1.53 (0.74–3.20)	1.47 (0.67–3.19)	0.39	
Copper								
Pre	193/190	1.00	1.41 (0.69–2.89)	1.92 (0.93–3.98)	1.48 (0.73–3.01)	1.22 (0.60–2.48)	0.67	0.57
Post	208/241	1.00	0.86 (0.47–1.60)	0.84 (0.44–1.58)	0.82 (0.44–1.52)	0.93 (0.49–1.75)	0.76	
Chromium								
Pre	193/190	1.00	0.93 (0.45–1.94)	0.95 (0.46–1.96)	1.06 (0.53–2.15)	0.47 (0.23–0.96)	0.08	0.02
Post	208/241	1.00	1.45 (0.76–2.75)	1.41 (0.75–2.64)	1.91 (1.00–3.63)	1.71 (0.87–3.35)	0.07	
Iron								
Pre	193/190	1.00	0.89 (0.44–1.80)	1.37 (0.67–2.81)	0.94 (0.45–1.96)	0.45 (0.21–0.95)	0.07	0.08
Post	208/241	1.00	1.28 (0.70–2.33)	1.29 (0.70–2.40)	0.90 (0.47–1.71)	1.56 (0.80–3.03)	0.50	
Zinc								
Pre	193/190	1.00	0.79 (0.39–1.62)	1.03 (0.53–2.01)	0.72 (0.36–1.42)	1.29 (0.62–2.71)	0.77	0.59
Post	208/241	1.00	1.31 (0.65–2.64)	1.97 (0.98–3.93)	2.58 (1.30–5.12)	1.32 (0.68–2.54)	0.20	

* From unconditional logistic regression models containing terms for age, date of nail return, smoking, age at first birth, parity, history of benign breast disease, history of breast cancer in mother, history of breast cancer in a sister, age at menarche, body mass index, and alcohol consumption.

† Menopausal status was defined at baseline from the 1982 questionnaire.

‡ Subjects missing information on smoking, parity, or menopausal status were excluded from this analysis, as described in table 2.

§ Quintile cutpoints are defined as in table 2.

‖ Numbers in parentheses, 95% confidence interval.

tors of iron status examined (serum iron, total iron binding capacity, or transferrin saturation) was found to be associated with breast cancer risk. Although the relative risk was elevated in the highest quintile of toenail iron among postmenopausal women in the current study, this was compatible with chance. In addition, this association was weakened considerably after controlling for toenail chromium levels. Among premenopausal women, we observed a significant inverse association between toenail iron levels and breast cancer risk.

Hair levels of chromium have been shown to reflect chromium status (17), suggesting that nails (which are biologically similar to hair) may also provide a useful indicator of chromium status. Occupational exposure to certain chromium compounds has been associated with an increased risk of lung cancer, and chromium has been shown to be genotoxic (10). However, virtually no data exist on dietary exposure to chromium and cancer risk. In a case-control study of nail chromium levels and laryngeal, esophageal, and oral cancers, chromium levels were found to be positively associated with laryngeal and esophageal but not oral cancers (34). We observed no overall association between chromium levels and breast cancer; however, a significant interaction was present with menopausal status. Although the positive association among postmenopausal women between toenail chromium levels and breast cancer is intriguing, caution must be applied in interpreting this finding, as this result may have arisen by chance. Similarly, the statistically significant inverse association in the highest quintile of chromium among premenopausal women was not hypothesized a priori, and, to our knowledge, no plausible biologic mechanism exists to explain this inverse association. Thus, these findings should be regarded as preliminary and should be tested in other studies. Because the reproducibility of chromium over a 6-year period was only moderate in this cohort ($r = 0.33$) (19), it is possible that our results may be attenuated by the within-person variability in this measurement.

Hair levels of zinc may reflect zinc status (17); however, virtually no data exist on the validity of nail zinc levels. The epidemiologic data on zinc and cancer are limited. Case-control studies utilizing blood zinc levels have yielded inconsistent results and are difficult to interpret, as zinc levels may be altered as a result of the cancer (35). In a case-control study of prostate cancer, zinc consumption was significantly positively associated with the risk of prostate cancer in men 70 years old or older (36), while in another case-control study, zinc consumption was found to be significantly inversely associated with melanoma risk (37). In one prospective study, blood zinc levels were found to be nonsignificantly inversely related to the risk of cancer death (11). In another prospective study, no association was found between blood zinc levels and lung or stomach cancer risk (38). We did not observe evidence of any association between zinc and breast cancer overall.

Few data are available on the validity of nail copper levels as a measure of copper status. Nail copper levels were shown to be elevated in two of three patients with Wilson's disease, an abnormality in hepatic excretion of copper resulting in toxic accumulations of this element (18). In these two male patients, nail copper levels were found to be greater than 20 $\mu g/g$, whereas among six normal males, the mean was 14.8 (standard deviation, 3.8) $\mu g/g$. In two prospective studies, investigators have observed a U-shaped relation between serum copper and cancer death (11) and between plasma copper and breast cancer incidence (12). However, in another prospective study of serum copper and cancer, null results were obtained after excluding cases diagnosed early in the follow-up period (13). We did not observe any notable associations between toenail copper levels and breast cancer. However, our results may be attenuated by within-person variability in toenail copper levels, given that the reproducibility of these levels was shown to be relatively low among members of this cohort ($r = 0.26$) (19).

In summary, we did not observe any clear associations between breast cancer and toenail levels of arsenic, iron, chromium, copper, or zinc in our overall analyses. At levels of exposure experienced by women in the general US population, arsenic is unlikely to have an important adverse effect on breast cancer risk. Chromium was found to be marginally significantly positively related to breast cancer risk among postmenopausal women and inversely associated with breast cancer risk among premenopausal women, and further data on these relations are needed. Although the validity of toenail levels of copper, chromium, iron, and zinc may need additional confirmation, it is unlikely that any strong relation between levels of these elements and the risk of breast cancer exists, particularly at levels commonly resulting from nonoccupational exposures among middle-aged women.

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