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# Prospective evaluation of serum sarcosine and risk of prostate cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

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**Metabolomic profiling has identified, sarcosine, a derivative of the amino acid glycine, as an important metabolite involved in the etiology or natural history of prostate cancer. We examined the association between serum sarcosine levels and risk of prostate cancer in 1122 cases (813 non-aggressive and 309 aggressive) and 1112 controls in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Sarcosine was quantified using high-throughput liquid chromatography–mass spectrometry. A significantly increased risk of prostate cancer was observed with increasing levels of sarcosine (odds ratio [OR] for the highest quartile of exposure [Q4] versus the lowest quartile [Q1] = 1.30, 95% confidence interval [CI]: 1.02, 1.65; *P*-trend 0.03). When stratified by disease aggressiveness, we observed a stronger association for non-aggressive cases (OR for Q4 versus Q1 = 1.44, 95% CI: 1.11, 1.88; *P*-trend 0.006) but no association for aggressive prostate cancer (OR for Q4 versus Q1 = 1.03, 95% CI: 0.73, 1.47; *P*-trend 0.89). Although not statistically significant, temporal analyses showed a stronger association between sarcosine and prostate cancer for serum collected closer to diagnosis, suggesting that sarcosine may be an early biomarker of disease. Interestingly, the association between sarcosine and prostate cancer risk was stronger among men with diabetes (OR = 2.66, 95% CI: 1.04, 6.84) compared with those without reported diabetes (OR = 1.23, 95% CI: 0.95–1.59, *P*-interaction = 0.01). This study found that elevated levels of serum sarcosine are associated with an increased prostate cancer risk and evidence to suggest that sarcosine may be an early biomarker for this disease.**

## Introduction

Recently, the utility of measuring prostate-specific antigen (PSA) for the early detection of prostate cancer has been questioned. Two large trials have reported little or no difference in prostate cancer deaths between screened and non-screened individuals (1,2) and the United States Preventive Services Task Force recently recommended against routine PSA screening (3). Thus, new clues about the etiology or the natural history of prostate cancer are needed.

**Abbreviations:** BMI, body mass index; CI, confidence interval; CV, coefficients of variation; OR, odds ratio; PLCO, Prostate, Lung, Colorectal and Ovarian; PSA, prostate-specific antigen.

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Sreekumar *et al.* (4) reported on the application of metabolomics in the discovery of a potentially new prostate cancer biomarker. In this study, amino acid metabolism was identified as an important pathway in cancer progression and sarcosine, a derivative of the amino acid glycine, was specifically identified as an important metabolite in prostate cancer. The authors showed differential levels of sarcosine in prostate tissue samples compared with benign prostate samples as well as differential urinary sarcosine levels between biopsy-positive and negative individuals. Additional experiments in prostate cancer cell lines also indicated that sarcosine imparted an invasive phenotype to benign epithelial cells suggesting a role in aggressive disease etiology. Since this report, other studies have attempted to replicate these findings with mixed results (5–14). It is difficult, however, to compare and draw conclusions from these studies because they used different study designs, had small populations (<150 subjects/samples), investigated different sample types (tissue, urine and serum) and utilized a variety of analytical methods to measure sarcosine (15–17).

Thus, we conducted a large, prospective epidemiologic study to evaluate the role of serum sarcosine and risk of prostate cancer in a case–control study of 1122 cases and 1112 controls nested in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.

## Methods

### *Study population*

Participants were selected from the PLCO Cancer Screening Trial, a randomized, controlled, multisite trial to test the efficacy of screening methods for these four cancers. The details of this trial have been described elsewhere (18,19). Briefly, the PLCO trial participants were individuals 55–74 years old without a history of prostate, lung, colorectal or ovarian cancer, who were enrolled between 1993 and 2001. Participants were randomized to either the screening or control arm of the trial. Men randomized to the screening arm were offered a PSA test and digital rectal exam at baseline and annually thereafter for 3 years, followed by 2 years of screening with PSA alone. Men with abnormal screening results were referred to their primary care physician for follow-up. Prostate cancer diagnoses were ascertained from diagnostic follow-up of screening results, mailed annual questionnaires and the National Death Index to enhance completeness. All cases of prostate cancer were confirmed by review of medical records. The study protocol was approved by the institutional review board at each study center and the National Cancer Institute, and participants provided informed consent.

For the current nested case–control study, we included men enrolled in the screening arm of PLCO who were diagnosed with prostate cancer at least 1 year after the first screen, between 1995 and 2009. Controls were male participants in the screening arm without a diagnosis of prostate cancer at the time of case diagnosis, frequency matched to the cases by age at entry into the study at 5 year intervals (55–59, 60–64, 65–69 and 70–74), year of enrollment and number of years of follow-up. All subjects for the nested case–control study were non-Hispanic white men. Participants also had to have completed a baseline risk factor questionnaire and dietary questionnaire, signed a consent form, and for controls, returned at least one Annual Study Update. A total of 2287 prostate cancer cases and controls from the PLCO cohort were initially selected for the proposed study.

### *Sarcosine assay and quality control*

Blood samples were collected at baseline for PLCO subjects (and at least 1 year prior to diagnosis for cases) and stored at −80°C until analysis time, when they were thawed at room temperature. Serum sarcosine (ng/ml) and alanine (ng/ml) levels were quantified using high-throughput liquid chromatography–mass spectrometry as described previously (20). Masked quality control samples ($n$ = 260) were interspersed among assay batches to evaluate intrabatch and interbatch variability. Intrabatch and interbatch coefficients of variation (CV) were used to assess the impact of problematic assay batches. The average intrabatch sarcosine CV was 4% (range = 0.03–15.7%) and the interbatch sarcosine CVs ranged from 8.5 to 12.3%. For alanine, the average intrabatch CV was 3.8% (range = 0.33–14.9%) and the interbatch CVs ranged from 7.4–11.9%. One sarcosine batch ($N$ = 35 subjects) was excluded because the intrabatch CV

was >20% and there was insufficient serum to rerun the batch. In addition, one subject with an extreme sarcosine measurement was excluded (>10 standard deviations), and 17 subjects had missing sarcosine levels due to a centrifuge problem, leaving 2234 subjects (1122 cases and 1112 controls) for analysis.

*Statistical analysis*

Univariate comparison between sarcosine levels in cases and controls was evaluated using the Wilcoxon rank-sum test. Differences between categorical variables were evaluated using chi-squared tests. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between prostate cancer and baseline serum sarcosine. Aggressive disease was defined as Gleason grade ≥8 or stage III/IV or fatal prostate cancer. Sarcosine was normalized to alanine (as in Sreekumar *et al.* (4)) and log transformed for all regression analyses. Quartiles of sarcosine were produced by dividing the frequency distribution of the control group at the 25th, 50th and 75th percentiles. Confounding variables were added to the model if they significantly contributed to the model at the alpha 0.05 level or if their addition changed the parameter estimates by >10%. The following confounding variables were included in the final model: age at selection (in years as a continuous variable), study center, family history of prostate cancer (yes/no/missing), ever self-reported history of diabetes (yes/no/missing) and cigarette smoking status (never/former/current/pipe or cigar). Adjustments for other variables such as year of enrollment, number of years of follow-up, body mass index (BMI), education, marital status, alcohol intake, use on non-steroidal anti-inflammatory drugs, history of hypertension, history of cardiovascular disease, serum testosterone measurements (for a subset), serum C-peptide measurements (for a subset), PSA level at blood draw, assay batch, number of years blood was stored frozen, hour of blood collection, and dietary and/or supplemental intake of folate, vitamins B12, B6, riboflavin and methionine did not result in significant changes in the observed associations and thus were not included in the final model. Examination of potential effect modification between the above listed variables and sarcosine was considered by including the cross-product terms (modeled categorically) and the main effect terms in regression models, and the statistical significance of the interaction was evaluated by comparing nested models with and without the cross-product terms using a likelihood ratio test. These interactions were largely exploratory and only interactions with established prostate cancer risk factors or factors likely to affect sarcosine levels are presented. To assess the performance of sarcosine in predicting prostate cancer incidence, we estimated *C*-statistics and 95% CIs for models including age, family history, study center, smoking history and diabetes history using linear predictors for sarcosine and PSA. The *C*-statistic, which is equivalent to the area under the receiver operating characteristic curve measures, the ability of the model to discriminate between outcomes with $C = 1$ indicating perfect discrimination and $C = 0.5$ showing no discrimination. All analyses were done using SAS statistical software, version 9.2 (SAS Institute).

## Results

Unadjusted median levels of sarcosine were similar in cases compared with controls ($P_{\text{-wilcoxon}} = 0.16$), with the highest value observed among non-aggressive cases (Table I). There were no significant differences between cases and controls with respect to age and BMI; however, there were small differences in study center, and cases were

**Table I.** Characteristics of prostate cancer cases and controls in the PLCO Cancer Screening Trial

| Characteristic | Cases *n* (%) | | | Controls *n* (%) | *P*-value[b] |
|---|---|---|---|---|---|
| | All | Non-aggressive | Aggressive[a] | | |
| | *N* = 1122 | *N* = 813 | *N* = 309 | *N* = 1112 | |
| Sarcosine[c] | | | | | |
| Median (range) | 3.088 (0.206–12.289) | 3.112 (0.206–12.289) | 2.995 (1.115–9.391) | 3.022 (1.192–10.235) | 0.16 |
| Follow-up time (years) | | | | | |
| Mean (range) | 3.3 (1.1–11.7) | 3.2 (1.1–11.6) | 3.4 (1.1–11.7) | 3.4 (1.2–11.5) | 0.86 |
| Age at selection | | | | | |
| Mean (SD) | 68.3 (5.4) | 68.2 (5.3) | 68.7 (5.5) | 68.1 (5.5) | 0.37 |
| Center | | | | | |
| Colorado | 118 (10.5) | 89 (11.0) | 29 (9.4) | 116 (10.4) | |
| Georgetown | 86 (7.7) | 59 (7.3) | 27 (8.7) | 59 (5.3) | |
| Henry Ford | 118 (10.5) | 80 (9.8) | 38 (12.3) | 111 (10.0) | |
| Minnesota | 203 (18.1) | 139 (17.1) | 64 (20.7) | 291 (26.2) | |
| Washington | 92 (8.2) | 72 (8.9) | 20 (6.5) | 88 (7.9) | |
| Pittsburgh | 160 (14.3) | 119 (14.6) | 41 (13.3) | 139 (12.5) | |
| Utah | 166 (14.8) | 115 (14.2) | 51 (16.5) | 116 (10.4) | |
| Marshfield | 166 (14.8) | 129 (15.9) | 37 (12.0) | 175 (15.7) | |
| Alabama | 13 (1.2) | 11 (1.4) | 2 (0.7) | 17 (1.5) | <0.001 |
| Family history of PCa | | | | | |
| No | 899 (80.1) | 645 (79.3) | 254 (82.2) | 997 (89.7) | |
| Yes | 203 (18.1) | 154 (18.9) | 49 (15.9) | 97 (8.7) | |
| Missing | 20 (1.8) | 14 (1.7) | 6 (1.9) | 18 (1.6) | <0.001 |
| Smoking status | | | | | |
| Never | 398 (35.4) | 288 (35.7) | 109 (35.3) | 313 (28.2) | |
| Former | 545 (48.6) | 384 (47.6) | 154 (49.8) | 577 (51.9) | |
| Current | 78 (7.0) | 61 (7.6) | 18 (5.8) | 122 (11.0) | 0.001 |
| Pipe/cigar | 101 (9.0) | 73 (9.1) | 28 (9.1) | 100 (9.0) | |
| Baseline diabetes | | | | | |
| No | 1049 (93.5) | 761 (93.6) | 288 (93.2) | 998 (89.8) | |
| Yes | 69 (6.2) | 48 (5.9) | 21 (6.8) | 109 (9.8) | |
| Missing | 4 (0.4) | 4 (0.5) | — | 5 (0.5) | 0.006 |
| BMI (kg/m$^2$) | | | | | |
| <25 | 295 (26.3) | 224 (27.6) | 71 (23.0) | 282 (25.4) | |
| 25–29 | 596 (53.1) | 424 (52.2) | 172 (55.7) | 563 (50.6) | |
| 30+ | 220 (19.6) | 157 (19.3) | 63 (20.4) | 256 (23.0) | |
| Missing | 11 (1.0) | 8 (1.0) | 3 (1.0) | 11 (1.0) | 0.22 |

PCa, prostate cancer; SD, standard deviation.
[a]Aggressive prostate cancer defined as stage III/IV or Gleason ≥8 or fatal prostate cancer.
[b]*P*-values for continuous variables based on Wilcoxon signed-rank test or *t*-test and chi-square test for categorical variables. Comparison is between all cases and controls.
[c]Sarcosine normalized to alanine × 1000.

more likely to have a family history of prostate cancer, more likely to be never smokers, and less likely to report a history of diabetes compared with controls (Table I).

Table II shows the association between sarcosine and prostate cancer overall and by stage of disease and Gleason score. There was a significantly increased risk of prostate cancer with increasing levels of sarcosine (OR for the highest quartile of exposure [Q4] versus the lowest quartile [Q1] = 1.30, 95% CI: 1.02, 1.65; *P*-trend 0.03). When stratified by disease aggressiveness, there was a stronger association for non-aggressive cases (OR for Q4 versus Q1 = 1.44, 95% CI: 1.11, 1.88; *P*-trend 0.006) than for aggressive prostate cancer (OR for Q4 versus Q1 = 1.03, 95% CI: 0.73, 1.47; *P*-trend 0.89, *P*-heterogeneity = 0.02). Similarly, there were significant associations between sarcosine and lower stage and Gleason score of prostate cancer (Table II).

Stratified associations between sarcosine and prostate cancer overall and by disease aggressiveness are shown in Table III. The association between sarcosine and prostate cancer overall was stronger among men with diabetes (OR = 2.66, 95% CI: 1.04, 6.84) compared with those without reported diabetes (OR = 1.23, 95% CI: 0.95, 1.59, *P*-interaction = 0.01). This difference persisted for non-aggressive disease with a stronger association among men with

**Table II.** Association between sarcosine and prostate cancer in the PLCO Cancer Screening Trial

| Sarcosine | Continuous | Q1 | | Q2 | | Q3 | | Q4 | | *P*-trend |
|---|---|---|---|---|---|---|---|---|---|---|
| | OR[a,b] (95% CI) | Ca/Co | OR | Ca/Co | OR[b] (95% CI) | Ca/Co | OR[b] (95% CI) | Ca/Co | OR[b] (95% CI) | |
| All cases | 1.29 (1.01, 1.66) | 268/278 | REF | 264/278 | 1.03 (0.80, 1.31) | 265/278 | 1.06 (0.83, 1.35) | 325/278 | 1.30 (1.02, 1.65) | 0.03 |
| Non-aggressive | 1.45 (1.11, 1.91) | 180/278 | REF | 193/278 | 1.13 (0.86, 1.48) | 199/278 | 1.19 (0.90, 1.55) | 241/278 | 1.44 (1.11, 1.88) | 0.006 |
| Aggressive[c] | 0.96 (0.65, 1.42) | 88/278 | REF | 71/278 | 0.84 (0.58, 1.21) | 66/278 | 0.78 (0.54, 1.13) | 84/278 | 1.03 (0.73, 1.47) | 0.89 |
| Stage | | | | | | | | | | |
| I/II | 1.38 (1.06, 1.80) | 206/278 | REF | 217/278 | 1.10 (0.85, 1.43) | 224/278 | 1.18 (0.91, 1.53) | 268/278 | 1.40 (1.08, 1.81) | 0.008 |
| III/IV | 0.98 (0.62, 1.54) | 62/278 | REF | 47/278 | 0.79 (0.52, 1.21) | 41/278 | 0.66 (0.43, 1.02) | 57/278 | 0.98 (0.65, 1.47) | 0.81 |
| Gleason | | | | | | | | | | |
| <8 | 1.38 (1.06, 1.79) | 222/278 | REF | 227/278 | 1.07 (0.83, 1.38) | 230/278 | 1.09 (0.85, 1.42) | 283/278 | 1.36 (1.06, 1.74) | 0.005 |
| ≥8 | 0.88 (0.51, 1.51) | 41/278 | REF | 32/278 | 0.80 (0.48, 1.32) | 31/278 | 0.83 (0.50, 1.39) | 38/278 | 1.01 (0.62, 1.64) | 0.91 |

Ca/Co, cases/controls; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4.
[a]OR of prostate cancer per one log unit increase in sarcosine.
[b]Adjusted for age at selection, center, family history of prostate cancer, diabetes and smoking.
[c]Aggressive prostate cancer defined as stage III/IV or Gleason ≥8 or fatal prostate cancer.

**Table III.** Stratified associations between sarcosine and prostate cancer by disease aggressiveness in the PLCO Cancer Screening Trial

| Sarcosine | All cases | | Non-aggressive | | Aggressive[a] | |
|---|---|---|---|---|---|---|
| | Ca/Co | OR[b,c] (95% CI) | Ca/Co | OR[b,c] (95% CI) | Ca/Co | OR[b,c] (95% CI) |
| Baseline diabetes | | | | | | |
| No | 1049/998 | 1.23 (0.95, 1.59) | 761/998 | 1.35 (1.02, 1.80) | 288/998 | 0.92 (0.62, 1.39) |
| Yes | 69/109 | 2.66 (1.04, 6.84) | 48/109 | 3.85 (1.33, 11.18) | 21/109 | 1.30 (0.28, 6.04) |
| *P*-interaction | | 0.01 | | 0.05 | | 0.98 |
| Tobacco smoking | | | | | | |
| Never | 398/313 | 0.73 (0.45, 1.16) | 289/313 | 0.70 (0.42, 1.18) | 109/313 | 0.71 (0.36, 1.43) |
| Former | 545/577 | 1.64 (1.15, 2.35) | 391/577 | 1.94 (1.31, 2.89) | 154/577 | 1.19 (0.69, 2.07) |
| Current | 78/122 | 1.82 (0.79, 4.17) | 60/122 | 1.95 (0.80, 4.76) | 18/122 | 1.47 (0.31, 7.03) |
| *P*-interaction | | 0.03 | | 0.01 | | 0.51 |
| Family history of PCa | | | | | | |
| No | 899/977 | 1.29 (0.99, 1.70) | 645/997 | 1.49 (1.10, 2.00) | 254/997 | 0.92 (0.61, 1.41) |
| Yes | 203/97 | 1.07 (0.52, 2.21) | 154/97 | 1.12 (0.52, 2.40) | 49/97 | 0.94 (0.29, 2.99) |
| *P*-interaction | | 0.90 | | 0.75 | | 0.77 |
| BMI (kg/m$^2$) | | | | | | |
| <25 | 295/279 | 1.13 (0.69, 1.85) | 224/279 | 1.26 (0.74, 2.14) | 71/279 | 0.89 (0.39, 2.02) |
| 25–29 | 596/563 | 1.17 (0.82, 1.66) | 424/563 | 1.29 (0.88, 1.89) | 172/563 | 0.91 (0.53, 1.55) |
| 30+ | 220/256 | 1.74 (0.96, 3.15) | 157/256 | 2.09 (1.07, 4.05) | 63/256 | 0.95 (0.37, 2.45) |
| *P*-interaction | | 0.43 | | 0.33 | | 0.95 |
| Blood draw to diagnosis | | | | | | |
| <2 years | 263/263 | 1.80 (1.07, 3.01) | 216/263 | 1.83 (1.06, 3.15) | 47/263 | 1.74 (0.62, 4.85) |
| 2–3 years | 245/236 | 1.33 (0.77, 2.31) | 188/236 | 1.58 (0.85, 2.94) | 57/236 | 0.82 (0.32, 2.09) |
| 3–5 years | 310/309 | 1.19 (0.74, 1.92) | 227/309 | 1.24 (0.73, 2.09) | 83/309 | 1.05 (0.50, 2.20) |
| >5 years | 303/302 | 1.03 (0.63, 1.70) | 181/302 | 1.30 (0.72, 2.35) | 122/302 | 0.67 (0.33, 1.35) |
| *P*-interaction | | 0.50 | | 0.88 | | 0.27 |
| Age at blood draw | | | | | | |
| 55–59 years | 182/216 | 1.50 (0.84, 2.67) | 125/216 | 1.69 (0.88, 3.24) | 57/216 | 1.30 (0.51, 3.31) |
| 60–64 years | 379/330 | 0.85 (0.55, 1.32) | 270/330 | 0.94 (0.58, 1.53) | 109/330 | 0.59 (0.30, 1.19) |
| 65–69 years | 358/349 | 1.43 (0.90, 2.27) | 263/349 | 1.65 (0.98, 2.78) | 95/349 | 0.98 (0.48, 1.98) |
| 70+ years | 202/217 | 2.15 (1.17, 3.97) | 154/217 | 2.52 (1.31, 4.86) | 48/217 | 1.09 (0.38, 3.14) |
| *P*-interaction | | 0.08 | | 0.13 | | 0.65 |

Ca/Co, cases/controls; PCa, prostate cancer.
[a]Aggressive prostate cancer defined as stage III/IV or Gleason ≥8 or fatal prostate.
[b]OR of prostate cancer per one log unit increase in sarcosine.
[c]Adjusted for age at selection, center, family history of prostate cancer, diabetes and smoking where appropriate.

diabetes (OR = 3.85, 95% CI: 1.33, 11.18, *P*-interaction = 0.05). Adjustment for additional factors, such as BMI, testosterone and C-peptide levels, did not substantially alter the observed association among men with diabetes. When we looked at the association between sarcosine (standardized to alanine) and diabetes, a significant inverse association was observed (OR = 0.53, 95% CI: 0.33, 0.85, for all subjects, data not shown) after adjustment for age at selection, center, family history of prostate cancer, BMI and smoking, whereas the association between alanine and diabetes was elevated (OR = 1.90, 95% CI: 0.98, 3.69, for all subjects, data not shown). Significant interactions were also observed for all cases and non-aggressive cases for smoking status (*P*-interaction = 0.03 and 0.01, respectively) with elevated levels of sarcosine associated with higher risks among former and current smokers but not among never smokers. There were no significant interactions observed between sarcosine and BMI; however, risks tended to be larger among obese subjects consistent with the effect modification observed for diabetes (Table III). Temporal analyses showed a stronger association between sarcosine and prostate cancer when blood was collected closer to diagnosis, with the strongest associations observed when blood was collected within 2 years of diagnosis (OR for all cases = 1.80, 95% CI: 1.07, 3.01; OR for non-aggressive cases = 1.83, 95% CI: 1.06, 3.15). None of these interactions, however, were statistically significant.

To evaluate the predictive ability of sarcosine, we estimated the *C*-statistic for a model including age, family history, study center, smoking history, diabetes history and serum sarcosine. The *C*-statistic for the model containing sarcosine was modest (0.623, 95% CI: 0.600, 0.646) indicating predictive value beyond random chance; however, this was not superior to the model containing PSA level at blood draw (*C*-statistic = 0.850, 95% CI: 0.834, 0.866). Furthermore, the model containing known prostate cancer risk factors, without sarcosine, appeared to contribute to most of the predictive value (*C*-statistic = 0.620, 95% CI: 0.597, 0.643). In addition, the combination of PSA and sarcosine within the same model did not provide better predictive ability compared with PSA alone (*C*-statistic = 0.850, 95% CI: 0.834, 0.866; *P*-value for comparison = 0.76, data not shown).

## Discussion

In this nested case–control study of sarcosine and prostate cancer risk, we observed elevated pre-diagnostic serum sarcosine levels among prostate cases compared with controls after adjustment for confounding variables. This association was observed primarily among non-aggressive prostate cancer cases, with no association among aggressive prostate cancer cases. Further, temporal analyses indicated a stronger association between sarcosine and prostate cancer when blood was collected closer to diagnosis, suggesting that sarcosine may be an early biomarker of disease; however, this was not a statistically significant interaction. Interestingly, for men who reported having diabetes, the association between sarcosine and prostate cancer risk was significantly stronger.

Since the original report by Sreekumar *et al.* (4) linking sarcosine to prostate cancer progression, several studies, mostly cross-sectional, have evaluated sarcosine as a potential biomarker for prostate cancer, and some have evaluated whether sarcosine is etiologically relevant for disease progression. Of these, only four studies used serum; one study ($N < 60$ participants) found no association between serum sarcosine levels and prostate cancer (12). Another study observed no difference in serum sarcosine levels between recurrent versus non-recurrent prostate cancer patients (11). A third study found that serum sarcosine did not distinguish early and advanced stages of prostate cancer (14). A fourth study from Italy showed that median serum sarcosine levels were significantly higher in cases compared with controls when restricted to subjects with a PSA < 4 ng/ml; the authors further identified higher median serum sarcosine levels in this subgroup for men with low and intermediate grade cancer (21). Consistent with the Italian study, we found an association between serum sarcosine levels and non-aggressive prostate cancer (lower stage and grade). In this study, serum sarcosine was associated with a stronger effect when blood was drawn within 2 years of diagnosis with decreasing magnitude of effect as time from blood draw increased. Although the interaction was not statistically significant, this pattern is consistent with an early biomarker of disease. We did not observe an association with aggressive prostate cancer, so it is possible that sarcosine is a marker of only non-aggressive prostate cancer. Due to the PSA screening in the trial, most cases observed closer to blood draw were non-aggressive prostate cancer cases. It is also possible, however, given the modest effect size and small sample of aggressive tumors, there was not enough power to detect an association for aggressive disease. Interestingly, approximately 40% of aggressive cases had blood drawn >5 years from diagnosis. Thus, the lack of association between sarcosine and aggressive prostate cancer could also be due to the under-representation of cases with blood collection close to diagnosis in our sample. These results are somewhat contradictory to the findings of Sreekumar *et al.* (4), which found greater sarcosine levels in metastatic tumor tissue compared with organ-confined disease. Thus, additional work is needed to assess the role of sarcosine in progression and disease severity.

As this study population is part of the screened arm of the PLCO Cancer Screening Trial, we were able to evaluate predictive models with sarcosine and PSA with the caveat that men with PSA levels above 4 ng/ml were referred to their physician for diagnostic work-up as part of the trial. The model containing sarcosine had modest predictive ability to detect incident prostate cancer; however, it was not superior to PSA. It has also been suggested that sarcosine might perform better than PSA when restricting samples to those in the clinical gray zone (PSA 2–10 ng/ml); however, we did not observe this to be the case in our sample (data not shown). Furthermore, additional analyses considering PSA and sarcosine in combination did not improve predictive ability beyond that of PSA. More work is needed, in other populations, to confirm and assess the generalizability of these findings.

We observed a stronger association between elevated serum sarcosine levels and prostate cancer risk among men with a self-reported history of diabetes. Although this could be a spurious finding and replication is needed, the relationship between diabetes and prostate cancer makes this finding intriguing. Studies investigating the association between diabetes and prostate cancer have shown that diabetes is inversely associated with prostate cancer (22–24). Recently, a study evaluating amino acid profiles and diabetes linked higher serum levels of alanine to increased type 2 diabetes risk (*P*-value = 0.04; Supplementary Table 1 of Wang *et al.* (25)); however, sarcosine levels were not evaluated. Similar to Wang e*t al.* (25), we found an increase in the risk of diabetes associated with alanine, suggesting that our findings are not atypical. Decreased testosterone and C-peptide levels (a marker of insulin secretion) have been implicated as mechanisms to explain the decreased prostate cancer risk among men with diabetes (26–28). Adjustment of regression models for these two markers did not change the observed results for the association between sarcosine and prostate cancer overall or for the subgroup of men with diabetes, suggesting that sarcosine may independently predict risk in this subgroup via a different mechanism. Androgens or alterations in cellular methylation have been suggested as intermediaries for sarcosine in the development of prostate cancer (29,30). Still, the mechanism by which sarcosine might be more strongly associated with prostate cancer among diabetic men is unclear. In addition, because both sarcosine and alanine may be related to diabetes, future work to explore the biological mechanisms behind the effect modification observed for prostate cancer may need to consider these two metabolites independently and together.

We also observed a stronger association between serum sarcosine levels and prostate cancer risk among former and current smokers. Smoking has more consistently been linked with prostate cancer mortality rather than with incidence, suggesting that smoking may contribute to prostate cancer progression (31,32). If sarcosine is acting as a marker of disease progression among former and current smokers, this connection would be consistent with the observation by Sreekumar *et al.* (4) showing a similar role for sarcosine in the progression of prostate cancer. The exact mechanism is unclear however, but smoking has been observed to cause aberrant CpG hypermethylation of multiple genes in prostate tissue (33)

and to impact global methylation in other cancer sites (34,35). Thus, it is possible that sarcosine levels may reflect, or perhaps be enhanced by, altered methylation profiles in susceptible subgroups like smokers.

Our study had several strengths and limitations. We observed interactions with diabetes and smoking and some indication that the association between sarcosine and prostate cancer may be restricted to the time closest to diagnosis. However, these subgroup findings need to be replicated to rule out the possibility of chance findings. Because little is known about sarcosine, we explored interactions with a number of factors, which may have led to spurious associations due to multiple testing; further studies are needed to confirm or refute these subgroup findings. In addition, we were unable to differentiate between type 1 and type 2 diabetes or about treatment for diabetes in this study; however, the majority of men reporting positive histories likely had type 2 diabetes based on the age of the participants and prevalence of this condition. Strengths of our study include its prospective design, the use of pre-diagnostic serum and large sample size to evaluate the association between serum sarcosine and prostate cancer. In addition, we have used a validated assay with high sensitivity and reproducibility (35), to accurately quantify sarcosine, a major criticism of previous work. Further, we were able to control and consider a wide range of potential confounding factors due to the detailed information collected in the PLCO cohort on risk factors and PSA screening results.

In conclusion, we identified a positive association between elevated serum sarcosine and prostate cancer in this large, prospective analysis. Our study suggests that serum sarcosine may be an early biomarker of this disease, specifically non-aggressive disease, and may have a stronger effect among men with diabetes and among smokers. These intriguing findings warrant additional follow-up in other large prospective studies and in other race/ethnic groups.

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