Profiles of circulating fatty acids are population-specific and linked to prostate cancer

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Article

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Abstract

High fatty acid intake is thought to increase cancer risk. This relationship remains poorly explored in African-descent populations. We examined 24 circulating fatty acids in 2,934 men, including 1,431 prostate cancer cases and 1,503 population controls from Ghana and African Americans and European Americans from the United States, using CLIA-certified mass spectrometry-based assays. We investigated associations with prostate cancer, lifestyle factors, and the fatty acid desaturase (FADS) genetic locus. Levels of circulating fatty acid varied robustly between the three population groups, particularly trans, omega-3 and omega-6 fatty acids. Yet, trans fatty acids, namely elaidic, palmitelaidic, and linoelaidic acids, whose levels were higher in populations from the United States compared to Ghanaian men, were associated with increased odds of prostate cancer among all men. FADS1/2 germline genetic variants and lifestyle explained some of the variation in fatty acid levels, with the FADS1/2 locus showing population-specific associations, suggesting differences in genetic control.

Introduction

Prostate cancer has a high global incidence and mortality\(^1,2\). Still, the factors that cause prostate cancer remain incompletely understood. Men of African ancestry, including African American (AA) and Ghanaian men, have a disproportionately higher burden of lethal prostate cancer when compared to European American (EA) men\(^1,3\). This increased mortality burden could be partially attributed to unique risk factor profiles by population group, as well as distinct inflammatory and immune responses that drive cancer aggressiveness and impact survival, as we have shown\(^4\).

The role of fatty acids in prostate cancer has been studied extensively, but the observations are conflicting, and a consensus of the effects of fatty acids on prostate cancer risk has yet to be achieved\(^5,6\). Previous studies have identified relationships between certain fatty acid structural groups and prostate cancer. A case-cohort study examining associations between plasma phospholipid fatty acids and prostate cancer risk among participants in the Selenium and Vitamin E Cancer Prevention Trial (SELECT) found an increased prostate cancer risk among men with high circulating concentrations of long-chain omega-3 polyunsaturated fatty acids\(^7,8\). Additional investigations have shown relationships between saturated fatty acids and the risk of advanced or fatal prostate cancer\(^9\). These studies support the involvement of fatty acids in prostate tumorigenesis, although racial/ethnic differences in fatty acid intake were not explored in these large studies because of a lack of diversity in the study populations.

The goal of our study was therefore to characterize the relationship between circulating fatty acids and prostate cancer in the ethnically diverse NCI-Maryland and NCI-Ghana Prostate Cancer Case-Control studies, with an over-representation of men of African descent. In addition, we explored how circulating fatty acid levels may relate to demographic, lifestyle, and germline genetic factors, and to an immune-oncology marker profile that we previously obtained for all men in the above studies. With this approach, we observed disparate patterns in circulating fatty acid levels among the participants from the three population groups and report relationships of the fatty acid levels with germline genetic variants in the
FADS1/2 locus, diet, clinico-socio-demographic characteristics, and immune-oncology marker-defined pathways. Importantly, we also observed a consistent association of circulating trans fatty acid levels with increased odds of prostate cancer.

Results

Serum fatty acid levels are different between Ghanaian men and men from the United States

It was the aim of our study to gain knowledge whether serum fatty acid profiles and their association with prostate cancer are different between Ghanaian, AA, and EA men. We utilized two case-control studies with an overrepresentation of men of African ancestry: the NCI-Ghana and NCI-Maryland Prostate Cancer Case-Control Studies. Both studies have been previously described\(^\text{10-12}\). Participant characteristics are shown in Supplementary Table 1. A CLIA-certified, mass spectrometry-based assay was applied to measure concentrations of 24 fatty acids (Supplementary Table 2) in sera from 2,934 participants, including 1,431 prostate cancer cases (585 Ghanaian, 407 AA, 439 EA) and 1,503 population controls (658 Ghanaian, 381 AA, 464 EA). To control for any batch effects, the serum samples were assayed in a random order along with 5% blind duplicates. All 24 fatty acids were detected in 100% of the samples tested.

To uncover global differences in circulating fatty acid profiles between the three groups of men in our study, we applied unsupervised hierarchical clustering to examine how the levels of the 24 fatty acids may associate with population groups (Fig. 1). We performed this analysis separately for controls (Fig. 1a) and cases (Fig. 1b). Among the controls, fatty acid levels tended to cluster by population group, with marked differences between Ghanaian, AA, and EA participants. A similar pattern was observed among cases, but the separation into Ghanaian men as one group and the AA and EA men as the other group was not as robust. These findings are consistent with the observed differences in median absolute concentrations for the 24 fatty acids between Ghanaian, AA, and EA healthy controls based on group comparisons, with Bonferroni-corrected significance testing to address multiple comparisons (Supplementary Table 3).

In another approach to characterize dissimilarities in circulating fatty acids between these groups of men, we investigated differences in fatty acid levels after grouping them into five structurally distinct classes: saturated, trans, cis-monounsaturated, omega-3, and omega-6 fatty acids. An initial analysis showed that circulating levels of these fatty acid classes are disparate between Ghanaian, AA, and EA healthy controls (Supplementary Table 4). We then compared one group of men to another (i.e., Ghanaian vs. AA, Ghanaian vs. EA men, or AA vs. EA men) and calculated fatty acid level ratios from these comparisons (Fig. 1c-e). The analysis was performed for both controls and cases and particularly highlights the significantly higher concentrations of circulating omega-3 fatty acids in Ghanaian men, among both controls and cases, when compared to EA and AA men (Figs. 1c and d). A more in-depth statistical evaluation of these comparisons that also included the serum total fatty acid level as an additional variable and Bonferroni adjustments can be found in Supplementary Table 5. In contrast to the omega-
3 fatty acid observations, trans and omega-6 fatty acid levels were consistently higher in EA and AA men, in both controls and cases, when compared to Ghanaian men (Figs. 1c and d). We did not find these stark differences in an analysis that compared EA with AA men (Fig. 1e). However, trans and cis monounsaturated fatty acid levels tended to be higher in EA compared to AA men among both controls and cases. Lastly, due to the elevated concentration of circulating omega-3 fatty acids in Ghanaian men, together with their rather low serum concentrations of omega-6 fatty acids, these men had the lowest omega 6:3 fatty acid ratio. Notable, the omega 6:3 fatty acid ratio may have implications for prostate cancer progression. A low ratio has been associated with a delay in such progression\textsuperscript{13}.

**Association of socio-demographic and clinical characteristics with circulating fatty acids**

We investigated the association of various socio-demographic and clinical characteristics that have been reported to be associated with prostate cancer (age, BMI, education, smoking, diabetes, and aspirin use) with serum levels of circulating fatty acids using a multivariable linear regression model, with adjustment for multiple comparisons (Fig. 2, Supplementary Data 1). Aspirin use and education level had only few relationships with circulating fatty acids. Levels of saturated fatty acids tended to be negatively associated with age among EA men while omega-6 fatty acids were negatively associated with age in both AA and EA men (Fig. 2). However, there was no relationship with age among the Ghanaian men. In contrast, BMI strongly associated with most of the circulating fatty acids among these men, but less so among US men. Lastly, smoking showed positive associations with two omega-6 fatty acids, namely docosapentaenoic-n6 and docosatetraenoic acids, among Ghanaian, AA, and EA men.

**Association of dietary factors with circulating fatty acids**

Next, we examined the relative contribution of diet to the concentration of circulating fatty acids. Previously, we collected nutritional data via a brief supplemental questionnaire from participants in the NCI-Maryland study. We applied variance analysis to evaluate the relationship between concentrations of individual fatty acids and diet using the questionnaire data on meat and fat consumption (Supplementary Data 2-4). Few notable associations were observed. In agreement with the literature, fish consumption was significantly associated with the variability of omega-3 and omega-6 fatty acid levels (Supplementary Fig. 1, Supplementary Data 2-4), accounting for 6.7% and 4.2% of the variability in the levels of docosahexanoic acid in AA cases and controls, and 7.2% and 12.3% of the variability in EA cases and controls, respectively (Supplementary Fig. 1, Supplementary Data 2-4). Frequent intake of bacon fat or drippings during the 2-year period prior to interview significantly, albeit modestly, explained the variability in the concentration of all three trans fatty acids only among cases: palmitelaidic (AA cases: 4.5%, EA cases: 5.0%), elaidic (AA cases: 2.9 %, EA cases: 3.7%), and linoelaidic (AA cases: 2.8%, EA cases: 3.9%) (Supplementary Fig. 2, Supplementary Data 2 and 4). Thus, dietary differences appear to account for at least some of the variability in the concentration of circulating fatty acids in our cohorts, yet the detected effect sizes were small.

**Association of fatty acid desaturase 1 and 2 (FADS1/2) locus with circulating fatty acid levels**
It has been shown that the levels of circulating fatty acids are partly under genetic control\textsuperscript{14,15}. Thus, we examined how germline genetic factors may relate to fatty acid concentrations in our diverse cohorts focusing on key examples from the published literature. We concentrated our efforts on single nucleotide polymorphisms in the \textit{FADS1/2} locus that have been found to influence fatty acid levels in humans\textsuperscript{14,15}. In European descent individuals, this gene cluster has been shown to have the strongest effect among all genetic loci on the levels of certain fatty acids, namely omega-3 and omega-6 polyunsaturated fatty acids. We selected three SNPs covering the \textit{FADS1/2} locus (\textit{Supplementary Table 6}). We then tested if the levels of each of the 24 fatty acids in blood circulation are influenced by the selected SNPs in our cohort. We found that the SNPs had significant associations with the levels of several omega-6 fatty acids including arachidonic, dihomo-gamma-linolenic, docosatetraenoic (or adrenic), and $\gamma$-linolenic acids in EA men, consistent with the literature, explaining up to 19% of their variance in this population. However, with the exception of rs174556 SNP in \textit{FADS1} gene explaining a small fraction of the variability (4.7%) in circulating arachidonic acid level among AA cases, these SNPs did not influence the levels of omega-6 fatty acids in AA or Ghanaian men (\textit{Figs. 3 and 4, Supplementary Fig. 3, Supplementary Tables 7-12}). The observation suggests population differences in the genetic control of circulating fatty acids.

\textbf{Association of fatty acid concentrations with prostate cancer across population groups}

We next assessed the association of individual fatty acids and fatty acid classes with prostate cancer in all men, and then stratified by population group (\textit{Table 1, Supplementary Tables 13 and 14}). In all men combined and across the three population groups, only \textit{trans} fatty acids, including elaidic, palmitelaidic, and linoelaidic acids, were consistently associated with increased odds of having prostate cancer. This key finding and a few other associations are further summarized in \textit{Fig. 5}, emphasizing how the three \textit{trans} fatty acids – elaidic, palmitelaidic, and linoelaidic acids – are associated with prostate cancer across Ghanaian, AA, and EA men.

The link between \textit{trans} fatty acids and prostate cancer was further investigated using multivariable logistic regression analyses with adjustments for potential confounders. Here, we divided each \textit{trans} fatty acid concentration into tertiles, termed low, intermediate, and high, and assessed the associations with prostate cancer across the three population groups (\textit{Table 2}). We found a significant dose-dependent increase in the odds of having prostate cancer with increasing elaidic, palmitelaidic, and linoelaidic fatty acid concentrations in all three population groups. Notably, although Ghanaian men were found to have the lowest mean \textit{trans} fatty acid concentration when compared to AA and EA men (\textit{Supplementary Fig. 4}), they still experienced significantly increased odds of developing prostate cancer with increasing \textit{trans} fatty acid levels.

To assess if fatty acid levels were associated with disease severity, we correlated individual fatty acids and fatty acid classes with National Comprehensive Cancer Network (NCCN) risk scores that describe disease severity\textsuperscript{16}, which were obtained for the AA and EA patients in the NCI-Maryland study\textsuperscript{4}. In this analysis, only palmitoleic acid showed a positive dose-dependent relationship with increasing NCCN risk scores, even after adjusting for multiple testing ($P_{trend} = 0.002$, \textit{Supplementary Table 15}).
Relationship between circulating immune-oncological markers and fatty acids

Circulating fatty acids may influence the immune environment. For the NCI-Maryland and NCI-Ghana studies, 82 immune-oncological markers have previously been measured and grouped into pathways: apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), vasculature. Thus, we assessed whether there was a relationship between the immune-oncology marker-defined pathways and circulating fatty acid levels. For this analysis, we grouped the fatty acids into the five classes as already described and assessed the relationships separately for men with and without prostate cancer. The correlation heat maps for our control population revealed significant positive correlations between circulating omega-3 fatty acid levels and the immune-oncology marker-defined pathway activity scores, with the only exception being autophagy (Fig. 6a, Bonferroni-corrected $P < 0.01$). We further observed significant negative correlations between trans fatty acids, omega-6 fatty acids, and the omega 6:3 ratio and the pathway activity scores representing apoptosis, chemotaxis, inflammation, and tumor immunity (Fig. 6a, Bonferroni-corrected $P < 0.01$ for all). Similar relationships were also found among men with prostate cancer: here omega-3 fatty acid levels positively correlated with almost all immune-oncological pathways whereas the omega 6:3 ratio negatively correlated with pathway activities representing chemotaxis, inflammation, tumor immunity, and vasculature (Fig. 6b; Bonferroni-corrected $P < 0.01$ for all). The observations suggest generally increased immune-oncology marker-defined pathway activities when circulating omega-3 fatty acid levels are high or the omega 6:3 ratio is low, and decreased pathway activity scores when levels of circulating trans and omega-6 fatty acids are elevated.

Discussion

Our study is the first to comprehensively characterize circulating fatty acid levels across three distinct population groups, Ghanaian, AA, and EA men, and to assess their associations with prostate cancer. Circulating fatty acid levels are surrogates for their prostate tissue concentrations, as it was shown recently. Here, we report that circulating fatty acid levels are different between Ghanaian men and AA and EA men from the United States, particularly trans, omega-3 and omega-6 fatty acids. This may relate to differences in diet, with a lower intake of trans fatty acids among Ghanaian men since they eat fewer processed foods. Yet, as a main finding, our study suggests a significant positive association of trans fatty acid intake with prostate cancer in these three groups of men. Another key observation indicates formerly unrecognized population differences in the genetic control of circulating fatty acids. Multiple studies have shown that common genetic variants in the FADS1/2 gene cluster exert a significant effect on the circulating levels of polyunsaturated fatty acids in European descent populations. While we replicated these findings for omega-6 fatty acids among EA men in our study, we did not find the same variants to be associated with omega-6 fatty acids in either AA or Ghanaian men.

Serum fatty acid levels were different between Ghanaian men and AA and EA men from the United States, with omega-3 fatty acids being consistently elevated in Ghanaian men, in both controls and cases,
whereas *trans* and omega-6 fatty acid levels were consistently higher in EA and AA, in both controls and cases. We did not find these differences comparing AA with EA men, suggesting that most of the fatty acid-related disparities between Ghanaian men and men from the United States are not due to ancestral genetic factors, but rather due to food intake differences. Still, with our food intake survey data for the men in the NCI-Maryland study, we could not identify a food source that robustly associated with circulating fatty acid levels. Our survey may not have captured well the increased intake of processed food in the United States, which is known to be a major source of *trans* fatty acids. The omega-3 fatty acids that were elevated in Ghanaian men are typically plant- or marine-derived. They are important ingredients of a healthy, traditional diet and are thought to have anti-inflammatory effects that provide cardiovascular benefits. Their potentially beneficial role in cancer development remains uncertain and an association with prostate cancer has not been established. We are not aware that others have described differences in circulating fatty acid levels between Ghanaian men and men from the United States. However, one study reported plasma fatty acid levels for 48 AA cases and 96 controls and 66 Nigerian cases and 226 controls. While not as comprehensive in design as our study, the authors also reported that *trans* and ω-6 fatty acid levels were higher in AA men than in the indigenous African men, here Nigerian men, whereas the omega-3 fatty acid levels were elevated in the Nigerian men, consistent with our findings.

We observed that circulating elevated *trans* fatty acid levels were associated with increased odds of prostate cancer in the three distinct population groups of Ghanaian, AA, and EA men, identifying *trans* fatty acids as potential prostate cancer risk factors independent of ancestry or geographic location. This observation agrees with the findings from a recent meta-analysis linking elevated *trans* fatty acids to an increased risk of prostate cancer. *Trans* fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration, and are commonly found in fast foods, highly processed snacks, baked goods, and hydrogenated oils such as margarine. As these foods are common in the Western diet, high consumption of *trans* fatty acids have long been studied as a risk factor for cancer and adverse cardiovascular events. Our study identified significant and consistent associations with prostate cancer for all *trans* fatty acids (16:1, 18:1, 18:2) tested across our three population groups. Multiple investigators have studied the association of these *trans* fatty acids with prostate cancer and most, but not all, reported positive associations. A study from the β-Carotene and Retinol Efficacy Trial (CARET), examining the association between individual *trans* fatty acids and prostate cancer risk, showed that C:18 *trans* fatty acids, such as elaidic and linoelaidic acids, were associated with increased prostate cancer risk, but they did not see a similar association in C:16 *trans* fatty acids.

Fatty acid levels are under genetic control. Although the dietary intake of fatty acids is a key factor in determining their levels in our body, multiple genome-wide association studies have shown that polymorphic genetic loci can strongly influence the levels of certain fatty acids in circulation. We found that SNPs in the *FADS1/2* locus explain up to 19% of the variability of serum arachidonic acid levels, consistent with prior reports. However, we could not find an association between the same SNPs
and arachidonic acid or other omega-6 fatty acids in either AA or Ghanaian men. Previously, a SNP in *FADS1* (rs174548) was shown to be associated with omega-3 fatty acid levels across different ancestries, but data for omega-6 fatty acid levels were not provided by these authors\(^1\). The lack of association in our study is not explained by a lack of statistical precision in estimation, as the AA and Ghana cohorts were similar sized or even larger than the EA cohort, and two of the studied SNPs (rs174577 and rs174583) did not show large differences in their genotype distribution between the three cohorts (Supplementary Table 6). In contrast, the fatty acid level-associated rs174556 SNP was indeed more common in EA men. Noteworthy, arachidonic acid can be further metabolized by the cyclooxygenase and 5-lipoxygenase enzymes, resulting in the synthesis of pro-inflammatory eicosanoids. One eicosanoid, the pro-metastatic thromboxane A2, has recently been linked by our group to an increased risk of lethal prostate cancer among African American men\(^3\). Nonetheless, the *FADS1/2* locus has not been previously linked to prostate cancer in most studies\(^3\)

*Trans* fatty acids have been widely shown to be strongly associated with systemic inflammation, including increased levels of inflammatory cytokines such as interleukin (IL)-1β, IL-1, IL-6, tumor necrosis factor 1/2, and monocyte chemoattractant protein 1 in patients with heart failure\(^4\). In contrast, other studies reported that high intake of *trans* fatty acids have little or no association with inflammatory markers\(^5,^6\). We investigated whether circulating fatty acid levels associate with serum proteome-defined pathways, namely apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), and vasculature. We found that omega-3 fatty acid levels may influence these pathways by increasing their activities. In contrast, *trans* and omega-6 fatty acid levels appeared to have opposite effects. Because our study observed only a weak negative correlation between *trans* fatty acids and these pathways, mostly in the control population, any *trans* fatty acid-mediated effects may only partially, if at all, contribute to the positive association between *trans* fatty acids and prostate cancer.

Our study has strengths and limitations. The major strength is the large sample size, the measurement of 24 fatty acids with CLIA-certified technology, and the inclusion of men from Ghana and the United States, with both AA and EA men in the latter cohort. For the fatty acid measurements, we collected blood samples from all participants. Although blood sample collection in Ghana adhered to a protocol that followed standards of practice in the United States, serum preparation methods and shipping may have influenced the performance of the fatty acid measurements. Nevertheless, the differences in fatty acid levels comparing men from the United States with men in Ghana in our study were consistent with the differences previously reported for AA men in the United States in comparison to Nigerian men\(^7\).

In conclusion, our findings point to a population-specific fatty acid profile that may impact prostate cancer development. Elevated serum *trans* fatty acid levels were associated with increased odds of prostate cancer in the Ghanaian, AA, and EA men, thereby identifying *trans* fatty acids as potential prostate cancer risk factors, independent of ancestry or geographic location. Although the extent to which fatty acids are prostate cancer risk factors remains controversial, the relationship between fatty acids,
immune function, and prostate cancer, specifically in men of African descent, merits further exploration to determine causal relationships.

**Methods**

NCI-Maryland prostate cancer case-control study. This study has been previously described\textsuperscript{10,11}. It was designed to examine the contribution of environmental exposures and ancestry-related factors to the excessive prostate cancer burden among AA men in the Baltimore metropolitan region. The study was approved by the NCI (protocol # 05-C-N021) and the University of Maryland (protocol #0298229) Institutional Review Boards, and all participants signed an informed consent. Men with prostate cancer were recruited at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center. A total of 976 cases (489 AA and 487 EA men) were enrolled into this study between 2005 and 2015. Controls were identified through the Maryland Department of Motor Vehicle Administration database and were frequency-matched to cases on age and race. A total of 1,033 population controls were recruited (485 AA and 548 EA men). At the time of enrollment, both cases and controls were administered a survey by a trained interviewer and a blood sample was collected. Serum samples were available for 846 cases (407 AA and 439 EA) and 845 controls (381 AA and 464 EA), and therefore only these individuals contributed to this study. Most of the 846 cases (85\%) were enrolled within a year of the disease diagnosis, with a median of 5.1 months between disease diagnosis and blood collection.

NCI-Ghana prostate cancer case-control study. This case-control study has been previously described\textsuperscript{12}. It was designed to study lifestyle, environmental, and genetic risk factors for prostate cancer in indigenous African men. The study was approved by Institutional Review Boards at the University of Ghana (protocol #001/01–02) and at the National Cancer Institute (protocol #02CN240). Prior to study enrollment, all participants signed an informed consent. Prostate cancer patients were recruited at Korle Bu Teaching Hospital in Accra, Ghana between 2008 and 2012. The cases were diagnosed using Digital Rectal Exam (DRE) and PSA tests, followed by biopsy confirmation. Immediately after diagnosis and before treatment, cases were consented and asked to submit a blood specimen and questionnaire data. Controls were identified through probability sampling using the 2000 Ghana Population and Housing Census data to recruit approximately 1,000 men aged 50–74 years in the Greater Accra region between 2004 and 2006. These men were confirmed to not have prostate cancer by PSA testing and DRE. Serum samples were available for 585 prostate cancer cases and 658 population controls; hence, only these individuals were used for the study herein.

Serum sample processing. The participants in the two studies provided blood samples at time of recruitment. For the NCI-Maryland study, most blood samples were processed the same day, but always within 48 hours, after storage in a refrigerator. For the NCI-Ghana study, blood samples were processed within 6 hours. In this study, only population controls provided overnight-fasting blood. Serum was prepared using standard procedures and aliquots were stored at -80\textdegree{} C. Serum samples were shipped from Ghana to the NCI in dry ice boxes.
Serum fatty acid measurement. Absolute (µg/mL) serum concentrations of 24 fatty acids were measured using gas chromatography (GC) with flame ionization detection by a CLIA-certified laboratory, OmegaQuant Analytics, using the following procedure. Serum (and an internal standard) was transferred to a screw-cap glass vial, dried down with a speed-vac and BTM (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v) (Sigma-Aldrich, St. Louis, MO) was added. The vial was briefly vortexed and heated in a hot bath at 100°C for 45 minutes. After cooling, hexane (EMD Chemicals, USA) and HPLC grade water was added, the tubes were recapped, vortexed and centrifuged help to separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness:Supelco, Bellefonte, PA). Fatty acids were identified by comparison with a standard mixture of fatty acids (GLC OQ-A, NuCheckPrep, Elysian, MN) which was also used to determine individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); trans (16:1, 18:1, 18:2); cis monounsaturated (16:1, 18:1, 20:1, 24:1); cis n-6 polyunsaturated or omega-6 (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); and cis n-3 polyunsaturated or omega-3 (18:3, 20:5, 22:5, 22:6) (Supplementary Table 2). The chromatographic conditions used in this study were sufficient to isolate the C16:1 trans isomers and the C18:2 Δ9t-12c, 9t-12t, and 9c-12t isomers, which was reported as C18:2n6t. However, each individual C18:1 trans molecular species (i.e., C18:1 Δ6 thru Δ13) could not be segregated but appeared as two blended peaks that eluted just before oleic acid. The areas of these two peaks were summed and referred to as C18:1 trans. The serum samples from the NCI-MD study (846 cases and 845 controls) and the NCI-Ghana study (585 cases and 658 controls) were completely randomized and assayed in that order. In addition to the built-in internal controls, 5% blinded duplicates were randomly selected and were randomized along with the original set of samples. The median Coefficient of Variation (CV) calculated based on the 156 blind duplicates was 5.1% across the 24 fatty acids where the CVs among duplicates for 20 out of the 24 markers were <15% (Supplementary Table 16). Fatty acids were assessed individually as well as grouped into five distinct chemical classes/structural groups: saturated, trans, cis-monounsaturated, omega-3, and omega-6 fatty acids (Supplementary Table 2). OmegaQuant Analytics provided measurements for the 24 fatty acids and assigned them a classification (saturated, trans, etc.). We created an abundance level score for each class by adding together concentrations of individual fatty acids that were grouped together (e.g., trans fatty acid class includes the combined concentrations for elaidic, palmitelaidic, and linoelaidic acids). Total fatty acids and the omega 6:3 ratio, an important indicator of dietary health\textsuperscript{13,38}, were also evaluated.

Association of clinical/socio-demographic characteristics with circulating fatty acids. In similar fashion to our previous proteomic study on these cohorts\textsuperscript{4}, we assessed the associations of age, BMI, education, smoking, diabetes, and aspirin use with the abundance of individual circulating fatty acids (as continuous values) by means of multivariable linear regression models implemented by the function lm in the base R package stats (version 3.6.1). For each fatty acid, we fitted the formula “fatty acid ~ age + bmi + education + smoking + diabetes + aspirin”, which yielded the model's F-statistic and associated F-statistic p value, as well as the intercept and regression coefficients with their associated standard errors.
(SE) and $P$ values. F-statistic $P$ values were adjusted by FDR across all models; moreover, within each model, regression coefficient $P$ values were also FDR-adjusted. Full regression results for each cohort are provided as Supplementary Data 1. A fatty acid (as response variable) was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded an FDR-adjusted $P$ value $< 0.05$ on the F-statistic. If this condition was satisfied, the association between the target fatty acid and each individual covariable was characterized by the corresponding FDR-adjusted $P$ value and coefficient.

Serum protein measurement. Serum levels of 82 immuno-oncology panel proteins were measured simultaneously using a proprietary multiplex Proximal Extension Assay (PEA) by Olink Proteomics (Boston) as previously described\textsuperscript{11}. Briefly, serum samples from NCI-MD study (846 cases and 845 controls) and NCI-Ghana study (585 cases and 658 controls) were completely randomized and were assayed in that order. 5% blinded duplicates were randomly selected and were randomized along with the original set of samples. Olink utilizes a relative quantification unit, Normalized Protein eXpression (NPX), which is in a log$_2$-format. Protein levels were intensity normalized to adjust for batch effects. Ninety-five percent of the samples passed a stringent quality control (NCI-MD study: 819 cases and 828 controls; NCI-Ghana study: 489 cases and 654 controls) – with coefficients of variation (CV) among duplicates at $< 10\%$ for every marker.

Functional annotation of serum proteins and biological processes scores. Proteins were grouped into six biological processes as previously described (i.e., apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), and vasculature\textsuperscript{11}. A description of the 82 immuno-oncology proteins and their assignment to pathways can be found in Supplementary Table 17. Apoptosis, autophagy, chemotaxis, suppression of tumor immunity, promotion of tumor immunity, or vasculature scores were calculated for each study participant as the mean z-score value for the proteins belonging to the respective biological process. The association between each immune-oncological biological pathway and fatty acid class were evaluated using pairwise linear correlation tests.

Analysis of variance. Variance analysis for the levels of each of the 24 fatty acids were simultaneously assessed as a function of demographic, clinical, and genetic factors in men with prostate cancer from the NCI-Maryland and NCI-Ghana studies. Data on dietary intake was available only for the participants of the NCI-Maryland study. The analyses were implemented by the function aov in the base stats R package (version 3.6.1).

FADS1/2 locus and circulating fatty acid levels. We assessed the association of germline genotypes in the fatty acid desaturase 1 and 2 locus ($FADS1$, rs174556; $FADS2$, rs174577 and rs174583) with the levels of the 24 individual fatty acids. The genotyping data came from the Infinium HumanOmni5-Quad BeadChip array and were generated for the NCI-Maryland and NCI-Ghana studies at the Cancer Genomics Research Laboratory/NCI-Leidos, a genotyping core facility with NCI-DCEG. All samples passed stringent quality control measures for GWAS data. Data for the NCI-Ghana study have previously been reported\textsuperscript{12}. 

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FADS1/2 genotypes were selected based on both coverage of the FADS1/2 locus by the array and literature reports showing that this genetic locus and the genotypes influence circulating fatty acid levels in European descent subjects\textsuperscript{14,15}. More information about the selected single nucleotide polymorphisms (SNPs) and their frequency by population group can be found in Supplementary Table 6.

Unsupervised Hierarchical Clustering of Fatty Acids. Heat maps and dendrograms showing unsupervised hierarchical clustering of 24 individual fatty acids were generated using JMP 14.0.

Statistical analysis. Data analyses were performed using Stata/SE 16.0, JMP 14.0, and R statistical packages. All statistical tests were two-sided. An association was considered statistically significant with $P < 0.05$ or Bonferroni-corrected significance threshold in instances where correction for multiple testing was required. Student's t-tests were used to compare fatty acid mean concentrations by population group. Unconditional logistic regression was used to compute the odds ratios (OR) and 95% confidence intervals (CI) to assess the association of circulating levels of fatty acids with prostate cancer. All tests used continuous fatty acid concentration data unless otherwise noted. We adjusted for potential confounding factors: age at study entry (years), body mass index at study enrollment (BMI, kg/m2), education (high school or less, some college, college, professional school), diabetes(yes/no), smoking history (never, former, current), and aspirin use (regular user, yes/no) where appropriate.

Declarations

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References


Tables

Tables 1-2 are available in the supplementary files section.

Figures
Circulating fatty acid levels by fatty acid class and population group in the NCI-Maryland and NCI-Ghana cohorts. Heatmaps depicting unsupervised hierarchical clustering using absolute (mean, μg/mL) concentrations from individual fatty acids in (a) controls (n=1,503) and (b) cases (n=1,431) with categorization into three population groups. Green indicates lower concentrations and red indicates higher concentrations for each participant. Samples were labeled by population group and includes
Ghanaian (red, n=1,243), AA (green, n=788), and EA (blue, n=903) men. Individual fatty acids were color-coded by fatty acid class. Red boxes indicate areas of fatty acid concentrations that significantly differ by population group; (c-e) show ratios of mean concentrations by fatty acid class for controls (blue) and cases (red) comparing (c) Ghanaian vs. EA, (d) Ghanaian vs. AA, and (e) AA vs. EA men. Student's t-test was used for significance testing of differences in fatty acid levels (by class) between population groups (see Supplemental Table 5 for Bonferroni adjustment). *, significantly different. AA = African American, EA = European American.

Figure 2

Association of socio-demographic and clinical characteristics with circulating fatty acids in Ghanaian, AA, and EA men with and without prostate cancer. The association of the 24 fatty acids (as continuous variables) with age, BMI, education, smoking, diabetes, and aspirin use was assessed in prostate cancer cases [Ghanaian (n=585), AA (n=407), and EA (n=439)] and controls [Ghanaian (n=658), AA (n=381), and EA (n=464)] using a multivariable linear regression test. *P* values were adjusted for multiple comparisons. An analyte was considered significantly associated with socio-demographic and clinical
covariables if the multivariable model yielded a false discovery rate (FDR)-adjusted $P < 0.05$ on the F-statistic. Blue represents a negative association while red represents a positive association. The significance level (FDR-adjusted two-sided $P$ value-based) for each association is color-coded. AA = African American, EA = European American.

Figure 3

**Influence of a single nucleotide polymorphism (SNP) in the FADS1 gene on circulating levels of fatty acids.** Variance analysis for the levels of each of the 24 fatty acids are assessed as a function of a SNP (rs174556) in FADS1 among (a) Ghanaian controls (n=602) and cases (n=511), (b) AA controls (n=350) and cases (n=344), and (c) EA controls (n=394) and cases (n=362). The red plot represents the proportion of variance in the levels of the fatty acids that can be explained by rs174556 while the grey plot represents the residual variance that remains to be explained by other factors. Asterisks (*) indicate variances in fatty acid levels that are significantly explained by the SNP. Bonferroni-adjusted significance threshold of $P = 0.002$ is used to account for multiple testing. The fatty acids are color-coded to indicate their fatty acid class. AA = African American, EA = European American.
Influence of a single nucleotide polymorphism (SNP) in the *FADS2* gene on circulating levels of fatty acids. Variance analysis for the levels of each of the 24 fatty acids are assessed as a function of a SNP (rs174583) in *FADS2* among (a) Ghanaian controls (n=602) and cases (n=511), (b) AA controls (n=350) and cases (n=344), and (c) EA controls (n=394) and cases (n=362). The red plot represents the proportion of variance in the levels of the fatty acids that can be explained by rs174583 while the grey plot represents the residual variance that remains to be explained by other factors. Asterisks (*) indicate variances in fatty acid levels that are significantly explained by the SNP. Bonferroni-adjusted significance threshold of $P = 0.002$ is used to account for multiple testing. The fatty acids are color-coded to indicate their fatty acid class. AA = African American, EA = European American.
Figure 5

Volcano plot highlighting circulating fatty acids with the most significant differences in their serum levels comparing cases with controls. Shown are Volcano plots defined by fold difference between cases and controls (log2 [fold change]) (x-axis) and the P-value from a Student's t-test (-log10 [p-value]) (y-axis) assessing differences for (a) Ghanaian (n=1,243), (b) AA (n=788), and (c) EA (n=903) men. The horizontal dashed line represents a Bonferroni-adjusted P-value ($P_B$) of 0.002 with differences above this.
line being significant after adjustment for multiple comparisons. The vertical dashed line represents a fold change of 1, meaning the fatty acid levels are equal in cases and controls. Fatty acids found to the right of the vertical dashed line are elevated in the cases whereas those to the left are lower among them when compared to the controls. Only the names of the fatty acids that are significantly elevated in cases are shown. FC= Fold Change, AA = African American, EA = European American.

Figure 6

Correlations between serum proteome-defined pathway activity scores and levels of circulating fatty acids grouped by class. Heat maps detailing the correlations between seven pathway activities and seven fatty acid classes in (a) controls (n=1,503) and (b) cases (n=1,431). Red = positive correlation, green = negative correlation. Pairwise linear correlation tests were used to investigate the associations, with asterisks (*) indicating significant relationships using a Bonferroni corrected $P$ value of 0.007 as threshold. For the definition of pathway activity scores see methods.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf
- SupplementaryData1.xlsx
- SupplementaryData2.xlsx
- SupplementaryData3.xlsx
- SupplementaryData4.xlsx
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