Serum Levels of Phytanic Acid Are Associated With Prostate Cancer Risk

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BACKGROUND. Recent findings of over-expression of the *AMACR* gene in prostate cancer and association between sequence variants in the *AMACR* gene and prostate cancer risk, along with the well established findings of association between prostate cancer risk and over-consumption of dairy products and red meat, indirectly suggest that phytanic acid, which primarily comes from dietary intake of dairy and red meat and requires the AMACR enzyme for its metabolism, may be associated with prostate cancer risk. In this small case-control study, we assessed the association between phytanic acid levels and prostate cancer risk.
METHODS. One hundred and four prostate cancer patients and controls were recruited in North Carolina. Serum levels of phytanic acid were measured using a gas liquid chromatography/mass spectroscopy analysis, and a food frequency questionnaire was administered to each individual to assess dietary intake.
RESULTS. Three key findings are reported. First, there was a high correlation between two independent measurements of phytanic acid levels from the same individuals and the levels of phytanic acid were within the expected range, suggesting that serum levels of phytanic acid levels can be reliably measured in large epidemiological studies. Second, serum levels of phytanic acid among prostate cancer patients were significantly higher than that of unaffected controls, suggesting an association between phytanic acid and prostate cancer risk. Lastly, there was a significantly positive correlation between serum levels of phytanic acid and dietary intake of dairy and red meat servings during the year prior to the serum measurement.
CONCLUSIONS. Although the results from our study suggest phytanic acid levels may be associated with prostate cancer risk, they were based on a study with a small sample size. Much larger studies are required to confirm these important findings. *Prostate 63: 209–214, 2005.*
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KEY WORDS: **prostate cancer; phytanic acid; association; diet; *AMACR***

INTRODUCTION

Phytanic acid is a methyl-branched fatty acid present in the human diet. It has recently received a great deal of attention in prostate cancer research because of several findings from gene expression experiments, epidemiological and genetic association studies. First, α-methylacyl-CoA racemase (AMACR), an enzyme that is absolutely required for the

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metabolism of phytanic acid in humans, has been consistently found to be over-expressed in high-grade prostatic intraepithelial neoplasia (HGPIN) and in a majority of prostate cancers [12,16,24]. Analysis of *AMACR* mRNA levels revealed an average up-regulation of ~9-fold in clinical prostate cancer specimens compared with normal specimens [16]. The up-regulation of AMACR in atrophic prostate, HGPIN, localized and metastatic PCa was further confirmed at the protein level by both Western blot and immunohistochemical analysis in two independent studies [16,24]. Because of these consistent findings, AMACR is now considered an excellent molecular marker for prostate cancer and has been widely investigated in clinical diagnosis, in conjunction with other basal cell markers [20,21,30–32].

Second, in addition to being an excellent molecular marker for the diagnosis of prostate cancer, AMACR may be associated with prostate cancer risk. By sequencing exons, exon–intron junctions, and the promoter region of AMACR in germline DNA samples of 96 probands from hereditary prostate cancer (HPC) families, we have identified multiple sequence variants in this gene, including several nonsynonymous mutations [29]. Alleles in a subset of sequence variants and mutations strongly co-segregate with prostate cancer in HPC families. In addition, alleles of several variants are over-represented among 159 probands of HPC families and 245 sporadic prostate cancer patients, compared with 211 controls without a diagnosis of prostate cancer.

Finally, over-consumption of red meat and dairy products, the major source of phytanic acid in human diet has been consistently found to be associated with prostate cancer risk. Sixteen of 22 case-control and cohort studies show a positive relationship between meat consumption and prostate cancer risk, with all but one showing risk ratios of 1.3 or more (reviewed by Kolonel [14]). The association between red meat and prostate cancer risk is even stronger, with seven out of eight studies that distinguished red meat as a group reporting a positive association. Similarly, 12 of 14 case-control studies and 7 of 9 cohort studies observed a positive association between dairy products and prostate cancer risk (reviewed by Chan and Giovannucci [8]). Men with the highest intakes of dairy products have approximately twice the risk of developing prostate cancer and a fourfold increased risk of developing metastatic or fatal prostate cancer compared to men who consume low amounts of dairy products [10,11]. The importance of phytanic acid in this observed association between prostate cancer risk and dietary intake of red meat and dairy products is inferred because phytanic acid cannot be synthesized endogenously and because AMACR, an enzyme that is required for the metabolism of phytanic acid in humans, has been implicated in prostate cancer. To date, there is no epidemiological study that has specifically assessed the association between phytanic acid and prostate cancer risk.

Epidemiological studies that attempt to directly assess the association between phytanic acid and prostate cancer risk are hindered by a lack of validated methods to measure phytanic acid levels in large-scale epidemiological studies. In this study, we assessed the reliability of measuring serum levels of phytanic acid using a gas liquid chromatography/mass spectroscopy analysis, tested the association between serum levels of phytanic acid and prostate cancer risk, and evaluated relationships between serum levels of phytanic acid and dietary intake.

METHODS

Study Subjects

Prostate cancer patients and control subjects studied in this project were from a pilot study designed to examine the feasibility of recruiting subjects to a large-scale population-based prostate cancer case-control study in North Carolina. Prostate cancer patients were randomly selected from men diagnosed with prostate cancer between July 1, 1999 and June 30, 2001 who lived within an eight county contiguous region in the Central North Carolina, based on a database from The North Carolina Central Cancer Registry (NCCCR). For each patient identified, control subjects were matched from data provided by the Highway Safety Research Center (HSRC) based on county of residence, race, and age (equal to or less than 5 years older than cases). Among the subjects contacted, we obtained questionnaire data, including food frequency, and blood samples for 49 cases and 55 controls. The mean age was 65 and 69 for cases and controls, respectively. About 45% cases and 44% of controls were African-Americans (AA), as the outcome of over-sampling of AA cases. The remaining subjects were European Americans (EA). All subjects participating in this study gave full informed consent.

Food Frequency Questionnaire (FFQ)

The 1998 version of the FFQ developed by Dr. Gladys Block (Block Dietary Data Systems Berkeley, California) was administered by an interviewer to each participant. This semi-quantitative FFQ was developed with portion size estimates and lists of foods derived from national surveys of representative samples of the American adult population, thus improving the precision of nutrient estimates produced by FFQs [5,6,7,28]. The validity of the FFQ has been tested in a variety of

studies and average correlations for energy-adjusted nutrients have ranged from 0.6 to 0.7 [6,17,23]. For this study, participants were asked to recall their dietary intake over the year prior to diagnosis to minimize the impact of prostate cancer diagnosis on the dietary assessment.

Blood Samples

Non-fasting blood was drawn into five 10 ml vacutainers, including one serum separator tube. To promote consistency among subjects, we developed a blood handling and transport protocol. According to this protocol, samples were to be kept at room temperature and delivered to our laboratories within 4 hr of the blood draw.

Measurement of Phytanic Acid

Extraction and derivitization of serum samples for analysis of phytanic acid was performed using gas liquid chromatography/mass spectroscopy analysis [3]. Plasma (40 μl) was subjected to saponification using 1 ml ethanolic KOH (5%) at 60°C for 1 hr with triheneicosanoic acid as internal standard. Non-saponifiable components were extracted in hexane and discarded after the addition on 1 ml of dionized water. The ethanolic phase was acidified by the addition of 100 μl glacial acetic acid. The free fatty acid moieties were extracted into hexane and converted to the more volatile fatty acid methyl esters (FAME) [18]. The FAME were extracted into isooctane, dried under nitrogen stream at room temperature, redissolved in 25 μl of isooctane and 1 μl was injected onto a GLC column.

The separation of FAME and the analysis of phytanic acid was performed on a Trace 2000 GC from ThermoQuest with an AS 2000 autosampler and a Finnigan trace mass spec. We used a 30 m × 0.25 mm DB-WAXetr column with a temperature program of 100°C for 2 min, then to 200°C at 16°C per min, held at 200°C for 1 min, then 200°–240° at 4°C per min, 240° for 1 min, then to 270° at 20°C per min and finally held at that temperature for 10 min. The column had a constant gas flow of 1.5 ml/min. The injector temperature was 270°C (splitless). For the mass spectrometer, the interface was 270°C, the source temperature 200°C, filament emission current 250 μA and the mass spec used positive ion electron impact. The mass range acquired was 50–450 m/z and full scan time acquired 5–30 min. This method can identify and detect phytanic acid methyl ester (rt = 10.70 min), and achieve a separation of this unique fatty acid from other fatty acids that might be found in serum. This included baseline separation from C17:0 and C16:1. The fatty acid C20:0, having a mass identical to that of phytanic acid, had a retention time of 17.09 min. We used phytanic acid purchased from Maytrea Chemicals treated as described above. A standard response curve was generated and tested by adding known amounts of phytanic acid into well characterized FAME complex standard mixes and analyzing these mixes as described above. Final quantitative determination was made using Excalibur software from Finigan. The reason for using the mass spec for analysis of phytanic acid is to be absolutely sure that the quantity of this unique fatty acid is accurately measured and not affected by contamination of other fatty acids and to also assure maximum sensitivity.

Statistical Analysis

Pearson product-moment correlation coefficients were calculated to quantitate the linear association between the two independent measurements of phytanic acid levels from the same individual. Spearman rank correlations were calculated to quantitate the monotonic association between phytanic acid levels and the amount of food and nutrient intake. The differences in the serum levels of phytanic acid between cases and controls and between EA and AA were tested using either a Student's *t*-test (2-sided) after logarithmic transformation of phytanic acid.

RESULTS

Two assays from the same blood specimen for each of the 104 study participants were subjected to gas liquid chromatography/mass spectroscopy analysis to obtain repeat measurements of serum phytanic acid levels. As shown in Figure 1, there was a strong correlation between the two measurements, with an estimated correlation coefficient (r) of 0.95 ($P < 0.0001$). The

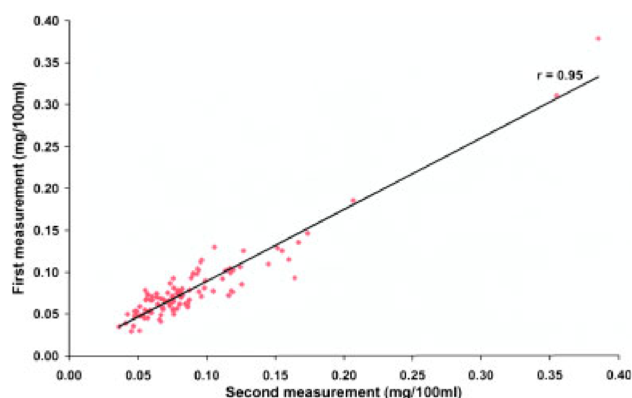

Fig. 1. Plot of two repeat measurements of serum levels of phytanic acid levels using the same blood specimen from each of the 104 study subjects. These repeat measures were obtained in two independent assays using gas liquid chromatography analysis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mean phytanic acid level (average of two measurements) among the 104 subjects was 0.09 mg/100 ml, with a range from 0.03 to 0.38 mg/100 ml, similar to the levels observed from 200 non-fasted healthy male donors in the study of Avigan [2,3].

Serum levels of phytanic acid decreased slightly, but this was not statistically significant, with age, $r = -0.13$ ($P = 0.18$). The mean (±SD) serum levels of phytanic acid were 0.09 ± 0.05 mg/100 ml in EA and 0.08 ± 0.05 mg/100 ml in AA. The difference was not statistically significant using the *t*-test ($P = 0.66$). However, the mean serum levels of phytanic acid were higher in prostate cancer patients (0.10 ± 0.06 mg/100 ml) than in unaffected controls (0.08 ± 0.03 mg/100 ml), and this difference was significant ($P = 0.04$).

We also tested the correlation between serum levels of phytanic acid and usual intake of several food items and nutrients, as estimated from the FFQ (Table I). Interestingly, we observed a significant positive correlation between serum levels of phytanic acid and dairy servings, one of the major sources of phytanic acid in the diet, $r_s = 0.24$ ($P = 0.01$). Similarly, a significant positive correlation was observed between phytanic acid levels and vitamin D intake, $r_s = 0.21$ ($P = 0.03$), likely due to the amount of dairy servings and the vitamin D enrichment of dairy foods. We also observed a correlation between serum levels of phytanic acid and meat servings, another major source of phytanic acid in the diet $r_s = 0.16$ ($P = 0.09$), although this finding was not statistically significant. No other significant correlations were noted.

DISCUSSION

Among several suggested risk factors for prostate cancer, positive family history of the disease and over consumption of dairy products and red meat have been consistently implicated. In particular, several intriguing findings implicate phytanic acid and the *AMACR* gene in prostate cancer risk, including the association of sequence variants in the *AMACR* gene and prostate cancer risk, over-expression of *AMACR* mRNA and protein in prostate cancer and HGPIN, and positive association between over-consumption of dairy products and red meat. The fact that phytanic acid primarily comes from dairy and red meat consumption, and its metabolism depends entirely on the AMACR enzyme, may account for these observations. However, no direct test for association between phytanic acid levels and prostate cancer risk has been reported, partly because of the lack of a reliable method to measure phytanic acid levels in large-scale epidemiological studies. This study, by assessing the reliability of a method to measure serum levels of phytanic acid and by measuring this in prostate cancer patients and unaffected controls, represents the first step in directly testing for association between phytanic acid levels and prostate cancer risk.

TABLE I. Correlation Between Serum Phytanic Acid Levels and Dietary Variables Estimated From FFQ (N = 103)

Dietary variables	Spearman correlation	*P*-value
Kcal	0.04	0.72
Fat	0.08	0.39
Protein	0.15	0.14
Carb	−0.08	0.45
Vit D	0.21	0.03
Lycopene	0.05	0.62
Dairy serving	0.24	0.01
Meat serving	0.16	0.09
Fat serving	−0.05	0.61

There are three major findings from this study. First, our results suggest phytanic acid levels in serum can be reliably measured in large epidemiological studies using a GC/Mass Spec analysis, based on the high correlation between two independent measurements of phytanic acid levels from the same individuals, and the fact that these measured levels of phytanic acid are within the expected range. Second, a statistically significant increase in phytanic acid levels of prostate cancer patients compared to unaffected controls directly suggests an association between phytanic acid and prostate cancer risk. Lastly, the significantly positive correlation between serum levels of phytanic acid and reported dietary intake of dairy and red meat servings to the serum measurement suggests that phytanic acid levels estimated from both serum measurement and FFQ can be used in epidemiological studies.

However, caution is required in interpreting our findings. Probably the most important limitation of this study is its small sample size. Although a type II error (false negative) is not a concern because significant findings were obtained, the results could be due to statistical fluctuations commonly associated with small sample size. Confirmation of our findings in larger studies is required. In this context, our findings provide an important basis for tests of specific hypotheses in future studies.

There are also several issues inherent to case control study designs that should be considered. It is possible that the observed differences in phytanic acid levels are in some part a result of confounding differences between cases and controls that could not be controlled by statistical adjustment or detailed analyses. In addition, because sera were collected from prostate cancer cases after diagnosis, the resulting data that shows differences between cases and controls might be the

product of changes in diet brought on by lifestyle changes following diagnosis or the effects of treatment. This could be in part addressed through replication of the result in larger case control studies. However, a prospective cohort study would be the ideal approach to deal with these potential drawbacks. Importantly, our study has shown the feasibility and importance of measuring phytanic acid, and this can be incorporated in either a large scale association study or in a prospective design.

Another potential consideration is that the enzyme activity and gene expression of AMACR were not measured in this study. This does leave an experimental gap between dietary intake of fatty acids and the resulting serum levels of phytanic acid. Clearly, an evaluation of AMACR gene expression and enzyme activity will be an important series of experiments in understanding the interaction between dietary fatty acids and AMACR.

It should also be noted that we observed some inconsistency between the dietary data on meat versus dairy products, both of which are thought to provide the bulk of phytanic acid in the diet. While the association of phytanic acid measurement and dairy intake support our hypothesis, there was not a significant association with overall meat consumption. The findings for dietary meat did trend toward an association with serum phytanic acid, although there is no way to know whether this trend might either intensify or weaken in a larger study. On the other hand, the lack of a significant association for dietary meat in our study does not invalidate the observed association with dietary dairy intake. Finally, the use of overall meat consumption was not an ideal dietary variable because red meat actually contains phytanic acid, while other forms of meat do not; thus larger studies in the future will be better suited to subdividing red meat from all meat intake.

The exact mechanism by which phytanic acid may be linked to prostate cancer is unknown, although there are several possible mechanisms. One potential mechanism is through the reactive oxygen species generated in the β-oxidation of phytanic acid. Phytanic acid is a substrate for peroxisomal β-oxidation after undergoing α-oxidation [26]. An important distinction between β-oxidation that occurs in the peroxisome as compared with that of the mitochondrion is that in the former site, hydrogen peroxide is produced at each round of β-oxidation. This hydrogen peroxide and the accompanying reactive oxygen species potentially generated by peroxisomal β-oxidation has been proposed to play a critical role in the transformation process that has been observed in rodent models of liver carcinogenesis [22,25,27]. Another potential mechanism is the role of phytanic acid in several signal transduction pathways which regulate cell proliferation and differentiation. Phytanic acid and its metabolic product, pristanic acid, are known to bind and activate at least two types of nuclear hormone receptors: retinoid acid receptors (RARs) [13,15] and peroxisome proliferator-activated receptors alpha (PPARα) [9], at the physiological levels of this fatty acid found in blood. Both RAR and PPAR pathways have been implicated in carcinogenesis for various tissues [1]. In addition, an interaction between AMACR and phytanic acid could also contribute to the development of prostate cancer. A recent study found that branched-chain fatty acids, including phytanic acid and pristanic acid themselves can enhance AMACR protein expression in LNCaP cells in vitro [19].

The association between phytanic acid levels and prostate cancer risk, if confirmed in large independent studies, may provide an important clue to better understanding the etiology of prostate cancer and to improve our ability to prevent prostate cancer. This association may account for the consistent finding of over-expression of AMACR mRNA and protein levels in prostate cancer. Therefore, further investigation of AMACR and phytanic acid not only may improve their utility in the diagnosis of prostate cancer as excellent molecular markers, but also may advance our knowledge on their roles in the development of prostate cancer. Additionally, this has the potential to lend support to the findings of epidemiologic studies that have reported dairy and red meat consumption as risk factors for prostate cancer by elucidating the specific component of the foods that are responsible for the increased risk, further strengthening the cancer prevention message that dietary modification can indeed reduce prostate cancer risk. The effectiveness of this message may be further improved when considering the host effect due to genetic variants of the *AMACR* gene. Considering the complexity of prostate cancer, studies that consider the interaction of specific risk factors are needed.

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