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Dietary Influences on Tissue Concentrations of Phytanic Acid and AMACR Expression in the Benign Human Prostate

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Abstract

BACKGROUND—Alpha-methylacyl-CoA racemase (AMACR) is an enzyme involved in fatty acid metabolism that is markedly over-expressed in virtually all prostate cancers (PCa), relative to benign tissue. One of AMACR's primary substrates, phytanic acid, is derived predominately from red meat and dairy product consumption. Epidemiological evidence suggests links between dairy/red meat intake, as well as phytanic acid levels, and elevated PCa risk. This study investigates the relationships among dietary intake, serum and tissue concentrations of phytanic acid, and AMACR expression (mRNA and protein) in the histologically benign human prostate.

METHODS—Men undergoing radical prostatectomy for the treatment of localized disease provided a food frequency questionnaire (n = 68), fasting blood (n = 35), benign fresh frozen prostate tissue (n = 26), and formalin-fixed paraffin-embedded (FFPE) sections (n = 67). Serum and tissue phytanic acid concentrations were obtained by gas chromatography–mass spectrometry. We extracted RNA from epithelial cells using laser capture microdissection and quantified mRNA expression of AMACR and other genes involved in the peroxisomal phytanic acid metabolism pathway via qRT-PCR. Immunohistochemistry for AMACR was performed on FFPE sections and subsequently quantified via digital image analysis. Associations between diet, serum, and tissue phytanic acid levels, as well as AMACR and other gene expression levels were assessed by partial Spearman correlation coefficients.

RESULTS—High-fat dairy intake was the strongest predictor of circulating phytanic acid concentrations (r = 0.35, *P* = 0.04). Tissue phytanic acid concentrations were not associated with any dietary sources and were only weakly correlated with serum levels (r = 0.29, *P* = 0.15). AMACR gene expression was not associated with serum phytanic acid (r = 0.13, *P* = 0.47), prostatic phytanic acid concentrations (r = 0.03, *P* = 0.88), or AMACR protein expression (r = −0.16, *P* = 0.20).

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CONCLUSIONS—Our data underscore the complexity of the relationship between AMACR and its substrates and do not support the unifying hypothesis that excess levels of dietary phytanic acid are responsible for both the overexpression of AMACR in prostate cancer and the potential association between PCa risk and intake of dairy foods and red meat.

Keywords

phytanic acid; AMACR; prostate cancer; diet; biomarker

INTRODUCTION

Studies of migrants and a large body of laboratory and epidemiological evidence suggest that a Westernized diet rich in animal fat and protein plays an important role in prostate carcinogenesis [1–8]. The responsible dietary factors are not well established, but both case–control and cohort studies have shown associations between prostate cancer (PCa) risk and intake of red meat and dairy foods [4,6,7]. Alpha-methylacyl-CoA racemase (AMACR) is an enzyme that is strongly overexpressed in a number of cancers, notably in prostate and colorectal cancer [9]. AMACR is overexpressed in virtually all PCa cases, with low levels present in normal prostatic tissues [10,11]. AMACR is essential in the peroxisomal metabolism of phytanic acid—a 20-carbon saturated branched chain fatty acid that humans obtain by consuming meat and dairy products from ruminant animals, whose gut fermentation is able to release the phytanic acid precursor phytol from chlorophyll [12]. Our group has reported that in radical prostatectomy specimens, benign glands near a focus of cancer had a higher expression of AMACR than distant glands, indicating the existence of a field effect for AMACR in prostate carcinogenesis [13]. These observations suggest that alterations in AMACR expression occur very early in cancer development and that they constitute a characteristic of high-risk but morphologically benign tissue [14]. Addition of phytanic acid to cultured prostate cancer cells increases AMACR expression [15]; therefore, we and others have hypothesized that excess levels of phytanic acid could explain the overexpression of AMACR in PCa while at the same time strengthening support for the epidemiological association of dairy and red meat intake with risk.

Dietary branched-chain fatty acids contribute to several processes that may have great relevance to prostate carcinogenesis, including oxidative stress and nuclear receptor signaling. After ingestion, phytanic acid is transported into peroxisomes via sterol carrier protein 2 (SCP2) (Fig. 1) [16]. Unlike most fatty acids, phytanic acid cannot be metabolized by β-oxidation; thus, it first undergoes removal of one carbon by α-oxidation in peroxisomes to form pristanic acid, which is isomerized by AMACR, rapidly β-oxidized, and eventually fully broken down to CO_2 and water in mitochondria. During multiple rounds of β-oxidation, this metabolic process generates reactive oxygen species with the potential to create molecular damage [16]. An inherited defect in this pathway causes Refsum disease, which results from the accumulation of branched chain fatty acids [17]. Catalase (CAT), an antioxidant enzyme, is present in peroxisomes to counteract free radicals. Downstream proteins include D-bifunctional protein (DBP), an enzyme involved in peroxisomal β-oxidation. Finally, both phytanic and pristanic acid are ligands for retinoid X receptor-alpha (RXRα) and peroxisome proliferator-activated receptor- alpha (PPARα), which are

transcription factors with a host of downstream effects on cellular metabolism, proliferation and apoptosis.

It is not yet clear whether AMACR is an “innocent bystander” or whether it or its dietary substrates in red meat and dairy food lie within the causal pathway leading to the development of PCa. The primary aim of this study was to quantify relationships between dietary intake, serum, and prostatic phytanic acid concentrations, mRNA and protein expression of AMACR and other genes in the phytanic acid metabolic pathway in benign prostate tissue. We postulated that men with a higher intake of ruminant animal products would have higher serum and prostatic levels of phytanic acid and consequently higher AMACR expression levels in benign prostate tissue. Pentadecanoic and heptadecanoic saturated fatty acids, which are found in ruminant animal products and have been suggested as biomarkers for dairy intake in serum and adipose tissue, were also tested as alternate measures of dairy intake and thus phytanic acid levels in the blood and tissue [18–20].

MATERIALSANDMETHODS

Study Population

A total of 81 men who underwent radical prostatectomy for the treatment of localized PCa were included in this study. Thirty-nine men were recruited from the University of Illinois Hospital at Chicago (UIC) or the Jesse Brown Veterans Affairs Medical Center (JBVAMC) in Chicago. A pre-surgical research visit was completed to obtain dietary and lifestyle data, medical history and a fasting blood sample. Immediately after surgery, a pathologist obtained fresh samples of benign tissue distant from tumor foci during gross examination of the prostatectomy specimens. Complete diet history, formalin-fixed paraffin-embedded (FFPE) blocks were obtained from 39 Chicago subjects, and fresh frozen benign prostate tissue was available for 26 Chicago subjects.

Existing fresh frozen prostate tissue, FFPE sections, and medical, lifestyle, and diet data from 42 men enrolled in a previous study at Henry Ford Health Systems (HFHS) in Detroit were also included. These men provided fresh frozen prostate tissue and medical/ lifestyle and diet history questionnaires; however, fasting blood samples were not available. Specimens and data were combined across all three study sites. Patients receiving exogenous hormones, or neoadjuvant anti-hormone therapy were excluded. The UIC, JBVAMC, and HFHS institutional review boards approved the study.

Laser Capture Microdissection (LCM) and RNA Isolation

LCM was utilized to quantify mRNA expression in frozen prostate tissue as described by Nonn et al. [21]. Hematoxylin and eosin stained slides were reviewed by a study pathologist to determine areas of benign glandular tissue from the peripheral zone of the prostate. Four 8 μm frozen sections from each patient were cut and placed on RNAse-free polyethylene naphthalate slides (Leica, Buffalo Grove, IL). Slides were fixed in 100% ethanol for 15 min 1 day prior to LCM collection and stored at −80°C before staining with 0.5% toludine blue. A Leica LMD-ASLMD instrument was used to collect 150–200 microdissected acini of benign epithelium into Eppendorf caps containing 50 μl digestion buffer (Life Technologies, Carlsbad, CA) that were stored overnight at −80°C prior to RNA isolation. RNA was

extracted with the RecoverAll® kit (Life Technologies) using the protocol provided by the manufacturer. RNA quality and quantity were evaluated using a NanoDrop® spectrophotometer.

qRT-PCR

RNA (50 ng) was reverse-transcribed using the VilocDNA kit (Life Technologies). cDNA was preamplified according to manufacturer's protocol using TaqMan® Pre-AMP master mix and TaqMan® assays. Assays included six genes of interest: AMACR, DBP, SCP2, PPARα, RXRα, and CAT. Three housekeeping genes were also measured: beta-actin (ACTB), tata-box binding protein (TBP), and hypoxanthine phosphoribosyltransferase 1 (HPRT-1).

The resulting pre-amplified product was diluted 1:20 and served as a template for the individual TaqMan® qPCR reactions, which were performed on an HT7900 instrument. Each reaction was completed in triplicate and genes for each subject were run on the same plate. No-template control reactions were included on every plate to evaluate contamination. Expression was normalized to the housekeeping genes, and the ΔCT method was utilized to quantify gene expression.

Dietary Assessment

A 100-item Block 98.2 or Block Brief Food Frequency Questionnaire (FFQ) was completed by Chicago participants (NutritionQuest, Berkeley, CA). HFHS participants completed an FFQ developed by the Nutrition Assessment Shared Resources of the Fred Hutchinson Cancer Research Center [22]. All three questionnaires captured information on the frequency of consumption and portion sizes of foods consumed during the previous year. The FFQs provided estimates of total daily energy, macronutrient and micronutrient intakes, as well as consumption of specific foods and food groups, including red meat, dairy foods and fish, in grams per day. Branched chain fatty acids have been identified in some oil-rich fish, presumably as a result of phytoplankton in the food chain [14]. Meat and dairy items were indexed as either high- or low-fat, since only the former contain significant amounts of branched chain fatty acid.

Serum and Tissue Phytanic Acid Assays

Singlicate measurement of fatty acids in fasting serum samples was performed by capillary gas chromatography, mass spectrometry following derivatization of total lipid fatty acids at the Peroxisomal Diseases Laboratory of the Kennedy Krieger Institute, as described in detail by Lagerstedt et al [23]. In brief, fatty acids were quantified by selective ion monitoring in ratio to stable isotope-labeled internal standards. A four deuterium labeled standard was used for phytanic acid quantitation. Each fatty acid was treated as a percentage of the total lipid levels. Mean intra- and inter-batch coefficients of variation (CV) for phytanic acid in serum samples were 1.3% and 12.7%, respectively, based on anonymous replicates from a quality control serum pool.

The protocol for tissue fatty acid analysis was similar; however, due to the limited volume of tissue available, lipids were extracted from samples before analysis. The glycerol-linked

fraction was measured because phytanic acid is mainly contained in glycerides and glycerophospholipids [24]. An average of 339mg of prostate tissue per subject was provided by the Chicago subjects, and a minimum of 100mg was considered desirable for phytanic acid quantification. Samples from Detroit subjects were too small to be analyzed. Results for individual fatty acids were expressed as their percentage of total tissue lipids. Mean intra-batch CV for phytanic acid in tissue was 18.6%, based on non-identical but adjacent samples from a random subject within each batch.

Immunohistochemistry

A tumor and normal FFPE block was selected for each subject. Slides were stained with AMACR (Dako, 1:100, Clone 13H4) and the assay was titrated to detect variation of expression in normal and tumor regions. Briefly, IHC was carried out as follows: 5 μm sections were cut on to charged slides and deparaffinized. After rehydration, antigen retrieval was carried out using a pressure cooker for 15 min. Slides were incubated for 60 min with the primary antibody, followed by incubation in a ready-to-use anti-rabbit secondary antibody from BioCare®. Color reaction was developed using diaminobenzidine (DAB) as the chromagen and slides were counterstained with hematoxylin. Positive and negative controls were run in each batch.

Slides were scanned at 20x on an Aperio (Leica) ScanScope® CS whole-slide digital microscope. A digital draw tool was used to demarcate normal, PIN, and tumor regions. The Genie® module in the Aperio system is a machine learning program that classifies each pixel in an image according to a set of hand-drawn, pre-classified training images provided by a trained individual. We created three classes of training images: epithelium, stroma, and blank slide. The resulting classes were determined to be highly accurate in the training set images, and were then applied to the study slides. The positive pixel algorithm within ImageScope® was utilized for digital image analysis. We determined the percent positive pixels and pixel stain intensity in the epithelium of the normal, PIN, and tumor compartments.

Statistical Analysis

Frequency distributions of dietary intake, mRNA levels, and serum and tissue fatty acid concentrations were examined for normality and outlier values. A difference in distribution amongst the three types of FFQs was noted, thus results are adjusted for FFQ type. Scatterplots and Spearman rank correlation coefficients were used to examine relationships among the variables of interest. A *P*-value of ≤0.05 (two-sided) was considered statistically significant. Analyses were performed using SAS Version 9.2 (SAS, Inc., Cary, NC).

RESULTS

Table I shows selected demographics and clinical characteristics of the study participants in Chicago and Detroit. Over three-quarters of the participants were overweight or obese (78%). The majority (58%) were African American and had a history of hypertension (64%) as reported by medical records. The average pre-surgical PSA was 9 ng/ml. About half of

the men (46%) had a Gleason score ≤6. Serum and tissue phytanic acid were not associated with Gleason grade.

Table II shows that no correlation was observed between total dairy intake and serum phytanic acid levels; however, when we restricted analysis to high-fat dairy foods, we observed a positive and significant correlation with serum phytanic concentrations (r = 0.36, $P = 0.04$). High-fat ruminant meat intake by itself was not associated with serum phytanic acid levels (r = 0.07, $P = 0.68$), and adding this meat intake to high-fat dairy intake did not materially change the aforementioned significant correlation with serum. Fish intake did not show any association with phytanic concentrations in serum. Concentrations of pristanic acid, the direct substrate of AMACR, were correlated with phytanic levels in serum and tissue (r = 0.64 and r = 0.43, respectively) and gave similar results; thus they are not discussed further.

Tissue phytanic acid concentrations did not correlate with combined high-fat dairy and meat intake, nor with any other dietary measure or serum biomarker. Furthermore, Figure 2 shows that serum and tissue phytanic concentrations were only weakly correlated (r = 0.29, $P = 0.15$).

Pentadecanoic and heptadecanoic acids were strongly correlated with each other in both serum and prostatic tissue (r = 0.69, $P < 0.01$ and r = 0.95, $P < 0.01$); therefore, we only show results for heptadecanoic acid. No association was seen between high-fat dairy intake and serum (r = 0.02, $P = 0.93$) or tissue (r = 0.07, $P = 0.76$) heptadecanoic concentration in this population. However, phytanic acid concentrations were strongly correlated with heptadecanoic acid concentration in both the serum and tissue (Fig. 3a and b). Furthermore, Figure 3c shows that serum and tissue levels of heptadecanoic acid were positively correlated (r = 0.67, $P < 0.01$). Phytanic acid concentrations in the prostate range from 0.05% to 0.30%, whereas heptadecanoic acid concentrations range from 5.49% to 38.00%—approximately a 100-fold difference.

AMACR expression at the mRNA level was positively correlated with expression of other genes in the peroxisomal phytanic acid metabolism pathway. Overall, there was strong evidence for inter-correlated expression among genes in the pathway, as shown in Table III. As was the case for tissue phytanic acid concentration, we found that AMACR mRNA expression was not associated with combined high-fat dairy and meat intake. Table IV shows that ruminant meat intake alone was marginally correlated with AMACR mRNA (r = 0.23, $P = 0.06$). However, we did not observe a stronger relationship with high-fat ruminant meat—a better estimate of phytanic acid intake—and thus this observation is probably due to chance. Overall, Table IV indicates no clear patterns of association between dietary determinants of phytanic acid and expression of genes associated with peroxisomal phytanic acid metabolism and function. Specifically, whole milk intake was inversely associated with catalase mRNA expression (r = −0.24, $P = 0.05$), however it was positively associated with PPARα mRNA expression (r = 0.25, $P = 0.04$). Serum phytanic acid concentration was not associated with AMACR, SCP2, DBP, CAT, and PPARα mRNA expression in benign prostate, but was positively correlated with mRNA expression of RXRα (r = 0.41, $P = 0.03$) (Table V). Tissue phytanic acid concentrations were not correlated with mRNA expression

for AMACR or any of its pathway partners; similar findings were observed for tissue heptadecanoic acid (data not shown).

AMACR protein expression significantly increased between normal (mean % positivity = 0.07; 95% CI, 0.06–0.08), PIN (mean % positivity = 0.12; 95% CI, 0.10–0.13), and tumor (mean % positivity = 0.17; 95% CI, 0.16–0.18) compartments. Overall there was no clear pattern of association between AMACR protein expression and dietary determinants of phytanic acid (data not shown). AMACR protein expression in the normal compartment was not correlated with mRNA expression ($r = -0.16$, $P = 0.20$). However, AMACR protein expression in the normal compartment was inversely and significantly correlated with SCP2 ($r = -0.25$, $P = 0.05$) and RXRα ($r = -0.37$, $P = 0.01$) mRNA levels. AMACR protein expression did not correlate with tissue phytanic levels ($r = -0.09$, $P = 0.46$).

DISCUSSION

This is the first study, to our knowledge, to investigate the interrelationships among intake of ruminant dairy and meat products, serum and tissue phytanic acid concentrations, and AMACR expression in the benign prostate. Our results show that among the dietary sources of branched chain fatty acids, only high-fat dairy intake was correlated with serum phytanic acid concentration. However, tissue concentrations of phytanic acid were not discernibly associated with any dietary source and were only weakly correlated with serum levels. Moreover, we observed no significant association of diet, serum or tissue phytanic acid levels, with AMACR gene or protein expression in the benign prostate. We found, as expected, that AMACR gene expression was highly correlated with expression of other genes in related pathways, and thus no overall patterns suggesting a relationship between these genes and food intake or fatty acid levels emerged. Taken together, the present results do not provide support for the hypothesis that excess levels of phytanic acid in the diet could explain both the overexpression of AMACR in prostate cancer and the epidemiological association between prostate cancer risk and intake of dairy foods and red meat.

The present data confirm previous findings in which serum phytanic acid levels were correlated with dietary intake of high-fat dairy food [2,25,26]. Allen et al. [2] found butter intake to be significantly correlated to serum phytanic acid ($r = 0.44$, $P < .01$) in the EPIC subcohort from the UK. The association we observed was not attributable to butter intake, but most likely due to other dairy components such as cheese, yogurt or ice cream. This discrepancy may be explained by differences in patterns of dairy fat consumption between countries, and variation in the branched chain fatty acid concentration across and even within food categories.

The lack of correlation between dietary intake and tissue phytanic acid, supported by the weak correlation between serum and tissue phytanic acid, suggests that metabolic activity leading to a high rate of turnover within prostate tissue might be an important factor. The tissues evaluated in this study were histologically normal but from cancerous prostates; therefore it is conceivable that the samples reflect a microenvironment affected by field cancerization. Measurement error, resulting from the FFQ approach to measuring usual intake, and the technical difficulty of assaying low abundance fatty acids in tissue samples,

also could have attenuated true relationships. However, we observed strong correlations between prostate and serum concentrations of relatively low abundance long chain omega-3 fatty acids, which are determined largely by diet and supplement use. For example, serum-tissue correlations were r = 0.75 ($P < 0.01$) for docosahexaenoic acid, and r = 0.52 ($P = 0.01$) for docosapentaenoic acid.

We observed that levels of heptadecanoic acid, an alternative biomarker for dairy intake, were correlated with phytanic acid in serum (as previously reported by Allen et al.) and in prostate tissue [2]. Since heptadecanoic levels were not correlated with other fatty acids such as linoleic and eicosapentanoic acid, we assume these relationships are due to common food sources as opposed to sample artifacts. Serum and tissue levels of heptadecanoic were also highly correlated with each other. However, we found no discernible relationship between dairy intake and heptadecanoic acid in either serum or tissue. Once again, this raises the possibility that true relationships were attenuated by measurement error involving dietary intake. However, recently reported correlations for dairy intake and pentadecanoic acid, such as those from the Multi-Ethnic Study of Atherosclerosis (MESA), are rather small (r = 0.13–0.22) and thus could have been missed in a small study such as ours [27]. More importantly, our results indicate that heptadecanoic acid, although approximately 100 times more abundant than phytanic acid, is not a superior biomarker to phytanic for identifying a relationship between dairy intake and AMACR expression in the prostate.

Phytanic acid has been shown to bind and activate the nuclear receptors PPARα and RXRα [28–32]. These receptors play a role in a variety of cellular processes, including adipogenesis, lipid homeostasis, fatty acid, and glucose metabolism [30]. The observation that RXRα mRNA expression was associated with serum but not tissue phytanic concentrations could be explained by chance or by an effect of local factors within the prostate.

Overall there was no association found between dietary intake and mRNA gene expression of AMACR or its pathway partners. Whole milk consumption was inversely associated with catalase and positively associated with PPARα mRNA expression. These findings could be due to chance because of multiple hypothesis testing; however, Suhara et al. [33] reported that high fat cow's milk products increased the activity of PPARα and RXRα in a reporter gene assay. Activation of PPARα promotes fatty acid catabolism and insulin sensitivity, which could favor tumor growth in the prostate microenvironment, although PPAR activation could also be invoked to explain epidemiological findings that milk consumption is inversely related to risk of type 2 diabetes [34].

AMACR protein expression was significantly increased between normal, PIN, and tumor compartments as seen in previous studies [13]. However, we observed no association between AMACR mRNA and protein expression in the benign prostate. The in vitro study by Mobley et al. demonstrated that treatment with phytanic acid markedly increased AMACR protein levels in androgen-sensitive LNCaP cells but had little effect on mRNA expression, indicating that post-transcriptional modifications or effects on protein half-life might be operative [15]. Our quantitative IHC results are consistent with this, and further studies are needed to explore possible post-translational processes and mechanisms.

The present study, which to our knowledge is the first to measure phytanic acid concentrations in prostate tissue, benefited from LCM to collect a homogeneous histologically normal epithelial cell population for gene expression analysis. Additionally, quantitative image analysis is an accurate and reproducible way to evaluate IHC in these RP specimens. However, certain limitations of this study are acknowledged, including a relatively small sample size that limits power and the ability to control for potential confounders. Additionally, the FFQs utilized did not allow us to discriminate between fatty and non-fatty fish while only the former is a source of phytanic acid [14]. Of further concern is the high CV (18%) for tissue phytanic acid concentration, which suggests poor reproducibility. However, this could be attributed to the heterogeneity of the prostate tissue, because repeat samples were not taken from precisely the same tissue location and hence were not strictly identical.

Although the reason for AMACR overexpression in prostate cancer, and its potential link to the etiology of this disease, remains unresolved, we believe there are promising avenues for future research. It is conceivable that AMACR participates in a broad program altering fatty acid metabolism in prostate cancer, a program designed to meet increased demands for energy production and biosynthesis. Most cancer cells rely on glycolysis as a primary source of energy for proliferation and growth, however, prostate cancer utilizes fatty acid oxidation as its predominant source of bioenergy [35]. Due to AMACR upregulation, branched chain fatty acids such as phytanic acid—and possibly other yet unidentified dietary substrates as well—are oxidized to acetyl-CoA, which then can be used either to produce ATP via the Krebs cycle, or as building blocks for lipid synthesis [36]. Indeed, our data showed that RNA expression in benign tissue for AMACR and fatty acid synthase (FASN) were moderately to strongly correlated ($r = 0.59$, $P < 0.01$), suggesting that they are coordinately regulated in high-risk tissue. AMACR is known to be pleotropic with respect to its substrates; for example, it also plays an important in role in the metabolism of bile acids and nonsteroidal anti-inflammatory drugs, such as ibuprofen [37]. Enhanced peroxisomal β-oxidation, as reflected by AMACR upregulation, might confer a growth advantage on prostate cancer cells, not only from catabolism of branched chain fatty acid substrates, but also from its involvement in initial oxidation of very long chain fatty acids and fatty acid derivatives that cannot be metabolized in mitochondria [38]. Therefore, manipulation of AMACR expression in cultured benign and malignant prostate cells could reveal important new substrates that are linked to cell growth and also clarify AMACR's role in metabolic adaptation during carcinogenesis.

CONCLUSION

In conclusion, we found that there is no simple chain of association linking dairy intake to phytanic acid concentrations in the prostate and to AMACR expression in benign tissue, despite evidence that dairy intake and serum levels are linked and in vitro data indicating upregulation of AMACR expression when phytanic acid is added to cultured PCa cells [15]. These results do not support a direct relationship between local prostatic phytanic acid concentration and AMACR expression. Studies that examine temporality and distribution of phytanic acid concentration in the prostate are warranted, as are studies examining the effects of manipulating AMACR expression in both benign and malignant cultured cells.

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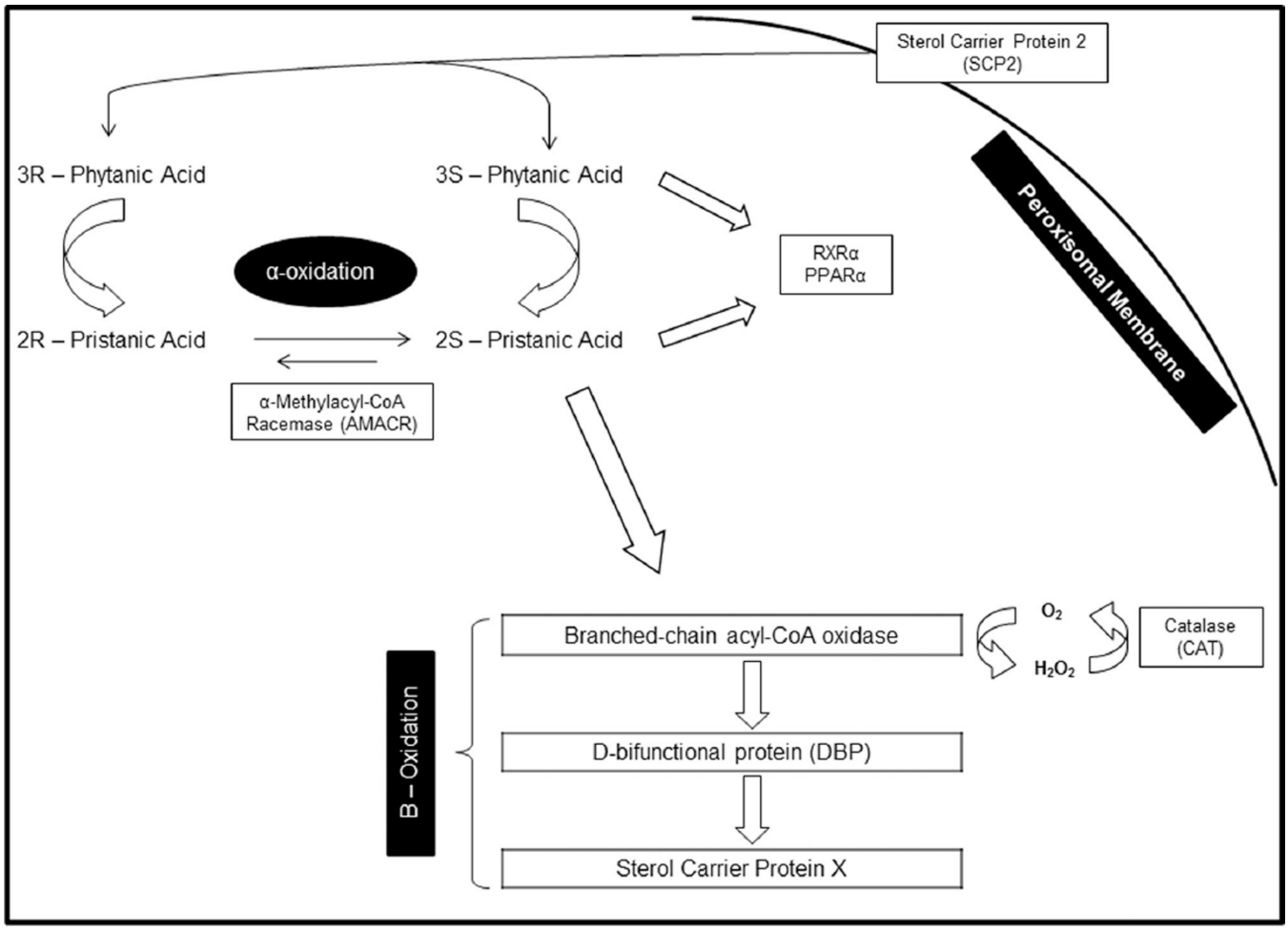

Fig. 1.
Role of AMACR in the metabolism of phytanic acid.

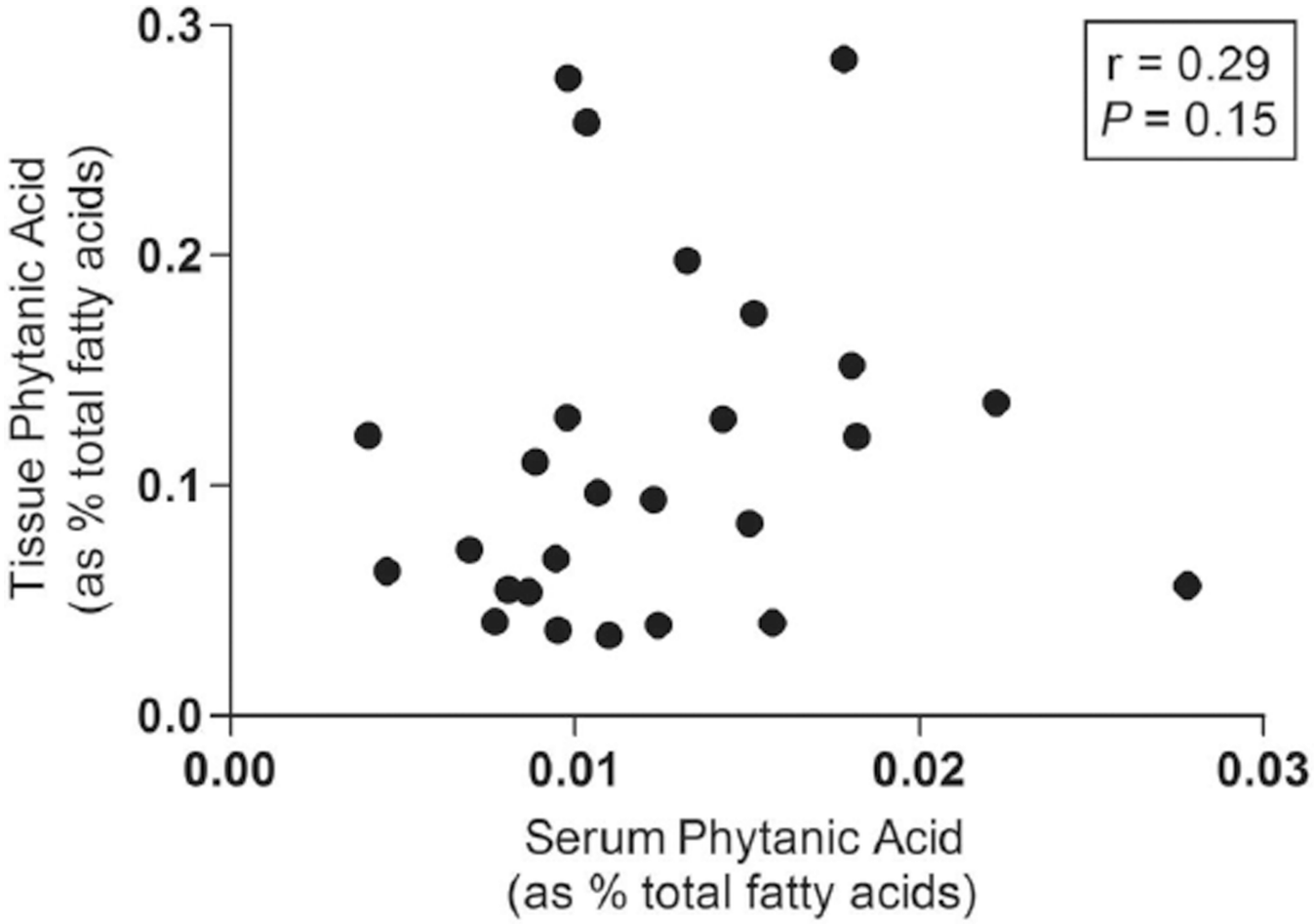

Fig. 2.
Scatter plot showing correlation (Spearman r) between serum and tissue phytanic acid.

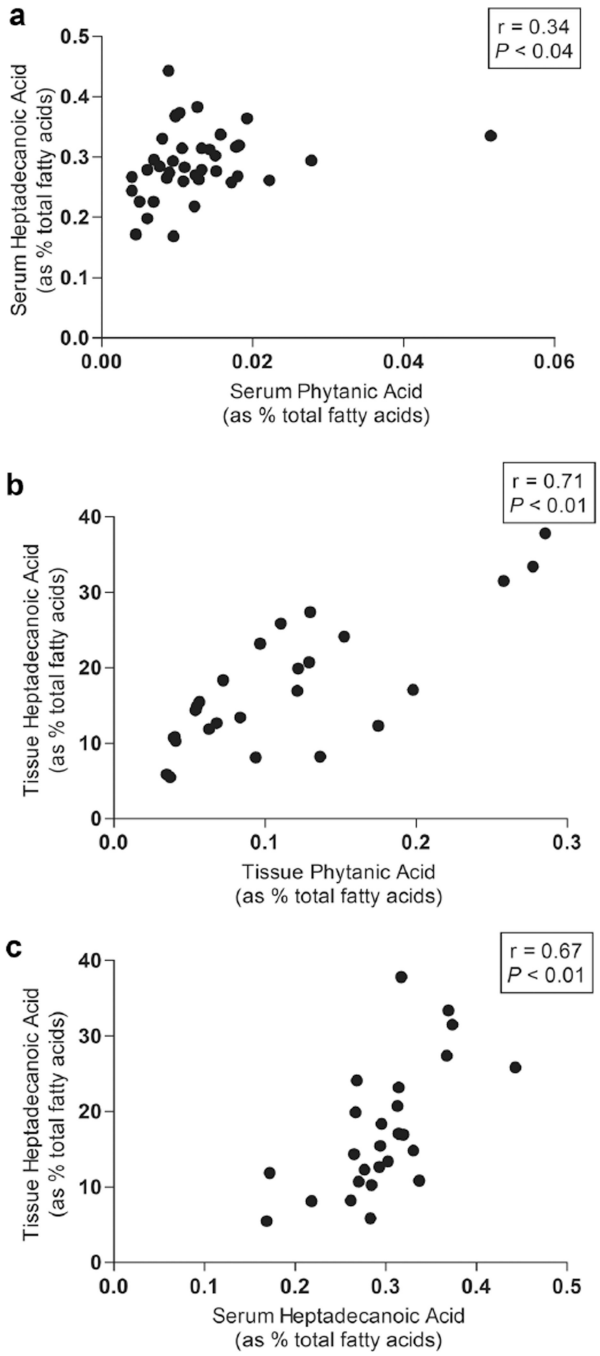

Fig. 3.

Scatterplots showing correlations (Spearman r) between (a) serum phytanic and pentadecanoic acid, (b) tissue phytanic and pentadecanoic acid, and (c) serum pentadecanoic acid and tissue pentadecanoic acid.

TABLE I

Selected Characteristics of the Study Population*

	Chicago (n = 39)	Detroit (n = 42)	All (n = 81)
	Mean (SD)	Mean (SD)	Mean (SD)
Age	62.5 (5.8)	61.7 (6.2)	62.1 (6.0)
PSA level, mean (ng/ml)	8.2 (5.6)	9.6 (7.5)	9.0 (6.7)
	n (%)	n (%)	n (%)
BMI (kg/m^2)			
<25	8 (20)	9 (21)	17 (21)
25–30	15 (40)	22 (52)	37 (46)
≥30	15 (40)	11 (26)	26 (33)
Race			
White	11 (28)	23 (55)	34 (42)
Black	28 (72)	19 (45)	47 (58)
Hypertension			
Yes	28 (72)	24 (57)	52 (64)
No	11 (28)	18 (43)	29 (36)
BPH			
Yes	5 (13)	9 (21)	14 (17)
No	34 (87)	33 (79)	67 (83)
Diabetes			
Yes	13 (33)	4 (10)	17 (21)
No	26 (67)	38 (90)	64 (79)
Gleason score			
≤6	23 (59)	14 (33)	37 (46)
3 + 4	11 (28)	8 (19)	19 (23)
4 + 3	3 (8)	6 (14)	9 (11)
8–10	2 (5)	14 (33)	16 (20)
Pathologic stage			
T2a	0 (0)	1 (2)	1 (1)
T2b	4 (10)	27 (64)	31 (38)
T2c	21 (54)	1 (2)	22 (27)
T3	2 (5)	0 (0)	2 (2)
T3a	5 (13)	9 (21)	14 (17)
T3b	2 (5)	4 (10)	6 (7)
Missing	5 (13)	0 (0)	5 (6)

*Not all Chicago participants had a value for BMI (n = 38), age (n = 38), and PSA (n = 35).

TABLE II

Partial Spearman Correlation Coefficients Between Dietary Intake and Tissue Concentrations of Phytanic Acid in Serum and Prostatic Tissue*

	Serum phytanic (% FA) (n = 35)		Serum heptadecanoic (% FA) (n = 35)		Tissue phytanic (% FA) (n = 24)	
Intake (g/day)	**r**	***P*-value**	**r**	***P*-value**	**r**	***P*-value**
Dairy						
Total	0.03	0.85	0.02	0.93	0.02	0.91
High-fat	0.35	0.04	−0.02	0.88	0.07	0.74
Whole milk	−0.17	0.33	−0.06	0.70	0.06	0.79
Butter	−0.03	0.87	−0.11	0.53	−0.32	0.14
Meat (ruminant)						
Total	0.06	0.75	0.15	0.38	0.15	0.50
High-fat	0.07	0.68	0.00	0.99	0.15	0.50
Combined high-fat						
Dairy and meat	0.36	0.04	0.006	0.97	0.11	0.59

* The data have been adjusted for the three different types of FFQ.

TABLE III

Spearman Correlations Between Expression Levels of Genes Involved in Peroxisomal Phytanic Acid Metabolismor Function (n = 70)*

	AMACR	SCP2	CAT	DBP	PPARα
AMACR					
SCP2	0.58				
	<0.01				
CAT	0.65	0.71			
	<0.01	<0.01			
DBP	0.57	0.42	0.75		
	<0.01	<0.01	<0.01		
PPARα	0.43	0.52	0.64	0.59	
	<0.01	<0.01	<0.01	<0.01	
RXRα	0.18	0.65	0.4	0.11	0.3
	0.14	<0.01	<0.01	0.37	0.01

*ΔCT method was used. Gene expression is normalized to housekeeping genes.

TABLE IV

Partial Spearman Correlation Coefficients Between Dietary Intake and mRNA Expression Levels in Benign Prostate Tissue (n = 68)*

	AMACR		SCP2		DBP		CAT		RXRα		PPARα	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Dairy												
Total	−0.07	0.55	0.03	0.80	−0.09	0.46	−0.02	0.85	0.20	0.11	−0.14	0.26
High-fat	0.03	0.84	0.07	0.58	−0.02	0.87	0.05	0.67	0.16	0.20	−0.17	0.17
Whole milk	−0.15	0.24	−0.15	0.24	−0.21	0.09	−0.24	0.05	0.05	0.71	0.25	0.04
Butter	0.14	0.25	−0.01	0.91	−0.08	0.50	−0.01	0.92	0.01	0.93	−0.06	0.59
Meat (ruminant)												
Total	0.23	0.06	0.07	0.55	0.22	0.07	0.09	0.49	0.08	0.53	0.07	0.58
High-fat	0.21	0.09	0.02	0.90	0.15	0.23	0.02	0.90	−0.03	0.81	−0.01	0.95
Combined high-fat												
Dairy and meat	0.13	0.31	0.11	0.35	0.10	0.42	0.13	0.33	0.16	0.19	−0.13	0.30

*The data have been adjusted for the three different types of FFQ.

TABLE V

Spearman Correlation Coeffıcients Between Serum and Tissue Phytanic Acid Levels and mRNA Expression in Benign Prostate Tissue

	Serum phytanic (n = 31)		Tissue phytanic (n = 26)	
mRNA	**r**	***P*-value**	**r**	***P*-value**
AMACR	0.13	0.47	0.03	0.88
SCP2	0.22	0.23	0.09	0.66
DBP	0.26	0.16	0.24	0.23
CAT	0.12	0.51	0.14	0.48
RXRα	0.41	0.02	0.11	0.61
PPARα	0.00	0.97	0.21	0.31