Published in final edited form as:

Metabolomics. 2014 October 1; 10(5): 1036–1041. doi:10.1007/s11306-014-0643-0.

1-stearoylglycerol is associated with risk of prostate cancer: results from serum metabolomic profiling

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Abstract

Although prostate cancer is the most commonly diagnosed cancer among men in developed populations, recent recommendations against routine prostate-specific antigen screening have cast doubt on its utility for early detection. We compared the metabolomic profiles of prospectively collected fasting serum from 74 prostate cancer cases and 74 controls selected from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of male smokers. Circulating 1-stearoylglycerol (1-SG, or 1-monostearin) was statistically significantly inversely associated with risk of prostate cancer after Bonferroni correction for multiple comparisons (i.e., 420 identified metabolites) (OR=0.34, 95% CI=0.20 – 0.58, $p=6.3 \times 10^{-5}$). The magnitude of this association did not differ by disease aggressiveness and was observed for cases diagnosed up to 23 years after blood collection. Similar but somewhat weaker prostate cancer risk signals were also evident for glycerol and alpha-ketoglutarate. In this population, men with higher serum 1-SG were less likely to develop prostate cancer, supporting a role for dysregulation of lipid metabolism in this malignancy. Additional studies are needed to retest the association and to examine 1-SG for its potential as a prostate cancer early detection marker.

Keywords

Metabolomics; prostatic neoplasms; prospective study; nested case-control study; early detection; biomarker

Introduction

Although prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer death in most developed populations, there are no well-established modifiable risk factors for the disease (Brawley 2012). Further, several organizations have

recommended against routine prostate-specific antigen (PSA) screening, the one available early detection marker, because of over-diagnosis and over-treatment (Moyer, Force 2012). Emerging metabolomic technologies that measure an array of low molecular weight biochemicals in blood and other tissue have the potential to identify compounds relevant to cancer etiology and early detection (Nicholson, Lindon 2008; O'Connell 2012). As recently reviewed, a few studies have compared the metabolomic profiles of prostate tissue, urine, or blood from men with and without prostate cancer, and by disease aggressiveness (Trock 2011), and another study specifically compared the plasma metabolomic profiles of men with stage 2 prostate cancer to those of healthy volunteers (Lokhov, Dashtiev, Moshkovskii, Archakov 2010). To our knowledge, however, no studies to date have examined pre-diagnostic metabolomic profiles in circulation years in advance of disease in relation to subsequent risk of prostate cancer. We report here discovery within a prospective nested case-control study of a novel metabolite associated with prostate cancer up to two decades prior to diagnosis.

Materials and Methods

Details of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study have been described (1994). Briefly, 29,133 Caucasian male smokers from southwestern Finland were recruited between 1985 and 1988. Men were between 50-69 years old at baseline and smoked at least 5 cigarettes per day, as part of the study eligibility criteria. Men who were enrolled in the trial were assigned to one of four intervention groups based on a 2×2 factorial design: 1) α -tocopherol (dl- α -tocopheryl-acetate, 50 mg/day), 2) β -carotene (20 mg/day), 3) both supplements, or 4) placebo. Trial participants took the study capsules for 5-8 years (median 6.1 years), until death, or until the trial ended on April 30, 1993. At enrollment, participants completed questionnaires about general risk factors, smoking, and medical history as well as a validated food-frequency questionnaire, and had their height and weight measured and an overnight fasting serum sample collected (protected from light and stored at -70°C until assayed). Written informed consent was obtained from all participants, and the trial was approved by institutional review boards at both the Finnish National Public Health Institute and the US National Cancer Institute.

The present analysis includes 74 confirmed cases of incident prostate adenocarcinoma diagnosed from 1-23 years after blood collection matched 1:1 with controls on age (± 1 year) and date of baseline blood collection (± 30 days). Aggressive cases were over-sampled (n=53) to permit their examination separately and defined as TNM stage III-IV, AJCC stage 3, or Gleason grade ≥ 8 (Gleason data available for all but one case). Serum metabolomic profiling was conducted at Metabolon, Inc. (Durham, N.C.) using ultrahigh performance liquid chromatography/mass spectroscopy (LC-MS) and gas chromatography/mass spectroscopy (GC-MS). Raw data were extracted, peak-identified, and quality control (QC) processed on the assay platform as previously described (Dehaven, Evans, Dai, Lawton 2010; Evans, DeHaven, Barrett, Mitchell, Milgram 2009). After excluding 206 metabolites not detected in all batches, there were 420 identified compounds for analysis. Each batch included blinded duplicate QC samples. The median and interquartile range of the intraclass correlation coefficients (ICC) across the metabolites was 0.84 (0.57–0.95). These ICCs are similar to those previously observed for blood samples analyzed by the same laboratory

(Sampson, Boca, Shu, Stolzenberg-Solomon, Matthews, Hsing, Tan et al. 2013). Serum total prostate specific antigen (PSA) concentration was measured for 122 of the participants in this study in the laboratory of Dr. Alan Remaley in the NIH Clinical Center. Each batch included blinded duplicate QC samples, with the CVs ranging from 5-10%.

For each metabolite and subject, signal strength was divided by the batch median value to standardize for batch variability; these normalized levels were then log-transformed and missing values were assigned the minimum non-missing value. We estimated the association between log-metabolite levels and prostate cancer by unmatched logistic regression; results from matched analyses were very similar. Multivariable models were adjusted for age, batch, trial supplement assignment, first-degree family history of prostate cancer, and physical activity. We also examined height, body mass index, smoking, education, intakes of total energy, fruit, vegetables, red meat, alcohol, and caffeine, and use of supplements, but they were not associated with prostate cancer (all $p \geq 0.16$, Table 1) and were not included in the model. A threshold for statistical significance of 0.000119 was applied based on a Bonferroni correction for 420 tests. It should be noted that this is a highly conservative (i.e., stringent) threshold because of intercorrelation between several metabolites. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, N.C.), and all statistical tests were 2-sided.

Results

Characteristics of the study population by case status are shown in Table 1. No factors appeared to differ by case status, with the possible exception of lower physical activity among the cases.

The top twenty metabolites associated with overall, non-aggressive, and aggressive prostate cancer after multivariable adjustment are shown in Table 2 (results for all identified metabolites are presented in Online Resource 1). We found that 1-stearoylglycerol (1-SG, or 1-monostearin) was statistically significantly inversely associated with risk of overall prostate cancer, and the magnitude of this association did not differ appreciably by disease aggressiveness (Table 2). The ICC for 1-SG was 0.77. An inverse association with the related compound glycerol was also observed, although it was not statistically significant after correction for multiple comparisons. The association between 1-SG and risk of prostate cancer persisted and was more highly ranked among cases diagnosed >5 years after blood collection (OR=0.20, 95% CI=0.07–0.57, $p=0.0024$). Further adjustment for serum total cholesterol did not alter our findings (data not shown). Another highly-ranked metabolite in our analysis was alpha-ketoglutarate (Table 2), along with an unnamed compound that could not be definitively structurally elucidated but appeared related to alpha-ketoglutarate (OR=0.49, 95% CI=0.33–0.72, $p=0.0003$). None of these compounds were strongly correlated with serum total PSA concentration (1-SG: $r=-0.15$, $p=0.10$; glycerol: $r=0.01$, $p=0.93$; alpha-ketoglutarate: $r=-0.16$, $p=0.08$).

Discussion

1-SG is a monoacylglycerol, a class of compounds that can be products of lipid catabolism and converted to free fatty acids by monoacylglycerol lipase (MAGL). A recent study found markedly lower expression of 1-SG in two subtypes of hepatocellular carcinoma tumor versus adjacent normal tissue (Beyoglu, Imbeaud, Maurhofer, Bioulac-Sage, Zucman-Rossi, Dufour, Idle 2013), suggesting that lipid catabolism is an important feature of these tumor subtypes. This is consistent with the known reprogramming of lipid biosynthetic pathways in cancer cells that provides energy and metabolites to facilitate growth, proliferation and progression (Menendez 2010). Recent studies have also demonstrated a role for MAGL in cancer pathogenesis and, in particular, in prostate cancer aggressiveness, with greater MAGL expression in more aggressive cell lines (e.g., SKOV3, C8161, 231MFP, PC3, and DU145), and attenuation of aggressive characteristics by pharmacological disruption of, or through RNA-interference with, its activity (Nomura, Lombardi, Chang, Niessen, Ward, Long, Hoover et al. 2011; Nomura, Long, Niessen, Hoover, Ng, Cravatt 2010). Our observation of lower 1-SG concentrations in pre-diagnostic serum of men with prostate cancer relative to controls is consistent with such changes in tumor metabolism; further research will be required to determine whether this finding is specific to prostate cancer or to carcinogenesis more generally. Although the three prior prostate cancer-circulating metabolite analyses did not find a similar association for 1-SG, none of these studies reported 1-SG among their identified metabolites and none were prospective, with blood collected and stored years in advance of the diagnosis of cancer (Lokhov, Dashtiev, Moshkovskii, Archakov 2010; Sreekumar, Poisson, Rajendiran, Khan, Cao, Yu, Laxman et al. 2009; Thysell, Surowiec, Hornberg, Crnalic, Widmark, Johansson, Stattin et al. 2010). Also, we and others were not able to replicate the findings of these previous studies; for example, the tumor tissue sarcosine finding of an early study (Sreekumar, Poisson, Rajendiran, Khan, Cao, Yu, Laxman et al. 2009), and the significant associations with good predictive values for two metabolites, acylcarnitine and arachidonoyl amine (Lokhov, Dashtiev, Moshkovskii, Archakov 2010), which were not identified by our LC-MS/GC-MS metabolomic platform. Differences across studies such as those resulting from case selection (e.g., stage of disease), biospecimens (e.g., plasma or tumor tissue, and preparation), temporality (i.e., when samples are collected relative to diagnosis), and a wide range of metabolomic platforms and identifiable molecular species make cross-study comparisons challenging. The inherently dynamic nature of the human metabolome likely also plays a role. A strength of the present study was the use of fasting serum specimens that were collected up to twenty years in advance of prostate cancer diagnoses and excellent laboratory quality control as indicated by ICC's; yet, based on the parent study, we were only able to examine smokers which will require broadening in future studies.

Concluding Remarks

In this study of smokers, we found 1-SG to be inversely associated with risk of prostate cancer up to 23 years prior to diagnosis, supporting a role for dysregulation of lipid metabolism in prostate carcinogenesis. Further studies are needed to reexamine our findings, including particularly whether the 1-SG metabolite signal strength varies during a 10 or 20 year sub-clinical, prediagnostic period and may have potential utility as a prostate cancer

early detection biomarker. Studies with serial blood sampling prior to diagnosis will be very informative in this regard. Whether the observed signal for 1-SG is independent of or correlated with PSA concentrations should also be examined.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health (U.S. Public Health Service), and U.S. Public Health Service contracts N01-CN-45165, N01-RC-45035, N01-RC-37004, HHSN261201000006C, HHSN261200800001E.

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Table 1Age-adjusted^a baseline^b characteristics by case-control status

	Controls	Cases	<i>p-value</i>
N	74	74	
Age (years)	59.1	59.3	<i>matched</i>
Height (cm)	173	174	<i>0.16</i>
Weight (kg)	78.3	79.5	<i>0.91</i>
BMI (kg/m²)	26.1	26.1	<i>0.77</i>
Serum cholesterol (mmol/L)	6.3	6.3	<i>0.63</i>
Serum α-tocopherol (mg/L)	11.9	12.1	<i>0.48</i>
Serum β-carotene (μg/L)	235	213	<i>0.99</i>
Serum retinol (μg/L)	609	595	<i>0.35</i>
Cigarettes per day	19.1	19.6	<i>0.81</i>
Years of smoking	38.2	37.6	<i>0.91</i>
Family history of prostate cancer (%)	6.7	4.3	<i>0.69</i>
Physically active (%)	25.7	14.9	<i>0.10</i>
Dietary intake per day			
Total energy (kcal)	2,707	2,810	<i>0.32</i>
Fruit (g)	235	192	<i>0.31</i>
Vegetables (g)	289	287	<i>0.83</i>
Red meat (g)	71.2	75.0	<i>0.38</i>
Alcohol (ethanol, g)	16.9	15.4	<i>0.64</i>
Supplement use			
Vitamin A (%)	13.5	13.5	<i>1.0</i>
Vitamin D (%)	8.1	8.1	<i>1.0</i>
Calcium (%)	12.2	8.1	<i>0.41</i>

^aValues are means unless otherwise indicated.^bAll characteristics are from the baseline questionnaire except family history which was collected during follow-up and is available for 107 men in this study.

Table 2

Top twenty metabolites associated with overall, non-aggressive, and aggressive prostate cancer in the ATBC Study

	Metabolite	Chemical Class	Odds Ratio (95% CI) ^a	p-value
Overall Prostate Cancer (74 cases/74 controls)	1-stearoylglycerol (1-monostearin)	Lipid	0.34 (0.20 – 0.58)	6.3E-05
	Glycerol	Lipid	0.47 (0.31 – 0.72)	0.0005
	alpha-ketoglutarate	Energy	0.53 (0.35 – 0.81)	0.0030
	myristoleate (14:1n5)	Lipid	0.58 (0.40 – 0.85)	0.0050
	10-heptadecenoate (17:1n7)	Lipid	0.59 (0.41 – 0.86)	0.0056
	palmitoleate (16:1n7)	Lipid	0.60 (0.41 – 0.87)	0.0069
	5-methylthioadenosine (MTA)	Amino acid	1.64 (1.14 – 2.34)	0.0071
	phenylalanylleucine	Peptide	1.64 (1.14 – 2.36)	0.0071
	myristate (14:0)	Lipid	0.60 (0.41 – 0.87)	0.0073
	21-hydroxypregnenolone disulfate	Lipid	0.59 (0.41 – 0.87)	0.0075
	linolenate [alpha or gamma; (18:3n3 or 6)]	Lipid	0.61 (0.43 – 0.88)	0.0082
	Methionine	Amino acid	0.56 (0.37 – 0.87)	0.0089
	phenylalanine	Amino acid	0.62 (0.43 – 0.89)	0.0095
	Valerate	Lipid	1.66 (1.13 – 2.43)	0.0097
	oleate (18:1n9)	Lipid	0.62 (0.43 – 0.90)	0.0107
	Xanthine	Nucleotide	0.62 (0.42 – 0.90)	0.0114
	Sphinganine	Lipid	0.63 (0.44 – 0.90)	0.0117
	3-methyl-2-oxobutyrate	Amino acid	0.60 (0.40 – 0.90)	0.0127
	Glycerate	Carbohydrate	0.62 (0.43 – 0.90)	0.0128
	propionylcarnitine	Lipid	0.61 (0.42 – 0.90)	0.0136
Non-Aggressive Prostate Cancer (20 cases/74 controls)	Glycerol	Lipid	0.33 (0.15 – 0.71)	0.0044
	10-heptadecenoate (17:1n7)	Lipid	0.42 (0.22 – 0.77)	0.0051
	palmitoleate (16:1n7)	Lipid	0.40 (0.20 – 0.77)	0.0062
	myristoleate (14:1n5)	Lipid	0.41 (0.21 – 0.80)	0.0090
	oleate (18:1n9)	Lipid	0.43 (0.22 – 0.81)	0.0093
	margarate (17:0)	Lipid	0.42 (0.22 – 0.82)	0.0106
	10-nonadecenoate (19:1n9)	Lipid	0.44 (0.23 – 0.84)	0.0122
	myristate (14:0)	Lipid	0.44 (0.23 – 0.84)	0.0127
	palmitate (16:0)	Lipid	0.47 (0.26 – 0.86)	0.0150
	pentadecanoate (15:0)	Lipid	0.48 (0.26 – 0.87)	0.0150
	linolenate [alpha or gamma; (18:3n3 or 6)]	Lipid	0.48 (0.26 – 0.87)	0.0157
	Lysine	Amino acid	0.36 (0.15 – 0.83)	0.0164
	21-hydroxypregnenolone disulfate	Lipid	0.46 (0.24 – 0.88)	0.0191
	1-stearoylglycerol (1-monostearin)	Lipid	0.29 (0.10 – 0.82)	0.0199
	2-arachidonoylglycerophosphoethanolamine	Lipid	0.48 (0.25 – 0.93)	0.0289
	5-dodecenoate (12:1n7)	Lipid	0.50 (0.26 – 0.93)	0.0291
	azelate (nonanedioate)	Lipid	0.49 (0.26 – 0.94)	0.0304

	Metabolite	Chemical Class	Odds Ratio (95% CI) ^a	p-value
Aggressive Prostate Cancer (53 cases/74 controls)	linoleate (18:2n6)	Lipid	0.50 (0.27 – 0.94)	0.0307
	stearate (18:0)	Lipid	0.49 (0.25 – 0.94)	0.0330
	eicosenoate (20:1n9 or 11)	Lipid	0.52 (0.29 – 0.95)	0.0341
	1-stearoylglycerol (1-monostearin)	Lipid	0.36 (0.21 – 0.63)	0.0003
	alpha-ketoglutarate	Energy	0.47 (0.30 – 0.74)	0.0010
	Valerate	Lipid	1.99 (1.29 – 3.07)	0.0017
	Glycerol	Lipid	0.51 (0.32 – 0.80)	0.0036
	phenol sulfate	Amino acid	1.88 (1.23 – 2.87)	0.0037
	3-methyl-2-oxobutyrate	Amino acid	0.54 (0.35 – 0.82)	0.0043
	5-methylthioadenosine (MTA)	Amino acid	1.81 (1.20 – 2.72)	0.0044
	propionylcarnitine	Lipid	0.53 (0.33 – 0.84)	0.0075
	Methionine	Amino acid	0.53 (0.33 – 0.86)	0.0093
	Heme	Cofactors and vitamins	0.59 (0.39 – 0.88)	0.0103
	Xanthine	Nucleotide	0.59 (0.40 – 0.89)	0.0110
	phenylalanylleucine	Peptide	1.63 (1.11 – 2.41)	0.0130
	Isovalerate	Lipid	1.65 (1.10 – 2.45)	0.0143
	Alanine	Amino acid	0.62 (0.42 – 0.92)	0.0167
	Ribose	Carbohydrate	0.62 (0.42 – 0.92)	0.0178
	Sphinganine	Lipid	0.61 (0.41 – 0.92)	0.0182
	Glycocholate	Lipid	0.64 (0.44 – 0.94)	0.0210
	3-hydroxyisobutyrate	Amino acid	0.64 (0.44 – 0.94)	0.0242
	pregnen-diol disulfate	Lipid	0.66 (0.45 – 0.96)	0.0294
	phenylalanyltryptophan	Peptide	1.77 (1.06 – 2.95)	0.0299

^aThe odds ratio per 1 standard deviation increase in metabolite level. Analyses are adjusted for age, batch, trial supplement assignment, family history of prostate cancer, and physical activity.