Fatty Acid Synthase Polymorphisms, Tumor Expression, Body Mass Index, Prostate Cancer Risk, and Survival

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ABSTRACT

Purpose

Fatty acid synthase (FASN) regulates de novo lipogenesis, body weight, and tumor growth. We examined whether common germline single nucleotide polymorphisms (SNPs) in the *FASN* gene affect prostate cancer (PCa) risk or PCa-specific mortality and whether these effects vary by body mass index (BMI).

Methods

In a prospective nested case-control study of 1,331 white patients with PCa and 1,267 age-matched controls, we examined associations of five common SNPs within *FASN* (and 5 kb upstream/downstream, $R^2 > 0.8$) with PCa incidence and, among patients, PCa-specific death and tested for an interaction with BMI. Survival analyses were repeated for tumor *FASN* expression ($n = 909$).

Results

Four of the five SNPs were associated with lethal PCa. SNP rs1127678 was significantly related to higher BMI and interacted with BMI for both PCa risk ($P_{\text{interaction}} = .004$) and PCa mortality ($P_{\text{interaction}} = .056$). Among overweight men ($\text{BMI} \geq 25 \text{ kg/m}^2$), but not leaner men, the homozygous variant allele carried a relative risk of advanced PCa of 2.49 (95% CI, 1.00 to 6.23) compared with lean men with the wild type. Overweight patients carrying the variant allele had a 2.04 (95% CI, 1.31 to 3.17) times higher risk of PCa mortality. Similarly, overweight patients with elevated tumor *FASN* expression had a 2.73 (95% CI, 1.05 to 7.08) times higher risk of lethal PCa ($P_{\text{interaction}} = .02$).

Conclusion

FASN germline polymorphisms were significantly associated with risk of lethal PCa. Significant interactions of BMI with *FASN* polymorphisms and *FASN* tumor expression suggest *FASN* as a potential link between obesity and poor PCa outcome and raise the possibility that *FASN* inhibition could reduce PCa-specific mortality, particularly in overweight men.

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INTRODUCTION

In tumor cells, most fatty acids are synthesized de novo by fatty acid synthase (FASN) to meet the excessive bioenergetic and structural demands of proliferation.¹ FASN is overexpressed in prostate tumors, both at the mRNA and protein level, and is highest in metastatic tumors.² Overexpression of FASN is associated with more aggressive disease, whereas treatment with FASN inhibitors decreases prostate tumor cell proliferation and induces cell death.^{3,4} Thus, *FASN* may act as an oncogene in epithelial tumors including prostate cancer (PCa).^{5,6}

Obesity is associated with aggressive PCa, poor outcomes after prostatectomy⁷ or radia-

tion,⁸ and increased PCa-specific mortality,⁹ but mechanisms underlying these effects are not well characterized. Two studies link *FASN* polymorphisms with obesity in humans, and *FASN* inhibition rapidly reduces fat stores in mice, clearly demonstrating that *FASN* impacts energy homeostasis.¹⁰⁻¹² Links between *FASN* polymorphisms and obesity and between *FASN* expression and aggressive PCa raise the possibility that *FASN* could play a role in the interaction between obesity and PCa lethality. In this study, we examine whether inherited variation in the *FASN* gene or variations in tumor *FASN* expression impact PCa lethality and whether these effects vary by body mass index (BMI).

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METHODS

Study Population for Genetic Analyses

This analysis is nested in the Physicians' Health Study (PHS), a randomized, double-blind, placebo-controlled trial of aspirin and β -carotene among 22,071 healthy US male physicians, age 40 to 84 years in 1982. BMI was reported at baseline and categorized as normal ($< 25 \text{ kg/m}^2$) or overweight ($\geq 25 \text{ kg/m}^2$).^{13,14} All patients with PCa were verified through medical record and pathology review with high follow-up for cancer incidence (96%) and mortality (98%).

In 1982, 14,916 men provided blood samples. We used a nested case-control design (1,331 patient cases and 1,267 controls), with controls selected by risk set sampling and matched on age at baseline and follow-up time. We restricted this analysis to self-reported whites (93% of the cohort) to reduce the potential for population stratification. Forty men who were diagnosed after they had previously been matched as controls in the case-control analysis were included in both the case-control analyses (controlling for follow-up time) and the outcomes analyses. During follow-up through March 1, 2008, 161 men died of PCa. The median follow-up time was 9.6 years (range, 0 to 25.3 years). All participants provided written informed consent for inclusion. This investigation was approved by the Human Subjects Committee at Brigham and Women's Hospital.

Single Nucleotide Polymorphisms, DNA, and Genotyping

Using the HapMap database (National Center for Biotechnology Information Build 35) and the Web-based Tagger application (<http://broad.harvard.edu/mpg/tagger/>), five single nucleotide polymorphisms (SNPs) captured genetic variation (with $R^2 > 0.80$) within *FASN* and 5 kb upstream/downstream (Fig 1). Selection criteria excluded SNPs with a minor allele frequency less than 5% in the HapMap CEU population. DNA was extracted from whole blood. Genotyping was performed with iPLEX (Sequenom, San Diego, CA) matrix-assisted laser desorption/ionization-time of flight mass spectrometry technology at the Partners HealthCare Center for Personalized Genetic Medicine. All SNPs had genotype completion rates greater than 91%.

Protein Expression and PCa Prognosis

To analyze the relationship between tumor *FASN* protein expression and development of lethal PCa (defined as death caused by PCa or development of bony metastases), we identified men treated with radical prostatectomy for whom we obtained formalin-fixed paraffin-embedded surgical tumor specimens ($n = 909$) from the PHS or from the Health Professionals Follow-Up Study, a prospective cohort study of 51,529 US male health professionals begun in 1986.¹⁵ Among patient cases with tumor specimens, 72 patients developed lethal PCa (55 patients with PCa-specific deaths and 17 others with bony metastases). Follow-up began at diagnosis and ended on March 1, 2008 (PHS); May 31, 2008 (Health Professionals Follow-Up Study); or the date of PCa death or death as a result of other causes, whichever was first. The median follow-up time was 10.8 years (range, 0.1 to 21.1 years). Nine tissue microarrays (TMAs) with at least three tumor tissue cores from the targeted areas of each specimen were constructed, arraying 0.6-mm cores per patient.

Four micrometer-thick sections were cut from each TMA on charged slides and used for immunohistochemical analysis. Sections were incubated using a rabbit polyclonal antibody for *FASN* (Assay Designs, Ann Arbor, MI; diluted 1:400), as previously described.⁶

Stained TMA slides were scanned at low magnification using the Ariol instrument SL-50 (Applied Imaging, Grand Rapids, MI), and the auto core-mapping function automatically located and centered the selection area over the tissue cores for high-power scanning. Every core was reviewed by a pathologist to ensure matching between the TMA map and the actual cell composition of the core. Normal prostatic glands were excluded from analysis by manual circling. Image analysis was performed using the following two output variables: region score (positive area/positive + negative area) and median positive intensity. Region score represents the percentage of positive cells, and median positive intensity is a numerical measure of the staining intensity. These two variables were combined to obtain a single *FASN* protein expression score using the following equation: score = log (region score $\times$ median positive intensity).

Fig 1. Five tagging single nucleotide polymorphisms (SNPs) identified in or within 5,000 kb up- and downstream of the fatty acid synthase (*FASN*) gene.

Statistical Analysis

The major analyses included a nested case-control design assessing the five *FASN* polymorphisms in relation to risk of developing PCa; a survival analysis assessing the associations of the five SNPs with risk of progression to fatal PCa among men diagnosed with the disease; and a survival analysis of *FASN* expression in tumor and progression to fatal outcome. We also assessed interactions between baseline BMI and *FASN* gene or tumor expression in all three main analyses.

The tag SNP selection algorithm makes assumptions based on single SNP analysis as appropriate analytic strategy; therefore, we focused on the single SNP analysis. We explored haplotype analysis but did not observe any associations more significant (or different) or insightful from what we had found in the single SNP analysis. Therefore, we present data only from the single SNP analysis. Using Pearson's goodness-of-fit test, none of the SNPs violated Hardy-Weinberg equilibrium ($P > .05$). SNPs were analyzed under additive model for the risk and survival analysis and the dominant model for test of interactions because of limited sample size in subgroups.

Patient cases and controls were matched by age and follow-up duration but not by race. Because excluding nonwhites or conducting subgroup analysis led to losing some case-control pairs, we used an unconditional logistic regression model to assess the risk of incident PCa according to genotype, adjusting for the matching factors (age at random assignment, smoking status, and follow-up time). We also conducted a subgroup analysis comparing patient cases with Gleason score ≥ 8 or clinically advanced stage (T3, T4, N1, or M1) with all controls.

Among patients with PCa, we performed a survival analysis of the *FASN* SNPs using a Kaplan-Meier analysis and Cox regression model adjusting for

Table 1. Baseline Demographics and Clinical Characteristics of Patients With Prostate Cancer and Controls in the Physicians' Health Study

Characteristic	Patients With Cancer (n = 1,331)		Controls (n = 1,267)	
	No.	%	No.	%
Age at study onset, years				
Mean	57.8		57.5	
Standard deviation	8.4		8.4	
Age at diagnosis, years				
Mean	70.1			
Standard deviation	7.4			
Diagnosis to prostate cancer death/censoring, years				
Median	9.6			
Range	0.0-25.3			
Deaths as a result of prostate cancer	161	12.1		
Clinical stage				
T1/T2	1,107	88.4		
T3	64	5.1		
T4/N1/M1	81	6.5		
Unknown	79			
Gleason score				
2-6	797	61.6		
7	337	26.0		
8-10	160	12.4		
Unknown	37			
Baseline smoking status				
Former	571	43.0	532	42.0
Current	114	8.6	99	7.8
Body mass index, kg/m ²				
Mean	24.7		24.7	
Standard deviation	2.4		2.6	

age at diagnosis and follow-up time. The outcome was death as a result of PCa (n = 161); follow-up began at diagnosis, and individuals were censored at the time of non-PCa death, loss to follow-up, or the end of follow-up. In secondary analyses, we further adjusted for baseline BMI, Gleason score, and clinical stage.

We used linear regression analysis to determine whether SNPs predict BMI levels, adjusting for the matching factors and case-control status. In a subgroup of 183 patient cases, we also assessed whether FASN expression in PCa tumors vary by FASN genotype using linear regression models controlling for TMA batch.

To assess whether the associations of FASN SNPs and PCa risk and survival were modified by BMI, we created interaction terms of BMI

(< $\nu \geq 25$ kg/m²) and genotype. In an unconditional logistic regression model, we used an additive model for incidence, and in a Cox regression model, we used a codominant model for survival as a result of limited sample size. The fit was compared with models containing only the main effects using a likelihood ratio test.

To assess associations of FASN protein expression and development of lethal PCa, we dichotomized FASN expression as high versus low, defined as the top two tertiles versus the bottom tertile, based on a prior finding that nearly two thirds of prostate tumors overexpress FASN by immunohistochemistry compared with normal prostate tissue.¹⁶ We also created an interaction term of BMI and used a Cox regression model for lethal PCa, which included the BMI (< $\nu \geq 25$ kg/m²) indicator variable, the FASN expression, and the interaction term. All statistics were calculated using SAS (version 9.1.3; SAS Institute, Cary, NC), with a two-sided significance level of $P = .05$.

RESULTS

Association of SNPs With PCa Incidence and FASN Expression

Demographics and clinical characteristics of the PHS patients with PCa and controls are listed in Table 1. Among the five FASN tagging SNPs, we found a significant inverse association of the variant A allele of SNP rs8066956 with the overall incidence of PCa; the relative risk (RR) per A allelic number increase was 0.84 (95% CI, 0.74 to 0.96; $P_{\text{trend}} = .01$). The association was stronger for advanced disease (stage T3, T4, N1, or M1), with an RR of 0.65 (95% CI, 0.47 to 0.91; $P_{\text{trend}} = .01$; Table 2). In addition, the T variant allele of SNP rs6502051 was also significantly associated with a lower risk of advanced PCa (per-allele RR = 0.77; 95% CI, 0.59 to 0.99; $P_{\text{trend}} = .04$). SNPs rs1127678, rs4246444, and rs12949488 were not significantly associated with risk of incident PCa (overall or by stage or grade).

In a subgroup of 183 patients with PCa with both genotyping and PCa tissue data, men homozygous for the AA variant of rs8066956 had significantly lower expression of FASN in their tumors (Fig 2A), which supports the link between the A variant allele and lower PCa risk. Other FASN SNPs were not significantly associated with expression (Appendix Table A1, online only).

Association of SNPs With PCa Mortality

Survival analysis among the patients with PCa showed inverse associations between SNPs rs4246444 and rs6502051 and PCa-specific mortality. Compared with the CC wild type of rs4246444, the hazard ratio (HR) for PCa mortality for the homozygous AA variant was 0.40 (95% CI, 0.16 to 0.98) adjusting for age at diagnosis, baseline BMI,

Table 2. Genotype and Allele Frequencies for Tagging SNPs in the FASN Gene and Adjusted RRs* and 95% CIs of Incident PCa

SNP	Genotype	MAF (%)		Overall PCa			Advanced PCa†		
		Patients	Controls	RR	95% CI	P_{trend}	RR	95% CI	P_{trend}
rs1127678	G>A	21.9	21.1	1.05	0.92 to 1.20	.51	1.11	0.83 to 1.50	.47
rs6502051	G>T	45.9	47.7	0.93	0.83 to 1.04	.19	0.77	0.59 to 0.99	.04
rs4246444	C>A	28.0	29.3	0.92	0.82 to 1.04	.20	0.94	0.71 to 1.24	.67
rs12949488	G>A	21.5	21.3	1.02	0.90 to 1.17	.73	0.90	0.66 to 1.23	.51
rs8066956	G>A	22.6	25.6	0.84	0.74 to 0.96	.01	0.65	0.47 to 0.91	.01

Abbreviations: SNP, single nucleotide polymorphism; FASN, fatty acid synthase; RR, relative risk; PCa, prostate cancer; MAF, minor allele frequency.

*RR associated with variant allele carrying status adjusted for age at random assignment, smoking status, and follow-up time.

†Advanced PCa was defined as clinical stage T3/N0/M0, T4/N0/M0, N1, or M1.

Fig 2. (A) Age- and tissue microarray (TMA)-adjusted mean fatty acid synthase (FASN) expression in prostate tumor according to FASN polymorphism (rs8066956; $n = 183$). (B) Body mass index (BMI) according to FASN polymorphism (rs1127678; $n = 2,598$) adjusted for age, smoking, and case-control status. (C) Significant interaction ($P_{\text{interaction}} = .004$) between BMI and FASN polymorphism (rs1127678) with risk of developing advanced prostate cancer (1,331 patients and 1,267 controls). RR, relative risk.

time between enrollment and PCa diagnosis, clinical stage, and Gleason score. The per-allele HR was 0.72 (95% CI, 0.55 to 0.95; $P_{\text{trend}} = .02$). Similarly, the corresponding per-allele HR for the T variant of rs6502051 was 0.81 (95% CI, 0.64 to 1.03; $P_{\text{trend}} = .08$; Table 3). These data suggest that the associations were independent of BMI and known PCa characteristics.

FASN Gene, BMI, and Interaction With PCa Risk and Mortality

Overall carriers of the homozygous AA (v GG) variant of rs1127678 had an approximately 2% ($P = .05$) higher prediagnostic BMI (Fig 2B). Among overweight (BMI ≥ 25 kg/m²) patients with PCa who died, the median BMI was significantly higher in those with

the AA genotype (BMI = 28.8 kg/m²) than those with GG wild type (BMI = 26.7 kg/m², $P = .004$; Appendix Table A2, online only). Similarly, this SNP interacted with BMI both for risk of advanced PCa and PCa-specific mortality. Specifically, among men who were overweight or obese (BMI ≥ 25 kg/m²), but not among leaner men (BMI < 25 kg/m²), carriers of the homozygous variant AA had a 2.49 times (95% CI, 1.00 to 6.23 times) increased risk of developing advanced PCa ($P_{\text{interaction}} = .004$; Fig 2C).

Among overweight patients with PCa, the 10-year cumulative PCa mortality was higher for those with a variant allele (AA or AG) of rs1127678 compared with the GG wild type (11% v 8%, respectively); the corresponding HR was 2.04 (95% CI, 1.31 to 3.17) for the risk of PCa mortality among overweight or obese patients with the variant allele (Fig 3A). The HR remained statistically significant after further controlling for clinical stage and Gleason score (HR = 1.78; 95% CI, 1.14 to 2.78). Among patients who were not overweight, the 10-year cumulative PCa mortality was essentially the same (6%) for men with PCa with and without the variant alleles (HR = 1.00; 95% CI, 0.62 to 1.60; $P_{\text{interaction}} = .056$).

Interaction Between FASN Expression and BMI and PCa Mortality

Overall, tumor FASN expression was not associated with either BMI ($P = .7$) or time to lethal PCa ($P = .4$). However, we found a significant interaction ($P_{\text{interaction}} = .02$) between tumor FASN expression and BMI for time to lethal PCa. Among 424 men with PCa who were overweight, a high FASN expression was associated with a significantly increased risk of lethal PCa (high v low FASN expression: HR = 2.73; 95% CI, 1.05 to 7.08), with a 10-year cumulative incidence of lethal PCa of 8% for high FASN expression versus 2% for low FASN expression (Fig 3B). However, among men who were not overweight, FASN expression level was not significantly associated with lethal PCa (HR = 0.72; 95% CI, 0.38 to 1.35).

Controlling for possible batch differences by adjusting for TMA had no material effect on any of the estimates. After controlling for pathologic Gleason score, pathologic stage, and prostate-specific antigen level at diagnosis, which were all strong predictors of lethal outcome, the interaction between FASN expression and BMI on the development of lethal PCa remained statistically significant ($P_{\text{interaction}} = .05$). All the associations listed here are summarized in Appendix Table A3 (online only).

DISCUSSION

Higher BMI is associated with worse PCa outcomes, and there is ample literature to suggest that FASN overexpression is associated with more aggressive cancers. In this prospective study, we found that among four of the five common tagging SNPs in the FASN gene, there were several associations with PCa risk, BMI, FASN overexpression, and PCa death, which together suggest that FASN might be an important mediator of the link between obesity and PCa progression.

The mechanism by which FASN exerts its influence might have been clearest if a single SNP could be linked to all of the relevant outcomes. However, the data showed that although rs8066956 and rs6502051 were related to the risk of advanced PCa and expression of FASN in tumor, a different SNP (rs1127678) was related to BMI and also had a strong interaction with BMI with regard to PCa risk and

Table 3. Adjusted Cox Proportional HR of PCa-Specific Mortality Among 1,331 Patients

SNP and Genotype	PCa Deaths		Censored		Adjusted for Age			Adjusted for Age, BMI, Stage, and Grade		
	No.	%	No.	%	HR	95% CI	P_{trend}	HR	95% CI	P_{trend}
rs6502051										
GG	54	34.6	311	28.5	1.00	Reference		1.00	Reference	
GT	76	48.7	544	49.9	0.80	0.57 to 1.14		0.71	0.50 to 1.02	
TT	26	16.7	236	21.6	0.66	0.41 to 1.06		0.70	0.43 to 1.13	
Per allele					0.81	0.65 to 1.02	.07	0.81	0.64 to 1.03	.08
rs4246444										
CC	86	54.8	579	51.4	1.00	Reference		1.00	Reference	
CA	66	42.0	454	40.3	0.93	0.68 to 1.29		0.78	0.56 to 1.09	
AA	5	3.2	94	8.3	0.33	0.13 to 0.82		0.40	0.16 to 0.98	
Per allele					0.77	0.59 to 1.00	.05	0.72	0.55 to 0.95	.02

Abbreviations: HR, hazard ratio; PCa, prostate cancer; BMI, body mass index.

mortality. Two other SNPs (rs6502051 and rs4246444) also had relevance to PCa mortality. Although no single SNP was related to all of the outcomes (Appendix Table A3), our current data and prior literature suggest that there may be multiple biologic pathways in which

FASN polymorphisms may be influencing PCa outcomes (Appendix Fig 1, online only). Specifically, *FASN* polymorphisms (eg, rs8066956) may be working at the level of the local tumor to increase intratumoral *FASN* expression, which in turn leads to more aggressive PCa. Alternatively, certain SNPs may directly lead to aggressive PCa (such as rs4246444 and rs6502051) or indirectly lead to aggressive PCa (such as rs1127678) by promoting obesity through central homeostatic mechanisms (Appendix Fig A1; Appendix Table A2).

One of the most striking findings in our study came from mirror image patterns of interactions of BMI with germline *FASN* polymorphism or with *FASN* protein expression in tumor. Among overweight or obese patients but not among normal-weight men, the rs1127678 variant allele was associated with a higher likelihood of PCa mortality ($P_{\text{interaction}} = .056$). Independently, high *FASN* protein expression in tumor was also associated with an increased risk of lethal disease among overweight patients but not among patients with normal weight ($P_{\text{interaction}} = .02$). A similar interaction between BMI and *FASN* expression has been reported in patients with colon cancer; *FASN* expression in tumor was associated with increased mortality in patients with a high BMI but not among leaner patients.¹⁷ These observations strongly suggest that this *FASN*-obesity-mortality interaction is genuine and not necessarily tissue specific.

The interaction between *FASN* and BMI is also consistent with our previous finding of a strong positive association of plasma C-peptide⁹ and inverse association of adiponectin¹⁸ with PCa mortality primarily in men with BMI ≥ 25 kg/m². On the basis of these results, one may speculate that in a metabolically permissive environment characterized by higher BMI, more abundant glucose, higher insulin/C-peptide levels, and low adiponectin,¹⁸ *FASN* may have an increased ability to exert its influence on either tumorigenesis or tumor maintenance. One possible mediator of this effect is adenosine monophosphate kinase (AMPK), which plays a crucial part in systemic energy balance by integrating nutritional and hormonal signals in peripheral tissues and in the hypothalamus. When AMPK is activated and functioning properly, it inhibits *FASN* activity.¹ However, in the setting of hyperinsulinemia and obesity, AMPK is usually impaired,¹⁹ which allows *FASN* activity to proceed unchecked. Nevertheless, although this mechanism might predict higher *FASN* expression in overweight men, more work is needed to explain why

Fig 3. Cumulative prostate cancer (PCa)-specific mortality after diagnosis by (A) fatty acid synthase (*FASN*) genotype (rs1127678) among patients with baseline body mass index (BMI) ≥ 25 kg/m² ($P_{\text{interaction}} = .056$) and (B) *FASN* tumor expression among patients with baseline BMI ≥ 25 kg/m² ($P_{\text{interaction}} = .02$).

only overweight men seem to face a worse prognosis when FASN is overexpressed. As Ogino et al¹⁷ have postulated in colon cancer, cells with FASN upregulation may depend on excess energy for growth, leading to increased aggressiveness among obese patients.

If confirmed, our findings raise the possibility of new treatment paradigms for interventions. FASN inhibitors, including orlistat, a treatment for obesity, seem to have antitumor activity in preclinical models,^{20,21} and derivatives of this drug with improved absorption could potentially be tested as adjuvant treatment for FASN-overexpressing prostate tumors, particularly among overweight men. Insulin and high-carbohydrate/low-fat diets result in upregulation of FASN,²²⁻²⁴ whereas exercise and energy restriction downregulate FASN.²⁵⁻²⁷ Therefore, dietary and lifestyle interventions may have the potential to improve PCa survival among overweight men whose tumors overexpress FASN, possibly by reducing FASN expression and its activity on tumorigenesis.

The associations of germline polymorphisms with phenotype (BMI and FASN expression) and PCa risk and survival, as well as their interactions, lend strength to a causal interpretation. Rather than being isolated associations, multiple links among FASN polymorphisms, expression, BMI, and lethal PCa seem to be mutually reinforcing. Optimal analysis allowed a precise, reproducible score for FASN expression that is not subject to variation in human interpretation. Also, the large number of patients with up to 25 years of follow-up for lethal PCa (rather than surrogate outcomes such as prostate-specific antigen recurrence) allowed enough power to discern the significant interaction between FASN's impact on survival and BMI, which could be missed in smaller studies. Finally, follow-up was collected prospectively and was 98% complete, which minimizes follow-up bias.

However, the small numbers in certain subpopulations limited statistical power to test for interactions and did not allow for separate derivation and validation data sets. In addition, the SNP analyses were limited to white patients. Therefore, these findings require further testing in other settings and in other ethnic groups.

In summary, common genetic polymorphisms in the FASN gene were associated with BMI, risk of advanced PCa, and PCa-specific

mortality. At both the gene level and the tumor protein level, FASN interacts with BMI such that it mainly increases the risk of advanced PCa and subsequent PCa mortality among overweight men. Although additional studies are needed, these results implicate FASN as a potential mediator between obesity and poor PCa outcomes and raise the possibility that FASN inhibition, particularly among overweight men, may reduce PCa mortality.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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