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ORIGINAL REPORT

Cohort Study of Fatty Acid Synthase Expression and Patient Survival in Colon Cancer

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S.O. had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
Energy balance seems to be important in the pathogenesis of colon cancer. Fatty acid synthase (FASN) is physiologically regulated by energy balance and is often upregulated in colorectal cancer. Nonetheless, the influence of FASN expression on patient outcome is uncertain.

Patients and Methods
Using the database of 647 patients with colon cancer in two independent cohort studies, FASN overexpression was detected in 84 tumors (13%) by immunohistochemistry. Cox proportional hazards models calculated hazard ratios (HRs) of colon cancer–specific and overall mortalities, adjusted for patient characteristics and related tumoral features, including *KRAS, BRAF*, p53, microsatellite instability and the CpG island methylation phenotype.

Results
There were 279 deaths, including 160 colon cancer–specific deaths. FASN overexpression was associated with a significant reduction in colon cancer–specific mortality by both univariate and multivariate analyses (adjusted HR, 0.41; 95% CI, 0.19 to 0.89) and an insignificant trend toward improved overall mortality (adjusted HR, 0.75; 95% CI, 0.50 to 1.13). Notably, the effect of FASN expression on mortality might be different according to body mass index (BMI; $P_{interaction} = .019$); the adjusted HR of overall mortality for FASN overexpression was 0.63 (95% CI, 0.39 to 1.02) among patients with BMI less than 27.5 kg/m^2 and 2.91 (95% CI, 1.19 to 7.12) among those with BMI $\geq$ 27.5 kg/m^2. Moreover, the adverse effect of moderate overweight/obesity on overall survival was limited to FASN-positive tumors (adjusted HR, 4.10; 95% CI, 1.14 to 14.8; BMI $\geq$ 27.5 kg/m^2 *v* $<$ 27.5 kg/m^2).

Conclusion
Among nonobese patients with colon cancer, tumoral FASN overexpression is associated with improved survival, whereas among moderately overweight or obese patients (BMI $\geq$ 27.5 kg/m^2), FASN overexpression may predict a worse outcome.

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INTRODUCTION

Energy balance, or the ability to maintain body weight by balancing energy intake with energy expenditure, seems to be important in the pathogenesis of human cancers, including colon cancer.[1,2] Animal studies find that restricting energy intake reduces tumor development.[3] In humans, prospective observational studies demonstrate that obesity increases the risk of colon cancer, whereas regular physical activity is associated with a reduced risk.[4] Studies of patients with colon cancer indicate that regular physical activity and lean body mass significantly reduce the risk of cancer recurrence and mortality.[5-9]

Fatty acid synthase (FASN) plays an important role in de novo lipogenesis and is physiologically regulated by energy balance.[1,10] High-carbohydrate/low-fat diets upregulate FASN, whereas exercise and energy restriction downregulate FASN.[11] A missense mutation of the *FASN* gene is protective against obesity,[12] suggesting the important role of FASN in regulating energy retention. FASN overexpression is commonly observed in human cancers,[10,13-15] including colon cancer.[16-19] Downregulation of FASN results in increased apoptosis in cancer cells.[20] Preclinical studies suggest that FASN inhibitors, such as C75 and orlistat, may possess both chemopreventive and antitumor activity.[21-23] Although FASN expression has been associated with patient outcome in breast, ovarian, and prostate cancers,[13-15] the influence of FASN expression on survival among patients with colon cancer remains uncertain.[16,18]

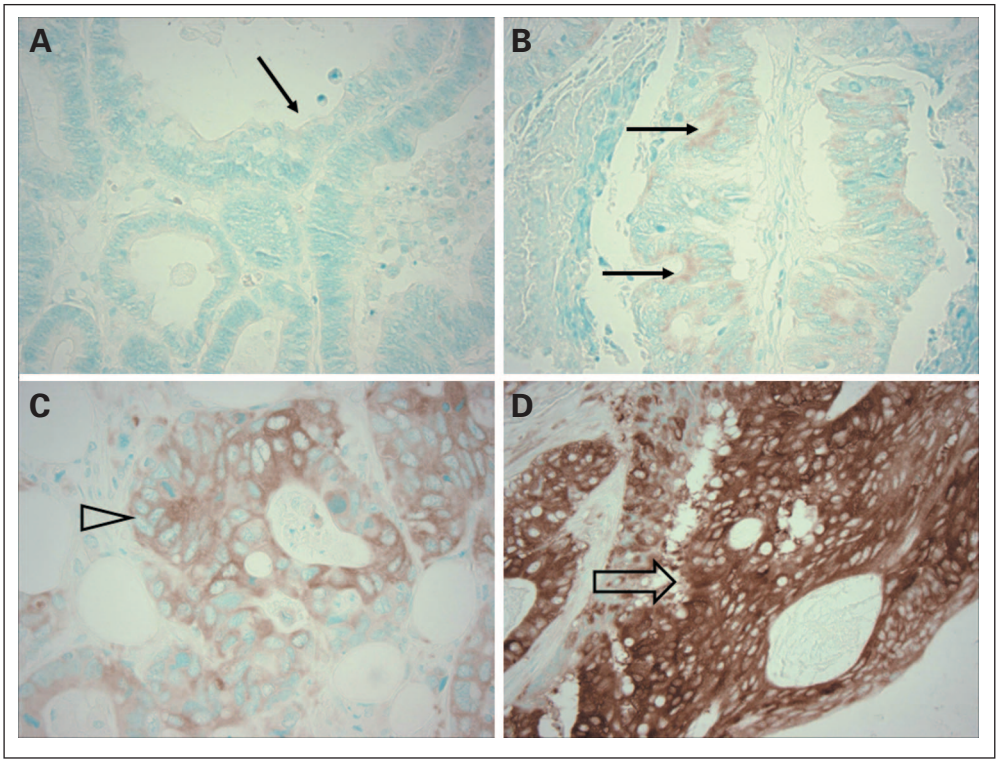

Fig 1. Fatty acid synthase (FASN) expression in colon cancer. (A) No FASN expression in colon cancer (arrow). (B) Little FASN expression in colon cancer (negative for FASN overexpression; arrows). (C) Weak FASN expression in colon cancer (negative for FASN overexpression; open arrowhead). (D) Strong FASN expression in colon cancer (positive for FASN overexpression; open arrow).

We therefore examined the impact of tumoral FASN expression on clinical outcome among patients with colon cancer in two cohort studies (the Nurses' Health Study and the Health Professionals Follow-Up Study). Because detailed data on patient characteristics and other major tumoral molecular events were recorded in this population, we were able to analyze the independent effects of tumoral FASN expression as well as the joint effect of body mass index (BMI) and FASN expression on patient outcome.

PATIENTS AND METHODS

Study Population

We used the databases of two large prospective cohort studies, the Nurses' Health Study (N = 121,700 women observed since 1976)[24,25] and the Health Professional Follow-Up Study (N = 51,500 men observed since 1986).[25] Every 2 years, participants have been sent follow-up questionnaires to update information on potential risk factors and to identify newly diagnosed cancer and other diseases. We calculated body mass index (BMI, kg/m^2) using self-reported height from the baseline questionnaire and weight from the biennial questionnaire that immediately preceded the diagnosis of colon cancer. In validation studies in both cohorts, self-reported anthropometric measures were well correlated with measurements by trained technicians ($r > 0.96$).[26] Informed consent was obtained from all patients in this study. This study was approved by the Human Subjects Committees at Brigham and Women's Hospital and the Harvard School of Public Health.

Measurement of Colon Cancer and Mortality

On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of colon cancer during the previous 2 years. When a participant (or next of kin for decedents) reported colon cancer, we sought permission to obtain medical records. Study physicians, although blinded to exposure data, reviewed all records related to colon cancer and recorded American Joint Committee on Cancer tumor stage and tumor location. For nonresponders, we searched the National Death Index to discover deaths and ascertain any diagnosis of colon cancer that contributed to death or was a secondary diagnosis. Approximately 96% of all incident colon cancer cases were identified through these methods. We collected paraffin-embedded tissue blocks from hospitals where patients with colon cancer underwent resections of primary tumors.[25] Tissue sections from all colon cancer cases were reviewed by a pathologist (S.O.). Tumor grade was categorized as high ($\leq$ 50% glandular area) or low ($>$ 50% glandular area). On the basis of availability of tissue samples, we included a total of 647 colon cancer cases diagnosed up to 2002.

Patients were observed until death or June 2006, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the National Death Index.[27] The cause of death was assigned by physicians blinded to other clinical and lifestyle information. In rare patients who died as a result of colon cancer not previously reported, we obtained medical records with permission from next of kin. More than 98% of deaths in the cohorts were identified by these methods.

Immunohistochemistry for FASN and p53

Tissue microarrays were constructed and immunohistochemistry for FASN and p53 was performed as previously described.[17,19,28] FASN expression was categorized as negative (no or weak expression) or positive (strong expression; Fig 1). Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically stained slides were interpreted by a pathologist (S.O.) blinded to other data. Random samples of 246 and 118 tumors were examined for FASN and p53, respectively, by a second observer (K.N.) unaware of other data, and the concordances between the two observers were 0.93 for FASN ($\kappa = 0.57$, $P < .0001$) and 0.87 for *p53* ($\kappa = 0.75$, $P < .0001$).

DNA Extraction, KRAS/BRAF *Sequencing, and Microsatellite Instability Analysis*

DNA from paraffin-embedded tissue was extracted, and sequencing of *KRAS* codons 12 to 13 and *BRAF* codon 600 were performed as previously

Table 1. Clinical and Molecular Features of Colon Cancer According to FASN Overexpression

Clinical or Molecular Feature	All Cases		FASN Negative		FASN Positive		*P*
	No.	%	No.	%	No.	%	
Total No.	647		563		84		
Sex							.68
Male, HPFS	287	44	248	44	39	46	
Female, NHS	360	56	315	56	45	54	
Age, years							.37
Mean	66.6		66.7		65.7		
SD	8.3		8.0		10.0		
Prediagnosis BMI, kg/m^2							.002
Mean	26.5		26.6		25.2		
SD	4.7		4.8		3.7		
BMI, kg/m^2							.04
< 25	262	42	219	40	43	52	
25-27.5	155	25	132	24	23	28	
27.5-30	98	16	89	16	9	11	
≥ 30	108	17	101	19	7	8.5	
Year of diagnosis							.77
Before 1990	100	15	86	15	14	17	
1990 to 1999	471	73	409	73	62	74	
2000 to 2002	76	12	68	12	8	9.5	
Tumor location*							.17
Proximal	371	58	317	57	54	65	
Distal	268	42	239	43	29	35	
Tumor stage							.01
I	135	21	118	21	17	20	
II	222	34	181	32	41	49	
III	162	25	143	25	19	23	
IV	82	13	79	14	3	3.6	
Unknown	46	7.1	42	7.5	4	4.8	
Tumor grade							.78
Low	573	89	499	89	74	88	
High	71	11	61	11	10	12	
MSI							0.01
Low/MSS	514	81	455	83	59	71	
High	119	19	95	17	24	29	
CIMP							.39
Low/0	523	81	458	81	65	77	
High	124	19	105	19	19	23	
KRAS mutation							.70
Negative	403	63	352	64	51	61	
Positive	233	37	201	36	32	39	
BRAF mutation							.73
Negative	520	84	451	84	69	85	
Positive	100	16	88	16	12	15	
p53 expression							.83
Negative	393	61	342	61	51	62	
Positive	250	39	219	39	31	38	

Abbreviations: FASN, fatty acid synthase; HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study; SD, standard deviation; BMI, body mass index; MSI, microsatellite instability; MSS, microsatellite stable; CIMP, CpG island methylator phenotype.
*Proximal colon includes cecum to transverse colon, and distal colon includes splenic flexure to sigmoid colon.

described.[29,30] Microsatellite insability (MSI) status was determined as previously described,[17] using a 10-marker panel (D2S123, D5S346, D17S250, BAT25, BAT26,[31] BAT40, D18S55, D18S56, D18S67, and D18S487). MSI high was defined as the presence of instability in ≥ 30% of the markers.

Real-Time Polymerase Chain Reaction for Quantitative DNA Methylation Analysis

Sodium bisulfite treatment on DNA and subsequent quantitative polymerase chain reaction were performed as previously described[32] to quantify DNA methylation in eight CpG island methylation phenotype (CIMP)–specific markers (*CACNA1G*, *CDKN2A* (*p16*), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*).[33-35] CIMP high was defined as six or more of eight methylated markers using the eight-marker CIMP panel; CIMP low/0 was defined as five or fewer than eight methylated markers, according to the previously established criteria.[34]

Statistical Analysis

We used Cox proportional hazards models to calculate hazard ratios (HRs) of death according to tumoral FASN status, adjusted for age, sex, BMI, year of diagnosis, tumor location, stage, grade, and statuses of MSI, CIMP,

KRAS, *BRAF*, and p53. For analyses of colon cancer–specific mortality, death as a result of colon cancer was the end point, and deaths as a result of other causes were censored. Among the covariates in multivariate Cox models, age and year of diagnosis were used as continuous variables, and the other covariates were used as categoric variables. In secondary analyses, we examined the joint effects of FASN expression and BMI on mortality. To maximize statistical power for these subgroup analyses as well as to effectively demonstrate influence of FASN overexpression in moderately overweight to obese patients, we categorized BMI dichotomously as less than 27.5 kg/m^2 and $\geq$ 27.5 kg/m^2, as this represented the midpoint between the upper limit of normal BMI and obesity, as defined by WHO.[36] We also assessed the effect of FASN on survival in a range of categories of BMI ($<$ 25 kg/m^2, 25 to 27.5 kg/m^2, 27.5 to 30 kg/m^2, and $\geq$ 30 kg/m^2). For the purposes of this analysis, a single multivariate Cox regression model computed HRs (in FASN-positive tumors compared with FASN-negative tumors) in different strata of the BMI categories under the assumption that coefficients for all covariates stay constant across the BMI categories. This method maximized power in analysis of multiple strata while adequately adjusting for covariates. When there was missing information on BMI (3.7% missing), tumor location (1.2% missing), stage (7.1% missing), grade (0.5% missing), MSI (2.2% missing), p53 (0.6% missing), *KRAS* (1.7% missing), or *BRAF* (4.1% missing), we assigned a separate ("missing") indicator variable and included those cases in multivariate Cox models. We confirmed that excluding cases with a missing variable did not significantly alter results (data not shown). An interaction was assessed by including the cross-product of two variables of interest in a Cox model, and the likelihood ratio test was performed. To assess an interaction of FASN and stage, we dichotomized tumor stage (I to II *v* III to IV) and also dealt with stage as a linear ordinal variable (from I to IV) to confirm no significant effect modification. To assess an interaction of FASN and BMI, we used BMI categories ($<$ 25 kg/m^2, 25 to 27.5 kg/m^2, 27.5 to 30 kg/m^2, and $\geq$ 30 kg/m^2) as a linear ordinal variable. The Kaplan-Meier method and the log-rank test were used to assess a difference in survival time distributions. The χ^2 test was used to examine an association between categoric variables. The *t* test assuming unequal variances was used to compare mean age and BMI. All analyses used SAS version 9.1 (SAS Institute, Cary, NC), and all *P* values were two-sided.

RESULTS

FASN Expression in Colon Cancer and Patient Survival

Among 647 eligible patients with available colon cancer specimens, there were 279 deaths, including 160 colon cancer–specific deaths. Among all tumors, 84 (13%) demonstrated FASN overexpression, whereas 563 (87%) were negative for FASN overexpression. Table 1 lists clinical and molecular features of colon cancer according to FASN expression status. When compared with patients with FASN-negative cancers, those with FASN-positive tumors were less likely to present with stage IV disease ($P = .01$) and more likely to exhibit MSI high ($P = .01$).

We assessed the influence of FASN expression on patient survival (Fig 2). Five-year colon cancer–specific survival was 76% among patients with FASN-negative tumors and 95% among patients with FASN-positive tumors (log-rank $P = .0002$). Similarly, 5-year overall survival was 71% among patients with FASN-negative tumors and 88% among patients with FASN-positive tumors (log-rank $P = .008$).

In the Cox regression model, compared with patients with FASN-negative tumors, those with FASN-positive tumors experienced a significant reduction in unadjusted colon cancer–specific mortality (HR, 0.27; 95% CI, 0.12 to 0.57) as well as overall mortality (HR, 0.59; 95% CI, 0.40 to 0.88; Table 2). After adjusting for potential confounders, FASN positivity remained as a significant predictor of improved colon cancer–specific mortality (HR, 0.41; 95% CI, 0.19 to 0.89), with a trend toward an improved overall mortality (HR, 0.75; 95% CI, 0.50 to 1.13). The attenuation in the effect of FASN overexpression in the multivariate analysis was principally the result of adjusting for tumor stage; when we simply adjusted for tumor stage, the FASN overexpression was associated with HR of 0.37 (95% CI, 0.17 to 0.80) for colon cancer–specific mortality and HR of 0.71 (95% CI, 0.48 to 1.07) for overall mortality.

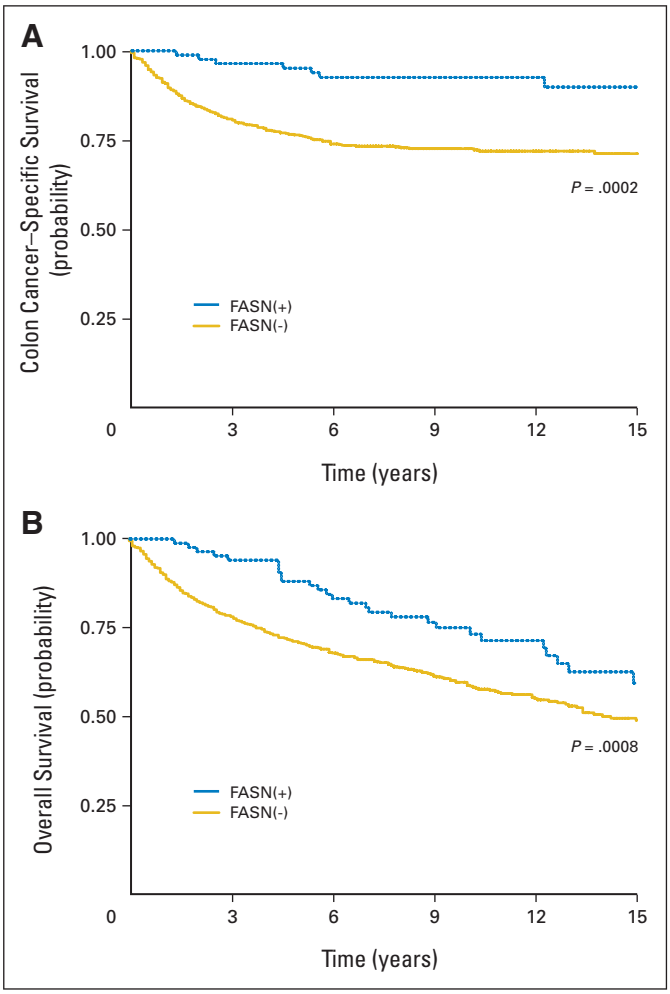

Fig 2. Kaplan-Meier curves for (A) colon cancer–specific survival and (B) overall survival according to tumoral fatty acid synthase (FASN) expression.

Interactive Effect of FASN and BMI on Patient Survival

We examined whether the effect of FASN expression on patient survival was modified by BMI (as reported within 2 years before the diagnosis of colon cancer). The effect of FASN expression on patient survival seemed to differ according to BMI (*P* for interaction = 0.019 in analysis of overall mortality). Multivariate HRs for overall mortality in FASN-positive tumors (compared with FASN-negative tumors) across a range of BMI categories were as follows: 0.55 (95% CI, 0.30 to 1.03) in BMI less than 25 kg/m^2, 0.68 (95% CI, 0.32 to 1.46) in BMI 25 to 27.5 kg/m^2, 1.70 (95% CI, 0.51 to 5.68) in BMI 27.5 to 30 kg/m^2, and 2.41 (95% CI, 0.82 to 7.08) in BMI $\geq$ 30 kg/m^2. Thus the adverse effect of FASN overexpression seemed to be limited to patients with BMI $\geq$ 27.5 kg/m^2,

Table 2. FASN Expression and Patient Survival in Colon Cancer

FASN Overexpression	Total No.	Colon Cancer–Specific Mortality					Overall Mortality				
		Deaths/ Person-Years	Univariate		Multivariate		Deaths/ Person-Years	Univariate		Multivariate	
			HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI
Negative	563	153/4,817	1	Referent	1	Referent	252/4817	1	Referent	1	Referent
Positive	84	7/915	0.27	0.12 to 0.57	0.41	0.19 to 0.89	27/915	0.59	0.40 to 0.88	0.75	0.50 to 1.13

The multivariate Cox model includes age, year of diagnosis, sex, body mass index, tumor location, stage, tumor grade, and statuses of *KRAS*, *BRAF*, p53, microsatellite instability, and the CpG island methylator phenotype.
Abbreviations: FASN, fatty acid synthase; HR, hazard ratio.

whereas there was no apparent adverse effect of FASN overexpression among patients with BMI less than 27.5 kg/m^2. Next we dichotomized patients according to BMI (< 27.5 kg/m^2 $v \geq 27.5$ kg/m^2). In patients with BMI less than 27.5 kg/m^2 (normal weight and minimally overweight), FASN positivity was associated with significantly improved cancer-specific mortality (adjusted HR, 0.37; 95% CI, 0.15 to 0.92) and a trend toward improved overall mortality (adjusted HR, 0.63; 95% CI, 0.39 to 1.02; Table 3). In contrast, among patients with BMI $\geq$ 27.5 kg/m^2 (moderately overweight and obese), FASN positivity was associated with higher overall mortality (adjusted HR, 2.91; 95% CI, 1.19 to 7.12). Similar results of an interactive effect of FASN and BMI on survival were observed among patients with prostate cancer in the Health Professionals Follow-up Study and Physicians Health Study cohorts (Nguyen et al, unpublished data).

We examined whether the effect of BMI on survival was modified by FASN status (Table 3). Among patients with FASN-negative tumors, moderate overweight or obesity (BMI $\geq$ 27.5 kg/m^2) was not significantly associated with increased mortality. In contrast, among patients with FASN-positive tumors, those with BMI $\geq$ 27.5 kg/m^2 experienced a significantly higher overall mortality (HR, 4.10; 95% CI, 1.14 to 14.8) when compared with those with BMI less than 27.5 kg/m^2.

FASN Expression, Other Patient and Tumoral Variables, and Prognosis

Finally, we examined whether the effect of FASN expression on mortality was modified by other patient or disease characteristics. Notably, the effect of FASN expression did not significantly differ between the male cohort (Health Professionals Follow-Up Study) and the female cohort (Nurses' Health Study; $P_{\text{interaction}} = .14$). The inverse relation between FASN positivity and mortality persisted across strata of other clinical and tumoral variables, and all point estimates of HRs for colon cancer–specific mortality were less than 1 (Fig 3). There was no evidence for significant effect modification by any of these variables (all $P_{\text{interaction}} > .13$).

DISCUSSION

In this large cohort of patients with colon cancer, we found that tumoral FASN overexpression was a significant predictor of reduced

Table 3. Joint Effects of FASN Expression and BMI on Patient Survival in Colon Cancer

Variable	Total No.	Colon Cancer–Specific Mortality			Overall Mortality		
			Multivariate			Multivariate	
		Death/Person-Years	HR	95% CI	Deaths/Person-Years	HR	95% CI
Effect of FASN by strata of BMI							
BMI < 27.5 kg/m^2							
FASN negative	351	98/2,995	1	Referent	163/2,995	1	Referent
FASN positive	66	5/749	0.37	0.15 to 0.92	20/749	0.63	0.39 to 1.02
BMI $\geq$ 27.5 kg/m^2							
FASN negative	190	47/1,658	1	Referent	78/1,658	1	Referent
FASN positive	16	2/147	1.31	0.29 to 5.90	7/147	2.91	1.19 to 7.12
Effect of BMI by strata of FASN							
FASN negative							
BMI < 27.5 kg/m^2	351	98/2,995	1	Referent	163/2,995	1	Referent
BMI $\geq$ 27.5 kg/m^2	190	47/1,658	0.91	0.63 to 1.30	78/1,658	0.86	0.65 to 1.14
FASN positive							
BMI < 27.5 kg/m^2	66	5/749	1*	Referent	20/749	1	Referent
BMI $\geq$ 27.5 kg/m^2	16	2/147	2.94*	0.48 to 18.0	7/147	4.10	1.14 to 14.8

NOTE. The multivariate Cox model includes age, year of diagnosis, sex, tumor location, stage, tumor grade, and statuses of *KRAS*, *BRAF*, p53, microsatellite instability, and the CpG island methylator phenotype.
Abbreviations: FASN, fatty acid synthase; BMI, body mass index; HR, hazard ratio.
*Adjusted for stage, as a result of the only seven total deaths in this subset analysis.

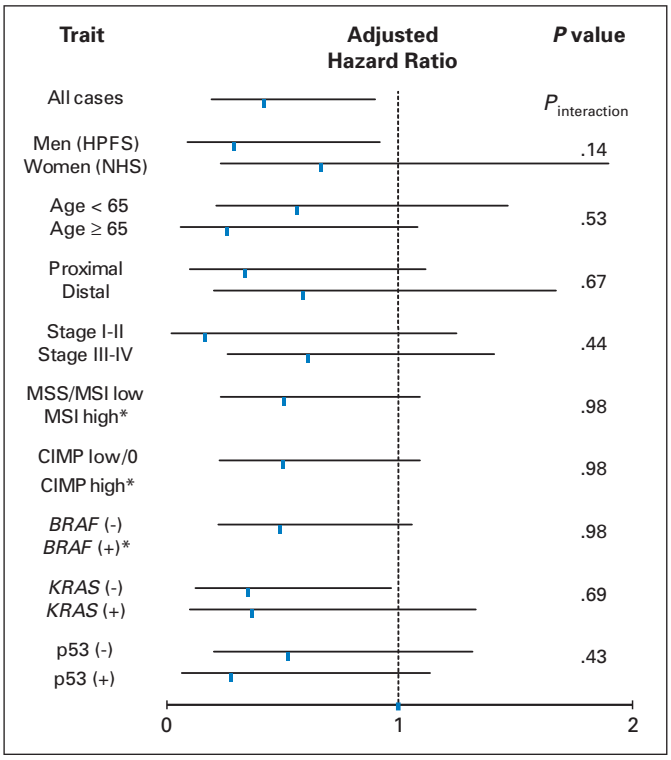

Fig 3. Stratified analysis of fatty acid synthase (FASN) and colon cancer–specific mortality. Adjusted hazard ratios (HRs) with 95% CI for FASN-positive tumors (compared with FASN-negative tumors) in various strata are shown. Adjusted HRs are consistently less than 1, indicating lower mortalities associated with FASN-positive tumors. *95% CI is not shown because of no colon cancer–specific deaths among patients with FASN-positive tumors that were microsatellite instability (MSI) high, CpG island methylation phenotype (CIMP) high, or *BRAF* positive. HPFS, Health Professionals Follow-Up Study; MSS, microsatellite stable; NHS, Nurses' Health Study.

cancer-specific mortality, independent of various clinical and molecular variables including tumor stage and statuses of MSI, *KRAS*, *BRAF*, and p53. Moreover, the improved outcome associated with FASN overexpression was similar across strata of most patient and disease characteristics and was consistent across the two independent prospective cohort studies in this analysis.

Notably, the effect of FASN overexpression on clinical outcome seemed to be modified by patient BMI. Among normal weight and minimally overweight patients (BMI < 27.5 kg/m^2), FASN overexpression was associated with a reduction in mortality, whereas among moderately overweight and obese patients (BMI ≥ 27.5 kg/m^2), FASN overexpression conferred a significant increased in mortality. Similar findings on FASN expression, BMI, and survival were observed among patients with prostate cancer in the Health Professionals Follow-Up Study and Physicians Health Study cohorts (Nguyen et al, unpublished data). These data suggest that influence of FASN overexpression differs substantially according to the host milieu in which the overexpression occurs. Moreover, consistent with other studies among patients with colon cancer,[6-9] higher BMI was associated with an increased mortality, although the deleterious effect of obesity was limited to patients with FASN overexpression.

FASN has been implicated in cancer pathogenesis.[1,10] FASN inhibitors, such as C75 and orlistat, have been shown to exhibit antitumor activity.[21,22,37] Downregulation of FASN through its enhanced proteasomal degradation results in increased apoptosis, indicating an addiction of tumor cells to FASN.[20] Recent data suggest that increased FASN activity in tumor cells is important for function of endoplasmic reticulum to maintain membrane biogenesis.[38] These data collectively support that FASN acts as an oncoprotein. In fact, FASN overexpression is commonly observed in human cancers,[10,13-15] including colon cancer.[16,18,19] Importantly, FASN overexpression confers chemoresistance in breast cancer cells, and inhibition of FASN may enhance the effect of chemotherapy.[39,40] FASN overexpression has been reported to confer a significantly worse outcome in patients with breast, ovarian, lung, and prostate cancers,[1,13-15] although none of these studies assessed patient BMI. Among patients with colorectal cancer, no significant relation between FASN expression and survival was demonstrated in two smaller studies.[16,18]

Potential prognostic factors and markers have been investigated in colon cancer.[41-46] Dietary factors and altered energy balance represent important risk factors for cancer incidence, recurrence, and death.[7-9] Among patients with colon cancer, obesity has been associated with poor clinical outcome.[7-9] However, mechanisms of how obesity or altered energy balance influence cancer prognosis are poorly understood. Accumulating evidence suggests that FASN may play a role in the link between dietary and energy factors and pathogenesis of neoplasia; FASN is regulated by energy balance and FASN alterations seem to be important in carcinogenesis.[1,10,11] Our current results are particularly intriguing in that the negative influence of obesity on survival may be limited to patients with FASN-overexpressing tumors. Our data support the importance of FASN in determining biologic behavior of colon cancer as well as a significant interaction between energy balance and tumor biology on clinical outcome. One may speculate that colon cancer cells with FASN upregulation may depend on excess energy for growth, leading to more aggressive tumor behavior among obese patients.

Although FASN has been considered to act as an oncoprotein,[20-22,37] its overexpression seems to mark a subtype of colon cancer that is associated with indolent behavior among relatively normal-weight patients. It should be noted that upregulation of a particular oncoprotein or downregulation of a particular tumor suppressor does not necessarily imply a poor clinical outcome.[47] For example, although MSI has been shown to mutate and inactivate a number of tumor suppressors, leading to cancer development, MSI-high colon cancer has been consistently associated with better outcome.[48,49] It is plausible that, among normal-weight patients, colon cancer without FASN upregulation develops through an alternative nonenergy dependent pathway, which is associated with poor clinical outcome.

Our study has several advantages, including a large number of colon cancers derived from the two prospective cohorts as well as extensive data on patient characteristics, disease characteristics, and other important tumoral molecular events. Thus we have been able to demonstrate an effect of FASN on patient survival, independent of clinical and other tumoral characteristics.

In our cohorts, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use differed according to tumoral FASN status, especially because such data were not available to patients or treating physicians. In addition, beyond cause of mortality, data on cancer recurrences were not available in these

cohorts. However, given that the median survival for metastatic colon cancer was 10 to 12 months during much of the time period of this study,[5] colon cancer–specific survival should be a reasonable surrogate for cancer-specific outcomes.

To date, there is no standardized classification scheme for FASN expression in colon cancer. Nevertheless, previous studies have demonstrated that the results of mRNA expression microarray and immunoblot analyses correlate well with immunohistochemical grading of FASN.[13,50] In validation studies of the central, blinded review of tumor specimens, we observed substantial interobserver agreement (93%). Moreover, any random misclassification of FASN overexpression would be expected to bias our results toward the null hypothesis.

In conclusion, this large prospective study of patients with colon cancer suggests that FASN upregulation is a significant independent predictor of improved survival among nonobese patients with colon cancer, whereas among moderately overweight or obese patients (BMI $\geq$ 27.5 kg/m^2), FASN overexpression may predict a worse outcome. Concurrently, the influence of obesity on patient survival may be modified by tumoral FASN expression. Our finding may have significant clinical implications and may offer a potential mechanism by which excess energy balance may influence tumorigenesis and cancer progression.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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