Serum Adiponectin Concentrations and Tissue Expression of Adiponectin Receptors Are Reduced in Patients with Prostate Cancer: A Case Control Study

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Abstract

Purpose: **Adiponectin, an adipocyte-secreted hormone with insulin-sensitizing effects, has been inversely associated with several hormonally dependent malignancies, including breast, endometrial, and colorectal cancer. Few studies have examined serum adiponectin in relation to prostate cancer, and expression of adiponectin receptors has previously not been assessed in prostate tumors.**

Experimental Design: **We collected plasma samples and covariate data in the context of a case-control study of 300 Greek men, including 75 prostate cancer cases, 75 patients with benign prostatic hyperplasia (BPH), and 150 healthy controls. Prostate tissue samples were taken from 72 cases and 27 noncases and examined for relative expression of adiponectin receptors AdipoR1 and AdipoR2 using immunohistochemistry.**

Results: **Prostate cancer patients had significantly lower plasma adiponectin concentrations as compared with men with BPH and healthy controls (7.4 ± 5.0 versus 11.5 ± 6.4 and 12.8 ± 8.0 ng/mL, respectively). Men in the top two quartiles of adiponectin had a 71% to 73% reduced risk of prostate cancer as compared with men in the lowest quartile after adjusting for age, body mass index, and additional potential confounders. We found no similar relationship between adiponectin and risk of BPH. Results from immunohistochemistry experiments show weaker expression of adiponectin receptors AdipoR1 and AdipoR2 in cancerous versus healthy prostate tissue.**

Conclusions: **Higher serum adiponectin is associated with a marked reduction in risk of prostate cancer, but not BPH, independently of other risk factors. Malignant prostate tissue samples have reduced expression of adiponectin receptors as compared with benign prostate tissue. These results support a role for adiponectin in the pathogenesis of prostate cancer.** (Cancer Epidemiol Biomarkers Prev 2007;16(2):308–13)

Introduction

Recent epidemiologic evidence indicates an association between serum adiponectin, an endogenous insulin-sensitizing adipocyte-secreted cytokine (1) that has established anti-inflammatory and antiatherosclerotic effects (2-5), and risk of several cancers independently of classic risk factors. These malignancies include breast (6-8) and endometrial (9, 10) cancer in women and colorectal cancer (11) in men, but few studies have examined the role of adiponectin in prostate cancer. There is a strong biological basis supporting such an association. Overweight and obesity are established risk factors for several cancers, including prostate (12), and adiposity also has a well-documented inverse association with plasma concentrations of adiponectin (1). In addition, adiponectin has been inversely associated with insulin and sex steroid levels (3), both of which have positive associations with risk of hormonally dependent cancers (13-15), such as prostate cancer. Specific effects of adiponectin on carcinogenesis may include reduction of reactive oxidative species, inhibition of angiogenesis, and regulation of growth factors that mediate cellular proliferation and apoptosis (16).

No previous study has examined relative expression of adiponectin or its receptors in prostate cancer tissues. Preliminary data from a small case-control study of prostate cancer patients, patients with benign prostatic hyperplasia (BPH), and healthy subjects found significantly reduced adiponectin concentrations in men with prostate cancer relative to BPH and healthy controls (17). However, the small size of that study limited the ability to obtain precise risk estimates and adjust for confounding factors. Thus, further research is warranted to confirm the observed association between adiponectin concentrations and risk of prostate cancer, to assess whether this association is independent of confounding variables, and to examine for the first time expression of adiponectin receptors in prostate cancer cells. With this aim, we measured serum adiponectin and obtained covariate data from 300 Greek men in the context of a case-control study, which included 75 prostate cancer cases, 75 patients BPH, and 150 healthy controls. We also took tissue samples from 72 cases, 11 subjects with BPH, and 16 healthy controls to compare expression of adiponectin receptors in malignant and normal tissues. Consistent with previous biological and observational data, we hypothesized that higher adiponectin concentrations would confer a reduced risk of prostate cancer and that tumor tissues would express adiponectin receptors less strongly than prostate tissues from BPH patients and healthy controls.

Materials and Methods

Study Subjects. Over 1 year, we recruited 75 patients with newly diagnosed prostate cancer at the Laiko University

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Note: K. Michalakis and C.J. Williams contributed equally in the production of the manuscript.

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Hospital, Athens, Greece. Diagnosis was based on pathohistologic confirmation of prostate cancer in all patients. Patients with previous or present neoplastic disease at any other site, previous prostate cancer, or other major chronic disease were excluded from the study. Five (6.7%) patients had metastases at the time of diagnosis. Follow-up information was available from hospital charts of all patients. BPH and control subjects were recruited among men who were screened at the same clinic using the same diagnostic criteria during the same time period but displayed no evidence of prostate cancer on the basis of clinical examination (PR exam), U/S imaging, and/or biopsy tests. Of the men identified, all agreed to participate in the study.

Serum Measurements. Fasting blood samples were obtained in the morning and immediately centrifuged and stored at −70°C until assaying. Serum concentrations were determined by RIAs (Linco Research, St. Charles, MO) for adiponectin and double-antibody RIAs (Diagnostic Systems Laboratories, Webster, TX) for insulin. For total adiponectin, the intra-assay coefficient of variation was 1.78% to 6.21%, the interassay coefficient of variation was 6.90% to 9.25%, and the sensitivity of the assay was 1 ng/mL. For insulin, the intra-assay coefficient of variation was 4.5% to 8.3%, the interassay was 4.7% to 12.2%, and the sensitivity of the assay was 1.3 μIU/mL. Total testosterone was quantified via Coat-a-Count RIA (Diagnostic Product Corporation, Los Angeles, CA) according to protocol, with sensitivity of 4 ng/dL and interassay coefficient of variation of 5.9% to 12%. Samples were measured in three batches for each assay using the same standards and controls. Medical and technical assistants were blinded with respect to case or control status.

Immunohistochemistry. For localization of AdipoR1 and R2 expression in normal and cancerous tissue, a total of 22 archival formalin-fixed, paraffin-embedded prostate cancer specimens, 11 samples from patients with BPH, and 16 samples from healthy control subjects who also provided serum were available for assessment. Specimens were examined by one of the coinvestigators who was blinded to case or control status and other data of this study. Immunohistochemical staining for AdipoR1 and AdipoR2 was done on 4-μm sections of two representative paraffin blocks from each sample. The deparaffinized sections were pretreated in citrate buffer (pH 6.0) with microwave oven heating for 15 min. Endogenous peroxidase was blocked by exposure of the slides to 3% H_2O_2. Sections were incubated overnight at 4°C with a primary rabbit polyclonal antibody against AdipoR1 and AdipoR2 (Phoenix Pharmaceuticals, Inc., Belmont, CA) at 1:40 dilution. The streptavidin-biotin complex method was used with Visualisation Kit LSAB (Dako K0690) and with 3,3′-diaminobenzidine as chromogen. The stain was evaluated and scored according to its intensity separately for the membranous and cytoplasmic distribution in a scale from 0 to +++. For negative control, primary antibody was replaced by PBS.

An additional 56 formalin-fixed paraffin-embedded prostate adenocarcinoma tissue samples from American men were purchased in the form of tissue array slides mounted to standard silanized slides (Imgenex, San Diego, CA). Also available were healthy prostate tissue (nontumor) samples from 11 of the 56 men who contributed tumor tissue. The average age of these patients was 65.4 ± 5.2 years. Of the cases, 1 (1.8%) had stage I disease, 12 (21.4%) stage II, 29 (51.8%) stage III, and 13 (23.2%) were stage IV. The 5-μm paraffin sections were deparaffinized, rehydrated, microwaved for 25 min in 10 mmol/L citrate buffer, and incubated for 30 min in methanol containing 0.5% H_2O_2. After incubation in 16% normal goat serum for 1 h at room temperature, the slides were incubated for 1 h with the primary antibodies at room temperature. The primary antibodies used were the rabbit anti-human AdipoR1 (raised against amino acid residues 357-375) antiserum and the rabbit anti-human AdipoR2 (raised against amino acid residues 374-386) antiserum (both from Phoenix Pharmaceuticals) used at 1:500 and 1:200 dilutions, respectively. The secondary antibody was a biotinylated antirabbit antibody (1:400 dilution) and was applied for 30 min at room temperature, followed by the Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA) for 30 min. The horseradish peroxidase reaction was developed with diaminobenzidine and the slides were counterstained with hematoxylin. Intensity and distribution of positive staining was evaluated on a scale of 0 to +++ by an expert pathologist. Comparison of expression by immunohistochemistry using a χ^2 test of proportions between the two samples of prostate cancer cases revealed no systematic differences between the techniques ($P = 0.65$ for AdipoR1 and $P = 0.98$ for AdipoR2).

Statistical Analyses. Descriptive characteristics of the prostate cancer cases, patients with BPH, and control subjects are expressed as proportions and mean or median values ± SD (or SE). Comparisons between groups were conducted using χ^2 tests for categorical variables and one-way ANOVA with Bonferroni corrections for post hoc pairwise comparisons for continuous measures. Log transformations were done on biomarker and hormonal variables before analysis to achieve normal distribution. Comparisons of the original continuous data were repeated using Kruskal-Wallis tests for nonnormally distributed variables, and similar results were obtained (not reported). Spearman correlation coefficients are reported for adiponectin with anthropometric and biochemical measures. Probability of prostate cancer case status by quartile of adiponectin concentration was modeled using simple and multivariate unconditional logistic regression to produce crude and adjusted risk estimates, respectively. Missing values on continuous measures were imputed based on observed values using previously used methods (11) and were modeled as a distinct group for categorical variables. Excluding subjects with missing data from analyses produced similar but less precise results (data not presented). Comparisons of cases and controls on expression of AdipoR1 and AdipoR2 were conducted using χ^2 test to assess differences in intensity. A two-sided level of $\alpha = 0.05$ was used to determine statistical significance of reported associations. All analyses were done using SPSS 11.5 (Texas Instruments, Chicago, IL) and SAS version 8.2 for UNIX (SAS Institute, Cary, NC).

Results

Men with prostate cancer or BPH tended to be older than control subjects (Table 1). In addition, prostate cancer cases had significantly lower adiponectin concentrations than healthy control subjects despite also having significantly decreased body mass index (BMI). Cases also had higher prostate-specific antigen compared with subjects with BPH or controls. The three groups were similar with regard to other anthropometric variables and had comparable lipid and metabolic profiles and similar age-standardized testosterone. Among noncase subjects, adiponectin was positively correlated with high-density lipoprotein ($r = 0.23$, $P < 0.01$) and was negatively associated with BMI ($r = -0.14$, $P = 0.05$) and triglycerides ($r = -0.23$, $P < 0.01$). Adiponectin was not significantly associated with age ($r = 0.09$, $P = 0.22$) for subjects without prostate cancer. Correlations were very similar among cases with respect to age and BMI.

We then examined risk of prostate cancer by quartile of adiponectin concentration (Table 2). In analyses adjusted only for age, men in the top two quartiles of adiponectin had a 69% to 73% reduced risk of prostate cancer as compared with men in the lowest quartile of adiponectin concentration (odds ratio, 0.27, for quartile 3 versus quartile 1; 0.31, for quartile 4 versus quartile 1; $P = 0.03$). Multivariate adjustment for BMI, smoking status, alcohol use, insulin, and testosterone strengthened the

Table 1. Descriptive characteristics of prostate cancer cases (n = 75), subjects with BPH (n = 75), and controls subjects (n = 150)

Variable	Cases	BPH	Controls	P
Stage, n (%)				
I	8 (11)			
II	45 (63)			
III	13 (18)			
IV	5 (7)			
Gleason grade, n (%)				
Low (2, 3, 4)	5 (7)			
Intermediate (5, 6, 7)	48 (67)			
High (8, 9, 10)	19 (26)			
PSA, ng/mL (median ± SE)	11.6 ± 4.9*,†	5.9 ± 0.4	2.4 ± 0.3	<0.01
Testosterone, ng/dL (age-standardized median ± SE)	314 ± 21	336 ± 21	298 ± 15	0.06
Descriptive characteristics				
Age, y (mean ± SD)	74.0 ± 8.2*	70.0 ± 6.5*	64.0 ± 8.3	<0.01
BMI, kg/m^2 (mean ± SD)	26.2 ± 3.2*	27.0 ± 3.5	27.6 ± 3.5	0.04
Current smoker, n/N (%)	20/66 (30)	20/72 (28)	44/120 (37)	0.40
Alcohol use, n/N (%)	9/43 (21)	17/61 (28)	19/72 (26)	0.71
Metabolic (mean ± SD)				
Adiponectin, ng/mL	7.4 ± 5.0*,†	11.5 ± 6.4	12.8 ± 8.0	0.04
Insulin, μIU/mL	19.0 ± 27.0	13.1 ± 10.9	19.5 ± 23.3	0.15
Glucose, mg/dL	109 ± 40	105 ± 36	111 ± 40	0.60
Lipid profile (mean ± SD)				
Total cholesterol, mg/dL	198 ± 43	197 ± 44	207 ± 45	0.35
HDL cholesterol, mg/dL	51 ± 14	52 ± 13	49 ± 14	0.31
LDL cholesterol, mg/dL	128 ± 39	124 ± 39	138 ± 42	0.11
Triglycerides, mg/dL	124 ± 78	132 ± 86	132 ± 55	0.30

Abbreviations: PSA, prostate-specific antigen; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*P < 0.05 for Bonferroni corrected post hoc comparison with controls.
†P < 0.05 for Bonferroni corrected post hoc comparison with BPH subjects.

observed reduction in risk, with a 71% to 73% reduced odds of prostate cancer for men with adiponectin concentrations above the median level relative to the bottom quartile. Adjusting further for lipids, triglycerides, or prostate-specific antigen did not substantially influence reported associations (not shown). We found no significant association between quartile of adiponectin and risk of BPH (Table 2). Adiponectin was significantly and inversely associated with stage of disease (P = 0.03) but did not correlate with disease grade (P = 0.50). Excluding patients with metastatic disease (n = 5) did not materially alter the relationship between adiponectin concentration and prostate cancer risk (data not shown).

Immunohistochemical staining shows that AdipoR1 and AdipoR2 may be expressed in both prostate tumor and healthy tissues (Fig. 1). Tumor tissues were less likely to show strong expression of either AdipoR1 (29%) as compared with prostate tissues from subjects who had BPH or were healthy, which displayed strong expression in 62% for AdipoR1 (P = 0.02). In addition, case tumor tissues were marginally more likely to show no or marginal expression of AdipoR2 (21%) as compared with tissues from BPH and control subjects (9%; P = 0.09; Table 3). The available nontumor prostate tissues from 11 of the cases positively expressed AdipoR1 in all samples, but AdipoR2 was expressed in only 55% of tissues. Age and prostate-specific antigen of the cases did not differ by level of expression for either AdipoR1 or AdipoR2 (Table 4). There was a trend toward increased expression of AdipoR1, but not AdipoR2, with high-grade disease (P = 0.07); however, stage of disease was not significantly associated with expression of either receptor (Table 4). Neither AdipoR1 nor AdipoR2 expression was different between tumor and healthy tissue in cases, but the numbers were too small to make firm conclusions.

Discussion

Although recent studies have examined the role of adiponectin as a molecular mediator of carcinogenesis in several hormonally dependent cancers (6-11), limited research has been conducted in relation to prostate cancer. As has been previously shown in breast, endometrial, and colorectal

Table 2. Odds ratios and 95% confidence intervals for risk of prostate cancer by quartile of adiponectin

	Quartile of adiponectin				P
	Q1	Q2	Q3	Q4	
Cases, % [(n)/total (N)]	24/73 (32)	26/73 (35)	13/74 (17)	12/73 (16)	
Adiponectin					
Median (range), ng/mL	3.4 (0.9–5.3)	6.8 (5.3–8.7)	10.7 (8.7–13.7)	18.7 (13.7–43.2)	
Cases vs control subjects					
Age adjusted	1.0	0.74 (0.28–1.94)	0.27 (0.11–0.67)	0.31 (0.13–0.77)	<0.01
Age and BMI adjusted	1.0	0.70 (0.27–1.86)	0.27 (0.11–0.67)	0.29 (0.12–0.73)	<0.01
Multivariate* adjusted	1.0	0.60 (0.21–1.71)	0.27 (0.10–0.72)	0.29 (0.10–0.82)	0.03
BPH vs control subjects					
Age adjusted	1.0	0.76 (0.34–1.70)	0.60 (0.25–1.39)	0.55 (0.23–1.28)	0.65
Age and BMI adjusted	1.0	0.60 (0.26–1.41)	0.58 (0.25–1.37)	0.73 (0.33–1.62)	0.62
Multivariate* adjusted	1.0	0.52 (0.20–1.34)	0.49 (0.19–1.22)	0.64 (0.27–1.50)	0.47

*Multivariate model included age, BMI, smoking status, alcohol use, insulin level, and testosterone.

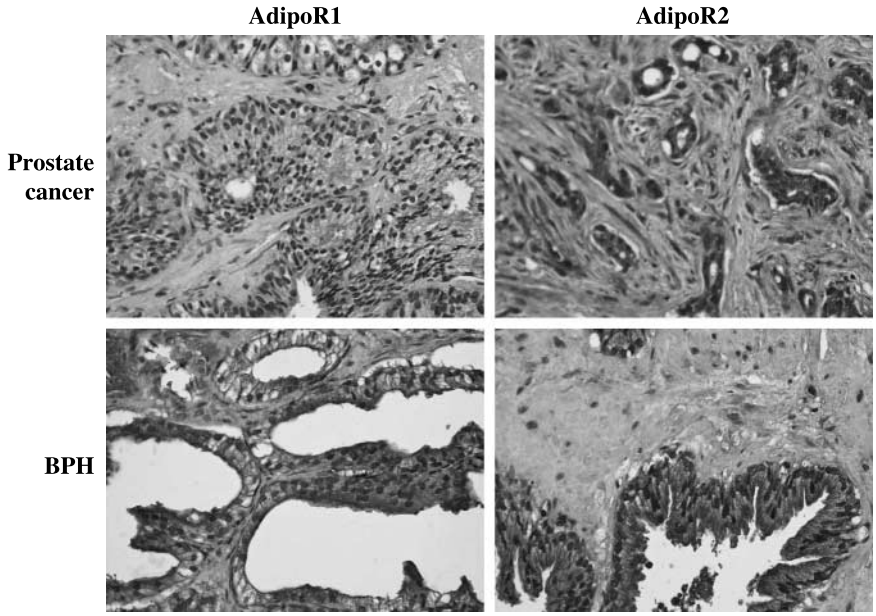

Figure 1. Expression of AdipoR1 and AdipoR2 in malignant and benign prostate tissues. Immunohistochemical staining of cancerous prostate tissues from cases and benign prostate tissues from BPH subjects (original magnification, ×400).

cancers, the results of this case-control study of 300 Greek men indicate a significantly reduced risk of prostate cancer with higher plasma adiponectin concentrations. Maximal benefit occurred at levels above the median range, with no additional risk reduction observed in men in the highest quartile of adiponectin over men in the second highest quartile. Similarly, men in the second lowest quartile of adiponectin concentration had comparable risk of prostate cancer as those in the bottom quartile, suggesting a cut point value of adiponectin around the median (8.7 ng/mL in our study population) above which risk of prostate cancer is reduced and below which it is elevated. We found a decrease in prostate cancer risk of ~73% for men with higher adiponectin levels but detected no similar association between adiponectin level and BPH, suggesting that the relationship between adiponectin and prostate cancer is specific to this disease. Immunohistochemistry experiments indicate that expression of adiponectin receptors AdipoR1 and AdipoR2 is reduced in tissues from prostate cancer cases as compared with tissues from BPH and control subjects. These results support a potential role of adiponectin in the carcinogenesis of prostate cancer, but more studies are needed to elucidate whether the adiponectin may act directly on prostate cancer cells and/or indirectly by causing changes in circulating androgen and other hormone levels (16).

To date, only two observational studies have examined adiponectin in relation to prostate cancer. Our results confirm findings of reduced adiponectin concentrations in prostate cancer from a smaller study (17) of 30 cases, 41 BPH subjects, and 41 healthy controls, and extend them by using a larger sample size in the case-control design to provide an estimate of the risk of prostate cancer associated with adiponectin and permitting statistical adjustment for the potential confounding effects of age, BMI, lifestyle factors, and hormonal variables. Results from a separate correlational study of 236 men having radical prostatectomy (18) found a negative association between adiponectin and grade of disease in overweight and obese subjects but not normal weight subjects, which is in line with another previous study reporting association between obesity and accelerated disease progression (19). In our sample of primarily overweight patients (65%), we found a significant inverse association between adiponectin and stage but not grade of disease. The results of these prior studies combined with our data suggest that adiponectin may play a role in prostate cancer development, but whether this role relates more closely to advanced disease or accelerated progression and whether the effect differs by level of adiposity need to be clarified by further research. Previous experimental work examining adiponectin receptors by Western blot analysis has shown expression of AdipoR1 and AdipoR2 in several prostate cancer cell lines (20). Results from our immunohistochemical analysis show not only that AdipoR1 and AdipoR2 may be expressed in prostate cancer tissue, which is consistent with

Table 3. Negative or marginal (0 or +/−), positive (+), and strong positive (++ or +++) expression of AdipoR1 and AdipoR2 in case tumor tissue samples (*n* = 72), case nontumor tissues (*n* = 11), and healthy tissues from BPH and control subjects (*n* = 27) as determined by immunohistochemistry

	Immunohistochemistry expression signal			*P*
	Negative or marginal	Positive	Strong positive	
AdipoR1				0.02
Case tumor tissue, *n* (%)	13 (19)	37 (53)	20 (29)	
Matched case nontumor tissue,* *n* (%)	0 (0)	8 (73)	3 (27)	
BPH and control tissue, *n* (%)	1 (5)	7 (33)	13 (62)	
AdipoR2				0.09
Case tumor tissue, *n* (%)	15 (21)	31 (43)	26 (36)	
Matched case nontumor tissue,* *n* (%)	5 (46)	5 (46)	1 (9)	
BPH and control tissue, *n* (%)	2 (9)	11 (48)	10 (44)	

NOTE: Row percentages may sum to >100% due to rounding.
*''Matched case nontumor tissue'' refers to noncancerous prostate tissue samples obtained from an area of the prostate not affected by cancer in 11 of the prostate cancer patients.

Table 4. Case characteristics (n = 72) by no or marginal expression (− or +/−), positive expression (+), or strong positive expression (++ or +++) of AdipoR1 and R2 by immunohistochemistry

	Immunohistochemistry expression signal			P
	Negative or marginal	Positive	Strongly positive	
AdipoR1				
n (%)	13 (17)	37 (49)	20 (26)	
Age, y (mean ± SD)	67.4 ± 4.0	65.8 ± 7.8	67.1 ± 7.5	0.79
Gleason score, n (%)				0.07
Low or intermediate grade (2-7)	10 (30)	16 (49)	7 (21)	
High grade (8-10)	2 (8)	15 (63)	10 (37)	
Stage, n (%)				0.64
I and II	6 (24)	13 (52)	6 (24)	
III and IV	7 (16)	36 (52)	14 (32)	
PSA (median ± SE)	10.1 ± 4.5	15.3 ± 7.6	14.5 ± 7.1	0.20
AdipoR2				
n (%)	15 (21)	31 (43)	26 (36)	
Age, y (mean ± SD)	64.9 ± 5.7	67.3 ± 6.4	67.0 ± 6.9	0.80
Gleason score, n (%)				0.22
Low or intermediate grade (2-7)	8 (22)	15 (43)	12 (34)	
High grade (8-10)	2 (7)	12 (44)	13 (48)	
Stage, n (%)				0.80
I and II	6 (21)	13 (46)	9 (32)	
III and IV	9 (21)	17 (40)	17 (40)	
PSA (median ± SE)	15.8 ± 4.8	12.1 ± 7.6	13.1 ± 5.3	0.31

NOTE: Column percentages may sum to >100% due to rounding.

previous *in vitro* results, but also that expression of adiponectin receptors in prostate cancer patients is reduced in relation to subjects without cancer. These data, combined with our serum adiponectin findings and previous study results, suggest that adiponectin and adiponectin receptors may have functional relevance in prostate cancer.

Other recent studies have shown potential mechanisms by which adiponectin may protect against prostate and other cancers. It has been suggested that, through its well-accepted insulin sensitizing effects, adiponectin has the potential to inhibit the progression of existent prostate cancer (18), but whether it has an effect on initiation of carcinogenesis has yet to be definitively established. Evidence suggests that adiponectin may prevent carcinogenesis via inhibition of neovascularization through the suppression of cellular proliferation, migration, and survival of endothelial cells (21). In addition to its effects on angiogenesis, adiponectin has also been shown to suppress growth and induce apoptosis in myelomonocyte leukemia cell lines (2) and androgen-dependent breast cancer cell lines (22). Whereas there was no evidence of induction of apoptosis, adiponectin was found to inhibit cellular proliferation of both androgen-dependent (LNCaP-FGC) and androgen-independent (DU145 and PC-3) prostate cancer cell lines (20). Additional research has shown that adiponectin mediates c-jun NH_2-terminal kinase and signal transducer and activator of transcription-3 signaling pathways in prostate cancer cell lines (23). Activation of c-jun NH_2-terminal kinase and suppression of signal transducer and activator of transcription-3 in DU145, PC-3, and LNCaP-FGC prostate cancer cells by adiponectin represent another mechanism by which adiponectin may have a direct effect on prostate cancer pathophysiology. These experimental results provide possible biological explanations of the observational association between adiponectin concentrations and prostate cancer risk.

High molecular weight adiponectin, but not other forms (full-length or globular), has been found to inhibit dehydrotestosterone-, leptin-, and insulin-like growth factor I–stimulated cell growth, as well as prostate cancer cell growth, and also enhanced the cell growth–inhibiting action of doxorubicin. In addition, it has been shown that testosterone decreases adiponectin and high molecular weight adiponectin in particular (24); however, in our study, we found no significant differences in age-standardized testosterone level between cases and controls. Thus, the low adiponectin levels observed in prostate cancer patients are not likely due to high testosterone, and adjusting for testosterone in multivariate analyses did not influence risk estimates. Another possible factor associated with the reduced adiponectin observed in prostate cancer cases may be altered fatty acid synthase and lipid metabolism, which has previously been observed in cancer patients (25). Adiponectin promotes fatty acid oxidation and circulating adiponectin correlates inversely with triglycerides and free fatty acid levels (26). Thus, it is possible that decreased adiponectin levels could result in increased fatty acid levels, which in turn could influence both apoptosis and proliferation of cancer cells. Dietary factors, such as intake of fats and cholesterol, could also, in part, mediate both adiponectin and lipid profile and may have influenced prostate cancer risk. We have previously shown that adiponectin is associated inversely with dietary glycemic load (27) and directly with adherence to a Mediterranean-pattern diet (28), but because in this study we did not conduct a dietary assessment, we could not determine whether nutritional factors may have played a role in the association between adiponectin and prostate cancer. However, because the relationship persisted adjustment for lipids, including triglycerides, the results of our study support a direct biological link between adiponectin and prostate cancer above and beyond its potential association with serum lipids. These findings suggest that more than one of the above mechanisms underline the association between adiponectin and prostate cancer, and whether diet may play a role in this relationship remains to be determined by future studies.

The observational nature of our study introduces some limitations. The cross-sectional design precludes establishment of a causal mechanism between adiponectin concentrations and development of prostate cancer. Prospective cohort studies are required to more fully explore this relationship. In addition, our case-control study was unmatched, necessitating reliance on statistical adjustment to control for differences in age, BMI, and other potential confounders in the relationship between adiponectin and prostate cancer. Adiponectin levels were lower in prostate cancer cases than controls, despite the lower mean BMI of cases, indicating that the association between adiponectin and prostate cancer is independent of overall adiposity. Further confirming our data, the correlation between adiponectin and BMI was negative, as

expected, when cases and noncases were analyzed separately. However, adiponectin has been shown to correlate more closely with central than overall obesity (3), and future studies should measure waist-to-hip ratio to determine any effect of additional confounding by central obesity. In addition, groups also differed by age, and whereas we used appropriate multivariate techniques to account for these variations, future case-control investigations should consider using a matched design. As with all studies, there is potential for inaccuracies in the measurement of covariate data, but such random misclassification could only attenuate reported associations, resulting in an underestimate of the true association.

Despite the confines of the design of our study, it is the first, to our knowledge, to have adequate sample size and information on potential confounders to allow for the estimation of risk of prostate cancer by level of adiponectin adjusted for known risk factors. We have also made the novel observation that adiponectin receptors AdipoR1 and AdipoR2 are expressed less strongly in prostate cancer tissues as compared with prostate samples from men without the disease. Although case tissue samples came from two populations (Greek and U.S.), our data showed no evidence of differential expression of adiponectin receptors between the groups and each showed weaker expression relative to BPH and control subjects. These data suggest that adiponectin plays a role in prostate cancer pathophysiology, but whether adiponectin has a direct effect on prostate cancer cells, in addition to potential indirect effects through altering endocrine profile, and whether reduced expression of adiponectin receptors in prostate cancer tissue is involved in the potential underlying mechanism remain to be studied by future prospective and experimental studies.

In summary, we show that higher plasma adiponectin concentrations are associated with a reduced risk of prostate cancer in this population of Greek men and that expression of adiponectin receptors is present, although reduced, in cancerous prostate tissue. These results suggest that adiponectin may be involved in the development of prostate cancer.

References

1. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–56.
2. Yokota T, Oritani K, Takahashi I, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000; 96:1723–32.
3. Gavrila A, Chan JL, Yiannakouris N, et al. Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab 2003;88: 4823–31.
4. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999; 100:2473–6.
5. Ouchi N, Kihara S, Arita Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001;103: 1057–63.
6. Mantzoros C, Petridou E, Dessypris N, et al. Adiponectin and breast cancer risk. J Clin Endocrinol Metab 2004;89:1102–7.
7. Miyoshi Y, Funahashi T, Kihara S, et al. Association of serum adiponectin levels with breast cancer risk. Clin Cancer Res 2003;9:5699–704.
8. Chen DC, Chung YF, Yeh YT, et al. Serum adiponectin and leptin levels in Taiwanese breast cancer patients. Cancer Lett 2006;237:109–14.
9. Dal Maso L, Augustin LS, Karalis A, et al. Circulating adiponectin and endometrial cancer risk. J Clin Endocrinol Metab 2004;89:1160–3.
10. Petridou E, Mantzoros C, Dessypris N, et al. Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. J Clin Endocrinol Metab 2003;88:993–7.
11. Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst 2005;97:1688–94.
12. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.
13. Giovannucci E. Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res 2003;35:694–704.
14. Key TJ, Allen NE, Verkasalo PK, Banks E. Energy balance and cancer: the role of sex hormones. Proc Nutr Soc 2001;60:81–9.
15. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 2001;60:91–106.
16. Kelesidis I, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. Br J Cancer 2006;94:1221–5.
17. Goktas S, Yilmaz MI, Caglar K, Sonmez A, Kilic S, Bedir S. Prostate cancer and adiponectin. Urology 2005;65:1168–72.
18. Freedland SJ, Sokoll LJ, Platz EA, et al. Association between serum adiponectin, and pathological stage and grade in men undergoing radical prostatectomy. J Urol 2005;174:1266–70.
19. Rodriguez C, Patel AV, Calle EE, Jacobs EJ, Chao A, Thun MJ. Body mass index, height, and prostate cancer mortality in two large cohorts of adult men in the United States. Cancer Epidemiol Biomarkers Prev 2001;10: 345–53.
20. Bub JD, Miyazaki T, Iwamoto Y. Adiponectin as a growth inhibitor in prostate cancer cells. Biochem Biophys Res Commun 2006;340:1158–66.
21. Brakenhielm E, Veitonmaki N, Cao R, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A 2004;101:2476–81.
22. Kang JH, Lee YY, Yu BY, et al. Adiponectin induces growth arrest and apoptosis of MDA-MB-231 breast cancer cell. Arch Pharm Res 2005; 28:1263–9.
23. Miyazaki T, Bub JD, Uzuki M, Iwamoto Y. Adiponectin activates c-Jun NH_2-terminal kinase and inhibits signal transducer and activator of transcription 3. Biochem Biophys Res Commun 2005;333:79–87.
24. Shaneyfelt T, Husein R, Bubley G, Mantzoros CS. Hormonal predictors of prostate cancer: a meta-analysis. J Clin Oncol 2000;18:847–53.
25. Baron A, Migita T, Tang D, Loda M. Fatty acid synthase: a metabolic oncogene in prostate cancer? J Cell Biochem 2004;91:47–53.
26. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439–51.
27. Qi L, Meigs JB, Liu S, Manson JE, Mantzoros C, Hu FB. Dietary fibers and glycemic load, obesity, and plasma adiponectin levels in women with type 2 diabetes. Diabetes Care 2006;29:1501–5.
28. Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FB. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. Am J Clin Nutr 2006;84: 328–35.