

## Prostate Cancer Risk and Serum Levels of Insulin and Leptin: a Population-Based Study

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**Background:** In a previous study of Chinese men, we found that men with a higher waist-to-hip ratio (WHR) have a higher prostate cancer risk. Because leptin and insulin are related to body fat distribution, we examined whether leptin and insulin were associated with prostate cancer risk. **Methods:** Blood samples were collected from 128 case patients with incident prostate cancer and from 306 healthy control subjects randomly selected from residents of Shanghai, China. Epidemiologic information and anthropometric measurements were collected in personal interviews. Serum leptin, insulin, and sex hormone levels were measured by radioimmunoassay, and insulin-like growth factor-I (IGF-I) was measured by enzyme-linked immunosorbent assay. Multiple logistic regression analyses were used to estimate odds ratios for prostate cancer in relation to serum insulin and leptin levels. All statistical tests were two-sided. **Results:** After adjustment for body mass index, WHR, IGF-I, and sex hormone levels, higher serum insulin levels were associated with a statistically significantly elevated risk of prostate cancer ( $P < .001$ ). Men in the highest tertile of insulin levels had a 2.56-fold (95% confidence interval [CI] = 1.38 to 4.75) risk of prostate cancer compared with men in the lowest tertile. Regardless of the tertile level of WHR, higher serum insulin levels were associated with an increased risk of prostate cancer: Men in the highest tertiles of WHR ( $>0.900$ ) and insulin ( $>8.83 \mu\text{U/mL}$ ) had 8.55 times (95% CI = 2.80 to 26.10) the prostate cancer risk of men in the lowest tertiles of both, and those in the lowest tertile of WHR ( $<0.873$ ) and highest tertile of insulin had 4.30 times (95% CI = 1.17 to 15.70) the risk. By contrast, the association between leptin levels and prostate cancer risk was not

statistically significant. **Conclusion:** Our results suggest that serum insulin levels may influence the risk of prostate cancer in Chinese men. Further research, especially prospective studies, is needed to confirm these findings in high-risk populations and to clarify the underlying mechanisms involved. [J Natl Cancer Inst 2001;93:783-9]

Although China has one of the lowest reported incidence rates of prostate cancer in the world, the incidence of this disease has increased steadily during the last few decades (1,2). The reasons for the rise in prostate cancer incidence in this low-risk population are unclear, but changes in lifestyle because of Westernization have been implicated (2). Westernization in developing countries usually involves improved socioeconomic status, increased intake of animal products, reduced levels of physical activity, and increased prevalence of obesity and metabolic disorders, such as diabetes and hypertension (3,4). Chinese men are generally considered to be relatively lean, with an average body mass index (BMI) of  $21.9 \text{ kg/m}^2$  compared with  $26 \text{ kg/m}^2$  in U.S. men. In an earlier report (5), we showed that abdominal adiposity, as measured by waist-to-hip ratio (WHR), is a risk factor for prostate cancer in Chinese men.

Abdominal adiposity has been linked to lower circulating levels of sex hormone-binding globulin (SHBG) and higher circulating levels of leptin, insulin, and free fatty acids (6-10). High amounts of visceral fat have also been associated with decreased insulin sensitivity, perhaps because of increased release of fatty acids from the abdominal depots (11).

There is a close relationship between circulating leptin and insulin levels and adiposity. Leptin increases the amount of adipose tissue in the body (12,13), regulates food intake and energy balance (14,15), and interacts with other endocrine systems (16,17), whereas insulin increases leptin gene expression, stimulates leptin protein production in rats, and regulates leptin and SHBG protein levels *in vivo* and *in vitro* (18-21).

Because of the potential role of insulin and leptin both in regulating body fat distribution and in hormonal metabolism, we examined the relationships of insulin and leptin with prostate cancer risk as part of a population-based, case-control study in China. Our goal was to clarify whether changes in serum levels of insulin and/or

leptin mediate the effect of abdominal obesity on prostate cancer risk.

## SUBJECTS AND METHODS

### Study Population

Details of the study population have been reported elsewhere (5,22,23). Briefly, case patients with primary prostate cancer (International Classification of Diseases code 185) (24) newly diagnosed from 1993 through 1995 were identified through a rapid reporting system. We identified a total of 268 eligible case patients who represented 95% of the prostate cancer patients diagnosed in urban Shanghai, China, during this period. Randomly selected control subjects were identified from the permanent residents of Shanghai through household registration records (6.5 million). We identified 495 healthy control subjects, who were frequency matched to the expected age distribution (5-year categories) of the case patients.

### Data Collection

In-person interviews were conducted to collect information on demographic characteristics, usual adult diet (reference period: 5 years before prostate cancer diagnosis), smoking history, intake of alcohol and other beverages, personal medical history, and body size. Case patients were interviewed in the hospital, and control subjects were interviewed in their homes. We interviewed 243 (91%) of the 268 eligible case patients and 472 (95%) of the 495 eligible control subjects. On average, case patients were interviewed within 20 days of diagnosis. After the interview, standing height, weight, and circumferences of waist, hip, and right upper arm were measured. Pathology slides of the prostate cancers from case patients were reviewed by pathologists from both Shanghai and the U.S. Armed Forces Institute of Pathology, Washington, DC, to confirm the prostate cancer diagnosis. After the review, four case patients were classified as having benign prostatic hyperplasia and were, therefore, excluded from the study, leaving 239 case patients for analysis. Written informed consent was obtained from all participating subjects, and the study was approved by the Institutional Review Boards at the U.S. National Cancer Institute (NCI), Bethesda, MD, and the Shanghai Cancer Institute.

### Blood Collection

A total of 20 mL of blood was collected from subjects who had fasted overnight. The blood

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samples were taken before treatment or surgery from 200 (83.7%) case patients and from 306 (69.5%) population control subjects. The refusal among control subjects was mostly because of cultural reasons. Immediately after collection, the blood was delivered to a central laboratory at the Shanghai Cancer Institute where the serum was separated from the clotted blood by centrifugation for 15 minutes at 2000g at room temperature. All processed serum samples were kept frozen at  $-70^{\circ}\text{C}$  at the Shanghai Cancer Institute until they were shipped in dry ice to the NCI repository in the United States and then stored at  $-70^{\circ}\text{C}$ .

## Laboratory Methods

We had sufficient sera from 128 case patients who had not yet received any treatment and from 306 control subjects for serum insulin and leptin assays. The serum samples were packed in dry ice and shipped from the NCI repository to the laboratory of F. Z. Stanczyk via overnight express. The samples arrived in good condition without any evidence of thawing and were analyzed for serum insulin, leptin, and sex hormone levels by one of the authors (F. Z. Stanczyk).

For the assays, samples were arranged in case-control pairs and were identified only by specimen-identification number. Laboratory personnel were blinded to the case-control status of the individual specimens. In addition, quality-control samples were included with the study samples to measure intra-assay and interassay variations. Leptin and insulin levels were measured by commercially available specific radioimmunoassay kits (Linco Research, St. Charles, MO), as described previously (25,26). Samples were assayed in duplicate. The limit of sensitivity was  $2\ \mu\text{U}/\text{mL}$  for the insulin assay and  $0.5\ \text{ng}/\text{mL}$  for the leptin assay. The intra-assay and interassay coefficients of variation were 4.0% and 6.0% for the insulin assay and 3.9% and 4.7% for the leptin assay, respectively. Sex hormones, including testosterone, dihydrotestosterone,  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol glucuronide, and SHBG, were also assayed by radioimmunoassay at the same laboratory (F. Z. Stanczyk), and insulin-like growth factor-I (IGF-I) and its binding proteins (IGFBP-1 and IGFBP-3) were assayed at Diagnostic System Laboratory (Webster, TX). Data on sex hormones and IGFs and their association with prostate cancer risk are presented in a separate report (23). Serum prostate-specific antigen (PSA) levels were measured by Dianon Systems, Inc. (Stratford, CT), by the PSA immunoassay, performed on the TOSOH AIA-1200 automated immunoassay instrument (Dianon Systems, Inc.).

## Statistical Analysis

The distributions of leptin, insulin, and WHR among control subjects were used to create tertiles for analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer risk in relation to serum levels of leptin and insulin were estimated by use of multiple logistic regression analysis (27), simultaneously controlling for several potential confounding factors: age at interview, educational level, BMI, and WHR. BMI, expressed as weight divided by the square of height ( $\text{kg}/\text{m}^2$ ), was used as a measure of overall obesity, and WHR was used as a measure of abdominal adiposity. BMI and WHR are physiologic correlates of insulin and leptin levels.

The age-adjusted regression models attempt to capture an insulin and/or leptin effect, which may or may not act through the obesity-insulin resistance or IGF-axis pathways. The BMI- and WHR-adjusted regression models are likely to capture any insulin or leptin effect that is independent of these pathways. IGF-I, IGFBP-1, and sex hormone levels are potentially associated with both prostate cancer risk and insulin levels; ORs in certain models were, therefore, further adjusted for these variables. In the multivariate models, most of the covariates, including age, BMI, and serum levels of sex hormones, were included as continuous variables. In selected analyses, we considered the case patients with localized or regional/remote disease as separate categories to evaluate whether the extent of cancer affected the exposure measurements and, therefore, the exposure-disease relationships. Spearman's rank-order correlation coefficients were used to measure the pairwise correlation between serum insulin, leptin, IGFs, and WHR. All statistical tests were two-sided.

## RESULTS

To determine whether serum levels of insulin and leptin affect the risk of prostate cancer in Chinese men, we conducted a population-based, case-control study. The age of case patients at prostate cancer diagnosis ranged from 50 to 94 years (median, 73 years). About two thirds of the case patients were diagnosed with advanced (regional/remote stage) cancer, and most (>60%) had moderately or poorly differentiated cancers. Most (>75%) case patients were symptomatic at diagnosis, with 77% having serum PSA levels greater than  $10\ \text{ng}/\text{mL}$  (median,  $87\ \text{ng}/\text{mL}$ ). Table 1 shows demographic information and anthropometric measures in case patients and control subjects. The

case patients had a higher caloric intake, had smaller hips and larger WHRs, and were less likely to be married, to have attended college, to smoke, or to drink alcohol. The prevalence of diabetes, hypertension, and liver cirrhosis was similar in case patients and control subjects.

We first determined whether there was a correlation between serum insulin and leptin levels among control subjects. Among the 306 control subjects, there was a statistically significant positive correlation between serum insulin and leptin levels ( $r = .52$ ;  $P < .001$ ) (Table 2). As shown in Table 2, serum insulin levels showed a statistically significant positive correlation with WHR, with serum levels of IGF-I, IGFBP-3, and  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol glucuronide, and with the ratio of testosterone to SHBG but showed a statistically significant negative correlation with serum levels of testosterone, dihydrotestosterone, SHBG, and IGFBP-1. Similar correlations were observed for serum leptin levels.

Age-adjusted mean serum levels of insulin were  $11.98\ \mu\text{U}/\text{mL}$  (95% CI =  $10.45$  to  $13.74$ ) and  $7.87\ \mu\text{U}/\text{mL}$  (95% CI =  $6.97$  to  $8.88$ ), respectively, among case patients and control subjects. Age-adjusted mean serum levels of leptin were  $3.32\ \text{ng}/\text{mL}$  (95% CI =  $2.88$  to  $3.83$ ) and  $3.00\ \text{ng}/\text{mL}$  (95% CI =  $2.64$  to  $3.40$ ), respectively, among case patients and control subjects. Mean serum levels of insulin in case patients were 52% higher than those in control subjects ( $P < .001$ ). Although mean serum levels of leptin in case patients were 10% higher than those

**Table 1.** Selected characteristics of prostate cancer case patients and population control subjects in China\*

Characteristic	Case patients (n = 128)		Population control subjects (n = 306)	
	Mean	SD	Mean	SD
Age, y	71.9	7.5	72.0	7.2
Total intake of calories, kcal/day	2434	674	2337	709
Height, cm	167.6	5.9	167.5	5.9
Weight, kg	60.8	8.0	61.4	9.9
Body mass index, $\text{kg}/\text{m}^2$	21.7	3.0	21.9	3.2
Waist circumference, cm	82.9	9.9	82.4	10.4
Hip circumference, cm	90.9	8.7	92.6	8.4
Waist-to-hip ratio	0.91	0.05	0.89	0.05
% married	87.5		91.8	
% with education greater than college	17.9		11.0	
% smokers	53.9		66.0	
% alcohol users	32.0		42.8	
% with diabetes	4.0		3.8	
% with hypertension	35.9		32.7	
% with liver cirrhosis	0.8		0.98	

\*SD = standard deviation.

**Table 2.** Spearman's correlation coefficients between serum insulin or leptin levels and selected factors among 306 population control subjects in China

Factor	Insulin	Leptin
Insulin	1.00	0.52
Leptin	0.52	1.00
Insulin-like growth factor-I	0.32	0.31
Insulin growth factor-binding protein-1	-0.39	-0.49
Insulin growth factor-binding protein-3	0.19*	0.27
Testosterone	-0.25	-0.30
Dihydrotestosterone	-0.21†	-0.26
5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol glucuronide	0.20‡	0.26
Sex hormone-binding globulin (SHBG)	-0.36	-0.35
Testosterone/SHBG ratio	0.26	0.18§
Body mass index	0.28	0.38
Waist-to-hip ratio	0.30	0.38

All statistical tests were two-sided. All *P* values <.001 unless noted.

\**P* = .009.

†*P* = .003.

‡*P* = .006.

§*P* = .002.

||Body mass index is calculated as weight divided by the square of height (kg/m<sup>2</sup>).

in control subjects, the difference was not statistically significant.

We next assessed whether serum levels of insulin or leptin were associated with prostate cancer risk (Table 3). ORs were sequentially adjusted first for age, next for anthropometric factors, and then for serum levels of IGF-I. After all adjustments, the men in the highest tertile of insulin levels had a 2.56-fold risk of prostate cancer (OR = 2.56; 95% CI = 1.38 to 4.75) relative to the men in the lowest tertile. After adjustment for age, the men in the highest tertile of leptin levels had an approximately twofold risk of prostate cancer (OR = 1.78; 95% CI = 1.07 to 2.95) relative to the men in the lowest tertile. This excess, however, disappeared after further adjustment for WHR and IGF-I.

For both insulin and leptin levels, all ORs for prostate cancer risk changed little after further adjustment for IGFBP-1, IGFBP-3, smoking, alcohol consumption, total caloric intake, and sex hormone levels (data not shown).

We next assessed the combined effect of increased levels of insulin, leptin, and WHR on prostate cancer risk. As shown in Table 4, at every level of WHR, increased insulin levels were associated with increased risks of prostate cancer in a statistically significant dose-response relationship (*P*<.001). Compared with the men in the lowest tertile of WHR levels (<0.873) and in the lowest tertile of insulin levels (<6.44  $\mu$ U/mL), the men in the lowest tertile of WHR levels and in the highest tertile of insulin levels (>8.81  $\mu$ U/

mL) had a more than fourfold risk of prostate cancer (OR = 4.30; 95% CI = 1.17 to 15.70), while the men in the highest tertile of WHR levels (>0.900) and in the highest tertile of insulin levels had a more than eightfold risk of prostate cancer (OR = 8.55; 95% CI = 2.80 to 26.10). In addition, increasing levels of WHR were associated with statistically significant excess risks of prostate cancer for each tertile of insulin levels. The associations between leptin levels and prostate cancer risk were inconsistent.

We also determined the ORs for the relationship between insulin and leptin levels and the stage at diagnosis of prostate cancer. Regardless of the stage at diagnosis, increased insulin levels were associated with excess risks of prostate cancer (the highest tertile of insulin versus the lowest tertile: OR for localized stage cancer = 1.81 [95% CI = 0.78 to 4.19]; OR for regional/remote stage cancer = 2.50 [95% CI = 1.22 to 8.13]). Higher levels of leptin were also associated with an increased risk of prostate cancer, but the trends were not statistically significant (data not shown). Risks of prostate cancer in relation to insulin levels were further examined by median levels of WHR, IGF-I, or IGFBP-1. Regardless of the levels of WHR and IGFBP-1, higher serum levels of insulin were associated with statistically significantly increased risks of prostate cancer. Men with higher (> median) serum insulin levels and lower WHR (< median) and IGF-I levels (< median) had a more than fourfold risk of prostate cancer (OR = 4.47; 95% CI = 1.50 to 13.31).

**Table 3.** Odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer risk in relation to serum levels of insulin and leptin in the population-based, case-control study in China

	No. of case patients/ control subjects	Adjusted for age		Further adjusted for education and anthropometric factors*		Further adjusted for IGF-I†	
		OR	95% CI	OR	95% CI	OR	95% CI
Tertile of insulin‡							
Low (<6.44 $\mu$ U/mL)	26/100	1.00§	—	1.00§	—	1.00§	—
Middle (6.44–8.83 $\mu$ U/mL)	28/102	1.07	0.59 to 1.96	1.03	0.55 to 1.94	0.99	0.52 to 1.86
High (>8.83 $\mu$ U/mL)	72/101	2.80	1.64 to 4.78	2.81	1.52 to 5.17	2.56	1.38 to 4.75
	<i>P</i> <sub>for trend</sub>		<.001		<.001		.001
Tertile of leptin‡							
Low (<2.30 ng/mL)	34/101	1.00§	—	1.00§	—	1.00§	—
Middle (2.31–4.04 ng/mL)	33/101	0.97	0.56 to 1.69	0.67	0.36 to 1.27	0.60	0.38 to 1.15
High (>4.04 ng/mL)	61/102	1.78	1.07 to 2.95	1.10	0.59 to 2.07	0.80	0.52 to 1.90
	<i>P</i> <sub>for trend</sub>		.02		.66		.95

\*Multivariate adjusted for age, education, body mass index, and waist-to-hip ratio. Leptin model included insulin, and insulin model included leptin.

†IGF-I = insulin-like growth factor-I.

‡Tertiles among control subjects were used as the cutoffs.

§Referent.

||All statistical tests were two-sided.

**Table 4.** Odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer in relation to serum levels of insulin, by tertile levels of waist-to-hip ratio (WHR) in the population-based, case-control study in China

	Tertile of WHR								
	Low (<0.873)			Middle (0.873–0.900)			High (>0.900)		
	No. of case patients/ control subjects	OR*	95% CI	No. of case patients/ control subjects	OR	95% CI	No. of case patients/ control subjects	OR	95% CI
Tertile of insulin ( $\mu\text{U}/\text{mL}$ ) <sup>†</sup>									
Low (<6.44)	5/42	1.00 <sup>‡</sup>	—	10/37	2.57	0.78 to 8.43	11/21	5.08	1.52 to 17.0
Middle (6.44–8.83)	7/41	1.74	0.49 to 6.12	9/30	2.40	0.71 to 8.05	12/31	3.50	1.07 to 11.50
High (>8.83)	9/18	4.30	1.17 to 15.70	22/31	6.83	2.18 to 21.4	40/52	8.55	2.80 to 26.10
Tertile of leptin ( $\text{ng}/\text{mL}$ ) <sup>†</sup>									
Low (<2.30)	9/55	1.00 <sup>‡</sup>	—	10/25	2.38	0.79 to 7.15	14/21	4.00	1.43 to 11.10
Middle (2.31–4.04)	7/24	1.34	0.41 to 4.40	12/46	0.93	0.32 to 2.68	14/31	2.19	0.78 to 6.12
High (>4.04)	5/22	0.97	0.25 to 3.68	20/28	3.18	1.18 to 8.60	36/52	2.89	1.10 to 7.57

\*Adjusted for age, education, body mass index, and insulin-like growth factor-I. Leptin model was further adjusted for insulin, and insulin model was further adjusted for leptin.

<sup>†</sup>Tertiles among population control subjects were used as cutoffs.

<sup>‡</sup>Referent.

## DISCUSSION

Results from this population-based, case-control study suggest that higher serum levels of insulin are associated with an increased risk of prostate cancer. To date, only one study has evaluated the role of insulin; it found no association with prostate cancer (28). One small case-control study (43 case patients and 48 healthy control subjects) (29) reported no association of leptin with prostate cancer risk, while one nested case-control study in Sweden reported a positive association with leptin, and a recent clinical survey (30) showed that higher plasma leptin levels were associated with larger (>0.5 cm<sup>3</sup>) tumor volume.

The observed association of insulin with prostate cancer risk is independent of overall and abdominal adiposity. Even among men in the lowest tertile of WHR (<0.873), those in the highest tertile of insulin levels had a fourfold risk of prostate cancer, suggesting that insulin may modulate the risk of prostate cancer through mechanisms other than obesity. One such mechanism may be the IGF-I axis. IGF-I has been implicated in the regulation of prostate epithelial cell proliferation and in the etiology of prostate cancer (31–34). Recently, we also reported a positive association of IGF-I with prostate cancer risk (23). Like IGF-I, insulin is a mitogen that appears to be a growth factor for prostatic epithelial cells and has an antiapoptotic effect (35,36). Insulin also decreases IGFBP-1 production and secretion, thereby increasing the bioavailability of IGF-I (37). Furthermore, because the receptors for

insulin and IGF-I are homologous, insulin can bind to and activate the IGF-I receptor (34). However, despite the potential relationship between insulin and IGFs, the observed insulin association with prostate cancer risk in our study was independent of IGF-I, IGFBP-1, and IGFBP-3 levels, and the statistically significant excess prostate cancer risk associated with high insulin levels (>7.53  $\mu\text{U}/\text{mL}$ ) persisted in the subgroup of men with low WHR (<0.89) and low IGF-I (<123  $\mu\text{U}/\text{mL}$ ) levels (OR = 4.47; 95% CI = 1.50 to 13.31).

The insulin-prostate cancer association among men with abdominal obesity (WHR >0.90) is of special interest because abdominal adiposity was reported (5) to be a strong risk factor for prostate cancer among Chinese men in the same case-control study. Abdominal obesity, especially visceral fat (intra-abdominal fat) obesity, is often associated with insulin resistance and androgen metabolism (6–9). Thus, in addition to affecting the IGF-I axis, insulin may affect prostate cancer risk through the obesity-sex hormone pathway. Insulin appears to regulate the production and metabolism of testosterone and SHBG (38). An *in vitro* study (39) showed that insulin suppresses the hepatic synthesis of SHBG, and cross-sectional studies (40,41) reported an inverse correlation between insulin and SHBG levels. Decreased levels of SHBG result in increased levels of the bioactive-free fraction of testosterone, which, in turn, may increase the risk of prostate cancer. Consistent with a study by Haffner (38), we found that serum levels of insulin correlated negatively with those of

total testosterone, dihydrotestosterone, and SHBG but positively with those of 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol glucuronide and the testosterone/SHBG ratio, which are surrogate markers of intraprostatic androgenicity (see Table 2). These results suggest that insulin may influence prostate cancer risk through changes in the hormonal milieu within the prostate gland.

However, the earlier reported (5) WHR association with prostate cancer risk does not seem to be explained totally by insulin, since, at each tertile of insulin, increasing levels of WHR were associated with an excess prostate cancer risk. Thus, other biologic mechanisms may be involved. It is unclear whether the underlying biologic mechanisms for a role of insulin in prostate cancer development in men with or without abdominal obesity are similar. Further research, especially prospective studies, is needed to confirm the insulin association and to elucidate the specific biologic mechanisms involved.

The reported lower prostate cancer risk among diabetic patients in some studies (42–45) but not in all (46) further supports a potential role for insulin in prostate cancer etiology. The basis for the reduced prostate cancer risk among diabetics is unclear, but potential explanations include lower levels of testosterone and IGF-I, as well as insulin insensitivity, in people with non-insulin-dependent diabetes mellitus (type II diabetes) (47–49). Type II diabetes is usually associated with abdominal obesity and insulin resistance (50). Metabolic changes in individuals with type II diabetes are complex; defects in both insulin secretion and action coex-

ist. Initially, insulin levels may be higher than normal in the prediabetic state because of insulin resistance, i.e., more insulin is produced to elicit a biologic response. Subsequently, serum insulin levels in type II diabetic patients (without insulin injection) may remain low because a dysfunction of the pancreatic beta cells can impair insulin secretion (51), even though these patients are markedly insulin resistant. Low serum levels of IGF-I and high serum levels of IGFBP-1 have been reported among diabetic patients (48), further supporting the possibility of a reduced prostate cancer risk among these patients. In our study, type II diabetes was not a risk factor for prostate cancer, and the exclusion of the 18 subjects with a history of diabetes from the analysis did not change the insulin results (data not shown). Although the precise mechanisms relating obesity, diabetes, and insulin resistance to prostate cancer risk are complex and presently unclear, it is important to clarify the interrelationships among these factors and their role in prostate cancer etiology because the prevalence of diabetes and obesity in both Western and Asian populations has increased over time (52–54).

Few studies have investigated the role of insulin in prostate cancer, in part because of the difficulty in measuring insulin sensitivity and resistance in epidemiologic studies. Moreover, serum levels of insulin fluctuate with glucose levels and food intake. The gold-standard method used to measure insulin resistance in clinical studies, the hyperinsulinemic euglycemic glucose clamp, is complex and labor intensive and is thus impractical in large-scale epidemiologic studies (55). Nevertheless, among healthy subjects without diabetes, a single measure of fasting insulin compared well with the clamp measurement (56), suggesting that fasting insulin levels can be used as a marker of insulin resistance. Because peripheral blood insulin levels depend on the rate of insulin secretion and metabolism, it is not clear from our study whether it is hyperinsulinemia *per se* or insulin resistance that is associated with the observed excess risk of prostate cancer. To examine this question further, future studies should also incorporate measurements of fasting glucose to use the homeostasis model (a mathematical model to assess insulin resistance and  $\beta$ -cell function based on insulin and glucose levels) to estimate insulin resistance more accurately (57).

Prostate cancer risk patterns associated with leptin were less consistent. In this study, the association of leptin with prostate cancer risk was confined largely to men with a WHR higher than 0.87, suggesting that leptin may interact with markers related to abdominal obesity, such as sex hormones or IGF-I, to increase the risk of prostate cancer. In fact, although leptin has a role in gonadotropin and androgen action in rats (58), the physiologic role of leptin in humans is less clear.

Possible limitations of our study were potential confounding of variables, the cross-sectional design, and possible differential measurement errors. Confounding is possible but should not account for the insulin or leptin findings entirely because the observed associations were independent of several known risk factors. However, confounding by unconsidered or unmeasured factors cannot be ruled out because insulin is linked with many other metabolic measurements. Thus, it is possible that increased levels of insulin are markers for other hormonal or metabolic aberrations that may affect the risk of prostate cancer.

The cross-sectional design is an inherent limitation of this study that hampers investigation of the temporal relationship between insulin or leptin levels and prostate cancer risk. In addition, the presence of cancer among case patients may potentially affect serum levels of insulin and leptin. However, such an effect, if any, should be minimal for several reasons. First, unlike IGF-I, which can be produced by prostatic cells, currently there are no data to suggest that insulin can be produced by either normal or tumor prostatic cells. Second, in general, anorexia and cachexia associated with advanced tumors can result in lower serum levels of insulin in prostate cancer patients and, therefore, in an underestimation of the true association between insulin levels and prostate cancer risk. Such an effect should have been minimal in our study because case patients had little weight loss (average, 1–2 pounds) and statistically significantly higher serum levels of insulin than control subjects. Third, prolonged administration of anabolic steroids can increase insulin levels. Such a treatment effect should have been negligible in our study because blood samples from case patients were collected before treatment. Finally, the small difference in leptin and insulin levels between case pa-

tients with localized prostate cancer and those with regional/remote stage prostate cancers in our study further suggests that the exposures in the study were not greatly influenced by the presence of cancer.

Differential measurement errors or variations are also unlikely explanations for the observed differences in insulin levels among case and control subjects because all serum samples were treated identically and because the study was designed and conducted with extreme caution to minimize laboratory variation. Selection bias was minimal because the response rate for the study was high, inclusion of subjects in the study was not related to survival, and there were no appreciable differences in demographic characteristics or in the prevalence of potential risk factors between case patients who gave blood and those who did not.

In summary, the results from our population-based, case-control study in China suggest that, independent of overall and abdominal adiposity, higher serum levels of insulin confer a higher risk of prostate cancer. Whether concomitant hormonal and metabolic alterations related to abdominal obesity and insulin resistance are the underlying mechanisms for the observed association is unclear. Future studies, especially prospective studies, are needed to confirm these results, to clarify the specific biologic mechanisms involved, and to determine the clinical usefulness of the insulin finding in predicting the risk of clinically significant prostate cancer. Whether the much lower insulin levels and obesity in Chinese men, relative to Western men, can help explain the substantial ethnic difference in prostate cancer risk also needs to be clarified.

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## NOTES

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