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Award Number: DAMD17-03-1-0315

TITLE: Estrogen Metabolism and Prostate Cancer Risk: A Prospective Study

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REPORT DATE: May 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2005	Annual (1 May 2004 - 20 Apr 2005)

4. TITLE AND SUBTITLE
Estrogen Metabolism and Prostate Cancer Risk: A Prospective Study

5. FUNDING NUMBERS
DAMD17-03-1-0315

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Prostate cancer is the most common cancer among men in the United States (IARC, 1995) and the second most common in the European Community (IARC, 1995). The causes of prostate cancer, however, remain largely unknown, with age, race, and family history being the only established risk factors (Nomura et al., 1997). The prostate gland has historically been considered the prototype of an androgen-dependent organ. However, there is evidence that estrogens may induce mitosis of prostatic epithelial cells in many species, including humans (Leav et al., 1978; Schulze et al., 1987).

The present report analyzes the association between prostate cancer and estrogen metabolism investigated in a case-control study. In particular, we tested the hypothesis that the pathway favoring 2-hydroxylation over 16α-hydroxylation may be associated with decrease in prostate cancer risk.

This is the annual report for the second year of the study activity. During the second year of activity, we completed the re-call of 1,150 participants of the Western New York prospective cohort with the identification of 41 incident prostate cancer cases. Among the participants who did not received diagnosis of prostate cancer, we identified 164 control subjects. The development of the database for the case-control analysis is on-going

The frozen urine samples have been identified in the biological specimen bank and in these days we are planning the shipment of the specimens to the laboratory for the hormone determinations.

14. SUBJECT TERMS
Prostate Cancer, estrogens, epidemiological study

15. NUMBER OF PAGES
57

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Prostate cancer is the most common cancer among men in the United States (IARC, 1995) and the second most common in the European Community (IARC, 1995). The causes of prostate cancer, however, remain largely unknown, with age, race, and family history being the only established risk factors (Nomura et al., 1997). The prostate gland has historically been considered the prototype of an androgen-dependent organ. However, there is evidence that estrogens may induce mitosis of prostatic epithelial cells in many species, including humans (Leav et al., 1978; Schulze et al., 1987).

The present report analyzes the association between prostate cancer and estrogen metabolism investigated in a case-control study. In particular, we tested the hypothesis that the pathway favoring 2-hydroxylation over 16α-hydroxylation may be associated with decrease in prostate cancer risk.

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BODY OF REPORT

In accordance with the Statement of Work, during the second budget year, we a) completed the follow-up of the cohort and b) b) defined the matched case-control pairs.

a) **Follow-up of the cohort**: We completed the re-call and follow-up of the Western-New York cohort (WNYHC) for the identification of the incident prostate cancer cases and their related control subjects. The follow-up was conducted in collaboration with another NIH-funded study, the "Epidemiology of Type 2 Diabetes" study, which is a prospective cohort study based on the same WNYHC cohort (RO1 DK 60587, Dr. R. Donahue, PI, Dr. P. Muti, Co-PI).

The re-call of the cohort started after January 2003. The re-call included participants without history of cancer, cardiovascular diseases, and clinically defined type-2 diabetes at baseline interview in 1996-2001. The re-call was also limited to those cohort participants provided with stored biological samples. Thus, we started the re-call and the follow-up process with a sample of **1,150** cohort participants.

Out of **1,150**, **52** were not eligible for medical reasons (too sick of diseases other than cancer, cardiovascular diseases, type 2 diabetes, mentioned before). **46** were deceased (for causes other than prostate cancer), **22** moved out of the Erie and Niagara Counties, **117** were not able to be contacted by mail and by phone. At the end , we obtained a sample of **913** re-called participants.

Among the **913** participants, **232** refused to participate in the study (however, they referred, in the short telephone interview, to have not been diagnosed with prostate cancer), **40** were scheduled, but then they cancelled the appointment, **8** were still in process at the end of the

follow-up period (September 30, 2004). Thus, **633** participants were available to participate in the study. Among those 633, we have identified **41** incident prostate cancer cases.

All procedures to re-call, to interview and to collect biological specimens from the WNYHC Study were similar to the procedures used for baseline recruitment of the cohort. All eligible participants were re-contacted initially by letter and then by phone (up to twelve callbacks). Participants were invited to attend our recruitment center at the Department of Social and Preventive Medicine for the clinical examination and to answer questions related to occurrence of prostate cancer diagnosis between the baseline examination and the re-call time.

b) Definition of the matched case-control pairs.

Case Identification of Incident Prostate Cancer Cases: Prostate cancer cases recruited in the study were men who have been diagnosed with incident cytologically and/or histologically confirmed prostate cancer after their recruitment (date at first interview) in the WNYHC Study and before the end of the cohort follow-up period (September, 30, 2004). Prostate cancer cases were identified by their own report at the re-call of the cohort. Their report has been validated with the clinical records for 32 cases, while the remaining 9 cases are on the process to be validated. At recruitment, each cohort member signed a consent form allowing us to ask copies of their clinical charts in cases of pathological events related to the WNYHC Study investigations. Thus, we are validating the information collected from participants through the access to their clinical charts.

Control Identification for the case-control study: Eligible controls were all men members of the WNYHC Study who, based on their report, were not diagnosed with prostate cancer at the time

of the diagnosis of the related case. For each prostate cancer case, four controls have been randomly chosen after matching for: a) age (within 3 years) and b) race.

To increase the power of the study (and to reduce effects of non-diagnosed prostate cancer cases among controls), **we used a 1:4 ratio for cases and controls,** thus the hormone determinations will be conducted on 41 prostate cancer cases and 164 control subjects.

At the present, we are retrieving the biological samples from the biorepository to be sent to the laboratory for the hormonal determination and define methods and procedures for the quality control analysis. At the same time we are developing the new database to collect the results of the hormone determinations to compose a final dataset for the final study analysis.

Publications and Presentations

At this time, there are no results or publications coming directly from this grant because we have still to complete the study. However, Dr. Muti has published or has in press research on hormone related prostate cancer using a previously collected data set on hormone and prostate cancer (the dataset was originated by a previously DOD funded study).

In 2004, she published a paper on the relation between Growth Hormone and Prostate Cancer (Fuhrman B, Barba M, Schunemann HJ, Hurd T, Quattrin T, Cartagena R, Carruba G, **Muti P.** *Basal growth hormone concentrations in blood and the risk for prostate cancer: A case-control study.* Prostate. 2005- Jan 21) and a paper on alcohol consumption and risk of prostate cancer (Barba M, McCann SE, Schunemann HJ, Stranges S, Fuhrman B, De Placido S, Carruba G, Freudenheim JL, Trevisan M, Russell M, Nochajski T, **Muti P.** *Lifetime total and beverage*

specific - alcohol intake and prostate cancer risk: a case-control study Nutr J. 2004; 3:23-29) .

In both cases the first authors are young collaborators of Dr. Muti.

In year 2004-2005, Dr. Muti has published other papers on hormones and cancer listed below:

1) Rinaldi S, Toniolo P, **Muti P**, Lundin A, Zeleniuch-Jacquotte A, Akhmedkhanov A, Micheli A, Lenner P, Dossus L, Krogh V, Shore RL, Koenig KL, Riboli E, Stattin P, Berrino F, Hallmans G, Lukanova A, Kaaks R IGF and IGFBP3 and breast cancer in young women: *A Pooled Reanalysis of three prospective studies European Journal of Cancer Prevention* (in press)

2) Carruba G, Cocciadiferro L, Bellavia V, Rizzo S, Tsatsanis C, Spandidos D, **Muti P**, Smith C, Mehta P, Castagnetta L. *Intercellular communication and human hepatocellular carcinoma* Ann N Y Acad Sci.1028:202-12

3) Bucca G , Carruba G , Saetta A , **Muti P**, Castagnetta L , Smith CP. *Gene expression profiling of human cancers* Ann N Y Acad Sci. 2004; 1028:28-37

4) Muti P. *The Role of Endogenous Hormones in the Etiology and Prevention of Breast Cancer: the Epidemiological Evidence.* Ann N Y Acad Sci. 2004; 1028:28-37

5) Micheli A, **Muti P**, Secreto G, Krogh V, Meneghini E, Sieri S, Venturelli E, Pala V, Berrino F. *Endogenous sex hormones and subsequent breast cancer in premenopausal women*. Int J Cancer 2004: 112 (2):312-318

She also presents new study results from other conducted studies at the on-coming Annual Meeting of the American Association for Cancer Research (2005):

1) Fuhrmann B*, Barba M, Krogh V, Micheli A, Berrino F, Muti P. *Insulin resistance is associated with elevated circulating androgens in postmenopausal women: A potential pathway for breast cancer etiology* Annual Meeting American Association for Cancer Research, Anaheim, California, April 2005

2) Platek M, Freudenheim JL, Quick S, Nie J, Muti P, McCann S, Trevisan M, Shields P, Edge **S** *Methylenetetrahydrofolate reductase (MTHFR) and risk of breast cancer: The Western New York Exposures and Breast Cancer Study (WEB Study)* Annual Meeting American Association for Cancer Research, Anaheim, California, April 2005

3) Barba M*, Mc Cann S, Stranges S, **Muti P**, Fuhrmann B, Trevisan M, Freudenheim J *Perinatal exposures and breast cancer risk: a case-control study* Annual Meeting American Association for Cancer Research, Anaheim, California, April 2005

Two of these studies have been submitted for publication.

In addition, Dr. Muti has several other manuscripts submitted for publication on hormone and related factors and cancer.

CONCLUSIONS

We have just begun the phase of hormone determinations for this grant; therefore, there are no conclusions to report at this time.

REFERENCES

Fuhrman B, Barba M, Schunemann HJ, Hurd T, Quattrin T, Cartagena R, Carruba G, **Muti P**. *Basal growth hormone concentrations in blood and the risk for prostate cancer: A case-control study.* Prostate. 2005- Jan 21 (Electronic publication ahead of print)

Barba M, McCann SE, Schunemann HJ, Stranges S, Fuhrman B, De Placido S, Carruba G, Freudenheim JL, Trevisan M, Russell M, Nochajski T, **Muti P**. *Lifetime total and beverage specific - alcohol intake and prostate cancer risk: a case-control study* Nutr J. 2004; 3:23-29

Rinaldi S, Toniolo P, **Muti P**, Lundin A, Zeleniuch-Jacquotte A, Akhmedkhanov A, Micheli A, Lenner P, Dossus L, Krogh V, Shore RL, Koenig KL, Riboli E, Stattin P, Berrino F, Hallmans G, Lukanova A, Kaaks R IGF and IGFBP3 and breast cancer in young women: *A Pooled Reanalysis of three prospective studies European Journal of Cancer Prevention* (in press)

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Micheli A, **Muti P**, Secreto G, Krogh V, Meneghini E, Sieri S, Venturelli E, Pala V, Berrino F. *Endogenous sex hormones and subsequent breast cancer in premenopausal women.* Int J Cancer 2004: 112 (2):312-318

APPENDIX

Appendix 1: **Fuhrman B, Barba M, Schunemann HJ, Hurd T, Quattrin T, Cartagena R, Carruba G, Muti P.** ***Basal growth hormone concentrations in blood and the risk for prostate cancer: A case-control study.*** **Prostate. 2005**

Appendix 2: **Barba M, McCann SE, Schunemann HJ, Stranges S, Fuhrman B, De Placido S, Carruba G, Freudenheim JL, Trevisan M, Russell M, Nochajski T, Muti P.** ***Lifetime total and beverage specific - alcohol intake and prostate cancer risk: a case-control study*** **Nutr J. 2004; 3:23-29**

Basal Growth Hormone Concentrations in Blood and the Risk for Prostate Cancer: a Case-Control Study

Barbara Fuhrman[1] , Maddalena Barba[1,2], Holger J Schünemann[3], Thelma Hurd[4], Teresa Quattrin[5], Ruben Cartagena [1,6],Giuseppe Carruba[7], & Paola Muti[1*].

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Abbreviated title: Growth Hormone and Prostate Cancer

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Acknowledgements. Supported in part by grant 1179818559 from Department of Defense, Prostate Cancer Program and grant 5K07CA89123 from the National Cancer Institute, and in part by the American Italian Cancer Foundation.

Abstract

Objective: To assess the relationship between basal serum growth hormone levels and prostate cancer risk.

Methods: We conducted a population-based case-control study; cases included 68 men, aged 45-85 years, diagnosed with incident, primary, histologically confirmed, and clinically apparent (stage B and higher) prostate cancer. Controls included 220 men, matched on age and residential area. Age, race, BMI, waist circumference, history of enlarged prostate, education and current smoking status, were all considered as possible confounders.

Results: We found a statistically significant trend of prostate cancer decreasing risk across increasing GH quintiles, in both crude (OR: 0.31 95% CI: 0.12-0.83, p for trend 0.01) and adjusted models (OR: 0.35 95% CI: 0.12-1.05, p for trend 0.03), in the highest compared to the lowest quintile, respectively.

Conclusions: Lower basal levels of growth hormone in serum are associated with increased prostate cancer risk. The inverse association may be explained by the negative feedback loop generated by IGF-1 produced by the tumor on GH secretion.

Key Words: Prostate cancer, growth hormone, epidemiological studies

Introduction

Prostate cancer researchers have long focused on the role of endogenous hormones in tumor biology and etiology. Recently, distinct lines of epidemiologic and basic science research have converged in the hypothesis that the somatotropic axis plays an important role in the development of prostate cancer.

The somatotropic axis is a set of neuroendocrine signaling pathways that regulates growth and development (1). Growth Hormone (GH) is the primary regulator of hepatic IGF-1 synthesis and plays an important role in regulating expression of Insulin-like Growth Factor Binding Protein – 3 (IGFBP-3), which modulates the availability of IGF-1 to its target tissue by binding approximately 90% of circulating IGF-I. GH secretion by the anterior pituitary represents the integration of a complex set of neuroendocrine signals; in turn, the actions of growth hormone at target tissues are important determinants of growth and body size (2). Pituitary GH secretion is pulsatile, with circulating peaks detectable in nocturnal hours, approximately 2 hours apart (3). The mean concentration of GH in serum between secretory spikes represents the "basal" levels of serum GH.

Over the past five years, a growing body of epidemiologic research has focused on the potential role of IGFs in the etiology of prostate cancer. Epidemiologic studies have been mainly investigating, with inconsistent results, the association between elevated serum levels of IGF-I and increased risk of prostate cancer (4-12). Although it is well known that GH is a major factor regulating IGF-I blood concentration, there is no evidence about how physiological mechanisms may change in presence of prostate cancer. From experimental studies (13-16), it appears that GH might be involved in regulating prostate function. The co-expression of GH and its receptor

demonstrated by Chopin and colleagues in prostate cancer cell lines (16) would enable an autocrine-paracrine pathway to exist in the prostate that would be able to stimulate growth, either directly or indirectly via IGF production. To our knowledge, GH involvement in prostate function has been extensively studied in laboratories models, but never in human beings.

In the present case-control study we examined the association between basal serum GH levels and risk for prostate cancer, in order to better understand the role of the possible contribution of the GH-IGF-I system to tumor pathogenesis.

Materials and Methods

Study Subjects: We conducted a case-control study of incident, primary, histologically confirmed prostate cancer cases in Erie and Niagara counties, NY, USA (the PROMEN study). Recruitment and enrollment of study participants were based on the same sources and criteria previously described in detail (17). All participants provided informed consent; the Human Subjects Review Board of the University at Buffalo, School of Medicine and Biomedical Science and each of the participating hospitals approved procedures for protection of human subjects in the study.

To exclude latent prostate carcinomas that one cannot distinguish from those that would not progress to clinical disease (real latent carcinoma) and those detected in a very early phase of their progression, the present study included only patients with clinically apparent disease [stage B and greater by the staging system proposed by Catalona (18)].

To standardize the stage of the disease across the hospitals, a screening form developed in the context of the PROMEN study was completed by a trained nurse case-finder using the hospital pathology records. The forms and hospital records were reviewed by the principal investigator (P. Muti) of the study.

In the course of the study period, from December 1998 to April 2001, 504 prostate cancer cases were identified. Of these 504, 163 met eligibility criteria, and were approved by the urologists and invited to join the PROMEN study. After being contacted, 50 men refused to participate. Thus, among the eligible participants, 70% (113/163) of the subjects participated in the study. Twenty-five prostate cancer cases did not provide blood samples and 20 cases had missing data items thus the present analysis is conducted on 68 cases.

In recruiting controls, since latent prostate carcinoma has a high prevalence in men over 50 (19), we evaluated serum prostate specific antigen (PSA). Those of them found to have a PSA level higher than 4 ng/ml were excluded from the control group, in accordance with the criterion adopted by the American College of Preventive Medicine (20), until the completion of further diagnostic procedures, that allowed us to clarify which of the two groups they truly belonged on the basis of their correct case-control status. We identified eight prostate cancer cases because of PSA determination in subjects who initially were recruited as controls.

Three hundred and seventeen over the 513 subjects contacted during the study period were willing to participate (60%). Blood samples were not available for 66 of these men, and 11 men had missing data items, thus the present analysis includes 240 controls.

Extensive data on demographics, smoking history and other study variables were collected by trained interviewers during in person computer assisted interviews and with self-administered

questionnaires. Heights, weight, and waist circumference were measured by trained personnel using standardized protocol. Body mass index (BMI), was calculated from measured height and weight.

Hormonal determinations: Blood specimens were collected between 7:00 a.m. and 9:00 A.M. in order to minimize intra-individual variation associated with time of day. Time and date of collection were recorded for each blood sample.

Serum specimens were split and stored in freezers at -80°C. All samples were handled identically and randomly located in laboratory runs. Laboratory personnel were blinded with regard to case/control status. The intra-assay coefficient of variation was 5.3%, and the inter-assay coefficient was 6.9%.

Growth Hormone levels were conducted using an immunometric assay kit (Immulite Growth Hormone; Diagnostic Products Corporation, Los Angeles, CA).

Prior to this study, we evaluated the reliability of growth hormone measures in 51 men who had been enrolled as controls for the current study. For each subject two blood samples were used, the second drawn exactly one-year after the first, at the same hour and minute of the day. After collection was completed, all samples were retrieved, and matched samples were assayed in the same runs. The intraclass correlation coefficient for matched samples was 0.86 ($p<0.01$), and the Spearman rank correlation coefficient for ranked GH levels was 0.80 ($p<0.01$), demonstrating good reliability of GH measures in both characterizing and ranking circulating GH levels (18).

Statistical Analysis: Questionnaire and biological data were analyzed using both SPSS version 10.0 (SPSS Inc., Chicago, IL) and SAS version 8.0 (SAS Corp., Cary, NC).

Distributions for all variables of interest were examined and for each continuous variable, the distribution among control subjects was used to group participants into tertiles for purposes of presentation. For continuous variables, t-tests, and for categorical variables, Pearson's chi square tests were used to assess the statistical significance of any associations between case/control status and participant characteristics.

The statistical significance of differences in levels for each participant characteristic was assessed using the one-way ANOVA with Tukey's correction for post-hoc comparisons. GH hormone levels were assessed both as a continuous variable and as a categorical variable, defined using the distribution among controls to group study subjects into quintiles.

Unconditional logistic regression was used to estimate crude and adjusted odds ratios associated with GH levels. Multivariate logistic models were run including as potential confounders age, race, BMI, waist circumference, current smoking status, history of enlarged prostate and education.

Results

Table 1 describes the characteristics of the participants in the study. Compared to controls, cases were significantly more likely to have a larger waist circumference ($p=0.01$), and to be African Americans ($p<0.01$). Non-significant associations were observed for age, BMI, history of enlarged prostate, familiar history of prostate cancer, level of education and current smoking status.

Since several anthropometric and lifestyle factors may play a role in prostate cancer etiopathogenesis, we evaluated associations between covariates and GH levels among control subjects (**Table 2**). Older participants had significantly higher levels of GH than younger aged groups ($p<0.01$). Waist circumference was significantly, inversely associated with basal growth hormone levels ($p< 0.01$). Participants in the highest tertile of BMI had lower serum GH levels but this association did not reach statistical significance ($p = 0.10$). Likewise, a near-significant association of current smoking with GH was observed ($p = 0.11$). Non-significant associations were observed as for remaining participants characteristics among controls.

Crude and adjusted estimates of prostate cancer risk by basal plasma GH quintiles, according to GH levels in the control group, are shown in **Table 3**.

Twenty-seven participants (17 cases and 10 control subjects) were excluded from this analysis because of missing data (e.g. BMI, waist circumference); they did not differ from included subjects as for GH basal levels.

In both the univariate and multivariate models, odds ratios decline with increasing GH quintiles (for highest quintile, OR_{crude}: 0.31 95% CI: 0.12-0.83 and $OR_{adjusted}$: 0.35 95% CI: 0.12-1.05) with a significant trend found for both crude and adjusted odds ratios by GH quintile ($p = 0.01$ and $p = 0.03$, respectively).

We also performed subgroup analyses by age and race.

Risk estimates for men aged 65 and older (cases = 54, controls = 207)) showed decreased prostate cancer risk across increasing GH levels tertiles, in both unadjusted and adjusted models (OR 0.28, 95% CI 0.10-0.82, p for trend = 0.02 and OR 0.29, 95% CI 0.10-0.86, p for trend =

0.02, respectively). Among participants younger than 65 years of age (cases = 14, controls = 33), we did not observe a statistically significant association between GH levels and prostate cancer.

Stratifying by race and defining categories based on GH levels medians, we observed similar results in the two ethnic groups. Among Caucasians (cases = 48, controls = 222), we found an inverse association between basal GH level and prostate cancer risk in both crude and adjusted models (OR: 0.45 95% CI 0.16-1.24, $p_{trend} = 0.04$, OR: 0.52 95% CI 0.17-1.57, $p = 0.048$, respectively). Among African Americans (cases = 20, controls = 18) there was evidence of the same association (For highest quintile, OR_{crude} 0.33 95% CI 0.06-1.88, $p_{trend} = 0.24$, $OR_{adjusted}$: 0.49, 95% CI 0.05-4.61, $p_{trend} = 0.55$).

DISCUSSION

The results of this case-control study suggest that basal levels of GH may be inversely related to risk of prostate cancer. There are two primary reasons that lead us to cautiously interpret these findings. First, the retrospective case-control design of the study bears the risk of bias. In this case a selection bias could have been generated by having restricted the controls to men with PSA < 4 ng/ml and, consequently, preferentially removed from the control group men with benign prostate hyperplasia (BPH). Since PSA $\geq$ 4 ng/ml appears to be associated with both prostate cancer and BPH, we could have artificially increased the difference in GH levels between the two groups. In such a case our results should have been biased in the opposite direction, since we could have expected higher GH levels in cases when compared to controls.

Second, because of the complexity of the biological function of the IGFs system, the observed relationship between basal GH level and prostate cancer status could be the expression of a

negative feedback loop, with elevated IGF-I circulating levels having a negative effect on GH pituitary secretion (3).

In spite of these two limitations, the study adds important evidence to the current knowledge about hormones in the etiology of prostate cancer. To our knowledge, among studies focusing on the relationship between the GH-IGF-I system and prostate cancer risk, this population-based case-control study is the first epidemiologic study to examine the relationship between GH levels and prostate adenocarcinoma. Our study is also characterized by an innovative recruiting strategy, that is, limiting eligibility for enrollment as a case to men who have been diagnosed with advanced cancer stages (stage B and higher). This approach has been helpful in reducing misclassification by eliminating early stage prostate cancers, as they are not distinguishable from latent diseases that may be prevalent among controls. With the same aim, subjects were eligible for recruitment as controls on the basis of a PSA determination, which helped to ensure that the control group was free from latent prostate cancer.

Additionally sample collection, handling, and laboratory procedures were standardized in order to minimize variability in GH measurement.

Our data show an increase in basal GH levels with increasing age among cases and controls. This is somewhat surprising based on the common paradigm that GH levels should decline with aging (21). Normative data are sparse for men in our study population age range (45-85 years); however our finding is in agreement with results from an Italian cross-sectional study, whose participants' ages were in same range (22).

Since in our study, differences in age between cases and controls approached statistical significance and GH levels were affected by age, we performed further analyses stratifying on

this variable. Growth Hormone levels showed a trend suggesting a protective effect among older men (≥ 65 years of age), but not among younger men (< 65 years of age). This may be due to the small number of younger men in our study sample.

There is a growing body of evidence about the potential role of growth factors in the etiopathogenesis of prostate cancer. A role for IGFs in cancer is supported by epidemiologic studies, which have found that high serum IGF-I concentration and low IGFBP-3 levels are associated with increased risk of several cancers, including breast, lung, colon-rectum and prostate (4, 23-25). However, to date, epidemiologic research on this topic has not been able to establish whether observed differences in IGF-I and its binding proteins circulating levels play a causal role in disease etiology or are caused themselves by the disease process. Two recent studies provide potential clues: Woodson and colleagues (9) observed concentrations of circulating IGF-1increasing over time in cases, but not in controls, providing evidence that higher IGF-I circulating levels could be a prostate cancer consequence, instead of a cause.

A case-control study showed a positive association between a GH gene promoter polymorphism and a higher colon rectal cancer risk, suggesting a possible major role of the somatotropic axis in affecting risk for this specific disease (25).

There are several reasons that could explain difficulties in addressing this important issue. The somatotropic axis is a complex set of pathways regulating growth and reproduction, with a complex interplay of each of its components. Further limits of circulating IGF-I measurements are due to the interaction and modulation of IGFBPs as well as by other hormones. Insulin can enhance GH stimulated IGF-I synthesis and can influence IGFBPs levels. At a tissue level, regulation is variable depending on the type of tissue. Besides, the somatotropic axis is deeply

influenced in its functioning by the availability of food and there is evidence showing that diet modulates the circulating levels of binding proteins and the receptors affinity (26).

Many aspects of the relationship between prostate cancer and IGFs remain still unclear, most of them concerning GH secretion. Recent laboratory evidence shows the presence of GH mRNA isoforms in prostate cancer cell lines (16), suggesting the possibility of an active role of prostate cancer in GH synthesis and secretion in vivo, but the extent at which this source might directly contribute to GH plasma levels is completely unknown .

GH pituitary secretion remain unclear as well. As already mentioned, lower basal GH levels in prostate cancer cases could suggest a negative association of GH serum concentration with prostate cancer, but they could also be explained by the negative feedback loop generated by IGF-I on GH secretion, or other disease effects on GH blood concentration. Given that GH pituitary secretion results from both a phasic and basal production, it is still to be established on which of them the negative loop could depend. Besides, if GH levels in patients are influenced by IGF-I secretion at a prostate level, the stromal components might have a role in determining the final effect, since we know that in healthy people they modulate prostatic hormones secretion (3).

The small sample size in our study limited our ability in detecting significant differences. Nevertheless, our findings underscore the importance of further research to clarify the possible role of the GH/IGF/IGFBP axis in the etiopathogenesis of prostate cancer.

Our need to reach a deeper knowledge about GH/IGF-I system Growth Hormone and its relationship with prostate cancer is undeniable from a public health perspective. Recently IGFs have been increasingly used in the treatment of pathologies, such as aging-related problems (27, 28), idiopathic stature disorders (29), and cardiac insufficiency (30). The establishment of a role

for GH in prostate cancer etiopathogenesis could have an important impact on the balance of costs-benefits for GH-based interventions and future guidelines in therapeutic and preventive management of some of the most socially relevant pathologies.

Acknowledgements. Supported in part by grant 1179818559 from Department of Defense, Prostate Cancer Program and grant 5K07CA89123 from the National Cancer Institute, and in part by the American Italian Cancer Foundation.

References

1. Partridge L and Gems D (2002) Mechanisms of ageing: public or private? *Nat Rev Genet* **3**: 165-75.

2. LeRoith D and Roberts CT, Jr (2003) The insulin-like growth factor system and cancer. *Cancer Lett* **195**: 127-37.

3. Muller EE, Locatelli V, Cocchi D (1999) Neuroendocrine control of growth hormone secretion. *Physiol Rev* **79**: 511-607.

4. Chan JM, Stampfer MJ, Giovannucci E, et al. (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* **279**: 563-6.

5. Chan JM, Stampfer MJ, Ma J, et al. (2002) Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *Journal of the National Cancer Institute* **94**: 1099-106.

6. Harman SM, Metter EJ, Blackman MR, Landis PK, Carter HB (2000) Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. *J Clin Endocrinol Metab* **85**: 4258-65.

7. Chokkalingam AP, Pollak M, Fillmore CM, et al. (2001) Insulin-like growth factors and prostate cancer: a population-based case-control study in China. *Cancer Epidemiology, Biomarkers & Prevention* **10**: 421-7.

8. Mantzoros CS, Tzonou A, Signorello LB, Stampfer M, Trichopoulos D, Adami HO (1997) Insulin-like growth factor 1 in relation to prostate cancer and benign prostatic hyperplasia. *British Journal of Cancer* **76**: 1115-8.

9. Woodson K, Tangrea JA, Pollak M, et al. (2003) Serum insulin-like growth factor I: tumor marker or etiologic factor? A prospective study of prostate cancer among Finnish men. *Cancer Res* **63**: 3991-4.

10. Stattin P, Bylund A, Rinaldi S, et al. (2000) Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. *Journal of the National Cancer Institute* **92**: 1910-7.

11. Lacey JV Jr, Hsing AW, Fillmore CM, Hoffman S, Helzlsouer KJ, Comstock GW (2001) Null association between insulin-like growth factors, insulin-like growth factor-binding proteins, and prostate cancer in a prospective study. *Cancer Epidemiol Biomarkers Prev* **10**: 1101-2.

12. Wolk A, Mantzoros CS, Andersson SO, et al. (1998) Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. *Journal of the National Cancer Institute* **90**: 911-5.

13. Reiter, E., Bonnet, P., Sente, B., Dombrowicz, D., De Leval, J., Closset, J., Hennen, G., 1992. Growth Hormon and prolactin stimulate androgen receptor. Mol. Cell. Endocrinol. **88**., 77-87.

14. Reiter, E., Kecha, O., Hennuy, B., Lardinois, S., Klug, M., Bruyninx, M., Closset, J., Hennen, G., 1995. Growth hormone directly affects the function of the different lobes of the rate prostate. Endocrinology **136**, 3338-3345.

15. Ballesteros, M., Leung, K.C., Ross, R.J. M., Iismaa, T. P., Ho, K. K. Y., 2000. Distribution and abundance of messenger ribonucleic acid for growth hormone receptor isoforms in human tissues. J. Clin. Endocrinol. Metab. 85, 2865-2871.

16. Chopin, L.K., Veveris-lowe, T. L., Phillips, A.F., Herington, A.C., 2002. Co-expression of GH and GHR isoforms in prostate cancer cell lines. Growth Horm. IGF Res. **12**, 126-136.

17. Muti P, Westerlind K, Wu T, et al. (2002) Urinary estrogen metabolites and prostate cancer: a case-control study in the United States. *Cancer Causes Control* **13**: 947-55.

18. Catalona WJ (1987) Diagnosis, staging, and surgical treatment of prostatic carcinoma. Arch Intern Med **147**:361-380.

19. Breslow N, Chan CW, Dhom G, et al (1977) Latent carcinoma of prostate at autopsy in seven areas. The International Agency for Research on Cancer, Lyons, France. *Int J Cancer* **20**: 680-8.

20. L. Ferrini and Steven H. Woolf. Screening for Prostate Cancer in American Men Am J Prev Med. July 1998.

21. Elmlinger MW, Dengler T, Weinstock C, Kuehnel W (2003) Endocrine Alterations in the Aging Male. *Clinical Chemistry & Laboratory Medicine.* **41(7)**:934-941.

22. Muti P, Gaddi P, Quattrin T (2002) Correspondence re: Chokkalingam A. Et al., Insulin-like Growth Factors and Prostate Cancer: A Population-based Case- Control Study in China. Cancer Epidemiol. Biomark. Prev., 10: 421,2001. *Cancer Epidemiology, Biomarkers & Prevention* **Vol. 11, 323**.

23. Hankinson SE, Willett WC, Colditz GA, et al. (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* **351**: 1393-6.

24. Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X (1999) Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. *J Natl Cancer Inst* **91**: 151-6.

25. Le Marchand, L., Donlon, T., Seifried, A., Keaks, R., Rinaldi, S., and Wilkens, L. Association of a common polymorphism in the human GH1 gene with colorectal Neoplasia. Journal of the National Cancer Institute, **94**: 434-60, 2002

26. Root AW (2004) Regulation of energy balance. *Current Opinion in Pediatrics.* **16(2)**:137-138.

27. Italian Association for Research on Brain Aging: Multicenter Study Group, Function of GH/IGF axis in aging: multicenter study in 152 healthy elderly subjects. *Aging- Clinical and Experimental Research* **9**: 185-192, 1997

28. Gurlek A, Gedik O (2001) Endogenous sex steroid, GH and IGF-I levels in normal elderly men: relationships with bone mineral density and markers of bone turnover. *J Endocrinol Invest* **24(6)**: 408-14.

29. Mehta A, Hindmarsh PC (2002) The Use of Somatropin (Recombinant Growth Hormone) in Children of Short Stature. *Pediatric Drugs* **4(1)**:37-47.

30. Modlinska A, Babinska A, Faran G, Sworczak K (2003). Clinical and metabolic effects of recombinant growth hormone in cardiac insufficiency. *Przegl Lek* **60** **(5)**:349-52.

Table 1: Participant Characteristics by Case-Control Status

	Cases	Controls
	Number (Column Percentage)	
Age		
45-64 years	14 (20.6)	33 (13.8)
65-74 years	42 (61.8)	145 (60.4)
75-85 years	12 (17.6)	62 (25.8)
Waist Circumference (cm) §*		
First (78-96)	19 (27.9)	81 (33.8)
Second (97-106)	14 (20.6)	81 (33.8)
Third (107-149)	35 (51.5)	78 (32.5)
Body Mass Index §		
First (18.1-26.4)	17 (25.0)	81 (33.8)
Second (26.5-30.0)	19 (27.9)	80 (33.3)
Third (30.0-46.0)	32 (47.1)	79 (32.9)
History of Enlarged Prostate		
Yes	35 (51.5)	102 (42.5)
No	33 (48.5)	138 (57.5)
Has First Degree Relative with Prostate Cancer		
Yes	9 (13.2)	22 (9.2)
No	59 (86.8)	218 (90.8)
African American **		
Yes	20 (29.4)	18 (7.5)
No	48 (70.6)	222 (92.5)
Education		

Did not complete high school	18 (26.5)	37 (15.4)
Completed high school	18 (26.5)	83 (34.6)
Some college or more advanced study	32 (47.1)	120 (50.0)
Current Smoking Status		
Yes	8 (11.8)	16 (6.7)
No	60 (88.2)	224 (93.3)

* $p < 0.05$; ** $p < 0.01$

§ Tertiles were defined using distribution among controls

Table 2: Plasma Growth Hormone Levels among Control Subjects by Participant Characteristics

	n	*Mean (s.d.)*
Age**		
45-64 years	33	0.27 (0.45)
65-74 years	145	1.40 (1.68)
75-85 years	62	1.45 (1.83)
Waist Circumference (cm) §**		
First (78-96)	81	1.75 (2.16)
Second (97-106)	81	1.02 (1.24)
Third (107-149)	79	1.00 (1.29)
Body Mass Index (tertiles) **§**		
First (18.1-26.4)	81	1.51 (1.77)
Second (26.5-30.0)	80	1.29 (1.86)
Third (30.0-46.0)	79	0.96 (1.23)
History of Enlarged Prostate		
Yes	102	1.47 (1.81)
No	138	1.10 (1.52)

Family History of Prostate Cancer		
Yes	22	1.30 (1.54)
No	218	1.25 (1.67)
African American		
Yes	18	1.38 (1.58)
No	222	1.25 (1.67)
Education		
Did not complete high school	37	1.31 (1.57)
High school graduate	83	1.11 (1.42)
Some college or more advanced study	120	1.35 (1.83)
Current Smoking Status		
Yes	16	0.62 (0.62)
No	224	1.30 (1.69)

* $p < 0.05$; ** $p < 0.01$

§ Tertiles were defined using distribution among controls

Table 3: Crude and Adjusted Estimates of Prostate Cancer Risk by Basal Plasma Growth Hormone Quintile [a]

Plasma Growth Hormone (ng/l)	Cases	Controls	OR	95% CI
Crude Estimates				
First quintile (0.05-0.09)	21	52	1.00	Reference
Second quintile (0.10-0.33)	17	44	0.96	0.45-2.04
Third quintile (0.34-0.83)	11	48	0.57	0.25-1.30

Fourth quintile (0.84-2.10)	13	**48**	0.67	0.30-1.49
Fifth quintile (2.15-19.95)	**6**	**48**	0.31	0.12-0.83
Totals:	68	240		
p_{trend}:	0.01			
Adjusted Estimates [b]				
First quintile (0.05-0.09)	21	52	1.00	Reference
Second quintile (0.10-0.33)	17	44	0.95	0.41-2.22
Third quintile (0.34-0.83)	11	48	0.50	0.20-1.26
Fourth quintile (0.84-2.10)	13	**48**	0.59	0.24-1.43
Fifth quintile (2.15-19.95)	**6**	**48**	0.35	0.12-1.05
Totals:	68	240		
p_{trend}:	0.03			

[a] cut-off points for quintiles were determined based on the distribution of GH levels among controls

[b] the multivariate model adjusted for age, race, BMI, and waist circumference (as continuous measures), as well as current smoking, history of enlarged prostate, and education.

[c] adjusted estimates excluded participants with missing data

LIFETIME TOTAL AND BEVERAGE SPECIFIC ALCOHOL INTAKE AND PROSTATE CANCER RISK: A CASE-CONTROL STUDY

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Abbreviated title:

Alcohol and Prostate Cancer

Acknowledgements. Supported in part by grant 1179818559 from Department of Defense, Prostate Cancer Program and grant 5K07CA89123 from the National Cancer Institute, and in part by an American Italian Cancer Foundation Fellowship.

ABSTRACT

Objective. We investigated lifetime alcohol consumption and prostate cancer risk in a case-control study conducted in Buffalo, NY (1998-2001).

Methods. The study included 88 men, aged 45 to 85 years with incident, histologically-confirmed prostate cancer and 272 controls. We conducted extensive in-person interviews regarding lifetime alcohol consumption and other epidemiologic data.

Results. Prostate cancer risk was not associated with lifetime intake of total and beverage specific ethanol. In addition we found no association with number of drinks per day (average drinks per day over the lifetime) or drinks per drinking day (average drinks per day on drinking days only over the lifetime). However, we observed an inverse association with the total number of drinking years. Men in the lowest tertile of total drinking years had a two-fold prostate cancer risk than men in the highest tertile (OR 2.16, 95% CI 0.98-4.78, p for trend <0.05).

Conclusions. Our results suggest that alcohol intake distribution across lifetime may play a more important role in prostate cancer etiology than total lifetime consumption.

Key words: alcohol, lifetime, prostate cancer

Introduction

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer death among men in the Western countries (1). Notwithstanding the importance of this malignancy, little is understood about its cause. To date the only well established risk factors are age, family history of disease, race and country of residence (2), while the body of the evidence about the role of alcohol intake is still controversial. Since alcohol consumption is a common lifestyle factor and potentially modifiable, the finding of an association with prostate cancer could have an important impact on public health.

Among the population-based case-control studies, those carried out by Heyes et al. (3) and Sharpe et al. (4) found an increased risk of prostate cancer associated with alcohol consumption. Risk increased with increasing frequency of alcohol consumption (3) and among those who drank regularly over a longer period (4). Sesso et al., in their prospective cohort study, confirmed the finding of a higher risk associated with alcohol consumption (5). However, numerous studies published since 1998 have not found an association between alcohol intake and prostate cancer (6-17). In a review by Breslow and Weed, only 6 of 32 studies reported a positive association between alcohol use and prostate cancer (18); however, they noted that many of the studies had biases that could have attenuated the risk estimates.

Although prostate cancer is known to have a long latency period, lifetime alcohol consumption was not addressed in the studies carried out until the late 1990s, and rarely in the more recent studies (18). Furthermore, investigators focusing on this topic have considered lifetime alcohol consumption as the average total amount of alcohol consumed over the lifetime, rarely taking into account such characteristics as number of drinks consumed on a typical

drinking day or other descriptions of drinking pattern. The distribution of an equivalent volume of alcohol across multiple drinking occasions rather than a single occasion (e.g., one drink per day vs. seven drinks on single day) is likely to have different physiologic effects and impact on cancer risk. Likewise, an examination of average total lifetime alcohol intake does not address the possibility that, although the total lifetime volume may not differ, the duration of intake may, thus effectively resulting in a higher dose over a shorter time period.

Alcohol may act as a carcinogen itself and may also modulate risk from other carcinogen exposures. It has been implicated in risk of cancer at a number of sites (19-20). In the present case-control study we examined the association between lifetime alcohol intake, duration of alcohol use, and drinks per day with risk of prostate cancer in western New York.

Material and methods

We conducted a case-control study of prostate cancer and hormones and alcohol intake (the PROMEN STUDY) in Erie and Niagara Counties, NY, USA, between December 1998 and April 2001. The methods for this study have been previously described in detail (21). Participants provided informed consent; the Institutional Review Board of the University at Buffalo, School of Medicine and Biomedical Science, and each of the participating hospitals approved the procedures for the protection of human subjects recruited for the study.

Cases were men aged 45 to 85 years with incident, primary, histologically confirmed prostate cancer. Men with a previous history of cancer (except non-melanoma skin cancer), or already on hormonal or chemotherapy treatment (current or in the 6 months prior to diagnosis), as well as those affected by chronic or acute liver diseases, were excluded. Cases aged 35-65 years were also required to have a driver's license, because we used driver's license records to identify age matched controls.

During the study period, 504 men were identified with incident prostate cancer. Of these, 336 men did not meet the eligibility criteria; we invited the remaining 163 patients to participate in the PROMEN study. After being contacted, 50 men refused to participate resulting in a participation rate of 70%. Ninety-six patients had complete data for the variables of interest.

Controls aged between 35 and 65 years were selected from a list of individuals holding a New York State driver's license and residing in Erie and Niagara Counties. Those aged 65 and over were selected from the rolls of the Health Care Financial Administration. As with cases, men on hormonal treatment (current or in the 6 months prior the diagnosis), or diagnosed with metabolic or endocrine disease were excluded, as well as participants with a previous story of cancer other than non-melanoma skin cancer. Since it is well known that latent prostatic carcinoma has a high prevalence in men over 50 (22-23), we evaluated prostate specific antigen (PSA) in the blood samples obtained from controls. Controls found to have a PSA value higher than 4 ng/ml were excluded from the control group, in accordance with the criterion established by the American Cancer Society Prostate Cancer Detection Project (24) until the completion of further diagnostic procedures to clarify their true case-control status.

During the study period, 1373 potential controls were contacted. One hundred and seventy nine of these individuals were deceased or were too ill to participate, 293 did not meet the eligibility criteria and we were not able to contact 272 persons. We identified eight prostate cancer cases as a result of PSA determination in subjects who initially were recruited as controls. Three hundred and seventeen of the remaining 513 subjects (60%) were enrolled and interviewed: 304 had complete data for analysis.

Extensive data on demographics, smoking history, alcohol consumption, and other study variables were collected by trained interviewers during in-person computer-assisted interviews

(25) and with self-administered questionnaires. Height, weight, waist and hip circumferences were measured by trained technicians using a standardized protocol. Body mass index (BMI) was calculated as weight in kilograms divided by square of the height in meters (kg/m^2). Waist to hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Alcohol intake

Detailed information on alcohol consumption throughout the lifetime was collected using the Cognitive Lifetime Drinking History (26-27). Prior to the interview, participants completed a lifetime events calendar on which they recorded the date and their age when significant events in their life occurred. The calendar was used during the interview to help them remember what they were doing during specified periods of their lives and whether drinking alcohol was involved. Participants reported the age when they started drinking alcohol regularly (at least once a month for six months) and when their drinking changed over the years. When changes were reported, participants were asked whether they continued regular drinking; if not, they were asked if they ever resumed regular drinking. Using this information, we defined intervals during each participant's life when drinking patterns were relatively homogeneous and computed the total number of drinking years and the total number of abstinent years. Lists of alcoholic beverages, beer, wine, wine coolers, and liquor, and models of glasses and bottles were used to help respondents recall what beverages they drank over their lifetimes; their usual drink size of each beverage; and whether drink size changed over their lifetimes. This provides information used to: (1) calculate absolute alcohol intakes and (2) tailor the computer-assisted interview to the each respondent's drinking history. Patterns of drinking were ascertained for intervals during which respondents drank weekly or more often by asking how often respondents drank on Fridays, Saturdays, Sundays, and weekdays, and how many drinks they usually had on each. For

intervals during which respondents drank less often than weekly, they were asked standard quantity and frequency questions. Quantity and frequency for times when they drank more than usual were assessed for all intervals, as was the frequency of intoxication; the proportion of drinks they had with meals/snack/without eating; and the proportion of drinks from beer, wine, wine coolers, and liquor.

Drinks per interval was estimated by multiplying quantity by frequency for days of the week and more than usual and adding. Drinks per interval was translated into ounces of ethanol per interval based on the proportion of drinks represented by specific beverages, respondents' beverage-specific drink size in ounces, and factors representing the average percent per ounce of absolute alcohol for a given beverage to estimates of drinks per interval. Factors used were 0.048, 0.12, 0.04 and 0.40, for beer, wine, wine cooler and hard liquor, respectively. These estimates were summed across drinking intervals to yield lifetime totals.

We considered several variables in these analyses: total number of years alcohol was consumed, number of drinks per day during the drinking years (total number of drinks/total number of days in drinking years), number of drinks per drinking day (total number of drinks/total number of days on which alcohol was consumed in drinking years), total lifetime ounces of ethanol and beverage-specific total lifetime ounces of ethanol. Because few participants consumed wine coolers, wine and wine coolers were combined. A drink was defined as 12 ounces of beer, 5 ounces of wine, and 1.5 ounces of liquor.

Statistical analysis

Statistical analyses were conducted using SPSS for Windows version 11.0. Differences between cases and controls in demographic characteristics and alcohol consumption were assessed using t-tests for continuous variables and χ^2 for categorical variables. Lifetime

abstainers, defined as those subjects who had less than 12 drinks in any one year over their lifetimes, were excluded from our analyses. The biological and social differences between lifetime abstainers and both former and current drinkers (28-29) and the very low number of these subjects in our sample (5 cases and 11 controls) represent the reasons for their exclusion from our analyses. Our final sample size for analysis included 88 cases and 272 controls.

In analyses of risk associated with lifetime alcohol intake, tertiles of total and beverage specific ounces and total drinking years were computed based on the distribution in the controls. For the beverage specific analyses, non-drinkers were those respondents not consuming that particular alcoholic beverage. For risk associated with drinks per day and drinks per drinking day, we categorized consumption as two drinks or less per day and greater than two drinks per day. Odds ratios (OR) and 95% confidence intervals (CI) for risk of prostate cancer associated with alcohol consumption were computed using unconditional logistic regression adjusting for age, cigarette smoking status, education, body mass index (BMI), and waist to hip ratio (WHRATIO). The beverage specific analyses were further mutually adjusted for the other beverages.

Results

Characteristics of the participants in the PROMEN study are shown in Table 1. Compared to cases, controls were slightly more educated (13.0 vs. 12.3 years) and more likely to be Caucasian (93.0% vs. 67%). No statistically significant differences between cases and controls were observed for age, body mass index, waist to hip ratio, smoking or drinking status.

Means and standard deviations for aspects of lifetime alcohol consumption for the sample overall and by current drinking status are shown in Table 2. Among drinkers overall and current drinkers, cases drank for fewer years than did controls (38.2 vs. 43.7 years, $p < 0.05$ and 41.3 vs.

46.8 years, $p < 0.05$, overall and current drinkers, respectively) and, consequently, had greater numbers of years abstaining. Few differences in lifetime total and beverage-specific ounces consumed, drinks per day, or drinks per drinking day were observed between cases and controls for drinkers overall or current drinkers. However, although not statistically significant, we observed several differences in alcohol consumption between cases and controls who were former drinkers. Among former drinkers, cases consumed more total ethanol, beer and liquor, more drinks per day and more drinks per drinking day, but consumed less ethanol from wine and wine coolers compared to controls.

Odds ratios and 95% confidence intervals for the risk of prostate cancer associated with lifetime alcohol consumption are shown in Table 3. We observed no associations with risk with lifetime ounces of total ethanol, beer, wine, or liquor. Risk associated with total drinking years, years of abstaining (ever/never), drinking status, drinks per day, and drinks per drinking day are shown in Table 4. Compared to the highest tertile of total drinking years, men in the lowest tertile had a marginally significant increased risk (OR 2.16, 95% CI 0.98-4.78, p for trend < 0.05) and, similarly, men reporting ever abstaining compared to those who never abstained had increased prostate cancer risk (OR 1.79, 95% CI 1.05-3.03). No associations with risk were observed for former vs. current drinkers, drinks per day, or drinks per drinking day.

Discussion

The assessment of lifetime alcohol consumption in cancer etiology has been predominantly expressed through the calculation of either total lifetime volume or an average volume per specified time period across the lifetime. Few investigations have evaluated lifetime drinking patterns in relation to prostate cancer risk. While methodological difficulties challenge the evaluation of drinking patterns, our results suggest that failure to take into account aspects of drinking pattern such as the relative duration and dose of consumption may reduce our ability to clearly elucidate the role alcohol may be playing in cancer development. Although we observed no associations with risk for total lifetime alcohol intake or when alcohol was expressed as average drinks per day or even average drinks per drinking day, our results suggest that the impact may differ when the same volume of alcohol consumption takes place in fewer drinking years over a lifetime.

Furthermore, it is notable that alcohol consumption was much higher among the cases compared with controls who were former drinkers. As alcohol consumption has been positively related to many causes of morbidity, a proportion of these men may have stopped drinking in response to poor health. Whether pre-existing morbid conditions or heavier drinking is related to subsequent development of prostate cancer remains to be clarified.

Our study has several strengths and limitations. A limitation of our study is the small sample size, especially for cases. However, because one of the original aims of the study was an examination of hormones and prostate cancer, both cases and controls were carefully identified. To eliminate the effect on hormone levels by treatment, cases were enrolled in the study prior to starting chemotherapy or hormone therapy; thus increasing the difficulty of case ascertainment. On the other hand, the exclusion of controls with high circulating PSA levels helped to reduce

misclassification and to ensure that the control group was free from prostate cancer. The data used in the present analysis were collected as a part of an in-person interview, and the questionnaire about lifetime alcohol consumption was very detailed allowing us to compute both the dose and frequency aspects of alcohol consumption.

Given the difficulties involved in measuring alcohol consumption, studies utilizing data collected before diagnosis would appear more likely to lead to valid inferences. Recently, Dennis in his meta-analysis (30) pointed out that in many of the published cohort studies alcohol consumption was assessed only at a baseline, often many years before the diagnosis, with no subsequent assessment to quantify changes in drinking pattern. While retrospective assessment of lifelong alcohol consumption at diagnosis may appear to be more likely to lead to recall bias, such an assessment may also be more likely to capture relevant attributes of exposure, such as overall duration of alcohol use and timing of potentially important changes in use, such as quitting. These differences are not always taken into account (30).

The plausibility of alcohol as a risk factor for prostate cancer relates to evidence that alcohol may act as a carcinogen or may modulate risk from other known carcinogens through generation of free radicals, affecting the metabolism of detoxification enzymes, impairment of immune system and depression of DNA repair enzymes (31). It remains unclear to what extent alcohol could affect the early phases of cancer development. Some studies suggest that the critical period of exposure may be as early as adolescence as the development of prostate gland begins prenatally, continuing until the end of puberty (32). If alcohol contributes to cancer promotion, duration and relative intensity of exposure during a specified period of time, instead of the total amount of the agent itself over the entire life time course may be important.

. Further studies focusing on lifetime exposure and more specifically on patterns of consumption may help in prevention of a disease with considerable public health impact.

Acknowledgements. Supported in part by grant 1179818559 from Department of Defense, Prostate Cancer Program and grant 5K07CA89123 from the National Cancer Institute, and in part by an American Italian Cancer Foundation Fellowship

REFERENCES

1. Kay T (1995) Epidemiologic characteristics of prostate cancer. Cancer **75 (Supp.7)**: 1766-1777

2. Bosland MC (2000) The role of steroid hormones in prostate carcinogenesis. Journal of the National Cancer Institute **27**:39-66.

3. Hayes RB, Brown LM, Shoenberg JB, et al. (1996). Alcohol use and prostate cancer risk in US blacks and whites. Am J Epidemiol **143**: 692-697.

4. Colin R. Sharpe & Jack Siemiatycki (2001) Case-control study of alcohol consumption and prostate cancer risk in Montreal, Canada Cancer Causes and Controls **12**: 589-598.

5. Howard D Sesso, Ralph S Paffenbarger J and I-Min Lee (2001) Alcohol consumption and risk of prostate cancer: The Harvard Alumni Health Study International Epidemiological Association **30:**749-755.

6. De Stefani E, Fierro L, Barrios E, Ronco A (1995) Tobacco, alcohol, diet and risk of prostate cancer. Tumori **81**:315-320.

7. Cerhan JR, Torner JC, Lynch CF, et al. (1997) Association of smoking, body max index, and physical activity, with risk of prostate cancer in the Iowa 65+ Rural Health Study (United States). Cancer causes & Controls **8**: 229-238.

8. Key TJA, Silcocks PB, Davey GK, Appleby PN, Bishpop DT (1997). A case-control study of diet and prostate cancer. Br J Cancer **76**: 678-687.

9. Jain MG, Hislop GT, Howe GR, Burch JD, Ghadirian P (1998). Alcohol and other beverage use and prostate cancer risk among Canadian men. Int J Cancer **78**: 707-711.

10. Lumey LH, Pittman B, Wyner EL (1998) Alcohol use and prostate cancer in US whites: no association in a confirmatory study. Prostate **36**: 250-255.

11. Breslow RA, Wideroff L, Graubard BI, et al. (1999) Alcohol and Prostate cancer in the NHANES I epidemiologic follow-up study. Ann Epidemiol **9**:254-261.

12. Hsiech CC, Thanos A, Mitropoulos D, Deliveliotis C, Mantzoros CS, Tricgopoulos D (1999). Risk factors for prostate cancer: a case-control study in Greece. Int J cancer **80**: 699-703.

13. Villeneuve PJ, Johnson KC, Kreiger N, Mao Y, et al. The Canadian Cancer Registries Epidemiology Research Group (1999). Risk factors for prostate cancer: results from the Canadian National Enhanced Cancer Surveillance System. Cancer Causes & Control **10**: 355-367.

14. Ellison LF (2000) Tea and other beverage consumption and prostate cancer risk: a Canadian retrospective cohort study. Eur J Cancer Prev **9**: 125-130.

15. Andersson BO, Baron J, Bergstrom R, Lindgren C, Wolk A, Adami HO (1996). Lifestyle factors and prostate cancer risk: a case-control study in Sweden. Cancer Epidemiol Biomarkers Prev **5**: 509-513.

16. Elizabeth A. Platz, Micheal f. Leitzmann et al. (2004) Alcohol Intake, Drinking Pattern, and Risk of Prostate Cancer in a Large Prospective Cohort Study Am J Epidemiol **159**:444-453

17 A. M. Hodge, Dallas R. English et al. (2004) Foods, nutrients and prostate cancer, Cancer Causes and Control **15 (1)**:11-20.

18. Breslow Rosalind et al. (1998) Review of Epidemiologic Studies of Alcohol and Prostate Cancer: 1971-1996. Nutrition and Cancer, **30 (1)**, 1-13.

19. Goldbohom RA, van den Brandt PA, van't Veer P, et al. (1994) Prospective study on alcohol consumption and the risk of cancer of the colon and rectum in the Netherlands. Cancer Causes & Control **5**:95-104.

20. Hirayama T. (1992) Life-style and cancer: from epidemiologic evidence to public behavior change to mortality reduction of target cancers J Natl Cancer Inst Monogr **12**:65-74.

21. Muti P, Westerlind K, Wu T, et al. (2002) Urinary estrogen metabolites and prostate cancer: a case-control study in the United States Cancer Causes & Controls **13**: 947-955.

22. Breslow N, Chan Cw, Dhorn G (1997). Latent carcinoma of the prostate at autopsy in seven areas. Int. J. Cancer **20**: 680-688.

23. Bostwick DG, Cooner WH, Denis L (1992). The association of benign prostatic hyperplasia and cancer of prostate. Cancer, **70**: 291-301.

24. Babaian RJ, Mettlin C, Kane R (1992). The relationship of prostate specific antigen to digital examination and transrectal ultrasonography. Findings of the American Cancer Society National Prostate Cancer Detection Project. Cancer **69**:1195-1200.

25. Hutchnison H ed: Sawtooth (1995) Ci3 system user manual. Version I.I. Evaston, IL: Sawtooth Software.

26. McCann SE, Marshall JR, Trevisan M, Russell M, Muti P, Markovic N, Chan AW, Freudenheim JL (1999) Recent alcohol intake as estimated by the Health Habits and history Questionnaire, the Harvard Semiquantitative Food Frequency Questionnaire, and more detailed alcohol intake questionnaire. Am J Epidemiol, **150**:334-40.

27. Russell M, Marshall JR, Trevisan M, Freudenheim JL, Chan AW, Markovic N, Vena JE, Priore RL (1997) Test-retest reliability of the cognitive lifetime drinking history . Am J Epidemiol, **146(11)**: 975-81.

28. Chick J. (1998) "Alcohol, health, and heart: implications for clinicians". Alcohol Alcohol 33**(6)**: 576-91.

29. Graham K. Schmidt G. (1998) 'The effects of drinking on health of older adults" Am J Drug Alcohol Abuse **24 (3)**:465-81.

30. Dennis LK (2000). Meta-analysis for combining relative risk of alcohol consumption and prostate cancer. Prostate **42**:56-66.28.

31. Poschl G, Stickel F, Wang XD, Seitz HK (2004 Feb) Alcohol and cancer: genetic and nutritional aspects. Proc Nutr Soc **63 (1)**: 65-7132.

32. Hayward SW. Cunhe GR, Dehiya R (1996). Normal development and carcinogenesis of prostate. A unifying hypothesis. Am NY Acad Sci **784**: 50-62.

Table 1. Characteristics of prostate cancer cases and controls, PROMEN Study

	Cases (n=88)	Controls (n=272)
	Mean (SD[a])	
Age, years	69.3 (8.4)	70.0 (6.3)
Education, years	12.3 (2.7)[b]	13.0 (2.8)
Body mass index, kg/m^2	29.2 (5.2)	28.6 (4.6)
Waist to hip ratio		
	Percent	
Race		
White	67.0[c]	93.4
Non white	33.0	6.6
Smoking status[d]		
Never	23.8	28.3
Former	61.4	61.8
Current	14.8	9.9
Drinking status[e]		
Non-current drinkers	36.4	23.5
Current drinkers	63.6	76.5

[a]standard deviation; [b] $p < 0.05$, t-tests for differences in means between cases and controls; [c] $p < 0.001$, χ^2 for differences in categorical variables between cases and controls; [d]smoking status at the time of diagnosis in cases or interview in controls; [e]drinking status in the 12-24 months prior to diagnosis or interview, non-current drinkers stopped drinking at least 12-24 months prior to interview

Table 2. Selected lifetime alcohol consumption characteristics among prostate cancer cases and controls, PROMEN Study

	All drinkers (n=360)		Former drinkers (n=96)		Current drinkers (n=264)	
	Cases (n=88)	Controls (n=272)	Cases (n=32)	Controls (n=64)	Cases (n=56)	Controls (n=208)
	Mean (SD)		Mean (SD)		Mean (SD)	
Total drinking years	38.2 [a] (16.5)	43.7 (14.9)	32.9 (18.5)	33.8 (17.2)	41.3 [a] (14.5)	46.8 (12.7)
Total abstaining years	11.4 [a] (15.0)	6.6 (12.5)	19.8 (16.4)	18.2 (15.3)	6.6 [a] (11.9)	3.0 (8.8)
Drinks per day	2.6 (7.3)	1.6 (3.4)	4.7 (11.6)	2.5 (5.8)	1.3 (1.7)	1.3 (2.2)
Drinks per drinking day	4.5 (7.3)	3.6 (4.3)	6.8 (11.3)	5.0 (6.3)	3.2 (2.5)	3.2 (3.4)
Total lifetime ethanol, ounces	12904.7 (18681.0)	11735.3 (12904.7)	19051.0 (26382.6)	13498.8 (21019.7)	9392.6 (11187.8)	11192.7 (16880.9)
Total lifetime ethanol from beer, ounces	6282.5 (11321.0)	6024.3 (9250.0)	7771.0 (15173.8)	5992.6 (12284.7)	5431.9 (8422.3)	6034.1 (8129.3)
Total lifetime ethanol from liquor, ounces	5654.2 (14571.6)	4067.2 (12815.8)	10307.0 (22051.6)	5233.7 (11480.5)	2995.5 (6480.2)	3708.3 (13204.5)
Total lifetime ethanol from wine/wine coolers, ounces	953.1 (2715.6)	1634.6 (4168.8)	958.9 (3588.4)	2271.0 (6154.0)	949.8 (2099.5)	1438.8 (3326.0)

[a] $p < 0.05$, t-tests for differences in means between cases and controls

TABLE 3. Odds ratios (OR)[a] and 95% confidence intervals (CI) for risk of prostate cancer associated with lifetime alcohol consumption

	Cases (n=88)	Controls (n=272)	Odds Ratios (95% CI)
Total lifetime ethanol, ounces			
≤ 2647.62	29	90	1.00
2647.62 - 11048.28	34	90	1.20 (0.65-2.23)
> 11048.28	25	92	0.83 (0.43-1.60)
Total lifetime ethanol from beer, ounces[b]			
≤ 1941.78	42	120	1.00
1941.78 - 6237.30	25	75	1.16 (0.62-2.16)
> 6237.30	21	77	0.89 (0.46-1.72)
Total lifetime ethanol from liquor, ounces[b]			
≤ 932.23	51	152	1.00
932.23 - 3976.79	15	59	0.71 (0.35-1.44)
> 3976.79	22	61	0.91 (0.47-1.76)
Total lifetime ethanol from wine and wine cooler, ounces[b]			
≤ 511.66	67	177	1.00
511.66 - 2283.00	10	47	0.76 (0.35-1.65)
> 2283.00	11	48	0.60 (0.27-1.30)

[a] Adjusted for race, age (years), smoke, education (years), BMI, WHRATIO; [b]further mutually adjusted for other beverages

TABLE 4. Odds ratios (OR) [a] and 95% confidence intervals (CI) for risk of prostate cancer associated with lifetime alcohol consumption: duration, drinking status, drinks per day, and drinks per drinking day.

	Cases (n=88)	Controls (n=272)	Odds Ratios (95% CI)
Total drinking years			
> 53	14	80	1.00
42 - 53	27	94	1.44 (0.66-3.14)
≤ 42	47	92	2.16[b] (0.98-4.78)
Ever abstained from drinking			
never abstained	39	173	1.00
ever abstained	49	99	1.79 [b] (1.05-3.03)
Drinking status [c]			
current drinkers	56	208	1.00
former drinkers	32	64	1.40 (0.77-2.53)
Drinks per day			
≤ 2	62	218	1.00
> 2	26	54	1.38 (0.76-2.51)
Drinks per drinking day			
≤ 2	24	106	1.00
> 2	64	166	1.57 (0.88-2.79)

[a] Adjusted for race age, smoke, education (years), BMI, WHRATIO; [b]p for trend < 0.05; [c]drinking status in the 12-24 months prior to diagnosis or interview. Former drinkers stopped drinking at least 12-24 months prior to interview.