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13. ABSTRACT (Maximum 200 Words)

There is experimental evidence showing that a sedentary life-style and high fat diet induce estrogen metabolism toward 16α hydroxylation leading to biologically potent metabolites (estriol and 16α hydroxyestrone). An active life-style and a low fat diet induce the alternative 2-hydroxylation with production of weak estrogen metabolites (2-hydroxyestrone). Potent estrogens may increase prostate cell division and increase prostate cancer risk. The research hypothesis is that the preferential induction of the 16α hydroxylation pathway in respect to the 2-hydroxylation, is associated with an increase risk of prostate cancer. We conduct a nested case–control study within the cohort of Western New York Health Study (WNYHS) to test this hypothesis. From 1994 to 2000, 2,158 men, randomly selected from the general population of Erie and Niagara Counties, participated in the WNYHS. At recruitment, information on lifestyle factors and anthropometric characteristics were collected together with a sample of blood and urine. After an average follow-up period of four years, we expect to observe 151 incident prostate cancer cases arising from the cohort. A total number of 604 control subjects (four for each prostate cancer case) will be identified and matched for age, race and recruitment period with the related prostate cancer cases.

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

Introduction

Although the prostate gland is commonly considered as a prototypic androgen-dependent tissue, there is convincing evidence that estrogens per se may induce mitosis of prostatic epithelial cells in many species (Leav et al.,1978; Schulze et al., 1987). In addition, the presence of estradiol binding sites has been detected in human prostatic tissue (Ekman et al., 1983; Donnelly et al., 1983). Previous work has indicated that estrogens may exert a direct effect on the proliferative activity of prostate epithelial cells (Orgebin-Crist et al., 1983; Castagnetta et al., 1995).

The relative importance of two major, mutually exclusive pathways for estradiol metabolism, 2-hydroxylation and 16α-hydroxylation has been postulated in the last twenty years (Dao, 1979). Estradiol is first reversibly converted to estrone by 17β oxidation; most of the estrone is then irreversibly oxidized to catechol estrogens, 2 hydroxyestrone or 16α hydroxyestrone, the initial metabolites of these two pathways. Experimental studies indicate that 16α-hydroxyestrone is a potent estrogen, genotoxic, and tumorigenic and that 2-hydroxyestrone is a weak estrogen and estrogen antagonist (Bradlow et al., 1985; Telang et al., 1992). Consequently, the ratio of 2-hydroxyestrone to 16α hydroxyestrone has been used as an indicator of the balance between the two pathways. 16α hydroxyestrone has been found to be elevated in women with breast and endometrial cancer (Fishman et al., 1984; Bradlow et al., 1986) and in women at high risk of breast cancer (Osborn et al., 1988). Environmental factors such as sedentary life-style and high fat diet have been associated with an increase in prostate cancer risk (Mettlin C., 1997) and in the same time they showed to induce estrogen metabolism toward 16α hydroxylation leading to biologically potent metabolites (estriol and 16α hydroxyestrone) (Longcope C., 1987). On the contrary, an active life-style and a low fat diet induce the alternative 2-hydroxylation with production of weak estrogen metabolites (2-hydroxyestrone).

Studies on migrants have indicated that environmental factors explain most of the large difference in prostate cancer mortality across countries and consequently play a major role in the etiology of prostate cancer. Preliminary results from a case-control study, conducted by our group on 96 prostate cancer cases and 304 control subjects, support

the study hypothesis. Prostate cancer cases observed a lower 2 hydroxyestrone to 16α-hydroxyestrone ratio than control subjects with an Odds Ratio of 0.60 (95%Confidence Interval:0.33-1.11, p for linear trend: 0.05).

This represents a new direction in the examination of the hormonal mechanisms responsible for the development of prostate cancer. It is expected that improved understanding of hormonal metabolism will improve our understanding of the etiology of prostate cancer, and allow for further refinement of preventive strategies for this disease.

Body

During the first five months of the budget year, we trained personnel, developed study protocols and implemented the study within the context of the cohort re-call conducted by the NIH funded "Epidemiology of Type 2 Diabetes". The funded study and the "Epidemiology of Type 2 Diabetes" are two integrated studies based on the active follow-up of the Western New York Cohort Study (the WNYHCS). The funded study focuses on the identification of prostate cancer cases to test the hypothesis that estrogen metabolism is related to prostate cancer risk. The "Epidemiology of Type 2 Diabetes" to evaluate molecular determinant of type 2 diabetes (RO1 DK 60587, Dr. R. Donahue, PI, Dr. P. Muti, Co-PI). The Western New York Cohort Study was originally developed as a series of population-based case-control studies on chronic diseases (cardiovascular diseases and cancers of the lung, breast and prostate), lifestyle factors and pattern of alcohol use at the Department of Social and Preventive Medicine, University at Buffalo, New York. These projects were funded by the NIAAA (AA098, Dr. Marcia Russel, PI) and by the Department of Defense (DAMD 170010417, Dr. Jo Freudenheim, PI; DAMD 1179818559, Dr. Muti, PI) in the mid 1990's. Control subjects recruited for those studies contributed to the development of the prospective cohort component of the Western New York Health Study and became the Western New York Health Cohort Study (WNYHCS). Since 1994, 4,321 population-based control subjects participated in the cohort, 2,158 of those were men (mean age 63.31, standard deviation 11.98). The on-going nested case-control study draws upon the participants in the Western New York Health Cohort Study. These healthy men were initially recruited and examined from 1994-2001 and provided an extensive array of information on lifestyle characteristics as well as samples of blood and urine.

The preliminary phase of the study took a longer period of time than the expected three months due to the complexity of the cohort recall, the recruitment and training of the field personnel and the logistic to implement two integrated studies. Between the two studies, we hired a Project Coordinator, a part time data manager, a part time administrative assistant, and three part time people to interview and recruit. Training has been provided for all interview staff and has been coordinated to allow a perfect integration between the two supporting studies. We developed a new questionnaire to update data on exposure and we reviewed interview techniques for standardization and the related training procedures.

In accordance with the Statement of Work, we prepared personnel and programs for the automatic data entry of the interview, and maintenance of files from the computer-assisted interview to coincide with procedures used at the time of the initial recruitment of the subjects in the study. Several databases have been created. The central

database for the prostate cancer study is written in Microsoft Access and contains information on all participants as well as prospected non-participants. Each form that a study participant fills out has a computer equivalent. Each of these databases is housed in the same location for security, backup, and data analysis purposes. These programs were written in Microsoft Access as well. Data validation, analysis, and compatibility (for integration with SPSS) have been written in Visual Basic. Training has also been provided for the identification and staging of prostate cancer cases in area hospitals as well as the private physician offices.

In the remaining seven months of the past year activity, we have seen 452 participants with the identification of 24 incident prostate cancer cases.
A total PSA determination has been done on all recalled participants to rule out any latent prostate cancer. Among all tested men, we have been able to identify 1 prostate cancer case, while 29 were the men showing abnormal PSA results. The information of prostate cancer diagnosis was provided to us by the primary physicians of the participants. The PSA results were sent to the participant's primary physician along with a letter to draw attention to the fact that it was outside of the normal range. The primary physician then referred the participant to an urologist where a biopsy was done to confirm prostate cancer.

At the present, we are also contacting the New York State Cancer Registry for an initial validation of our follow-up. We request to link our file of the WNYHC Study male participants with their file. This procedure will be completed in the next two months.

At the same time, we have developed a specific form for a standard staging of prostate cancer across all hospitals and private practices to verify and to classify the prostate cancer cases we identified until now.

Finally, we have finalized procedures for the ongoing maintenance of the biological specimen bank, tracking of samples, retrieval of the samples for the bioassays and re-mapping of the freezers.

Key Research Accomplishments

a) Development of protocols and instruments to implement the Study
b) Re-call of study 452 participants
c) Determination of PSA levels in all the re-called study participants
d) Identification of 24 incident prostate cancer cases among men who were clinically healthy at their first recruitment as controls in the study
e) Initial validation of the study follow-up through the New York State Cancer registry
f) Development of a new scheme for the collection of prostate cancer stage at diagnosis for all the incident cancer cases observed in the cohort

Reportable Outcomes

We do not have direct outcomes to report at this time, but we have presented data on prostate and breast cancer at several conferences.

Publications and Presentations

In May 2004, there are no results or publications coming directly from this grant because we have just begun data collection. However, Dr. Muti has published or has in press research on hormone related cancer. In 2004, she presented results regarding hormone activity and cancer risk at the Annual Meeting of the American Association for Cancer Research (1, 2, 3). In particular, the second and third studies were conducted on datasets derived from a previously DOD funded study on prostate cancer. The second study analyzed the relation between chronic alcohol intake and risk of prostate cancer, while the third has found, for the first time in a population-based research, that basal growth hormone is related to prostate cancer. All these studies have been submitted for publication (please see appendix for the manuscript).

Furthermore, Dr. Muti has in publication a manuscript on hormones and breast cancer (4-New York Academy of Science, *in press*)

Conclusions

We have just begun data collection for this grant; therefore, there are no conclusions to report at this time. Recall of participants is underway.

References

1) **Muti P**, Pasanisi P, Bellati C, Venturelli E, Krogh V, Mastroianni A, Berselli G, Berrino F *Serum testosterone levels and breast cancer recurrence* Annual Meeting Am Ass Cancer Research Orlando, March 27-31, 2004

2) ***Barba M,** Mc Cann S, Stranges S, Schünemann H, De Placido S, Carruba G, Freudenheim JL, Trevisan M, Muti P. *Lifetime total and beverage specific alcohol intake and prostate cancer risk,* Annual Meeting Am Ass Cancer Research Orlando, March 27-31, 2004

3) ***Fuhrman B**, Hurd TC, Schünemann HJ, Quattrin T, Barba M, Muti P *Growth hormone and prostate cancer risk: a case-control study*, Annual Meeting Am Ass Cancer Research Orlando, March 27-31, 2004

4) **Muti P.** *The Role of Endogenous Hormones in the Etiology and Prevention of Breast Cancer: the Epidemiological Evidence.* New York Academy of Science *(in press)*

*Presentations by students and postdoctoral fellows under the supervision of Dr. Muti

Appendices

1. Basal Growth Hormone Concentrations in Blood and the Risk for Prostate Cancer: a Case-Control Study
2. Lifetime Total and Beverage Specific Alcohol Intake and Prostate Cancer Risk: A Case-Control Study

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

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Abbreviated title: Growth Hormone and Prostate Cancer

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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 88 men, aged 45-85 years, diagnosed with incident, primary, histologically confirmed, and clinically apparent (stage B and higher) prostate cancer. Controls included 251 men, matched on age and residential area. Age, race, BMI, waist-to-hip ratio, family history of prostate cancer, 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: 0.42 95% CI: 0.19-0.95, p for trend 0.02) and adjusted models (OR: 0.40 95% CI: 0.16-1.01, p for trend 0.03), in the lowest compared to the highest quintile, respectively.

Conclusions: Lower basal levels of growth hormone in serum suggest an increased prostate cancer risk. The inverse association may be explained by the negative feedback loop generated by IGF-1 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, two 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). Insulin-like Growth Factor-I (IGF-1) is an important hormone in the pathway, conveying centrally regulated signals to the tissue level; it is also an autocrine / paracrine growth factor that can be synthesized at the target tissue. IGF-1 is a mitogen that stimulates cell proliferation and inhibits apoptosis. In addition, IGF-I and the other members of the IGF family are known to interact with other factors important to cancer development and progression, including sex steroid hormones, other growth factors and proteins involved in tumor suppression (2).

Growth Hormone (GH) is the primary regulator of hepatic IGF-1 synthesis and plays an important role in the 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 (3). Pituitary GH secretion is pulsatile, with circulating peaks detectable in nocturnal hours, approximately 2 hours apart (4). The mean concentration of GH in serum beyond the 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. Recent epidemiologic studies suggest an association between elevated blood levels of IGF-I and increased risk of prostate cancer, although data are inconsistent across the studies (5-13). Of three retrospective case-control studies, all showed statistically significant associations between case-control status and circulating IGF-1 levels. These studies reported higher mean serum IGF-1 levels in cases versus controls, and significantly elevated risk for disease in men having higher IGF-1 levels (8, 9, 13). In contrast, prospective studies have had mixed results (5, 7, 11, 12).

A causal link has not been conclusively established between IGFs and prostate cancer and it is still not clear whether elevated serum IGFs levels observed in prostate cancer patients are in the causal pathway or are simply a reflection of the presence of the tumor.

The biological function of IGF-1 could be consistent with either of the following hypotheses. It is possible that observed differences between men with and without prostate cancer could reflect a tissue-level phenomenon, with tumor tissue producing IGF-I to fuel its growth in an autocrine fashion. It is also possible that system-level variations in somatotropic hormone levels may play a causal role in prostate cancer etiopathology, being therefore important factors in determining prostate cancer risk at the population level.

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). The methods for this study have been previously described in detail (14). 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.

Prostate cancer patients were between 45 and 85 years of age. Interview and blood collection were done before any cancer treatment for all prostate cancer cases.

Men with a previous history of cancer (except non-melanoma skin cancer), or already on hormonal or chemotherapy treatment (current or in the six months prior to diagnosis), as well as those affected by chronic or acute liver diseases, were excluded. Cases were also requested to have a driver's license if they were between 35 and 65 years of age, because we used driver's license records to identify controls aged 35-65 years as described below.

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 Catalina (15)].

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 thus the present analysis is conducted on 88 subjects.

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 history of cancer other than non-melanoma skin cancer. Since latent prostate carcinoma has a high prevalence in men over 50 (16), we evaluated prostate specific antigen (PSA) in all the blood samples obtained from controls. Controls found to have a PSA level 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 (17) until the completion of further diagnostic procedures to clarify their true case-control status. We identified eight prostate cancer cases because of PSA determination in subjects who initially were recruited as controls.

During the study period, 1,373 potential controls were contacted. One hundred and seventy nine of these potential candidates were deceased or too ill to participate, 293 were not eligible, and we were not able to contact 273 individuals (wrong address, and wrong telephone number). Three hundred and seventeen of the remaining 513 subjects

(60%) were enrolled and interviewed. Blood samples were not available for 66 of these men, thus the present analysis includes 251 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, waist to hip ratio were measured by trained personnel using standardized protocol. Body mass index (BMI), waist to hip ratio was calculated.

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., Chapel Hill, 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, first including all potential confounders namely all those that showed either a statistically significant association (history of enlarged prostate and current smoking status), or that suggested a trend and had a p value of less than 0.15 (age and waist-to-hip ratio) with both case-control status and growth hormone level.

Results

Table 1 describes the characteristics of the participants in the study. Compared to controls, cases were significantly more likely to have a history of enlarged prostate ($p<0.05$), to be African Americans ($p<0.01$), less educated ($p<0.05$), and current smokers ($p<0.05$). Non-significant associations were observed for age, BMI, and waist to hip ratio.

Since several anthropometric and lifestyle factors may play a role in prostate cancer etiopathogenesis, in order to identify potential covariates excluding the effects of disease status, we evaluated them only in control subjects (table 2). Older participants had significantly higher levels of GH than younger aged groups ($p<0.01$). Current smoking was significantly associated with participants in the highest tertile of BMI showed significantly lower serum GH levels ($p <0.05$). Current smoking was significantly associated with lower levels of GH ($p<0.01$). Non-significant associations were observed as for remaining participants characteristics among controls.

The risk effect of basal GH on prostate cancer is described in table 3 by quintiles of GH distribution in control subjects. Twenty-seven participants (17 cases and 10 control subjects) were excluded from this analysis because of missing data (e.g. BMI, WHRATIO). However, there was no difference in GH basal levels between participants with the missing variables and participants with all the conventional covariates. In both the univariate and multivariate models, odds ratios decline with increasing GH quintiles (OR: 0.42 95% CI: 0.19-0.95 and OR: 0.40 95% CI: 0.16-1.01) with a significant trend was found for crude and adjusted odds ratios by GH quintile.

We also performed sub group analyses by age and race. Risk estimates for men aged 65 and older (cases = 69, controls = 218)) showed decreased prostate cancer risk across increasing GH levels tertiles, in both unadjusted and adjusted models (OR 0.45, 95% CI 0.21-0.98, p for trend = 0.041).

Among participants younger than 65 years of age (cases = 19, 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 different results in the two ethnic groups. Among Caucasians (cases = 60, controls = 233), we found an inverse association between basal GH level and prostate cancer risk in both crude and adjusted models (OR: 0.62 95% CI 0.34-1.10, p = 0.10, OR: 0.47 95% CI 0.24-0.92, p = 0.029, respectively). Among African Americans (cases = 28, controls = 18) there was evidence of the same association only in the crude point estimates (OR 0.52 95% CI 0.16-1.71, p = 0.28, OR: 1.04, 95% CI 0.20-5.38, p = 0.96, respectively).

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 case-control design of the study bears the risk of bias. 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.(4).

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 (18). Normative data are sparse for men in our study population age range (60-80 years); however our finding is in agreement with results from an Italian cross-sectional study, whose participants' age was in same range (19).

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 ($\geq$65 years of age), but not among younger men ($<$ 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 (5, 20-22). 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 (10) 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 an 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. (23)

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 (24). Many aspects of the relationship between prostate cancer and IGFs remain still unclarified, most of them concerning GH secretion. 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. On the other hand, GH pituitary secretion results from both a phasic and basal production and it are still unknown 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, it remains still unclear to what the stromal components are responsible for the final effect, since we know that in healthy people they modulate prostatic hormones secretion (4).

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 (19, 25), idiopathic stature disorders (26), and cardiac insufficiency (27). 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.

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References

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

2. Yu H and Berkel H (1999) Insulin-like growth factors and cancer. *Journal of the Louisiana State Medical Society* **151**: 218-23.

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

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

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

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

16. 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.

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

18. 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**.

19. 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

20. 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.

21. 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.

22. 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

23. Webb SM, Casanueva F, Wass JAH (2002) Oncological Complications of Excess GH in Acromegaly. *Pituitary* **5(1)**:21-25.

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

25. 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.

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

27. 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: Participants Case-Control Status by Tertiles of Distribution in Control Subjects

	Cases	Controls
	Subject Number (Percentiles)	
Age		
45-64 years	19 (21.6)	33 (13.1)
65-74 years	45 (51.1)	152 (60.6)
75-85 years	24 (27.3)	66 (26.3)
Waist-to-Hip Ratio (tertiles)		
First (0.75-0.92)	25 (29.8)	92 (33.3)
Second (0.93-0.98)	22 (26.2)	81 (32.4)
Third (0.99-1.30)	37 (44.0)	77 (30.8)
Body Mass Index (tertiles)		
First (18.17-26.35)	19 (25.3)	81 (32.9)
Second (26.36-29.98)	22 (29.3)	83 (33.7)
Third (29.99-49.56)	34 (45.3)	82 (33.3)
History of Enlarged Prostate *		
Yes	47 (54.0)	106 (42.2)
No	40 (46.0)	145 (57.8)

Family History of Prostate Cancer		
Yes	12 (13.6)	22 (8.8)
No	76 (86.4)	229 (91.2)
African American **		
Yes	28 (31.8)	18 (7.2)
No	60 (68.2)	233 (92.8)
Education*		
Did not complete high school	22 (27.8)	37 (15.1)
Completed high school	22 (27.8)	86 (35.1)
Some college or more advanced study	35 (44.3)	122 (49.8)
Current Smoking Status*		
Yes	13 (14.8)	18 (7.2)
No	75 (85.2)	233 (92.8)

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

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	152	1.36 (1.66)
75-85 years	66	1.46 (1.87)
Waist-to-Hip Ratio (tertiles)		
First (0.75-0.92)	92	1.56 (1.92)
Second (0.93-0.98)	81	1.00 (1.31)
Third (0.99-1.30)	77	1.14 (1.60)
Body Mass Index (tertiles)*		
First (18.17-26.35)	81	1.58 (1.83)
Second (26.36-29.98)	83	1.26 (1.84)
Third (29.99-49.56)	82	0.94 (1.21)
History of Enlarged Prostate		
Yes	106	1.45 (1.79)
No	145	1.10 (1.54)

Family History of Prostate Cancer		
Yes	22	1.30 (1.54)
No	229	1.24 (1.67)
African American		
Yes	18	1.38 (1.58)
No	233	1.24 (1.66)
Education		
Did not complete high school	37	1.31 (1.57)
High school graduate	86	1.10 (1.40)
Some college or more advanced study	122	1.33 (1.82)
Current Smoking Status**		
Yes	18	0.57 (0.61)
No	233	1.30 (1.70)

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

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)	25	48	1.00	Reference
Second quintile (0.10-0.33)	22	53	0.80	0.40-1.59
Third quintile (0.34-0.83)	15	50	0.58	0.27-1.22
Fourth quintile (0.84-2.10)	15	50	0.58	0.27-1.22
Fifth quintile (2.15-19.95)	11	50	0.42	0.19-0.95
Totals:	88	251		
ptrend:	0.02			

Adjusted Estimates [b]				
First quintile (0.05-0.09)	20	46	1.00	Reference
Second quintile (0.10-0.33)	19	51	0.79	0.34-1.82
Third quintile (0.34-0.83)	12	47	0.51	0.20-1.28
Fourth quintile (0.84-2.10)	13	48	0.54	0.22-1.34
Fifth quintile (2.15-19.95)	7	49	0.34	0.11-0.99
Totals:	71	241[c]		
ptrend:	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, BMI, WHR, current smoking, and education.

[c] point 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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ABSTRACT

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

Methods. Eighty-eight men, aged 45 to 85 years with incident, histologically-confirmed prostate cancer and 272 controls frequency-matched to cases on age and county of residence were included. We conducted extensive in-person interviews regarding lifetime alcohol consumption and other epidemiologic data. We estimated odds ratios (OR) and 95% confidence intervals (CI) for risk of prostate cancer with unconditional logistic regression adjusting for age, race, smoking status, BMI, waist to hip ratio and alcohol intake.

Results. Risk of prostate cancer was not associated with lifetime ounces of total ethanol or ethanol from beer, wine, or liquor. Prostate cancer risk was inversely associated with the total number of drinking years and directly associated with the total number of abstaining years; compared to men in the highest tertile of total years drinking, men in the lowest tertile had a twofold risk of prostate cancer (OR 2.16, 95% CI 0.98-4.78, p for trend <0.05). Similarly, men who reported ever abstaining from alcohol consumption compared to those never abstaining had an increased prostate cancer risk (OR 1.77, 95% CI 1.05-2.98, p for trend < 0.05). No association with prostate cancer risk was observed with number of drinks per usual day (average drinks per day over the lifetime) or with number of drinks per usual drinking day (average drinks per day on drinking days only over the lifetime).

Conclusions. Our results suggest that in evaluating the relationship between lifetime alcohol intake and risk of prostate cancer, the manner in which alcohol intake is distributed across the

lifetime may play a more important role in prostate cancer etiology than total lifetime consumption.

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 a relevant impact on public health. Although this issue has been addressed in a number of studies, in a review by Breslow and Weed, only 6 of 32 studies reported a positive association between alcohol use and prostate cancer (3); however, they noted that many of the studies had biases that could have attenuated the risk estimates.

Among the population-based case-control studies, those carried out by Heyes et al. (4) and Sharpe et al. (5) found an increased risk of prostate cancer associated with alcohol consumption. Risk increased with increasing frequency of alcohol consumption (4) and among those who drank regularly over a longer period (5). Sesso et al., in their prospective cohort study, confirmed the finding of a higher risk associated with alcohol consumption (6). However, numerous studies published since 1998 have not found an association between alcohol intake and prostate cancer (7-19).

Although prostate cancer is known to have a long latency period, lifetime alcohol consumption is an issue not addressed in the studies carried out until the late 1990s, and rarely in the most recent studies (3). Furthermore, the authors 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 Friday) 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 (20-21). In the present case-control study we examined the association between lifetime alcohol intake, duration of alcohol use, and drinks per usual day and usual drinking day and risk of prostate cancer in western New York.

Material and methods

Data were collected as a part of a case-control study of prostate cancer and hormones (the PROMEN STUDY) conducted in Erie and Niagara Counties, NY, USA, between December 1998 and April 2001. The methods for this study have been previously described in detail (22). 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 were also

requested to have a driver's license, since we used driver's license records to identify controls aged 35-65 years.

During the study period, 504 men were identified with incident prostate cancer. Of these, 336 men did not meet the eligibility criteria; the remaining 163 patients were approved by the urologists and invited to join the PROMEN study. After being contacted, 50 men refused to participate resulting in a participation rate of 70%. Ninety-six 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 (23-24), 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 (25) until the completion of further diagnostic procedures to clarify their true case-control status. We identified eight prostate cancer cases as a result of PSA determination in subjects who initially were recruited as controls.

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. 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 (28) 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 (29-30). 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 whether any difference occurred in the way they had been drinking over the years. Using this information, we defined intervals during each participant's life when drinking patterns were homogeneous, to compute the total number of the drinking years based on the sum of the years of drinking and, eventually, the total number of years of abstinence. For each interval, participants were asked about the quantity and the frequency of different alcoholic beverages (beer, wine, wine coolers, and liquor). Participants defined their usual drink size for each beverage consumed relative to sample bottles of various sizes and sample glasses with drink sizes marked on the side to improve the accuracy of their estimates. Participants consuming more

than one type of beverage were asked what proportions of all beverages consumed were accounted for by each reported beverage. Additionally, we asked questions for occasional (less than weekly) versus regular consumers of alcohol (at least weekly), and for occasions where alcohol was consumed in greater than usual amounts. Weekly consumers of alcohol were asked detailed questions concerning frequency of use and number of drinks per drinking occasion for each beverage type for Friday, Saturday, Sunday and weekdays for all reported beverages. Additionally, detailed questions concerning frequency of use and number of drinks per drinking occasion were asked for periods during which alcohol was consumed in "more than usual" amounts.

Lifetime alcohol consumption in ounces was calculated as the sum across all reported drinking intervals of the product of the reported beverage-specific drink size in ounces times the number of drinks per drinking occasion times frequency of consumption multiplied by the alcohol factors appropriate to each beverage. The factors used were 0.048, 0.12, 0.04 and 0.40, for beer, wine, wine cooler and hard liquor, respectively.

We considered several variables in these analyses: total number of years alcohol was consumed, number of drinks per usual day during the drinking years (total number of drinks/total number of days in drinking years), number of drinks per usual 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 1and a half ounces of liquor.

Statistical analysis

Statistical analyses were conducted using SPSS for Windows version 11.0. Differences between cases and controls in the demographic and alcohol variables were assessed using t-tests for continuous variables and χ^2 for categorical variables. Lifetime abstainers, defined as those subjects who never had at least 12 drinks in any one year over their lifetime, were excluded from our analyses. The biological and social differences between lifetime abstainers and both former and current drinkers (26, 27) 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 classified as those respondents not consuming that particular alcoholic beverage. For risk associated with drinks per usual day and drinks per usual drinking day, we categorized consumption as two or less drinks per day and greater than two drinks per day. Odds ratios (OR) and 95% confidence intervals (CI) for risk of prostate cancer associated with the alcohol variables 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

The characteristics of the participants in the PROMEN study are shown in Table 1. Controls compared to cases were slightly more educated (13.0 vs 12.3 years) and significantly 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 and 41.3 vs. 46.8 years, 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 these consumption variables between cases and controls who were former drinkers. Among former drinkers, cases consumed more total ethanol, beer and liquor, more drinks per usual 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 usual days, 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 usual day, or drinks per usual 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 average volume per a specified time period across the lifetime. Few investigations have emphasized a characterization of drinking pattern. While there are methodological difficulties inherent in measuring 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 associated with 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. Because the original 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 PSA circulating levels helped to ensure that the control group

was free from prostate cancer, reducing misclassification as controls those men who were affected by latent prostate cancer. The data used in the present analysis were collected as a part of an in-person interview and the questionnaire about the lifetime alcohol consumption was very detailed allowing us to compute both the quantitative and frequency aspects of alcohol consumption. Even though our power to detect differences was limited, our findings nevertheless suggest the importance of considering different aspects of lifetime alcohol consumption in evaluating prostate cancer risk.

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 (34) 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 into account in previous studies (34).

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 (37-38). 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 (39) as the development of prostate gland begins prenatally, continuing until the end of puberty (40). 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 (41). Further studies focusing on lifetime exposure and more specifically on patterns of consumption may help in prevention of a disease with such a considerable public health impact.

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REFERENCES

1. Kay T (1995) Epidemiologic characteristics of prostate cancer. Cancer 75 (Supp.7): 1766-1777
2. Bosland MC. The role of steroid hormones in prostate carcinogenesis. Journal of the National Cancer Institute. 27:39-66, 2000.
3. Breslow Rosalind et al. Review of Epidemiologic Studies of Alcohol and Prostate Cancer: 1971-1996. Nutrition and Cancer, 30 (1), 1-13, 1998.
4. 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.
5. Colin R. Sharpe & Jack Siemiatycki. Case-control study of alcohol consumption and prostate cancer risk in Montreal, Canada. Cancer Causes and Controls 12: 589-598, 2001.
6. Howard D Sesso, Ralph S Paffenbarger J and I-Min Lee. Alcohol consumption and risk of prostate cancer: The Harvard Alumni Health Study. International Epidemiological Association 2001; 30:749-755.
7. De Stefani E, Fierro L, Barrios E, Ronco A (1995) Tobacco, alcohol, diet and risk of prostate cancer. Tumori 81:315-320.
8. 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.
9. 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.
10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

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

16. 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.

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

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

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

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

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

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

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

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

25. 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.

26. Chick J. "Alcohol, health, and heart: implications for clinicians". Alcohol Alcohol. 1998 Nov-Dec; 33(6): 576-91.

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

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

29. McCann SE, Marshall JR, Trevisan M, Russell M, Muti P, Markovic N, Chan AW, Freudenheim JL: 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 1999, 150:334-40.

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

31. Lopez-Velez M, Martinez-Martinez F, Del Valle-Ribes C. The study of phenolic compounds as natural antioxidants in wine. Critical Reviews in Food Science & Nutrition 43 (3): 233-44, 2003.

32. Lairon D, Amiot MJ. Flavonoids in food and natural antioxidants in wine. Current Opinion in Lipidology. 10 (1): 23-8, 1999 Feb.

33. Schuurman AG, Goldbohm RA, van den Brandt PA (1999). A prospective cohort study on consumption of alcoholic beverages in relation to prostate cancer incidence (The Netherlands). Cancer causes & Controls 10: 597-605.

34. Dennis LK (2000). Meta-analysis for combining relative risk of alcohol consumption and prostate cancer. Prostate 42:56-66.

35. 31) Mc Cann SE, Sempos C, Freudenheim JL, Muti P, Russell M, Nochajski T, Ram M, Hovey K, Trevisan M. Alcoholic beverage preference and characteristics of drinkers and non drinkers in western New York (United States). Nutrition, Metabolism, and Cardiovascular Disease 2003; 13 (1): 2-11. Alcohol Clin Exp Res, vol 27, n I, 2003: pp 88-92

36. Schlesselman, JJ: "Proof" of cause and effect in epidemiologic studies: criteria for judgment. Prev. Med 16, 195-210, 1987.

37. Jensen, OM, Paine, SL, McMicheal, AJ, and Ewetz, M: Alcohol. In Cancer Epidemiology and Prevention, 2nd ed, D Schottenfeld and JF Fraumeni (eds). New York: Oxford University Press, 1996, pp 290-318.

38. World Health Organization International agency for Research on Cancer. Evaluation of Carcinogenic risks to Humans: Alcohol Drinking. Lyon, France: Int Agency Res Cancer, 1988. (IARC Monogr 44).

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

40. Weed, DL, and Gorelic, LS: The practice of causal inference in cancer epidemiology. Cancer Epidemiol Biomarkers Prev 5, 303-311, 1996.

41. Donato F, Tagger A, Gelatti U. et al. The effect of lifetime intake and hepatitis virus in men and women. American Journal of Epidemiology. 155(4): 323-31, 2002 Feb. 15.

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 usual 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 - 1048.28	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 usual day			
≤ 2	62	218	1.00
> 2	26	54	1.38 (0.76-2.51)
Drinks per usual 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.