Hormonal profile of diabetic men and the potential link to prostate cancer

Jocelyn S. Kasper · Yan Liu · Michael N. Pollak · Nader Rifai · Edward Giovannucci

Abstract
Objective Previous studies suggest men with diabetes may be at reduced risk for prostate cancer as compared to men without diabetes. To investigate potential biological mechanisms, hormonal profiles of diabetic men and non-diabetic controls were compared.
Methods In the Health Professionals Follow-Up Study, plasma levels of C-peptide, testosterone, sex-hormone binding globulin, insulin-like growth factor-1, and insulin-like growth factor binding protein-3 were determined in 171 diabetic men and 3,001 non-diabetic controls. Multiple linear regression analysis was conducted and least square means were calculated for hormones of interest.
Results Plasma levels of several hormones either $\leq$1, 1.1–6, 6.1–14.9, or $\geq$15 years after diagnosis with diabetes were examined. As time since diabetes diagnosis increased, plasma levels of C-peptide and IGFBP-3 significantly decreased (p for trend: C-peptide =.05, IGFBP-3 =.03). While testosterone and SHBG levels both significantly increased with increasing time since diabetes diagnosis (p for trend: testosterone =.02, SHBG =.002), the ratio of testosterone to SHBG decreased, suggesting a reduction in bioavailable testosterone. Plasma IGF-1 levels were lower in diabetics than non-diabetics, but no significant time trend was noted.
Conclusion This study of hormonal profiles of diabetic versus non-diabetic men identified changes in diabetic men that may be consistent with reduced prostate cancer risk.

Keywords Diabetes · Prostate cancer · Testosterone · C-peptide · SHBG · IGF-1 · IGFBP-3

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Introduction

Diabetes mellitus (DM) has been associated with increased risk of numerous cancers including cancers of the pancreas, liver, biliary tract, endometrium, kidney, colon, esophagus, and bladder [1–5]. Studies investigating the association between diabetes and prostate cancer, however, have shown that diabetes may be associated with lower risk for this cancer [6, 7]. Hormone-related mechanisms including sex steroid hormones, insulin, and insulin-like growth factors have been proposed to explain this reduced prostate cancer risk. Some researchers propose that testosterone (T) and sex hormone binding globulin (SHBG), C-peptide—a marker of circulating insulin, and insulin-like growth factor-1 (IGF-1)

and one of its binding proteins (IGFBP-3) are associated with both diabetes and prostate cancer and could explain the reduction in prostate cancer risk observed in diabetic patients [6, 7]. In order to study these associations, we measured circulating levels of T, SHBG, C-peptide, IGF-1, and IGFBP-3 in both diabetic and non-diabetic participants from Health Professionals Follow-Up Study (HPES). We previously observed an inverse relationship between diabetes and prostate cancer risk in this cohort [8].

Materials and methods

Study population

The study population was drawn from the Health Professionals Follow-Up Study, an ongoing prospective investigation of the causes of cancer and other chronic diseases. The HPFS is composed of 51,529 U.S. male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians born between 1 January 1910 and 31 December 1946. These men responded to a mailed questionnaire in 1986. The questionnaire included information about diet, physical activity, age, marital status, body measures, ancestry, medications, medical history, and smoking history. Every other year thereafter, they provided updated information on exposures and diseases, and every four years they provided an updated diet questionnaire. At baseline, we excluded men with diagnosed cancer (except for nonmelanoma skin cancer), men with more than 70 food items missing, and men whose energy intake was outside the range of 800–4,200 kcal/day (a total of 3% of the entire cohort).

Between 1993 and 1995, 18,018 of the men in the cohort provided blood specimens, which were collected in tubes containing sodium EDTA and shipped by overnight courier while chilled. Upon receipt by our laboratory, the blood was centrifuged, aliquotted into plasma, erythrocytes, and buffy coat, and stored in liquid nitrogen freezers. The processing was usually complete within several hours of receipt of the specimens so that the vast majority of samples were frozen within 29 h of venipuncture. Cryotubes are stored in the vapor phase of liquid nitrogen freezers; the highest temperature is −130°C and is as cold as −196°C near the nitrogen liquid. We have conducted studies that confirm degradation of the proposed analytes is minimal during the processing, storing and shipping. From these stored frozen plasma samples, we examined hormonal levels in 3,172 men based on exposure of diabetes mellitus history. The men were free of diagnosed cancer when they provided the blood sample, and they did not vary appreciably from other men in the cohort on diet and lifestyle factors. Bloods were all drawn between 1993 and 1995 and were processed for studies of biomarkers in the same laboratories at one of seven different times until the year 2000. T and SHBG were processed for five separate sub-studies investigating prostate cancer in 1996, 1998, or 2000, benign prostatic hyperplasia, and studying biomarkers by race. IGF-1 and IGFBP-3 were processed for six separate sub-studies (same studies as T and SHBG but an additional one for colon cancer outcomes). C-peptide was processed in five sub-studies investigating prostate cancer in 1996, 1998, or 2000, colon cancer, and alcohol-effects on biomarkers.

Assessment of diabetes mellitus

On the baseline 1986 questionnaire and on the subsequent biennial questionnaires, we asked the men if and when they had ever been diagnosed with diabetes mellitus (either insulin-dependent or non-insulin-dependent). Beginning with new cases diagnosed after return of the 1986 questionnaire, we confirmed the diagnosis through an additional questionnaire sent to those who reported new cases. We classified a person as having diabetes if he met one of the following criteria according to those of the National Diabetes Data Group: (1) report of one or more classic symptoms (thirst, polyuria, weight loss, hunger, or pruritus), plus fasting plasma glucose at least 140 mg/dl or random plasma glucose at least 200 mg/dl; or (2) at least two elevated plasma glucose concentrations on different occasions (fasting at least 140 mg/dl or random at least 200 mg/dl or concentrations at least 200 mg/dl after two hours on oral glucose tolerance testing); or (3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). Among men who reported diabetes and returned a supplementary questionnaire, we confirmed 94% of the initial reports. The validity of self-reported diabetes using the same supplementary questionnaire has been examined in the Nurses' Health Study, a parallel population of female health professionals. Medical records were obtained from 62 women, who were selected at random, reporting diabetes and classified as definite diabetes by a supplementary questionnaire. Sixty-one of the 62 women classified as having diabetes by questionnaire response were confirmed by medical record review.

Hormone assessments

After selecting appropriate specimens, the specimens were retrieved, aliquotted and sent to the laboratory blinded to any exposure information. We have worked with each of the selected laboratories, because each has been found to have high assay precision. We continued to monitor precision by including masked QC samples along with our samples.

Men in our analysis did not specify whether they had type I or type II diabetes. Based on the age of participants at DM diagnosis and the distribution of type 1 diabetics versus type 2 diabetics in the general population, it was assumed that most diabetic study participants were type 2 diabetics.

Assays for T and SHBG

Plasma concentrations of T and SHBG were measured in the laboratory of Dr. Nader Rifai at Children's Hospital Boston, MA using the following methods: total T—a chemiluminescent immunoassay (Elecsys autoanalyzer, Roche Diagnostics, Indianapolis, IN) and SHBG—coated-tube non-competitive immunoradiometric assay (Diagnostic Systems Laboratory, Webster, TX). The mean intra-pair coefficients of variation calculated from blinded quality control ranged between 4.9% and 5.5% for T and between 8.1% and 10.7% for SHBG. We assessed the intra-person consistency of T and SHBG over time by measuring for 144 men from the HPFS who were free of a cancer diagnosis plasma concentrations in specimens collected in 1993 and again in 1997 (mean of 3.03 ± 0.46 years apart) [9]. Adjusting for age and race the Spearman correlation coefficients between the two time points were 0.68 for total T and 0.74 for SHBG (all $p \leq 0.0001$).

Assays for C-peptide, IGF-1, and IGFBP-3

Plasma levels of C-peptide, IGF-1, and IGFBP-3 were assayed by Dr. Pollack. Plasma levels of C-peptide were assayed by radioimmunoassay (Linco Research, St. Charles, MO), an assay with little or no cross-reactivity with proinsulin [10]. For plasma IGF-1 and IGFBP-3, ELISAs were used with reagents provided by Diagnostic Systems Laboratory (Webster Texas). The mean intra-assay coefficients of variation for IGF-1 and IGFBP-3 from blinded control samples ranged from 2.6 to 13.2% and from 3.5 to 11.6%, respectively. For C-peptide, the mean intra-assay coefficient of variation was <10%. We found that IGF-1 and IGFBP-3 assessed an average three years apart from the same man in the HPFS yield acceptable correlations ($r = 0.70$ and $r = 0.68$, respectively) indicating a single measure of each is informative [11].

Statistical analyses

In order to statistically test if a specified plasma factor is different between any of the groups, we used multiple linear regression analysis. The hormones investigated were T, SHBG, C-peptide, IGF-1, and IGFBP-3 and the main exposure of interest was DM status—either diabetics or non-diabetics at time of blood draw. The diabetics were further divided into four groups based on years since DM diagnosis at time of blood draw. Group-duration was chosen in order to have similar size groups; group 1 = diagnosed ≤1 year prior to blood draw ($n = 39$, 22.8%), group 2 = diagnosed >1 and ≤6 years prior to blood draw ($n = 53$, 31%), group 3 = diagnosed >6 and ≤15 years prior to blood draw ($n = 42$, 24.6%), and group 4 = diagnosed ≥15 years prior to blood draw ($n = 37$, 21.6%).

The multivariate linear regression model calculated the least-squares means by adjusting for the following co-variables: age (continuous), BMI (continuous), quintiles of total calories, fasting hours (four discrete groups—fasting for less than three hours, three hours to less than six hours, six hours to less than nine hours, or nine or greater hours), time of day of blood draw (four discrete groups—12 am–6 am, 6:01 am–noon, 12:01 pm–6 pm, 6:01 pm–11:59 pm), and one of seven batches as described in "study population" (discrete; batch refers to the sub-study for which the bloods were originally processed).

We also examined the Spearman correlation coefficients for each combination of T, SHBG, IGF-1, IGFBP-3, and C-peptide. Correlation coefficients between hormone variables were determined for both diabetics and non-diabetics separately.

Results

Blood samples were collected from 3,172 men in the Health Professionals Follow-Up Study between 1993 and 1995. Of these, 171 men were diagnosed with DM and the remaining 3,001 were non-diabetic controls. The mean age of diabetic cases and non-diabetic controls was 64.2 and 67.8 years, respectively. Table 1 presents crude results for C-peptide, T, SHBG, IGF-1, and IGFBP-3 for the various groups of participants. "All-diabetics" include all 171 cases of diabetes that were diagnosed before the blood specimens were drawn. The unadjusted crude data suggests that in diabetics, C-peptide levels are slightly elevated over non-diabetics while T, SHBG, IGF-1, and IGFBP-3 are all slightly decreased in diabetics. When diabetics were divided into groups based on years since diagnosis, the crude data suggests that as time since DM diagnosis increases, C-peptide, IGF-1, and IGFBP-3 levels may decrease and both T and SHBG levels appear to increase.

The graphs shown in Fig. 1 provide multivariate-adjusted mean hormone levels for non-diabetics, all diabetics, and for diabetics divided into four groups as described for Table 1. p-Values for the trend from group one to group four are also presented on these graphs. The mean C-peptide level for all-diabetic men is slightly lower than for non-diabetics (Fig. 1a). When the men with DM were divided into groups based on years-since diagnosis,

Table 1 Unadjusted values for selected characteristics of men divided into groups by DM-status

	Group 0 (non-diabetics) $n = 3{,}001$	All diabetics $n = 171$	Group 1 (diagnosed for $\leq$1 year) $n = 39$	Group 2 (diagnosed for >1 year and $\leq$6 years) $n = 53$	Group 3 (diagnosed for >6 years and $\leq$15 years) $n = 42$	Group 4 (diagnosed for $\geq$15 years) $n = 37$
Mean age	64.2	67.8	65.6	68.7	68.4	68.2
Mean BMI	25.6	27.7	28.7	28.2	27.6	26.0
Unadjusted mean hormone levels						
C-peptide (ng/ml)	2.41	2.82	3.28	3.39	2.37	1.86
Testosterone (ng/ml)	4.82	4.13	3.61	3.83	4.09	4.98
SHBG (nmol/l)	74.3	69.3	52.7	60.7	79.1	84.1
IGF-1 (ng/ml)	184.5	180.9	187.3	187.3	182.8	163.1
IGFBP-3 (ng/ml)	3,603.7	3,521.9	3,921.7	3,526.4	3,547.5	3,112.1

we observed a significant trend for changes in C-peptide levels ($p = .05$). Men diagnosed with diabetes $\leq$1 year have levels of C-peptide that are elevated over the non-diabetics, diabetic men diagnosed 1–6 years have similar levels to non-diabetics, men diagnosed >6–15 years prior to blood draw have slightly higher C-peptide levels than non-diabetics, and those diagnosed for $\geq$15 years prior to blood draw have substantially lower levels than non-diabetics. Total T levels in diabetic men are also slightly lower than in non-diabetic men (Fig. 1b). When T levels were examined by DM-group, T levels initially dropped off but with increasing time since DM diagnosis, they returned to slightly higher-than-normal levels (p for trend $= .02$). SHBG levels show a similar pattern as T levels (Fig. 1c). The men with DM have a slightly lower level of this hormone than non-diabetic controls. Levels of SHBG increase significantly as time since DM-diagnosis increases (p for trend $= .002$). In addition we observed that with increasing time since DM-diagnosis, the T:SHBG ratio decreases significantly (p for trend $= .02$) (Fig. 1d). Although there are no significant changes in IGF-1 levels (p for trend $= .38$), IGFBP-3 appears to peak slightly when patients are first diagnosed and then they significantly decrease as time since diagnosis increases (p for trend $= .03$). The ratio of IGF1:IGFBP-3 does not change significantly as time since DM diagnosis increases ($p = .17$).

In Table 2 correlation coefficients are presented between hormone levels for non-diabetics and all-diabetics for the same hormones and ratios as above. There is a significant negative correlation between C-peptide levels and T that appears to be slightly stronger in diabetics ($-.53$, $p = <.0001$) than in non-diabetics ($-.30$, $p = <.0001$). Similarly, there is a significant inverse correlation between SHBG and C-peptide in diabetics ($-.22$, $p = .04$) but not in the non-diabetic ($-.04$, $p = .18$) controls. Correlation coefficients pertaining to IGF-1 and IGFBP-3 do not appear to vary significantly between diabetics and non-diabetics.

Discussion

Our study of the hormonal profiles in diabetic versus non-diabetic men identified changes in hormonal profiles in diabetic men that may be consistent with a reduction in prostate cancer risk. The levels for C-peptide, T, SHBG, and IGFBP-3 appear to change consistently in one direction as time since DM-diagnosis increases. IGF-1 levels appear to be decreased in diabetics as compared to non-diabetics but there is no significant time trend following years since diagnosis.

C-peptide

This study suggests that many type 2 diabetics are initially hyperinsulinemic, but with worsening DM, the levels of insulin in some patients decline. In the present study we measured C-peptide levels in men who have been diagnosed with DM for varying lengths of time. C-peptide is a peptide which is made when proinsulin is converted to insulin. Since there is one C-peptide molecule for each insulin molecule, C-peptide is used as a marker of insulin secretion. Our data demonstrates that for patients diagnosed with DM within one year prior to the blood draw, C-peptide levels were higher than in non-diabetics. As time since diagnosis increased, however, C-peptide levels decreased leading eventually to a hypoinsulinemic state. This suggests that the longer a man lives with DM, the lower his insulin levels become. The p-value for the trend from group one through group four was statistically significant ($p = .05$). Since some studies suggest that the longer a man is diabetic, the more reduced his prostate cancer risk is [8, 12, 13], our results support a model whereby decreasing insulin over time may contribute to the decrease in prostate cancer risk. Further supporting this model, previous studies indicate that insulin is positively associated with the growth of both normal and cancerous

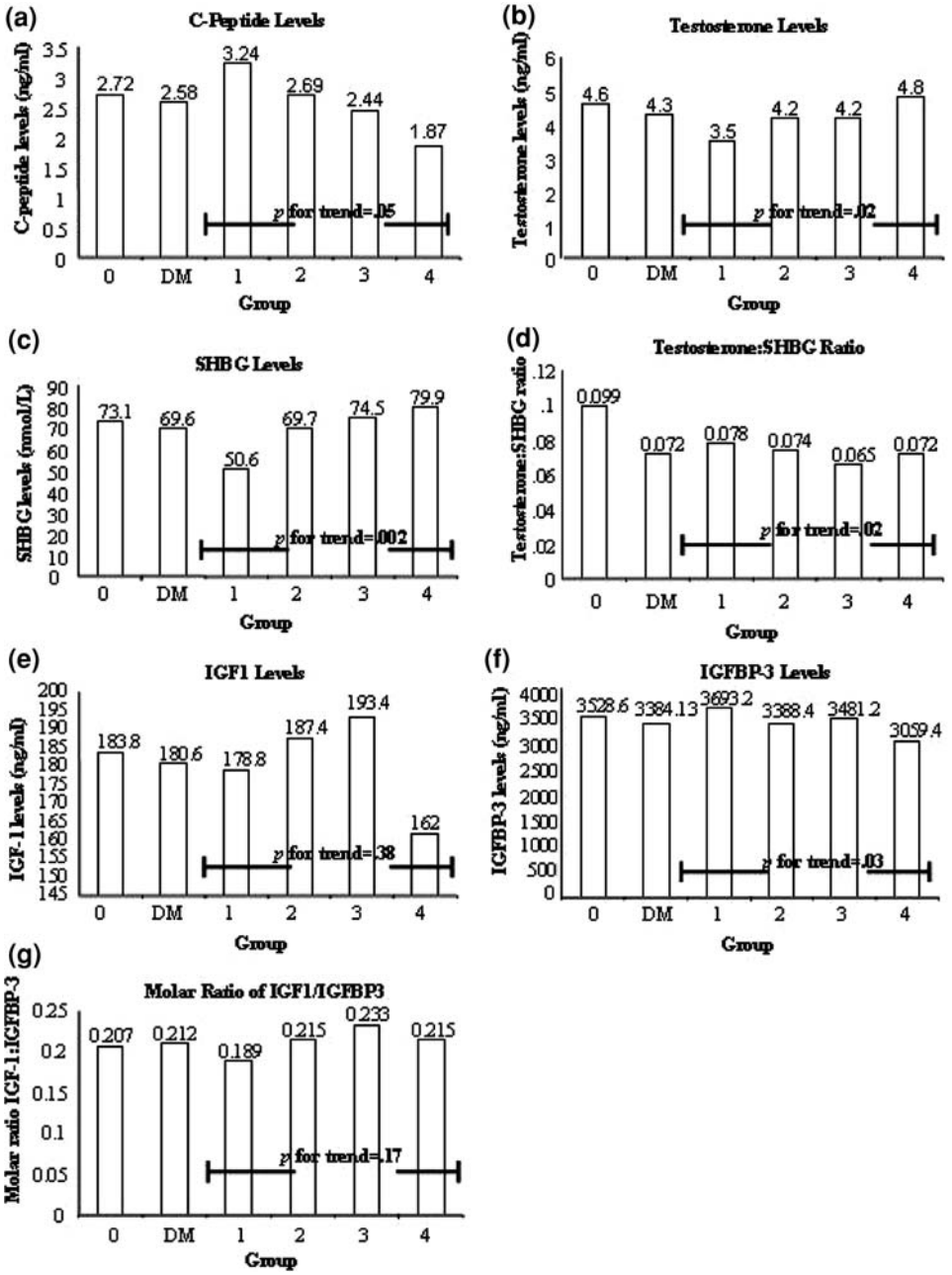

Fig. 1 Multivariate-adjusted mean hormone levels (adjusted for group, age, batch, time of day blood was withdrawn, fastin hours, BMI, and calories). Group names on x-axis: 0 = non-diabetics, DM = all diabetics, 1 = ≤1 year since DM diagnosis, 2 = >1 & ≤6 years since DM diagnosis, 3 = >6 & <15 years since DM diagnosis, 4 = ≥15 years since DM diagnosis. Note jump in scale in (**e**)

Table 2 Spearman correlation coefficients between C-peptide, Testosterone, SHBG, IGF-1, and IGFBP-3 for both diabetics and non-diabetics

	All-diabetics				Non-diabetics			
	T	SHBG	IGF-1	IGFBP-3	T	SHBG	IGF-1	IGFBP-3
C-peptide	−.53 (<.0001)	−.22 (.04)	.15 (.09)	.06 (.50)	−.30 (<.0001)	−.04 (.18)	.07 (.003)	.06 (.02)
T		.50 (<.0001)	−.09 (.36)	−.16 (.11)		.43 (<.0001)	−.08 (.002)	−.15 (<.0001)
SHBG			−.21 (.03)	−.60 (<.0001)			−.25 (<.0001)	−.60 (<.0001)
IGF-1				.65 (<.0001)				.57 (<.0001)

Each correlation coefficient is followed by the *p*-value which is reported in the parentheses

prostate cells. Insulin has been shown to be a growth factor for prostatic epithelium in vitro [14], to stimulate growth of a rat prostate cancer cell line in vitro [15], and is associated with higher incident [16] and recurrent [17] prostate cancer. Therefore, decreased insulin may have a growth-inhibitory effect on these cells and if long-term diabetic patients

experience reduced levels of circulating insulin, they may be at a reduced risk of developing prostate cancer. In addition, there is evidence that higher serum insulin levels are associated with poor outcome in prostate cancer [18]. While some prospective studies demonstrate an association between hyperinsulinemia and prostate cancer risk [19], several studies do not support a role for insulin in prostate cancer risk [20, 21].

Sex-hormones

The data presented in this study demonstrate that although total T decreases overall in diabetic men compared to non-diabetic men, we did not observe that total T levels decreased as time since diagnosis increased. Total T levels initially dropped off but over time they return to slightly higher-than-normal levels. Also, SHBG levels increased significantly as time since DM diagnosis increases, consistent with the well-described inverse relationship between SHBG and insulin [22, 23]. The ratio of T to SHBG decreased over time since diagnosis indicating that although there is more total T, less may be available.In addition, the correlation coefficient between C-peptide and both T and SHBG was slightly stronger in diabetics than non-diabetics further suggesting that there is a role for these hormones in DM progression and possibly reduction in prostate cancer risk. Unlike for diabetics, we did not observe a statistically significant inverse association between C-peptide and SHBG in the controls; the reason for this is unclear, but could possibly relate to the greater variation in C-peptide in the diabetics.

One hypothesis for why diabetics are at decreased risk for prostate cancer is that with worsening DM, decreased levels of T provide an environment that is not conducive to proliferation of prostate cancer cells. Published literature contains data both supporting and refuting this hypothesis. Some prospective studies suggest that increased T may be a risk factor for prostate cancer [24] and others, including a recent meta-analysis, report either no or small associations between serum sex-hormone levels, including T, and subsequent prostate cancer risk [9, 25–30]. Several studies suggest that men with low T levels may be at increased risk for more poorly-differentiated cancers of the prostate [31–33]. Since high T levels may increase the risk of prostate cancer, if patients with DM have low T levels, they may also be at decreased risk of prostate cancer. In addition to changes in T, low levels of SHBG have been shown to be associated with increased risk of prostate cancer [24, 30]. Some animal and human studies support our hypothesis that in diabetics, there is less bioavailable T in the serum. Jackson and Hutson showed that diabetic rats have reduced T levels [34]. Similarly, studies in men show that as blood glucose levels increase, T levels decrease [35, 36].

Insulin-like growth factors

Another proposed hypothesis for how hypoinsulinemia may decrease prostate carcinogenesis is by limiting the bioavailability of IGF-I. In an insulin-deficient environment, IGF-binding protein I is up-regulated, presumably resulting in less bioavailable IGF-I [37–39]. Furthermore, several prospective studies show that IGF-I is an important risk factor for prostate cancer [40–43]. IGF-I is a growth regulator that has been shown to be associated with carcinogenesis [44], and activation of IGF-I receptors stimulates the proliferation of prostate cancer cells in an IGF-I-dose-dependent manner [41, 45]. IGF-1 circulates bound to binding proteins, the most prevalent of which is IGFBP-3. When bound to IGF-1, IGFBP-3 may decrease the bioavailability of IGF-1. However, when IGF-1 is bound by IGFBP-3, IGF-1 is protected from degradation. We hypothesized that in diabetic men, IGF-1 levels may decrease over time since DM diagnosis and that this could be mediated through changes in IGFBP-3 levels. Our data demonstrates that in this group of men, both IGF-1 and IGFBP-3 are decreased in the all-diabetic group compared to the non-diabetics although it was only significant for IGFBP-3 (p = .05). When we evaluated time since DM-diagnosis, IGFBP-3 displayed a slight though significant downward trend as time increased. There was neither a significant trend for IGF-1 nor is there for the molar ratio between these two proteins. Further suggesting that IGF-1 levels are not significantly different between the diabetics and controls in our study, when we compared the correlation coefficients for C-peptide and IGF-1 or IGFBP-3, we observed no significant difference between diabetics and controls. Future studies should examine IGFBP-1 as having a role in prostate carcinogenesis.

Our study has several limitations. A major limitation is that in the time-since-diagnosis groups, the sample size was relatively small. This reduced our power and ability to further divide these groups. Nonetheless, we were able to see a significant trend through the groups for four of the five hormones. In addition, blood samples were processed in one of seven batches and only one sample was used per participant. Other limitations to our study are both that we used self-reported DM and we grouped all diabetics together instead of categorizing type 1 versus type 2 diabetics. Based on the age of participants and the distribution of type 1 diabetics versus type 2 diabetics in the general population, it was assumed that most diabetic study participants were type 2 diabetics. Future studies that investigate the association between type 1 or type 2 diabetics and prostate cancer separately will provide further insight into the specific biological mechanisms behind prostate cancer. One alternate explanation for the hormonal changes seen in diabetics over time is that upon DM diagnosis, a patient

may alter his life-style or begin a treatment regimen in order to control his DM. These changes may affect his hormone levels and ultimately his potential for prostate cancer may be altered.

Our study supports the hypothesis that varying hormonal profiles over time since DM diagnosis may influence prostate carcinogenesis. As our results suggest that the hormonal profiles of diabetics are changing over time since DM diagnosis, future studies of DM and prostate cancer should carefully examine the temporal relationships between these two diseases. It may also be informative for future studies to examine the relationship between DM and cancers of varying grades and stages. The overall health effect of DM is not beneficial but these data provide us unique insight into furthering our understanding of the process of prostate cells becoming malignant.

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