RELATIONSHIPS BETWEEN BASELINE FASTING GLUCOSE AND INSULIN STATUS, TYPE 2 DIABETES, AND INCIDENT PROSTATE CANCER IN THE OSTEOPOROTIC FRACTURES IN MEN STUDY (MrOS)

By

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CERTIFICATE OF APPROVAL

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Abstract

With the rapid increase of obesity and cancer in the United States, it is imperative for scientists to understand how these two entities may be related to possibly increase risk of cancer. Incidence of type 2 diabetes and of prostate cancer have been increasing over the past 8 years, suggesting a possible shared mechanism of risk or association between the two. Previous epidemiological studies found that those with type 2 diabetes have a decreased risk of prostate cancer, while other epidemiological studies show that those with a history of type 2 diabetes may have a increased risk of other cancers such as lung and colorectal cancer. However, many of these studies have not evaluated an association between type 2 diabetes and fasting glucose and insulin levels and incident prostate cancer as the outcome in a large, prospective fashion and therefore, require further investigation. In addition, previous studies have not examined pre-diabetes as determined by fasting glucose and insulin levels in a prospective fashion and the relationship between these factors and prostate cancer risk. Analyzing these exposures (fasting glucose, insulin, and type 2 diabetes) is crucial to determining risk factors for and the biological mechanisms behind the onset of prostate cancer. We determined the relative risk estimates for the relationships between baseline fasting glucose and insulin levels and type 2 diabetes with incident prostate cancer in 5,995 men in the Osteoporotic Fractures in Men Study (MrOS) over 8 years of follow up. Compared to men with normal fasting glucose levels and no history of diabetes, we found no increased risk for incident prostate cancer in men with impaired fasting glucose levels (HR = 1.18; 95% CI 0.94, 1.50). Men with higher fasting insulin level had an increased risk of prostate cancer (HR = 1.52; 95% CI 1.09, 2.13) compared to those with lower fasting insulin level, but men with type 2 diabetes had a decreased risk of prostate cancer (HR = 0.56; 95% CI 0.37 - 0.85). The finding of increased prostate cancer risk in men with higher

fasting insulin levels fits with current biologic models linking enhanced insulin signaling with development of prostate cancer. Finding that older men with type 2 diabetes are at a decreased risk of prostate cancer compared to those without diabetes suggests that other biological factors involved in glucose metabolism and obesity also play important roles in the relationship between obesity and prostate cancer.

Chapter 1

Introduction

Prostate Cancer: Definition and Risk Factors

Prostate cancer is the second leading cause of cancer death in American men and is the most commonly diagnosed non- skin cancer in men in the United States In 2009, the American Cancer Society estimated that about 192,280 new cases of prostate cancer in the United States were diagnosed and 27,360 men will die from prostate cancer[1].

The prostate is composed of branching tubuloalveolar glands arranged in lobules and surrounded by a stroma[1]. The prostate gland consists of multiple acinal units, each comprising an epithelial compartment made of epithelial, basal, and neuroendrocrine cells and a stromal compartment that includes fibroblasts and smooth-muscle cells. Both prostate epithelial cells and stromal cells express androgen receptors and depend on androgens, such as testosterone, for growth[1]. The prostate gland has four distinct glandular regions, two of which are the source of most prostate cancers. Approximately 64% of prostate cancers develop in the peripheral zone, located in the posterior portion of the gland that surrounds the urethra with the remainder occurring in a region known as the transition zone[2].

Clinically, physicians use various screening methods to identify and diagnose prostate cancer. Some common ways of detecting prostate cancer are the digital rectal exam (DRE) and by checking serum levels of prostate specific antigen (PSA). PSA and acid phosphatase are produced in the epithelial cells of the prostate gland. PSA is a single-chain glycoprotein that hydrolyzes peptide bonds and as a result, liquefies semen. A PSA level of 4-10 ng/mL may signify a benign or cancerous prostate while a PSA level of greater than 10 ng/mL often signifies

the presence of prostate cancer[2]. After a prostate biopsy reveals cancerous tumor, Gleason scores are used to classify the patterns of malignant cells. A number between 1 and 5 is assigned to the predominant cellular pattern and another number between 1 and 5 is assigned to the second most common cellular pattern. The sum of these two numbers is the Gleason score.

Previous studies have identified some non-modifiable risk factors for prostate cancer, including older age, race and family history [2, 3, 4]. African-American males compared to white males have a greater number of prostatic intraepithelial neoplasia (PIN) lesions[3], which are precursors to cancer and larger tumors[4]. This may be related to higher testosterone levels in African Americans[3]. African American men with prostate cancer have higher rate of mortality compared to white men with prostate cancer[3]. With regard to family history as a risk factor, the risk of being diagnosed with prostate cancer increases by a factor of two if one first-degree relative is affected and by four if two or more first-degree relatives are affected[1].

Previous studies have also identified modifiable risk factors. Environmental factors such as high consumption of dietary fats may increase risk of prostate cancer[1]. Migration studies have demonstrated that prostate cancer risk in Asian men increases when they move to Western environments, which may be due to increased consumption of fat [5]. Potential protective factors include consumption of antioxidant nutrients such as lycopene in tomatoes, vitamin E and selenium intake, and use of drugs that inhibit cholesterol biosynthesis, such as statins [6, 7].

Because prostate cancer is a hormone dependent cancer, scientists have considered how the presence and interactions of hormones with one another affect risk of prostate cancer. Increased risk of prostate cancer may also occur in patients with decreased blood levels of sex-hormone binding globulin (SHBG) and increased levels of testosterone and insulin-like growth

factor (IGF-1)[8-12]. IGF-1 production is dependent primarily on growth hormone, and secondarily on nutrition and insulin[11, 13, 14]. The activity of IGF-1 is regulated in different ways by IGF-binding proteins (IGFBP-1-6). Most of the circulating IGF-1 and IGFBP-1, 2, and 3 are produced in the liver. In serum, IGF-1 is bound to IGFBP-3, a growth hormone dependent storage protein[15-17]. However, only the free fraction of IGF-1 is biologically active and this comprises less than 1% of the total IGF-1 in serum. Insulin can increase IGF-1 bioactivity by decreasing the synthesis and plasma levels of IGFBP-1 and IGFBP-2[14, 18].

Peptide hormones such as IGF-1 may stimulate the cell growth and tumorogenesis through several pathways, including mitosis[11, 13, 14] and angiogenesis. Angiogenesis, the process of forming new blood vessels from pre-existing ones, is stimulated by IGF-1 since it increases vascular endothelial growth factor (VEGF) production in prostate cancer cells[19]. In human prostate cancer cell lines, one study showed that androgen-independent cell lines PC-3 and DU-145 had expressed specific binding sites for IGF-1[20]. The IGF-1 receptor concentrations of androgen-independent cell lines were significantly higher than those of androgen-dependent cell lines though androgen itself appeared to have no effect on the expression of IGF-1 receptors or the secretion of IGF-1 in human prostate cancer cell lines[20].

Insulin is similar to IGF-1 and IGF-2 and has been postulated to also play a role in tumorogenesis. Insulin, however, differs from IGF-1 and IGF-2 in several ways (**Table 1**) [21]. One difference is that each is present in different quantities in the plasma. The physiologic role of insulin also differs from IGF-1 and IGF-2, where insulin is primarily responsible for control of glucose metabolism, IGF-1 is responsible for skeletal and cartilage growth, and IGF-II is responsible for growth during fetal development.

Table 1: Insulin and Insulin-Like Growth Factors			
Property	**Insulin**	**IGF-1**	**IGF-II**
Number of amino acids	51	70	67
Source	Pancreatic B cells	Liver and other tissues	Diverse tissues
Level Regulated by	Glucose	Growth hormone after birth, nutritional status	Unknown
Plasma levels	0.3-2 ng/mL	10-700 ng/ml; peaks at puberty	300-800 ng/mL
Plasma-binding proteins	No	Yes	Yes
Major Physiologic Role	Control of metabolism	Skeletal and cartilage growth	Growth during fetal development

Insulin enhances the growth hormone stimulated synthesis of IGF-1 and IGFBP-3. In the Northern Sweden Health and Disease Cohort study, prostate cancer cases were found to have higher levels of IGF-1 and insulin than non-cases[11]. Glucose and insulin are also thought to be key players in the development of prostate cancer, especially insulin which is considered to be a growth-stimulatory factor through IGF-1. High insulin levels often reduce IGF binding proteins, therefore increasing IGF-1 levels and promoting angiogenesis within tumors[22, 23]. However, insulin may not promote de novo tumor development[22-24]. To further understand how glucose and insulin affect incident prostate cancer, one can make use of epidemiologic designs to test relationships between fasting serum glucose and insulin levels and diagnosis of type 2 diabetes in patients who develop prostate cancer over time.

Glucose utilization in normal, healthy individuals

In patients without glucose impairment or type 2 diabetes, several metabolic mechanisms occur simultaneously to maintain glucose homeostasis. From beta islet cells located in the pancreas, insulin is released in response to changes in plasma levels of energy substrates such as

nutrients (glucose and amino acids), hormones (glucagon-like peptide GLP-1), somatostatin and epinephrine (which inhibit insulin secretion), and neurotransmitters (norepinephrine and acetylcholine). High glucose levels, such as those that occur after ingestion of a meal, stimulate release of insulin from the pancreas[25]. Increased insulin levels also promote glucose uptake in skeletal muscle and fat as well as enhanced protein synthesis[25]. The major portion of postprandial glucose is taken up and utilized by skeletal muscle, an effect of insulin-dependent glucose uptake. Other tissues, such as the brain, utilize glucose in an insulin-independent fashion[25].

Once insulin is secreted into the portal venous system by pancreatic beta islet cells, approximately 50% is degraded by the liver[26]. Insulin that is not degraded by the liver enters the systemic circulation where it binds to receptors in target tissues and stimulates intrinsic tyrosine kinase activity[9]. This leads to phosphorylation of intracellular signaling molecules, such as insulin receptor substrates (IRS). IRS and other adaptor proteins initiate a complex cascade of reactions, resulting in the widespread metabolic and mitogenic effects of insulin[26]. As an example, activation of the phosphatidylinositol-3'-kinase (PI-3-kinase) pathway stimulates translocation of glucose transporters (e.g., GLUT4) to the cell surface. This event is crucial for glucose uptake by skeletal muscle and fat[11], which in turn convert the glucose into usable energy by creating ATP. Activation of other insulin receptor signaling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes in insulin-responsive cells[9].

Normal fasting glucose, impaired fasting glucose, and type 2 diabetes: a continuum.

Insulin resistance is the inability of peripheral target tissues to respond properly to normal

circulating concentrations of insulin[25]. To maintain normoglycemia, the pancreas compensates by secreting increased amounts of insulin. However, compensating for insulin resistance by an increase in insulin release is effective only temporarily[25]. As insulin resistance increases over time, individuals may develop impaired fasting glucose. For those with impaired fasting glucose, the peripheral tissues in their bodies' exhibits reduced sensitivity to insulin. This inefficiency represents an overall decrease in maximum glucose utilization, which is 30–60% lower than in normal individuals[11]. Increased hepatic glucose output predominantly accounts for increased fasting plasma glucose levels[9]. In skeletal muscle, there is a greater impairment in non-oxidative glucose usage than in oxidative glucose metabolism through glycolysis[9].

The precise molecular mechanism leading to insulin resistance in type 2 diabetes has not been elucidated. We know that insulin receptor levels and tyrosine kinase activity in skeletal muscle are reduced[25]. Defects in insulin-regulated phosphorylation and dephosphorylation may play the predominant role in insulin resistance, which affects other signaling pathways[26]. For example, a PI-3-kinase signaling defect may reduce translocation of GLUT4 to the plasma membrane. This reduces the amount of glucose that is taken up by skeletal muscle, and ultimately impairs production of ATP for energy. Other abnormalities include the accumulation of lipid within skeletal myocytes, which may impair mitochondrial oxidative phosphorylation and reduce insulin-stimulated mitochondrial ATP production[9].

While we may not know the precise molecular mechanisms leading to type 2 diabetes, failure or the exhaustion of the pancreatic beta islet cells results in decreased insulin secretion, and the combination of insulin resistance and impaired cell function characterizes clinical type 2 diabetes[25]. Therefore, insulin resistance occurs as a continuum in which insulin receptors on peripheral target tissues become less sensitive. As islet cell secretion of insulin begins to reduce,

an individual will develop impaired fasting glucose. When insulin receptors are even less sensitive to insulin, and pancreatic beta islet cell insulin secretion reduces to a greater extent, type 2 diabetes occurs. Therefore, type 2 diabetes is the result of inadequate responsiveness of the cells to glucose. This is later followed by a net reduction in pancreatic beta cell mass and a decreased responsiveness of peripheral tissues to insulin action[25]. While actual levels of circulating insulin may not be different among type 2 diabetics and non-diabetics, in response to a glucose load (or meal) type 2 diabetics secrete considerably less insulin than non-diabetics. When peripheral tissues experience reduced sensitivity to insulin, this results in higher levels of fasting glucose in type 2 diabetics.

Currently, type 2 diabetes is clinically diagnosed by fasting glucose levels $\geq$ 126 mg/dl. Impaired fasting glucose is considered to be in the range of 100-126 mg/dl while normal fasting glucose levels are typically < 100 mg/dl. As of 2009, it is estimated that 11.2% of all men over 20 years of age have diabetes in the United States[27]. Risk factors associated with type 2 diabetes include older age, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity[28]. Both visceral and central obesity are very common in those with type 2 diabetes.

IGF-1 is a peptide hormone that may vary in quantity depending whether or not an individual has impaired fasting glucose or type 2 diabetes. As previously discussed, the peptide hormone IGF-1 plays a crucial role in the development of other diseases such as cancer by promoting mitosis. IGF-1 production is dependent on growth hormone, nutrition, and insulin[17] but IGF-1 activity is regulated by IGF-binding proteins[29]. Insulin and IGF-1 can bind to each other's receptors but with low affinity at high levels[30]. IGF-1 is a more potent mitogen with stronger anti-apoptotic activity than insulin. IGF-1 plays a major role in regulation of cell

replication, differentiation, and survival whereas insulin has stronger metabolic activity than IGF-1[31, 32]. IGF-1 levels may influence glucose homeostasis since compensatory changes in IGF-1 levels may be adaptive in the presence of insulin resistance[33]. In a cross-sectional study, Brugts *et.al* observed progressively increasing levels of circulating IGF-1 for those with normal and impaired fasting glucose, which peaked at a fasting glucose < 7.0 mmol/l[29]. However, circulating IGF-1 levels dropped in patients with type 2 diabetes with fasting glucose > 7 mmol/l[29]. In other cross sectional studies, elevated levels of free IGF-1 and reduced levels of IGFBP-1 were found in those with pre-diabetes and those with type 2 diabetes[34]. However, one small prospective cohort study showed an inverse association between IGF-1 and subsequent 2 hour glucose concentrations, but not fasting glucose[35]. Metabolic syndrome, which often occurs in conjunction with type 2 diabetes, was also previously studied in relation to IGF-1 levels. The components of metabolic syndrome are abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance, proinflammatory state, and prothormbic state[36]. In one study, participants with up to three components of metabolic syndrome had the highest IGF-1 levels, but IGF-1 levels declined for individuals with all five components of metabolic syndrome[29]. Another study found a similar result: In a population of Swedish men, IGF-1 was inversely correlated to diabetes duration ($r= -0.19$; $p < 0.05$) and higher levels of fasting blood glucose ($r = -0.23$; $p<0.01$)[15]. One explanation is that those with impaired fasting glucose or pre-diabetes experience insulin-mediated suppression of IGFBP-1 levels[34], resulting in high IGF-1 levels. Those with type 2 diabetes eventually develop hepatic insulin resistance and hyperinsulinemia-induced growth hormone resistance, resulting in IGF-1 reduction. The biological mechanism for why there is a sudden shift in IGF-1 levels as individuals progress from pre-diabetes to type 2 diabetes is unclear.

The Role of Glucose, Insulin, Type 2 Diabetes in the Development of Prostate Cancer

Fasting Glucose, Insulin, and Prostate cancer

Albanes and others found that risk of prostate cancer varied inconsistently with glucose concentration in a case-cohort study[37]. In the Baltimore longitudinal study of aging, Hubbard and others showed that fasting insulin and glucose levels were unrelated to prostate cancer risk in a population of 823 men, and the authors concluded that larger prospective studies are warranted to measure these parameters further[24]. In a study consisting of Finnish men, Albanes and others found increased insulin levels were associated with statistically significant increased risk of prostate cancer[25]. In Swedish men, high levels of C-peptide (an indicator for the presence of insulin), HOMA-IR (a calculated value that measures the ratio of fasting glucose and insulin), and leptin were associated with decreased risk of prostate cancer in a matched case-control study[27].

Type 2 diabetes mellitus and prostate cancer

Some studies show that those with higher, possibly diabetic, levels of fasting glucose (>126 mg/dl) have decreased insulin response (lower IGF-1), lower testosterone levels, and therefore, lower risk of prostate cancer. However, this finding has not been replicated in many prospective cohort studies. Darbinian and others saw that individuals with type 2 diabetes had a lower risk of prostate cancer (RR = 0.71; 95% CI 0.62-0.79) compared to those with a 1-hour serum glucose < 140 mg/dl[9] in a prospective fashion[9]. Leitzmann and others found through a prospective cohort study that men with a history of diabetes had a 14% lower baseline PSA concentration than those without a history of diabetes[38], though PSA is not a risk factor for prostate cancer, it is simply a biological marker that may indicate whether or not an individual

has prostate cancer, as stated previously. We have summarized the changes in hormone levels, including sex-hormone binding globulin (SHBG) and testosterone in addition to insulin and IGF-1, across glucose tolerance status based on findings of some epidemiological studies in Table 2. These include several cross-sectional studies and prospective cohort analysis [9, 10, 29, 30, 32-34, 39].

Table 2: Relative levels of Hormones across Glucose Tolerance Status

	Normal	Impaired	Type 2 Diabetes
IGF-1	↑	↑	↓
Insulin	↑	↑	↓
SHBG	↓	↓	↑
Testosterone	↑	↑	↓

Based on the findings of many studies and our knowledge of how these same hormones are related to prostate cancer risk, we can see that some biological characteristics of diabetes may be associated with a decreased risk of prostate cancer, including low levels of IGF-1 and testosterone, increased levels of sex hormone binding globulin (SHBG), and low levels of PSA. In addition, many type 2 diabetic patients are obese, and in obese patients (BMI > 30 kg/m^2) free testosterone is converted to serum estradiol by aromatase in adipocytes [40]. An increase in serum estradiol triggers the increase in SHBG [17]. This may confer a protective effect for prostate cancer risk. The findings of epidemiological studies also support the observation that there is a shift in the risk of developing prostate cancer: those with normal or impaired fasting glucose have an increased risk of prostate cancer, while those with type 2 diabetes have a decreased risk of prostate cancer[9, 10, 37, 41-44].

Calton and others found an inverse association between risk of incident prostate cancer

for those with self-reported and physician diagnosed type two diabetes in the NIH-AARP study[41]. In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, Leitzmann and others also found an inverse association between self-reported diabetes and non-aggressive prostate cancer, as determined through medical record review[17]. In a multiethnic cohort study, Waters and others found an inverse association for those with self-reported diabetes and prostate cancer. The risk ratio was closer to one, but still protective, for African-American men compared to men of European descent (RR = 0.89 vs. RR = 0.65 ; Chi squared $p<0.0001$)[44]. Pierce and others found a no association between diabetes that was self reported and risk of localized prostate cancer[26]. Those with type 2 diabetes verified through medical records and self-assessment questionnaires were found to have decreased risk of both low grade and high grade prostate cancer in the Prostate Cancer Prevention Trial, a randomized study to test whether the 5α-reductase inhibitor finasteride could reduce the incidence of prostate cancer over a 7-year period [28]. A diagnosis of type 2 diabetes was found to lower risk of prostate cancer mortality in a study by Smith, *et. al.* where all patients received external beam radiation therapy to the whole pelvis. The intervention was whether patients received no further therapy or adjuvant goserlin therapy for 24 months (2009).

Tables 3 summarizes the proposed biological reasons for why there may be a increased risk of prostate cancer associated with impaired and normal fasting glucose levels while those who have type 2 diabetes, may have a decreased risk of prostate cancer[10, 41, 43-45]. Table 4 summarizes previous literature findings across the exposures of fasting glucose and fasting insulin, type 2 diabetes mellitus, and incident prostate cancer.

Table 3: Relative levels of Hormones across Normal and Impaired Fasting glucose status, Type 2 Diabetics and those with Increased/Decreased Prostate cancer risk

	Increased Risk of Prostate Cancer	Impaired Fasting Glucose	Normal Fasting Glucose	Decreased Risk of Prostate Cancer	Type 2 Diabetes
IGF-1	↑	↑	↑	↓	↓
Insulin	↑	↑	↑	↓	↓
SHBG	↓	↓	↓	↑	↑
Testosterone	↑	↑	↑	↓	↓

Table 4: Summary of Selected Literature Findings between Various Exposures and Incident Prostate Cancer as outcome

Author, year	Exposure	Type of study	Age-adjusted finding	Increase or Decrease Prostate Cancer Risk
Albanes, 2009	Fasting glucose	Case-cohort	Null	Null
Albanes, 2009	Fasting insulin quartiles	Case-cohort	OR = 1.50, 1.75, 2.55 (p = 0.02)	Increase
Hubbard, 2008	Fasting glucose and insulin	Prospective	Null	Null
Darbinian, 2008	Fasting glucose (untreated type 2 diabetes)	Prospective	RR = 0.71 (95% CI 0.62, 0.79)	Decrease
Leitzmann, 2008	Type 2 diabetes	Prospective	RR = 0.80 (95% CI 0.68, 0.95)	Decrease
Pierce, 2008	Type 2 diabetes	Case-control	OR = 0.98 (95% CI 0.76, 1.27)	Null
Waters, 2008	Type 2 diabetes	Prospective	RR = 0.81 (95% CI 0.74, 0.87)	Decrease
Calton, 2007	Type 2 diabetes	Prospective	RR = 0.69 (95% CI 0.64, 0.74)	Decrease
Meyers, 2007	Type 2 diabetes	Case-control	OR = 0.47 (95% CI 0.22, 0.94)	Decrease

In summary, understanding the relationships between glucose and insulin levels, and diabetes status, and prostate cancer risk requires further study. Many previous case-control and cross-sectional studies are not able to establish a temporal sequence between the exposure and outcome. Previous large prospective studies rely simply on self-reported type 2 diabetes, which may be prone to bias.

There has not been a single study that examines the relationships between type 2 diabetes, including all levels of fasting glucose and insulin, and prostate cancer risk within a single study population. Studies that have investigated fasting glucose levels and prostate cancer

risk have not found statistically significant findings because they have been underpowered. Comparing categories of fasting glucose and insulin levels such as untreated diabetics to those with impaired fasting glucose and normal levels may help to elucidate more risk factors of prostate cancer. Studying the association between type 2 diabetes and prostate cancer prospectively using clinically confirmed cases of each disease may also help to understand the risk factors and enhance the validity of the findings.

Therefore, the objectives of this study are 1) to determine the association between fasting glucose (untreated diabetics, impaired, and normal) and prostate cancer risk 2) to determine the association between fasting insulin (higher, lower) and prostate cancer risk. 3) to determine the association between type 2 diabetes and prostate cancer risk. We hypothesize that those with fasting glucose ≥126 mg/dl, who we will classify as untreated diabetics, will have a decreased risk of prostate cancer compared to those with a normal level of fasting glucose (<100 mg/dl). Those with impaired level of fasting glucose (100-126 mg/dl) will have an increased risk of prostate cancer compared to those with normal level of fasting glucose. Those with higher level of fasting insulin (≥ 5.5 μIU/mmol) will have an increased risk of prostate cancer compared to those with lower level of fasting insulin (< 5.5 μIU/mmol). We hypothesize that those with type 2 diabetes will have a decreased risk of prostate cancer compared to those without type 2 diabetes.

Chapter 2

Methods

Participants

The Osteoporotic Fractures in Men Study (MrOS) is a prospective, observational study of risk factors for fractures and was also designed to examine potential risk factors for prostate cancer. The subjects included community dwelling, ambulatory men ≥ 65 years of age who were able to walk without assistance of another, had an absence of bilateral hip replacements; had the ability to provide self-reported data, lived near a clinical site for duration of the study; had an absence of a medical condition that would result in death, and had the ability to sign an informed consent Participants were recruited from six cities (Palo Alto and San Diego CA; Birmingham AL; Pittsburgh PA; Minneapolis MN; and Portland OR) and approximately 1,000 participants were recruited from each center making a total study sample of 5,995 men. Each center had various recruitment strategies including use of motor vehicle and voter registration records to make age-appropriate mailings; community and senior newspaper advertisements; and word of mouth[29]. The minimal exclusion criteria allowed for a large sample size and generalizability of results to the US male population.

The Institutional Review Board at each center approved the study, and all participants completed informed consent forms. Data collected from each study center were de-identified such that information could not be traced to the participants name or medical record prior to submitting the information to a repository. The appropriate committees for the MrOS study reviewed the analysis plan for the specific aims of this project.

Fasting Glucose, Insulin, and Type 2 Diabetes measurements

Participants at the baseline visit completed self-administered questionnaires, a clinic visit, and at least the anthropometric, Dual energy X-ray absorptiometry (DEXA), and vertebral X-ray procedures. The baseline exam took place between March 2000 and April 2002, and baseline measures were repeated in 2005. Fasting morning phlebotomy was performed for all participants at the baseline visit to obtain serum, plasma, and whole blood specimens[29]. Blood specimens were processed and stored (-120^{o}C). All participants that had more 10 vials of serum stored centrally were included in this ancillary study.

Assays were performed in January 2006 in Dr. Santica Marcovina's Northwest Lipid Metabolism and Diabetes Research Laboratories at the University of Washington. This lab has a coefficient of variation for both glucose and insulin measures based on blind duplicates as follows: glucose-interassay CV% < 3.0%, insulin-interassay and intraassay CV%< 10.0%. The operational definition of diabetes created by the MrOS study is as follows: fasting (≥ 8 hours) glucose ≥ 126 mg/dl or self-reported prevalent diabetes at baseline or using hypoglycemic medications at baseline and insulin use. Self-reported prevalent diabetes was obtained through self-assessment questionnaires. Participants who were considered to have self-reported history of diabetes answered "yes" to the question "Has a doctor or other health care provider ever told you that you had or have diabetes?"

Measurement of Incident Prostate Cancer

Diagnoses of incident prostate cancer were obtained through self-report from participants who completed a Tri-Annual Questionnaire sent every four months. Participants who did not

return the questionnaire were contacted by study staff and obtained this information from in-person or telephone interviews. For each reported prostate cancer case, medical records were requested from the hospital or clinic for adjudication. Reports included pathology reports for initial diagnosis of prostate cancer, PSA lab reports before diagnosis, clinical notes ordering biopsy, post-diagnosis studies reports, and post-diagnosis clinic reports[29]. All information was collected and recorded centrally at the MrOS coordinating center at the University of California, San Francisco and California Pacific Medical Center Research Institute without knowledge of diabetes or other risk factors. Those with prevalent prostate cancer at baseline were excluded from these analyses (n=707). The endpoints analyzed in this study include prostate cancer reports that had been adjudicated as of July 28, 2008.

Other Baseline Measurements

Baseline measures included height, weight, body composition, ankle-arm blood pressure, grip strength, leg power, visual acuity, and cognitive function[29]. Self-administered questionnaires included medical history, physical activity, diet, and lifestyle and demographic characteristics.

Race and ethnicity were self-reported and included the following categories: Caucasian; Black/African-American; Asian; Hispanic or Latino; Native Hawaiian/Pacific Islander; American Indian/Alaskan Native. A binary variable for race comparing white and non-white participants was also created to avoid the issue of small cell size in statistical analysis. Weight was measured for each participant without shoes using a balance beam scale, while height was measured with a Harpenden stadiometer (DyFed, UK). Body mass index (BMI) was calculated using both of these measurements.

Self-reported alcoholic beverage consumption was analyzed as current average number of

drinks consumed per week, and smoking history was assessed categorically (current, past, or former smoker). Both prescription and non-prescription medications were brought to the clinic and recorded. Medications of interest included anti-androgen, androgen, statin, hypotensive agents, insulin, and hypoglycemic medications.

Anthropometric measurements were obtained through DEXA, and measurements of interest include total body mass, total body fat mass, total body lean mass, BMI, and total percentage of body fat. In addition to assays for fasting glucose and insulin, other clinical findings such as levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), triglycerides, and total cholesterol were assessed.

Statistical Analysis

Descriptive statistics

Incidence rate for prostate cancer events was calculated as the number of new prostate cancer cases divided by the total number of person-years of follow-up. Person-years were calculated in days from date of study entry to the date of prostate cancer diagnosis, death, or last contact with the participant. Baseline characteristics for men with and without incident prostate cancer were compared using a Pearson's chi-square test for categorical data and Student's t-test for continuous data. Significance level was set to alpha = 0.05. Baseline data were compared across diabetes status (diabetics vs. non-diabetics) and levels of fasting glucose status (untreated diabetic vs. impaired vs. normal). Participants with data on fasting glucose status were categorized as follows: Untreated diabetic (fasting glucose > 126 mg/dl); Impaired (fasting glucose 100-126 mg/dl); Normal (fasting glucose < 100 mg/dl). These cutoff values are generally accepted as clinically significant when diagnosing diabetes and impaired glucose

tolerance[9]. Participants with data on fasting insulin status were categorized as follows: Higher (fasting insulin > 5.5 μIU/mmol); Lower (fasting insulin < 5.5 μIU/mmol). There is no gold standard for clinically significant cutoff values for fasting insulin as it is a test that is not typically performed to diagnose diabetes. Fasting insulin levels which fall into a range which may suggest impairment or diabetes are heavily dependent on the individual's age and race. Due to lack of a cutoff value for fasting insulin levels in the literature for our sample, we chose a cutoff value based on the median, and categorized those levels above the median value to suggest high level and those that fell below the median to suggest low fasting insulin level. Therefore, we recognize that these results will have limitations in interpretation for clinical standards.

In order the analyze the influence of fasting glucose and fasting insulin alone on prostate cancer incidence, those with self-reported diabetes at baseline and those who were taking diabetic medications at baseline were excluded from these analyses. Including baseline diabetics and those who are on medications may lead to an inaccurate analysis of baseline fasting glucose and fasting insulin measures since preexisting disease and diabetes medications influence serum glucose and insulin levels as negative confounders.

Survival Analysis and Cox Proportional Hazards Regression

Cox proportional hazards regression was used to estimate the relative risk of prostate cancer based on each predictor variable. Survival analysis follows the time interval for each participant beginning from baseline to the outcome or other endpoint such as death or loss to follow-up[30].

In using the Cox model to compute hazard ratios (HR), an estimate of relative risk, the baseline hazards cancel out, and the estimate is simply the exponentiation of the sum of each

coefficient times the difference between the set of predictors for one individual and the set of predictors for the other individual. To assess the association between fasting glucose status and risk of prostate cancer, two new binary variables were created. The first binary variable analyzed those who were untreated diabetics as determined by their fasting glucose level, compared to those who had a normal level of fasting glucose. The second binary variable analyzed those who were at an impaired level of fasting glucose compared to those who had a normal level of fasting glucose. We created an additional binary variable to assess the relationship of fasting insulin status on incident prostate cancer in those with higher and lower insulin levels. To assess the association between diabetes and prostate cancer risk, there is only one predictor of interest: exposure to diabetes and unexposed to diabetes. Hazard ratios were presented along with confidence intervals and p-values to assess the significance of association. Separate models were created for each predictor variable. Additional analyses investigated the association between diabetic and impaired levels of fasting glucose, fasting insulin levels, and prostate cancer risk.

Covariate/Confounder Assessment

Confounders are variables that are associated with both the exposure and the outcome, but are not on the causal pathway between the exposure and outcome. To assess for confounding, a single covariate was added to the crude Cox Proportional Hazards model for each exposure of interest: diabetes, fasting glucose level for untreated diabetics, impaired level of fasting glucose, and higher fasting insulin level. If the addition of the covariate to the crude model changed the crude hazard ratio estimate of the primary exposure by more than 10% in either direction, then the covariate is considered a confounder.

All covariates from the baseline visit were analyzed as potential confounders and assessed through univariate Cox Proportional Hazard models. Confounders identified in previous studies include age, race, alcohol consumption, and BMI[3]. Because many anthropometric measurements such as total body mass and trunk fat mass, accurately capture levels of obesity, all of these covariates, which included total body mass, total body fat mass, total body lean mass, and trunk fat mass, were analyzed as potential confounders. In addition, few studies have analyzed lipid measures, such as HDL, as potential confounders. From a biological standpoint, cholesterol and other lipid measures play a role in glucose metabolism and may also influence initiation or progression of cancer. For this reason, lipid measures were also analyzed as potential confounders through univariate Cox proportional hazards models. Other known factors to influence diabetes or fasting glucose and insulin, and prostate cancer include statins, antihypertensive medications, diabetes medications, and family history of prostate cancer. All of these were assessed as both categorical and continuous covariates where applicable, and each was added to the univariate model separately. To test for the significance of possible interaction terms, the likelihood ratio (LR) statistic was used where a p-value of less than 0.05 indicates significant interaction. Interaction terms were modeled in an additive fashion.

Multivariable Modeling

Prior to multivariable modeling for the main predictors of our exposures of interest, collinearity was assessed among the identified statistically and biologically significant covariates and confounders that were similar in nature. For example, LDL may be collinear with triglycerides as both are lipid components of total cholesterol. Collinearity of all significant covariates was assessed using Pearson's correlation coefficient analysis. For two covariates that exhibit collinearity, the covariate which has a clearer physiologic role to the main predictor and

outcome was included in the final model. The final Cox proportional hazard regression models included the main predictor of interest as well as all statistically significant and biologically significant confounding terms. Each model was assessed for fit by analyzing Schoenfield residuals.

The final model for high fasting glucose level in untreated diabetics as a predictor of incident prostate cancer was adjusted for age, race, antidepressant and corticosteroid use as categorical variables, and LDL and triglycerides as continuous measures. The final model for impaired fasting glucose level as a predictor of incident prostate cancer was adjusted for age, race, antidepressant use, and corticosteroid use, all as categorical variables. The final model for higher fasting insulin level was adjusted for age, race, and HDL all as categorical variables. The final model for diabetes as a predictor of incident prostate cancer was adjusted for age, race, alcohol use, HDL. All of these confounders were analyzed as categorical variables.

Data were analyzed using Stata Statistical Software version 10.0

Chapter 3

Results

Table 5 presents baseline characteristics of the 5,995 MrOS study participants evaluated in this study. Men in the MrOS study were generally white (90.76%) and overweight (BMI 27.38 kg/m^2). Of the participants who self-reported their smoking status (n=5,559), 3.40% were current smokers. At the baseline exam, 10.89% of participants had self-reported diabetes and 11.79% had a diagnosis of prostate cancer (n=707). The operational definition of diabetes used in this analysis was an individual who self-reported diabetes at baseline or used hypoglycemic medications or insulin, or had a serum fasting glucose greater than or equal to 126 mg/dl. Those with prostate cancer at baseline were excluded from all analyses.

Table 5: Selected characteristics of the MrOS study population used in this analysis

		Mean ± SD (%)
Age		73.66 ± 5.87
Race (%)		
	White	90.76
	Non-White	9.24
Education (%)		
	Elementary	1.9
	High school	21.93
	College	41.22
	Graduate school	34.95
Currently smoking (%)		3.40
Family history of prostate cancer (%)		14.60
BMI (kg/m^2)		27.38 ± 3.83
Diabetes (%)		10.89
Hypoglycemic medication (%)		8.01
Insulin use (%		1.33

Descriptive analyses results

Table 6a presents results of a similar descriptive statistical analysis with the main predictor as various levels of fasting glucose (untreated diabetics, impaired, and normal). Here, we excluded the participants with self-reported diabetes and those who take insulin and hypoglycemic medications. There are many statistically significant differences among baseline demographic characteristics for those with untreated diabetes, impaired, or normal level of fasting glucose. The percentage of current smokers was low among those with untreated diabetes, impaired, and normal level of fasting glucose but those in the impaired fasting glucose group were more likely to be past smokers than those in untreated diabetes and normal fasting glucose groups ($p=0.0003$). The average consumption of alcohol measured by drinks per week was slightly higher in the impaired fasting glucose group compared to untreated diabetes and normal fasting glucose groups ($p=0.008$). Among men with an impaired level of fasting glucose, the reported percentage of family history of prostate cancer was higher (14.1%) compared to those with normal (13.9%) and untreated diabetes fasting glucose (10.9%), but these differences were not statistically significant ($p=0.163$). The common statistically significantly different demographic characteristics for all levels of fasting glucose and prostate cancer were age as a categorical variable, alcohol as a categorical variable, and family history of prostate cancer.

TABLE 6A: Comparison of Baseline Demographics across Fasting Glucose levels

Demographic	Fasting glucose level			P
Mean ± SD, N(%)	Untreated Diabetic (%) N=603	Impaired (%) N=1804	Normal (%) N=2280	
Age	73.9 ± 5.6	73.3 ± 5.8	73.3 ± 5.9	0.03
Age, years				0.144
64-69	164 (27.2)	553 (30.7)	745 (32.6)	
70-74	178 (29.5)	546 (30.3)	640 (28.0)	
75-79	144 (23.9)	425 (23.6)	524 (22.9)	
>80	117 (19.4)	280 (15.5)	377 (16.5)	
Race				<0.001
White	512 (84.9)	1653 (91.6)	2142 (93.7)	
Non-White	91 (15.1)	151 (8.4)	144 (6.3)	
Highest Level of Education				<0.001
Elementary	9 (1.5)	34 (1.9)	47 (2.1)	
High school	118 (19.6)	425 (23.6)	452 (19.8)	
College	259 (43.0)	764 (42.4)	913 (40.0)	
Graduate School	217 (36.0)	581 (32.2)	874 (38.2)	
Smoking status				0.0003
Never	239 (39.6)	622 (34.5)	909 (39.8)	
Past	344 (57.0)	1114 (61.8)	1289 (56.4)	
Current	20 (3.3)	68 (3.8)	87 (3.8)	
Alcohol	4.1 ± 6.2	4.8 ± 7.1	4.2 ± 6.4	0.008
Average drinks/week				0.047
<10	521 (86.4)	1514 (84.0)	1982 (86.9)	
10-20	65 (10.8)	196 (10.9)	220 (9.7)	
>20	17 (2.8)	92 (5.1)	78 (3.4)	
Family history of prostate cancer	66 (10.9)	255 (14.1)	317 (13.9)	0.163

Table 6b compares baseline demographic characteristics with respect to fasting insulin levels among those higher compared to those with lower levels. Many characteristics such as age, race, alcohol consumption, and family history of prostate cancer showed statistically significant differences across each level of fasting insulin.

The distribution of categorical age and categorical alcohol consumption is interesting when comparing the two levels of fasting insulin. Men with higher insulin level were more likely to be in the highest age group of > 80 years (50.8%) compared to only 17.3% of men with

lower level of fasting insulin in this same age group. The majority of men in each insulin level consumed less than 10 alcoholic beverages per week (88.0% in higher group and 85.2% in lower group; p = 0.093). Men with lower fasting insulin levels reported higher percentage of family history of prostate cancer compared to higher levels (16.7% vs. 14.1%; p<0.03). The common statistically significantly different demographic characteristics for all levels of fasting insulin and prostate cancer were age as categorical variable and family history of prostate cancer.

TABLE 6B: Comparison of Baseline Demographics across Fasting Insulin Levels

Demographic	Fasting Insulin level		P
Mean ± SD, N(%)	Higher (%) N=906	Lower (%) N=3702	
Age	72.6 ± 5.5	73.5 ± 5.9	<0.001
Age, years			<0.001
64-69	330 (20.2)	1132 (30.0)	
70-74	279 (17.1)	1085 (28.8)	
75-79	194 (11.9)	899 (23.9)	
>80	829 (50.8)	652 (17.3)	
Race			<0.001
White	811 (87.7)	3496 (97.8)	
Non-White	114 (12.3)	272 (7.2)	
Highest Level of Education			0.487
Elementary	19 (2.05)	71 (1.9)	
High school	193 (20.9)	802 (21.3)	
College	380 (42.0)	1547 (41.1)	
Graduate School	324 (35.0)	1348 (35.8)	
Smoking status			0.19
Never	325 (35.2)	1445 (38.4)	
Past	565 (61.2)	2182 (57.9)	
Current	34 (3.7)	141 (3.7)	
Alcohol	3.8 ± 6.3	4.62 ± 6.7	0.0017
Average drinks/week			0.093
<10	813 (88.0)	3204 (85.2)	
10-230	80 (8.7)	401 (10.7)	
>20	31 (3.4)	156 (4.2)	
Family history of prostate cancer	104 (14.1)	534 (16.7)	0.03

Table 6c describes demographic characteristics of participants who had what we define as type 2 diabetes compared to those who did not. We also report demographic characteristics among those with and without incident prostate cancer. These comparisons were performed using Pearson's chi-squared test for categorical variables and a Student's t-test for continuous variables. All variables are represented continuously as mean values and as categorical variables using clinically defined cutoff values if available, or quartiles. Percentages for each category of the variable were determined using a two by two table approach. For example, the number of those with diabetes who are 64 to 69 years old is 228. This value is divided by the total number of those with diabetes as the denominator (N = 788); therefore, the percentage is 28.9%.

TABLE 6C: Comparison of Baseline Demographics across Type 2 Diabetes and Incident Prostate Cancer Status

Demographic	Type 2 Diabetes		P	Prostate Cancer		P
Mean	Yes (%)	No (%)		Yes (%)	No (%)	
	N = 788	N=4151		N=375	N=4911	
Age	73.3 ± 5.6	73.6 ± 5.9	0.18	72.2 ± 5.0	73.5± 5.9	<0.001
Age, years			0.44			<0.001
64-69	228 (28.9)	1313 (31.6)		131 (34.9)	1507 (30.7)	
70-74	229 (29.1)	1198 (28.9)		132 (35.2)	1401 (20.5)	
75-79	200 (25.4)	966 (23.3)		75 (20.0)	1169 (23.8)	
>80	131 (16.6)	674 (16.2)		37 (9.9)	834 (17.0)	
Race			<0.001			0.921
White	679 (86.2)	3850 (92.7)		339 (90.4)	4466 (90.9)	
Non-White	109 (13.8)	301 (7.3)		36 (10.6)	445 (9.1)	
Highest level of education			<0.001			0.621
Elementary	20 (2.5)	82 (2.0)		9 (2.4)	95 (1.9)	
High school	235 (29.8)	894 (21.5)		74 (19.7)	1093 (22.3)	
College	314 (39.9)	1695 (40.8)		151 (40.3)	2025 (41.2)	
Graduate School	219 (27.8)	1482 (35.7)		549 (1.5)	1290 (26.2)	
Smoking status			0.017			0.324
Never	269 (34.1)	1556 (37.5)		153 (40.8)	1815 (36.9)	
Past	497 (63.1)	2438 (58.7)		210 (56.0)	2915 (59.4)	
Current	22 (2.8)	156 (3.8)		12 (3.2)	180 (3.7)	
Alcohol, mean ± SD	3.25 ± 6.1	4.46 ± 6.8	<0.001	4.15 ± 6.1	4.25 ± 6.7	0.779
Average drinks/week			0.657			0.102
<10	707 (89.8)	3546 (85.6)		318 (85.5)	4237 (86.4)	
10-20	60 (7.6)	422 (10.2)		40 (10.8)	478 (9.8)	
>20	20 (2.5)	175 (4.2)		14 (3.8)	190 (3.9)	
Family history of prostate cancer	90 (11.4))	573 (13.8))	0.077	69 (18.4)	631 (12.8)	0.005

There were many statistically significant differences in demographic characteristics among those with and without diabetes. The distribution of diabetics to non-diabetics overall differs with respect to binary race ($p<0.001$). Comparing the percentages of non-diabetics to diabetics with regard to smoking status, there appears to be approximately equal percentage across each category although the result of the overall chi-squared test suggest the difference is statistically significant ($p =0.017$). For example, 34.1% of diabetics never smoked and 37.5% of non-diabetics also never smoked. A small percentage of diabetics reported a family history of prostate cancer compared to non-diabetics and this difference was statistically significant (13.6% vs. 86.4%; $p=0.077$) using a liberal alpha value of 0.25

Table 6c also presents a comparison of demographic characteristics among those who did and did not develop prostate cancer. Age was statistically significantly different among those with and without prostate cancer, where those who developed prostate cancer tended to be in the low to mid range (64-74) of age compared to those who did not develop prostate cancer ($p<0.001$). The percentage of those with a family history of prostate cancer was higher in those who developed prostate cancer (18.4%) compared to those who did not develop the disease (12.86%; $p=0.0005$). The common demographic characteristics which were statistically significant across diabetes status and incident prostate cancer status were age as continuous measure and family history of prostate cancer.

Table 7a, **7b**, and **7c** compare anthropometric measurements by fasting glucose, fasting insulin, type 2 diabetes, , and prostate cancer status.

With respect to fasting glucose status, those with untreated diabetes and impaired levels of fasting glucose generally have higher averages of many anthropometric measures (BMI, weight, total body mass, etc.) compared to those with a normal level of fasting glucose ($p<0.001$). Common anthropometric measures which were statistically significantly different across fasting glucose status, and prostate cancer status, include total body lean mass, total body mass, and trunk fat mass using a liberal alpha significance level of 0.25 for the preliminary analysis.

TABLE 7A: Comparison of Select Anthropometric measures across Fasting Glucose levels

Characteristic	Fasting glucose level			P
Mean ± SD or N(%)	**Untreated Diabetic** (%) N=603	**Impaired** (%) N=1804	**Normal** (%) N=2280	
BMI (kg/m^2)	27.7 ± 28.3	28.0 ± 28.0	26.4 ± 26.4	<0.001
Underweight	4 (6.6)	12 (6.6)	33 (1.4)	<0.001
Normal	147 (24.4)	341 (18.9)	764 (33.5)	
Overweight	297 (49.3)	946 (52.5)	1135 (49.8)	
Obese	145 (24.0)	469 (26.0)	315 (13.8)	
Total body fat mass (kg)	22.3 ± 7.3	22.9 ± 7.1	20.0 ± 6.7	<0.001
Q1 (4.32-16.82)	139 (23.1)	336 (18.6)	773 (33.9)	<0.001
Q2 (16.83-20.89)	137 (22.7)	415 (23.0)	611 (26.7)	
Q3 (20.90-25.82)	148 (24.5)	505 (28.0)	485 (21.3)	
Q4 (25.83-61.60)	176 (29.2)	530 (29.4)	397 (17.4)	
Total body lean mass (kg)	58.6 ± 7.9	58.6 ± 7.6	57.1 ± 7.1	<0.001
Q1 (34.32-52.76)	149 (24.7)	404 (22.4)	630 (27.6)	<0.001
Q2 (52.77-57.39)	126 (20.9)	440 (24.4)	611 (26.7)	
Q3 (57.40-62.74)	149 (24.7)	453 (25.1)	562 (24.6)	
Q4 (62.75-98.43)	179 (29.7)	490 (27.2)	465 (20.4)	
Total body mass (kg)	82.3 ± 13.6	83.2 ± 13.0	78.7 ± 11.9	<0.001
Q1 (46.80-72.16)	147 (24.4)	358 (19.9)	709 (31.1)	<0.001
Q2 (72.17-80.13)	138 (22.9)	419 (23.3)	627 (27.5)	
Q3 (80.14-89.00)	134 (22.2)	476 (26.4)	534 (23.4)	
Q4 (89.01-137.08)	184 (30.5)	534 (29.6)	397 (17.4)	
Trunk fat mass (g)	1.27E3 ± 4.4E3	1.29E4 ± 4.2E3	1.11E4 ± 2.05E3	<0.001
Q1 (< 9.2E3)	137 (22.7)	330 (18.3)	758 (33.2)	<0.001
Q2 (9.21E3-1.2E4)	137 (22.7)	425 (23.6)	625 (27.4)	
Q3 (1.21E4-1.43E4)	149 (24.7)	501 (27.8)	509 (22.3)	
Q4 (1.44E4-3.2E4)	180 (30.0)	533 (29.6)	379 (16.6)	
Trunk Fat Mass/Total body fat mass	566.4	565.1	550.3	<0.001

TABLE 7B: Comparison of Select Anthropometric measures across Fasting Insulin levels

Characteristic	Fasting Insulin level		P
Mean ± SD or N(%)	Higher	Lower	
	(%) N=906	(%) N=3702	
BMI (kg/m^2)	29.2 ± 4.3	26.0 ± 3.4	<0.001
Underweight	4 (0.4)	45 (1.2)	<0.001
Normal	133 (14.7)	1119 (30.2)	
Overweight	410 (45.3)	1968 (53.2)	
Obese	359 (39.6)	570 (15.4)	
Total body fat mass (kg)	25.3 ± 8.0	20.5 ± 6.4	<0.001
Q1 (4.32-16.82)	139 (14.2)	1118 (29.9)	<0.001
Q2 (16.83-20.89)	161 (17.5)	1002 (26.8)	
Q3 (20.90-25.82)	227 (24.7)	911 (24.4)	
Q4 (25.83-61.60)	400 (43.6)	703 (18.8)	
Total body lean mass (kg)	60.4 ± 8.0	57.2 ± 7.0	<0.001
Q1 (34.32-52.76)	156 (17.0)	1027 (27.5)	<0.001
Q2 (52.77-57.39)	191 (20.8)	986 (26.4)	
Q3 (57.40-62.74)	231 (25.1)	933 (25.0)	
Q4 (62.75-98.43)	342 (37.2)	792 (21.2)	
Total body mass (kg)	87.1 ± 14.6	79.4 ± 11.6	<0.001
Q1 (46.80-72.16)	137 (14.9)	1077 (28.8)	<0.001
Q2 (72.17-80.13)	177 (19.2)	1007 (26.9)	
Q3 (80.14-89.00)	213 (23.2)	931 (25.0)	
Q4 (89.01-137.08)	393 (42.7)	722 (19.3)	
Trunk fat mass (g)	1.27E3 ± 4.4E3	1.29E4 ± 4.2E3	<0.001
Q1 (< 9.2E3)	129 (14.0)	1096 (29.3)	<0.001
Q2 (9.21E3-1.2E4)	157 (17.1)	1030 (27.5)	
Q3 (1.21E4-1.43E4)	226 (24.6)	933 (24.9)	
Q4 (1.44E4-3.2E4)	408 (44.4)	684 (18.3)	
Trunk Fat Mass/Total body fat mass	566.4	565.1	<0.001

All anthropometric measurements were statistically significantly different among those with higher levels of fasting insulin compared to those with lower levels. All mean values of the covariates seen in this table were higher among those with higher insulin levels compared to those with lower levels. With regard to the distribution of BMI, those in the higher group as well as those in the lower group were more likely to be overweight than obese, underweight, or a normal BMI ($p<0.001$). There were higher percentages of men with anthropometric covariates in the fourth quartile range for men with higher insulin group compared to those with lower insulin levels. Common anthropometric measures which were statistically significantly different across fasting insulin status, and prostate cancer status, include total body lean mass, total body

mass, and trunk fat mass using a conservative alpha significance level of 0.25 for the preliminary analysis.

Comparing those with and without type 2 diabetes, all of the covariates presented in this table were statistically significant. All variables are presented as continuous measures and as quartiles with the exception of body mass index (BMI) which was separated into clinically defined categories of underweight, normal, overweight, and obese. In addition, those with diabetes tended to have higher averages of anthropometric characteristics (BMI, weight, total body fat mass, etc.) compared to those without diabetes ($p<0.001$). We do not see a similar trend across prostate cancer status. In fact, many anthropometric measurements were not different between those with and without prostate cancer using an alpha significance level of 0.05.

TABLE 7C: Comparison of Select Anthropometric measures across Type 2 Diabetes and Incident Prostate Cancer status

Characteristic mean ± SD, or N(%)	Type 2 Diabetes		P	Prostate Cancer		P
	Yes (%) N=778	No (%) N=4151		Yes (%) N=375	No (%) N=4910	
BMI (kg/m^2)	29.0 ± 4.2	27.1 ± 3.7	<0.001	27.55 ± 3.7	27.36 ± 3.8	0.362
Underweight	6 (0.8)	46 (1.1)	<0.001	2 (0.5)	53 (1.1)	0.622
Normal	121 (15.6)	1123 (27.1)		91 (24.2)	1253 (25.5)	
Overweight	361 (46.4)	2116 (51.0)		190 (50.7)	2469 (50.3)	
Obese	290 (37.3)	791 (19.1)		87 (23.2)	1048 (21.3)	
Total body fat mass (kg)	86.0 ± 7.5	80.6 ± 7.0	<0.001	21.7 ± 6.9	22.7 ± 7.1	0.827
Q 1 (4.32-16.82)	122 (15.5)	1128(27.2)	<0.001	97 (25.9)	1248 (25.4)	0.213
Q2 (16.83-20.89)	167 (21.2)	1048(25.2)		79 (21.1)	1226 (25.0)	
Q3 (20.90-25.82)	195 (24.7)	1000(24.1)		105 (28.0)	1176 (24.0)	
Q4 (25.83-61.60)	299 (37.9)	936 (22.5)		93 (24.8)	1217 (24.8)	
Total body lean mass (kg)	60.1 ± 8.0	57.8 ± 7.3	<0.001	58.7 ± 7.3	58.1± 7.5	0.128
Q1 (34.32-52.76)	141 (17.9)	1051(25.3)	<0.001	76 (20.3)	1211 (24.7)	0.21
Q2 (52.77-57.39)	155 (19.7)	1070(25.8)		103 (27.5)	1196 (24.4)	
Q3 (57.40-62.74)	208 (26.4)	1030(24.8)		93 (24.8)	1228 (25.0)	
Q4 (62.75-98.43)	280 (35.5)	965 (23.2)		101 (26.9)	1239 (25.2)	
Total body mass (kg)	86.0 ± 13.7	80.6 ± 12.5	<0.001	82.20 ± 12.6	81.34 ± 12.9	0.215
Q1 (46.80-72.16)	127 (16.1)	1087(26.2)	<0.001	75 (20.0)	1236 (25.2)	0.127
Q2 (72.17-80.13)	141 (17.9)	1063(25.6)		100 (26.7)	1196 (24.4)	
Q3 (80.14-89.00)	211 (26.8)	1025(24.7)		103 (27.5)	1205 (24.5)	
Q4 (89.01-137.08)	305 (38.7)	940 (22.6)		95 (25.3)	1236 (25.2)	
Trunk fat mass (g)	1.4E4 ± 4.4E3	1.2E4 ± 4.4E3	<0.001	1.2E4 ± 4.2E3	1.2E4 ± 4.3E4	0.435
Q1 (< 9.2E3)	109 (13.8)	1110(26.7)	<0.001	91 (24.3)	1222 (24.9)	0.227
Q2 (9.21E3-1.2E4)	152 (19.3)	1069(25.7)		80 (21.3)	1233 (25.1)	
Q (1.21E4-1.43E4)	207 (26.3)	1020(24.6)		107 (28.5)	1206 (24.6)	
Q4 (1.44E4-3.2E4)	316 (40.1)	922 (22.2)		96 (25.6)	1217 (24.8)	
Trunk Fat Mass/Total body fat mass, mean	577.1	556.6	<0.001	565.6	559.8	0.049

Lipid profile measures and medication use for different levels of fasting glucose are presented in **Table 8a**. Lipid profile measures were all statistically significantly different across all three fasting glucose groups. Mean values of total cholesterol, LDL, and HDL were all lower for those with untreated diabetes and impaired levels compared to those with normal fasting glucose levels ($p<0.0001$). However, mean triglycerides was much higher in those with untreated diabetics and impaired levels compared to normal fasting glucose ($p<0.0001$). There was an interesting distribution of participants who fell into various levels of LDL. For example, in the untreated diabetics fasting glucose group, the majority of men had very high LDL (57.5%) but in the impaired fasting glucose group, the majority of men had borderline high LDL (37.4%). Another interesting trend was seen for HDL, where the untreated diabetics fasting glucose group had a greater distribution of men in the optimal level of HDL but those in impaired level fell mostly in the high risk HDL category. For fasting insulin levels, there was a greater percentage of those who fell into the highest quartile of fasting insulin for those who were untreated diabetic and impaired compared to those with normal glucose tolerance (84.6% vs. 33.8% vs. 15.3%; $p< 0.001$). There were small percentages of men who used antidepressants, androgens, and hypotensive medications. Approximately 25% of men within each level of fasting glucose used statins.

TABLE 8A: Comparison of Select Clinical Findings across Fasting Glucose levels

Clinical measure or medication use	Fasting Glucose Level			P
Mean ± SD or N(%)	Untreated Diabetic (%) N=603	Impaired (%) N=1804	Normal (%) N=2280	
Total cholesterol (mg/dl)	192.8 ± 35.4	193.8 ± 34.0	195.2 ± 34.1	0.271
Desirable (< 200)	161 (26.7)	1064 (59.0)	1276 (56.0)	<0.001
Borderline (200-239)	73 (12.1)	555 (30.8)	765 (33.6)	
High Risk (>240)	368 (61.0)	175 (9.7)	237 (10.4)	
LDL (mg/dl)	110 ± 31.6	114.4 ± 31.0	117.5 ± 30.2	<0.001
Optimal (<100)	103 (17.1)	571 (31.6)	631 (27.7)	<0.001
Near Optimal (100-129)	88 (14.6)	674 (37.4)	887 (38.9)	
Near High (130-159)	49 (8.1)	411 (22.8)	557 (24.4)	
High (160-189)	16 (2.7)	120 (6.7)	167 (7.3)	
Very High (>190)	347 (57.5)	28 (1.6)	44 (1.9)	
HDL (mg/dl)	45.8 ± 14.0	47.7 ± 13.5	51.3 ± 15.2	<0.001
High risk (<40)	107 (17.7)	605 (33.6)	534 (23.4)	<0.001
Less Risk (50-59)	42 (7.0)	377 (20.9)	532 (23.3)	
Optimal (>60)	380 (63.0)	303 (16.8)	529 (23.2)	
Triglyceride (mg/dl)	188 ± 134.4	158.2 ± 100.1	132.3 ± 77.4	<0.001
Normal (<150)	118 (20.0)	1060 (58.8)	1642 (72.0)	<0.001
Borderline high (150-199)	65 (10.8)	332 (18.4)	319 (14.0)	
High (200-499)	68 (11.3)	380 (21.1)	295 (12.9)	
Very high (>500)	352 (58.4)	32 (1.8)	30 (1.3)	
HOMA	97.7 ± 110.5	50.5 ± 32.2	31.2 ± 21.1	<0.001
Fasting Insulin (µIU/mmol)	14.8 ± 16.3	10.5 ± 6.3	7.6 ± 4.8	< 0.001
Q1 (0-5.2)	25 (4.2)	255 (14.4)	801 (35.4)	<0.001
Q2 (5.3-7.5)	23 (3.8)	436 (24.7)	614 (27.2)	
Q3 (7.6-11.1)	44 (7.4)	478 (27.1)	500 (22.1)	
Q4 (>11.2)	507 (84.6)	598 (33.8)	346 (15.3)	
Antiandrogen use	1 (0.2)	0 (0.0)	0 (0.0)	0.121
Androgen use	9 (1.5)	15 (0.8)	25 (1.1)	0.677
Hypotensive Use	30 (5.0)	85 (4.7)	71 (3.1)	<0.001
Statin use	167 (27.7)	471 (26.1)	525 (23.0)	<0.001
Hypoglycemic	0 (0.00)	0 (0.0)	0 (0.0)	<0.001
Insulin use	0 (0.0)	0 (0.0)	0 (0.0)	0.163
Antidepressant use	31 (5.1)	108 (6.0)	114 (5.0)	0.345
Corticosteroid use	17 (2.8)	25 (1.4)	50 (2.2)	0.05

Lipid profile measures and medication use for different levels of fasting insulin are presented in **Table 8b**. Lipid profile measures were all statistically significantly different across all three fasting insulin groups. While mean values of total cholesterol, LDL, and HDL were lower for those in the higher fasting insulin group compared to those with lower insulin levels, mean triglycerides were much higher in the higher fasting insulin group compared to those with lower insulin, and these were statistically significant differences ($p<0.001$). A greater percentage

of men in the higher group were more likely to have optimal HDL compared to those with lower insulin level in which HDL levels were more or less equally distributed. Antiandrogen, hypotensive, statin, antidepressant, and corticosteroid use were all statistically significantly different among insulin levels using a conservative alpha level of 0.25.

TABLE 8B: Comparison of Select Clinical Findings across Fasting Insulin levels

Clinical measure or medication use	Fasting Insulin Level		
Mean ± SD or N(%)	**Higher** (%) N=603	**Lower** (%) N=1804	**P**
Total cholesterol (mg/dl)	191.7 ± 34.4	194.9 ± 34.2	0.037
Desirable (< 200)	351 (38.0)	2150 (57.3)	<0.001
Borderline (200-239)	187 (20.2)	1206 (32.2)	
High Risk (>240)	386 (41.8)	394 (10.5)	
LDL (mg/dl)	109.5 ± 31.5	116.8 ± 30.3	<0.001
Optimal (<100)	216 (23.4)	1089 (28.9)	<0.001
Near Optimal (100-129)	216 (23.4)	1433 (38.0)	
Near High (130-159)	120 (13.0)	897 (23.8)	
High (160-189)	23 (2.5)	280 (7.4)	
Very High (>190)	350 (37.8)	69 (1.8)	
HDL (mg/dl)	41.3 ± 11.8	50.7 ± 14.6	<0.001
High risk (<40)	311 (40.9)	935 (35.0)	<0.001
Less Risk (50-59)	63 (8.3)	888 (33.5)	
Optimal (>60)	286 (50.8)	826 (31.2)	
Triglyceride (mg/dl)	205.8 ± 128.3	137.2 ± 80.6	<0.001
Normal (<150)	218 (23.6)	2602 (69.1)	<0.001
Borderline high (150-199)	143 (15.5)	573 (15.2)	
High (200-499)	197 (21.3)	546 (14.5)	
Very high (>500)	367 (39.7)	47 (8.8)	
HOMA	97.7 ± 110.5	50.5 ± 32.2	<0.001
Antiandrogen use	0(100.0)	0 (100.0)	0.121
Androgen use	10 (1.1)	39 (1.1)	0.925
Hypotensive Use	50 (5.6)	136 (3.8)	0.014
Statin use	260 (29.1)	903 (25.0)	0.014
Antidepressant use	101 (6.5)	201 (5.7)	0.246
Corticosteroid use	42 (2.7)	68 (1.9)	0.075

Clinical findings across type 2 diabetes status and prostate cancer status are displayed in **Table 8c**. Fasting lipid profile measures include total cholesterol, HDL, LDL, and triglycerides.

All of these measurements were statistically significantly different between type 2 diabetics and non-diabetics.

TABLE 8C: Comparison of Select Clinical Findings across Type 2 Diabetes and Incident Prostate Cancer status

Characteristic	Type 2 Diabetes		P	Prostate Cancer		P
Mean ± SD or N(%)	Yes (%) N=788	No (%) N=4151		Yes (%) N=375	No (%) N=4910	
Total cholesterol (mg/dl)	184.5 ± 34.9	194.6 ± 33.9	<0.001	194.0 ± 36.1	193.0 ± 34.2	0.583
Desirable (< 200)	529 (67.1)	2373 (57.2)	<0.001	211 (56.3)	2695 (54.9)	0.989
Borderline (200-239)	183 (23.2)	1340 (32.3)		106 (28.3)	1418 (28.9)	
High Risk (>240)	75 (9.5)	419 (10.1)		58 (15.5)	778 (15.8)	
LDL (mg/dl)	102.9± 31.1	116.1± 30.5	<0.001	114.3 ± 31.7	114.0 ± 30.9	0.862
Optimal (<100)	366 (46.4)	1222 (29.4)	<0.001	112 (29.9)	1481 (30.2)	0.857
Near Optimal (100-129)	255 (32.4)	1581 (38.1)		138 (36.8)	1697 (34.6)	
Near High (130-159)	101 (12.8)	982 (23.7)		70 (18.7)	1015 (20.7)	
High (160-189)	36 (4.7)	291 (7.0)		22 (5.9)	305 (6.2)	
Very High (>190)	30 (3.8)	75 (1.8)		33 (8.8)	413 (8.4)	
HDL (mg/dl)	43.8 ± 13.6	49.7 ± 14.6	<0.001	114.3 ± 16.5	114.0 ± 14.4	0.862
High risk (<40)	354 (62.5)	1156 (39.5)	<0.001	103 (37.9)	1412 (39.6)	0.813
Less Risk (50-59)	109 (30.8)	921 (31.5)		73 (33.6)	958 (26.9)	
Optimal (>60)	103 (29.1)	848 (29.0)		96 (35.3)	1197 (35.3)	
Triglyceride (mg/dl)	189.50 ± 133.7	143.9 ± 88.1	<0.001	148.0 ± 86.5	151.3 ± 99.0	0.539
Normal (<150)	360 (45.7)	2733 (65.8)	<0.001	224 (59.7)	2871 (58.5)	0.505
Near high (150-199)	159 (20.2)	666 (16.0)		57 (15.2)	770 (15.7)	
High (200-499)	217 (27.6)	688 (16.6)		69 (18.4)	838 (17.1)	
Very high (>500)	52 (6.6)	64 (1.5)		25 (6.7)	432 (8.8)	
HOMA	86.5 ± 99.7	39.7 ± 27.6	<0.001	45.9 ± 40.9	46.6 ± 49.4	0.802
Fasting Insulin (µIU/mmol)	13.1 ± 12.4	8.9 ± 5.7	<0.001	9.6 ± 6.8	9.5 ± 7.3	0.809
Q1 (0-5.3)	119 (15.2)	1,056 (25.8)	<0.001	76 (20.5)	1,099 (22.7)	0.216
Q2 (5.4-7.7)	126 (16.1)	1,056 (25.8)		92 (24.9)	1,089 (22.5)	
Q3 (7.8-11.4)	154 (19.7)	1,016 (24.9)		94 (25.4)	1,075 (22.2)	
Q4 (>11.5)	384 (49.0)	960 (23.5)		108 (29.2)	1,585 (32.7)	
Antiandrogen use	1 (0.1)	0 (0.0)	0.749	0 (0.0)	1 (0.00)	0.784
Androgen use	10 (1.3)	41 (1.0)	0.305	4 (1.1)	52 (1.1)	0.963
Hypotensive Use	70 (8.9)	157 (3.8)	<0.001	19 (5.1)	218 (4.4)	0.523
Statin use	270 (34.3)	1011 (24.4)	<0.001	112 (29.9)	1265 (25.8)	0.05
Hypoglycemic use	434 (55.1)	0 (0.0)	<0.001	19 (5.1)	415 (8.5)	0.026
Insulin use	71 (9.0)	0 (0)	<0.001	5 (1.3)	66 (1.3)	0.984
Antidepressant use	63 (8.0)	224 (5.4)	0.004	19 (5.1)	285 (5.8)	0.63
Corticosteroid use	18 (2.3)	78 (1.9)	0.439	3 (0.8)	104 (2.1)	0.086

Most notably, there was a high percentage of diabetics that had low HDL (62.5%). However, comparing diabetics to non-diabetics, the average HDL is very similar but still statistically significantly different (43.8 mg/dl vs. 49.7 mg/dl; $p<0.0001$). Non-diabetics tended to have higher averages of total cholesterol and LDL, but lower mean level of triglycerides ($p<0.0001$ for all). There were statistically significant differences across fasting insulin levels

between type 2 diabetics and non-diabetics as expected, where those with type 2 diabetes had higher fasting insulin levels than non-diabetics ($p<0.001$).

There were no statistically significant differences between those with and without prostate cancer for lipid profile or fasting insulin levels. In terms of medication use, there was a very small percentage of diabetics who used antiandrogens, androgens, insulin, and antidepressants but a larger percentage were taking statins and hypoglycemic medications. There were statistically significant differences among medication use between diabetics and non-diabetics ($p<0.0001$) with the exception of oral corticosteroid use which was not statistically significant ($p=0.439$). Statin use, hypoglycemic medication, and oral corticosteroid use were the only categories of medications where there was a statistically significant difference between those with and without prostate cancer.

Overall, the greatest variation in covariates was seen among clinical findings and medication use when comparing diabetics to non-diabetics, when comparing men who had untreated diabetic, impaired, and normal fasting glucose levels, and when comparing men who had higher and lower fasting insulin levels. By this, we mean the greatest number of statistically significant differences, judged by $p<0.25$, were seen among covariates which fell within the category of clinical findings and medication use. For the comparison of prostate cancer and non-prostate cancer, the greatest variation in covariates was found among anthropometric measurements.

Confounder and interaction variable assessment

To assess the relationship between glucose tolerance and prostate cancer for potential confounders, we used Cox Proportional Hazards regression with incident prostate cancer as the

outcome, fasting glucose or fasting insulin levels as the main effect, and the covariates seen in **Tables 6a-8a** as the covariate. We conducted this analysis for both continuous and categorical variables. Adding a single covariate to the model regressed with untreated type 2 diabetes, we obtained the hazard ratio for diabetes and compared this with the crude diabetes hazard ratio through a percent change. Confounders were recognized as those covariates, which changed the main predictor by more than 10%. We then assessed each covariate as an effect modifier or interaction variable. By definition, effect modification exists when the effect estimate between a single exposure and outcome differ by levels of the third factor. This third factor is the effect modifier. Interaction terms were created by multiplying the covariate by the main predictor, and statistical significance was assessed by including the main predictor, single covariate, and interaction term in the Cox Proportional Hazards model. If the hazard ratio of the main predictor, and the interaction term did not have statistical significance, we concluded that the particular covariate was not an effect modifier. These results are summarized in **Table 9c.**

Table 9a and **9b** display confounder variable assessment for untreated diabetic fasting glucose level, and the impaired fasting glucose level. A similar process is followed for fasting glucose level, however we created a binary variable for untreated diabetics fasting glucose level and impaired glucose level. The covariates are ranked according to the greatest difference in percent change compared to the crude estimate of the hazard ratio.

TABLE 9A: Confounder assessment using Cox Proportional Hazards Regression Univariate Modeling for the Relationship between Fasting glucose level in Untreated Diabetics and Incident Prostate Cancer

Potential confounder	Untreated Diabetic	95% CI	% Difference from crude	Interaction Term (Y/N)
	Fasting Glucose HR			
Total cholesterol continuous	**0.43**	**0.20, 0.87**	**35.8**	N
Triglycerides continuous	**0.45**	**0.22, 0.92**	**32.8**	N
Race	**0.79**	**0.58, 1.07**	**17.9**	N
Antidepressant	**0.75**	**0.50, 1.12**	**11.9**	N
LDL group	**0.59**	**0.38, 0.93**	**11.9**	N
Coricosteroid use	**0.74**	**0.50, 1.10**	**10.4**	N
Antiandrogen use	0.73	0.49, 1.09	9	N
Androgen use	0.73	0.49, 1.10	9	N
Hypotensive medication use	0.73	0.49, 1.10	9	N
Statin use	0.72	0.49, 1.08	7.5	N
Family history of PC	0.71	0.46, 1.10	6	N
Age group	0.69	0.47, 1.03	3	N
HDL Group	0.69	0.48, 0.98	3	N
Age continuous	0.69	0.47, 1.03	3	N
White	0.65	0.44, 0.97	3	N
Total cholesterol	0.65	0.42, 1.00	3	N
Alcohol	0.68	0.46, 1.00	1.5	N
Triglycerides	0.68	0.42, 1.10	1.5	N
BMI	0.68	0.46, 1.02	1.5	N
Alcohol continuous	0.68	0.46, 1.00	1.5	N
Total body fat continuous	0.68	0.46, 1.01	1.5	N
Total body mass	0.66	0.45, 0.98	1.5	N
Trunk fat mass	0.66	0.45, 0.99	1.5	N
Total body lean continuous	0.66	0.45, 0.99	1.5	N
Total lean mass	0.67	0.45, 0.99	0	N
Smoking status	0.67	0.45, 0.99	0	N
Total body fat	0.67	0.45, 1.01	0	N
Education	0.67	0.45, 1.00	0	N
BMI continuous	0.67	0.45, 0.99	0	N
Total body mass continuous	0.67	0.45, 0.99	0	N
Trunk fat mass continuous	0.67	0.45, 1.00	0	N
Crude	0.67	0.45, 1.00	0	N

TABLE 9B: Confounder assessment using Cox Proportional Hazards Regression Univariate Modeling for the Relationship between Impaired Fasting glucose level and Incident Prostate Cancer

Potential confounder	Untreated diabetic fasting glucose HR	95% CI	% Difference from crude	Interaction present (Y/N)?
Corticosteroid use	**0.74**	**0.50, 1.10**	**36.8**	N
Antidepressant use	**0.75**	**0.50, 1.12**	**35.9**	N
Antiandrogen use	1.22	0.97, 1.53	4.3	N
Androgen use	1.22	0.97, 1.53	4.3	N
Hypotensive med use	1.22	0.97, 1.53	4.3	N
Total body mass	1.12	0.90, 1.41	4.3	N
Statin use	1.21	0.96, 1.52	3.4	N
Race	1.13	0.91, 1.41	3.4	N
Trunk fat mass	1.14	0.91, 1.43	2.6	N
BMI continuous	1.14	0.91, 1.43	2.6	N
Trunk fat mass continuous	1.14	0.90, 1.43	2.6	N
BMI	1.15	0.91, 1.44	1.7	N
Total body fat	1.15	0.92, 1.45	1.7	N
HDL continuous	1.19	0.95, 1.49	1.7	N
Total body mass continuous	1.15	0.91, 1.44	1.7	N
Smoking status	1.18	0.94, 1.47	0.9	N
White	1.16	0.93, 1.45	0.9	N
Total cholesterol	1.16	0.93, 1.46	0.9	N
Triglycerides continuous	1.18	0.94, 1.47	0.9	N
Total body fat continuous	1.16	0.92, 1.46	0.9	N
Total body lean mass continuous	1.16	0.93, 1.45	0.9	N
Alcohol continuous	1.18	0.94, 1.47	0.9	N
Alcohol	1.17	0.93, 1.46	0	N
Height continuous	1.17	0.93, 1.46	0	N
HDL	1.17	0.90, 1.53	0	N
Total lean mass	1.17	0.94, 1.47	0	N
Family history of PC	1.17	0.92, 1.49	0	N
Age	1.17	0.93, 1.46	0	N
Age continuous	1.17	0.93, 1.46	0	N
total cholesterol continuous	1.17	0.94, 1.46	0	N
LDL	1.17	0.93, 1.46	0	N
Triglycerides	1.17	0.93, 1.46	0	N
LDL continuous	1.17	0.94, 1.46	0	N
Crude	1.17	0.93, 1.46	0	N

LDL, total cholesterol, triglycerides (all as continuous), race, antidepressant use, LDL (as categorical), and corticosteroid use, were all confounders when untreated diabetic fasting glucose level was the main predictor. Because of high collinearity between LDL as a categorical and continuous variable, we decided to retain LDL as continuous measure in the model because it changed the crude estimate significantly more than the categorical estimate despite the fact that it too was statistically significant.

Corticosteroid and antidepressant use were found to be significant confounders when impaired fasting glucose level was the main predictor.

HDL was the only covariate determined to be a confounder in the relationship between higher fasting insulin level and incident prostate cancer.

TABLE 9C: Confounder assessment using Cox Proportional Hazards Regression Univariate Modeling for the Relationship between Higher Fasting Insulin Level and Incident Prostate Cancer

Potential confounder	HR	95% CI	% Difference from Crude	Interaction present (Y/N)?
HDL	**1.47**	**1.05, 2.06**	**23.5**	N
Trunk fat mass continuous	1.27	0.94, 1.70	6.7	N
Corticosteroid use	1.12	0.84, 1.49	5.9	N
Antidepressant use	1.12	0.84, 1.48	5.9	N
Antiandrogen use	1.12	0.85, 1.49	5.9	N
Total body mass continuous	1.26	0.94, 1.68	5.9	N
Family history of PC	1.12	0.83, 1.52	5.9	N
Androgen use	1.13	0.85, 1.49	5	N
Hypotensive med use	1.13	0.85, 1.50	5	N
Total body mass	1.25	0.93, 1.66	5	N
Race	1.13	0.91, 1.41	5	N
Trunk fat mass	1.25	0.94, 1.67	5	N
Statin use	1.14	0.86, 1.50	4.2	N
BMI continuous	1.14	0.91, 1.43	4.2	N
Total body lean mass continuous	1.24	0.92, 1.65	4.2	N
Total body fat continuous	1.23	0.91, 1.64	3.4	N
Age	1.23	0.93, 1.63	3.4	N
Age continuous	1.23	0.93, 1.63	3.4	N
BMI	1.22	0.91, 1.60	2.5	N
Total body fat	1.22	0.92, 1.62	2.5	N
Total lean mass	1.22	0.92, 1.62	2.5	N
White	1.21	0.91, 1.60	1.7	N
Height continuous	1.17	0.93, 1.46	1.7	N
Total cholesterol	1.18	0.88, 1.57	0.84	N
Triglycerides continuous	1.18	0.94, 1.47	0.84	N
Alcohol continuous	1.2	0.90, 1.58	0.84	N
total cholesterol continuous	1.18	0.84, 1.66	0.84	N
Triglycerides	1.2	0.87, 1.66	0.84	N
LDL continuous	1.18	0.84, 1.67	0.84	N
HDL continuous	1.19	0.95, 1.49	0	N
Smoking status	1.19	0.90, 1.50	0	N
Alcohol	1.19	0.89, 1.57	0	N
LDL	1.19	0.89, 1.60	0	N
Crude	1.19	0.90, 1.58	0	N

For type 2 diabetes as the main predictor, the confounders determined from this statistical perspective were alcohol, HDL, and age (all as categorical variables). We also included race as a confounder presented as a binary variable because this is commonly adjusted for in models found in previous literature as a known risk factor for prostate cancer.

TABLE 9D: Confounder assessment using Cox Proportional Hazards Regression Univariate Modeling for the Relationship between Type 2 Diabetes Mellitus and Incident Prostate Cancer

Potential confounder	Diabetes HR	95% CI	% Difference from crude	Interaction present? (Y/N)
Alcohol group	**1.12**	**0.64, 1.17**	**64.7**	N
HDL group	**0.57**	**0.38, 0.93**	**16.2**	N
Age group	**0.76**	**0.54, 1.09**	**11.8**	N
Statin use	0.66	0.47, 0.92	2.9	N
Trunk fat mass group	0.66	0.47, 0.92	2.9	N
Alcohol continuous	0.66	0.48, 0.92	2.9	N
BMI continuous	0.66	0.47, 0.92	2.9	N
Total body lean mass continuous	0.66	0.48, 0.92	2.9	N
Total body mass continuous	0.66	0.48, 0.92	2.9	N
Trunk fat mass continuous	0.66	0.48, 0.92	2.9	N
Age continuous	0.69	0.50, 0.96	1.5	N
Antidepressant Use	0.69	0.49, 0.97	1.5	N
Family history of prostate cancer	0.67	0.47, 0.96	1.5	N
Total body fat mass continuous	0.67	0.49, 0.94	1.5	N
Total cholesterol continuous	0.67	0.48, 0.94	1.5	N
LDL continuous	0.67	0.48, 0.94	1.5	N
Triglycerides continuous	0.67	0.48, 0.93	1.5	N
BMI	0.67	0.48, 0.95	1.5	N
Total body fat group	0.67	0.48, 0.93	1.5	N
Total lean group	0.67	0.48, 0.93	1.5	N
Total body mass group	0.67	0.48, 0.92	1.5	N
Antiandrogen use	0.67	0.48, 0.98	1.5	N
Androgen use	0.67	0.48, 0.92	1.5	N
BP med use	0.67	0.48, 0.94	1.5	N
Race	0.68	0.49, 0.94	0	N
White	0.68	0.49, 0.94	0	N
Smoking status	0.68	0.49, 0.95	0	N
Total cholesterol	0.68	0.49, 0.93	0	N
LDL	0.68	0.49, 0.93	0	N
Triglycerides	0.68	0.49, 0.93	0	N
HDL continuous	0.68	0.49, 0.95	0	N
Oral corticosteroid use	0.68	0.48, 0.95	0	N
Crude	0.68	0.49, 0.94	0	N

Multivariable modeling for each predictor with incident prostate cancer

All final models are summarized in **Table 10**. These models were assessed for fit through Schoenfield residuals and all models satisfied the proportional-hazards assumption (data not shown here).

Table 10: Prostate cancer incidence by fasting glucose, fasting insulin, and type 2 diabetes status in MrOS, 2000-2008

Predictor	Cases (no.)	Person-years (no.)	Incidence Rate (by 10,000 py)	HR	95% CI	p-value
Fasting glucose level in untreated diabetics*	**29**	**1.5 X 106**	**2.71**	0.51	0.25, 1.07	0.071
Impaired fasting glucose level**	**149**	**4.6 X 106**	**3.82**	1.18	0.94, 1.50	0.16
Normal fasting glucose**	**163**	**5.9 X 106**	**3.25**			
Higher fasting insulin level***	**283**	**9.6 X 106**	**2.94**	1.52	1.09, 2.13	0.008
Normal fasting insulin***	**58**	**2.3 X 106**	**2.47**			
Type 2 Diabetes******	**41**	**1.1 X 107**	**3.7**	0.56	0.37, 0.85	0.007
No Type 2 Diabetes******	**313**	**2.0 X 107**	**15.6**			

*Adjusted for age, race, LDL, triglycerides, antidepressants, and corticosteroid use
**Adjusted for age, race, antidepressants, and corticosteroid use
***Adjusted for age, race, and HDL
****Adjusted for age, race, HDL, and alcohol use (all as categorical)

Age and binary race as categorical variables were included in all models since these two covariates were commonly included in previous literature as known risk factors for prostate cancer. The final model for fasting glucose level in untreated diabetics was adjusted for the following variables: age, binary race, LDL continuous, triglycerides continuous, antidepressants, and corticosteroid use. The HR for high fasting glucose level is 0.51 (95% CI 0.25, 1.07; p = 0.071). The risk of prostate cancer for those with untreated diabetes indicated by high fasting glucose level is 0.51 times the risk of prostate cancer for those who are at a normal level of fasting glucose. This also suggests that there is a non-significant inverse trend for the high level of fasting glucose and prostate cancer risk. However, we cannot reject the null hypothesis because the confident interval contains HR = 1.00. Additional studies are needed to assess this relationship.

The final model for fasting glucose status for the impaired level was adjusted for the following variables: age, binary race, antidepressants, and corticosteroid use. The risk of

prostate cancer among those who have an impaired fasting glucose is 1.18 times the risk of prostate cancer among those with a normal glucose level (95% CI 0.94, 1.50; p =0.161). There is no association between risk of prostate cancer and impaired fasting glucose.

The final model for those with higher fasting insulin levels was adjusted for the following variables: age, binary race, HDL. The risk of prostate cancer among those with higher fasting insulin levels is 1.52 times the risk of prostate cancer among those with lower insulin level (95% CI 1.09, 2.13; p=0.008). This suggests that there is an increased risk of prostate cancer among those with a higher level of insulin and this risk is statistically significant.

The final model for type 2 diabetes was adjusted for the confounders identified through the statistical sense also. To summarize, the final model included the following: alcohol, HDL, age, and race. I did not adjust for hypoglycemic or insulin medications as these were used to identify the diabetes exposure status of participants. The type 2 diabetes hazard ratio (HR) was 0.56 (95% CI 0.37, 0.85; p = 0.007). The risk of prostate cancer among those who have type 2 diabetes is 0.56 times the risk of prostate cancer among those without type 2 diabetes. This suggests that type 2 diabetes may be protective against risk of prostate cancer. Because the confidence interval does not contain the null hypothesis (HR=1.00), we conclude that this estimate is statistically significant.

Chapter 4

Discussion

We used a prospective cohort design and Cox Proportional Hazards regression analysis to address the following aims: to describe the association between levels of fasting glucose and incident prostate cancer; to describe the association between levels of fasting insulin and incident prostate cancer; and to describe the association between baseline type 2 diabetes and incident prostate cancer.

Fasting Glucose and Incident Prostate Cancer

There are many different hypotheses for the etiology of prostate cancer, and we described one predominant hypothesis, which focuses on insulin and IGF-1 as being key players in the development of prostate cancer, in this analysis. Prior to conducting Cox Proportional Hazards regression to address the first study aim, we excluded individuals who self-reported type 2 diabetes at baseline and those who were currently taking insulin or hypoglycemic medications at the study entry because these two characteristics would influence the levels of glucose and insulin in the human body. We found that there was no relationship between fasting glucose levels and incident prostate cancer. This finding was consistent with that of a few case-control and prospective cohort studies[37, 43]. Hubbard and others showed that fasting glucose levels were unrelated to prostate cancer risk in a population of 823 men, and authors concluded that larger prospective studies are warranted to measure these parameters further[24]. There have not been many studies, which examine the relation of fasting glucose levels and prostate cancer risk prospectively. One prospective cohort study by Jee and others examined the influence of serum glucose on prostate cancer mortality adjusted for age, and found a non-significant increase risk

of death from prostate cancer in those with high fasting serum glucose[31]. Due to the lack of previous studies on this subject, we were unaware of a priori confounders that we may force into the final model with the exception of age and binary race.

Fasting Insulin levels and Prostate Cancer

The final model for the relationship between higher fasting insulin level and incident prostate cancer was adjusted for age, race, and HDL. There was an increased risk of prostate cancer associated with individuals who had a fasting insulin level ≥ 5.5 μIU/mmol compared to those with fasting insulin levels less than this median value. This finding was consistent with that of a prospective cohort study of Finnish men[37, 43]. There have been few prospective studies, which examine the relationship between fasting insulin and incident prostate cancer. However, there have been a few case-control studies which found a non-statistically significant increased risk of prostate cancer with increasing levels of fasting insulin adjusted for age and BMI[25, 35]. In a case-control study consisting of Finnish men, Albanes and others found increased insulin levels were associated with statistically significant increased risk of prostate cancer [25]

Type 2 Diabetes and Incident Prostate Cancer

With respect to the third study aim, we found that there was a statistically significant relationship between type 2 diabetes and decreased risk of prostate cancer after adjusting for age, race, alcohol, and HDL. Age and alcohol were both positive confounders while HDL was a negative confounder. Binary race did not influence the crude hazard ratio by more than 10%, but was included as a confounder since it is a known risk factor for both type 2 diabetes and prostate cancer.

While there were statistically significant differences with respect to many anthropometric measurements in diabetics and non-diabetics, this was not the case in comparing those who did and did not develop prostate cancer. Our finding of an inverse association between type 2 diabetes and risk of prostate cancer was previously reported in many other studies as mentioned previously. These include case-control, cross-sectional, and a few prospective cohort studies[9, 10, 44, 45]. For example, Calton and others found a decreased risk of prostate cancer for those with type 2 diabetes in the NIH-AARP prospective cohort trial[41]. However, many previous prospective cohort studies use self-reported diabetes as the exposure, which may not be as accurate as type 2 diabetes captured through multiple methods such as physician confirmed diagnosis, fasting blood glucose test, or use of medications. Because our analysis utilized an operational definition of diabetes using self-reported diabetes, fasting blood glucose above 126 mg/dl, or use of hypoglycemic or insulin medications, it is likely that we are accurately able to capture those who truly have diabetes.

Proposed Biological Mechanism for Results

The biological mechanism for the relationship between fasting glucose, fasting insulin, type 2 diabetes, and prostate cancer continues to be a topic of debate due to inconclusive results from previous animal and human studies. However, there are many hypothesized biological reasons for why we sometimes see a change in the direction of risk of prostate cancer as individuals' progress from pre-diabetic stage (impaired fasting glucose) to development of type 2 diabetes. In this study, we found that there was no relationship between levels of fasting glucose (untreated diabetes, impaired fasting glucose) and prostate cancer risk. However, we did find a significant, positive association between higher fasting insulin levels and prostate cancer risk. One mechanism that may explain the observed association involves the role of IGF-1. As we

previously stated, IGF-1 production is primarily dependent on growth hormone and secondly on nutrition and insulin[11, 13, 14]. IGF-1 is a more potent mitogen with stronger anti-apoptotic activity than insulin, and plays a major role in cell replication[31, 32]. Insulin can increase IGF-1 bioactivity by decreasing the synthesis and plasma levels of IGFBP-1 and IGFBP-2, which are two binding proteins. Therefore perhaps individuals with higher fasting insulin also happen to have increased IGF-1 bioactivity, and hence have increased risk of prostate cancer. However, we cannot say whether or not individuals with higher fasting insulin also have a degree of insulin resistance since typically, fasting insulin is not used to diagnose impaired fasting glucose or type 2 diabetes. In fact, fasting insulin levels may not be different among type 2 diabetics and non-diabetics; type 2 diabetics simply secrete less insulin in response to a glucose load compared to non-diabetics so they exhibit reduced intracellular signaling through insulin[25].

We found that there was a statistically significant inverse association between type 2 diabetes and incident prostate cancer. As we previously stated, type 2 diabetes results from exhaustion of the pancreatic cell resulting in decreased insulin secretion and decreased responsiveness of peripheral tissues to insulin action[25]. Therefore, type 2 diabetics have a combination of decreased insulin secretion and a degree of insulin resistance. Previous studies hypothesize that IGF-1 levels may influence glucose homeostasis. Hepatic insulin resistance and hyperinsulinemia-induced growth hormone resistance are two features which may be common in those with type 2 diabetes[25, 26, 29]. Chronic hyperinsulinemia induces growth hormone receptor resistance and reduces growth hormone expression and signaling at receptor and post-receptor levels[12, 46]. IGF-1 levels are influenced by growth hormone and insulin; with hepatic insulin resistance and hyperinsulinemia-induced growth hormone resistance, IGF-1 levels may be lower in type 2 diabetics compared to non-diabetics[29]. Some epidemiological studies add support to

this biological mechanism: in a population of Swedish men, IGF-1 was inversely correlated to diabetes duration. A cross sectional study showed that circulating IGF-1 levels were lower in type 2 diabetics compared to those with impaired fasting glucose or normal fasting glucose[29]. Some biological characteristics of type 2 diabetes were found to be associated with a decreased risk in prostate cancer, including low levels of IGF-1 and testosterone, increased levels of sex hormone binding globulin (SHBG), and low levels of PSA in some studies[9, 37, 43] compared to non-diabetics.

In summary, we can infer that while higher fasting insulin levels leads to increased risk of prostate cancer, once type 2 diabetes is diagnosed prostate cancer risk decreases in men. Although further studies are needed to establish the biological mechanism for the observed relationship, it is plausible that our findings reflect an inverse "U-shaped" curve between insulin levels and prostate cancer risk such that cancer risk increases during the compensatory rise in insulin (and IGF-1) to development of insulin resistance, but then declines when islet cell compensatory capacity drops off with the progression from impaired fasting glucose to a frankly diabetic state.

Study Limitations

This analysis does have limitations.

Non-differential misclassification bias may have occurred. In this type of bias, inaccuracies or measurement error occurs equally in both exposure and disease groups. In creating categorical variables, such as alcohol consumption or smoking status, random misclassification may have occurred if we did not adequately capture all participants who reported this data. This would result in an underestimation of the true reported association.

Response bias may also be present for variables which were collected through self-assessment questionnaires. This is often pertinent in questions associated with some social stigma, such as alcohol and smoking. It is likely that participants tend to underreport the amount of alcohol they consume, or the number of cigarettes they smoke per week. This would result in either an over or underestimation of the reported association only if smoking status and alcohol use are related to both diabetes or fasting glucose, and prostate cancer. With regard to self-reported type 2 diabetes, it is unlikely that participants will be biased in their response based on the way in which the question was asked, "Has a physician ever diagnosed you with diabetes?" There is no response bias possible in the collection of incident prostate cancer cases since these were recorded by physician and medical records, unless they did not report a diagnosis. There was no physician review of medical records if the participant did not self-report the diagnosis. Undiagnosed prostate cancer in this sample of older men may have occurred, which would result in misclassification bias. Anthropometric and clinical findings were ascertained through blood testing and medical instrument; therefore, these findings would not be subject to response bias, but measurement error is possible. Since this was a multi-centered study, it was important for research staff to follow a consistent protocol in collection of data and chemical assays for lipid measures, and fasting glucose and insulin measures to reduce non-differential misclassification bias.

Loss to follow-up is the biggest disadvantage to prospective cohort studies with long follow-up periods. Non-response, non-participation, and loss to follow-up only biases results if the reasons for loss to follow-up are related to both the exposure and the outcome. One way to assess whether or not this is present is by exploring the distribution of missing values with respect to demographic characteristics, anthropometric measurements, and clinical findings. While there

were different numbers of missing values for each covariate, the distribution of nonparticipants were similar to those that did participate in the study (data not shown here). There is little evidence to suspect that individuals chose not to participate because of awareness of diabetes or fasting glucose and insulin level and its possible relation to prostate cancer. Another way to assess this is to survey the non-respondents and determine reasons for loss to follow-up.

Residual confounding may also affect our reported hazard ratios for all four exposures regressed with incident prostate cancer as the outcome: type 2 diabetes, fasting glucose level in untreated diabetics, impaired fasting glucose level, and higher fasting insulin level. The primary reason to control for confounding is to ensure that the comparison group is as close to the exposure group as possible with respect to all other factors related to the disease except for the exposure. If there really is no association between disease and exposure, the disease rates in the exposed and unexposed groups will be the same. As mentioned earlier, there were a few covariates such as HDL that were distributed differently in those with and without diabetes at a statistically significant level as determined by chi-squared analysis, but were not different in those with and without prostate cancer. We included HDL in the final model even though the distribution of HDL levels may be similar among those with and without disease. A similar situation may occur in identifying confounders for high glucose in untreated diabetics and impaired fasting glucose levels. Here, residual confounding may be present since we did not conduct chi squared or t-test for high fasting glucose or impaired fasting glucose as binary variables. We may not have adequately adjusted for confounders that were statistically significantly different among exposure and disease groups.

With respect to external validity, the study only recruited men over the age of 65. In addition, the majority of participants were white and overweight. Although we controlled for

age and race, we cannot be certain that these results can be generalized to a larger, more diverse population without further analysis. While we used clinically significant cutoff values to create categories for fasting glucose status, we did not do so for our fasting insulin predictor. Due to lack of clinically significant cutoff values for fasting insulin levels in the literature for our sample, we chose the median as the cutoff value, and categorized those levels above the median value to suggest a high level and those that fell below the value to suggest low fasting insulin level. Therefore, we recognize that these results will have limitations in clinical interpretation. In addition, we only measured incident prostate cancer but did not report information on Gleason score or other staging. Thus, it is not possible to determine the relationship of diabetes and fasting glucose and insulin levels with regard to the severity of prostate cancer in this particular study.

Study Strengths

Prospective cohort studies have several advantages. For this particular research question, data from the MrOS study was readily available, de-identified, and cleaned for the purpose of analysis.

In prospective cohort studies, selection bias is less of an issue than it is in case-control studies since the exposure (type 2 diabetes or fasting glucose level) were assessed prior to the occurrence of disease (prostate cancer). With regard to our specific aims, we are able to establish a temporal sequence between the exposure of diabetes or fasting glucose and insulin level, and the disease of incident prostate cancer. However, there may have been participants with undiagnosed prostate cancer at entry. PSA screening was not performed at entry, so we are unable to confidently conclude that participants were free of prostate cancer at baseline. Much

of the data is available from the participants directly, such as blood glucose level, anthropometric, and lipid measurements. While there is some bias associated with self-report, using physician and medical-record confirmed diagnosis of prostate cancer substantially reduces concerns for non-differential misclassification bias. The MrOS study has a large number of participants, and with regard to the variables of interest to these particular aims, missing information was minimal. After a systematic analysis of alternative explanations and addressing bias, we find that the statistical findings confirm our hypothesis that individuals with type 2 diabetes may be at a lower risk of prostate cancer than those without diabetes. Higher fasting insulin levels are associated with an increased risk of prostate cancer compared to those with lower fasting insulin levels.

Chapter 5

Summary and Conclusions

Using a large prospective cohort study design, we found that there was no association between fasting glucose levels and prostate cancer risk in this study. While we found that individuals with higher fasting insulin levels are at a significant increased risk of prostate cancer compared to those with lower fasting insulin levels, further studies are needed to accurately define cutoff points for insulin levels to determine at which point they may be predisposed to type 2 diabetes so that this result may be clinically applicable. Alternatively, analyzing fasting insulin level as a continuous measure or in quartiles as a main predictor may help to better quantify the increased risk related to prostate cancer. Measuring IGF-1 directly in patients with and without type 2 diabetes may also help to better understand the biological mechanism between diabetes and prostate cancer. In this analysis, we show that type 2 diabetics may be at a lower risk of prostate cancer compared to non-diabetics, and this result was statistically significant.

Comment [JS1]: Or maybe it would be more important to consider at what point they reflect adverse IGF-1 levels??

While our results confirm the findings of previous studies in this subject, we cannot establish a cause and effect relationship between type 2 diabetes and prostate cancer using epidemiologic evidence. We hope that our results warrant further research into understanding the biological mechanism of the proposed association, and that eventually we can create clinical guidelines that are applicable to the general population in order to reduce the incidence of prostate cancer.

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