Type 2 diabetes, metformin, and the risk of mortality in patients with prostate cancer

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Abstract

Background: While several observational studies have reported a lower incidence of prostate cancer in patients with type 2 diabetes, few observational studies have investigated whether type 2 diabetes is also associated with a decreased risk of prostate cancer mortality. Additionally, recent experimental and observational studies have suggested that metformin, a first-line oral hypoglycemic agent, has antineoplastic activity. To date, there are limited studies assessing the effects of type 2 diabetes and its metformin treatment on the incidence of prostate cancer mortality.

Objective: This thesis has two objectives. The first objective is to determine whether type 2 diabetes influences prostate cancer mortality or all-cause mortality. The second objective is to assess if the use of metformin after a prostate cancer diagnosis is associated with decreased risks of these prostate cancer outcomes.

Research Design and Methods: Two observational studies were conducted, each corresponding to a thesis objective. Both studies used four electronic databases from the United Kingdom: the National Cancer Registry, Clinical Practice Research Datalink, Hospital Episodes Statistics database, and the Office for National Statistics. For both studies, we assembled a cohort of men newly-diagnosed with non-metastatic prostate cancer between April 1, 1998 and December 31, 2009, and followed until October 1, 2012. With respect to the first objective, Cox proportional hazard models were used to estimate

adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) of prostate cancer mortality and all-cause mortality comparing patients with to without type 2 diabetes. For the second objective, we conducted a nested case-control analysis and used conditional logistic regression to estimate adjusted rate ratios (RRs) with 95% CIs for the association between the use of metformin after a prostate cancer diagnosis and the risk of mortality. For both studies, the models were adjusted for a number of potential confounders, which included excessive alcohol use, smoking, comorbidities, and prostate cancer-related variables.

Results: Type 2 diabetes was associated with a 24% increase risk in prostate cancer mortality (HR: 1.24, 95% CI: 1.04-1.47) and a 25% increase risk in all-cause mortality (HR: 1.25, 95% CI: 1.12-1.41). The use of metformin after prostate cancer diagnosis was not significantly associated with a decreased risk of cancer-related mortality (RR: 1.09, 95% CI: 0.51-2.33) or all-cause mortality (RR: 0.79, 95% CI: 0.50-1.23).

Conclusion: Type 2 diabetes was associated with an increased incidence of prostate cancer mortality and all-cause mortality. Furthermore, metformin use after prostate cancer diagnosis was not associated with a significantly decreased risk of prostate cancer mortality and all-cause mortality. Given the inverse association between type 2 diabetes and prostate cancer incidence, different mechanisms may be at play with respect to prostate cancer mortality. Moreover, further research is warranted to confirm the effects of metformin on mortality.

Keywords: Type 2 Diabetes (T2D), Metformin, Oral hypoglycemic agents (OHAs), Prostate Cancer, United Kingdom Cancer Registry, Clinical Practice Research Datalink (CPRD), Hospital Episodes Statistics (HES), and Office of National Statistics (ONS)

Résumé

Problématique: Bien que de nombreuses études aient signalé une incidence plus réduite de cancer de la prostate chez les patients diabétiques de type 2, peu d'études observationnelles ont investiguée si le diabète de type 2 est aussi associé avec une réduction du risque de mortalité relié au cancer de la prostate. De plus, de récentes études expérimentales et observationnelles ont suggéré que la metformine, un agent hypoglycémique oral de première ligne, à une activité antinéoplasique. Jusqu'à présent, il existe peu d'études sur les effets du diabète de type 2 et de son traitement à la metformine sur l'incidence de mortalité du cancer de la prostate.

Objectif: Cette thèse a deux objectifs. Le premier est de déterminer si le diabète de type 2 est associé à l'incidence de mortalité du cancer de la prostate et de toute cause. Le deuxième objectif est déterminé si l'usage de la metformine après un cancer de la prostate est associé à un meilleur pronostique du cancer de la prostate.

Recherche et méthode: Deux études observationnelles ont été menées, chacune correspondant à un objectif de cette thèse. Ces deux études ont utilisées quatre bases de données électroniques parvenant de la Grande Bretagne, dont le *National Cancer Registry*, le *Clinical Practice Research Datalink*, le *Hospital Episode Statistics database*, et le *Office for National Statistics*. Pour chaque étude, nous avons assemblé une cohorte d'hommes nouvellement diagnostiqués avec un cancer de la prostate non-métastatique

entre le 1er avril 1998 et le 31 décembre 2009 et suivis jusqu'au 1er octobre 2012. En ce qui concerne le 1er objectif, les modèles proportionnels de risque de Cox ont été utilisés pour estimer des rapports de risque (RR) avec à des intervalles de confiance (IC) à 95% de la mortalité reliée au cancer de la prostate et de toute autre cause, comparant les patients avec le diabète de type 2 à ceux sans cette maladie. En ce qui concerne le deuxième objectif, nous avons mené une analyse cas-témoins niché dans une cohorte. Pour cette analyse, la régression logistique conditionnelle a été utilisée pour estimer les rapports de taux (RTs) ajustés avec des ICs à 95% pour estimer l'association entre l'utilisation de la metformine après un diagnostic du cancer de la prostate et le risque de mortalité. Pour ces deux études, les modèles ont été ajustés pour plusieurs variables potentiellement confondantes, incluant l'usage excessif d'alcool, le tabagisme, les comorbidités et autres variables reliées au cancer de la prostate.

Résultats: Le diabète type 2 a été associé à un risque de mortalité accru de 24% relié au cancer de la prostate (RR: 1.24, 95% IC: 1.04-1.47) et de 25% relié à toute autre cause de mortalité (RR: 1.25, 95% IC: 1.12-1.41). L'usage de la metformine après le diagnostic du cancer de la prostate n'était pas associé de manière significative à un risque moindre de mortalité relié au cancer (RT: 1.09, 95% IC: 0.51-2.33) ou toute autre cause de mortalité (RT: 0.79, 95% IC: 0.50-1.23).

Conclusions: Le diabète type 2 était associé à une incidence élevée de mortalité du cancer de la prostate et de toute autre cause de mortalité. De plus, l'usage de la metformine lors du cancer de la prostate n'était pas associé de manière significative à une réduction du risque de mortalité du cancer de la prostate et de toute autre cause de mortalité. Sur une base de l'association inverse entre le diabète de type 2 et l'incidence du cancer de la prostate, il est possible que différents mécanismes sont invoqués en ce qui concerne la mortalité du cancer de la prostate. D'autres études seront nécessaires pour confirmer les effets de la metformine sur la mortalité.

Mots clés: Diabète type 2 (T2D), Metformine, Agents hypoglycémiants, Cancer de la prostate, *National Cancer Registry*, *Clinical Practice Research Datalink (CPRD)*, *Hospital Episode Statistics database (HES)*, et le *Office for National Statistics (ONS)*

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Contribution of authors

Drs. Samy Suissa and Laurent Azoulay contributed to the study concept and design, to analysis and interpretation of data, to drafting of the manuscript, and to critical revision of the manuscript for important intellectual content. Hui Yin and Dr. Michael Pollak contributed to the analysis and interpretation of data and critical revision of the manuscript. Dr. Laurent Azoulay supervised the study and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Abbreviations

ATP	5’ adenosine triphosphate
AMPK	5’ adenosine monophosphate activated protein kinase
BMI	Body mass index
CI	Confidence intervals
CPRD	Clinical practice research datalink
DMFS	Distant metastasis free survival
Hba1c	Glycosylated hemoglobin levels
HR	Hazard ratio
ICD-10	International Classification of Diseases, 10th revision
LKB1	Liver kinase-B1
MRR	Mortality rate ratio
mTOR	Mechanistic target of rapamycin
NSAIDs	Non-steroidal anti-inflammatory drugs
OHAs	Oral hypoglycaemic agents
OPCS-4	Office of population censuses and surveys classification of interventions and procedures, 4th version
OR	Odds ratio
OS	Overall survival
PCSM	Prostate cancer-specific mortality
PSA	Prostate-specific antigen
PSA-RFS	Prostate-specific antigen recurrence-free survival
RCT	Randomized controlled trials
RR	Rate ratio
SD	Standard deviation
T2D	Type 2 diabetes
TZD	Thiazolidinedione
VEGF	Vascular endothelial growth factor
WHO	World health organization

Chapter 1: Introduction

Over the years, there has been considerable interest in the relationship between type 2 diabetes and cancer [1]. Indeed, observational studies have found that patients with type 2 diabetes have a higher risk of several cancers, including pancreatic, colorectal, breast, endometrial, bladder, and primary liver cancer [1-9], with the exception of prostate cancer, where an inverse relationship has been reported [10, 11]. Type 2 diabetes durations ranging between 10 to 20 years have been associated with a 25-50% decreased risk of prostate cancer [11, 12]. Although the biological mechanism for this inverse association remains unclear, it has been hypothesized that it may be due to a decrease in bioavailable testosterone levels in patients with type 2 diabetes, leading to a decrease in prostate tumor growth [11, 13], the latter being an important contributor to prostate cell division and tumor proliferation [14-16]. Thus, lower free testosterone levels in patients with type 2 diabetes may contribute to the lower risk of prostate cancer [17-20].

While prior research has assessed the association between type 2 diabetes and prostate cancer incidence [11, 21], several observational studies have been conducted to determine whether the type 2 diabetes is also associated with improved prostate cancer prognosis [2, 4, 5, 22-26]. Overall, these studies have not confirmed a decreased risk [2, 4, 5, 22-26],

but had methodological shortcomings, such as lack of adjustment for important confounders.

In parallel, several observational studies have reported that metformin, a first-line anti-diabetic agent, has antineoplastic activity that could potentially decrease the risk of prostate cancer [6, 27], although not all studies agree [28, 29]. Experimental studies have proposed direct and indirect mechanisms for this possible decreased risk [27]. In terms of direct mechanisms, it has been proposed that metformin inhibits cellular energy production, inducing energetic stress, thereby limiting tumor growth [27]. On the other hand, metformin may limit tumor growth indirectly in liver cells by inhibiting gluconeogenesis, which reduces circulating glucose levels and consequently reduces circulating insulin levels [30-32]. Reducing insulin levels in hyperinsulinaemic patients, a common characteristic in patients with type 2 diabetes, may contribute to an antiproliferative effect on cancer cells [33].

To date, observational studies assessing the association between type 2 diabetes and its metformin treatment on prostate cancer outcomes have a number of methodological limitations [23, 34-36]. As such, carefully-designed studies are needed to better understand the relationship between type 2 diabetes and its metformin treatment on mortality.

Chapter 2: Literature Review

The following chapter is divided into five sections. The first section describes type 2 diabetes and its complications, as well as the different anti-diabetic therapies available. The second section describes the association between type 2 diabetes and cancer. The third section provides an overview of prostate cancer, along with its related treatment options and its association with type 2 diabetes. The fourth section details the antiproliferative effects of metformin. In the final section, a review of the scientific literature will describe the association between type 2 diabetes and its metformin treatment on mortality.

2.1 Type 2 diabetes

2.1.1 Epidemiology of type 2 diabetes

According to the Health Canada, type 2 diabetes is one of the fastest growing diseases, with an incidence of more than 60,000 people per year [37]. The increasing epidemic in Canada as well as in other western countries has been partly attributed to the aging population, a higher prevalence of obesity, and an increase in sedentary lifestyles [38]. Overall, type 2 diabetes affects over 30 million people worldwide, contributing to chronic complications and premature death [39]. Furthermore, this disease reduces quality of life, and is associated with a higher risk of heart disease, stroke, kidney disease, blindness, and erectile dysfunction [38]. In Canada, the cost

of patients with type 2 diabetes can range from 1,000 to 15,000 dollars yearly [38].

2.1.2 Pathophysiology of type 2 diabetes

Type 2 diabetes is generally caused by a combination of lifestyle characteristics and genetic factors [40]. Obese patients with a genetic predisposition to the disease commonly develop type 2 diabetes [41]. Common symptoms of the disease are excess thirst, frequent urination, hunger, and weight loss [42]. Patients are generally diagnosed with the disease when a combination of the following results are present: a frequent fasting plasma glucose of 126 mg/dL or more, a glycated hemoglobin (HbA_{1c}) greater than 6.5%, or a random blood sugar greater than 200 mg/dL [41]. HbA_{1c} levels represent plasma levels of glycated hemoglobin and are considered a stable measure of blood glucose levels in individuals that are not required to fast [43].

Type 2 diabetes is characterized by insulin resistance, where cells of classic insulin target tissues such as liver, muscle, and fat no longer respond to the normal extent of the insulin produced by β cells of the pancreas [44]. In these conditions, plasma glucose levels abnormally increase, leading to the diagnosis [44]. This occurs initially even though there is an attempt at compensation by increased insulin secretion and hyperinsulinemia [44]. Overtime, the β cell insulin production falls, and this situation often leads to a requirement for the use of exogenous insulin [45]. Thus, the goal of the

different anti-diabetic treatments is to either increase insulin sensitivity or production in order to improve glucose absorption.

2.1.3 Anti-diabetic agents

Type 2 diabetes is treated differently at different stages of the disease. The first-line treatment for patients with the disease is diet and exercise. Since obesity is an important contributor to the development of type 2 diabetes, weight loss can reduce the risk of development and progression of the disease [41]. Furthermore, regular exercise has been shown to prevent type 2 diabetes, while increasing insulin sensitivity [41].When diet and exercise fail, there are pharmacologic options available to patients. These include biguanides, thiazolidinediones, sulfonylureas, glucagon-like peptide (GLP) 1 based therapies, and insulins. Biguanides and thiazolidinediones are considered sensitizers, where glucose production is inhibited in order to increase sensitivity to insulin [3]. On the other hand, sulfonylureas and GLP-1 based therapies belong to the secretagogue family, triggering insulin release by the β cells in the pancreas [46]. Another class of drugs, α-glucosidase inhibitors, are usually taken after a meal and regulate the absorption of carbohydrates in the intestines [41].

Biguanides, such as metformin, specifically translocate glucose transport proteins to the plasma membrane of hepatic and muscle cells [41]. The benefit of these drugs is targeting the cell's sensitivity to insulin, as well as lowering elevated glucose levels without causing hypoglycemia [41].

Furthermore, biguanides maintain weight, improve cholesterol levels, and effectively lower HbA_{1c} levels [41]. These drugs are commonly given as monotherapy, as well as in combination with other anti-diabetic agents [41]. Biguanides are generally well tolerated, however patients exposed to these drugs can be at risk of lactic acidosis, especially if renal complications are present [41].

Sulfonylureas are known as insulin secretagogues, since they stimulate insulin secretion by binding to the cell membrane of β cells of the pancreas [47]. These drugs have been associated with increased mortality, a higher risk of cardiovascular events, and a higher risk of cancer incidence [48, 49].

Thiazolidinediones also enhance insulin sensitivity by binding to the peroxisome proliferator activator receptor-γ, thereby reducing free fatty acid levels [41]. These drugs are prescribed with caution, since they have been associated with an increased risk of cardiovascular outcomes and certain cancers such as hepatocellular bladder cancers [50-52].

Another class of drugs, α-glucosidase inhibitors, act by slowing the absorption of carbohydrates by the intestines after a meal [41]. Furthermore, GLP-1-based therapies have been recently favored, as they enhance glucose-dependent insulin secretion, slow down gastric emptying, regulate postprandial glucagon, and reduce food intake [53]. Finally, the administration of exogenous human insulin or related drugs is given to

patients with advanced type 2 diabetes or to patients who do not support the standard oral hypoglycemic treatments [47].

Table 1 below presents a summary of the different pharmacological agents used in the treatment of type 2 diabetes.

Table 1: Summary of anti-diabetic medications

Class	Class	Mechanism of action
Sulfonylureas	Secretagogues	Stimulate insulin secretion
GLP-1 based therapies	Secretagogues	Stimulate insulin secretion
Biguanides	Sensitizers	Enhance insulin sensitivity
Thiazolidinediones	Sensitizers	Enhance insulin sensitivity
α-glucosidase inhibitors	α-glucosidase inhibitors	Slow absorption of glucose in small intestines
Insulins	Insulins	Mimic endogenous insulin

2.2 Association between type 2 diabetes and cancer incidence

Type 2 diabetes is a complex disease that has been linked to other comorbidities, such as cancer [8, 54, 55]. Indeed, a number of observational studies and meta-analyses have confirmed an association between type 2 diabetes and an increased risk of several cancers (ranging from a two-to three-fold increased risk), including those of the liver, kidney, endometrium, bladder, and breast [9, 56-59].

The association between type 2 diabetes and cancer is likely mediated by common risk factors such as hyperinsulinaemia [60], obesity [61], hypertension [61], frequent infections in the urinary tract [3], sex hormone abnormalities [62], and immune disorders [63]. Furthermore, the increased risk may depend on duration and severity of type 2 diabetes, as well as the maintenance of this disease [3]. Interestingly, while type 2 diabetes has been associated with an increased risk of several cancers, an inverse association has been reported with prostate cancer [21]. The latter cancer will be the subject of the next sections of this thesis.

2.3 Prostate cancer

Prostate cancer is considered the most common cancer diagnosed among men in Canada, and by the end of 2013, an estimated 23,600 men will be diagnosed and 3,900 will die from this disease [64]. The causes of this cancer remain unclear, although there has been some speculation that androgens, diet, physical activity, sexual factors, inflammation, obesity, and in a large part genetic factors may contribute to the risk of developing this disease [65, 66]. Furthermore, based on a 1997 study, the estimated life-time cost of prostate cancer in Canada was approximately $9.76 billion in 1997 [67]. Therefore, lowering the risk of prostate cancer would have beneficial effects on both an individual and societal level.

Prostate tumor growth initiates in the prostate gland and is generally diagnosed in patients with high prostate-specific antigen (PSA) levels (PSA

≥10) [68] or by digital rectal examination (DRE) [66]. Among those diagnosed with prostate cancer, approximately 10% of men develop a locally advanced disease [69]. Generally, there are no symptoms of the disease, however more advanced prostate cancers may initiate anxiety, frequent urination, pain while urinating, as well as blood in the urine [66]. Prostate cancer is considered an androgen-dependent cancer, where genetic factors play an important role in androgen biosynthesis and metabolism [65]. Therefore, a treatment strategy is to lower androgen levels which translate in a decreased progression of the disease.

2.3.1 Treatment options

Due to the slow-growing nature of prostate cancer, patients initially diagnosed with low-grade prostate cancer remain untreated in a period known as watchful waiting [68]. Patients with low-risk localised prostate cancer with a clinical stage T1c, a Gleason score of 3+3, and a PSA density <0.15 ng/ml are placed under active surveillance, where biopsies and blood test are taken regularly [68]. If there are signs of tumor progression, patients are then treated with radical radiotherapy or radical prostatectomy [68]. Patients with aggressive cancer may undergo chemotherapy or androgen deprivation therapy as salvage treatments [68].

2.3.2 Association between type 2 diabetes and prostate cancer

As described earlier, type 2 diabetes has been associated with a decreased risk in prostate cancer [21]. One suggested mechanism associated

with this decreased risk has been attributed to the decreased testosterone levels in patients with type 2 diabetes [70, 71]. High testosterone levels have been shown to induce prostate cancer in rats, while also enhancing cancer proliferation [65]. Furthermore, experimental models have shown that increased insulin levels may be associated with the decreased testosterone levels in patients with type 2 diabetes [72, 73]. While there are a plethora of observational studies on the association between type 2 diabetes and prostate cancer incidence [74], only a few studies have assessed the association between type 2 diabetes and prostate cancer mortality [2, 4, 5], distant metastasis [22, 23], and all-cause mortality [24-26].

2.4 Anti-tumor effects of metformin

Due to the interest in studying the association between type 2 diabetes and cancer [1, 3, 12, 75], there has also been interest in investigating the association between anti-diabetic treatments and cancer risk and mortality [3, 23, 28, 34, 36, 76, 77].

Experimental studies have found that metformin inhibits adenosine-5'-triphosphate (ATP) production in the mitochondria via the liver kinase-B1 (LKB1)-5' adenosine monophosphate-activated protein kinase (AMPK) signalling pathway [6]. By activating this pathway important in regulating cellular energy homeostasis, metformin inhibits gluconeogenesis, thereby decreasing blood glucose concentrations [6]. Consequently, insulin levels decrease, which effectively resolves the hyperinsulinaemia found in patients

with type 2 diabetes [27]. By effectively inhibiting gluconeogenesis and cellular energy production, metformin has indirectly shown antineoplastic activity in experimental models [78, 79]. Furthermore, *in vivo* and *in vitro* models have confirmed metformin's antineoplastic mechanism directly in tumor cells that thrive in hyperinsulinemic and hyperglycemic environments [78, 79]. Due to the important clinical implications of these findings, the effect of metformin on cancer prognosis deserves further research.

2.5 Observational studies

2.5.1 Type 2 diabetes and the risk of prostate cancer outcomes

To date, eight observational studies have assessed the association between type 2 diabetes and prostate cancer outcomes. A detailed summary of these studies are summarized in Table 2.

In 2010, D'amico et al. [2] investigated the association between pre-existing diabetes and prostate cancer specific mortality. Data was obtained from the Chicago Prostate Cancer Center between 1997 and 2007. The cohort consisted of 5,279 diabetic and non-diabetic men treated with either brachytherapy or neoadjuvant external beam radiotherapy. In the first analysis, logistic regression was used to analyze the effect of pre-existing diabetes on prostate cancer specific mortality, while Cox regression analyses were conducted with respect to non-prostate cancer specific mortality. The results concluded that pre-existing diabetes was not significantly associated with an increased risk of prostate cancer specific mortality (HR: 1.28, 95% CI: 0.54-3.03) relative to men without pre-existing diabetes. However, pre-existing diabetes was associated with an increased risk in non-prostate cancer-specific mortality (HR: 1.53, 95% CI: 1.13-2.07). Limitations of this study include the small number of study participants with both prostate cancer and pre-existing diabetes (n=608). Furthermore, the analysis did not include some important confounders, such as smoking, alcohol use, body mass index (BMI), and comorbidities such as myocardial infarction.

In a cohort of 102,651 men with type 2 diabetes between 1995 and 2006, Tseng [5] found that Asian diabetic men had an increased risk of prostate cancer mortality; this trend increased for younger diabetic men (mortality rate ratio (MRR): 1.55 95% CI: 1.29-1.86; 2.68 95% CI: 2.29-3.13; 6.84 95% CI: 5.34-8.75; for ages > 75, 65-74, and 40-64 years). The MRR was age-standardized and was not adjusted for potentially important confounding factors [80].

Using a nationwide population based Swedish database of 1,016,105 cancer patients accrued between 1961 and 2008, Liu et al. [4] reported an overall higher risk of cancer-specific mortality for patients with type 2 diabetes compared to non-diabetic patients. The authors also reported a higher risk of prostate cancer mortality among patients with type 2 diabetes relative to no diabetes (HR: 1.32, 95% CI: 1.23-1.41). A limitation of this study was that it included only patients hospitalized for type 2 diabetes, thus representing a more severe population that may not be generalizable. Furthermore, the analyses did not adjust for prostate cancer-related variables, such as tumor grade or treatments received.

In 2012, Currie et al. [25] published a large retrospective cohort study using data obtained from greater than 350 United Kingdom (UK) primary care practices. The study investigated the effects of type 2 diabetes on all-cause mortality in patients newly diagnosed with any cancer between 1990 and 2009. Cox proportional hazard models were used to estimate hazard

ratios (HRs) and 95% confidence intervals (CIs). After adjusting for age at baseline, sex, smoking history, Charlson comorbidity index, and year of diagnosis, the study found an increased risk of all-cause mortality in patients with type 2 diabetes relative to patients without type 2 diabetes (HR: 1.19, 95% CI: 1.08-1.31). A limitation to this study is the lack of adjustment for important prostate cancer-related treatments, which are indicative of prostate cancer severity [81]. Furthermore, a more informative outcome should have been prostate cancer mortality, as the increased risk observed with all-cause mortality in patients with type 2 diabetes was expected.

Yeh et al. [26] conducted a prospective study on the association between treated diabetes and cancer mortality on patients accrued between 1989 and 2006. A total of 599 patients with type 2 diabetes and 17,681 non-diabetics contributed to the analysis. Overall, diabetes was associated with an increased risk of cancer-related mortality (HR: 1.36, 95% CI: 1.02-1.81). Moreover, type 2 diabetes was associated with an increased risk of all-cause mortality among patients with prostate cancer (HR: 2.32, 95% CI: 1.29-4.19). The limitations of this study include the self-reported data for diabetes status. Individuals were considered diabetic if they reported having taken any diabetic medication within the previous 48 hours. Other baseline characteristics such as weight and height were self-reported, which may have introduced information bias.

Shetti et al.[24] studied the association between diabetes and men with clinically localized prostate cancer treated with brachytherapy. The study lasted from April 1995 to May 2006 and included 1,624 subjects with clinically localized prostate cancer. In this study, 199 patients had diabetes and were more likely to die of cardiovascular disease than of prostate cancer (HR: 1.54; p-value: 0.01). In fact, no patients with diabetes died of prostate cancer. A limitation of this study included a lack of information on diabetes duration.

In 2005, Chan et al. [22] assessed the association between diabetes and risk of prostate cancer recurrence. Using data from CaPSURE, a community-based prostate cancer registry study, 691 men with diabetes and diagnosed with prostate cancer were identified between 1989 and 2002. Kaplan Meier log-rank tests and Cox proportional hazard models assessed the prostate cancer recurrence rates among patients with and without diabetes. After adjusting for age, race, education, PSA, T-Stage, Gleason groups, and BMI, patients in the low prognostic risk group were found to have a higher hazard of recurrence, although numbers were small in the low prognostic risk group (HR: 3.79, 95% CI: 1.28-11.19). Limitations of this study included the lack of information on tumor grade at time of prostate cancer diagnosis. This study could not conclude any association on the risk of prostate cancer mortality among diabetics relative to non-diabetics due to insufficient power and lack of supporting data. Furthermore, prostate cancer-

related variables were not included in the model, which may have introduced some confounding [81].

In 2010, Patel et al.[23] conducted a retrospective study analyzing the relationship between diabetes and metformin use with outcomes after radical prostatectomy for clinically localized cancer. Data was obtained from Columbia University Urologic Oncology Database between 1990 and 2009. A total of 616 patients were assessed, including diabetic metformin users, non-metformin users, and non-diabetic controls. Two non-diabetic controls were matched to one diabetic patient by a 5-year risk of biochemical recurrence, which was estimated by the preoperative Kattan nomogram scoring system. The outcome was a biochemical recurrence, defined as 1 PSA value >0.2 ng/ml. In the statistical analysis, the Kaplan Meier method and Cox proportional hazard model were used to assess time to biochemical recurrence and other variables that could independently predict a biochemical recurrence. Diabetes was found to increase the risk of biochemical recurrence relative to non-diabetics (HR: 1.55, 95% CI: 1.03-2.33). Limitations of this study include lack of information on other diabetic medications. Also, this study did not assess the duration of metformin use, nor did it distinguish between type 1 and type 2 diabetes.

Table 2: Cohort studies on the association between type 2 diabetes and prostate cancer outcomes

Authors	Publication year	Sample Size	Main Outcome	Point Estimate (95% Confidence Interval)
Chan et al.[22]	2005	6,722	Prostate cancer recurrence	HR 3.79 (1.28-11.19)*
Patel et al.[23]	2010	616	Biochemical recurrence	HR 1.55 (1.03-2.33)
D'Amico et al.[2]	2010	5,279	Prostate cancer mortality	HR: 1.28 (0.54-3.03)
D'Amico et al.[2]	2010	5,279	Non-prostate cancer-specific mortality	HR: 1.53 (1.13-2.07)
Tseng[5]	2011	102,651	Prostate cancer mortality	MRR: 1.55 (1.29-1.80)† 2.60 (2.29-3.13)‡ 6.84 (5.34-8.70)§
Liu et al.[4]	2012	1,016,105	Prostate cancer mortality	HR: 1.32 (1.23-1.41)
Liu et al.[4]	2012	1,016,105	All cancer mortality	HR: 1.38 (1.35-1.41)
Yeh et al.[26]	2012	18,280	Cancer mortality	HR 1.36 (1.02-1.81)
Yeh at al.[26]	2012	18,280	All-cause mortality	HR 2.32 (1.29-4.19)
Currie et al.[25]	2012	112,408	All-cause mortality	HR: 1.19 (1.08-1.31)
Shetti et al.[24]	2012	1,624	Overall Survival	HR: 1.54 (p-value: 0.01)

Abbreviations: HR, hazard ratio; MRR, mortality rate ratio
* men in low prognostic risk group
† for age > 75
‡ age 65-74
§ age 40-64

2.5.2 Metformin and the risk of prostate cancer outcomes

To date, four observational studies have assessed the association between metformin and the risk of prostate cancer outcomes. A detailed summary of these studies can be found in Table 3.

In 2010, Patel et al. [23] conducted a retrospective study analyzing the relationship between diabetes and metformin use with outcomes after radical prostatectomy for clinically localized cancer. Data was obtained from Columbia University Urologic Oncology Database between 1990 and 2009. A total of 616 patients were assessed, including diabetic metformin users, non-metformin users, and non-diabetic controls. Two non-diabetic controls were matched to one diabetic patient by a 5-year risk of biochemical recurrence, which was estimated by the preoperative Kattan nomogram scoring system. The outcome was a biochemical recurrence, defined as 1 PSA value >0.2 ng/ml. In the statistical analysis, the Kaplan Meier method and Cox proportional hazard model were used to assess time to biochemical recurrence, as well as other variables that could independently predict a biochemical recurrence. After adjusting for variables in the model, metformin was not found to have a significant effect on biochemical recurrence relative to non-metformin use (HR: 0.94, 95% CI: 0.6-1.5). Limitations to this study include lack of information on other diabetes medications. Also, this study did not assess the duration of metformin use. Lastly, misclassification may have occurred between type 1 and type 2 diabetes.

A recent case-control study by Hitron et al. [34] analyzed the association between metformin and the development of prostate cancer. Using the Kentucky Medicaid Database, cases and controls were diagnosed with prostate cancer between 2000 and 2005, followed until 2009. Concerning exposure, cases were divided into two groups: type 2 diabetics with elevated serum insulin exposure and type 2 diabetics without elevated serum exposure. Elevated insulin exposure was defined as sulfonylureas, insulin (>0.8 unites/kg/day), or combination therapy for more than two thirds of the study period. Physiologic insulin exposure was defined as metformin, TZDs, insulin (<0.8 units/kg/day), or combination therapy for more than two thirds of the study period. One case was matched to two controls by age. The measured outcome was determined by the time to tumor progression, which was confirmed by a Gleason score >7. Kaplan Meier survival curves and Cox proportional hazard models were used to compute the time to event and hazard ratios. With the non-diabetics as a reference group, physiologic insulin exposure (including metformin) had a non-significant decrease in prostate cancer progression (OR: 0.62, 95% CI: 0.22-1.73). With elevated insulin and type 2 diabetes as a reference group, physiologic insulin exposure had a non-significant decrease in prostate cancer progression (OR: 0.58, 95% CI: 0.22-1.53). Some limitations of this study include the lack of information on duration and dose of anti-diabetic medication. Each diabetic drug was classified as exposed and unexposed, when individuals could have been exposed for shorter or longer durations.

Furthermore, this study did not assess latency of the outcome, which is necessary when assessing the effect of a drug on cancer outcomes.

In 2011, He et al. [36] analyzed data from prostate cancer patients with and without pre-existing treated type 2 diabetes between 1999 and 2008. Those on diet-controlled diabetes were excluded from the analysis. Age and race were adjusted for in the multivariate Cox regression analysis and the Kaplan-Meier analysis. The exposure was classified as ever-users and never-users for TZDs, metformin, and the combination therapy (TZDs and metformin). Metformin was found to have a 45% risk reduction in prostate cancer mortality (HR: 0.55, 95% CI: 0.32-0.96). A major limitation of this study is immortal time bias. This bias was introduced by misclassifying the time between cohort entry and first metformin as exposed instead of unexposed, which may explain the exaggerated risk reductions. Furthermore, dose and duration of metformin use were not considered in the analysis.

In a recent study published by Spratt et al. [35], 2,901 men treated with external-beam radiation therapy for localized prostate cancer were included in the study. The study assessed the effect of metformin on tumor outcomes and on the development of castration-resistant disease. The outcomes were determined at the end of radiation treatment, including prostate-specific antigen recurrence-free survival (PSA-RFS), distant metastasis-free survival (DMFS), prostate cancer-specific mortality (PCSM), and overall survival (OS). The study looked at three different groups:

metformin users, diabetic patients treated with medication other than metformin, and non-diabetic patients taking metformin. When analyzing metformin use and prostate-specific antigen recurrence as an outcome, Cox proportional hazards models were used to compare non-diabetics to the reference metformin group (HR: 1.44, 95% CI: 0.96-2.13) and diabetics non-metformin group to the reference metformin group (HR: 1.99, 95% CI: 1.24-3.18). With respect to distant metastasis, non-diabetics had a more severe prognosis compared to the reference metformin group (HR: 1.75, 95% CI: 0.90-3.41), although non-significant. The diabetic non-metformin group had a significantly worse outcome compared to the metformin group (HR: 3.68, 95% CI: 1.78-7.62). Patients without diabetes as well as those with diabetes and not exposed to metformin had increased risks of prostate cancer mortality compared to metformin users (HR: 2.68, 95% CI: 0.85-8.44 and HR: 5.15, 95% CI: 1.53-17.35, respectively). Similar results were observed for overall survival (no diabetes versus metformin [HR 1.38, 95% CI: 0.9-2.11] and diabetic non-metformin group versus metformin [HR 2.25, 95% CI: 1.38-3.61]).

This study has several limitations, including the study's definition of metformin exposure. Specifically, metformin exposure was assessed at the time of prostate cancer diagnosis or any time after radiation therapy. Given metformin is a time-varying exposure, the time independent analysis used introduced immortal time bias, which explained the large risk reductions.

Furthermore, the study did not assess metformin duration and dosage and its cumulative effect on prostate cancer outcomes.

Table 3: Observational studies on the association between metformin use and prostate cancer outcomes

Authors	Publication Year	Study Design	Sample Size	Main Outcome	Point Estimate (95% Confidence Interval)
Patel et al.[23]	2010	Cohort	616	Biochemical recurrence	HR: 0.94 (0.6-1.5)
He et al.[36]	2011	Cohort	233	Prostate Cancer Mortality	HR: 0.55 (0.32-0.96)
Hitron et al.[34]	2012	Case-control	722	Prostate cancer progression	OR: 0.62 (0.22-1.73)*
Spratt et al.[82]	2012	Cohort	2,901	Prostate-specific antigen-recurrence-free survival	HR: 1.99 (1.24-3.18)†
Spratt et al.[82]	2012	Cohort	2,901	Distant metastasis-free survival	HR: 3.68 (1.78-7.62)†
Spratt et al.[82]	2012	Cohort	2,901	Prostate cancer-specific mortality	HR: 5.15 (1.53-17.35) ‡
Spratt et al.[82]	2012	Cohort	2,901	Overall survival	HR: 1.38 (0.90-2.11)†

Abbreviations: HR, hazard ratio: OR, odds ratio;
*Physiologic insulin exposure (including metformin) relative to non-diabetes
‡Metformin group compared to diabetic non-metformin group
†Diabetic non-metformin group compared to the metformin group

Chapter 3: Objectives and hypotheses

3.1 Objectives

This thesis has two main objectives and several secondary aims:

1. **Objective 1: To assess the association between type 2 diabetes and mortality among patients with prostate cancer**

   Aim 1: To assess whether type 2 diabetes is associated with the incidence of prostate cancer mortality.

   Aim 2: To assess whether type 2 diabetes is associated with the incidence of all-cause mortality.

   Aim 3: To assess the association between duration of type 2 diabetes and prostate cancer mortality.

2. **Objective 2: To assess the association between metformin use after prostate cancer diagnosis and mortality among patients with type 2 diabetes**

   Aim 1: To assess whether metformin use after prostate cancer diagnosis is associated with an incidence of prostate cancer mortality.

   Aim 2: To assess whether metformin use after prostate cancer diagnosis is associated with an incidence of all-cause mortality.

   Aim 3: To assess the association between cumulative duration and dose of metformin use after prostate cancer diagnosis and prostate cancer mortality.

3.2 Hypotheses

1. **Hypotheses for objective 1:**

   Aim 1: Type 2 diabetes is associated with the incidence of prostate cancer mortality.

   Aim 2: Type 2 diabetes is associated with the incidence of all-cause mortality.

   Aim 3: Duration of type 2 diabetes is associated with prostate cancer mortality.

2. **Hypothesis for objective 2:**

   Aim 1: Metformin use after prostate cancer diagnosis is associated with a decreased incidence of prostate cancer mortality.

   Aim 2: Metformin use after prostate cancer diagnosis is associated with a decreased incidence of all-cause mortality.

   Aim 3: Cumulative duration and dose of metformin use after prostate cancer diagnosis is associated with prostate cancer mortality.

Chapter 4: Type 2 diabetes and the risk of mortality among patients with prostate cancer

The following chapter presents the methods and results of objective 1 on the association between type 2 diabetes and the risk of mortality. This manuscript is currently under review in Diabetologia.

The topic presented in this paper will first be introduced with some necessary background information on type 2 diabetes and cancer. The methods will then cover comprehensive information on the data sources and study population. The statistical analysis and results are then described in detail. A thorough discussion will then provide critical information, such as future implications for research.

Type 2 diabetes and the risk of mortality among patients with prostate cancer

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4.1 Abstract

Aims/Hypothesis: The aim of this study was to determine whether type 2 diabetes is associated with the incidence of prostate cancer mortality and all-cause mortality.

Methods: This study was conducted by linking four databases from the United Kingdom: the National Cancer Registry, the Clinical Practice Research Datalink, the Hospital Episodes Statistics database, and the Office for National Statistics database. The cohort consisted of men newly-diagnosed with non-metastatic prostate cancer between April 1, 1998 and December 31, 2009, followed until October 1, 2012. Cox proportional hazard models were used to estimate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) of prostate cancer mortality and all-cause mortality comparing patients with to without type 2 diabetes. All models were adjusted for a number of potential confounders, which included excessive alcohol use, smoking, comorbidities, and prostate cancer-related variables.

Results: The cohort consisted of 11,920 patients, which included 1132 (9.5%) with pre-existing type 2 diabetes. During a mean follow-up of 4.7 (SD: 3.0) years, there were 3605 deaths (incidence rate: 6.4% per year) including 1792 from prostate cancer (incidence rate: 3.3% per year). Type 2 diabetes was associated with a 24% increased risk of prostate cancer mortality (HR: 1.24, 95% CI: 1.04-1.47) and a 25% increased risk in all-cause mortality (HR: 1.25, 95% CI: 1.12-1.41).

Conclusions/interpretation: The results of this large population-based study indicate that type 2 diabetes is associated with an increased risk of prostate cancer mortality, which may signal an association between hyperinsulinaemia or other diabetes-associated metabolic derangements and cancer aggressivity.

Key words: Type 2 diabetes, prostate cancer, mortality, hazard ratios

Abbreviations: Clinical Practice Research Datalink (CPRD), Hospital Episodes Statistics (HES), International Classification of Diseases, 10th revision (ICD-10), non-steroidal anti-inflammatory drugs (NSAIDs), office of national statistics (ONS), Office of Population Censuses and Surveys classification of interventions and procedures, 4th version (OPCS-4), Prostate-specific antigen (PSA), United Kingdom (UK)

4.2 Introduction

Over the years, a number of observational studies have associated type 2 diabetes with an increased risk of several cancers [1], with the exception of prostate cancer where an inverse relationship has been reported [10]. This may be related to the fact that type 2 diabetes is associated with reduced circulating and presumably prostatic levels of androgens [83]. While a number of observational studies have investigated the association between type 2 diabetes and prostate cancer incidence [74], several have assessed the association between this condition and prostate cancer outcomes [2, 4, 5, 22-26]. Furthermore, the results of these studies contrast with those on prostate cancer incidence, in that type 2 diabetes was associated increased risks of prostate cancer mortality [4, 5], recurrence [22, 23] and all-cause mortality [24-26].

While the results of these observational studies have been relatively consistent, residual confounding is always a concern, given that patients with type 2 diabetes may have risk factors, such as obesity and smoking that may potentially confound the association with mortality. Furthermore, only one of the previous studies assessed the association between diabetes duration on mortality [5].

Thus, the primary objective of this population-based study was to determine whether type 2 diabetes is associated with the incidence of prostate cancer mortality in men newly-diagnosed with prostate cancer. A

secondary objective was to determine whether this condition is also associated with the incidence of all-cause mortality.

4.3 Methods

4.3.1 Data sources

This study was conducted by linking four large electronic databases from the United Kingdom (UK), the UK National Cancer Registry, the Clinical Practice Research Datalink (CPRD) (previously known as the General Practice Research Database), the Hospital Episode Statistics (HES) database, and the Office for National Statistics (ONS) database.

The UK National Cancer Registry includes information on the tumour site of primary growth (coded using the International Classification of Diseases, 10th revision [ICD-10]), as well as information on tumour characteristics (such as grade, stage, and primary treatments received). The CPRD is a general practice database comprising the medical records for more than 12 million people enrolled in more than 650 general practices. The geographic distribution of the practices participating in the CPRD has been shown to be representative of the UK population, and age and sex distributions of patients in the CPRD are similar to those reported by the National Population Census [84-86]. General practitioners are trained to record medical information including demographic data, medical diagnoses, procedures, and deaths using a standardized form. The database records information on body mass index (BMI), smoking, and excessive alcohol use,

and prescriptions issued by general practitioners are automatically transcribed into the computer record. Read codes are used to enter medical diagnoses and procedures, which is the standard clinical terminology system used in general practice in the UK [84, 87], and a coded drug dictionary based on the UK Prescription Pricing Authority Dictionary is used for recording prescriptions [88]. Data in the CPRD are regularly audited, and diagnoses and drug exposures recorded in the CPRD have been validated and shown to be of high quality [85, 87-90].

The HES database contains details of all inpatient encounters in National Health Services hospitals in England since 1997. This database contains dates of hospital admissions, outpatient visits, primary and secondary diagnoses (coded using the ICD-10 classification), and related procedures (coded using the ICD-10 classification and Office of Population Censuses and Surveys classification of interventions and procedures, 4th version [OPCS-4]). Finally, the ONS contains the electronic death certificates of all citizens living in England and Wales, and was used to identify the underlying cause of death (coded using the ICD-10 classification) for all patients who died during follow-up.

The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD and the Research Ethics Committee of the Jewish General Hospital, Montreal, Canada.

4.3.2 Study population

Using the UK National Cancer Registry, we identified all men diagnosed for the first time with prostate cancer (ICD-10: C61) between April 1, 1998 and December 31, 2009, with follow-up until October 1, 2012. We excluded patients with less than one year of 'up-to-standard' medical history in the CPRD prior to diagnosis, those with metastases at the time of diagnosis, patients previously diagnosed with type 1 diabetes, as well as those with less than six months of follow-up. The latter was necessary to exclude those who died from prostate cancer soon after their cancer diagnosis, suggesting that they were already metastatic at the time of diagnosis.

Thus, cohort entry was set to the six months after the prostate cancer diagnosis, and patients were followed until one of the study outcomes (prostate cancer mortality [primary outcome] and all-cause mortality [secondary outcome]), end of registration with the general practice, or the end of the study period (October 1, 2012), whichever came first.

4.3.3 Assessment of type 2 diabetes

For all patients included in the cohort, we determined whether they had pre-existing type 2 diabetes. This was assessed by searching for either diagnoses of type 2 diabetes or prescriptions of anti-diabetic drugs (metformin, sulfonylureas, thiazolidinediones (TZDs), insulins, and others) at any time prior to the prostate cancer diagnosis. Patients deemed to have type

2 diabetes were then categorized, into tertiles, according to their duration of their disease prior to the prostate cancer diagnosis. This was defined as the time between the earliest of either a first-ever recorded diagnosis of type 2 diabetes or a prescription of an anti-diabetic drug and the prostate cancer diagnosis.

4.3.4 Statistical analysis

Descriptive statistics were used to describe the baseline characteristics of patients with and without pre-existing type 2 diabetes. Kaplan-Meier curves were constructed comparing the cumulative incidence of prostate cancer mortality between patients with and without pre-existing type 2 diabetes. Cox proportional hazards models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) of the outcomes under study, comparing patients with to without pre-existing type 2 diabetes. In a first model, we assessed whether type 2 diabetes was associated with the incidence of prostate cancer mortality, which was considered the primary outcome. We also assessed whether prostate cancer mortality varied with duration of type 2 diabetes. In a secondary model, we determined whether type 2 diabetes was associated with the incidence of all-cause mortality.

All the models were adjusted for the following potential confounders measured prior to the prostate cancer diagnosis: age, ethnicity (white, black, other, unknown), excessive alcohol use (based on alcohol-related disorders such as alcoholism, alcoholic cirrhosis of the liver, alcoholic hepatitis and

failure), smoking status (ever, never, unknown), obesity (BMI ≥ 30 kg/m^2), use of antihypertensive drugs (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, diuretics, other antihypertensive drugs), non-steroidal anti-inflammatory drugs (NSAIDs), statins, 5-alpha reductase inhibitors, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, peripheral artery disease), previous cancer (other than nonmelanoma skin cancer), prostate-specific antigen (PSA) levels (last measurement prior to the prostate cancer diagnosis), and Gleason score. Tumour stage was not included as a covariate since it was missing for over 90% of the patients. The models were further adjusted for the following prostate cancer-related interventions measured in the six-month time window between prostate cancer diagnosis and cohort entry: radical prostatectomy, radiation therapy, chemotherapy, and androgen deprivation therapy. For variables with missing data (such as smoking, BMI, and PSA), an 'unknown' category was created and analysed as such in the models. For all models, we verified the proportional hazards assumption for each variable using Schoenfeld residuals and found no violations [91].

4.3.4.1 Sensitivity and secondary analyses

To account for the possibility that some patients in the non-diabetes group may have developed type 2 diabetes during follow-up, which would have the effect of diluting the HRs, we conducted a sensitivity analysis

censoring such patients at the time of a first diagnosis of type 2 diabetes or prescription of an anti-diabetic agent occurring during follow-up.

We conducted two additional secondary analyses. In the first, we assessed the association between type 2 diabetes and non-prostate cancer mortality. In the second analysis, we assessed whether obesity (BMI≥30 kg/m^2) and age (≥75 years) were effect modifiers of the association between type 2 diabetes and the primary outcome of prostate cancer mortality. Effect modification was assessed by including interaction terms between these variables and the type 2 diabetes indicator variable in the models. All analyses were conducted with SAS version 9.2 (SAS Institute, Cary, NC).

4.4 Results

A total of 11,920 men newly-diagnosed with non-metastatic prostate cancer were included in the study (Figure 1), which included 1,132 (9.5%) with pre-existing type 2 diabetes. During a mean follow-up of 4.7 (standard deviation [SD]: 3.0) years, there were 3605 deaths (incidence rate: 6.4% per year) including 1,792 from prostate cancer (incidence rate: 3.3% per year).

Table 4 presents the characteristics of patients with and without pre-existing type 2 diabetes. Compared to patients without type 2 diabetes, those with the condition were more likely to have used alcohol excessively, to have been smokers, obese, had a higher prevalence of comorbidities, higher Gleason scores, higher baseline PSA levels, and were more likely to have used antihypertensives, aspirin, other NSAIDs, and statins prior to their prostate cancer diagnosis.

Figure 2 presents Kaplan-Meier curves for prostate cancer mortality of patients with and without pre-existing type 2 diabetes. Patients with pre-existing type 2 diabetes had a lower 5-year survival than patients without type 2 diabetes (81.4% versus 85.4%).

The results of the primary analysis are presented in Table 5. The prostate cancer mortality rate among patients with type 2 diabetes was 4.2% (95% CI: 3.7-4.9) per year, compared to 3.1% (95% CI: 3.0-3.3) per year in patients without the condition. In multivariate analyses, type 2 diabetes was associated with an increased risk in prostate cancer mortality (adjusted HR:

1.24, 95% CI: 1.04-1.47). Similar findings were obtained with the secondary outcome of all-cause mortality (Appendix Table 10).

In a secondary model assessing the effect of the duration of type 2 diabetes, the risk of prostate cancer mortality increased in the first two tertile categories (<2.95 years HR: 1.26, 95% CI: 0.98-1.61; 2.95-7.90 years HR: 1.50, 95% CI: 1.16-1.94) and then declined towards the null at the last tertile category (HR: 0.97, 95% CI: 0.72-1.30) (Table 5). Similar patterns were observed with all-cause mortality (Appendix Table 10).

4.4.1 Sensitivity and secondary analyses

In a sensitivity analysis, censoring the 54 (0.005%) patients who developed type 2 diabetes in the non-diabetes group did not materially change the HR (Appendix Table 11). In secondary analyses, type 2 diabetes was associated with an increased risk of non-prostate cancer mortality, which progressively increased with longer durations of type 2 diabetes (Appendix Table 12). Finally, obesity (BMI≥30 kg/m^2) and age (≥75 years) were not found to be effect modifiers of the association between type 2 diabetes and prostate cancer mortality (Appendix Tables 13 and 14).

4.5 Discussion

The results of this population-based study indicate that type 2 diabetes is associated with an increased risk of cancer-related mortality and all-cause mortality among patients with prostate cancer. Our findings of an association of type 2 diabetes with more aggressive behaviour of prostate cancer are of particular interest in the context of the previously reported inverse association between type 2 diabetes and prostate cancer incidence [74]. While the biological basis of the contrasting associations of type 2 diabetes on prostate cancer incidence and prognosis remain unclear, it is possible that the incidence effect is driven dominantly by the relatively low androgen levels in diabetics compared to non-diabetics, while the prognosis effect is driven by the proposed stimulatory effects of hyperinsulinemia on prostate cancer behaviour [72, 92, 93]. This would be consistent with the view that hyperinsulinemia and/or other metabolic effects of diabetes are not carcinogenic, but rather encourage progression of pre-existing cancers [33].

Although all of the previous observational studies suggest an increased risk of adverse prostate cancer outcomes among patients with type 2 diabetes [2, 4, 5, 22-26], only three studies assessed the association between type 2 diabetes and prostate cancer mortality [2, 4, 5]. In the first study [2], type 2 diabetes was not associated with an increased risk of prostate cancer mortality (HR: 1.28, 95% CI: 0.54-3.03). However, that study was likely underpowered, with only six prostate cancer mortality events

among patients with pre-existing type 2 diabetes. We note that the HR is similar in magnitude to the one estimated in this study. In the second study, type 2 diabetes was associated with a statistically significant increased risk of prostate cancer mortality (HR: 1.38, 95% CI: 1.35-1.41), though that study was limited to patients hospitalized for type 2 diabetes, thus limiting the generalizability of the results [4]. Finally, in the third study [5], the age-standardized mortality rate ratio was 1.55 (95% CI: 1.29-1.80), 2.60 (95% CI: 2.29-3.13), and 6.84 (95% CI: 5.34-8.70) for ages ≥75, 65-74, and 40-64 years, respectively. However, this analysis did not adjust for potentially important confounders, and thus the use of mortality rate ratios can lead to biased estimates of the true risk [94].

With respect to the secondary outcome, previous studies have associated type 2 diabetes with an increased risk in all-cause mortality [24-26], although there were differences in the reported magnitude of the effects. Such discrepancies can be due to certain methodological limitations, such as relying on patient self-report, and no exclusion of patients with type 1 diabetes [22, 23]. Furthermore, seven of the studies did not assess the effect of diabetes duration [2, 4, 22-26], an important variable necessary to understand the potential biological mechanisms that may be at play. Indeed, in this study, we conducted a secondary analysis where we assessed diabetes duration on the risk of prostate cancer outcomes. Patients in the first two tertiles of type 2 diabetes duration were found to have an increased risk in prostate cancer mortality relative to patients with no diabetes. However,

patients that had type 2 diabetes for more than 7.9 years had a lower risk in prostate cancer mortality compared to patients with no diabetes (HR: 0.97, 95% CI: 0.72-1.30). This surprising finding is likely due to competing risks bias [95], a situation where patients with longstanding type 2 diabetes were more likely to die early from non-cancer causes such as those cardiovascular in nature. This effect was confirmed in a secondary analysis where the risk of dying from a non-prostate cancer cause was the highest in patients with longstanding type 2 diabetes.

This cohort study has several strengths. Firstly, we avoided selection bias by conducting analyses within a large population-based representative cohort of patients with both type 2 diabetes and prostate cancer followed for up to 14 years. Additionally, information on exposure and confounders are prospectively collected in the CPRD, eliminating the likelihood of recall bias. By linking four electronic databases from the UK, we were able to obtain patient medical histories (including diagnoses and treatments), lifestyle measurements (smoking, excessive alcohol use, and BMI), and cancer-related variables (Gleason scores, PSA levels, and prostate cancer-related treatments). As such, we were able to adjust the models for a number of important potential confounders.

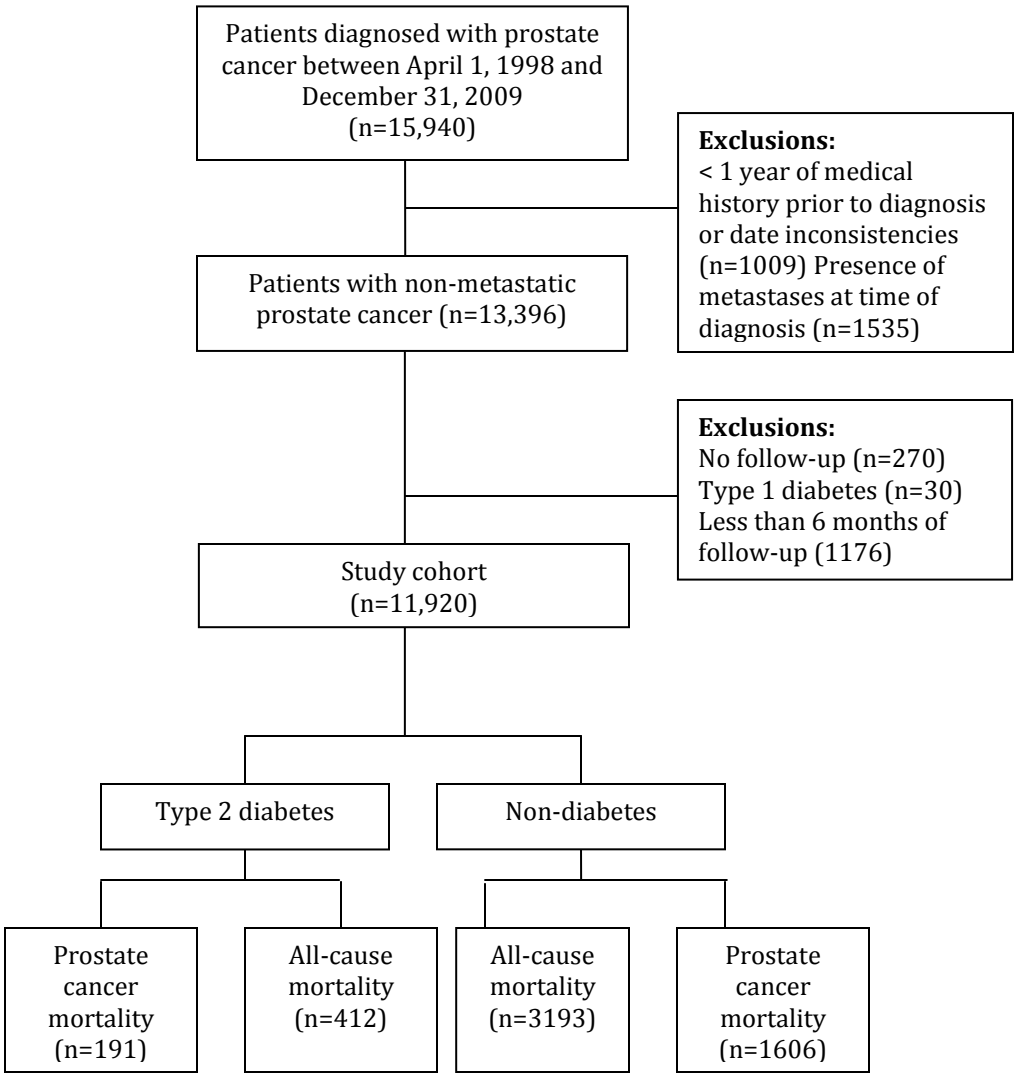
This study has some limitations. We were not able to adjust for tumour stage because it was missing for the vast majority of patients. However, the models were adjusted prostate cancer-related treatments, which are highly correlated with tumour grade and stage [96]. Furthermore,

as with any observational study, residual confounding needs to be considered. However, we adjusted the models for many important potential confounders, which should have minimized this bias. Lastly, misclassification of our primary outcome of prostate cancer is a possibility. However contrary to other cancers, prostate cancer mortality has been reported to be well recorded in deaths certificates [97].

In summary, type 2 diabetes was associated with an increased risk of prostate and all-cause mortality, which is consistent with the findings of the previous observational studies that have considered these outcomes. This association should raise clinician awareness that patients with prevalent type 2 diabetes may have worse prognosis, and may thus require more aggressive prostate cancer and diabetes treatment regimens.

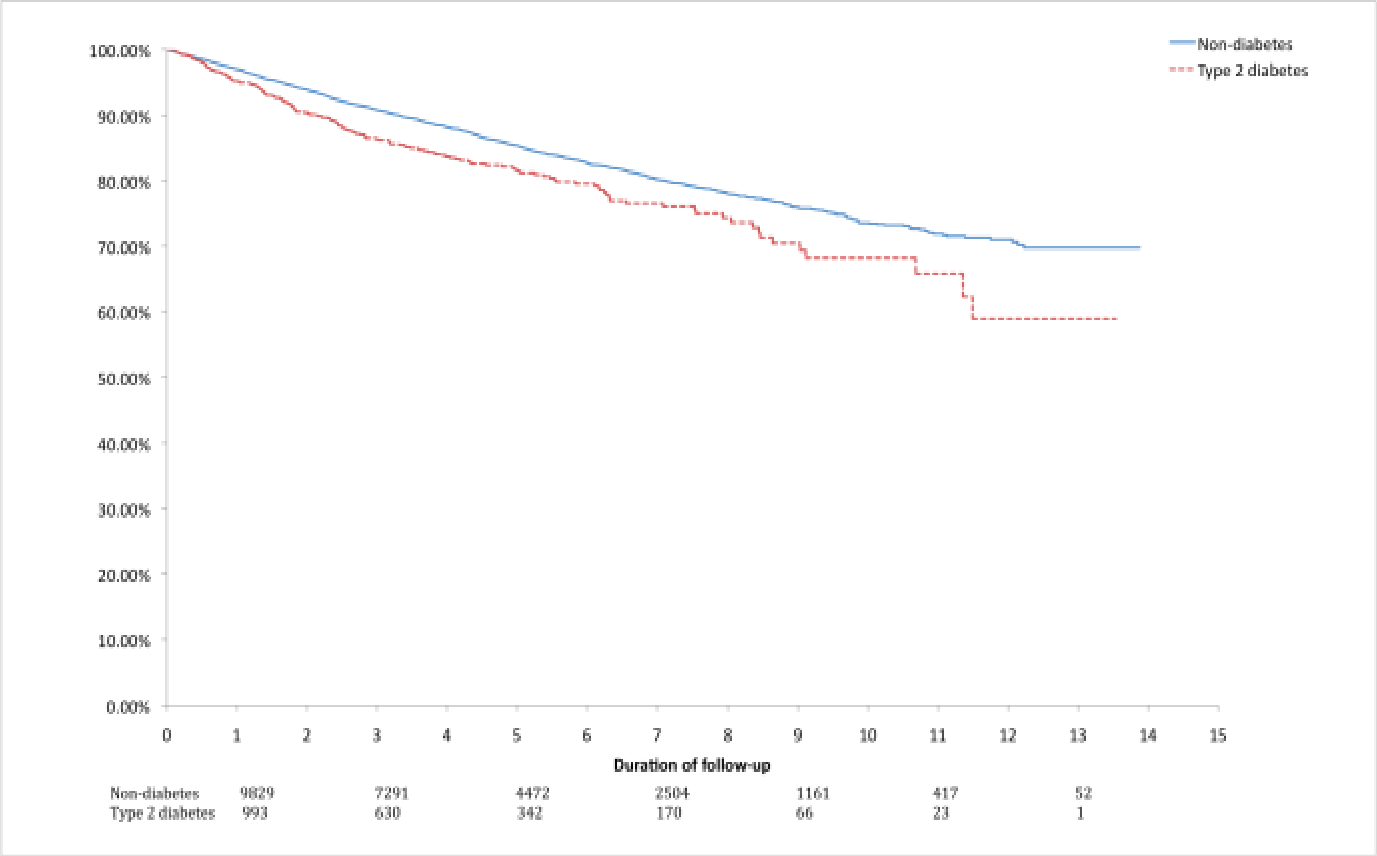
4.6 Figures and Tables

Figure 1



Study flow chart: Type 2 diabetes and the risk of mortality

Figure 2



Kaplan-Meier curve assessing the cumulative incidence of prostate cancer mortality between patients with and without pre-existing type 2 diabetes

Table 4: Baseline characteristics of patients with and without type 2 diabetes

Characteristics	Type 2 diabetes (n=1132)	No diabetes (n=10,788)
Age, n (%)	73.4 (7.9)	71.3 (9.0)
Ethnicity, n (%)		
White	959 (84.7)	9528 (88.3)
Black	36 (3.2)	99 (0.9)
Other	31 (2.7)	107 (1.0)
Unknown	106 (9.4)	1054 (9.8)
Excessive alcohol use, n (%)	141 (12.5)	741 (6.9)
Smoking status, n (%)		
Never	332 (29.3)	4403 (40.8)
Ever	773 (68.3)	5789 (53.7)
Unknown	27 (2.4)	596 (5.5)
Body mass index, n (%)		
≤25 kg/m^2	255 (22.5)	3608 (33.4)
25-30 kg/m^2	544 (48.1)	4672 (43.3)
≥ 30 kg/m^2	321 (28.4)	1669 (15.5)
Unknown	12 (1.1)	839 (7.8)
Co-morbidities, n (%)		
Chronic kidney disease	173 (15.3)	651 (6.0)
Myocardial infarction	160 (14.1)	865 (8.0)
Ischemic stroke	78 (6.9)	427 (4.0)
Transient ischemic attack	79 (6.8)	531 (4.9)
Peripheral artery disease	742 (65.6)	1146 (10.6)
Previous cancer, n (%)	191 (16.9)	1690 (15.7)
Prostate-specific antigen, n (%)		
< 4 ng/mL	84 (7.4)	609 (5.7)
4-10 ng/mL	228 (20.1)	2811 (26.1)
>10 ng/mL	505 (44.6)	4390 (40.7)
Unknown	315 (27.8)	2978 (27.6)
Gleason score, n (%)		
2-6	266 (23.5)	2688 (24.9)
7	222 (19.6)	2022 (18.7)
≥8	156 (13.8)	1214 (11.3)
Unknown	488 (43.1)	4864 (45.1)
Angiotensin converting enzyme inhibitors, n (%)	669 (59.1)	2658 (24.6)
Angiotensin receptor blockers, n (%)	200 (17.7)	760 (7.0)
Calcium channel blocker, n (%)	509 (45.0)	2857 (26.5)
Beta blockers, n (%)	492 (43.5)	2759 (25.6)
Diuretics, n (%)	593 (52.4)	3665 (34.0)
Antihypertensive, n (%)	930 (82.2)	5508 (51.1)
Other antihypertensive, n (%)	26 (2.2)	94 (0.9)
Aspirin, n (%)	719 (63.5)	3524 (32.7)
Other non-steroidal anti-inflammatory drugs, n (%)	608 (52.4)	5291 (49.1)
Statin use, n (%)	702 (62.0)	2722 (25.2)
5-alpha reductase inhibitors, n (%)	104 (9.2)	706 (6.5)
Radical prostatectomy	553 (48.9)	5458 (50.6)
Radiation therapy	185 (16.3)	1955 (18.1)
Chemotherapy	43 (3.8)	374 (3.5)
Androgen deprivation therapy	6216 (54.4)	5518 (51.2)

Abbreviations: SD, standard deviation

Table 5: Crude and adjusted hazard ratios for the association between type 2 diabetes and the incidence of prostate cancer mortality

Pre-existing type 2 diabetes	Patients	Cases	Person-Years	Rate/100 per year (95% CI)	Crude HR	Adjusted HR (95% CI)*
Absent	10,788	1606	51,705	3.1 (3.0-3.3)	1.00	1.00 (reference)
Present	1132	191	4,506	4.2 (3.7-4.9)	1.35	1.24 (1.04-1.47)
Duration of type 2 diabetes§, (years)						
<2.95	377	70	1,671	4.2 (3.3-5.3)	1.34	1.26 (0.98-1.61)
2.95-7.90	379	70	1,492	4.7 (3.7-5.9)	1.50	1.50 (1.16-1.94)
≥7.90	376	51	1,343	3.8 (2.9-5.0)	1.21	0.97 (0.72-1.30)

Abbreviations: CI, confidence interval; HR, hazard ratio

*Adjusted for age, ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, and peripheral artery disease), prostate-specific antigen levels, Gleason score, ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, statins, and the following prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

§Based on tertile categories.

Chapter 5: Metformin and the risk of mortality among patients with type 2 diabetes

The following chapter describes objective 2: The association between metformin use after prostate cancer diagnosis and the risk of mortality.

The following topic will first be introduced with some necessary background information on metformin use and cancer. The methods will then cover comprehensive information on the data sources and study population. The statistical analysis and results are then described in detail. A thorough discussion will then provide critical information, such as future implications for research.

Metformin use in patients with type 2 diabetes and the risk of mortality among patients with type 2 diabetes

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5.1 Abstract

Objective: Given the conflicting results from observational studies published to date, we assessed whether the use of metformin after a prostate cancer diagnosis is associated with a decreased risk of prostate cancer mortality and all-cause mortality, using a design that addressed the sources of bias.

Research design and methods: This study was conducted using the UK Cancer Registry, Clinical Practice Research Datalink, Hospital Episodes Database, and the Office of National Statistics. The cohort consisted of men with a history of treated type 2 diabetes, newly-diagnosed with non-metastatic prostate cancer between April 1, 1998 and December 31, 2009, and followed until October 1, 2012 or the occurrence of prostate cancer mortality and all-cause mortality. Nested case-control analyses were performed, where exposure was defined as use of metformin during the time to risk-set. Conditional logistic regression was used to estimate adjusted rate ratios (RRs) of each outcome with 95% confidence intervals (CIs).

Results: The cohort consisted of 935 men with type 2 diabetes and prostate cancer, followed for a mean 3.7 years during which 258 deaths occurred, including 112 from prostate cancer. Overall, the use of metformin after the prostate cancer diagnosis was not associated with a decreased risk of cancer-related mortality (RR: 1.09, 95% CI: 0.51-2.33) or all-cause mortality (RR: 0.79, 95% CI: 0.50-1.23).

Conclusions: The use of metformin after a prostate cancer diagnosis was not associated with a decreased risk of cancer-related mortality and all-cause mortality.

5.2 Introduction

Metformin is a safe and effective treatment that improves elevated insulin and glucose levels in patients with type 2 diabetes [27, 98]. In recent years, there has been interest in the antineoplastic activity of this compound demonstrated in several *in vitro* models [6, 33]. Proposed mechanisms of action begin with metformin inhibiting ATP production in the mitochondria, resulting in energetic stress [27]. Energetic stress results in the activation of AMPK which inhibits mTOR, and minimizes cellular energy consumption, thus inhibiting tumor growth [27]. Apart from this 'direct' mode of action, metformin may also act by lowering circulating levels of mitogens such as insulin or other cytokines that can stimulate tumor growth [27].

With respect to prostate cancer, observational studies investigating whether the use of metformin is associated with a decreased incidence have produced mixed results [99, 100]. However, there has been renewed interest in the effect of this drug on prostate cancer outcomes with four observational studies investigating the effects of metformin on prostate cancer mortality, distant metastasis, and all-cause mortality [23, 34, 36, 82]. In two studies, the use of metformin was associated with strong decreased risks (ranging between 45% to 80% risk reductions) of several prostate cancer outcomes [36, 82], while the other two studies reported non-significant findings [23, 34]. However, these studies had important methodological shortcomings, which included immortal time bias [34, 36, 82], a bias previously described in this literature [101, 102], and no consideration of latency time windows

and reverse causality [23, 34, 36, 82].

Given the methodological limitations of the few observational studies conducted to date, the primary objective of this population-based study is to determine whether the use of metformin after a prostate cancer diagnosis is associated with a decreased risk of cancer-related mortality. A secondary objective is to determine whether the use of this drug is associated with a decreased risk of all-cause mortality.

5.3 Methods

5.3.1 Data sources

This study was conducted by linking four large electronic databases from the United Kingdom (UK), the UK National Cancer Registry, the Clinical Practice Research Datalink (CPRD) (previously known as the General Practice Research Database), the Hospital Episode Statistics (HES) database, and the Office for National Statistics (ONS) database.

The UK National Cancer Registry contains tumour information, including site of primary growth (coded using the International Classification of Diseases, 10th revision [ICD-10]), grade, stage, and primary treatment received. The CPRD contains the complete medical record for more than 12 million people enrolled in more than 650 general practices. The geographic distribution of the practices participating in the CPRD has been shown to be representative of the UK population, and age and sex distributions of patients in the CPRD are similar to those reported by the National Population Census

[84-86]. Participating general practitioners have been trained to record medical information including demographic data, medical diagnoses, procedures, and deaths using a standardized form. Prescriptions written by CPRD physicians are automatically transcribed into the computer record. In addition, unlike administrative databases, the CPRD collects information regarding lifestyle variables such as body mass index (BMI), and quantitative and qualitative data pertaining to smoking and alcohol use. Read codes are used to enter medical diagnoses and procedures, which is the standard clinical terminology system used in general practice in the UK [84, 87], and a coded drug dictionary based on the UK Prescription Pricing Authority Dictionary is used for recording prescriptions [88]. The data collected are audited regularly and the participating general practices are subjected to a number of quality checks. Data recorded in the CPRD have been previously validated and proven to be of high quality [85, 87-90].

The HES database is a data warehouse containing details of all inpatient encounters in National Health Services hospitals in England since 1997. This database contains dates of hospital admissions, primary and secondary diagnoses (coded using the ICD-10 classification), and related procedures (coded using the ICD-10 classification and Office of Population Censuses and Surveys classification of interventions and procedures, 4th version [OPCS-4]). Finally, the ONS contains the electronic death certificates of all citizens living in the UK. This database was used to identify the

underlying cause of death (coded using the ICD-10 classification) for all patients who died during follow-up.

The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD and the Research Ethics Committee of the Jewish General Hospital, Montreal, Canada.

5.3.2 Study population

Using the UK National Cancer Registry, we identified all patients newly-diagnosed with prostate cancer (ICD-10 code: C61) between April 1, 1998 and December 31, 2009, followed until October 1, 2012. Cohort entry corresponded to the date of the prostate cancer diagnosis. We excluded patients with less than one year of 'up-to-standard' medical history in the CPRD prior to cohort entry, as well as patients diagnosed with metastatic disease (as identified in the UK National Cancer Registry, CPRD, or HES). Furthermore, the cohort was restricted to patients who had used anti-diabetic agents (metformin, sulfonylureas, thiazolidinediones, insulins, and other agents) in the year prior to cohort entry. This latter restriction was necessary to ensure that all patients had type 2 diabetes to minimize confounding by indication. Patients meeting the study inclusion criteria were followed until one of the outcomes of interest: prostate cancer mortality [primary outcome] and all-cause mortality [secondary outcomes], end of registration with the general practice, or the end of the study period (October 1, 2012), whichever came first.

5.3.3 Case-control selection

Two nested case-control analyses were conducted to assess the association between post-diagnostic use (i.e. after the prostate cancer diagnosis) of metformin and prostate cancer mortality and all-cause mortality. This approach was used due to the time-varying nature of metformin exposure and is computationally more efficient than a time-dependent survival analysis [103]. This approach produces odds ratios (ORs) that are unbiased estimators of rate ratios (RRs) [103-105].

From the cohort defined above, we identified all cases of prostate cancer mortality and all-cause mortality occurring during follow-up. The date of each case's outcome (prostate cancer mortality and all-cause mortality) defined the index date. Up to 10 controls were randomly selected from the case's risk set, after matching on year of birth, year of cohort entry, and duration of follow-up. By definition, all controls were alive, and registered with their general practice when matched to a given case. All analyses were restricted to cases and matched controls with at least one year of medical history prior to index date. This was to ensure a minimum exposure history for cases and matched controls.

5.3.4 Exposure to metformin

For cases and controls, we obtained prescriptions for all anti-diabetic agents prescribed between cohort entry and index date. We excluded exposures in the year immediately prior to index date in order to take into

account a biologically meaningful latency time window, and to minimize reverse causality, where early signs or symptoms of cancer may influence the initiation or termination of a treatment.

Exposure to metformin was defined in three ways. In the first approach, patients were considered exposed to metformin after their prostate cancer diagnosis if they received at least one prescription between cohort entry and the year prior to index date. For the second and third approach, we determined whether there was a dose-response relationship between metformin and the primary and secondary outcomes. Therefore, for patients deemed to be post-diagnostic users of metformin, we calculated their cumulative duration of use by summing the durations of each metformin prescription between cohort entry and the index date. Finally in the third approach, cumulative dose was computed by multiplying the daily dose of each metformin prescription by its specified duration of use. Thus, cumulative dose was calculated by summing the total quantities received between cohort entry and index date. Cumulative duration and dose were categorized in tertiles based on the distribution in the controls.

5.3.5 Statistical analysis

Conditional logistic regression was used to estimate RRs with 95% confidence intervals (CIs) of the two outcomes in relation to the post-diagnostic use of metformin. For the primary analysis, we evaluated whether post-diagnostic use of metformin was associated with a decreased risk of

prostate cancer mortality. In a secondary analysis, we determined whether post-diagnostic use of metformin was associated with a decreased risk of all-cause mortality. We also evaluated whether there was a dose-response relationship in terms of cumulative duration of use and cumulative dose for the primary and secondary outcomes of interest.

In addition to year of birth, year of cohort entry, and duration of follow-up on which the logistic regression was conditioned, the models were adjusted for the following potential confounders measured prior to cohort entry: excessive alcohol use (based on alcohol-related disorders such as alcoholism, alcoholic cirrhosis of the liver, alcoholic hepatitis and failure), smoking status (ever, never, unknown), obesity (BMI ≥ 30 kg/m^2), hemoglobin A1c (HbA1c) (last measure prior to cohort entry), pre-diagnostic use of anti-diabetic agents (metformin, sulfonylureas, thiazolidinediones, insulins, and other agents, entered individually in the models), Charlson comorbidity index, prostate-specific antigen (PSA) levels (last measure prior to cohort entry), Gleason score, and post-diagnostic use of other anti-diabetic drugs (measured between cohort entry and the year prior to index date). Tumor stage was not included as a covariate since it was missing for over 90% of the patients. In a secondary analysis, the models were additionally adjusted for the following prostate cancer-related variables measured between cohort entry and the year prior to index date: PSA testing activity (defined as the total number of tests performed), prostatectomy, radiation therapy, chemotherapy, and androgen deprivation therapy.

5.3.5.1 Sensitivity and secondary analyses

For all of the analyses described above, we applied a one year lag period prior to index date to account for a latency time window as well as to minimize reverse causality. Since the length of the true latency window is unknown, we performed a sensitivity analysis by varying that lag period to two years.

We also conducted secondary analyses to determine whether pre-diagnostic use of metformin, obesity (BMI≥30 kg/m^2), and age≥75 years acted as effect modifiers of the association between post-diagnostic use of metformin and the primary outcome of prostate cancer mortality. This was assessed by including interaction terms between these variables and post-diagnostic metformin use in the models. All analyses were conducted with SAS version 9.2 (SAS Institute, Cary, NC).

5.4 Results

A total of 935 men newly-diagnosed with non-metastatic prostate cancer with a history of anti-diabetic drug use were included in the study (Figure 3). The mean follow-up was 3.7 (standard deviation [SD]: 2.8) years, during which there were 258 deaths (incidence rate: 7.5% (95% CI: 6.6-8.4) per year), including 112 from prostate cancer (incidence rate: 3.2% (95% CI: 2.7-3.9) per year).

Table 6 presents the characteristics of the cases and matched controls for the primary outcome of prostate cancer mortality. Compared to controls, cases were more likely to have used alcohol excessively, to have been smokers, and obese. As expected, cases had higher PSA levels at cohort entry, higher Gleason scores, higher PSA testing activity, and were more likely to have used androgen deprivation therapy compared to controls.

The results of the primary analysis are presented in Table 7. Compared to non-use, post-diagnostic use of metformin was not associated with a decreased risk of prostate cancer mortality (adjusted RR: 1.09, 95% CI: 0.51-2.33). Similar null findings were obtained with the secondary outcome of all-cause mortality (Appendix Table 15). In the sensitivity analysis, varying the lag period prior to index date to two years did not materially change the results of the primary analysis (Appendix Table 16).

In a secondary analysis, the highest tertile category of metformin cumulative duration of use was associated with an increased risk of prostate cancer mortality (Table 8). Specifically, after 938 days of use, metformin was

associated with approximately a three-fold increased risk (RR: 3.20, 95% CI: 1.00-10.24). For cumulative dose, none of the RRs were statistically significant. No dose-response relationship in terms of cumulative duration and dose were observed for all-cause mortality (Appendix Table 15).

5.4.1 Subgroup analyses

In subgroup analyses, we explored whether the use of metformin before prostate cancer diagnosis, obesity (BMI≥30 kg/m^2), and age ≥75 years were effect modifiers of the association between post-diagnostic use of metformin and prostate cancer mortality. Overall, none of these variables were found to modify the association (Table 9).

5.5 Discussion

The results of this population-based study indicate that the use of metformin after a prostate cancer diagnosis is not associated with a decreased risk of prostate cancer mortality. Similar findings were observed with the secondary outcome of all-cause mortality.

Overall, the results of this study are inconsistent with the favourable effects of metformin on neoplasia observed in previous laboratory models [6, 33], and contrast with the results of the few observational studies conducted on this topic [23, 34, 36, 82]. Indeed, of the four observational studies conducted to date [23, 34, 36, 82], only two found a statistically significant decreased risk of prostate cancer outcomes [36, 82]. However, these studies had several methodological shortcomings. In the latter two studies, immortal time bias was introduced by not considering exposure in a time-dependent fashion. This bias was introduced by misclassifying the time between cohort entry and first metformin prescription as exposed, which greatly exaggerated the potential effects of metformin [36, 82]. In another study, the authors investigated the effect of post-diagnostic use of metformin, thiazolidinediones, sulfonylureas, and low doses of insulin [34]. The combination of those drugs was associated with a nonsignificant decreased risk in prostate cancer mortality. As with the two aforementioned studies [36, 82], immortal time bias was introduced by not considering metformin exposure as a time-dependent variable. Finally, none of the four observational studies conducted on this topic considered latency [23, 34, 36,

82], which is necessary for any study assessing the effect of a drug on cancer outcomes.

An unexpected finding of this study was the three-fold increased risk of prostate cancer mortality (RR: 3.20, 95% CI: 1.00-10.24) associated with the highest tertile of metformin cumulative duration of use. However, such an elevated risk was not observed with all-cause mortality. It is quite plausible that patients treated with long term metformin use may have had metabolic or clinical characteristics associated with an adverse prostate cancer outcome. For example, some clinicians may prefer to avoid insulin and maintain oral agent diabetes treatment in their patients who are seen clinically to have aggressive cancer. Thus, it is possible that patients were maintained on metformin or switched to this therapy as part of the palliative approach, resulting in what appears to be worse outcomes associated with longer durations of use. On the other hand, as previously reviewed (1), there are some models where metformin leads to increased vascular endothelial growth factor (VEGF) production by tumor cells, which could theoretically worsen prognosis. Thus, the apparent long-term adverse effect of metformin observed in this study requires further investigation.

This nested case-control study has several strengths. Firstly, by linking four electronic databases from the UK, we were able to obtain complete patient medical histories (including medication use, diagnoses, and treatments), lifestyle measurements (smoking, excessive alcohol use, and BMI), and cancer-related variables (Gleason scores, PSA levels, and prostate

cancer treatments). Therefore, we were able to adjust for a number of important potential confounders. Secondly, information in the CPRD database is prospectively collected, eliminating the likelihood of recall bias. Thirdly, controls were matched to cases using risk set sampling, and thus post-diagnostic use of metformin and other covariates measured during follow-up were assessed in a time-dependent fashion, eliminating the possibility of immortal time bias which affected some of the previous studies [34, 36, 82].

This nested case-control study has some limitations. First, drug information in the CPRD represents prescriptions written by general practitioners. As such, it is unknown whether prescriptions were actually filled at the pharmacy and whether patients fully complied with the treatment regimen. Furthermore, tumor stage was not included as a covariate since it was incomplete in the UK National Cancer Registry, and there was missing information of Gleason scores. However, we adjusted for prostate cancer-related treatments (such as prostatectomy, radiation therapy, androgen deprivation therapy, and chemotherapy), which are closely correlated with tumor characteristics [68]. Thus, we believe that this lack of information did not affect the validity of our study. Furthermore, despite adjusting the models for a number of potential confounders, residual confounding may still be present. Moreover, some variables such as smoking and BMI had missing information. However, within a cohort of patients with treated type 2 diabetes, it is unclear if the missing information is differential

between users of metformin and users of other anti-diabetic agents. in this cohort of patients with type 2 diabetes. Lastly, misclassification of the primary outcome of prostate cancer mortality is a possibility, although prostate cancer mortality was previously shown to be generally well recorded in death certificates [97].

The combination of two chronic diseases, type 2 diabetes and prostate cancer, is a major public health concern [106]. Contrary to previous studies that have found associations suggestive of a decreased risk [23, 34, 36, 82], this study did not find an association between use of metformin and mortality. A phase III randomized controlled trial (RCT) comparing metformin to placebo for men with early prostate cancer who meet specific criteria for active surveillance rather than immediate treatment has been initiated [110], and other RCTs for prostate cancer prevention or treatment of advanced metastatic disease have also been proposed. While these RCTS may provide more definitive evidence on the effects of metformin on mortality, our results do indicate that caution must be used in basing the rationale for conducting such RCTs solely on prior observational studies.

5.6 Figures and tables

Figure 3: Study flow chart: Metformin and the risk of mortality

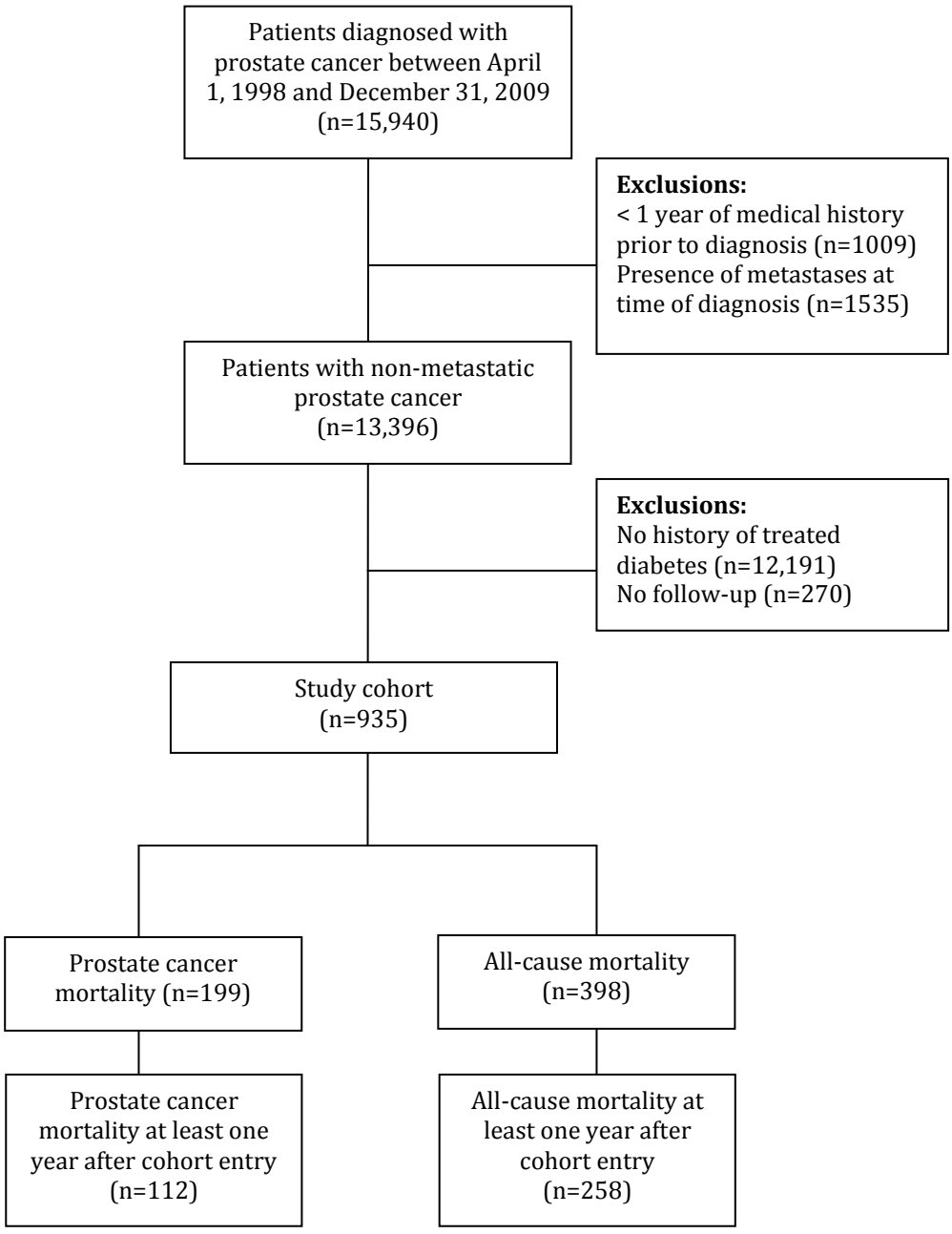

Table 6: Characteristics of prostate cancer mortality cases and matched controls

Characteristics	Cases (n=112)	Controls (n=268)
At index date		
Age (years), mean (SD)*	75.5 (8.1)	75.5 (7.6)
Duration of follow-up, mean (SD)*	3.4 (2.3)	3.4 (2.3)
At cohort entry		
Excessive alcohol use, n (%)	14 (12.5)	25 (9.3)
Smoking status, n (%)		
Never	29 (25.9)	91 (34.0)
Ever	79 (70.5)	170 (63.4)
Unknown	4 (3.6)	7 (2.6)
Body mass index, n (%)		
<30 kg/m^2	75 (67.0)	195 (72.8)
≥ 30 kg/m^2	36 (32.1)	72 (26.9)
Unknown	1 (0.9)	1 (0.4)
Hemoglobin A1C, n (%)		
≤7% (53 mmol/mol)	53 (47.3)	148 (55.2)
>7% (53 mmol/mol)	30 (26.8)	89 (33.2)
Unknown	29 (25.9)	31 (11.6)
Metformin, n (%)	78 (69.6)	194 (72.4)
Sulfonylureas, n (%)	80 (71.4)	184 (68.7)
Thiazolidinedione, n (%)	13 (11.6)	28 (10.5)
Insulins, n (%)	21 (18.8)	53 (19.8)
Other anti-diabetic drugs, n (%)	8 (7.1)	16 (6.0)
Charlson score, mean (SD)	1.92 (0.8)	1.93 (0.8)
Prostate-specific antigen, n (%)		
< 4 ng/mL	1 (0.9)	24 (9.0)
4-10 ng/mL	12 (10.7)	49 (18.3)
>10 ng/mL	57 (50.9)	121 (45.2)
Unknown	42 (37.5)	74 (27.6)
Gleason score, n (%)		
2-4	2 (1.8)	10 (3.7)
5-7	23 (20.54)	102 (38.1)
≥8	29 (25.9)	47 (17.5)
Unknown	58 (51.8)	109 (40.7)
During follow-up		
Prostate-specific antigen testing activity, mean (SD)	3.1 (4.3)	2.1 (3.0)
Prostatectomy, n (%)	55 (49.1)	149 (55.6)
Radiation therapy, n (%)	16 (14.3)	50 (18.7)
Chemotherapy, n (%)	4 (3.6)	8 (3.0)
Androgen deprivation therapy, n (%)	105 (93.8)	184 (68.7)

* Matching factors along with year of cohort entry.

Table 7: Post-diagnostic use of metformin and the risk of prostate cancer mortality

Metformin exposure	Cases (n=112)	Controls (n=268)	Crude RR*	Model 1 Adjusted RR (95% CI)†	Model 2 Adjusted RR (95% CI)‡
No use after prostate cancer diagnosis, n (%)	41 (36.6)	97 (36.2)	1.00	1.00 (reference)	1.00 (reference)
Use after prostate cancer diagnosis, n (%)	71 (63.4)	171 (63.8)	1.23	1.12 (0.56-2.25)	1.09 (0.51-2.33)

Abbreviations: RR, rate ratio; CI, confidence interval.

* Cases and controls matched on year of birth, year of cohort entry, and duration of follow-up.

† Model 1 was adjusted for excessive alcohol use, smoking, obesity, Hemoglobin A1C, pre-diagnostic use of metformin, sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs, Charlson score, prostate-specific antigen, Gleason score, and post-diagnostic use of sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs.

‡ Model 2 included the variables in Model 1 and was additionally adjusted for prostate-specific antigen testing activity, prostatectomy, radiation therapy, chemotherapy, and use of androgen deprivation therapy.

Table 8: Cumulative duration and dose of metformin use and the risk of prostate cancer mortality

Metformin exposure	Cases (n=112)	Controls (n=268)	Crude RR*	Model 1 Adjusted RR (95% CI)†	Model 2 Adjusted RR (95% CI)‡
No use after prostate cancer diagnosis, n (%)	41 (36.6)	97 (36.2)	1.00	1.00 (reference)	1.00 (reference)
Cumulative duration (days)§, n (%)					
1-537	18 (16.1)	57 (21.3)	1.09	0.98 (0.38-2.58)	1.03 (0.36-2.96)
537-938	15 (13.4)	55 (20.5)	0.73	0.65 (0.26-1.64)	0.54 (0.20-1.44)
≥938	38 (33.9)	59 (22.0)	2.37	2.62 (0.91-7.50)	3.20 (1.00-10.24)
Cumulative dose (mg)§, n (%)					
1-514,385	23 (20.5)	57 (21.3)	1.32	1.30 (0.56-3.00)	1.36 (0.54-3.43)
514,385-991,840	14 (12.5)	55 (20.5)	0.99	0.80 (0.33-1.96)	0.66 (0.25-1.78)
≥991,840	34 (30.4)	59 (22.0)	1.34	1.26 (0.52-3.06)	1.28 (0.49-3.33)

Abbreviations: RR, rate ratio; CI, confidence interval.

* Cases and controls matched on year of birth, year of cohort entry, and duration of follow-up.

† Model 1 was adjusted for excessive alcohol use, smoking, obesity, Hemoglobin A1C, pre-diagnostic use of metformin, sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs, Charlson score, prostate-specific antigen, Gleason score, and post-diagnostic use of sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs.

‡ Model 2 included the variables in Model 1 and was additionally adjusted for prostate-specific antigen testing activity, prostatectomy, radiation therapy, chemotherapy, and use of androgen deprivation therapy.

§ Based on tertile categories.

Table 9: Potential effect measure modifiers of the association between post-diagnostic use of metformin and prostate cancer mortality

Characteristic	Characteristic absent Adjusted RR (95% CI)*	Characteristic present Adjusted RR (95% CI)*	p-value for interaction
Model 1†			
Pre-diagnostic use of metformin	1.65 (0.49-5.52)	0.91 (0.38-2.18)	0.45
Obesity (body mass index ≥30 kg/m^2)	1.16 (0.53-2.51)	1.11 (0.34-3.66)	0.95
Age ≥75 years	1.07 (0.40-2.83)	1.16 (0.51-2.64)	0.89
Model 2‡			
Pre-diagnostic use of metformin	1.96 (0.56-6.83)	0.75 (0.28-1.99)	0.25
Obesity (body mass index ≥30 kg/m^2)	1.12 (0.48-2.65)	1.07 (0.30-3.82)	0.95
Age ≥75 years	1.07 (0.37-3.07)	1.09 (0.45-2.66)	0.98

Abbreviations: RR, rate ratio; CI, confidence interval.
* Cases and controls matched on year of birth, year of cohort entry, and duration of follow-up.
† Model 1 was adjusted for excessive alcohol use, smoking, obesity, Hemoglobin A1C, pre-diagnostic use of metformin, sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs, Charlson score, prostate-specific antigen, Gleason score, and post-diagnostic use of sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs.
‡ Model 2 included the variables in Model 1 and was additionally adjusted for prostate-specific antigen testing activity, prostatectomy, radiation therapy, chemotherapy, and use of androgen deprivation therapy.

Chapter 6: General Discussion

The results provided in this thesis illustrate the association between type 2 diabetes and its metformin treatment on mortality in patients with type 2 diabetes.

In the first objective, type 2 diabetes was found to be associated with an increased risk of prostate cancer mortality and all-cause mortality. This finding contrasts with the inverse relationship previously reported between type 2 diabetes and prostate cancer incidence [74]. The decreased risk of prostate cancer incidence observed in patients with type 2 diabetes may be related to lower testosterone levels, limiting the growth of tumors in the prostate [70, 71]. Furthermore, experimental models have confirmed that hyperinsulinemia may be associated with the decreased testosterone levels in patients with type 2 diabetes [72, 73]. However, different mechanisms may be at play with respect to cancer-related mortality. It is possible that certain diabetic characteristics may contribute to a worse prognosis of prostate cancer. Recent studies have shown that obesity and hyperinsulinemia are associated with worse prostate cancer outcomes [60, 61, 92].

In a secondary analysis, shorter diabetes durations were found to be associated with an increased risk of prostate cancer mortality. Alternatively, longer type 2 diabetes durations (highest tertile >7.9 years) were associated with null findings (HR: 0.97, 95% CI: 0.72-1.30). This surprising result may

be explained by competing risks, where patients with type 2 diabetes are dying from other causes [95]. In order to confirm this possibility, another analysis assessed the duration of type 2 diabetes on non-prostate cancer mortality. As expected, patients with longer durations of diabetes were associated with an increased risk of non-prostate cancer mortality.

The results of this study, along with the previous observational studies [2, 4, 5, 22-26], suggest an increased risk of mortality among patients newly-diagnosed with prostate cancer with pre-existing type 2 diabetes. While only three studies assessed the association between type 2 diabetes and prostate cancer mortality [2, 4, 5], one study was likely underpowered [2] and another study reported age-standardized mortality rate ratios from an Asian population [5]. Furthermore, only one study assessed the duration of type 2 diabetes in two groups (<10 years and ≥10 years) on the risk of mortality [5]. Overall, our study confirms an association between type 2 diabetes and an increased risk of prostate cancer mortality and all-cause mortality.

In the secondary objective of this thesis, metformin use after prostate cancer diagnosis was not significantly associated with a decreased risk of prostate cancer mortality. The point estimate obtained with respect to prostate cancer mortality was however suggestive of a modest increased risk with a wide confidence interval (RR: 1.09, 95% CI: 0.51-2.33). Although these results do not reject the null hypothesis of a decreased risk of prostate cancer

mortality, they do not suggest an increased risk of prostate cancer mortality. In a secondary analysis, the highest tertile of metformin cumulative duration (≥938 days) and dose (≥991,840 mg) categories were associated with an increased risk of prostate cancer mortality. Given this increased risk was not observed with all-cause mortality, it may be plausible that patients with longer cumulative durations and doses of metformin had other metabolic complications affiliated with worse mortality. Consequently, physicians may have preferred to keep high-risk patients on metformin, as opposed to second and third line diabetes treatments as part of palliative approach strategy. Alternatively, the apparent increased risk may be due to metformin activating AMPK, which can activate the vascular endothelial growth factor (VEGF), a protein that favors cell survival [107, 108].

Further research is needed to confirm the association between metformin and mortality. While the previous studies on this topic suggest metformin is associated with large risk reductions [23, 34, 36, 82], immortal time bias is likely the cause of some of those spurious associations [36, 82, 102]. This bias occurs when a time varying exposure is analyzed in a time independent fashion [109]. Therefore, the time between cohort entry and first exposure is misclassified as exposed time, underestimating the true risk. Furthermore, none of the prior observational studies considered latency, which is important when determining the effects of a drug on cancer outcomes [23, 34, 36, 82].

Strengths of both studies include the population-based study design and a follow-up time for up to 14 years. Moreover, the CPRD contains information that is recorded prospectively, eliminating the possibility of recall bias, where patients with the disease may recall information differentially from patients without the disease. Additionally, unlike some of the previous observational studies, this study linked four electronic databases from the UK, enabling adjustment for lifestyle variables including smoking, BMI, and excessive alcohol use and prostate cancer related treatments, such as prostatectomy, chemotherapy, androgen deprivation therapy, and radiation therapy. Finally, sensitivity analyses were conducted to ensure the validity and reliability of the results.

Strengths specific to the association between post-diagnostic metformin use and prostate cancer mortality include the nested case-control design, where controls were matched to cases on age and follow-up time using risk set sampling. Furthermore, due to the time varying nature of metformin exposure, this design assessed the exposure in a time-dependent manner in order to avoid immortal time bias and exposure misclassification. Furthermore, the cohort was composed of patients newly-diagnosed with non-metastatic prostate cancer, and cases and controls were matched on time since diagnosis. This method ensured that both cases and matched controls were comparable on the length of their disease at the time of the risk.

Both studies had some limitations as well. Tumor stage was missing in over 90% of the study population. However, the models adjusted for prostate cancer treatments, which have been shown to be highly correlated with tumor characteristics [96]. Furthermore, like with any observational study, residual confounding always needs to be considered. However, the models were adjusted for many important confounders, which we believe minimized this bias. Thirdly, there was missing information on BMI and smoking. However, within a cohort of men with treated diabetes, it is unclear if the missing information would be differential between metformin and non-metformin users. Additionally, possible misclassification with the outcome of interest, prostate cancer mortality, needs to be considered. However, prostate cancer has been shown to be well recorded in death certificates [97].

Overall, these observational studies have shown an association between type 2 diabetes and mortality, as well as metformin use after prostate cancer diagnosis and mortality. Given the results of the association between type 2 diabetes and mortality in the first study do not contradict the prior observational and experimental models, perhaps further observational research is not necessary. Alternatively, further research is necessary to confirm the association between metformin use and cancer-related mortality.

Chapter 7: Conclusion

Type 2 diabetes is associated with an increased risk of mortality among patients with prostate cancer. Clinicians should be aware of this association when considering different diabetes and prostate cancer treatments in order to delay progression of either disease. Additionally, metformin use after prostate cancer diagnosis is not associated with a decreased risk of prostate cancer mortality.

The results obtained in this study do not justify the initiation of clinical or randomized control trials. Currently however, phase III randomized controlled trials are being conducted with respect to metformin use versus placebo effects on men with early prostate cancer [110]. While such research may yield important data, our results suggest that these trials should incorporate early stopping rules, especially if observational data report no benefit.

Important future research should address the discrepancy of dosage between observational and experimental studies. Reasonable next steps include measuring antineoplastic effects of metformin in experimental models adjusted to use doses that achieve serum metformin levels similar to those seen in diabetes treatment or conducting phase I clinical trials to determine if the drug exposure levels used in the experimental models can be achieved clinically.

Chapter 8: References

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Chapter 9: Appendix

9.1 Ethics Approval

9.1.1 ISAC approvals

ISAC EVALUATION OF PROTOCOLS FOR RESEARCH INVOLVING CPRD DATA

FEED-BACK TO APPLICANTS

CONFIDENTIAL			***by e-mail***
PROTOCOL NO:	13_058		
PROTOCOL TITLE:	The impact of type 2 diabetes on prostate cancer mortality and recurrence		
APPLICANT:	Samy Suissa, Professor, McGill University		
APPROVED ☐	**APPROVED SUBJECT TO MINOR AMENDMENT** **(resubmission not required)** ☒	**REVISION/ RESUBMISSION REQUESTED** ☐	**REJECTED** ☐

INSTRUCTIONS:

Please include your response/s to the Reviewer's feedback below <u>only</u> if you are required to Revise/ Resubmit your protocol.

Protocols with an outcome of 'Approved' or 'Approved subject to minor amendments' <u>do not</u> require resubmission to the ISAC

REVIEWER COMMENTS:

Protocol 13_058 is approved with the following comments:

1. It appears to us that the answer to Question 9 on the application form should be "Disease Epidemiology" rather than "Adverse Drug Reaction".

2. Given that your objective relates specifically to Type 2 diabetes, you will need to consider how patients who have or may have Type I diabetes will be handled. As written on page 8, your definition of Type 2 diabetes is likely to include some patients on insulin only who actually have Type 1 diabetes.

DATE OF ISAC FEEDBACK:	05 April 2013
DATE OF APPLICANT FEEDBACK:	

ISAC EVALUATION OF PROTOCOLS FOR RESEARCH INVOLVING CPRD DATA

FEED-BACK TO APPLICANTS

<table>
<tr><td colspan="4">CONFIDENTIAL by e-mail</td></tr>
<tr><td>PROTOCOL NO:</td><td colspan="3">13_022R</td></tr>
<tr><td>PROTOCOL TITLE:</td><td colspan="3">Metformin use in prostate cancer and the risk of death in patients with type 2 diabetes</td></tr>
<tr><td>APPLICANT:</td><td colspan="3">Samy Suissa, Professor, McGill University</td></tr>
<tr><td>APPROVED
☒</td><td>APPROVED SUBJECT TO MINOR AMENDMENT
(resubmission not required)
☐</td><td>REVISION/ RESUBMISSION REQUESTED
☐</td><td>REJECTED
☐</td></tr>
<tr><td colspan="4">INSTRUCTIONS:

Please include your response/s to the Reviewer's feedback below <u>only</u> if you are required to Revise/ Resubmit your protocol.

Protocols with an outcome of 'Approved' or 'Approved subject to minor amendments' <u>do not</u> require resubmission to the ISAC

REVIEWER COMMENTS:

Protocol 13_022R is approved.</td></tr>
<tr><td colspan="2">DATE OF ISAC FEEDBACK:</td><td colspan="2">12 March 2013</td></tr>
<tr><td colspan="2">DATE OF APPLICANT FEEDBACK:</td><td colspan="2"></td></tr>
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9.2 Appendix Tables and Figures

9.2.1 Type 2 diabetes and the risk of mortality among patients with prostate cancer

Table 10: Crude and adjusted hazard ratios for the association between type 2 diabetes and all-cause mortality

Pre-existing type 2 diabetes	Patients	Cases	Person-Years	Rate/100 per year (95% CI)	Crude HR	Adjusted HR (95% CI)*
Absent	10,788	3193	51,705	6.2 (6.0-6.4)	1.00	1.00 (reference)
Present	1132	412	4,506	9.1 (8.3-10.1)	1.49	1.25 (1.12-1.41)
Duration of type 2 diabetes§, (years)						
<2.95	377	127	1,671	7.6 (6.4-9.0)	1.24	1.11 (0.93-1.33)
2.95-7.90	379	147	1,492	9.9 (8.4-11.6)	1.61	1.44 (1.20-1.71)
≥7.90	376	138	1,343	10.3 (8.7-12.1)	1.69	1.25 (1.04-1.50)

Abbreviations: CI, confidence interval; HR, hazard ratio

*Adjusted for age, ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, and peripheral artery disease), prostate-specific antigen levels, Gleason score, ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, statins, and the following prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

§Based on tertile categories.

Table 11: Sensitivity analysis on association between type 2 diabetes and prostate cancer mortality with censored patients who developed diabetes during follow-up

Pre-existing type 2 diabetes	Patients	Cases	Person-Years	Rate/100 per year (95% CI)	Crude HR	Adjusted HR (95% CI)*
Absent	10,734	1601	49,790	3.2 (3.1-3.4)	1.00	1.00 (reference)
Present	1132	191	4,343	4.4 (3.8-5.1)	1.32	1.22 (1.03-1.45)

Abbreviations: CI, confidence interval; HR, hazard ratio

*Adjusted for age, ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, and peripheral artery disease), prostate-specific antigen levels, Gleason score, ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, statins, and the following prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

Table 12: Type 2 diabetes and non-prostate cancer mortality

Pre-existing type 2 diabetes	Patients	Cases	Person-Years	Rate/100 per year (95% CI)	Crude HR	Adjusted HR (95% CI)*
Absent	10,788	1587	51,705	3.1 (2.9-3.2)	1.00	1.00 (reference)
Present	1132	221	4,506	4.9 (4.3-5.8)	1.66	1.28 (1.09-1.50)
Duration of type 2 diabetes§, (years)						
<2.95	307	57	1,671	3.4 (2.6-4.4)	1.17	1.04 (0.80-1.37)
2.95-7.90	309	77	1,492	5.2 (4.1-6.4)	1.73	1.35 (1.05-1.72)
≥7.90	325	87	1,343	6.5 (5.2-8.0)	2.17	1.56 (0.15-1.85)

Abbreviations: CI, confidence interval; HR, hazard ratio

*Adjusted for age, ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, and peripheral artery disease), prostate-specific antigen levels, Gleason score, ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, statins, and the following prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

§Based on tertile categories.

Table 13: Effect measure modification by body mass index [BMI] on the association between type 2 diabetes and mortality

Outcome	BMI<30 (kg/m^2) Adjusted HR (95% CI)	BMI≥30 (kg/m^2) Adjusted HR (95% CI)	P-value for interaction
Prostate cancer mortality	1.16 (0.96-1.41)	1.30 (0.94-1.81)	0.54
All-cause mortality	1.19 (1.04-1.35)	1.21 (0.97-1.51)	0.89

Abbreviations: CI, confidence interval; HR, hazard ratio

*Adjusted for age, ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities (chronic kidney disease, myocardial infarction, ischemic stroke, transient ischemic attack, and peripheral artery disease), prostate-specific antigen levels, Gleason score, ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, statins, and the following prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

Table 14: Effect measure modification by age on the association between type 2 diabetes and mortality

Outcome	<60 years Adjusted RR (95% CI)	60-75 years Adjusted RR (95% CI)	≥75 years Adjusted RR (95% CI)	P-value for interaction
Prostate Cancer Mortality	1.71 (0.69-4.24)	1.16 (0.88-1.51)	1.28 (1.04-1.57)	0.54
All-cause mortality	1.51 (0.70-3.25)	1.28 (1.07-1.54)	1.23 (1.06-1.41)	0.60

Adjusted for ethnicity, excessive alcohol use, obesity, smoking, previous cancer, comorbidities, psa levels, gleason grade, ace inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, 5-alpha reductase inhibitors, and use of non-steroidal anti-inflammatory drugs, antihypertensive drugs, and statins.
Prostate cancer related variables: prostatectomy, radiation, androgen deprivation therapy, and chemotherapy.

9.2.2 Metformin and the risk of mortality among patients with type 2 diabetes

Table 15: Post-diagnostic use of metformin and the risk of all-cause mortality

Metformin exposure	Cases (n=258)	Controls (n=613)	Crude RR*	Model 1 Adjusted RR (95% CI)†	Model 2 Adjusted RR (95% CI) ‡
No use after prostate cancer diagnosis, n (%)	103 (39.9)	215 (35.1)	1.00	1.00 (reference)	1.00 (reference)
Use after prostate cancer diagnosis, n (%)	155 (60.1)	398 (64.9)	0.88	0.79 (0.51-1.23)	1.79 (0.50-1.23)
Cumulative duration (days)§, n (%)					
1-587	41 (15.9)	134 (21.9)	0.82	0.75 (0.42-1.33)	0.74 (0.41-1.34)
587-1116	48 (18.6)	128 (20.9)	0.85	0.74 (0.43-1.28)	0.74 (0.42-1.28)
≥1116	66 (25.6)	136 (22.2)	1.00	0.96 (0.51-1.81)	0.95 (0.50-1.83)
Cumulative dose (mg)§, n (%)					
1-562,500	55 (21.3)	132 (21.5)	0.97	0.83 (0.50-1.36)	0.84 (0.51-1.39)
562,500-1,125,000	43 (16.7)	130 (21.2)	0.84	0.78 (0.45-1.36)	0.78 (0.44-1.37)
≥1,125,000	57 (22.1)	136 (22.2)	0.81	0.74 (0.41-1.35)	0.70 (0.38-1.30)

Abbreviations: RR, rate ratio; CI, confidence interval.
* Cases and controls matched on year of birth, year of cohort entry, and duration of follow-up.
† Model 1 was adjusted for the following variables measured at cohort entry: excessive alcohol use, smoking, obesity, Hemoglobin A1C, pre-diagnostic use of metformin, sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs, Charlson score, prostate-specific antigen, and Gleason score. The model was also adjusted for the following variables measured during follow-up: post-diagnostic use of sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs.
‡ Model 2 was additionally adjusted for the following variables measured during follow-up: prostate-specific antigen testing activity, prostatectomy, radiation therapy, chemotherapy, and androgen deprivation therapy.
§ Based on tertile categories.

Table 16: Sensitivity analysis using a two-year lag for the association between post-diagnostic use of metformin and prostate cancer mortality

Metformin exposure	Cases (n=112)	Controls (n=268)	Crude RR*	Model 1 Adjusted RR (95% CI)†	Model 2 Adjusted RR (95% CI)‡
No use after prostate cancer diagnosis, n (%)	41 (36.6)	97 (36.2)	1.00	1.00 (reference)	1.00 (reference)
Use after prostate cancer diagnosis, n (%)	71 (63.4)	171 (63.8)	1.50	1.03 (0.42-2.52)	0.90 (0.32-2.57)

Abbreviations: RR, rate ratio; CI, confidence interval.

* Cases and controls matched on year of birth, year of cohort entry, and duration of follow-up.

† Model 1 was adjusted for the following variables measured at cohort entry: excessive alcohol use, smoking, obesity, Hemoglobin A1C, pre-diagnostic use of metformin, sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs, Charlson score, prostate-specific antigen, and Gleason score. The model was also adjusted for the following variables measured during follow-up: post-diagnostic use of sulfonylureas, thiazolidinediones, insulins, other anti-diabetic drugs.

‡ Model 2 was additionally adjusted for the following variables measured during follow-up: prostate-specific antigen testing activity, prostatectomy, radiation therapy, chemotherapy, and androgen deprivation therapy.