The associations between insulin, hypoglycemia, and dementia: Combating threats to internal validity in a series of population-based cohort studies

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

Wajd Alkabbani was the sole author for Chapters 1 and 6 which were written under the supervision of Dr. John-Michael Gamble and were not written for publication. This thesis consists in part of four manuscripts written for publication. Exceptions to sole authorship of material are as follows:

Research presented in Chapters 2, 3, 4, and 5:

This research was conducted at the University of Waterloo by Wajd Alkabbani under the supervision of Dr. John-Michael Gamble. Wajd Alkabbani conceived the study idea, designed the study, conducted all analyses, and wrote the first draft of the manuscript. Dr. Colleen Maxwell, Dr. Ruth Ann Marrie, Dr. Suzanne Tyas, Dr. Ilana Lega, and Dr. John-Michael Gamble contributed to design, methodology, and the final draft of the manuscript. All authors approve of the final published or submitted of the manuscript.

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Abstract

Although the association between type 2 diabetes and dementia is recognized, findings from the epidemiology literature on the effect of insulin and one of its side effects, hypoglycemia, are less clear. The currently available observational studies assessing these associations suffer from a wide range of methodological limitations that diminish their internal validity and lead to contradictory evidence. The aim of this thesis is to implement design and analysis techniques to combat bias and confounding in previous studies and to further extend knowledge on the risk of dementia associated with four interconnected diabetes-related exposures, each assessed in a separate study: 1) severe hypoglycemia, 2) age of severe hypoglycemia, 3) insulin use, and 4) the mediating effect of severe hypoglycemia from insulin use.

Herein, a series of cohort studies were conducted using population-based health administrative data (1996-2018) from British Columbia, Canada housed by Population Data BC. First, we identified individuals newly diagnosed with type 2 diabetes between 01 January 1998 and 31 December 2016. Each cohort was then designed based on the research question, wherein exposure was defined accordingly. For studies 1 and 2, the exposure of interest was severe hypoglycemia compared to no hypoglycemia. For studies 3 and 4, the exposure of interest was insulin initiation compared to initiating a non-insulin class. For all cohorts, the outcome of interest was all-cause dementia. Confounding adjustment techniques including inverse probability of treatment weighting (IPTW) were used in all studies. In each study, a wide range of sensitivity analyses were conducted to ensure the robustness of results.

Findings from study 1 confirm the previously reported higher risk of all-cause dementia with severe hypoglycemia after implementing exposure density sampling, a lag period, and IPTW (HR 1.83; 95% CI 1.31-2.57). Findings from study 2 show that the increased risk of dementia observed in study 1 is consistent whether hypoglycemia occurs in midlife (HR 2.85; 95% CI 1.72-4.72) or late life (HR 2.38; 95% CI 1.83-3.11). Conversely, findings from study 3 negate existing evidence and do not show an increased risk of dementia associated with insulin use (HR 1.14; 95% CI 0.81-1.60). Lastly,

findings from study 4 indicate a potential role of severe hypoglycemia as a mediator of the association between insulin and dementia (Natural Indirect Effect HR 1.04; 95% CI 1.01-1.08).

Collectively these studies provide further insight on the complex associations between insulin, hypoglycemia, and the risk of all-cause dementia to inform both clinicians and patients with type 2 diabetes on the need to prevent hypoglycemia. Importantly, these studies showcase the need for robust methodology when conducting observational studies for type 2 diabetes-related exposures.

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Dedication

To my parents who paved the way.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

A1C	-	Glycated Hemoglobin A1C
ACCORD	-	Action To Control Cardiovascular Risk In Diabetes
ACCORD-MIND	-	Action To Control Cardiovascular Risk In Diabetes – Memory IN Diabetes
AD	-	Alzheimer's Disease
aDCSI	-	Adapted Diabetes Complications Severity Index
ADL	-	Activities Of Daily Living
AGE	-	Advanced Products Of Glycosylation
ATC	-	Anatomical Therapeutic Code
BC	-	British Columbia
BCGP	-	British Columbia Generation Project
BPSD	-	Behavioural And Psychological Symptoms Of Dementia
CAIDE	-	Cardiovascular Risk Factors, Aging, And Dementia
CI	-	Confidence Interval
CVD	-	Cardiovascular Disease
DM	-	Diabetes Mellitus
DPP-4	-	Dipeptidyl-Peptidase-4
DSM	-	Diagnostic And Statistical Manual
DSST	-	Digit Symbol Substitution Test
GLP-1	-	Glucagon-Like Peptide
GPRD	-	General Practice Research
HDPS	-	High Dimensional Propensity Score
HR	-	Hazard Ratio
ICD-10-CA	-	International Classification of Disease-10-Canadian Adaptation
ICD-9-CM	-	International Classification of Disease-9-Clinical Modification
IDE	-	Insulin Degrading Enzyme
IPTW	-	Inverse Probability of Treatment Weighting
IQR	-	Interquartile Range
KPW	-	Kaiser Permanente Washington
MCI	-	Mild Cognitive Impairment

MMSE	-	Mini-Mental State Examination
NACRS	-	National Ambulatory Care Reporting System
NCD	-	Neurocognitive Disorder
NDE	-	Natural Direct Effect
NIE	-	Natural Indirect Effect
OR	-	Odds Ratio
ORIGIN	-	Outcome Reduction with Initial Glargine Intervention
PopData BC	-	Population Data British Columbia
PS	-	Propensity Score
RCT	-	Randomized Controlled Trial
RR	-	Risk Ratio, Relative Risk
SAS	-	Statistical Analysis Software
SD	-	Standard Deviation
SE	-	Standard Error
SES	-	Socioeconomic Status
SGLT-2	-	Sodium Glucose Cotransporter 2
SMD	-	Standardized Mean Difference
SU	-	Sulfonylureas
TBV	-	Total Brain Volume
TNF	-	Tumor Necrosis Factor
TZD	-	Thiazolidinediones
UKPDS	-	United Kingdom Prospective Diabetes
VD	-	Vascular Dementia
VHA	-	Veterans Health Affairs

Chapter 1
Type 2 diabetes and dementia: Background and relationship

1.1 Introduction

Both type 2 diabetes and dementia are heterogeneous complex chronic conditions recognized as global public health concerns. More than 536 million individuals are living with type 2 diabetes around the world based on the 10th edition of the International Diabetes Federation (IDF) report.[1] This estimate is expected to increase to 783.2 million by 2045, corresponding to more than US$ 414 billion in total diabetes-related health expenditures in North America only.[1] Dementia, an umbrella term describing a set of symptoms of progressive deterioration in cognition, is one of the most common causes of disability among seniors. Globally, more than 55 million people live with dementia, with projections showing this number to increase to 78 million in 2030 and 139 million in 2050.[2] The current estimated total global societal cost of dementia is US$ 1.3 trillion.[2]

Although on the surface, the disease processes of dementia and diabetes appear to differ, several complex and interconnected metabolic and inflammatory pathophysiological mechanisms by which diabetes is linked to dementia have been emerging from ongoing research. In fact, epidemiologic studies have shown diabetes to be one of multiple cardiometabolic risk factors of dementia.[3–5] Herein, an overview of the pathophysiology, management, and outcomes of both conditions is provided. The mechanisms that have been hypothesized to explain the association between type 2 diabetes and dementia are outlined. This chapter also highlights the currently available epidemiologic evidence linking type 2 diabetes and dementia, with a focus on the effect of insulin and one of its side effects, hypoglycemia. Lastly, previous observational studies assessing the associations between insulin, hypoglycemia, and the risk of incident dementia are reviewed to identify methodologic limitations and inform the project rationale and objectives.

1.2 Type 2 diabetes

1.2.1 Pathophysiology of type 2 diabetes

Diabetes Mellitus is a metabolic disorder that results from beta cell (β-cell) dysfunction to secrete insulin and/or the failure of insulin to exert its biological influence at the level of peripheral tissues (e.g., muscle, adipose, or hepatic tissues), thus leading to chronic high levels of glucose, or hyperglycaemia.[6] Diabetes often involves disturbances in the metabolism of carbohydrates, fat, and protein with consequences resulting in long-term complications.

Most cases of diabetes fall under two main categories, type 1 and type 2 diabetes. The hallmark of type 1, which represents 5-10% of diabetes cases, is an absolute deficiency of insulin secretion due to an autoimmune destruction of the β-cells of the pancreas.[6,7] Although it can occur at any age, type 1 diabetes is commonly diagnosed among children and adolescents. The other, much more prevalent category accounting for 90-95% of cases, is type 2 diabetes, also known as adult-onset diabetes. In this type, there is a resistance to insulin action with an inadequate compensatory response of insulin secretion.[6,7] The risk of developing type 2 diabetes increases with several factors, such as age, obesity, family history, lack of physical activity, presence of related comorbidities like hypertension, as well as certain drugs (e.g. glucocorticoids).[8] Other types of diabetes mellitus are gestational diabetes, which occurs temporarily during pregnancy, and secondary diabetes, that is attributed to the use of medications or other medical conditions, and lastly, genetic diabetes due to genetic dysfunction in insulin secretion.[6,7]

1.2.2 Pharmacotherapeutic options of type 2 diabetes

The field of diabetes therapies is dynamic and has seen many changes over the years, with new agents entering the market empowered by non-glycemic benefits while other agents have fallen down the list due

to safety concerns. There are currently eight classes of antihyperglycemic agents available in Canada, in addition to insulin.[9] A summary of the mechanism of action and adverse effect profile for these agents is reported in Table 1.1. Generally speaking, there are four major categories of mechanisms by which antihyperglycemic agents exert their effect: 1) stimulating insulin secretion; 2) increasing insulin sensitivity at the cell level; 3) mimicking the effect of incretin, a hormone involved in glucose metabolism; and 4) increasing elimination of glucose in urine. While all mechanisms impact the level of glucose in the body, only two, insulin secretagogues and insulin sensitizers, have a direct effect on blood-insulin levels.

1.2.3 Management of type 2 diabetes

Management of diabetes depends on the type; while type 1 management is exclusively by insulin, the management of type 2 is a stepwise approach that takes into account multiple treatment and patient-related factors. Since there is a relative rather than absolute insulin deficiency, patients often, at least initially, do not require insulin for survival or treatment.[9] Although insulin resistance may improve with weight reduction and/or pharmacological treatment, it is rarely restored to normal. The aim of diabetes management is not only to control the symptoms of hyperglycemia, such as polyuria, polydipsia, and fatigue, but also to reduce the risk of complications.[9]

At diagnosis and depending on how far away the glycated hemoglobin A1C (A1C) is from the individualized target level, typically 7%, patients can receive a wide range of recommendations that almost always include lifestyle changes and weight reduction.[9] Newly diagnosed patients with A1C level < 1.5% above target, receive pharmacological therapy if three months of behaviour intervention after diagnosis was not sufficient to reach the target A1C.[9] However, newly diagnosed patients with A1C

>1.5% above target, receive pharmacotherapy at diagnosis, with metformin as the most prescribed first-line agent. The use of insulin at diagnosis is rare and usually reserved for patients with a more severe first presentation.[9]

As the disease progresses physicians implement a tailored stepwise approach that takes into account several factors including A1C level, contraindications, presence of complications, kidney function, weight, age, frailty, and previous experience with side effects. The complexity of diabetes as a chronic condition and the availability of several medication classes, can lead to complex therapeutic scenarios throughout the course of the disease. This includes medication switching, discontinuation, reinitiating, and adding, which can lead to great diversity in patients' history and treatment trajectories. Generally speaking, diabetes medications can be classified into (1) a first-line class, often referring to metformin unless contraindicated; (2) second-line classes, which historically include both sulfonylureas and thiazolidinediones and more recently DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT-2 inhibitors; and (3) a third-line class, which often refers to insulin.

1.2.4 The efficacy and safety of insulin for type 2 diabetes

Insulin is a cornerstone therapeutic option for type 2 diabetes, and the initiation of insulin for some patients is inevitable despite life-style modification, including exercise and diet, as well as proper adherence to non-insulin therapy. Insulin regimens are also often complicated and individualized, considering the patient's weight, carbohydrate intake, and the type of insulin used. Initially basal insulin is introduced, and bolus insulin may be added at mealtimes if glycemic control is suboptimal after 3-6 months on basal insulin. Shared decision making through conversations between the physicians, pharmacists, and dietitians are recommended to help guide patients as they start their insulin regimen.

The efficacy and safety of insulin has been a long-standing debate. Insulin, in its various forms and regimens, has been the intervention of interest in an overwhelming number of trials looking at several short- and long-term outcomes. One aspect of interest to many trials was comparing the initiation of basal insulin to additional oral antihyperglycemic classes after failure to achieve glycemic control on two agents among patients with type 2 diabetes. A meta-analysis of these trials showed a small but significant improvement in A1C.[10] Notably, another systematic review and meta-analysis compared the use of insulin to hypoglycemic drugs or diet/placebo and concluded a lack of significant evidence of long-term efficacy of insulin on several clinical outcomes, including all-cause mortality and cardiovascular mortality.[11] However, there is significant evidence of harmful adverse effects, including hypoglycemia and weight gain.[11]

1.2.5 Hypoglycemia as an acute complication in type 2 diabetes

Hypoglycemia, or blood glucose levels below normal (< 4.0mmol/L), is characterized by autonomic symptoms, including increased palpitations, sweating, hunger, nausea as well as neuroglycopenic symptoms, such as difficulty concentrating, confusion, weakness, drowsiness, and vision changes.[12] Clinicians often use Whipple's triad to recognize hypoglycemia. Whipple's triad is a collection of three elements: 1) symptoms of hypoglycaemia, 2) low blood plasma glucose concentration, and 3) relief of symptoms when plasma glucose concentration is increased. Additionally, hypoglycemia can be categorized based on severity as: (1) mild, wherein autonomic symptoms are present, and patients are able to self-treat; (2) moderate, wherein both autonomic and neuroglycopenic symptoms are present; however, patients are still able to self-treat; and (3) severe, wherein patients need assistance from another person and unconsciousness may sometimes occur.[12] Plasma glucose typically drops below 2.8 mmol/L in severe

hypoglycemia.[12] Although hypoglycemia is more common in type 1 diabetes, patients with type 2 diabetes managed by insulin or sulfonylureas also experience this acute complication.

Notably, the progressive loss of β cells in type 2 diabetes prevents paracrine signaling between α and β cells leading to impaired glucagon release during hypoglycemia. Glucagon is a counter regulatory hormone that triggers the liver to release stored glucose. Therefore, there is an increased vulnerability to hypoglycemia with longer duration of insulin treatment in type 2 diabetes.[13] Indeed, the rate of self-reported severe hypoglycemic episodes occurred in 25% of patients who had been taking insulin for 5 years compared to 7% among those taking insulin for 2 years.[14]

The frequency of hypoglycemia varies in the literature due to the differences in the definition used, the age of the populations studied and the treatment modalities implicated. However, there is a general consensus on underreporting of hypoglycemia, specifically mild episodes due to limited ability to recognize the sign and symptoms and lack of glucose self-monitoring.[15] Moreover, decreased awareness of hypoglycemia has been reported in older adults, hence leading to lower reporting and higher risk of future severe episodes.[16] Additionally, frailty among older adults has been recognized as an important risk factor for hypoglycemia.[17,18] In fact, clinical diabetes guidelines recommend a higher A1C target and avoidance of insulin and sulfonylurea among frail patients to minimize the probability of hypoglycemia.[19] Recognizing and reporting of hypoglycemia in type 2 diabetes are necessary to ensure proper mitigation and avoidance strategies are implemented in order to avoid negative outcomes, including falls as well as cognitive impairment.

1.2.6 Long-term consequences of type 2 diabetes

Unlike in type 1, patients with type 2 diabetes do not initially experience overt symptoms because hyperglycemia develops gradually. Therefore, some patients with diabetes can go undiagnosed for years; however, these patients are still at increased risk of developing glucose-related complications.[20] These complications impact the physiological state of body systems and organs, making diabetes a risk factor for multiple health conditions. Generally, the effects of prolonged hyperglycemia are grouped into macrovascular complications (coronary artery disease, peripheral vascular disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). The risk of developing these complications is proportional to both the duration and severity of diabetes.[20] While diabetic retinopathy is responsible for almost 10,000 new cases of blindness every year in the United States alone, diabetic nephropathy is the leading cause of renal failure in the United States.[21] The association between diabetes and cardiovascular disease (CVD) and coronary heart disease is profound. In fact, the primary cause of death among patients with diabetes is due to CVD, which also accounts for the greatest component of health expenditures in people with diabetes.[22] Moreover, diabetes is considered an independent risk factor of stroke, increasing its risk by 150-400% among patients with diabetes, compared to those who do not have diabetes.[20] It is critical to note that the presence of these complications is indicative of diabetes severity; Therefore, diabetes severity is not exclusively assessed or defined by blood glucose levels, A1C levels, or the intensity of the treatment regimen.

In addition to these classic and established complications, neurocognitive consequences of diabetes have been of research interest for several years. Diabetes, its complications, and its treatment can induce transient or permanent cognitive abnormalities, due to acute and chronic disturbances of blood glucose homoeostasis.[23] Results showing significantly poorer cognitive function among patients with type 2

diabetes have been reported by several systematic reviews and meta-analyses. Herein, we summarize the most recent systematic reviews with the largest number of studies included.

A meta-analysis in 2014 showed that type 2 diabetes was associated with poorer performance in six cognitive domains summarized in Table 1.2, with the largest effects on measures of speed of information processing (n=16, Effect Size [95% CI] -0.33 [-0.41, -0.26]), executive functions (n=12, -0.33 [-0.42, -0.24]), and verbal memory (n=15, -0.28 [-0.37, -0.19]).[24] Moreover, in 2015, a meta-analysis of 15 studies indicated that in comparison to controls without diabetes, persons with type 2 diabetes showed deterioration of several cognitive domains including episodic memory (Standardized mean difference (SMD -0.51), and speed of processing (SMD -0.22).[25] Similarly, in 2019, Pelimanni, et al. examined possible differences in cognitive performance between middle-aged type 2 diabetes patients compared to healthy controls.[26] This meta-analysis included 12 studies and found that patients with diabetes performed worse than controls in several cognitive functions.[26] The largest differences were found in information processing speed (SMD -0.68, [95% CI -0.84, -0.52]), attention/concentration (SMD -0.55, [-0.80, -0.30]), executive functions (SMD -0.51, [-0.69, -0.34]), and working memory (SMD -0.51, [-0.51, -0.79]).[26]

Decrements in cognitive function in subjects with type 2 diabetes have also been associated with increased duration of diabetes and poor glycemic control.[27,28] One major study sought to directly determine if the level of glycemic control impacts cognitive performance.[29] The ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial is one of the landmark clinical trials for diabetes, in which the effects of intensive glycemic control strategies (intensive A1C control; target < 6% versus standard A1C control; target 7-7.9%) were assessed among individuals with type 2 diabetes at high risk for cardiovascular disease.[29] Among a subset of the ACCORD participants, several cognitive domains were

assessed in what is known as the ACCORD-MIND (Memory IN Diabetes) study.[29] In ACCORD-MIND, cognitive functions were assessed at baseline (n=2977), 20 months, and 40 months after randomisation. The primary cognitive outcome was performance on the Digit Symbol Substitution Test (DSST) at 40 months.[29] DSST is a test of psychomotor function, speed, learning, and working memory, during which subjects are shown digit-symbol pairs (e.g. 1/#, 2/*). The subjects are then given a list of digits, under which they are asked to write down the corresponding symbol as fast as possible. Additionally, for a subset of the ACCORD-MIND subjects (n=614), brain MRI was performed at baseline and 40 months to test for total brain volume (TBV).[29] Results from ACCORD-MIND show that DSST scores significantly declined in both intervention arms. However, there were no differences in mean DSST scores between the standard and intensive glycemic groups at 40 months; nevertheless there was a modest but significant beneficial effect on TBV (higher mean TBV) at 40 months among the intensive group compared to the standard group.[29]

Another landmark diabetes trial is the ORIGIN trial (Outcome Reduction with Initial Glargine Intervention), a multicentre randomised trial to assess the effects of insulin glargine versus standard care (i.e., treated on the basis of the investigator's best judgment and local guidelines) on cardiovascular morbidity and mortality.[30] Specifically, it was aimed to assess these outcomes in patients with a high risk of cardiovascular disease, 50 years and older, with impaired fasting glucose, impaired glucose tolerance, or early type 2 diabetes mellitus. The effects of the ORIGIN treatment regimens on measures of cognitive function were evaluated in a sub-study, with the aim to assess if early insulin intervention can reduce the risk of cognitive impairment.[31] Of all participants in the ORIGIN trial, 11685 completed a Mini-Mental State Examination (MMSE) and 3392 completed a DSST at baseline. After a median (IQR) follow-up of 6.2 (5.8, 6.7) years, results show that there was a decline in both MMSE and DSST scores over time for

both groups. However, there was no difference in the rate of change of cognitive test scores between the insulin glargine and standard care groups (for the MMSE 0.004, [95% CI -0.013, 0.022], P=0.39; and for the DSST -0.036, [-0.218, 0.145], P=0.34).[31] Although this trial is unique in implementing an early intervention, this however makes detecting potential cognitive decline, cognitive impairment, and dementia challenging, due to the younger population. With all the above-mentioned evidence relating diabetes to cognitive impairment, a link between diabetes and dementia is plausible.[32]

1.3 Dementia

1.3.1 Types and causes of dementia

Dementia, recently renamed major Neurocognitive Disorder (NCD), is a broad term to describe a set of neurological conditions, of which the major symptom is a decline in brain function.[33–36] The primary clinical deficit is in cognitive functions (Table 1.2) that is acquired rather than developmental. The syndrome of dementia may be caused by various underlying diseases, each characterized by a specific aggregation and pattern of signs and symptoms. The most common cause of dementia is Alzheimer disease (AD), contributing to 60-70% of cases.[2] Other common types of dementia include vascular, frontotemporal, and Lewy body dementia.[37] Alzheimer disease is characterized by the accumulation of amyloid plaques and neurofibrillary tau-based tangles in the brain.[33] Other neuropathological lesions are encountered in cases of Alzheimer disease, but the disease is mostly defined and recognized by these two cardinal lesions. These neuropathological features are thought to begin 10 to 20 years before obvious cognitive symptoms.[38] The second most prevalent cause of dementia is vascular dementia (VD), which may be caused by various types of vascular pathology in the brain, including infarctions to small and large vessels. Other frequent causes of dementia include frontotemporal lobar degeneration and dementia

with Lewy bodies. Distinguishing between the subtypes of dementia is often difficult, if not impossible, and is verified only by autopsy. Additionally, these subtypes can co-occur, in a condition known as mixed dementia.[39]

There are several risk factors of dementia, the strongest of which is age. The risk of AD rises rapidly with age, with the prevalence rising from 2.3% in those 65-74 years old, to over 34% in those 85 years of age and older.[40] Other risk factors include hypertension, hearing impairment, smoking, obesity, depression, physical inactivity, diabetes, low educational attainment, and low social contact.[3,4,40,41] In 2020, excessive alcohol consumption, traumatic brain injury, and air pollution were identified as additional modifiable risk factors.[5] Evidence from a systematic review of 34 prospective cohort studies, of which 24 were meta-analyzed (n=159,594), assessed the association between midlife modifiable risk factors and dementia.[42] Results revealed five modifiable factors associated with an increased risk of dementia, including diabetes mellitus (Relative Risk (RR), 1.69; 95% CI 1.38, 2.07), obesity (1.78; 95% CI 1.31, 2.41), current smoking (1.61; 95% CI 1.32, 1.95), hypercholesterolemia (1.57; 95% CI 1.19, 2.07), and hypertension (1.72; 95% CI 1.25, 2.37).[42]

1.3.2 Disease progress

Dementia is a progressive disease; it has been shown that neuropathological changes can occur as early as 20 years before symptoms are evident.[38] Patients with dementia first progress from normal baseline cognitive abilities through subtle changes, in what is known as the preclinical stage, to obvious symptoms of dysfunction, termed prodromal stage, and finally, to dementia.

Mild cognitive impairment (MCI), minor Neurocognitive Disorder (minor NCD), or prodromal dementia, is the stage that precedes dementia and is characterized by some evidence of cognitive

dysfunction in one of the cognitive domains summarized in Table 1.2, but without impact on daily functioning.[32] The criterion of maintenance or loss of independent functioning represents the key distinction between mild and major NCD, or dementia.[32] Eventually, as the disease progresses, some patients with MCI lose the ability to perform activities of daily living (ADL) independently and therefore progress to dementia.[32–35]

1.3.3 Diagnosis of dementia

According to DSM-5 diagnostic criteria, dementia is diagnosed based on evidence of significant cognitive decline from a previous level of performance in one or more of the cognitive domains.[32–35] Additionally, these cognitive deficits must be sufficient to interfere with independence in activities of daily living and they must not be attributable to another mental disorder.[33–36] The diagnosis is based on patient history, a series of neurological examinations, psychiatric evaluation, and brain imaging, including CT or MRI scan to assess evidence of stroke or bleeding and PET scans to show patterns of brain activity and the deposition of the amyloid protein.[33–36] Early-onset dementia is diagnosed before the age of 65, while most cases of dementia are diagnosed after the age of 65 years.[43]

1.3.4 Presentation and patterns of symptoms

The presentation and patterns of symptoms can vary based on the subtype of dementia; patients with AD usually present with loss of memory, especially for new information.[33–36] Later as the disease progresses, cortical function such as language and executive function become affected with evidence of behavioural and psychiatric disturbances.[33–36] These disturbances, commonly referred to as behavioural and psychological symptoms of dementia (BPSD), usually include agitation, depression, wandering, and aggression.[30] On the other hand, patients with vascular dementia present with gait disturbances, decline in

problem-solving skills, and apraxia, the difficulty with motor planning to perform tasks or movements when asked.[33] Frontotemporal dementia usually presents with language disturbances, while Lewy body dementia is sometimes characterized by visual hallucinations early in the disease process.[33] The overlap of these symptoms is common, which adds to the difficulty of distinguishing between the subtypes of dementia.[33]

1.4 Type 2 diabetes and dementia: proposed mechanisms

The coexistence of diabetes may increase the risk of progression from the mild cognitive impairment stage to dementia.[44] In fact, a systematic review and meta-analysis of 15 studies (n=6865) reported that the relative risk of progression from mild cognitive impairment to dementia in people with diabetes compared to those without diabetes is RR 1.53; 95% CI 1.20, 1.97.[45] The exact pathophysiological mechanism by which diabetes is linked to dementia is not fully elucidated. However, several possible hypotheses have been emerging from ongoing research, with key proposed complex and interconnected metabolic and inflammatory mechanisms including hyperinsulinemia, inflammatory signaling pathways, oxidative stress, vascular complications, and hypoglycemic insults (Figure 1.1).[46–50]

1.4.1 Glucose metabolism

Abnormal peripheral glucose concentration is related to dysregulation of brain glucose metabolism, which has been suggested as a factor in AD pathology. In 2018, An et al. measured brain glucose concentration within the autopsy cohort of the Baltimore Longitudinal Study of Aging, and assessed the associations between plasma glucose, measured prior to death, and brain tissue glucose.[51] Results from this study demonstrated that brain regions that are vulnerable to amyloid deposition and neurofibrillary pathology show significantly higher tissue glucose concentrations in AD.[51] Additionally, higher concentrations of

glucose in brain tissue are associated with greater severity of amyloid plaque deposition and increased phosphorylation of tau proteins, indicating that abnormalities in brain glucose homeostasis are intrinsic to AD pathogenesis.[51]

Notably, Crane et al. assessed the association between plasma glucose levels and the risk of dementia.[52] From 2067 dementia-free participants, 35264 clinical measurements of glucose levels were used and the primary outcome, dementia, was assessed every two years.[52] After a median follow-up time of 6.8 years, 524 participants (74 with diabetes and 450 without) developed dementia. Among those without diabetes, compared to normal glucose levels (5.5 mmol/L), higher average glucose levels (6.4 mmol/L) within the preceding 5 years were related to an increased risk of dementia, HR (95% CI) 1.18 (1.04, 1.33).[52] Likewise, among those with diabetes, compared to average glucose level (8.9 mmol/L), higher average glucose levels (10.5 mmol/L), were also related to an increased risk of dementia, HR 1.40 (1.12, 1.76). These results suggest that even among people without diabetes, higher glucose levels may be a risk factor for dementia.[52]

1.4.2 Hyperinsulinemia

Hyperinsulinemia, or high levels of insulin in the blood, has been proposed to be a risk factor for dementia independent of cerebrovascular disease. Since insulin can cross the blood brain barrier, higher levels of insulin in the brain are thought to compete with amyloid beta for the insulin degrading enzyme (IDE), eventually leading to lower amyloid clearance.[46] Also, Craft et al. has shown that high levels of peripheral hyperinsulinemia led to the downregulation of insulin uptake by the blood brain barrier due to saturation over physiologic levels, eventually leading to a reduction of insulin levels and insulin receptors in the brain and therefore a downregulation of IDE expression.[53] Ultimately, the low expression of IDE

leads to lower IDE mediated amyloid clearance. Moreover, insulin in the brain can increase the deposition of amyloid Beta and the phosphorylation of tau protein, which are central to the pathogenesis of Alzheimer disease.[54] Additionally, insulin receptors in the brain including the hippocampus and entorhinal cortex structures have been shown to be adversely affected among patients who have dementia.[54]

In 2004, Luchsinger et al evaluated the association between fasting insulin levels and the risk of Alzheimer disease in a cohort of 683 dementia-free participants 65 years and older living in Northern Manhattan.[55] Insulin levels were measured from frozen sera using solid-phase chemiluminescent enzyme immunoassay and dementia was diagnosed by a team of neurologists and psychiatrists based on DSM -5 criteria.[55] The prevalence of diabetes among the hyperinsulinemia group (> 27 [mu]IU/mL) was 29%, while it was 17% among the normal insulin group. Results showed that 149 persons developed dementia, 6 of which experienced a stroke, and that the risk of AD was higher among the hyperinsulinemia group, HR (95% CI) 1.9 (1.4, 2.7). The risk remained high in a diabetes-stratified analysis, HR 2.3 (1.5, 3.6) for those without diabetes and although attenuated in individuals with diabetes, HR 1.5 (0.8, 2.9) it was not statistically significant. Additionally, over time hyperinsulinemia was related to a significant decline in memory-related cognitive scores (β [SE]= -0.08 [0.03]; $P = 0.01$).[55]

In 2019, data from the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study was used to examine the effect of serum insulin on cognitive function. Baseline serum levels were measured from 269 dementia-free participants aged 65-79 years as well as several cognitive functions.[56] Seven years later, individuals' cognitive functions were re-evaluated. Results showed that baseline insulin resistance, estimated with the homeostasis model assessment for insulin resistance (HOMA-IR), values were related to worse performance in global cognition (β [standard error (SE)] = -0.05 [0.02]; $P = 0.04$) and

psychomotor speed (β [SE] -0.06 [.03]; P = [0.04]).[56] Raised serum insulin levels were associated with lower scores on global cognition (β [SE] -0.054 [0.03]; P = 0.04) and tended to relate to poorer performance in psychomotor speed (β [SE] = -0.06 [0.03]; P = 0.07).[56] These results suggest that hyperinsulinemia may be an independent predictor of cognitive performance.

1.4.3 Vascular complications

Type 2 diabetes is an independent risk factor for stroke and vascular complications; direct brain damage as a result of a stroke or clinically silent cerebral infarctions due to minor blood clots, is an established underlying cause of vascular dementia.[49,57] Several autopsy studies assessed the relation between diabetes, cerebrovascular lesions, and dementia.[58–60] These studies found that dementia patients with diabetes are more likely to have small clinically silent infarcts at autopsy, compared to those who do not have diabetes.[58–60] Additionally, there is long-term brain endothelial damage due to atherosclerosis and narrowing of brain blood vessels, which is possibly mediated by conditions that correlate with type 2 diabetes such as hypertension, obesity, and dyslipidemia.[49,57]

1.4.4 Hypoglycemia

Severe hypoglycemic events have also been linked to dementia.[44] Glucose is the fuel utilized by the central nervous system which cannot synthesize glucose nor store it; hence the brain requires a continuous supply of glucose from the peripheral circulation.[49,61–64] Therefore, disruption of the supply of exogenous glucose will rapidly cause functional disturbances in the brain. The consequences of this lack of supply include neuronal cell death, damage to receptors in brain regions, which are critical for learning and memory, as well as increased platelet aggregation and fibrinogen formation.[65]

However, results from the ACCORD-MIND trial, which was described earlier, showed that those with severe hypoglycemic attacks, compared to those without, had marginally significant less atrophy (less decrease in TBV) from baseline to 40 months (mean difference= -9.55; [95% CI -15.21, -3.90] vs. -15.38 [95% CI -16.64, -14.12], $P = 0.051$), and no significant increase of abnormal white matter volume, which is indicative of damage (mean difference= 2.06 [95% CI 1.71, 2.49] vs. 1.84 [95% CI 1.76, 1.91], $P = 0.247$).[66]

The relationship between hypoglycemia and dementia appears to be bidirectional and patients with unrecognized cognitive impairment may be more susceptible to severe hypoglycemia.[44] In fact, in a post hoc analysis of the ACCORD-MIND trial, cognitive decline over a 20-month period was associated with an increased risk of subsequent hypoglycemia in patients with type 2 diabetes, for both the standard and intensive glycemic control groups.[67]

1.4.5 Other mechanisms

Several other mechanisms have been proposed, such as oxidative stress, and advanced products of glycosylation (AGE), which have been reported to be significantly higher among patients with both diabetes and dementia, as well as inflammation.[64] Levels of several inflammatory mediators, such as C-reactive protein, cytokines, and tumor necrosis factor-α (TNF-α) are elevated in metabolic disorders, including obesity and diabetes.[48] This chronic inflammation has also been linked to progressive tissue damage in dementia.[47,48,64]

1.5 Type 2 diabetes and dementia: epidemiology

After several studies linked diabetes to a decline in cognitive function, a series of observational studies in the 1990s and early 2000s examined the association between type 2 diabetes and dementia.[68–73] These

were summarized by two systematic reviews and meta-analyses, Cheng, et al. in 2012[74] and Gudala et al. in 2013.[75] First, Cheng et al. meta analyzed 16 studies with a total of 5706 patients with diabetes compared to 36191 patients without diabetes and found the RR of Alzheimer disease was 1.5 (95% CI: 1.2, 1.8). For vascular dementia, the RR was 2.5 (95% CI: 2.1, 3.0) based on 10 studies with a total of 3519 subjects with diabetes and 23,026 subjects without diabetes.[74] Similarly, results from Gudala et al, in which a total of 28 observational studies were included, showed that the RR of developing all-cause dementia (*n* = 20 studies) was 1.73 (1.65, 1.82), Alzheimer's disease (*n* = 20 studies) was 1.56 (1.41, 1.73), and vascular dementia (*n* = 13 studies) was 2.27 (1.94, 2.66) in patients with diabetes mellitus.[75]

Notably, over the past 20 years, there has been an abundance in the number of observational studies assessing the risk of dementia attributed to type 2 diabetes using a wide range of data sources, including medical records,[76–78] surveys,[79] prospectively collected clinical data,[80–85] and administrative or claims databases.[86,87] Several epidemiological designs were also used, including but not limited to, cross-sectional, cohort (retrospective and prospective), as well as case-control studies. Even within the same study design, there was a wide variation in the design elements, such as exposure and outcome definitions. Despite such heterogeneities, there seems to be a positive association between type 2 diabetes and dementia with most adjusted risk estimates ranging from 1 to 3. The association seems to be stronger with vascular dementia with risk estimates ranging from 2 to 4 for most studies, nevertheless the association with Alzheimer disease is still significant in the majority of the studies. Research focused on the association between diabetes and other sub-types of dementia has not been conducted to the same extent. However, they are also reported to be less common among patients with diabetes compared to vascular dementia and Alzheimer disease.[80]

Beyond establishing an association between diabetes and dementia, research detailing the association between various diabetes-related factors and dementia has emerged. These factors include diabetes onset, duration, severity, complications, as well as management and pharmacotherapeutic options.

Notably, an earlier diabetes diagnosis has been linked to a higher risk of dementia, wherein every 5-year younger age of diabetes onset has been significantly associated with a 24% higher risk of dementia.[88] Moreover, a longer duration of type 2 diabetes has also been associated with a higher risk of dementia. At least two studies have reported a 30-40% increased risk of dementia for every 5-year increase in duration.[86,87] Other than age of onset and diabetes duration, diabetes severity defined using various definitions has been linked to an increased risk of dementia. Some studies have computed diabetes severity scores based on the presence of micro and macrovascular complications and found a dose-response relationship. For example, Chiu et al used the Taiwan claims data to examine the effect of diabetes severity on all-cause dementia. Diabetes severity was defined based on the adapted diabetes complications severity index (aDCSI), a 13-point score based on the presence of diabetes complications.[86] At diabetes diagnosis, compared to having no complications (aDCSI=0), the hazard ratio for dementia was 0.98 (0.94-1.02) when aDCSI=1, 1.12 (1.08-1.16) when aDCSI=2, 1.26 (1.19-1.33) when aDCSI=3, and 1.42 (1.35-1.50) in patients with an aDCSI>3.[86] Ten years after diagnosis, this dose-response risk was also observed.[86] Similarly, using national data from Taiwan, Kuo et al, found that the risk of dementia increased as the number of complications increased among the diabetes group, HR (95%CI), 1.73 (1.59, 1.89) for one complication, HR 2.01 (1.85, 2.19), for two complications, HR 2.24 (2.00, 2.50) for three complications, and HR 2.49 (2.06, 3.00) for four or more complications.[87] Other studies focused on comparing the presence of specific chronic complications compared to not having these complications

and found the risk of dementia to be roughly 30% higher with microvascular complications, 60% higher with cerebrovascular complications, and 21% higher with cardiovascular complications.[87]

However, the published epidemiological literature on the possible association between different classes of antihyperglycemic agents and the incidence of dementia is less conclusive. In the past decade, findings from several observational studies have been inconsistent and sometimes contradictory with effect sizes ranging from a 47% protective effect to 18% harmful effect for the same class. Most of the earlier studies with a main objective to assess the risk of dementia in diabetes either disregarded the effect of medications, adjusted for it in the analysis, or conducted a subgroup analysis, in which users of a specific class of medication were compared to patients without diabetes, or in a best-case scenario, to patients with diabetes not receiving any treatment. In 2018, a meta-analysis of both observational studies and RCTs by McMillan *et al*. showed that there was no increase in the risk of incident dementia associated with diabetes treatment in general (RR 1.01; 95% CI 0.93, 1.10); however, there seem to be a difference of risk by class of medication.[89] Results for metformin were reported to be inconclusive (RR 1.08; 95% CI 0.49, 2.36). While insulin was associated with an increased risk (RR 1.21; 95% CI 1.06, 1.39), thiazolidinediones were associated with a lower risk (RR 0.71; 95% CI 0.55, 0.93) and sulfonylureas (RR 0.96; 95% CI 0.69, 1.40) had no effect on the risk of dementia.[89] The authors recognized the high levels of heterogeneity and low quality of the studies.

Notably, Ye et al, looked specifically at the impact of insulin sensitizing agents on the incidence of dementia.[90] A total of nine studies (n=544,093) were meta-analyzed and showed that the RR (95% CI) of incident dementia was 0.78 (0.64, 0.95) for the use of any insulin sensitizers.[90] The RR was less for metformin 0.79 (0.62, 1.01), and thiazolidinediones 0.75 (0.56, 1.00), although not statically significant for either.[90]

Findings from a pooled alnysis of five cohort studies concluded that insulin use was associated with a 58% increased risk of incident all-cause dementia.[91] Additionally, a 2020 network meta-analysis of 17 studies (n=1,258,879) showed that insulin use was associated with higher risk of dementia when compared to metformin (HR 2.10; 95% CI 1.30, 3.30), sulfonylureas (HR 1.80; 95% CI 1.10, 2.90), thiazolidinediones (HR 2.20; 95% CI 1.40, 3.50), or DPP-4 inhibitors (HR 2.90; 95% CI 1.70, 5.10).[92] Further research is needed to establish whether the use of insulin has an adverse effect or only reflects the severity of diabetes.

1.6 Identifying the gaps in observational studies assessing insulin, hypoglycemia, and dementia

While methodological gaps exist in studies assessing different antihyperglycemic classes and dementia, herein we focus on summarizing threats to validity and knowledge gaps in observational studies focused on insulin use or hypoglycemia and the incidence of dementia.

1.6.1 Insulin and dementia in observational studies

Despite evidence of an increased risk of dementia with insulin use from the abovementioned meta-analyses[89,91,92], concerns about the methodology in the observational studies included in these meta-analyses remain. Thus far, at least 15 observational studies have assessed the association between exogenous insulin use and the risk of incident dementia, albeit none of which were designed specifically to answer this question as the primary objective.[68,69,87,89,93–100] A summary of all previous observational studies assessing the association between insulin and dementia is reported in Table 1.3. The threats to internal validity in most of these studies can be grouped into confounding by indication and disease

severity due to inappropriate population, specifically not restricting the population to those with type 2 diabetes, as well as inappropriate comparator.

Earlier studies have found a higher risk of dementia with insulin use compared to patients without diabetes, therefore introducing confounding by indication and more specifically confounding by diabetes itself and the accompanying cardio and cerebrovascular complication as well as co-morbidities. For example, in two studies Ott and colleagues used data from the Rotterdam study and found concerning high risk estimates, 3.2 and 4.3 with insulin use compared to no diabetes.[68,69] Importantly, the length of available data was unclear in one study and roughly only 2 years in the other.[68,69] Another example of an inappropriate comparator for insulin was metformin, which was used by Whitmer and colleagues, wherein a significant 28% increased risk was reported with insulin use.[99] Metformin however, is a first-line agent that is usually prescribed early in the disease stage while insulin is often prescribed as the disease progresses and after secondary failure on non-insulin classes. Therefore, despite accounting for the presence of diabetes, this design does not account for confounding by disease severity. Notably, comparing insulin use to no insulin use does not protect from this potential confounding; as present in several other studies that have reported an increased risk of dementia with insulin use.

Besides this design shortcoming, the short period of available data, failure to implement a latency period to allow for disease development to take place, and the inclusion of older adults aged 65 years and older only at baseline, can also lead to biased estimates wherein the risk of dementia is possibly overestimated.

1.6.2 Hypoglycemia and dementia in observational studies

In addition to examining the association between antihyperglycemic agents and dementia, the aforementioned systematic review by McMillan *et al*. also examined the association of hypoglycemia and dementia.[89] Three studies were meta analysed (n=19407) and showed that the risk of incident dementia associated with one or more severe hypoglycemic episode compared to no hypoglycemic episodes, (RR 1.77; 95% CI1.35, 2.33).[89] Similarly, a recent systematic review and meta-analysis of ten studies found severe hypoglycemic episodes to be associated with dementia in patients with diabetes (HR 1.44; 95% CI1.26, 1.65).[101]

A summary of all previous observational studies assessing the association between hypoglycemia and dementia is reported in Table 1.4. Almost all studies thus far have included patients with diabetes who were older than 60 and in one study[102] older than 70 years. Moreover, none of the studies examined if the association is stronger as patients approach a dementia diagnosis. This is important as the underlying cognitive impairment that takes place prior to an official dementia diagnosis can possibly increase the risk of hypoglycemia. The lack of accounting for this potential reverse causality can lead to biased estimates. Moreover, only more recent studies accounted for immortal time bias using a time-varying exposure definition, nonetheless index date was set at diabetes diagnosis with the covariate assessment period preceding diabetes diagnosis, potentially leading to underestimation of the presence of micro and macrovascular complications. Additionally, all studies adjusted for age; however, previous studies have not further explored the relationship between hypoglycemic events occurring at a younger age compared to an older age and dementia.

1.7 Research rationale

A reassessment of the associations between insulin, hypoglycemia and dementia is warranted given that the published literature on these associations suffers from methodological pitfalls and also overlooks the possibly mediating effects of hypoglycemia by which insulin may be linked to dementia. Although clinical trials are regarded as the 'gold standard' to discern the association between diabetes therapies and dementia, there are limitations to this design. Long periods of follow-up would be necessary and therefore costly and difficult in terms of retaining the study population. Additionally, restricted inclusion criteria for the specified populations in RCTs can limit the generalizability of results. Furthermore, some diabetes-related exposures, including hypoglycemic events are not amenable to randomization, deeming observational approaches necessary. Therefore, evidence from observational studies needs to not only be supported by multiple studies, but more importantly under the condition of robust methodology to ensure internal validity. Lastly, despite the abundance and length of health data available in Canada, some studies have assessed the association between diabetes and dementia, but none have explored questions concerning the effects of insulin and hypoglycemia among the Canadian population.

Beyond methodological considerations, providing insight on the role of insulin and hypoglycemia as potential risk factors of dementia is clinically important. Insulin is a cornerstone therapeutic option in type 2 diabetes. In addition, the initiation of insulin is often a profound decision for patients with type 2 diabetes, their prescribers, other healthcare providers and sometimes their family members and caregivers. Anxiety and concerns around the use of insulin and its potential side effects are often coupled with a feeling of guilt or failure by patients because they were 'unable' to manage their diabetes.[103] Therefore, evidence on a possible association between insulin and dementia, albeit based on studies

suffering from methodological shortcomings can exacerbate concerns about insulin use. Hence, such an association and the potential role of hypoglycemia need to be confirmed with robust evidence to inform clinical decisions.

Lastly, further understating of the pathways by which insulin, hypoglycemia and dementia are associated may help better understand the relationship, provide more individualized dementia risk assessment in patients with type 2 diabetes, and can also piece together clues about the etiology of dementia. Accordingly, the objective of this thesis is to address these questions.

1.8 Thesis objectives

The overarching goal of this project is to assess the association between insulin use, hypoglycemia, and incident dementia among patients with type 2 diabetes. Herein, I conducted four retrospective cohort studies while combating threats to internal validity through implementing design and analysis techniques. The specific research objectives are:

- **Objective 1:** To assess the association between **severe hypoglycemia** and the risk of incident dementia.
- **Objective 2:** To assess the association between mid-life and late-life **severe hypoglycemia** and the risk of incident dementia.
- **Objective 3:** To assess the association between **exogenous insulin use** and the risk of incident dementia.
- **Objective 4:** To explore the potential role of **hypoglycemia as a mediator** of dementia risk difference between those who initiate insulin versus non-insulin in type 2 diabetes.

Chapter 2
Hypoglycemia and the risk of dementia: A population-based cohort study using exposure density sampling

In this chapter, a population-based cohort study is conducted to reexamine the association between severe hypoglycemia and the risk of all incident dementia while implementing several design and analysis techniques to account for various threats to validity in previous studies.

Data resources and availability

All data were de-identified and no personal information was available at any point of the study. Access to data provided by the Data Steward(s) is subject to approval, but can be requested for research projects through the Data Steward(s) or their designated service providers. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). Ethics approval was also obtained from the University of Waterloo.

2.1 Abstract

Background: Previous studies have shown hypoglycemia to be associated with an increased risk of dementia; however, there are several design challenges to consider. The objective of this study is to assess the association between hypoglycemia and dementia while addressing these challenges using a lag period, exposure density sampling (EDS), and inverse probability of treatment weighting (IPTW).

Methods: A population-based cohort using data (1996-2018) from British Columbia, Canada. From a cohort of incident type 2 diabetes patients aged 40-70 years, we created a dynamic sub-cohort of hypoglycemia exposed (≥1 episode requiring hospitalization or a physician visit) and unexposed individuals using EDS, in which four unexposed individuals per one exposed were randomly selected into risk sets based on diabetes duration and age. Follow-up was until dementia diagnosis, death, emigration, or 31 December 2018. Those diagnosed with dementia within 2 years of follow-up were censored. We adjusted for confounding using IPTW and estimated the hazard ratio (HR; 95% CI) of dementia using weighted conditional cause-specific hazards risk models with death as a competing risk.

Results: Among 13,970 patients with incident type 2 diabetes, 2,794 experienced hypoglycemia. There were 329 dementia events over a median (IQR) follow-up of 5.03 (5.7) years. IPTW resulted in well balanced groups with weighted incidence rates (95% CI) of 4.59 (3.52-5.98)/1,000 person-years among exposed and 3.33 (2.58-3.88)/1,000 person-years among unexposed. The risk of dementia was higher among those with hypoglycemia (HR1.83; 95% CI, 1.31-2.57).

Conclusions: After addressing several methodological challenges, hypoglycemia contributes to an increased risk of all-cause dementia among patients with type 2 diabetes.

2.2 Key messages

- Among patients with type 2 diabetes, the risk of all-cause dementia is over 80% higher among individuals who experienced at least one hypoglycemic episode, compared to those who did not.
- The use of exposure density sampling, high-dimensional propensity scores, inverse probability of treatment weighting, and a range of lag periods was utilized to minimize threats to validity.
- These findings add insight on the modifiable risk factors of dementia in type 2 diabetes.

2.3 Introduction

Hypoglycemia is an acute complication of diabetes that often occurs as an adverse effect of exogenous insulin or other medications that increase endogenous insulin secretion, such as sulfonylureas.[1] Although most hypoglycemic episodes are mild and can be managed independently by patients, the InHypo-DM survey study found that approximately 38% of participants with type 2 diabetes had reported at least one hypoglycemic event that required the assistance of a third party to administer treatment with glucose or glucagon.[2] Additionally, an estimated 1% of type 2 diabetes patients treated with oral antihyperglycemics and 7% of those treated with insulin experience at least one severe hypoglycemic episode that requires an emergency department visit in their lifetime.[3] Although these estimates might be low, the global proportion of hypoglycemia-related deaths is 4.49/1,000 of total diabetes deaths.[4,5]

Glucose is the brain's primary source of energy and the reduction of glucose supply from the peripheral circulation to the brain can negatively impact cognitive function.[6] Although cognitive function is often restored when glucose supply is normalized, it has been hypothesized that severe hypoglycemic episodes can lead to platelet aggregation, fibrinogen formation, and irreversible damage, including neuronal cell death.[6-14]The ACCORD-MIND trial showed that severe hypoglycemic attacks were not associated with increases in brain atrophy or abnormal white matter volume, which is indicative of damage. .[15] However, these findings are limited given only 40 months of follow-up and a sample of 500 individuals.[15]

Given that hypoglycemia is not amenable to randomization and a long-follow up period is necessary to capture dementia, the weight of evidence assessing the association between hypoglycemia and dementia is from observational studies. In fact, findings from previous observational studies assessing this

association using different data sources, design elements, follow-up periods, and populations have consistently shown a higher risk of dementia with relative risk estimates ranging between 1.20 and 4.40,[16-22] albeit one study with a small sample size and an older population at baseline did not support such findings.[23]

Although robust methodology is critical for all observational studies, some design considerations are more critical when assessing the complex association between hypoglycemia and dementia. First, the relationship between hypoglycemia and dementia appears to be bidirectional and patients with unrecognized cognitive impairment may be more susceptible to severe hypoglycemia. [9,24] Despite that, some studies did not consider a possible lag period to account for this reverse causality.[16-20] That is, these studies did not require a specific censoring period of dementia events occurring after the hypoglycemia to account for hypoglycemia due to prodromal dementia. Additionally, a latency period between exposure to hypoglycemia and the development of dementia needs to be considered. Second, hypoglycemia is an adverse effect of diabetes therapies; therefore, exposure to hypoglycemia should be time-dependent, wherein most exposed individuals start as unexposed. While some studies considered hypoglycemia as a time-dependent exposure,[16-19,21,22] the covariate assessment period was often at diabetes diagnosis or study enrollment. Therefore, groups might be imbalanced on several confounding variables at the time of hypoglycemic episodes. For example, at diabetes diagnosis, groups might be well balanced on diabetes duration, therapy, and complications; however, this balance is not necessarily maintained at the time of hypoglycemia. In fact, those with more severe diabetes over time are more likely to receive insulin and therefore more likely to experience hypoglycemia. Third, most previous studies only adjusted for a

limited number of important confounders despite the complexity of the relationship, thus suffering from residual confounding.[16,17,22]

Herein, we utilize multiple design approaches to emulate a hypothetical trial and combat the aforementioned threats to validity to assess the association between hypoglycemic episodes and all-cause dementia using real-world data. Specifically, we use a lag period, exposure density sampling (EDS), and high-dimensional propensity scores with inverse-probability of treatment weights (IPTW) for confounding adjustment.

2.4 Methods

2.4.1 Study design and data source

This was a retrospective population-based cohort study using British Columbia's (BC) healthcare data from 01 January 1996 to 31 December 2018, obtained from the administrative databases within Population Data BC (https://www.popdata.bc.ca/data). This repository captures the encounters with the health care system for nearly all of BC's population that receives universal health care coverage through the provincial government.[25-29] These data have been validated and used extensively in health services research.[30-34]

We linked data across multiple databases using a de-identified personal health identification number. Several databases were used: (1) a population registry (Consolidation File) that captures date of birth, sex, and dates of health care coverage;[25] (2) the PharmaNet program, which includes drug dispensation date, name, drug identification number (DIN), and quantity.[26] This database captures all prescription drugs dispensed by community pharmacies to BC residents regardless of the type of insurance coverage (government-sponsored, private, or out-of-pocket), comprehensively capturing nonhospital drug use. The

provincial Pharmacare program provides complete coverage for eligible medications for residents after an income-based deductible has been met during the fiscal year; (3) the Medical Services Plan (MSP) database provided data on physician visits, including the service date and the International Classification of Diseases, 9th Revision [Clinical Modification] (ICD-9-CM) diagnosis code;[27] (4) the Discharge Abstract Database provided hospital admission and discharge dates and several diagnoses coded with ICD-10-Canadian Adaptation (CA) codes;[28] and (5) the Vital Events Deaths database provided the date of death.[29] We also acquired an area-level measure of socioeconomic status (SES) based on the first three characters of the postal code and aggregated neighbourhood-level income data from Census Geodata.[35]

2.4.2 Study population

First, we identified a cohort of patients newly diagnosed with type 2 diabetes between 01 January, 1998 through 31 December 2016. Using a washout period of 2 years, incident diabetes was defined based on the validated diabetes case-defining algorithm from the Canadian Chronic Disease Surveillance System, whereby diabetes is defined as the earliest occurrence of 2 physician claims (ICD-9 codes) or 1 hospitalization (ICD-10-CA) for diabetes within a 2-year period.[36] This definition has a 89.3% sensitivity (95% confidence interval (CI) 88.9 - 89.9), 97.6% specificity (95% CI 97.5 - 97.7), 81.9% positive predictive value (95% CI 81.3 - 82.4), and 98.7% negative predictive value (95% CI 98.6 - 98.7).[36,37]

Second, we applied the following inclusion criteria: (1) aged between 40-70 years at the date of diabetes onset, with the lower limit set to allow enough follow-up time to capture incident dementia, while the upper limit was set to account for a possible period of prodromal dementia and delayed diagnosis; (2) continuous registration in the population registry for at least 2 years prior to diabetes onset; (3) no receipt of any antihyperglycemic agents prior to diabetes onset; (4) no presence of a diagnostic

code indicating type 1 diabetes at any time or receipt of insulin monotherapy as first-line treatment; (5) no previous record of diagnostic codes indicating dementia or any cognitive impairment or a dispensation record for a cholinesterase inhibitor before diabetes diagnosis; and (6) no diagnosis of Down's syndrome due to the high risk of diabetes and dementia in Down's syndrome with genetic variation that we are unable to assess. ICD codes used to identify diabetes and inclusion criteria are reported in Supplementary Table S2.1.

2.4.3 Exposure definition and exposure density sampling

Hypoglycemia was defined as at least one hospitalization or a physician claim indicating hypoglycemia. The date of the first hypoglycemic episode is defined as the index date. ICD codes used to identify hypoglycemia are reported in Supplementary Table S2.1.

Since nearly all diabetes patients start as unexposed to hypoglycemia, we used EDS with replacement to create a dynamic sub-cohort.[38] EDS, a technique of dynamic matching at the time of exposure, allows for the estimation of the effect of a time-dependent exposure with minimal loss in precision and improved interpretability of the exposure effect, when compared to a full cohort analysis.[39] Importantly, time-dependent bias, which can lead to an underestimation of risk in a standard survival analysis with exposure as a time-dependent covariate, is avoided.[39,40] Specifically, we randomly selected four unexposed individuals for each exposed individual within risk-sets based on diabetes duration and age. Those selected as unexposed to hypoglycemia (controls) at one point were eligible to be exposed (cases) in the future (Figure 2.1). Index date was the date of hypoglycemia for those exposed and the date equivalent to that in diabetes duration for those unexposed (Figure 2.1). The latest index date

allowed was 31 December 2016 to allow for a minimum follow-up time of 2 years to capture dementia based on the validated algorithm used.

2.4.4 Outcome definition

Incident all-cause dementia was defined using a validated algorithm that requires one hospitalization code, three physician claims codes (at least 30 days apart in a two-year period), or a prescription filled for a cholinesterase inhibitor.[41] This definition has 79.3% sensitivity, 99.1% specificity, 80.4% positive predictive value, and 99.0% negative predictive value.[41] The outcome was restricted to all-cause dementia due to difficulty in ascertaining subtypes using administrative data.[41] ICD codes used to identify all-cause dementia are reported in Supplementary Table S2.1.

To account for a dementia latency period and minimize possible reverse causality (i.e., hypoglycemic episodes due to prodromal cognitive impairment prior to dementia diagnosis) a lag period between exposure and the development of dementia was required (Figure 2.2). Specifically, those who received a dementia diagnosis within 2 years of index date were censored. This approach has been used previously to minimize reverse causality or protopathic bias in multiple observational studies assessing the risk of dementia.[42-45]

2.4.5 Confounding mitigation

First, we used the high dimensional propensity score (hdps) algorithm to identify relevant potential confounders based on five dimensions (hospitalizations, procedures, medical diagnoses, medical services, and prescription medication claims) during the year before index date (Figure 2.2).[46] We identified the 200 most prevalent variables in each dimension and ranked them according to their frequency as once, sporadic or frequent. Then, we selected the top 500 variables for inclusion in the model to estimate the

propensity score, in addition to a list of 43 predefined variables, including: (1) demographic variables (age, sex and socioeconomic status [SES], defined as quintiles based on an area-level measure of SES based on the first three characters of the postal code and aggregated neighbourhood-level income data[35]); (2) indicators of healthcare utilization (number of distinct medications dispensed, hospitalizations, physician visits); (3) indicators of diabetes severity such as macrovascular complications (ischemic heart disease, heart failure, hypertension, dyslipidemia, stroke), microvascular complication (nephropathy, neuropathy, retinopathy, and peripheral vascular disease), antihyperglycemic agents (metformin, sulfonylureas, thiazolidinediones, glucagon-like peptide receptor agonists [GLP1-RA], dipeptidyl peptidase-4 [DPP-4] inhibitors, sodium glucose co-transporter-2 [SGLT-2] inhibitors, insulins, meglitinides, and acarbose), and treatment for macrovascular complications (angiotensin converting enzyme [ACE] inhibitors, angiotensin receptor blockers [ARBs], loop diuretics, thiazide diuretics, beta blockers, calcium channel blockers [CCB], and other antihypertensives, including methyldopa, hydralazine, and alpha blockers); (4) other morbidities (Parkinson disease, Huntington's disease, delirium, anxiety/mood disorder); (5) other prescription drug use (antidepressants, antipsychotics, opioids, migraine medications, Parkinson medications, antacids); and (6) index year. A multivariable logistic regression model was used to estimate the likelihood of experiencing a hypoglycemic episode.[42] Then, propensity scores were used to compute the inverse probability of treatment weight (IPTW) to balance possible confounding variables.[47] We used stabilized weights as they are preferred over raw weights to reduce the variance associated with any extreme weights.[48] No further truncation of weights was needed. Last, balance of baseline covariates after weighting was assessed using absolute standardized differences (ASD), with ASD >10% considered as an imbalance.[49] Since we used EDS,

individuals may appear more than once with different index dates. For those, hdps and IPTW were updated at each appearance.

2.4.6 Statistical analysis

Patients were followed from index date until the date of dementia diagnosis, death, emigration, end of provincial health coverage, or end of study period (31 December 2018), whichever occurred first. A conditional weighted cause-specific hazards model with death as a competing risk was used to estimate a hazard ratio (HR) and 95% CI of dementia associated with the hypoglycemic event.[50,51] Model assumptions including the proportional hazards assumption were tested using Schoenfeld residuals.[52] Two additional models were run, wherein we added interaction terms between the exposure variable and biological sex (female and male) or SES (quintiles) to assess for any effect modification. We further addressed the possible impact of the introduction of a government-sponsored reimbursement policy for cholinesterase inhibitors in Oct 2007, which has affected the number of physician visits with a diagnosis of Alzheimer's disease in BC. Specifically, we created and adjusted for a “before/after” variable to indicate if follow-up ended before or after Oct 2007.[53] We used robust variance (sandwich estimator) to calculate a confidence interval for all models.

2.4.7 Secondary and sensitivity analyses

As a secondary analysis we repeated the primary analysis within a high-risk population. Specifically, we restricted the population to those using diabetes medications that have a high risk of inducing hypoglycemia (sulfonylureas, meglitinides, or insulin). Those who experienced a hypoglycemic event before the initiation of any of these medications were excluded.

We also conducted several sensitivity analyses, wherein we varied the age of the included population, the exposure definition, and the lag period. First, we repeated the primary analysis using a cohort of patients with incident diabetes aged 50-60 years. Increasing the lower age limit at cohort entry requires less follow-up time to capture incident dementia, while decreasing the upper limit helps account for a longer prodromal period or delayed diagnoses. Second, hypoglycemic episodes that result in a hospitalization are more clinically severe than those reported in a physician visit; therefore, we changed the exposure definition wherein we stratified the hypoglycemia composite exposure definition to either hypoglycemia captured solely from hospitalizations or solely captured from physician claims. Third, we used 6 lag periods (1 year, 3-7 years), wherein those diagnosed with dementia were censored. Last, we calculated the E-value to quantify the minimum strength of amount of association between an unmeasured confounder such as smoking and the exposure/outcome for unmeasured confounding to explain away the main result.[54,55] All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

2.5 Results

2.5.1 Primary analysis

There were a total of 286,006 individuals with incident type 2 diabetes between 1998 and 2016, of whom 278,812 met the inclusion criteria (Figure 2.3). Our dynamic sub-cohort was comprised of 13,970 dementia-free patients, of whom 2,794 experienced a serious hypoglycemic episode between January 1998 and December 2016. There were a total of 116 all-cause dementia events over a median (IQR) follow-up period of 5.01 (5.55) years among those who experienced a hypoglycemic episode and 213 events over 5.07 (6.53) years among those who did not experience any hypoglycemic episodes. The unadjusted incidence rate (95% CI) of dementia was 7.19 (6.00-8.60) per 1000 person-years for those

exposed and 3.21 (2.80-3.67) per 1,000 person-years for those unexposed. Before any confounding mitigation, the risk of all cause-dementia was more than twice as high among those who experienced hypoglycemia compared to those who did not (crude HR, 2.73; 95%CI, 2.12-2.57).

Before ITPW, those who experienced a hypoglycemic episode were more likely to be on multiple medications and more likely to be on certain medications such as insulin, antidepressants, and opioids (Table 2.1). Additionally, they were more likely to have a more severe diabetes, as indicated by the higher frequency of several diabetes complications (Table 2.1). IPTW resulted in well balanced groups across all the included potential confounders (Table 2.1). The mean age (SD) was 62.97 (8.38) years for those exposed and 63.48 (9.53) for those unexposed. Distribution of other sociodemographic characteristics like sex and SES as well as several clinical characteristics were also well balanced (Table 2.1). Although diabetes duration was no longer well balanced after weighting with absolute standardized difference >10%, those exposed to hypoglycemia had a shorter duration (~ 8 months) compared to unexposed. Therefore, confounding by diabetes duration, a possible indicator of diabetes severity is towards the null. The weighted incidence rate (95% CI) of all-cause dementia was 4.59 (3.52-5.98) per 1000 person-years for those exposed and 3.33 (2.58-3.88) per 1,000 person-years for those unexposed (Table 2.2).

The risk of all-cause dementia was higher for those exposed to hypoglycemia compared to those who were not (weighted HR, 1.83; 95%, 1.31-2.57). As previously mentioned, we further adjusted for any potential impact on dementia diagnoses in BC due to the introduction of a cholinesterase inhibitor reimbursement policy in 2007. This further adjustment led to a similar risk estimate (Adjusted HR, 1.78; 95% CI, 1.27-2.49) (Table 2.2). Additionally, results from multiplicative interaction models were not statistically significant ($p>0.06$ for all) and did not suggest any effect modification by sex or SES.

2.5.2 Secondary and sensitivity analyses

From those who were using sulfonylurea, meglitinides, or insulin, we created a secondary sub-cohort of 9120 individuals, of whom 1824 experienced a hypoglycemic episode. The incidence rates and hazard ratio were slightly higher compared to the primary sub-cohort (Weighted HR, 1.98; 95% CI, 1.41-2.78 and Adjusted HR, 1.96; 95% CI, 1.39-2.77) (Table 2.2).

The overall conclusion of an increased risk of dementia associated with hypoglycemia was similar using both exposure definitions; however, it was higher when hypoglycemia was defined based on hospitalization records only, albeit with wider confidence intervals due to a smaller number of events (Table 2.2). Similarly, the risk of all-cause dementia among those who experienced hypoglycemia was higher compared to those unexposed when we used a restricted age range at cohort entry (Table 2.2). Importantly, both crude and weighted hazard ratios were consistent when we used lag periods up to 4 years. However, estimates were attenuated using longer lag periods (5-7 years) with weighted hazard ratios not reaching statistical significance (Figure 2.4). Finally, the minimum strength of association on the risk ratio scale required for an unmeasured confounder associated with the exposure as well as the outcome to explain away the association (i.e., the E-value) was 3.06 (Figure 2.5).

2.6 Discussion

Our study found an increased risk of all-cause dementia associated with hypoglycemia. This conclusion was consistent across secondary and sensitivity analyses. These findings are broadly consistent with previous studies that have assessed this association;[16-22] however, we used several design and analysis techniques in an effort to combat multiple threats to validity.

Importantly, this study is the first to use data from Canada and utilize the EDS approach to handle the time-dependent nature of the exposure. This approach addresses the limitations of previous studies by anchoring the index date and ascertainment period for the adjustment of confounders. Specifically, unlike previous studies, our covariate assessment period was after diabetes diagnosis but within 365 days before the index date (i.e., exposure to hypoglycemia or equivalent date for controls within the risk set). There at least three advantages to our approach.

First, this allowed enough time for diabetes complications to develop and exposure to several diabetes medications, particularly insulin, to take place. For example, in studies where covariates were assessed before diabetes diagnosis, the proportion of those using diabetes medications, particularly insulin, and those with a diabetes-related microvascular complications was low.[21]

Second, this approach emulates an RCT, wherein we were able to mimic randomization by modeling the exposure and the outcome separately. Although theoretically the causal inference positivity assumption is not violated as individuals can experience a hypoglycemic event before or at the time of diabetes diagnosis, it is highly unlikely. Therefore, modeling the probability of exposure at the time of diabetes diagnosis is suboptimal. The EDS approach allowed us to anchor the index date at the time of exposure to hypoglycemia, and therefore we were able to model the exposure using the hdps algorithm, wherein we included >500 covariates.

Third, we were able to utilize the hdps algorithm to derive propensity scores to calculate IPTW, which resulted in well-balanced exposure groups on several important covariates. This was evident by the reduction in the absolute standardized difference (ASD) between exposure groups for a wide range of potential confounders such as the number of distinct medications used, a measure of polypharmacy that

can increase the risk of both hypoglycemia and dementia. A similar reduction in ASD was observed with the use of insulin and other medications such as antidepressants, psychotropics, opioids and Parkinson disease medications, some of which have anticholinergic properties. Moreover, several other macro and micro-complications including stroke, ischemic heart disease, heart failure, nephropathy, retinopathy, neuropathy, and peripheral vascular disease, all of which are indicative of diabetes severity, were more likely to be present among those who experienced hypoglycemia before IPTW but not after. Indeed, this is clearly evident in the distribution of insulin use at baseline, which was much higher among the hypoglycemia group in most of the previous studies that adjusted for insulin use, but not in our analysis.[16,17,19,22]

Additionally, as a sensitivity analysis, we explored the effect of different lag periods on the association between hypoglycemia and dementia. Importantly, when a lag period was not considered, both the crude and weighted hazard ratios were higher compared to hazard ratios when a lag period was considered. This may be explained by potential reverse causality wherein hypoglycemia is an early manifestation of dementia that is yet to be diagnosed. This can occur due to several clinical scenarios including incorrect dosing of insulin or lack of dose adjustment despite weight loss or frailty. Weighted hazard ratios were consistently above 1 when all lag periods were used (1 to 7 years); however, estimates were no longer significant with the lower limit below 1 using longer lag periods (5-7 years). Although reverse causality remains possible, the risk estimates become less precise with longer lag periods occurring after 5 years of index date. Additionally, given the uncertainty on an optimal latency period needed for hypoglycemia to impact the development of dementia, longer lag periods can lead to an

underestimation of the effect of hypoglycemia. Therefore, neuroimaging studies as well as observational studies with longer follow-up times are needed for a more definitive conclusion.

In addition to these design nuances, our findings provide important clinical insights. Interestingly, the risk of dementia seems to be higher with more severe hypoglycemic episodes that required hospitalizations, compared to those captured using physician visits. This signals the need for further work to detail how the severity of the hypoglycemic episode can impact brain structure and lead to cognitive impairment. Moreover, despite plausible effect modification by SES, as access to healthcare after the occurrence of hypoglycemia might impact its cognitive consequences, we did not observe any evidence of difference in risk across quintiles of SES. However, Canada has a universal healthcare system and therefore this finding should not be generalized to populations with less accessible healthcare.

Our study has some limitations. First, we used data collected for administrative purposes; therefore, misclassification of type 2 diabetes, hypoglycemia, and dementia is possible. Validated algorithms and specific eligibility criteria were used to minimize misclassification bias. We are also only able to assess serious hypoglycemic events that require medical attention (hospitalization or a physician visit) and we are not able to capture milder hypoglycemic events. In addition, our outcome was limited to all-cause dementia and we were not able to accurately differentiate between subtypes. Second, although we used multiple lag periods up to 7 years after exposure index date, reverse causality remains possible due to the bidirectional nature of the relationship between hypoglycemia and dementia. Third, we were not able to include important clinical indicators such as hemoglobin A1c; however, we included several indicators for diabetes severity including macrovascular and microvascular complications and diabetes therapies. Our data also lacked information on lifestyle-related covariates, such as smoking, alcohol consumption

and education. Fourth, as with all observational studies, residual and unmeasured confounding remains possible, despite the use of hdps and IPTW. However, to fully explain the observed HR of 1.83, a confounder would have to be associated with both hypoglycemia and with dementia, each by a risk ratio of at least 3.06 in addition to the confounders we were able to measure and adjust for. Last, we did not test for a dose-response relationship among subjects with recurrent hypoglycemic episodes. Future studies should investigate this issue as it would improve our understanding of the underlying pathophysiology.

2.7 Conclusions

Using longitudinal population-level real-world data from over 20 years, we found that serious hypoglycemic episodes contribute to an increased risk of all-cause dementia. These findings add to the existing body of evidence and provide clinical and public health insight on the modifiable risk factors of dementia in type 2 diabetes. Importantly, this study provides an illustration of several design elements that need to be considered when studying this complex association.

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Chapter 3

Associations of mid- and late-life severe hypoglycemic episodes with incident dementia among patients with type 2 diabetes: A population-based cohort study

In this chapter, an extension of chapter two is conducted, wherein the association of mid- and late-life severe hypoglycemic episodes with incident dementia is examined to provide further clinical insight on this association and highlight the need to prevent hypoglycemia across the life-span of patients with type 2 diabetes.

Data resources and availability

All data were de-identified and no personal information was available at any point of the study. Access to data provided by the Data Steward(s) is subject to approval, but can be requested for research projects through the Data Steward(s) or their designated service providers. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). Ethics approval was also obtained from the University of Waterloo.

3.1 Abstract

Objective: Severe hypoglycemia is associated with an increased risk of dementia. We examined if the association is consistently present for mid-life and late-life hypoglycemia.

Methods: Using healthcare data from Population Data BC, we created a base cohort of patients with incident type 2 diabetes aged ≥40 years. Exposure was the first occurrence of severe hypoglycemia (hospitalization or physician visit). We assessed exposure vs no exposure in mid-life (age 45-64) and late-life (age 65-84) cohorts. Index date was the later of the 45th birthday (mid-life cohort), 65th birthday (late-life cohort) or diabetes diagnosis. Those with hypoglycemia or dementia before index date were excluded. Patients were followed from index date until dementia diagnosis, death, emigration or December 31, 2018. Exposure was modeled as time-dependent. We adjusted for confounding using propensity score weighting. Dementia risk was estimated using cause-specific hazards models with death as a competing risk.

Results: Of 221,683 patients in the mid-life cohort, 1,793 experienced their first severe hypoglycemic event. Over a median of 9.14 years, 3,117 dementia outcomes occurred (32 among exposed). Of 223,940 patients in the late-life cohort, 2,466 experienced their first severe hypoglycemic event. Over a median of 6.7 years, 15,997 dementia outcomes occurred (158 among exposed). The rate of dementia was higher for those with (vs. without) hypoglycemia in both the mid-life (hazard ratio, 95% confidence interval=2.85, 1.72-4.72) and late-life (2.38, 1.83-3.11) cohorts.

Conclusion: Both mid-life and late-life hypoglycemia were associated with approximately double the risk of dementia, indicating the need for prevention throughout the life-course of diabetes.

3.2 Article highlights

- Using data from British Columbia, Canada, that spans over 20 years, we assessed if severe hypoglycemia in mid-life or late-life is consistently associated with an increased risk of dementia.

- Both mid-life and late-life severe hypoglycemia was associated with a doubling in the risk of dementia. This did not differ by sex, socioeconomic status or the presence of diabetes complications.

- These findings indicate the importance of preventing hypoglycemia throughout the life-course of type 2 diabetes patients.

3.3 Introduction

Type 2 diabetes has been reported as a potential modifiable risk factor for dementia.[1,2] Beyond hyperglycemia and insulin resistance, diabetes is a heterogeneous condition with several chronic complications, some of which have been associated with vascular dementia, including stroke and hypertension.[3] Additionally, severe hypoglycemia has been associated with an increased risk of all-cause dementia among patients with diabetes.[4-11] This acute complication, characterized by a symptomatic decrease of blood glucose below 3.9 mmol/L, primarily results from diabetes management. Specifically, it is an adverse effect of concern for exogenous insulin and insulin secretagogues, including sulfonylureas and meglitinides. The risk is heightened with intensive management and longer duration of use.

Several observational studies have linked hypoglycemia requiring hospitalization among patients with type 2 diabetes to dementia, using different data sources and methodologies.[4-11] However, most of these studies focused on the occurrence and number of hypoglycemic episodes without considering the effect of age, except as a potential confounder. The potential for age to impact the association between hypoglycemia and dementia among adults with type 2 diabetes is an important knowledge gap.[4-11] For example, among children and adolescents, studies have found that hypoglycemia had quantitatively different effects on cognitive function depending on the age of exposure.[12,13] The timing of the hypoglycemic episodes might be of great relevance in children due to the critical role of glucose in providing energy needed for brain development.[13] Nevertheless, similar work investigating if the effect of hypoglycemia on cognition varies by the age of occurrence or time during a person's life-course has not been conducted among adults with type 2 diabetes.

Plausibly, the pathophysiological damage and consequences resulting from hypoglycemia might not be equivalent across all adults due to aging-related variations in cerebral glucose metabolism, brain vulnerability, ability to compensate, and adaptation cascades.[14-17] Research assessing the risk of dementia associated with modifiable risk factors, such as depression or diabetes onset, has looked further to assess if the risk differs based on the age at exposure.[18,19] These studies concluded that the risk is not constant and tends to differ based on when the exposure occurs in a person's lifetime.[18,19] For example, the risk of dementia seems to be higher when diabetes onset or depression occurs earlier in life.[18,19] Therefore, it can be hypothesized that the risk of dementia differs based on the age of occurrence of hypoglycemia among adults.

Given the aforementioned uncertainties, understanding if the risk of dementia differs based on the age of occurrence of severe hypoglycemia can provide clinicians with insights to guide therapeutic management, including the pharmacological options, dose, and HbA1c targets. Conversely, evidence of a consistent risk of dementia, independent of age of hypoglycemia occurrence, can contribute to future work aimed at understanding the complex risk factors of dementia, in addition to clinical and public health implications. Specifically, identifying if earlier exposure to a hypoglycemic insult poses a similar risk of dementia, can help direct clinical, educational, life-style, and public health measures targeting younger patients with diabetes. Therefore, the objective of this study was to examine the association of mid-life and late-life hypoglycemia with dementia.

3.4 Research design and methods

3.4.1 Study design and data source

We conducted a retrospective population-based cohort study using British Columbia's (BC) healthcare data from 01 January 1996 to 31 December 2018 obtained from the health administrative databases within Population Data BC (https://www.popdata.bc.ca/data). Population Data BC provides data on health system encounters for nearly all of BC's population that receives universal health care coverage through the provincial government.[20-24]

We linked six datasets through a de-identified personal health identification number to obtain several variables. Specifically, we obtained demographic variables, including sex, dates of birth and health care coverage from the population registry (Consolidation File).[20] We used variables related to outpatient prescriptions, including dispensation date and drug identification number (DIN) from the PharmaNet program.[21] This database is a comprehensive source of all non-hospital prescription drugs dispensed by community pharmacies to BC residents regardless of insurance coverage (government-sponsored, private, or out-of-pocket). We also obtained variables on physician visits, including the service date and the International Classification of Diseases, 9th Revision [Clinical Modification] (ICD-9-CM) diagnosis code from the Medical Services Plan (MSP) database.[22] Hospital admission-related variables, including up to 25 ICD-10-Canadian Adaptation (CA) diagnosis codes (ICD-9-CM before 2002) and the date of admission and discharge were captured from the Discharge Abstract Database.[23] We accessed dates of death from the Vital Events Deaths database.[24] Last, we acquired an area-level measure of socioeconomic status (SES) based on the first three characters of the postal code and aggregated neighbourhood-level income data from Census Geodata.[25]

3.4.2 Study population

First, we identified a base cohort of patients with incident type 2 diabetes between 01 January 1996 and 31 December 2016. Incident diabetes was defined based on the diabetes case-defining algorithm from the Canadian Chronic Disease Surveillance System, whereby diabetes is defined as the earliest occurrence of 2 physician claims (ICD-9 codes) within a 2-year period or 1 hospitalization (ICD-10-CA).[26] This definition has been validated (positive predictive value [PPV]=81.9% and negative predictive value [NPV]=98.7%) and used in diabetes research using administrative data.[26]

Second, we identified patients meeting the following inclusion criteria: (1) aged ≥ 40 years at the date of diabetes onset; (2) continuously registered in the population registry for at least 365 days before diabetes onset; (3) not dispensed any antihyperglycemic agents before diabetes onset; (4) no history of a diagnostic code indicating type 1 diabetes and not dispensed insulin monotherapy as first-line treatment; (5) without a previous record of diagnostic codes indicating dementia or cognitive impairment or a dispensation record for a cholinesterase inhibitor before diabetes diagnosis; and (6) not diagnosed with Down's syndrome, wherein there is a higher risk of diabetes and dementia with genetic variation that we are unable to assess. ICD codes used to identify diabetes and inclusion criteria are reported in supplementary Table S3.1.

3.4.3 Exposure assessment and life-course sub-cohorts

Our exposure of interest was the occurrence of one or more severe hypoglycemic episodes, defined as at least one hospitalization or a physician claim indicating hypoglycemia without previous hospitalization or claim in the entire period of available data. ICD codes used to identify hypoglycemia are reported in supplementary Table S3.1.

From the base cohort, we created 2 sub-cohorts. In the first, herein referred to as the "mid-life" cohort, the cohort entry date was the date of a patient's 45th birthday. We used an open-cohort design, wherein patients diagnosed with diabetes before their 45th birthday, i.e., "prevalent diabetes", as well as patients diagnosed after their 45th birthday, i.e., "incident diabetes", were included. The index date was the date of the 45th birthday or the date of diabetes onset, whichever occurred last, to account for the delayed entry of patients with incident diabetes. Patients with diagnostic codes indicating history of hypoglycemia or cognitive impairment before index date were excluded. Exposure to hypoglycemia was assessed from index date until their 64th birthday. Specifically, patients who experienced one or more hypoglycemic episodes from index date until their 64th birthday were considered exposed from the date of their first hypoglycemic episode while patients who did not experience any hypoglycemic episode from index date until their 64th birthday were considered unexposed. To avoid immortal time bias, person-time between index date and the occurrence of hypoglycemia was considered unexposed time.

In the second cohort, the "late-life" cohort, the cohort entry date was the date of a patient's 65th birthday. All design elements were similar to the mid-life cohort, including eligibility criteria of being hypoglycemia-free and dementia-free before index date (65th birthday for prevalent diabetes or diabetes onset for delayed entry with incident diabetes). Therefore, the late-life cohort was not independent of the mid-life cohort. Specifically, the late-life cohort consisted of those with incident diabetes after the age of 65 years in addition to those with prevalent diabetes who did not experience hypoglycemia, were not diagnosed with dementia, or censored before the age of 65 years, therefore including individuals from the mid-life cohort who met these criteria (Figure 3.1). Exposure to hypoglycemia was assessed from index date up until their 84th birthday. Therefore, patients who experienced one or more hypoglycemic episodes

from index date up until their 84th birthday were considered exposed from the date of their first hypoglycemic episode while patients who did not experience any hypoglycemic episode from index date until their 84th birthday were considered unexposed. Person-time was handled similarly to the mid-life cohort to avoid immortal time bias.

3.4.4 Outcome definition

A diagnosis of incident all-cause dementia was defined using a validated algorithm (PPV=80.4% and NPV=99.0%) that requires one hospitalization code, three physician claims codes (at least 30 days apart in a two-year period), or a prescription filled for a cholinesterase inhibitor.[27] We restricted the outcome to all-cause dementia, including all subtypes, due to difficulty in ascertaining dementia subtypes using administrative data. ICD codes used to identify all-cause dementia are reported in supplementary Table S3.1.

3.4.5 Covariates

Our covariate assessment period was 1 year before index date. Specifically, we used inverse probability of treatment weighting (IPTW) to create balance between those exposed and unexposed on an array of important confounders. To calculate the weights, we used a logistic model to estimate the propensity score, or the probability of exposure. The logistic model included 40 predefined variables that are potential confounders based on our clinical knowledge as well as previous observational studies assessing hypoglycemia and dementia[4-11], including: (1) demographic variables (age, sex, and SES, defined as area-level income quintiles based on postal codes); (2) diabetes duration; (3) proxies for diabetes severity such as macrovascular complications (ischemic heart disease, heart failure, hypertension, dyslipidemia, stroke, and peripheral vascular disease), microvascular complication (nephropathy, neuropathy, and retinopathy),

antihyperglycemic treatment (metformin, sulfonylurea, thiazolidinedione, GLP1-RA, DPP-4 inhibitor, SGLT-2 inhibitor, insulin, meglitinide, and acarbose), and treatment for macrovascular complications (ACE inhibitors, ARBs, loop diuretics, thiazide diuretics, beta blockers, CCB, and other antihypertensives); (4) other morbidities (Parkinson disease, Huntington's disease, delirium, anxiety/mood disorder); (5) other prescription drug use (antidepressants, antipsychotics, opioids, migraine medications, Parkinson medications, antacids); and (6) index calendar year to account for any time trends.

Additionally, we used the high dimensional propensity score (hdps) algorithm to identify a total of 500 empirical variables using five dimensions (hospitalizations, procedures, medical diagnoses, medical services, and prescription medication records). We stabilized the weights to reduce the variance associated with any extreme weights. Last, we assessed balance of baseline covariates after weighting using absolute standardized differences (ASD), with ASD >0.10 considered as significant imbalance.

3.4.6 Statistical analysis

Patients were followed from index date until the date of dementia diagnosis, death, emigration, end of provincial health coverage, or end of study period (31 December 2018), whichever occurred first. Exposure was treated as time-varying, wherein person-time from index date until exposure to hypoglycemia was considered unexposed person-time. Additionally, to minimize any reverse time causality, those diagnosed with dementia within 2 years after index date or the hypoglycemic episode were censored. This approach and lag period have been used previously in studies assessing risk factors for dementia.[9,28,]

We calculated the crude and weighted incidence rate of dementia by dividing the number of incident dementia cases over the total person-time in each cohort. We estimated the hazard ratio (HR) of dementia

and 95% CIs using a weighted cause-specific hazards model with death as a competing risk, which may provide conservative estimates of the association between hypoglycemia and dementia. Failure to account for the competing risk of death when assessing diseases of older adults can lead to biased overestimated associations.[29] We assessed model assumptions, including the proportional hazards assumption using Schoenfeld residuals. We used robust variance (sandwich estimator) to calculate the confidence intervals.

In October 2007, the BC government introduced a reimbursement policy for cholinesterase inhibitors which affected the number of physician visits with a diagnosis of AD in administrative data. To address this, we created and adjusted for a "before/after" variable to indicate if follow-up ended before or after October 2007.[30] All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

3.4.7 Secondary and sensitivity analyses

As a secondary analysis we assessed potential effect modification of the association between hypoglycemia and dementia in each cohort by sex, SES, and the presence of diabetes micro- or macrovascular complications at baseline. Specifically, we ran three additional models that included interaction terms added to the main effect terms. For the SES model, those with unknown income quintile (roughly 2-3%) were excluded from the analysis. For each model, we tested the statistical significance (p-value <0.05) of the interaction effect using the Wald test. If the interaction term was a significant predictor, we calculated the HR of dementia for each level of the potential effect modifier using a linear combination of parameters.

We conducted three types of sensitivity analyses. In the first sensitivity analysis, we increased the lag period, during which we censored dementia cases occurring after hypoglycemia, from 2 years to 4 years. In the second sensitivity analysis, we broadened the hypoglycemia definition to include 4 additional ICD-

10-CA codes, including non-diabetic hypoglycemia and hypoglycemia captured using 5-digit level codes (Supplementary Table S3.1). In the third sensitivity analysis, we changed the age cut-off period used to define the mid-life and late-life cohorts. Specifically, we changed the mid-life cohort from 45-64 years to 50-64 and 55-64 years and we changed the age cut-period for the late-life cohort from 65-84 years to 65-79 and 65-74 years. Raising the lower age limit at cohort entry requires less follow-up time to capture incident dementia. Conversely, lowering the upper limit helps account for delayed diagnoses and minimizes potential reverse causality. For example, an individual can have prodromal dementia that is not yet diagnosed at the age of 84 that led to a hypoglycemic event; lowering the upper age limit can help minimize this issue.

3.5 Results

A total of 358,090 patients met the inclusion criteria and were included in the base cohort (Figure 3.1).

3.5.1 Mid-life cohort

The mid-life cohort included 221,683 patients, of whom 0.81% (n=1,793) experienced one or more severe hypoglycemic episodes during the study period (Figure 3.1). Baseline characteristics before weighting are reported in Table 3.1. After weighting, patients in the mid-life cohort who experienced hypoglycemia (exposed) and those who did not (unexposed) were comparable across all characteristics including several expected to confound the association between hypoglycemia and dementia, such as insulin and sulfonylurea use, polypharmacy, and a range of macro- and microvascular complications (Table 3.2).

Over a median (IQR) follow-up of 4.90 (6.70) years, 32 patients were diagnosed with dementia among those who experienced hypoglycemia, while 3,085 were diagnosed with dementia over 9.18 (7.81) years among those who did not experience hypoglycemia (Table 3.3). The weighted incidence rate of all-

cause dementia was higher among those who experienced hypoglycemia (2.41, 95% CI=1.47-3.96 per 1000 person-years) compared to those who did not (1.45, 95% CI=1.39-1.50 per 1000 person-years). Results from survival models show that the occurrence of hypoglycemia in mid-life was associated with a higher risk of dementia compared to those without an episode of hypoglycemia (weighted HR=2.85, 95% CI=1.72-4.72) (Table 3.3).

3.5.2 Late-life cohort

There were 223,940 patients who entered the late-life cohort, of whom 1.10% (n=2,466) experienced one or more severe hypoglycemic episodes (Figure 3.1). Baseline characteristics before weighting are reported in Table 3.1. After weighting, patients who experienced hypoglycemia (exposed) and those who did not (unexposed) were comparable in the late-life cohort across all characteristics, including several important potential confounding variables, such as insulin and sulfonylurea use, polypharmacy, and a range of macro- and microvascular complications (Table 3.2).

Over a median follow-up of 2.90 (5.02) years, 158 patients were diagnosed with dementia among those who experienced hypoglycemia while 15,839 were diagnosed with dementia over 6.80 (6.53) years among those who did not experience hypoglycemia (Table 3.3). The weighted incidence rate of all-cause dementia was higher among those who experienced hypoglycemia (13.79, 95% CI=10.63-17.88 per 1000 person-years) compared to those who did not (9.48, 95% CI=9.34-9.63 per 1000 person-years). Results from survival models show that the occurrence of the first hypoglycemic episode in late life was associated with a higher risk of dementia compared to those without an episode of hypoglycemia (weighted HR=2.38, 95% CI=1.83-3.11) (Table 3.3).

3.5.3 Secondary and sensitivity analyses

Results from the secondary analyses did not indicate potential effect modification of the association of hypoglycemia on dementia by sex, SES, or the presence of diabetes micro- or macrovascular complications at baseline in the mid-life or late-life cohorts (Table 3.3).

The overall findings of the primary and secondary analyses were consistent across a range of sensitivity analyses that included changing the hypoglycemia definition, lag period, or the age cut-off used to define the cohorts, albeit some estimates had wider confidence intervals due to a smaller sample size (Table 3.3 and Figure 3.2).

3.6 Discussion

Findings from this cohort study show that the risk of all-cause dementia is higher among those with at least one episode of severe hypoglycemia, regardless of the timing of one's first serious hypoglycemic episode after the age of 45 years. Specifically, the risk of dementia is consistently more than doubled if a patient with diabetes experiences hypoglycemia in mid-life or late life compared to those who did not experience any severe hypoglycemia during that period. Moreover, this increased risk does not appear to differ for males compared to females, or by residence in lower compared to higher income areas.

Due to the complex, paradoxical, and bidirectional pathophysiological relationship between hypoglycemia and cognitive impairment, we hypothesized that the association between severe hypoglycemia and dementia is not consistent across an individual's life-course. The association may not be significant, or at least be attenuated, if hypoglycemia occurs during mid-life. This is potentially due to higher brain reserve (brain structural or physiological pre-morbid capacity) or resistance to injury in mid-life compared to late life, as well as reduced compensatory and adaptive mechanisms of the aging

brain.[16,18,31,32] Findings from multiple studies show that older stroke patients have higher morbidity, including cognitive deficits, and poorer functional recovery than younger stroke patients.[33] Similar to stroke, serious hypoglycemia is an acute event disrupting brain structure, hemostasis, and function; hence, a similar scenario is plausible. However, our results did not support this hypothesis. In fact, our findings indicate that the long-term cognitive consequences of hypoglycemia are similar regardless of whether hypoglycemia occurred earlier or later in life, potentially indicating long-lasting damage.

Previous work has shown that earlier onset of chronic conditions such as diabetes, depression, or hypertension, is associated with higher risk of dementia.[19,20,34] This may be explained by the longer duration spent under chronic stress and the complications induced by these conditions. Severe hypoglycemia, however, is an acute insult and can lead to irreversible damage including neuronal death, loss of gray matter volume and cortical atrophy in areas involved with cognitive functions.[12,35] Additionally, multiple mechanisms have been proposed linking glucose deprivation to an increase in amyloidogenesis and tau phosphorylation, both of which are important hallmarks in the pathophysiology of Alzheimer's disease.[36,37] It is also worth noting that severe hypoglycemia can lead to a proinflammatory state, characterized by increased platelet activation and decreased fibrinolysis, ultimately leading to a prothrombotic state in addition to increased blood pressure and changes in cardiac output and rhythm as well as stroke.[38] Hence, beyond a direct insult to the brain, hypoglycemia can lead to long-term changes that might contribute to the pathophysiology of dementia. Therefore, further neuropathology and imaging studies are needed to better understand both the immediate as well as the delayed consequences of hypoglycemia on the brain's structure and function, while taking into account

several concepts that relate to the aging brain process including brain reserve, resistance, and compensation.

Clinically, these findings have implications that relate to diabetes care. Hypoglycemia is a concern for patients with diabetes receiving insulin or insulin secretagogues across all age groups; however, this concern is heightened among older adults and frail individuals. This heightened concern is attributed to lowered awareness of hypoglycemia among older adults and more severe consequences of hypoglycemia, including falls that are more debilitating for older adults.[39] However, findings from this study provide insight on the equal necessity of preventing the occurrence of severe hypoglycemic episodes in adults in mid-life. Additionally, this study highlights the future need to assess frailty across a person's life-time, and how it can impact diabetes management, probability of experiencing hypoglycemia, and the risk of dementia in all ages.

Ultimately, identifying individuals who are at an increased risk of hypoglycemia irrespective of their age can help clinicians provide an individualized trade-off in the intensity of diabetes therapy while avoiding the risk of a severe hypoglycemic event. This can be achieved through treatment optimization, reducing inappropriate medication use, and life-style and educational interventions with an overarching goal of preserving cognitive function and preventing, or at least delaying, dementia. We believe our study to be one of the first to explore the association of mid- and late-life hypoglycemia with dementia using population-level data that span over 20 years. The available literature suggests that the association between hypoglycemia and dementia is bidirectional and that hypoglycemia is not associated with dementia but rather is a prodromal symptom or an early manifestation of cognitive impairment. This argument is especially of concern in previous studies that include older adults only. However, results

from our mid-life cohort, which has a maximum age of assessing hypoglycemia at 64 years, is less prone to this issue and therefore provides a more definitive answer to establish an association between hypoglycemia and dementia. We also used a lag period approach, wherein we censored those diagnosed with dementia within two years of experiencing hypoglycemia. This approach minimizes potential overestimation of the risk of hypoglycemia on dementia due to reverse causality. Moreover, we conducted secondary analyses to explore the role of two social determinants, sex and SES, as well as the presence of diabetes complications.

Our study has limitations. First, we used health administrative data; therefore, misclassification of type 2 diabetes and dementia is possible. This misclassification is expected to be non-differential and we used validated algorithms, when available, to minimize any misclassification bias. Second, our exposure was limited to very severe hypoglycemic episodes that would require hospitalization or a physician visit. Findings from the In-Hypo DM study showed the one-year incidence proportion of severe hypoglycemia among patients with type 2 diabetes to be 38%.[40] Therefore, we expect the number of those exposed in our study to be underestimated given that less severe hypoglycemia that can be managed independently was not captured. However, misclassification of potential exposed individuals as unexposed would bias estimates towards the null. Importantly, we conducted a sensitivity analysis, in which we used a broader definition of hypoglycemia. Nevertheless, large population-level cohort studies in mid-life and late-life that include reporting of minor and moderate hypoglycemic episodes are warranted. Third, our outcome was limited to all-cause dementia and we were not able to accurately differentiate between subtypes. Fourth, our median follow-up time among those who experienced hypoglycemia was roughly five and three years in the mid-life and late-life cohorts, respectively. Given the long prodromal period of

dementia that can last up to decades, we are unable to fully rule out the possibility of hypoglycemia as an early manifestation of dementia. Although we have used multiple approaches to minimize reverse causality, including a lag period and an upper-age limit, reverse causality remains possible given the bidirectional nature of the relationship between hypoglycemia and dementia. This issue of reverse causality would be more relevant to the late-life cohort than to the mid-life cohort. Last, despite adjusting for more than 500 variables, we were not able to include important clinical indicators such as hemoglobin A1c, although we included several indicators for diabetes severity including duration, macrovascular and microvascular complications, and diabetes therapies. Moreover, data on genetic determinants of dementia, including apolipoprotein E, were not available. Additionally, data did not include a variable to indicate frailty, which has an impact on both hypoglycemia and dementia.[39] Our data also lacked information on education, race/ethnicity, and lifestyle-related covariates, such as smoking and alcohol consumption. Hence, residual and unmeasured confounding remains possible, despite the use of hdps and IPTW.

3.7 Conclusions

Both mid-life and late-life hypoglycemia were associated with a higher risk of dementia in this population-based cohort study using data that spans over 20 years. These findings support the need to prevent hypoglycemia throughout the life-course of type 2 diabetes patients. Additionally, this finding indicates a possible long-lasting effect that can direct future research aimed at understanding the pathophysiological mechanisms by which severe hypoglycemia increases the risk of dementia.

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Chapter 4
Insulin use in type 2 diabetes and the risk of dementia: A comparative population-based cohort study

In this chapter, a population-based cohort study is conducted to reexamine the association between exogenous insulin use and the risk of all incident dementia while accounting for confounding by disease severity through design and analysis.

Data resources and availability

All data were de-identified and no personal information was available at any point of the study. Access to data provided by the Data Steward(s) is subject to approval, but can be requested for research projects through the Data Steward(s) or their designated service providers. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). Ethics approval was also obtained from the University of Waterloo.

4.1 Abstract:

Background: Evidence of an increased risk of dementia with insulin use in type 2 diabetes is weakened by confounding by indication and disease severity. Herein we reassess this association, while accounting for confounding through design and analysis.

Methods: We conducted a cohort study using administrative healthcare data from British Columbia, Canada housed by Population Data BC and identified patients with a new diagnosis of type 2 diabetes (1998-2016). To adjust for confounding through design, from a sub-cohort of those who received two non-insulin antihyperglycemic classes, we identified new users of insulin compared to new users of a non-insulin class. We adjusted for confounding using: (1) conventional multivariable adjustment and (2) inverse probability of treatment weighting (IPTW) based on the high-dimensional propensity score algorithm. The hazard ratio [HR] (95% confidence intervals [CI]) of dementia was estimated using cause-specific hazards models with death as a competing risk.

Findings: We identified 414,089 patients with type 2 diabetes, of whom 33,093 received two antihyperglycemic classes and initiated a third class (7,863 insulin and 25,230 non-insulin) between ages 40-70 years. A total of 257 dementia events occurred over 4.5-year median follow-up. The HR (95% CI) was 1.68 (1.29-2.20) before adjustment and 1.39 (1.05-1.86) after multivariable adjustment, which was further attenuated to 1.14 (0.81-1.60) after IPTW weighting.

Conclusions: After accounting for confounding through design and analysis, initiation of insulin after two antihyperglycemic classes was not associated with an increased risk of all-cause dementia, providing reassurance to prescribers and patients with type 2 diabetes.

4.2 Article highlights:

- Confounding by indication and disease severity was a major threat to validity in previous studies showing an increased risk of incident dementia with insulin use in type 2 diabetes.

- In this population-based cohort study, we adjusted for confounding through design by identifying new users of insulin compared to new users of a non-insulin class from a sub-cohort of those who received two non-insulin antihyperglycemic classes. We further adjusted for confounding using inverse probability of treatment weighting based on the high-dimensional propensity score algorithm.

- Insulin initiation after two antihyperglycemic classes was not associated with an increased risk of all-cause dementia. These findings show the complementary role of design and analysis in combating confounding in observational studies and provide reassurance to prescribers and patients with type 2 diabetes.

4.3 Introduction

The association between type 2 diabetes and dementia has been examined extensively with a general consensus linking the two conditions;[1,2] however, the literature on the possible association between the use of different classes of antihyperglycemic agents and the incidence of dementia is inconclusive.[3] Several classes of antihyperglycemic medications including metformin, thiazolidinediones and more recently the sodium-glucose cotransporter 2 (SGLT-2) inhibitors, have been linked to a lower risk of incident dementia by observational studies with supporting findings from pharmacology research proposing insulin-sensitizing, anti-inflammatory, and various cardio- and cerebrovascular mechanisms.[3-6] Conversely, insulin use has been found to increase the risk of dementia in several observational studies.[3,4] However, the pharmacological impact of insulin on the pathophysiology of dementia is not fully understood and mechanisms with conflicting directions have been hypothesized.

On one hand, the use of exogenous insulin has been suggested as a potential promising therapeutic option for dementia due to its neuromodulatory actions in the brain, including synaptic formation and remodeling, regulation of neurotransmitters, and amyloid clearance.[7] Indeed, several trials have been conducted to assess the efficacy of intranasal insulin in improving cognition. Unlike peripheral subcutaneous injections, the intranasal administration of insulin does not pose a risk of hypoglycemia as insulin penetrates the blood-brain barrier and enters the central nervous system without causing peripheral side effects.[8,9] Thus far, these trials have not yielded promising results.[10]

On the other hand, hyperinsulinemia is thought to be linked with Alzheimer's dementia due to the downregulation of the insulin degrading enzyme (IDE), for which both insulin and amyloid β are competing substrates.[11-13] Hyperinsulinemia leads to lower levels of insulin in the brain from

downregulation of insulin uptake across the blood brain barrier due to desensitization and saturation at supraphysiological levels.[13,14] These lower levels of insulin in the brain lead to decreased levels of IDE, and therefore decreased degradation and increased deposition of amyloid β-protein.[15] Additionally, peripherally injected insulin has been reported to cause rapid cerebral insulin receptor signal transduction leading to site-specific tau phosphorylation.[16] Hyperinsulinemia also plays an important pathogenic role in the development of atherothrombotic infarction and hence can be linked to stroke and vascular dementia.[17]

Due to the long follow-up period required and difficulty in retaining the study population, most evidence on the association of antihyperglycemic medications, including insulin, with the risk of dementia stems from observational studies rather than randomized clinical trials (RCTs). Specifically, results from a 2019 systematic review and meta-analysis of six cohort studies showed that insulin was associated with a 21% increased risk of dementia.[3] Similarly, a pooled analysis of five cohort studies showed insulin use to be associated with a 58% increased risk of new-onset dementia.[4]

Confounding by indication has been arguably the biggest challenge facing observational studies assessing the safety and effectiveness of medications in routine clinical care.[18] Indeed, multiple studies assessed the use of insulin compared to not having diabetes,[19-21] thereby introducing imbalance on several confounding factors, including diabetes itself and all the cardio- and cerebrovascular complications that accompany it. More granularly, type 2 diabetes is a chronic condition with several pharmacological options used at different disease stages throughout the life-course of a patient with diabetes; hence, concerns of confounding by severity of diabetes also require mitigation through design.[22,23] This issue is heightened with insulin, as it is often used for more severe diabetes in routine clinical care. Indeed,

confounding by disease severity has been shown to play a role in other pharmacoepidemiologic studies evaluating insulin and cardiovascular or cancer outcomes.[24,25] While the new user active comparator design has been adopted as a gold standard design to assess the effect of diabetes medications, the implementation of this design is more complex due to the lack of an obvious active comparator for insulin.[18,23]

We hypothesized that at least some of the previously reported positive association between insulin and dementia is explained by confounding by severity of indication. Herein we re-examine the association between insulin use and the risk of dementia while addressing this threat to validity through design and analysis.

4.4 Methods

4.4.1 Design and data source

We conducted a retrospective population-based cohort study using administrative healthcare data from British Columbia (BC), Canada (01 January 1996 to 31 December 2018). Given the universal health care coverage through the provincial government, data on all encounters with the health care system are captured and housed at Population Data BC (https://www.popdata.bc.ca/data).[26-30]

We linked data across six databases using a de-identified personal health identification number. These databases included: (1) the population registry (Consolidation File), which provided the date of birth, sex, and dates of health care coverage;[26] (2) the Medical Services Plan (MSP), which provided data on physician visits, including the service date and the International Classification of Diseases, 9th Revision [Clinical Modification] (ICD-9-CM) diagnosis code;[27] (3) the Discharge Abstract Database (DAD), which provided hospital admission and discharge dates and several diagnoses coded using the

ICD-10-Canadian Adaptation (CA) codes;[28] (4) the PharmaNet program, which includes the drug dispensation date, name, drug identification number (DIN), and day supply.[29] Importantly, all non-hospital prescription drugs dispensed by community pharmacies to BC residents regardless of the type of insurance coverage (government-sponsored, private, or out-of-pocket), are captured; (5) the Vital Events Deaths database, which provided the date of death;[30]and (6) Census Geodata, from which we acquired an area-level measure of socioeconomic status (SES) based on the first three characters of the postal code and aggregated neighbourhood-level income data.[31]

4.4.2 Study cohort

First, we created a cohort of patients with incident diabetes who were diagnosed between Jan 1, 1998 through 31 December 2016. Patients were required to have continuous registration in the population registry for at least 2 years prior to diabetes onset. We used this 2-year period as a washout period, during which patients did not receive any diabetes diagnosis codes, or any antihyperglycemic medications, to ensure diabetes was incident. We defined diabetes based on the validated case-defining algorithm from the Canadian Chronic Disease Surveillance System, whereby diabetes is defined as the earliest occurrence of 2 physician claims or 1 hospitalization captured by relevant ICD codes within a 2-year period.[32] This definition has an 81.9% positive predictive value and 98.7% negative predictive value.[32,33] To minimize capturing type 1 diabetes cases, we excluded those who received insulin monotherapy as first-line treatment.

4.4.3 Exposure

Our exposure contrast of interest was new insulin use compared to non-insulin use. To minimize confounding by severity of indication, we took multiple design approaches. As there is no single

clinically appropriate active comparator to insulin that is used at a similar disease stage, we created a sub-cohort of those who received two distinct non-insulin antihyperglycemic classes. From this sub-cohort, we identified new users of insulin compared to new users of a non-insulin class. The purpose of this approach was to provide more balanced exposure groups with regards to the diabetes disease stage, wherein both insulin and the comparator are used as third-line therapies. We used a new user design and the index date was either the date of insulin initiation (i.e., first prescription as a third-line) for those exposed or the date of initiating a third antihyperglycemic agent for those unexposed.

We then restricted the analytical cohort to those who met the following criteria: (1) aged between 40-70 years at index date, with the lower limit set to allow enough follow-up time to capture incident dementia, while the upper limit was set to account for a possible period of prodromal dementia and delayed diagnosis; (2) no previous record of diagnostic codes indicating dementia or any cognitive impairment, or a dispensation record for a cholinesterase inhibitor before index date; (3) no diagnosis of Down's syndrome due to the high risk of diabetes and dementia in Down's syndrome with genetic variation that we were unable to assess; and (4) a latest index date of 31 December 2016 to ensure a minimum follow-up of 2 years.

4.4.4 Outcome

Incident all-cause dementia was defined using a validated algorithm that requires one hospitalization code, three physician claims codes (at least 30 days apart in a two-year period), or a prescription filled for a cholinesterase inhibitor.[34] This definition has been validated using Canadian data with 80.4% positive predictive value and 99.0% negative predictive value.[34] We restricted the outcome to all-cause dementia and did not explore subtypes of dementia given the difficulty in ascertaining these subtypes using

administrative data and the possibility of mixed dementia.[34] Similar to previous pharmacoepidemiological studies, we used a lag period to account for existing dementia that is not yet diagnosed and to also allow for the disease process to occur.[35-37] Therefore, those who received a dementia diagnosis within 2 years of index date were censored.[35-37]

4.4.5 Primary analysis

We used two analytic approaches to minimize confounding. First, we adjusted for important potential confounders including age, biological sex, and SES as well as proxies of diabetes severity such as diabetes duration, the presence of micro- and macrovascular complications, and any previous hypoglycemic episodes. We also included other conditions, such as depression, and the use of other medications, including statins, antacids, and antihypertensives. These covariates were assessed within 365 days before index date. A full list of all included potential confounders is reported in a previous study.[38]

Second, we further improved covariate balance using inverse probability of treatment weighting based on high-dimensional propensity scores (hdps), which have been found to improve adjustment for confounding .[39,40] Specially, we augmented the aforementioned predefined variables with 500 additional variables that were empirically identified and prioritized through an automated technique which examines thousands of potential covariates from five dimensions (hospitalizations, procedures, medical diagnoses, medical services, and prescription medication claims). The empirical variables were also assessed within 365 days before index date. We then used multivariable logistic regression to model the probability of exposure to insulin based on the predefined and empirical covariates. The estimated propensity scores were then used to compute the inverse probability of treatment weight (IPTW), which were stabilized to reduce the variance associated with any extreme weights.[40,41] We assessed balance of baseline covariates

after weighting using absolute standardized differences (ASD), with ASD>0.10 considered significant imbalance.[42]

Patients were followed from index date until dementia diagnosis or 31 December 2018. We censored at the first occurrence of either emigration, end of registration, death, or switching from exposure groups. Specifically, for insulin users, we censored when patients discontinued insulin (>180-day gap) and for non-insulin users we censored when patients received insulin. The hazard ratio (HR, 95% confidence intervals [CI]) of dementia was estimated using cause-specific hazards models with death as a competing risk.

4.4.6 Sensitivity analyses

We conducted three sensitivity analyses. First, since sulfonylurea is also a class of diabetes medications that has been reported to increase the risk of dementia, we conducted a sensitivity analysis wherein we excluded those who initiated sulfonylurea as a third class in the non-insulin comparator group or those who were on sulfonylurea at the time of the third class initiation. Moreover, we censored those who received sulfonylureas after index date in both exposure groups. Sulfonylurea use before index date was adjusted for in the model. Second, we repeated all primary analyses using new insulin compared to new non-insulin use however as a fourth rather than a third therapy. Third, we changed the grace period used to define the exposure end from a gap of 180 days to 90 days or to a gap of any length. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

4.5 Results

We included 414,089 patients with newly diagnosed type-2 diabetes. Our final analytical cohort included 7,863 new users of insulin as a third-line class and 25,230 new users of a non-insulin third line class

(Figure 4.1). Before ITPW, the mean (SD) age was 57.3 (7.8) for insulin users and 57.0 (7.7) for non-insulin users, while the mean duration of diabetes was 6.1 for insulin users and 6.3 for non-insulin users at index date (Table 4.1). However, patients using insulin were more likely to have multiple hospitalizations, physician visits, and be on more distinct classes of medications, indicating higher use of the healthcare system. The frequency of several micro- and macrovascular complications, including ischemic heart disease, nephropathy and peripheral vascular disease, in addition to other morbidities such as anxiety was also higher among insulin users (Table 4.1). IPTW resulted in well-balanced groups across all the included potential confounders, wherein the ASD between insulin users and non-insulin users was < 0.10 (Table 4.1).

A total of 78 all-cause dementia events occurred over a median (IQR) follow-up period of 3.9 (5.8) years among insulin users and 179 events over 4.6 (4.4) years among non-insulin users (Table 4.2). The crude incidence rate (95% CI) of dementia was 2.13 (1.71-2.66) per 1,000 person-years for insulin users and 1.31 (1.13-1.51) per 1,000 person-years for non-insulin users (Table 4.2). The weighted incidence rates were 1.61 (1.24-2.09) per 1,000 person-years for insulin users and 1.43 (1.24-1.65) per 1,000 person-years for non-insulin users (Table 4.2).

Before any mitigation of confounding by analysis, the crude HR (95% CI) for all cause-dementia was 1.68 (1.29-2.20). After adjusting for baseline confounders using a multivariable regression model the estimate was attenuated (HR 1.39; 95% CI 1.05-1.86). Further adjustment through hdps weighting led to even more attenuation of estimates, with the association no longer reaching statistical significance (HR 1.14; 95% CI 0.81-1.60) (Table 4.2). These overall findings were consistent across all sensitivity analyses (Figure 4.2).

4.6 Discussion

Findings from this comparative cohort study show that insulin use is not associated with an increased risk of dementia. These results are in line with our hypothesis that at least some of the previously reported association between insulin use and dementia is likely explained by confounding of severity of diabetes. Indeed, results from our study negate findings assessing this association in most previous cohort studies that are weakened by methodological limitations in the design[19-21,43-48], but are in line with a more recent robust nested case-control study.[49] Specifically, in observational studies wherein insulin use was compared to not having diabetes, the risk estimates ranged from 1.40 to 4.30.[19-21] Restricting the cohort to diabetes patients in some studies attenuated the risk estimate, albeit still indicating an increased risk.[43-48] For example, Whitmer and colleagues compared the use of insulin to metformin and found a 28% increase in the risk of dementia among insulin users.[48] Metformin, however, is often used as the first-line therapeutic option for type 2 diabetes, while most clinical guidelines recommend the use of insulin later on as the diseases progresses and after failing to control hyperglycemia on multiple classes.[22] Individuals who receive insulin before trying other classes often have a higher HbA1c.[22] Therefore, imbalance on several measures relating to diabetes severity can be expected.

Our study highlights that when studying the effects of medications for chronic disease wherein there is escalation of medication classes throughout the life-course of the disease, such as in the case of type-2 diabetes, design approaches and the careful consideration of the comparator group are critical.[23] While insulin does not have a clear active-comparator, aligning the comparator group based on an indicator of diabetes severity is a possible approach. In this study, we used the therapeutic history to manage diabetes as an indicator of diabetes severity and the failure to control for hyperglycemia. This also allowed for a

distinct index date and therefore the covariate assessment period to cover a similar disease stage. While matching the cohort based on diabetes duration is also an option, diabetes duration may not be an optimal marker of diabetes severity. For example, an individual could have well-controlled diabetes while on metformin only for a duration of five years compared to someone who also had diabetes for five years but who had been on several medications. In fact, this is evident in our study: through design some balance between groups on various indicators of diabetes severity, including diabetes duration, retinopathy, and neuropathy, was achieved before weighting.

Nevertheless, adjusting for confounding through design only is not sufficient and a further adjustment through analysis is necessary.[50] Results from this study show that using a conventional multivariable adjusted model, estimates were attenuated compared to the crude model, albeit still indicating a significantly higher risk of dementia. This was despite including a wide range of potential predefined confounders based on clinical knowledge. Notably, results were no longer statistically significant after further adjustment through the use of inverse probability of treatment weighting approach based on hdps which included 500 more empirical variables.

This study shows an example of the complementary role of design and analysis to combat confounding by severity of indication in observational studies of chronic disease medications, highlighting the need for robust methodology to better answer important clinical questions pertaining to dementia risk. This is particularly true given that RCTs are not feasible to assess the safety of insulin on a long-term outcome such as dementia, rendering evidence from observational studies the main source of evidence to inform practice. Clinically, these results provide reassurance to health care providers and diabetes patients. As most patients with uncontrolled type diabetes 2 eventually start insulin therapy,

anxiety about insulin use is common. Future work should further detail the relationship between the different types and regimens of insulin as well as insulin dosing.

Our study has several strengths, including the implementation of a new-user design, mitigation of confounding through design and analysis, and the use of a comprehensive data source for all BC residents with a high level of insurance coverage and a span of over 20 years. Despite the strength of using administrative health care data, however, there are some limitations. First, there is a potential for misclassification of type 2 diabetes and dementia, albeit this misclassification would likely be non-differential and we used administrative health data and validated algorithms to define both conditions. Second, drug exposure was based on outpatient prescription dispensations, and therefore the actual consumption of medications can only be assumed. Third, we only assessed all-cause dementia as an outcome as it is difficult to accurately differentiate between subtypes. Fourth, data on lifestyle indicators; education; laboratory measures, such as HbA1c; as well as genetic determinants of dementia, including apolipoprotein E, were not available; therefore, residual and unmeasured confounding remains possible. Importantly, response to insulin differs based on the presence of the apolipoprotein E gene.[51] Fifth, we assessed third-line exposure to insulin which may impact the generalizability of results, albeit in a sensitivity analysis, we also defined exposure as fourth-line.

4.7 Conclusion

Findings from this population-based cohort study show that insulin use after two antihyperglycemic classes was not associated with an increased risk of all-cause dementia. These findings highlight the importance of adjusting for confounding through design and analysis techniques in observational studies.

Clinically, this overall conclusion provides reassurance to clinicians prescribing insulin and patients with type 2 diabetes.

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Chapter 5

Insulin, hypoglycemia, and dementia: A causal mediation analysis showcasing challenges and potential opportunities.

In this chapter, an extension of Chapter 4 is conducted, wherein the role of severe hypoglycemia as a potential mediator for the association between exogenous insulin use and the risk of all-cause incident dementia is explored.

Data resources and availability

All data were de-identified and no personal information was available at any point of the study. Access to data provided by the Data Steward(s) is subject to approval, but can be requested for research projects through the Data Steward(s) or their designated service providers. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). Ethics approval was also obtained from the University of Waterloo.

5.1 Introduction

In a previous cohort study (Chapter 4), we showed that insulin was not associated with an increased risk of all-cause dementia after adjusting for confounding. We illustrated how confounding by disease severity due to the lack of a clinically appropriate comparator could account for the higher risk of dementia with insulin use observed in previous observational studies. However, questions surrounding the role of hypoglycemia as a potential mediator of the insulin-dementia association remain.[1-3]

Severe hypoglycemia, defined based on hospitalization or a physician visit, has been consistently linked to an increased risk of dementia in observational studies using data from various routine-clinical settings in diverse populations.[4] Given the plausible pathophysiologic and pharmacologic pathways by which insulin may lead to hypoglycemia, which in turn has been linked to an increased risk of dementia, we believe a mediation analysis is worth conducting despite the absence of a total effect (i.e., the lack of an overall association between insulin and dementia). It has been argued that mediation analyses should be informed by clinical hypotheses that are based on *a priori* biological knowledge rather than by the total effect.[5]

In this paper, we apply recent advances in mediation analysis to further detail the direct (not through hypoglycemia) and the indirect (through hypoglycemia) effect of insulin on the risk of dementia.

5.2 Methods

5.2.1 Insulin and dementia cohort

We leveraged data from a previous cohort study (Chapter 4) of newly diagnosed type 2 diabetes patients without a history of dementia (n=414,089), wherein we used administrative healthcare data from British Columbia, Canada (1996-2018).[6-10] In that study, we minimized confounding by diabetes severity by first

restricting the cohort to those who received two distinct non-insulin antihyperglycemic classes of medications, from whom we identified 40 to 70-year-old new users of insulin (n= 7,863) or a non-insulin class (n=25,230) between 01 January 1998 and 31 December 2016. The outcome of interest was all-cause dementia defined using a validated algorithm.[11] The index date was the date of initiating insulin or a non-insulin class and patients were followed until dementia, death, emigration, switching between exposure groups or 31 December 2018. We used inverse probability of treatment weighting (IPTW) based on the high-dimensional propensity score algorithm to adjust for >500 potential confounders, including demographics, indicators of diabetes severity, previous medication use, and co-morbidities. We used Cox proportional hazards models to estimate the hazard ratio (HR) of dementia accounting for death as a competing event. We conducted two sensitivity analyses: (1) we varied the exposure definition; and (2) we excluded those who received sulfonylurea as the third non-insulin class and censored those who receive sulfonylurea during follow-up. Further details on the exposure, outcome, covariates, and analysis are reported in Chapter 4.

A total of 78 dementia events occurred over a median (IQR) follow-up period of 3.9 (5.8) years among insulin users (weighted incidence rate: 1.61; 95% CI 1.24-2.09 per 1,000 person-years) and 179 events over 4.6 (4.4) years among non-insulin users (1.43; 95% CI 1.24-1.65 per 1,000 person-years).

5.2.2 Mediator

We assessed severe hypoglycemic episodes between the index date and the end of follow-up. Hypoglycemia was defined based on primary or non-primary hospitalisation codes using ICD-10-CA codes (E15, E11.63, E13.63, E14.63, E16.0, E16.1, E16.2) and physician visits codes using ICD-9-CM (251.0, 251.1, 251.2). Hypoglycemic episodes occurring within one year before dementia were not

counted given the possibility of reverse causality, whereby undiagnosed dementia may increase the risk of hypoglycemia.

5.2.3 Statistical analysis

To assess the natural direct effect (NDE) and natural indirect effect (NIE) of insulin on the risk of dementia, we used the causal mediation analysis approach based on concepts of the potential outcomes framework for causal inference.[12,13] The use of this approach has increased dramatically over the last decade due to its advantages over naïve methods, including the multiplicative and additive method.[8]

To estimate the direct and indirect effects, we combined parameters from: (1) a logistic regression model for hypoglycemia (after treatment initiation) conditional on treatment (i.e., exposure to insulin); and (2) a Cox survival regression model for all-cause dementia conditional on treatment and hypoglycemia (after treatment initiation) allowing for exposure-mediator interaction. Both models were weighted by the stabilized IPTW. We used the delta method to obtain standard errors and 95% confidence intervals (95% CI). Given the large sample, the use of delta method standard errors may be preferred over bootstrapping because of computational efficiency.[14]

5.3 Results

In the primary analysis, the unadjusted total effect of insulin on dementia was HR; 95% CI 1.58; 1.21-2.07 with a NDE of 1.49; 1.14-1.97 and NIE of 1.06; 1.02-1.09. After IPTW, the total effect of insulin on dementia was HR; 95% CI 1.06; 0.78-1.44 with a NDE of 1.02; 0.74-1.40 and NIE of 1.04; 1.01-1.08 with a proportion mediated of 0.66 (Table 5.1). Regression coefficients are reported in Figure 5.1.

5.4 Discussion

Our results show a potential small indirect effect of insulin on dementia through hypoglycemia. This overall conclusion was consistent across the different exposure contrasts and definitions. This finding is clinically plausible given the existing evidence on the increased risk of all-cause dementia with severe hypoglycemia; nonetheless, caution in interpretation is warranted.

First, a small NIE is observed. Given the use of administrative healthcare data, only hypoglycemic episodes recorded in a physician visit or hospitalisation claim were captured, while mild and moderate episodes are not captured. Hence, we expect a differential underestimation of the mediator among those exposed to insulin, thereby an underestimation of the NIE. Longitudinal data on plasma glucose measurements that span over multiple decades would be a valuable source to ascertain all hypoglycemic episodes.

Second, a significant NIE in the absence of a total effect indicates 'inconsistent mediation', which suggests that the mediation effect has a different direction than other mediated or direct effects in the model.[5,10] Statistical power needed to detect a significant NIE is less than that needed for the test of total effect.[11] Therefore, it is more likely to find a significant NIE than a significant total effect, especially when these effects are not large.[16] Although our study was population-based at the provincial level, our restrictions to adjust for confounding by disease severity diminished the final sample size. Conducting a multi-centre analysis can help confirm these findings.

Third, despite using longitudinal data and excluding hypoglycemic episodes occurring within a year of dementia, reverse causality cannot be ruled out. Cognitive impairment before a diagnosis of dementia increases the risk of experiencing severe hypoglycemic episodes.[17] Further longitudinal data that include

detailed cognitive assessments along with neuroimaging results are necessary to better assess the possibility of reverse causality.

Fourth, an over-interpretation of the proportion mediated (NIE/NIE+NDE) is not recommended as this measure does not hold good statistical properties due to the mathematical possibility of 0 in the denominator.[18] Nonetheless, the proportions reported can provide valuable information on other potential protective pathways by which insulin is associated with dementia. Indeed, this is evident by findings from one of the sensitivity analyses, wherein the proportion mediated was >1 when we excluded sulfonylurea from the comparator group. This occurs when the size of the mediation effect is larger than the total effect; that is, when the directions of the main and mediated effects are opposite.[15,18] Pharmacological evidence to support a protective role of insulin on cognition exists.[19] Besides its effect on reducing A1C, insulin has neuromodulatory actions in the brain that are hypothesized to improve cognition, including synaptic formation and remodeling, regulation of neurotransmitters, and amyloid clearance.[14] Multiple clinical trials have been conducted to assess the role of intranasal administration of insulin, whereby hypoglycemia is avoided, in improving cognition.[20]

Fifth, in the presence of an unmeasured confounder that affects only the hypoglycemia-dementia relationship, the total effect of insulin on dementia will remain unbiased, but the indirect effect of insulin on dementia through hypoglycemia will be biased.[21] Clinically, a confounder that can affect the hypoglycemia-dementia relationship but not the insulin-dementia relationship is unlikely; however, it can never be ruled out.

Sixth, despite adjusting for a wide range of confounders, we did not have access to confounders that can affect the insulin-hypoglycemia, hypoglycemia-dementia, and insulin-dementia relationships, including lifestyle measures and indicators of frailty.[22,23]

Last, diabetes is a complex condition whereby the presence of time-varying covariates affected by previous exposure is expected. This includes changes to A1C and cardio- and cerebrovascular events. However, methods to conduct such complex analyses are currently too computationally obscure, hindering their use by most clinical researchers.[24]

Despite the added value of detailing pathways by which medications and outcomes are connected, the use of mediation analysis in pharmacoepidemiologic studies, especially those relating to diabetes, is rare. This is highly consequential when these pathways are through a preventable side effect, such as hypoglycemia. Notwithstanding the abovementioned challenges in implementing mediation analysis to detail the potential role of hypoglycemia, findings from this analysis provide further evidence for clinicians, patients with type 2 diabetes, and caregivers of the importance of preventing hypoglycemia.

5.5 Conclusions

A small indirect association between insulin and dementia through the mediating effect of severe hypoglycemia was observed, providing further evidence on the need to prevent severe hypoglycemia in patients with type 2 diabetes. Importantly, several challenges in the implementation of mediation analysis to detail the complex association between insulin, hypoglycemia, and dementia are highlighted, signaling many opportunities for future research.

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Chapter 6
Major conclusions and future directions

6.1 Summary of findings

This series of cohort studies has provided novel findings to better understand the relationships between insulin, hypoglycemia, and dementia. Findings from study 1 (Chapter 2) confirm the previously reported higher risk of all-cause dementia with severe hypoglycemia after implementing several design and analysis techniques to mitigate bias and confounding, including exposure density sampling, a lag period, and IPTW. Specifically, the weighted HR; 95% CI of dementia for those who experienced hypoglycemia, defined based on hospitalisations or physician claims, was roughly double (HR 1.83; 95% CI 1.31-2.57) compared to those who did not experience severe hypoglycemia. Findings from study 2 (chapter 3) show that the increased risk of dementia observed in study 1 is consistent whether hypoglycemia occurs in midlife (HR 2.85; 95% CI 1.72-4.72) or late life (HR 2.38; 95% 1.83-3.11). The subgroup analyses in both study 1 and study 2, showed that the hypoglycemia-dementia association was not modified by demographic factors, including sex, age, or socioeconomic status.

Conversely, findings from study 3 (Chapter 4) negate existing evidence and do not show an increased risk of dementia associated with insulin use (HR 1.14; 95% CI 0.81-1.60). Particularly, we improved on previous studies by adjusting for confounding through design by first restricting the population to those with type 2 diabetes who received two classes of non-insulin antihyperglycemic therapies and then defining the exposure as insulin initiation compared to initiating a non-insulin class, both as third therapies. This design approach along with the use of IPTW based on hdps resulted in the nullification of the previously reported increased risk of dementia with insulin use.[89,91,92] Lastly, findings

from study 4 (chapter 5) indicate a potential role of severe hypoglycemia as a mediator of the association between insulin and dementia, wherein the NIE was HR 1.04; 95% CI 1.01-1.08.

6.2 Contribution to literature

The contribution of this thesis is twofold; clinical and methodological. Clinically, these studies provide further insight on the role of two closely related exposures in type 2 diabetes, insulin and hypoglycemia, on the risk of all-cause dementia. Although the association between diabetes and dementia has received much attention in epidemiological studies, the details by which this association is purportedly mediated are often overlooked. Diabetes is a heterogeneous condition encompassing various factors, including those related to management, such as insulin and hypoglycemia. Therefore, the risk of dementia cannot be assumed to be equal in all diabetes patients. Hence, quantifying a potential increased risk of dementia associated with a particular diabetes-specific event or medication will help both prescribers and patients with diabetes to be cognizant of this issue. Indeed, it is through further understanding of the specific role of modifiable risk factors that clinicians may be able to intervene and improve patient outcomes.

This work provides insight on the importance of minimizing hypoglycemia to reduce the risk of dementia among those with type 2 diabetes. Identifying individuals who are at an increased risk of hypoglycemia can help clinicians provide an individualized trade-off between intensive diabetes therapy or less intensive therapy while avoiding the risk of a severe hypoglycemic event. Additionally, focus should be on the need for individualized educational sessions to help patients and their caregivers in managing their insulin and carbohydrate intake, recognizing symptoms of hypoglycemia, managing mild episodes of hypoglycemia independently, and deciding when to seek care.

While findings from this thesis provide insight to various stakeholders on the importance of minimizing hypoglycemia, they also provide reassurance on the safety of insulin as a class. Insulin is a cornerstone therapeutic option for type 2 diabetes and will continue to be a necessary addition to manage hyperglycemia for many patients. Providing further evidence that negates previous safety concerns based on a potentially spurious association informs our understanding of the benefit-risk profile of insulin with an aim to achieve better health outcomes to millions using insulin around the world.

Methodologically, this thesis illustrates the need for robust design and analysis when conducting observational studies for type 2 diabetes-related exposures and dementia. First, we reviewed previous observational studies assessing the hypoglycemia-dementia and insulin-dementia associations and identified methodologic gaps. We addressed these limitations utilizing established methods, such as exposure density sampling, lag periods, age restriction at baseline, high-dimensional propensity score, and inverse probability of treatment weighting. Second, we utilized advanced techniques, such as causal mediation analysis to further extend the current knowledge on the insulin-hypoglycemia-dementia association. Methods illustrated in this thesis to combat bias can also be implemented in future observational studies of other diabetes-related exposures and the risk of dementia. For example, similar to hypoglycemia, stroke and major cardiovascular events are also acute exposures in type 2 diabetes.[104] Several of the methodologic shortcomings that weakened the internal validity of the hypoglycemia-dementia studies can also be found in studies assessing stroke or cardiovascular events and the risk of dementia.[87,105] Another important example of a methodologic contribution to the field of pharmacoepidemiology of type 2 diabetes is illustrated in study 3 (Chapter 4), wherein we applied restriction to minimize confounding by diabetes severity. Specifically, we restricted the exposure

definition to insulin compared to no-insulin, from a sub-cohort of those who received two antihyperglycemic classes. This design approach can be implemented in studies that have been potentially weakened by confounding by diabetes severity, including those that found insulin use to increase the risk of various outcomes, including mortality and cancer.[106,107]

Taken all together, both the methods and findings of this program of work contribute to future research relating to health outcomes in those with type 2 diabetes as well as the field of epidemiology and pharmacoepidemiology of type 2 diabetes. This is in addition to furthering evidence on the complex association between diabetes and dementia that can signal future work.

6.3 Global limitations

Despite the methodologic nuances in these studies and the strengths and advantages of using administrative healthcare data, there are some limitations, most of which stem from the data source. Specifically, these cohort studies were based on diagnosis claims and pharmacy dispensation data; hence potential misclassification of each of the exposures and dementia is possible. For example, in all studies only hypoglycemic episodes that were coded in a hospitalisation or a physician claim were captured; hence self-managed episodes were misclassified leading to an underestimation of the association between hypoglycemia and dementia. Similarly, insulin use was based on outpatient dispensation data; hence actual use can only be assumed. Given that dementia is a long-term outcome with pathophysiological changes that can precede clinical manifestations and an official diagnosis, it is difficult to ascertain the actual incidence of dementia using this data source. The data source also lacked some important potential confounders including A1C, which can be an informative measure of diabetes severity, in addition to lacking data on genetics as well as life-style factors. Moreover, all studies in this thesis were

observational; hence residual measured and unmeasured confounding can never be ruled out. There are also limitations to the external validity of these findings. The study is conducted in a Canadian province with universal access to healthcare. Therefore, findings from this study may not be generalized to populations with a less accessible healthcare system.

We took multiple approaches to minimize these limitations, including the use of algorithms validated using Canadian data, when available, the use of a lag-period between exposure and dementia, as well as a wide range of sensitivity analyses to ensure the robustness of results from the primary analysis. Despite these limitations, this was a population-based database study allowing for a complete assessment from the real-world , without sampling errors. Moreover, the population of British Columbia is relatively stable, allowing us to follow individuals longitudinally over time with limited loss of follow-up.

6.4 Future directions

Although this program of research contributes to the existing literature, many questions around the associations between insulin, hypoglycemia, and the risk of dementia remain. Thus far, previous studies and the ones presented in this thesis have focused on severe hypoglycemia only. However, mild and self-managed hypoglycemic episodes are more common among patients with diabetes; therefore, it is important to extend the work to understand if mild or moderate hypoglycemic episodes are also associated with a higher risk of dementia. This includes exploring episode recurrence, timing, and management. Given the proposed pathophysiology by which hypoglycemia is associated with a higher risk of dementia, a dose-response association can be hypothesized, wherein more severe episodes of hypoglycemia can lead to more brain tissue damage. Nonetheless, routinely collected data on blood glucose measurements that span over decades would provide a valuable source to explore these nuanced

research questions. Similarly, a more detailed look at the association between insulin use and dementia is necessary. This includes studies comparing insulin types, regimens, and dosing. While more intense insulin regimens and higher doses may help achieve lower A1C targets and improve cardiovascular outcomes, they propose a higher risk of hypoglycemia. Hence, conflicting pathways by which insulin impacts the risk of dementia can be hypothesized. Therefore, there are several future research opportunities to better understand indirect pathways, by which insulin may affect the risk of dementia. As highlighted in chapter 5, other potential mediators, such as stroke, cardiovascular events and changes in A1C can also mediate or moderate the association between insulin and dementia. There are currently methodologic and computational challenges that hinder exploring such complex pathways; hence, many opportunities to help implement and integrate the causal mediation framework in the field of pharmacoepidemiology lie ahead.

In this program of research, we have considered severity of diabetes mostly as a potential source of confounding; however, diabetes severity is heterogeneous and can be measured and defined differently. Importantly, diabetes severity is also dynamic over the disease course. Hence, studies design to quantify the association between trajectories of diabetes severity over time and the impact of management, including therapeutic options and A1C targets are important to better guide clinicians to provide better care with a goal to minimize the risk of dementia.

Additionally, some subtle findings can also signal future work. For example, the use of antidepressants, anti-psychotics, as well as opioids was disproportionally higher among those who experienced hypoglycemia compared to those who did not in study 1 and 2. This indicates the need to better understand predictors of serious hypoglycemic events to be able to identify individuals who might

be at an increased risk of experiencing such an event. Therefore, further understanding on why and how these morbidities and medications, which are also associated with an increased risk of dementia, are strong predictors of serious hypoglycemic episodes is crucial. Ultimately, identifying individuals who are at an increased risk of hypoglycemia can help clinicians provide an individualized trade-off between intensive diabetes therapy or less intensive therapy while avoiding the risk of a severe hypoglycemic event.

6.5 Major conclusion

Insulin is not associated with an increased risk of dementia, providing reassurance to clinicians and patients with type 2 diabetes. However, evidence of an increased risk of dementia with severe hypoglycemia is consistently observed, indicating the need to minimize this preventable side effect. In the increasingly aging Canadian population and as the prevalence of diabetes continues to increase, we should not lose sight of the importance of preventing possible consequences among patients with diabetes, particularly dementia. Collectively, this program of research has detailed the role of two closely related exposures in type 2 diabetes, insulin and hypoglycemia, on the risk of dementia. Notably, established design and analysis methods were utilized to combat potential threats to internal validity with an ultimate aim to employ observational evidence to improve the health outcomes of patients with type 2 diabetes.

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Tables

Table 1.1: Summary of antihyperglycemic drug classes.

Class	Mechanism of action	Adverse effects
Sulfonylureas	Stimulate the pancreas to produce more insulin and decrease in-hepatic clearance of insulin (Insulin secretagogues)	Hypoglycemia
Meglitinides	Stimulate the pancreas to produce more insulin (Insulin secretagogues)	Hypoglycemia
Biguanides (metformin)	Reduce the production of glucose by the liver (Insulin sensitizers)	Diarrhea, metallic aftertaste, nausea
Thiazolidinediones (TZD)	Increase insulin sensitivity of the body cells and reduce the production of glucose by the liver (Insulin secretagogues)	Water retention, weight gain, increased risk of bladder cancer, increased risk of non-fatal heart attack
Alpha-glucosidases inhibitor	Slow the absorption of carbohydrates (sugar) ingested	Bloating and flatulence
Dipeptidyl-peptidase-4 (DPP-4) inhibitors	Intensify the effect of intestinal hormones (incretins) involved in the control of blood sugar	Pharyngitis, headache

Glucagon-like peptide-1 (GLP-1) agonist	Mimic the effect of certain intestinal hormones (incretins) involved in the control of blood sugar	Nausea, diarrhea, vomiting
Sodium glucose cotransporter 2 (SGLT2) inhibitors	Help eliminate glucose in the urine	Genital and urinary infections, more frequent urination

Table 1.2: Description of the main domains of neurocognitive function.

Cognitive domain	Description
Complex attention	Includes sustained attention, divided attention, selective attention and information processing speed
Executive function	Includes planning, decision making, working memory, responding to feedback, inhibition and mental flexibility
Learning and memory	Includes free recall, cued recall, recognition memory, semantic and autobiographical long-term memory, and implicit learning
Language	Includes object naming, word finding, fluency, grammar and syntax, and receptive language
Perceptual-motor function	Includes visual perception, visuoconstructional reasoning and perceptual-motor coordination
Social cognition	Includes recognition of emotions, theory of mind and insight

Table 1.3: Characteristics and results of observational studies assessing the association between exogenous insulin and dementia.

Author, year	Data source (country)	Design	Years of available data	Age at inclusion	Intervention	Control	Total dementia	Effect estimates [a] (95% CI)
Ott, 1996[68]	The Rotterdam study (Netherlands)	Cross-sectional	NA	55-99	Insulin	No diabetes	265	3.2 (1.4-7.5)
Ott, 1999[69]	The Rotterdam study (Netherlands)	Cohort	2.1	≥ 55	Insulin	No diabetes	126	4.3 (1.7 - 10.5)
Parikh, 2011[93]	Veterans Administration records (USA)	Cohort	4	≥ 65	Insulin	No insulin	14580	1.02 (0.98–1.06)
Imfeld, 2012[96]	United Kingdom–based General Practice Research Database (GPRD) (UK)	Case-control	10	≥ 65	Insulin	No insulin	786	AD: 1.01 (0.58-1.73
Fei, 2013[100]	Random sample from Tianjin city (China)	Not clear	NA	≥ 65	Insulin	Not clear	132	1.01 (0.97–1.06)

Whitmer, 2013[99]	Kaiser Permanente Northern California Diabetes Registry (USA)	Cohort	5	=>55	Insulin	metformin	1487	1.28 (1.1-1.6)
Bruce, 2014[85]	Fremantle Diabetes Study (Australia)	Cohort	14.7	≥ 50	Insulin	No insulin	17	NR
Huang, 2014 [108]	Taiwan's National Health Insurance database (Taiwan)	Cohort	11	NR	Insulin	Not clear	612	AD: 1.53 (0.98-2.39)
Heneka, 2015[94]	German mandatory public health insurance company AOK, (Germany)	Cohort	6	≥ 60	insulin	No insulin	13177	1.16 (1.46-1.77)
Ma, 2015[109]	Random sample from Tianjin city (China)	Cohort	4	≥ 65	Insulin	No treatment	634	1.01 (0.97-1.05)
Kuo, 2015[87]	National Health Insurance program in Taiwan (Taiwan)	Cohort	11	≥ 40	No insulin	No diabetes	NR	1.41 (1.29-1.55)

Yuan, 2015[89]	Medicare Current Beneficiary Survey (USA)	Cohort	2	≥ 65	Insulin	NR	NR	0.77 (0.40-1.48)
Bohlken, 2018[95]	Nationwide Disease Analyzer database (IQVIA) (Germany)	Cohort	4	≥ 60	Insulin	No insulin	8276	1.34 (1.24–1.44)
Wium-Andersen, 2019[97]	Danish National Diabetes Register (Denmark)	Case-control	18	NR	Insulin	No insulin	11619	0.93 (0.87-0.99)
Buchman, 2019[98]	German health claims data (Germany)	Cohort	5	≥ 60	Insulin	No diabetes	12,784	1.40 (1.31-1.50)

a; estimate of all-cause dementia unless otherwise specified.
Abbreviations: AD, Alzheimer disease; NAD, non-Alzheimer dementia; VD, vascular dementia.

Table 1.4: Characteristics and results of observational studies assessing the association between severe hypoglycemia and dementia.

Author, year	**Data source (country)**	**Design**	**Years of available data**	**Age at baseline**	**Total dementia**	**Exposure definition** [a]	**Effect estimate (95% CI)** [b]
Whitmer, 2009[110]	Kaiser Permanente Northern California Diabetes Registry (USA)	Cohort	27	≥ 65	1822	One episode	1.26 (1.08-1.47)
						Two episodes	1.80 (1.37-2.36)
						Three episodes	1.94 (1.42-2.65)
Yaffee, 2013[102]	Health, Aging, and Body Composition (Health ABC) Study (USA)	Cohort	12	70-79	148	Any	2.10 (1.00-4.40)
Haroon, 2015[111]	Ontario provincial health data (Canada)	Cohort	12	≥ 65	43029	Any	1.73 (1.62-1.84)
Chin, 2016[112]	Korea National Diabetes Program (KNDP) (Korea)	Cohort	3.4	≥ 60	52	One episode	2.69 (1.08-6.69)
						Two episodes	4.07 (1.10-15.05)
Mehta, 2017[113]	Clinical Practice Research	Cohort	12	>65	NR	One episode	1.26 (1.03–1.54)

	Datalink (CPRD) (UK)					Two episodes	1.50 (1.09–2.08)
Lee, 2019 [114]	Atherosclerosis Risk in Communities study (USA)	Cohort	12	Not clear	186	Any	2.54 (1.78, 3.63)
Kim, 2020[115]	National Health Insurance Service Senior cohort (Korea)	Cohort	13	>60	2934	Any	1.25 (1.16-1.34)
						One episode	1.17 (1.04-1.31)
						Two-three episodes	1.20 (1.01-1.42)
						More than three episodes	1.36 (1.06-1.74)
Li, 2021[116]	National Health Insurance program in Taiwan (Taiwan)	Cohort	10	NR	25444	Any	1.77 (1.69–1.85)
Zheng, 2021105	Clinical Practice Research Datalink (CPRD) (UK)	Cohort	30	≥ 50	28,627	Any	1.30 (1.22–1.39)

a; severe hypoglycemia defined using international classification of disease (ICD) codes compared to no hypoglycemia.
b; estimate of all-cause dementia unless otherwise specified.

Table 2.1: Baseline characteristics of the exposure groups before and after inverse probability of treatment weighting.

Characteristic	Before weighting			After weighting		
	Hypoglycemia	No hypoglycemia	ASD	Hypoglycemia	No hypoglycemia	ASD
Age, years, mean (SD)	63.25 (9.37)	63.25 (9.36)	<0.001	62.97 (8.38)	63.48 (9.53)	0.058
Female, n (%)	1279 (45.78)	5042 (45.11)	0.020	1037.14 (45.41)	5225.54 (45.04)	<0.001
Diabetes duration, years, mean (SD)	6.68 (4.69)	6.68 (4.69)	0.000	6.28 (4.09)	6.96 (4.86)	0.152
Socioeconomic status quintile, n (%)						
1 (Highest)	773 (27.67)	2425 (21.70)	0.243	502.98 (22.02)	2877.26 (24.80)	0.093
2	653 (23.37)	2417 (21.63)		528.22 (23.13)	2549.36 (21.98)	
3	521 (18.65)	2278 (20.38)		454.89 (19.92)	2243.08 (19.34)	
4	433 (15.50)	2063 (18.46)		397.92 (17.42)	2003.77 (17.27)	
5 (lowest)	348 (12.46)	1862 (16.66)		350.77 (15.36)	1779.81 (15.34)	
Missing	66 (2.36)	131 (1.17)		49.15 (2.15)	147.65 (1.27)	
Health care utilization						

Number of hospitalizations in year before index date, n (%)						
Zero	1630 (58.34)	8860 (79.28)	0.509	1645.33 (72.04)	8408.39 (72.48)	<0.001
One	558 (19.97)	1545 (13.82)		385.08 (16.86)	1934.07 (16.67)	
Two	295 (10.56)	495 (4.43)		149.62 (6.55)	847.39 (7.30)	
Three or more	311 (11.13)	276 (2.47)		103.89 (4.55)	411.08 (3.54)	
Number of physician visits in year before index date, n (%)						
Zero	S	284 (2.54)	0.324	33.82 (1.48)	229.58 (1.98)	0.135
One	S	91 (0.81)		14.29 (0.63)	76.75 (0.66)	
Two	S	92 (0.82)		10.11 (0.44)	76.69 (0.66)	
Three or more	2782 (99.57)	10709 (95.82)		2225.7 (97.45)	11217.90 (96.70)	
Number of distinct drugs in year before index date, n (%)						
Zero	51 (1.83)	631 (5.65)	0.351	92.49 (4.05)	539.59 (4.65)	0.048
One	47 (1.68)	606 (5.42)		97.79 (4.28)	5220.54 (4.49)	
Two	67 (2.40)	684 (6.12)		104.01 (4.55)	597.20 (5.15)	
Three or more	2629 (94.09)	9255 (82.81)		1989.64 (87.12)	9943.6 (85.71)	
Comorbidities in year before index date, n (%)						

Parkinson disease	17 (0.61)	45 (0.40)	0.029	12.66 (0.55)	47.09 (0.41)	0.021
Huntington’s disease	0 (0)	s	0.019	0	s	0.016
Delirium	91 (3.26)	32 (0.29)	0.227	22.61 (0.99)	99.24 (0.86)	0.014
Anxiety/mood disorder	1673 (59.88)	3536 (31.64)	0.591	954.70 (41.80)	4556.19 (39.27)	0.051
Hypertension	1136 (40.66)	4193 (37.52)	0.064	880.26 (38.54)	4606.45 (39.71)	0.024
Ischemic heart disease	563 (20.15)	1376 (12.31)	0.021	33.13 (14.59)	1653.94 (14.26)	0.009
Dyslipidemia	401 (14.35)	1321 (11.82)	0.075	286.00 (12.52)	1394.91 (12.02)	0.015
Heart failure	332 (11.88)	418 (3.74)	0.307	130.57 (5.72)	795.13 (6.85)	0.047
Stroke	172 (6.16)	297 (2.66)	0.171	86.65 (3.97)	494.14 (4.26)	0.024
Nephropathy	400 (14.32)	630 (5.64)	0.293	183.62 (8.04)	936.96 (8.08)	0.001
Neuropathy	128 (4.58)	181 (1.62)	0.172	61.71 (2.70)	234.35 (2.02)	0.045
Retinopathy	116 (4.15)	200 (1.79)	0.139	55.38 (2.42)	272.92 (2.35)	0.005
Peripheral vascular disease	367 (13.14)	262 (2.34)	0.412	130.28 (5.70)	761.69 (6.57)	0.036

Use of medications in year before or on index date, n (%)						
Antidepressants	844 (30.21)	2072 (18.54)	0.274	554.06 (24.26)	2471.79 (21.31)	0.070
Antipsychotics	955 (34.18)	2085 (18.66)	0.358	541.63 (23.71)	2787.43 (24.03)	0.007
Opioids	1097 (39.26)	2468 (22.08)	0.379	624.07 (27.32)	2947.77 (25.41)	0.043
Migraine medications	39 (1.40)	79 (0.79)	0.068	17.10 (0.75)	85.13 (0.73)	0.002
Parkinson’s medications	60 (2.15)	135 (1.21)	0.073	38.87 (1.70)	151.75 (1.31)	0.036
Antacids	1093 (39.12)	2530 (22.64)	0.363	630.42 (27.60)	3189.60 (27.49)	0.002
Metformin	1756 (62.85)	4998 (44.72)	0.370	1167.16 (51.10)	5804.37 (50.03)	0.021
Sulfonylurea	1363 (48.78)	1909 (17.08)	0.716	590.45 (25.85)	3062.32 (26.40)	0.012
Thiazolidinedione	132 (4.72)	262 (2.34)	0.129	66.75 (2.92)	356.84 (3.08)	0.009
GLP1-RA	40 (1.43)	131 (1.17)	0.023	26.56 (1.16)	145.18 (1.25)	0.008
DPP-4 inhibitor	144 (5.15)	504 (4.51)	0.030	113.36 (4.96)	543.39 (4.68)	0.013
SGLT-2 inhibitor	30 (1.07)	206 (1.84)	0.064	20.32 (0.89)	208.76 (1.80)	0.079

Insulin	560 (20.04)	588 (5.26)	0.456	221.42 (9.69)	1436.76 (12.38)	0.086
Meglitinides	19 (0.68)	52 (0.47)	0.028	12.83 (0.56)	56.05 (0.48)	0.011
Acarbose	21 (0.75)	47 (0.42)	0.043	8.89 (0.39)	49.29 (0.42)	0.005
Statins	1476 (52.83)	5232 (46.81)	0.120	1092.01 (47.81)	5664.86 (48.83)	0.020
ACE inhibitors	1262 (45.17)	3907 (34.96)	0.209	851.31 (37.27)	4407.62 (37.90)	0.015
ARBs	495 (17.72)	1976 (17.68)	<0.001	403.15 (17.65)	2059.23 (17.75)	0.003
Loop diuretics	475 (17.00)	611 (5.47)	0.371	187.74 (8.22)	1016.69 (8.76)	0.019
Thiazide diuretics	543 (19.43)	1912 (17.11)	0.060	436.39 (19.11)	1964.53 (16.93)	0.057
Beta blockers	766 (27.42)	2191 (19.60)	0.185	487.28 (21.34)	2500.06 (21.55)	0.005
CCB	656 (23.48)	2071 (18.53)	0.122	431.81 (18.91)	2474.37 (21.33)	0.060
Other antihypertensives	91 (3.26)	150 (1.34)	0.128	41.33 (1.81)	231.41 (1.99)	0.014

Abbreviations: ASD, absolute standardized difference; GLP1-RA, glucagon-like peptide-1 receptor agonist; DPP-4, dipeptidyl-peptidase 4; SGLT, sodium-glucose cotransporter; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.

s: suppressed number < 5 as per data provider requirements to ensure patient confidentiality/health privacy is maintained.

Table 2.2: Risk estimates of all-cause dementia associated with exposure to hypoglycemia from all analyses.

Exposure	No. of patients	No. of Events	Median follow-up in years (IQR)	Crude incidence rate [a] (95% CI)	Weighted incidence rate [a] (95% CI)	Crude HR (95% CI)	Weighted HR [b] (95% CI)	Adjusted HR [c] (95% CI)
Primary analysis								
Hypoglycemia	2794	116	5.01 (5.55)	7.19 (6.00-8.60)	4.59 (3.52-5.98)	2.73 (2.12-2.57)	1.83 (1.31-2.57)	1.78 (1.27-2.49)
No Hypoglycemia	11176	213	5.07 (6.35)	3.21 (2.80-3.67)	3.33 (2.58-3.88)	1.00	1.00	1.00
Secondary analysis (High-risk population)								
Hypoglycemia	1824	90	4.48 (5.21)	9.25 (7.54-11.34)	7.78 (4.51-11.34)	2.73 (2.08-3.57)	1.98 (1.41-2.78)	1.96 (1.39-2.77)
No Hypoglycemia	7296	212	5.48 (6.67)	4.57 (3.97-5.27)	4.95 (4.18-5.86)	1.00	1.00	1.00
Sensitivity analyses								
Population restricted to those with incident type 2 diabetes aged 50-60 years								

Hypoglycemia	1111	38	5.13 (5.69)	5.83 (4.25-7.98)	3.18 (1.43-7.01)	4.43 (2.68-7.30)	2.78 (1.50-5.15)	3.05 (1.65-5.63)
No Hypoglycemia	4444	46	5.22 (6.27)	1.72 (1.28-2.31)	2.06 (1.47-2.89)	1.00	1.00	1.00
Hypoglycemia defined based on hospitalizations only								
Hypoglycemia	290	15	2.89 (7.84)	10.83 (6.56-17.88)	10.64 (6.43-17.61)	3.11 (1.59-6.09)	3.13 (1.50-6.96)	2.88 (1.34-6.21)
No Hypoglycemia	1160	47	6.04 (7.40)	5.87 (4.43-7.77)	5.87 (4.43-7.77)	1.00	1.00	1.00
Hypoglycemia defined based on physician visits only								
Hypoglycemia	2570	104	5.09 (5.39)	6.90 (5.71-8.35)	4.57 (3.39-6.16)	2.48 (1.91-3.23)	1.55 (1.07-2.23)	1.55 (1.07-2.26)
No Hypoglycemia	10280	196	4.83 (6.15)	3.31 (2.87-3.81)	4.42 (3.27-5.97)	1.00	1.00	1.00

a: per 1,000 person years; b: Inverse probability of treatment weighted model (IPTW); c: IPTW adjusted for the impact of policy change in cholinesterase inhibitor coverage in British Columbia.

Table 3.1: Baseline characteristics of the exposure groups before propensity score weighting in the mid-life and late-life cohorts

Characteristics	Mid-life cohort			Late-life cohort		
	Hypoglycemia (n=1793)	No hypoglycemia (n=219,890)	ASD	Hypoglycemia (n=2466)	No hypoglycemia (n=221,474)	ASD
Age, mean (SD)[a]	51.61 (5.28)	54.71 (6.16)	0.534	68.98 (4.96)	69.91 (5.78)	0.173
Diabetes duration, mean (SD)[a]	0.37 (1.05)	0.25 (0.86)	0.125	2.34 (3.77)	2.18 (3.71)	0.043
Female, n (%)	839 (46.79)	95,251 (43.32)	0.080	1152 (46.72)	102,055 (46.08)	0.021
Socioeconomic status quintile, n (%)						
1 (Lowest)	847 (27.16)	47,743 (21.71)	0.172	602 (24.41)	48,553 (21.92)	0.179
2	417 (23.26)	47,163 (21.45)		527 (21.37)	46,731 (21.10)	
3	307 (19.97)	43,963 (19.99)		497 (20.15)	43,196 (19.50)	
4	297 (17.12)	40,897 (18.60)		434 (17.60)	40,807 (18.43)	
5 (Highest)	225 (12.55)	36,089 (16.41)		355 (14.40)	38,992 (17.61)	
Missing	60 (3.35)	4035 (1.84)		51 (2.07)	3195 (1.44)	
Health care utilization						

Number of distinct drugs in 100 days before index date, n (%)						
0	120 (6.69)	20,526 (9.33)	0.186	84 (3.41)	10,047 (4.54)	0.176
1	138 (7.70)	23,001 (10.46)		84 (3.41)	11,890 (5.37)	
2	147 (8.20)	25,511 (11.60)		123 (4.99)	16,087 (7.26)	
≥3	1388 (77.41)	150,852 (68.60)		2175 (88.20)	183,450 (82.83)	
Number of physician visits in 100 days before index date, n (%)						
0	29(1.62)	2710 (1.23)	0.164	21 (0.85)	2735 (1.23)	0.142
1	7 (0.39)	1396 (0.63)		21 (0.85)	954 (0.43)	
2	15 (0.84)	1498 (0.68)		13 (0.53)	994 (0.45)	
≥3	1742 (97.16)	214,286 (97.45)		2420 (98.13)	216,791 (97.89)	
Number of hospitalizations in 100 days before index date, n (%)						
0	1399 (78.03)	187,264 (85.16)	0.194	1757 (71.25)	168,542 (76.10)	0.122
1	234 (13.05)	23,027 (10.47)		420 (17.03)	33,871 (15.29)	
2	85 (4.74)	6067 (2.76)		161 (6.53)	11946 (5.39)	
≥3	75 (4.18)	3523 (1.60)		128 (5.19)	7115 (3.21)	

Comorbidities in year before index date, n (%)						
Parkinson disease	s	231 (0.11)	0.017	10 (0.41)	1031 (0.47)	0.009
Huntington’s disease	0	6 (<0.01)	0.007	0 (0)	9 (<0.01)	0.009
Delirium	9 (0.50)	414 (0.19)	0.054	13 (0.53)	1008 (0.46)	0.010
Anxiety/mood disorder	805 (44.90)	70,653 (32.13)	0.265	969 (39.29)	78681 (35.53)	0.078
Hypertension	553 (30.84)	82,472 (37.51)	0.141	1212 (49.15)	112,444 (50.77)	0.032
Ischemic heart disease	186 (10.37)	18,718 (8.51)	0.064	397 (16.10)	35270 (15.93)	0.005
Dyslipidemia	213 (11.88)	30,163 (13.72)	0.055	298 (12.08)	29013 (13.10)	0.031
Heart failure	52 (2.90)	4321 (1.97)	0.061	200 (8.11)	13825 (6.24)	0.072
Stroke	31 (1.73)	3034 (1.38)	0.028	99 (4.01)	8387 (3.79)	0.012
Nephropathy	44 (2.45)	3373 (1.53)	0.066	134 (5.43)	11695 (5.28)	0.007
Neuropathy	41 (2.29)	3069 (1.40)	0.066	56 (2.27)	3673 (1.66)	0.044
Retinopathy	24 (11.34)	2151 (0.98)	0.034	56 (2.27)	3777 (1.71)	0.041

Peripheral vascular disease	36 (2.01)	1757 (97.99)	0.034	95 (3.85)	7129 (3.22)	0.034
Use of medications in year before or on index date, n (%)						
Antidepressants	538 (30.01)	40,154 (18.26)	0.278	538 (21.82)	35396 (15.98)	0.149
Antipsychotics	502 (28.00)	38,333 (17.43)	0.254	593 (24.05)	45726 (20.65)	0.082
Opioids	605 (33.74)	47,498 (21.60)	0.274	731 (29.64)	47796 (21.58)	0.186
Migraine medications	49 (2.73)	2593 (1.18)	0.112	18 (0.73)	1187 (0.54)	0.024
Parkinson disease medications	33 (1.84)	1936 (0.88)	0.083	31 (1.26)	2534 (1.14)	0.010
Antacids	440 (24.54)	39,799 (18.10)	0.158	777 (31.51)	54294 (24.51)	0.156
Metformin	654 (36.48)	55,422 (25.20)	0.246	1040 (42.17)	61376 (27.71)	0.307
Sulfonylureas	221 (12.33)	10,792 (4.91)	0.267	647 (26.24)	19069 (8.61)	0.478
Thiazolidinediones	25 (1.39)	1226 (0.56)	0.085	60 (2.43)	2273 (1.03)	0.108
GLP1-RAs	s	412 (0.19)	0.019	10 (0.41)	823 (0.37)	0.005
DPP-4 inhibitors	s	694 (0.32)	0.018	30 (1.22)	2919 (1.32)	0.009

SGLT-2 inhibitors	s	330 (0.15)	0.029	7 (0.28)	801 (0.36)	0.013
Insulin	27 (1.51)	648 (0.29)	0.128	139 (5.64)	3823 (1.73)	0.209
Meglitinides	6 (0.33)	196 (0.09)	0.053	7 (0.28)	394 (0.18)	0.022
Acarbose	s	217 (0.10)	0.019	8 (0.32)	394 (0.18)	0.029
Statins	405 (22.59)	54,373 (24.73)	0.050	1017 (41.24)	94011 (42.45)	0.024
ACE inhibitors	387 (21.58)	49,971 (22.73)	0.027	1006 (40.79)	78434 (35.41	0.111
ARBs	100 (5.58)	19,042 (8.66)	0.120	399 (13.75)	34726 (15.68)	0.055
Loop diuretics	92 (5.13)	6327 (2.88)	0.115	307 (12.45)	17264 (7.80)	0.155
Thiazide diuretics	253 (14.11)	32,567 (14.81)	0.019	594 (24.09)	50827 (22.95)	0.027
Beta blockers	215 (11.99)	28,945 (13.16)	0.035	639 (25.91)	53924 (24.35)	0.036
CCBs	170 (9.48)	21,644 (9.84)	0.012	576 (23.36)	47053 (21.25)	0.051
Other antihypertensives	28 (1.56)	1914 (0.87)	0.063	61 (2.47)	3054 (1.38)	0.080

Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; ASD, absolute standardized difference; CCB, calcium channel blocker, DPP-4, dipeptidyl-peptidase 4; GLP1-RA, glucagon-like peptide-1 receptor agonist; SGLT, sodium-glucose cotransporter.

Table 3.2: Baseline characteristics of the exposure groups after propensity score weighting in the mid-life and late-life cohorts.

Characteristics	Mid-life cohort			Late-life cohort		
	Hypoglycemia (n=1793)	No hypoglycemia (n=219,890)	ASD	Hypoglycemia (n=2466)	No hypoglycemia (221,474)	ASD
Age, mean (SD)[a]	54.66 (5.60)	54.68 (6.17)	0.004	70.24 (5.43)	69.90 (5.78)	0.061
Diabetes duration, mean (SD)[a]	0.24 (0.91)	0.25 (0.88)	0.006	2.12 (3.85)	2.18 (3.71)	0.017
Female, n (%)	791.09 (45.11)	95,314.30 (43.35)	0.040	1108.90 (45.56)	102,073.00 (46.09)	0.001
Socioeconomic status quintile, n (%)						
1 (Lowest)	401.44 (22.89)	47,841.50 (21.76)	0.087	554.18 (21.96)	48,617.20 (21.95)	0.106
2	390.08 (22.25)	47,197.00 (21.46)		463.44 (19.04)	46,738.70 (21.10)	
3	322.31 (18.38)	43,911.90 (19.97)		475.28 (19.53)	43,212.80 (19.51)	
4	293.69 (16.75)	40,859.30 (18.58)		508.07 (20.87)	40,787 (18.42)	

5 (Highest)	309.75 (17.66)	36,020.70 (16.38)		404.46 (16.62)	38,913.60 (17.57)	
Missing	36.29 (2.07)	4061.19 (1.85)		28.70 (1.18)	3210.27 (1.45)	
Health care utilization						
Number of distinct drugs in 100 days before index date, n (%)						
0	190.35 (10.86)	20,479.00 (9.31)	0.068	106.59 (4.38)	10,019.50 (4.52)	0.048
1	178.97 (10.21)	22,951.90 (20.44)		109.45 (4.56)	11,842.10 (5.35)	
2	202.31 (11.54)	25,450.30 (11.57)		176.23 (7.24)	16,031.10 (7.24)	
≥3	1181.93 (67.40)	151,010 (68.67)		2041.42 (83.87)	183,587.00 (82.89)	
Number of physician visits in 100 days before index date, n (%)						
0	21.27 (1.21)	2716.90 (1.24)	0.001	29.89 (1.23)	2725.67 (1.23)	0.014
1	8.99 (0.51)	1391.65 (0.63)		12.75 (0.52)	955.44 (0.43)	
2	17.64 (1.01)	1500.80 (0.68)		10.87 (0.45)	995.89 (0.45)	
≥3	1705.67 (97.27)	214,282.00 (97.45)		2380.63 (97.74)	216,803 (97.89)	

Number of hospitalizations in 100 days before index date, n (%)						
0	1425.17 (81.27)	18,7136.00 (85.10)	0.153	1750.36 (71.91)	168,424.00 (76.05)	0.127
1	250.33 (14.28)	23,076.20 (10.49)		450.77 (15.31)	33,914.30 (15.31)	
2	38.77 (2.21)	6110.34 (2.78)		146.07 (6.00)	11,975.60 (5.41)	
≥3	39.29 (2.24)	3569.25 (1.62)		86.93 (3.57)	7165.48 (3.24)	
Comorbidities in year before index date, n (%)						
Parkinson disease	s	230.16	0.035	11.17 (0.46)	1029.52 (0.46)	0.001
Huntington’s disease	0	5.95 (<0.01)	0.007	0	8.90 (<0.01)	0.009
Delirium	9.75 (0.56)	419.39 (0.19)	0.060	12.87 (0.53)	1010.22 (0.46)	0.010
Anxiety/mood disorder	640.10 (36.50)	70,883.00 (32.24)	0.089	1009.99 (41.49)	78,779.30 (35.57)	0.121
Hypertension	632.49 (36.07)	82,354.40 (37.45)	0.029	1258.27 (51.69)	112,408 (50.75)	0.018
Ischemic heart disease	129.08 (7.36)	18,748.80 (8.53)	0.043	388.10 (15.94)	35,276.40 (15.93)	0.001

Dyslipidemia	212.02 (12.09)	30,130.70 (13.70)	0.048	309.39 (12.71)	28,989.10 (13.09)	0.011
Heart failure	45.62 (2.60)	4337.48 (1.97)	0.042	159.87 (6.57)	13,872.70 (6.26)	0.012
Stroke	29.77 (1.70)	3048.91 (1.39)	0.025	94.69 (3.89)	8392.92 (3.79)	0.005
Nephropathy	38.61 (2.20)	3390.44 (1.54)	0.049	103.36 (4.25)	11,699.20 (5.28)	0.048
Neuropathy	26.41 (1.51)	3085.81 (1.40)	0.009	46.66 (1.92)	3687.58 (1.66)	0.018
Retinopathy	23.84 (1.36)	2157.65 (0.98)	0.035	28.49 (1.17)	3790.49 (1.71)	0.045
Peripheral vascular disease	32.30 (1.84)	3435.02 (1.56)	0.022	75.02 (3.08)	7144.62 (3.23)	0.008
Use of medications in year before or on index date, n (%)						
Antidepressants	357.74 (20.41)	40,364.30 (18.36)	0.052	467.06 (19.19)	35,541.80 (16.05)	0.082
Antipsychotics	353.02 (20.13)	38,523.80 (17.52)	0.067	562.93 (23.13)	45,811.80 (20.68)	0.059
Opioids	406.55 (23.18)	47,719.20 (21.70)	0.036	542.95 (22.31)	47,997.00 (21.67)	0.015
Migraine medications	18.78 (1.07)	2621.91 (1.19)	0.011	9.06 (0.37)	1191.74 (0.54)	0.025

Parkinson disease medications	31.55 (1.80)	1953.56 (0.89)	0.079	36.07 (1.48)	2537.63 (1.15)	0.029
Antacids	341.95 (19.50)	39,916.20 (18.15)	0.034	698.97 (28.72)	54,469.30 (24.59)	0.093
Metformin	427.32 (24.37)	55,622.1 (25.30)	0.021	735.16 (30.20)	61,733.80 (27.87)	0.051
Sulfonylureas	99.12 (5.65)	10,926.50 (4.97)	0.030	223.82 (9.20)	19,506.80 (8.81)	0.013
Thiazolidinediones	9.63 (0.55)	1240.71 (0.56)	0.002	23.31 (0.96)	2307.75 (1.04)	0.008
GLP1-RAs	s	413.50 (0.19)	0.004	7.06 (0.29)	824.06 (0.37)	0.014
DPP-4 inhibitors	s	692.27 (0.31)	0.012	38.80 (1.59)	2916.42 (1.32)	0.023
SGLT-2 inhibitors	s	328.19 (0.15)	0.053	11.63 (0.48)	799.15 (0.36)	0.018
Insulin	8.42 (0.48)	670.81 (0.31)	0.028	56.79 (2.33)	3920.92 (1.77)	0.040
Meglitinides	s	200.62 (0.09)	0.016	s	396.43 (0.18)	0.007
Acarbose	s	218.28 (0.10)	0.025	s	400.82 (0.18)	0.001
Statins	366.53 (20.90)	54,334.70 (24.71)	0.091	984.99 (40.47)	93,983.60 (42.43)	0.040

ACE inhibitors	376.27 (21.46)	49,951.40 (22.72)	0.030	882.40 (36.25)	78,567.70 (35.47)	0.016
ARBs	159.52 (9.10)	18,988.20 (8.64)	0.016	370.73 (15.23)	34,679.00 (15.66)	0.011
Loop diuretics	60.14 (3.43)	6367.64 (2.90)	0.031	224.94 (9.24)	17,280.60 (7.85)	0.050
Thiazide diuretics	254.23 (14.50)	32,553.60 (14.80)	0.009	519.20 (21.36)	50,856.60 (22.96)	0.038
Beta blockers	217.58 (12.41)	28,922.80 (13.15)	0.022	604.95 (24.85)	53,966.70 (24.37)	0.011
CCBs	146.20 (8.34)	21,636.60 (9.84)	0.052	487.85 (20.04)	47,108.90 (21.27)	0.030
Other antihypertensives	22.93 (1.31)	1926.96 (0.88)	0.042	24.33 (1.00)	3080.56 (1.39)	0.036

Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; ASD, absolute standardized difference; CCB, calcium channel blocker, DPP-4, dipeptidyl-peptidase 4; GLP1-RA, glucagon-like peptide-1 receptor agonist; SGLT, sodium-glucose cotransporter.

Table 3.3: Association of first hypoglycemic episode in mid-life and late-life and the incidence of dementia.

Exposure	Total persons	No of events	Median follow-up time (IQR), years	Crude incidence rate [a]	Weighted incidence rate [a]	Crude HR 95% CI	Weighted HR 95% CI [b]	Adjusted HR 95% CI [c]	EM by sex [d]	EM by SES [d]	EM by complications [d,e]
Lag period of 2 years (Primary analysis)											
Mid-life cohort											
Hypoglycemia	1793	32	4.90 (6.70)	3.11 (2.21-4.39)	2.41 (1.47-3.96)	3.30 (2.32-4.70)	2.85 (1.72-4.72)	2.88 (1.72-4.81)	0.3166	0.3399	0.3160
No hypoglycemia	219,890	3085	9.18 (7.81)	1.45 (1.40-1.50)	1.45 (1.39-1.50)	1.00	1.00	1.00			
Late-life cohort											
Hypoglycemia	2466	158	2.90 (5.02)	16.36 (14.06-19.03)	13.79 (10.63-17.88)	2.40 (2.04-2.82)	2.38 (1.83-3.11)	2.63 (2.01-3.45)	0.6445	0.2139	0.6630
No hypoglycemia	221,474	15839	6.80 (6.53)	9.45 (9.31-9.60)	9.48 (9.34-9.63)	1.00	1.00	1.00			
Lag period of 4 years (Sensitivity analysis)											

Mid-life cohort											
Hypoglycemia	1793	25	4.90 (6.70)	2.43 (1.65-3.59)	1.46 (0.88-2.41)	3.04 (2.04-4.53)	2.10 (1.26-3.51)	2.11 (1.24-3.57)	0.4355	0.3025	0.2450
No hypoglycemia	219,890	2833	9.18 (7.81)	1.33 (1.28-1.38)	1.33 (1.28-1.38)	1.00	1.00	1.00			
Late-life cohort											
Hypoglycemia	2466	107	2.90 (5.02)	11.08 (9.23-13.30)	9.08 (6.56-12.57)	2.25 (1.84-2.74)	2.35 (1.68-3.30)	2.56 (1.82-3.60)	0.6466	0.6157	0.8049
No hypoglycemia	221,474	13028	6.80 (6.53)	7.78 (7.65-7.91)	7.80 (7.67-7.93)	1.00	1.00	1.00			
Broad hypoglycemia definition (sensitivity analysis)											
Mid-life cohort											
Hypoglycemia	2392	44	4.35 (6.58)	3.47 (2.58-4.65)	2.81 (1.82-4.35)	3.89 (2.88-5.26)	3.55 (2.28-5.53)	3.69 (2.36-5.77)	0.0685	0.7639	0.7296
No hypoglycemia	219284	3067	9.16 (7.8)	1.44 (1.39-1.49)	1.44 (1.39-1.49)	1.00	1.00	1.00			
Late-life cohort											

Hypoglycemia	3960	222	2.33 (4.76)	16.22 (14.28-18.43)	12.35 (9.98-15.28)	2.52 (2.20-2.89)	2.22 (1.78-2.77)	2.53 (2.02-3.16)	0.9523	0.2655	0.8622
No hypoglycemia	219842	15696	6.78 (6.52)	9.39 (9.25-9.54)	9.45 (9.30-9.59)	1.00	1.00	1.00			

a: per 1,000 person years; b: Inverse probability of treatment weighted model (IPTW); c: IPTW adjusted for the impact of policy change in cholinesterase inhibitor coverage in British Columbia; c: P-value for the interaction term between effect modifier of interest and hypoglycemia. EM; Effect modification

Table 4.1: Baseline characteristics of the insulin and non-insulin groups before and after inverse probability of treatment weighting.

Characteristic	Before weighting			After weighting		
	Insulin	Non-insulin	ASD	Insulin	Non-insulin	ASD
Age, years, mean (SD)	57.32 (7.84)	57.02 (7.69)	0.040	56.93 (7.76)	57.00 (7.77)	0.010
Female, n (%)	3163 (40.23)	9810 (38.88)	0.020	3091 (39.34)	10184 (39.87)	0.020
Diabetes duration, years, mean (SD)	6.13 (3.81)	6.33 (3.80)	0.053	6.10 (3.70)	6.24 (3.82)	0.037
Socioeconomic status quintile, n (%)						
1 (Highest)	1973 (25.09)	5268 (20.88)	0.136	1828 (23.26)	5639 (22.07)	0.031
2	1723 (21.91)	5396 (21.39)		1666 (21.20)	5568 (21.80)	
3	1531 (19.47)	5282 (20.94)		1554 (19.78)	5223 (20.45)	
4	1375 (17.49)	4891 (19.39)		1491 (18.98)	4799 (18.78)	
5 (lowest)	1092 (13.89)	4064 (16.11)		1204 (15.33)	3931 (15.39)	
Missing	169 (2.15)	329 (1.30)		114 (1.46)	386 (1.51)	
Health care utilization						

Number of hospitalizations in year before index date, n (%)						
0	5131 (65.25)	20817 (82.51)	0.447	6153 (78.29)	19736 (77.25)	0.036
1	1507 (19.17)	3074 (12.18)		1058 (13.46)	3529 (13.81)	
≥2	1225 (15.58)	1339 (5.31)		648 (8.25)	2282 (8.93)	
Number of physician visits in year before index date, n (%)						
0	141 (1.79)	524 (2.08)	0.281	169 (2.15)	512 (2.00)	0.047
1	500 (6.36)	2719 (10.78)		699 (8.90)	2447 (9.58)	
2	1128 (14.35)	5369 (21.28)		1389 (17.68)	4927 (19.29)	
≥3	6094 (77.50)	16618 (65.87)		5601 (71.27)	17660 (69.13)	
Number of distinct drugs in year before index date, n (%)						
0-3	471 (5.99)	2289 (9.07)	0.311	646 (8.22)	2126 (8.32)	0.051
4-7	2355 (29.95)	9993 (39.61)		2744 (34.92)	9343 (36.57)	
8-11	2433 (30.82)	7721 (30.60)		2356 (29.98)	7762 (30.38)	
≥12	2614 (33.24)	5227 (20.72)		2112 (26.88)	6316 (24.73)	
Comorbidities in year before index date, n (%)						

Parkinson disease	12 (0.15)	30 (0.12)	0.009	11 (0.13)	34 (0.13)	<0.001
Huntington's disease	0	0	0	0	0	0
Delirium	98 (1.25)	41 (0.16)	0.130	32 (0.42)	227 (0.89)	0.059
Anxiety/mood disorder	3625 (46.10)	7382 (29.26)	0.353	2685 (34.16)	8774 (34.34)	0.004
Hypertension	2259 (28.73)	7693 (30.49)	0.039	2384 (30.34)	7639 (29.90)	0.009
Ischemic heart disease	1351 (17.18)	2807 (11.13)	0.174	969 (12.32)	3183 (12.46)	0.017
Dyslipidemia	753 (9.58)	2730 (10.82)	0.041	868 (11.05)	2744 (10.74)	0.010
Heart failure	542 (6.89)	677 (2.68)	0.198	312 (3.98)	939 (3.69)	0.016
Stroke	300 (3.82)	506 (2.01)	0.108	605 (2.37)	207 (2.64)	0.017
Nephropathy	685 (8.71)	1129 (4.47)	0.171	433 (5.25)	1613 (6.31)	0.004
Neuropathy	251 (3.19)	543 (2.15)	0.064	207 (2.64)	630 (2.47)	0.011
Retinopathy	227 (2.89)	621 (2.46)	0.026	197 (2.51)	649 (2.54)	0.002
Peripheral vascular disease	657 (8.36)	705 (2.79)	0.244	342 (4.35)	1092 (4.28)	0.004
Use of medications in year before or on index date, n (%)						
Antidepressants	2210 (28.11)	4965 (19.68)	0.199	1776 (22.61)	5686 (22.26)	0.008

Antipsychotics	1956 (24.88)	4356 (17.27)	0.188	1559 (19.85)	5108 (20.00)	0.004
Opioids	2591 (32.95)	5977 (23.69)	0.207	2124 (27.03)	6741 (26.39)	0.014
Migraine medications	83 (1.06)	245 (0.97)	0.008	105 (1.34)	318 (1.25)	0.008
Antacids	2279 (28.98)	5378 (21.32)	0.177	1938 (24.66)	6075 (23.78)	0.021
Metformin	7103 (90.33)	22339 (88.54)	0.058	6927 (88.14)	22652 (88.67)	0.016
Sulfonylurea	6475 (82.35)	15285 (60.58)	0.497	4979 (63.36)	16846 (65.94)	0.054
Thiazolidinedione	241 (3.06)	2911 (11.54)	0.330	800 (10.18)	2426 (9.50)	0.023
GLP1-RA	59 (0.75)	352 (1.40)	0.063	126 (1.61)	317 (1.24)	0.031
DPP-4 inhibitor	290 (3.69)	2231 (9.24)	0.227	765 (9.74)	2023 (7.92)	0.064
SGLT-2 inhibitor	7 (0.09)	102 (0.40)	0.064	12 (0.16)	82 (0.32)	0.033
Meglitinides	89 (1.13)	426 (1.69)	0.047	136 (1.74)	395 (1.55)	0.015
Acarbose	154 (1.96)	350 (1.39)	0.044	114 (1.46)	383 (1.50)	0.003
Statins	4295 (54.62)	14703 (58.28)	0.073	4505 (57.32)	14536 (56.90)	0.009
ACE inhibitors	3910 (49.73)	11648 (46.17)	0.071	3721 (47.35)	12071 (47.25)	0.002
ARBs	1229 (15.63)	5096 (20.20)	0.119	1603 (20.40)	4829 (18.90)	0.038

Loop diuretics	835 (10.62)	1083 (4.29)	0.024	482 (6.14)	1731 (6.78)	0.026
Thiazide diuretics	1596 (20.30)	4665 (18.49)	0.046	1505 (19.16)	4878 (19.09)	0.002
Beta blockers	1895 (24.10)	4539 (17.99)	0.150	1562 (19.87)	5185 (20.29)	0.011
CCB	1498 (19.05)	4567 (18.10)	0.024	1593 (20.27)	4803 (18.80)	0.037
Other antihypertensives	173 (2.20)	320 (1.27)	0.071	104 (1.32)	496 (1.94)	0.049

Abbreviations: ASD, absolute standardized difference; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; DPP-4, dipeptidyl-peptidase 4; GLP1-RA, glucagon-like peptide-1 receptor agonist; SGLT, sodium-glucose cotransporter.

Table 4.2: Hazard ratio of the association between insulin use and the incidence of dementia in all cohorts from the primary analysis.

Exposure	Total Persons	No of events	Median follow-up time (IQR), years	Crude incidence rate [a]	Weighted incidence rate [a]	Crude HR 95% CI	Adjusted HR 95% CI [b]	Weighted HR 95% CI [c]
Third-line insulin vs any third-line non-insulin cohort								
Insulin	7863	78	3.94 (5.86)	2.13 (1.71-2.66)	1.61 (1.24-2.09)	1.68 (1.29-2.20)	1.39 (1.05-1.86)	1.14 (0.81-1.60)
Non-insulin	25230	179	4.60 (4.40)	1.31 (1.13-1.51)	1.43 (1.24-1.65)	1.00	1.00	1.00
Third-line insulin vs third-line non-insulin (excluding sulfonylureas) cohort								
Insulin	7863	78	3.94 (5.86)	2.13 (1.71-2.66)	1.61 (1.24-2.09)	1.63 (1.24-2.15)	1.41 (1.05-1.90	1.11 (0.77-1.59)
Non-insulin	19953	142	4.41 (4.24)	1.34 (1.14-1.58)	1.47 (1.25-1.71)	1.00	1.00	1.00
Fourth-line insulin vs any fourth-line non-insulin cohort								
Insulin	5326	55	4.68 (5.71)	1.96 (1.51-2.56)	2.05 (1.60-2.62)	1.38 (0.92-2.07)	1.53 (0.92-2.52)	1.15 (0.54-2.44)
Non-insulin	9707	50	3.93 (3.18)	1.13 (0.86-1.49)	1.39 (1.08-1.79)	1.00	1.00	1.00

a: per 1,000 person years.; b: Adjusted for predefined variables only; c: Inverse probability of treatment weighted model (IPTW).

Table 5.1: Crude and adjusted natural direct and indirect effects of exposure to insulin vs. a non-insulin class (both as third therapies) on the risk of dementia, mediated by hypoglycemia.

	Unadjusted Hazard Ratio (95% Confidence interval)			Adjusted Hazard Ratio (95% Confidence interval) and proportion mediated [c]			
Exposure definition	**Natural direct effect**	**Natural indirect effect**	**Total effect**	**Natural direct effect**	**Natural indirect effect**	**Total effect**	**Proportion mediated**
As treated exposure definition, without a gap > 180 days in treatment allowed							
Insulin vs no-insulin, both as third therapies [a]	1.49 (1.14-1.97)	1.06 (1.02-1.09)	1.58 (1.21-2.07)	1.02 (0.74-1.40)	1.04 (1.01-1.08)	1.06 (0.78-1.44)	0.66
Insulin vs no-insulin excluding sulfonylurea, both as third therapies [b]	1.45 (1.09-1.93)	1.08 (1.03-1.13)	1.56 (1.18-2.06)	0.99 (0.72-1.37)	1.04 (1.01-1.08)	1.03 (0.75-1.41)	1.34
As treated exposure definition, with any gaps in treatment allowed							
Insulin vs no-insulin, both as third therapies [b]	1.57 (1.24-1.98)	1.06 (1.03-1.10)	1.67 (1.33-2.10)	1.04 (0.80-1.35)	1.06 (1.01-1.10)	1.10 (0.86-1.41)	0.60
Insulin vs no-insulin excluding sulfonylurea, both as third therapies [b]	1.55 (1.22-1.98)	1.06 (1.02-1.09)	1.64 (1.29-2.09)	1.01 (0.77-1.32)	1.06 (1.01-1.10)	1.06 (0.82-1.38)	0.89

a: Primary analysis
b: Sensitivity analyses
c: Weighted based on inverse probability of treatment weighting

Figures

Figure 1.1: Simplified illustration of mechanisms linking diabetes to dementia.

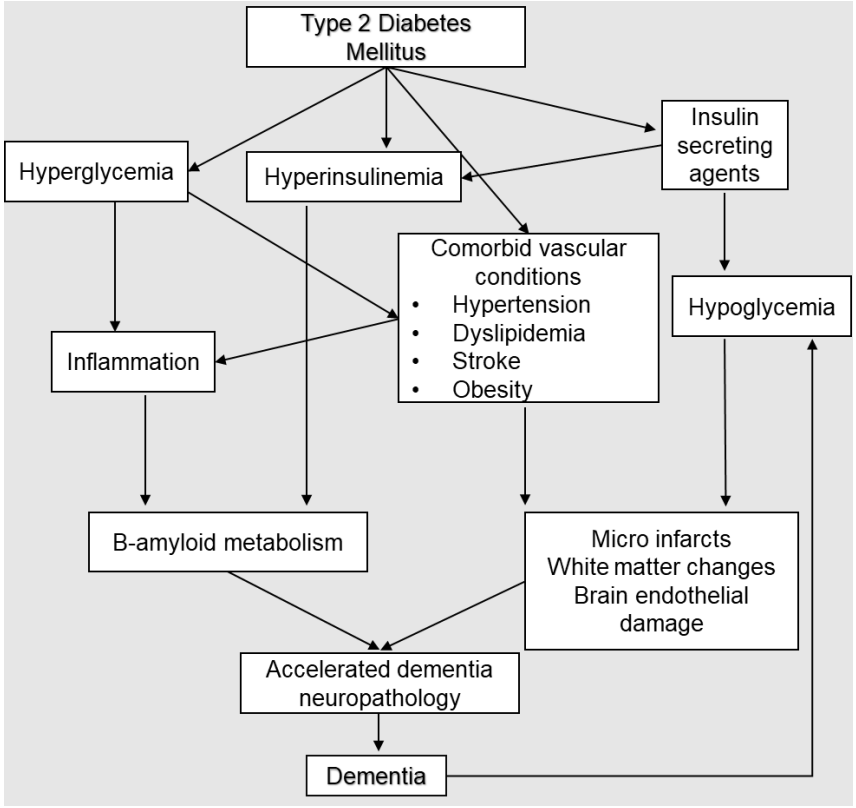


Figure 2.1: Illustration of exposure density sampling. Patients A and C meet the exposure criteria and are considered exposed. Patients C-E can be selected as unexposed for patient A. Patient E only can be selected as unexposed for patient C.

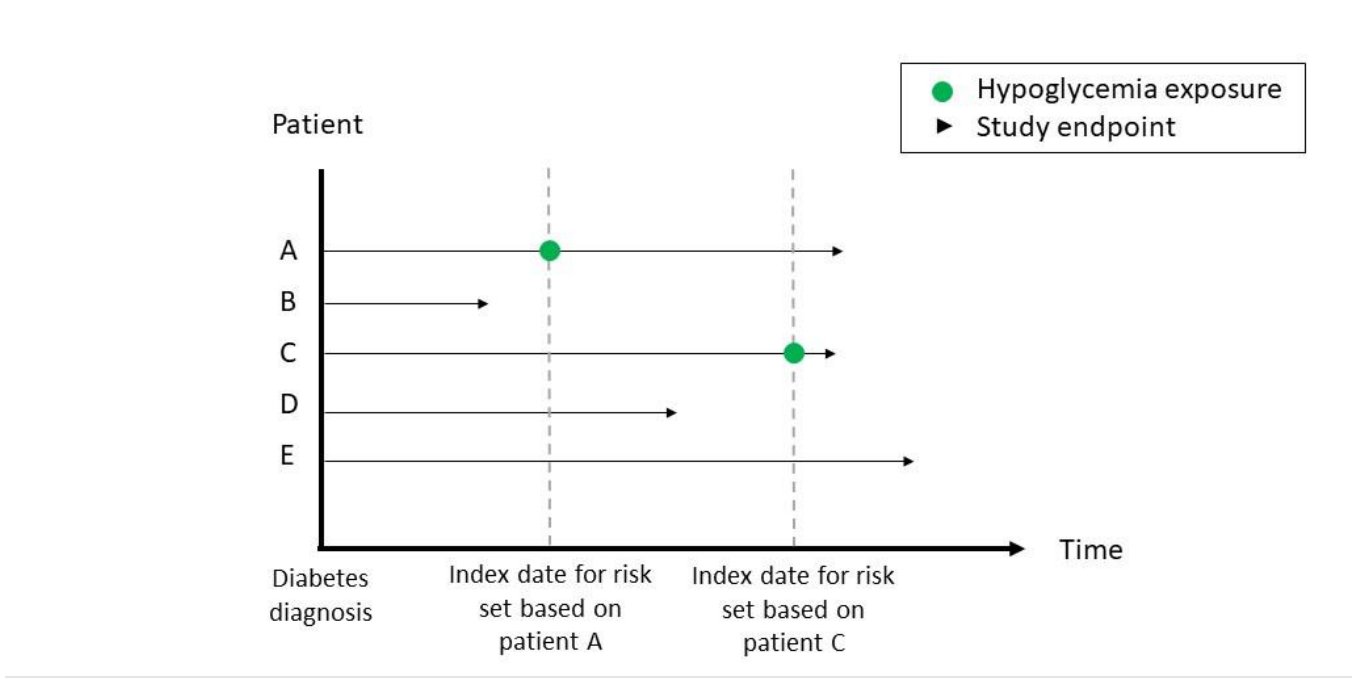


Figure 2.2: Illustration of study design, including cohort entry, index date, covariate assessment period, and lag period. DM, diabetes mellitus.

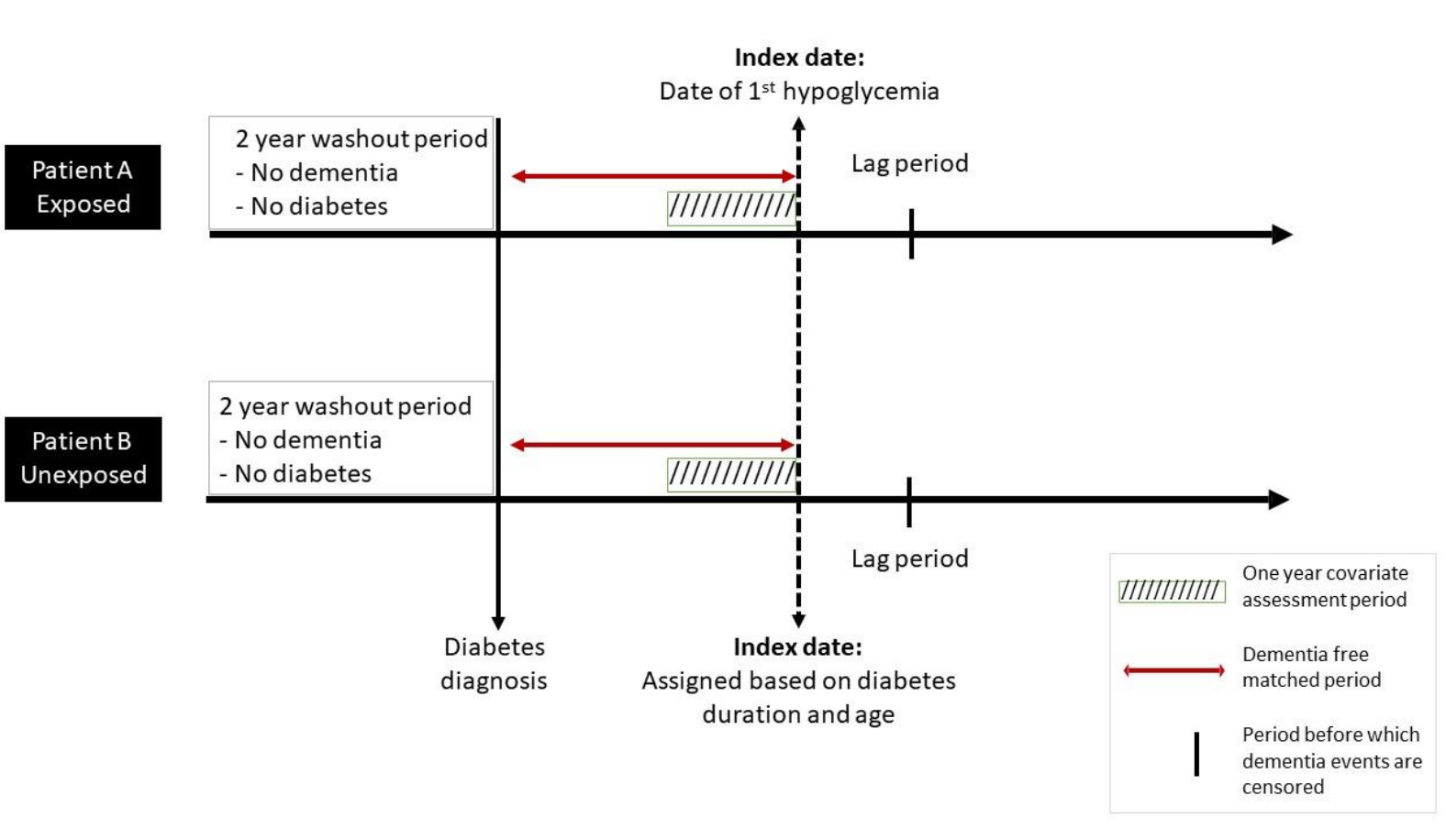


Figure 2.3: Flow chart of cohort study. a: Based on the Canadian Chronic Disease Surveillance System (1 hospitalization or 2 physician claims within 2 years), without a code indicating type 1 diabetes mellitus. b: At any time before diabetes diagnosis with a minimum of 2 years; patients may belong to more than one group.

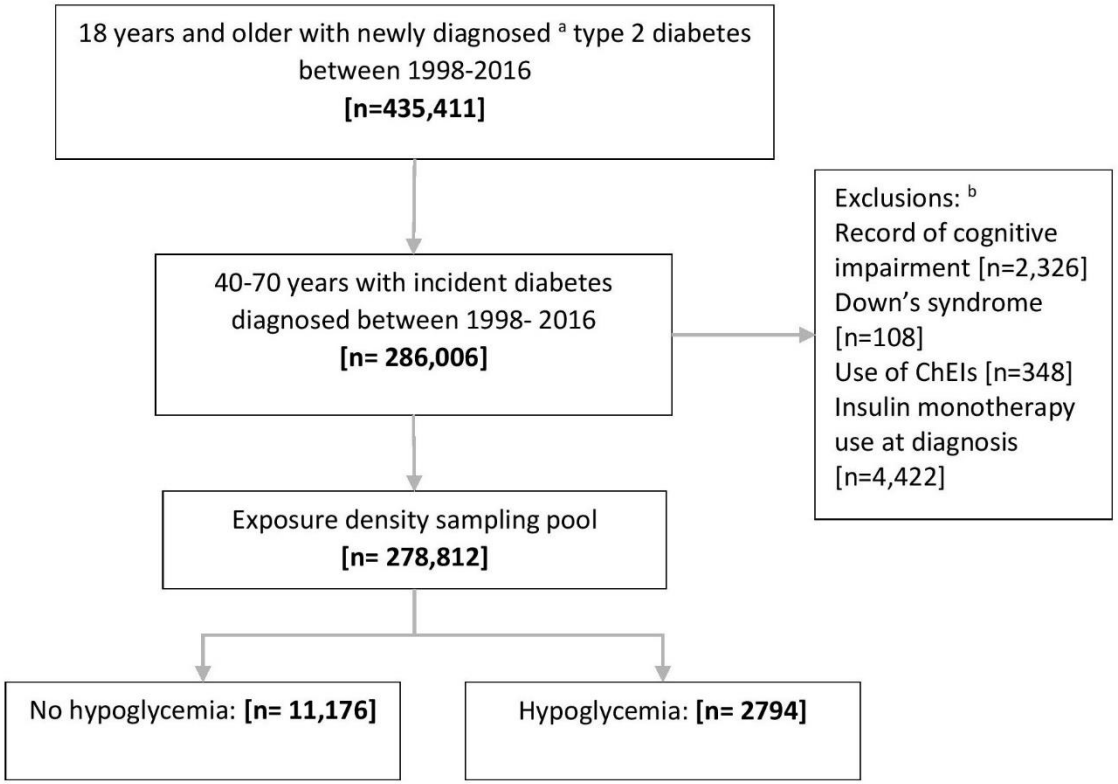


Figure 2.4A: Crude hazard ratios (95% confidence intervals) of main analysis using different lag periods.

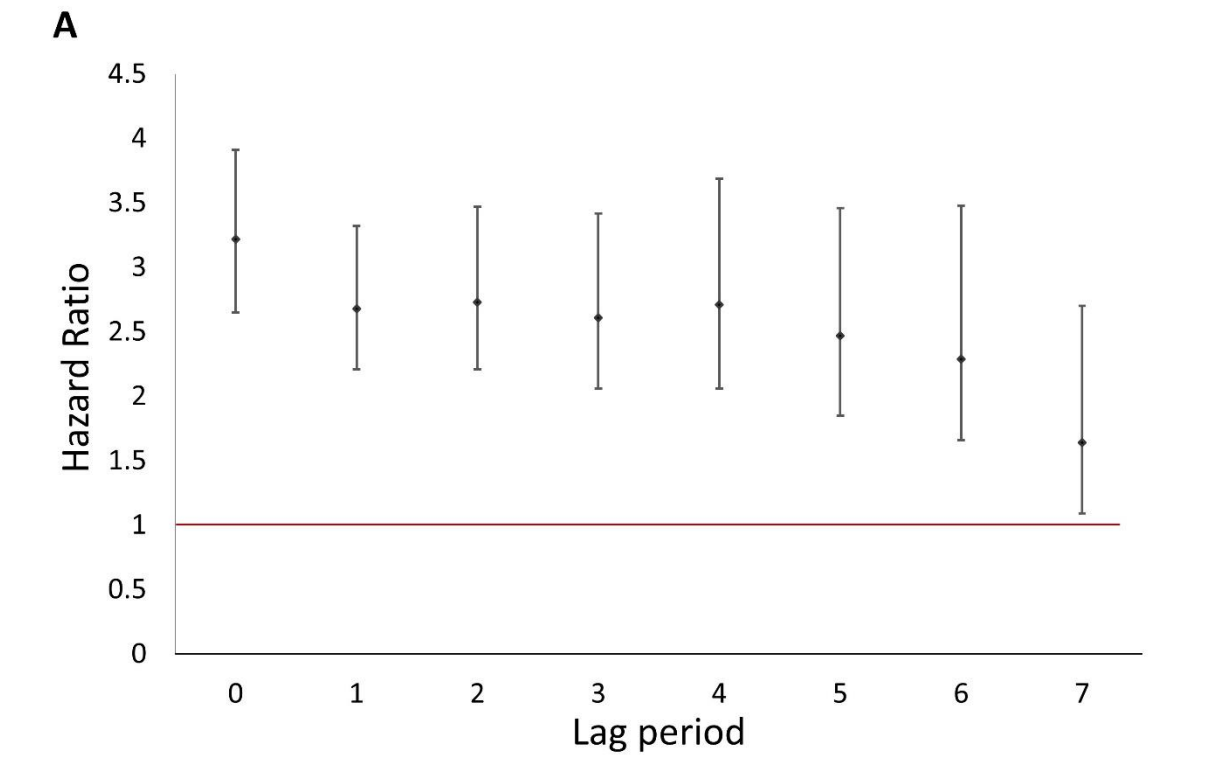


Figure 2.4B: Weighted hazard ratios (95% confidence intervals) of main analysis using different lag periods.

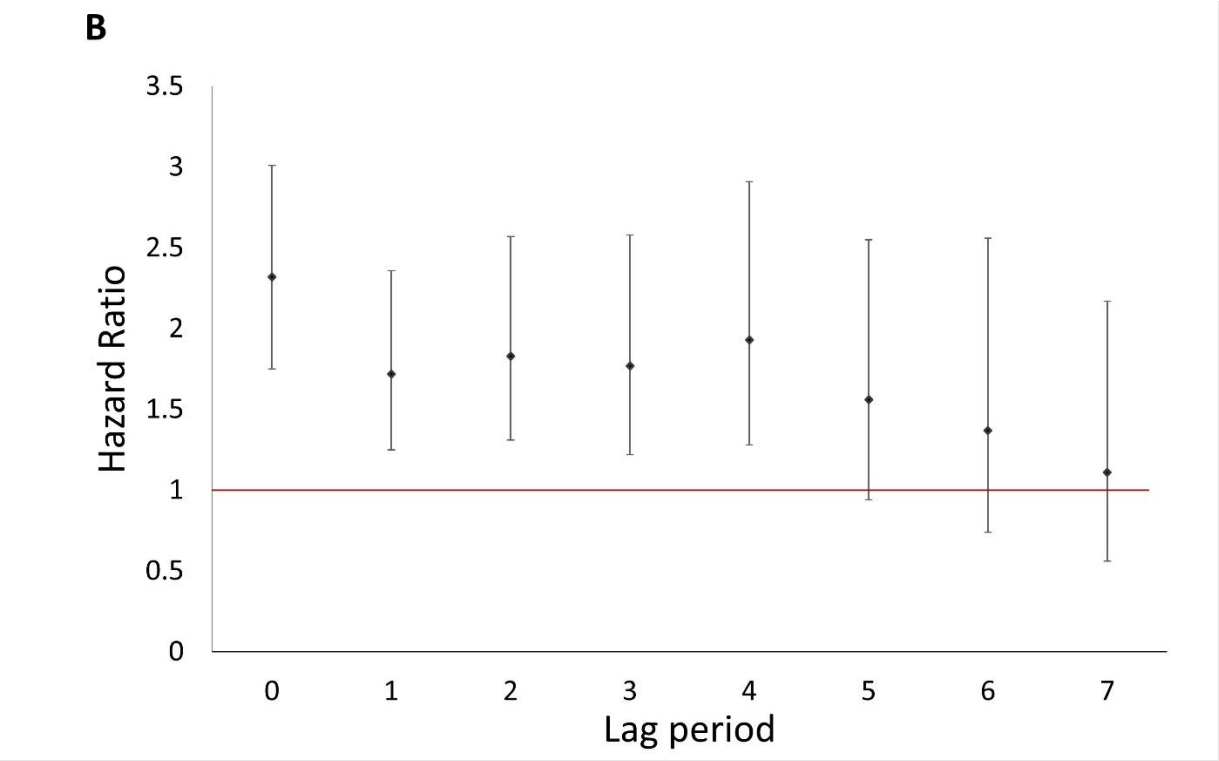


Figure 2.5: Joint values of the minimum strength of association between an unmeasured confounder and hypoglycemia and an unmeasured confounder and all-cause dementia to fully explain away the observed point estimate of the main analysis.

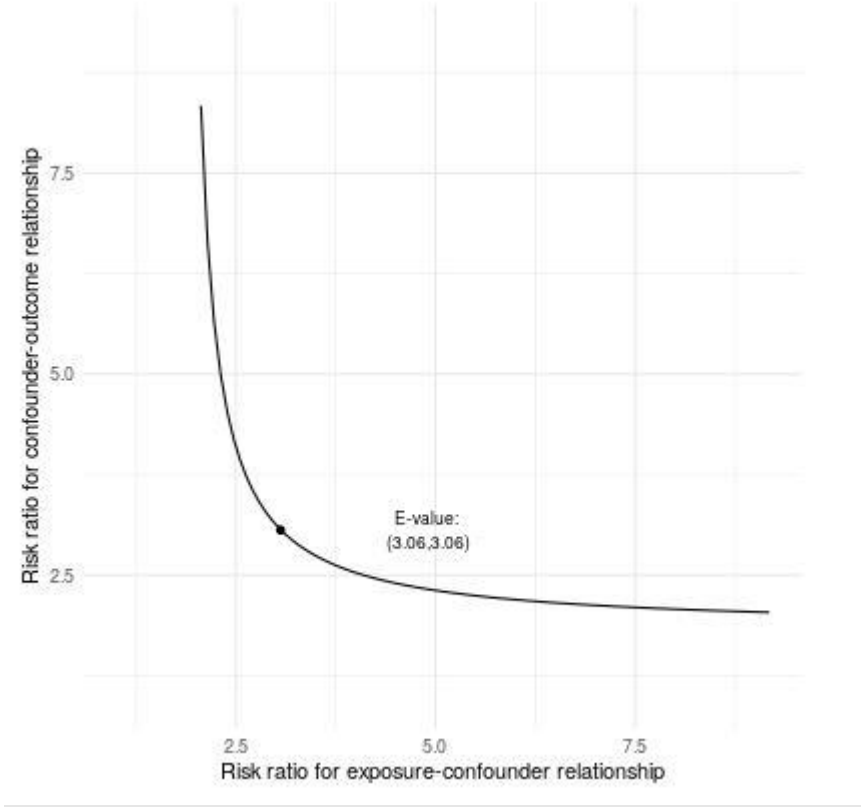


Figure 3.1: Flow chart of cohort study. a: Based on the Canadian Chronic Disease Surveillance System. b: At any time before diabetes diagnosis with a minimum of 2 years; patients (n=204) may belong to more than one group. c: late-life cohort consists of those with incident diabetes ≥ 65 years and those without hypoglycemia who did not have dementia or were censored before age of 65 years.

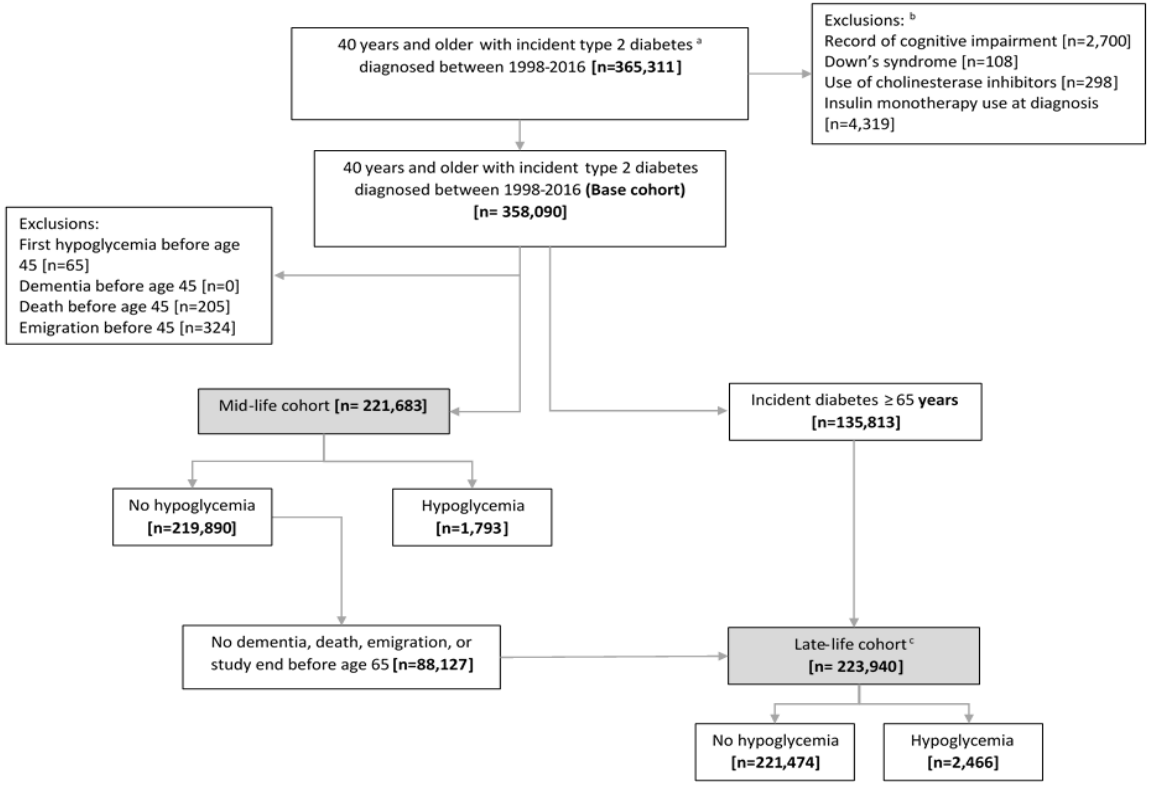


Figure 3.2: Weighted hazard ratios and 95% confidence intervals of dementia using different age cut-offs to define mid-life and late-life cohort.

	Total with Hypoglycemia	No of events among hypoglycemia	Total without hypoglycemia	No of events among no hypoglycemia
Increasing the lower age limit and decreasing the upper age limit by 5 years				
Mid-life cohort (50-64)	1563	29	206,443	3051
Late-life cohort (65-79)	1992	124	203,345	13129
Increasing the lower age limit and decreasing the upper age limit by 10 years				
Mid-life cohort (55-64)	1157	23	179,503	2980
Late-life cohort (65-74)	1328	76	174,106	9064

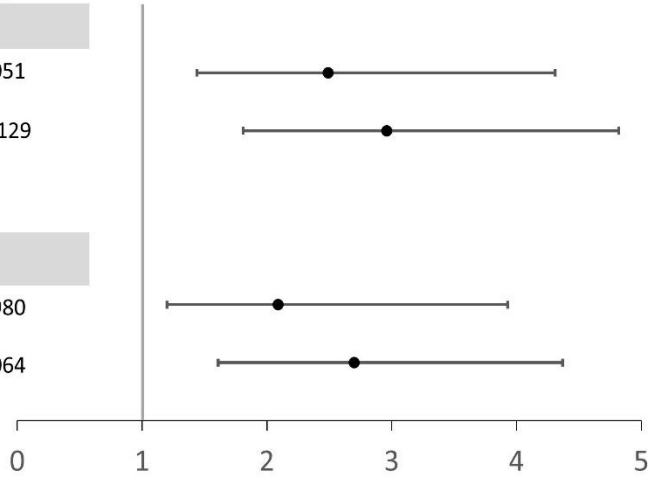


Figure 4.1: Flow chart illustrating the cohort construction for new insulin and new non-insulin use as a third therapy. a indicates 319 patients may belong to more than one exclusion criteria; [b] indicates 2052 patients may belong to more than exclusion criteria.

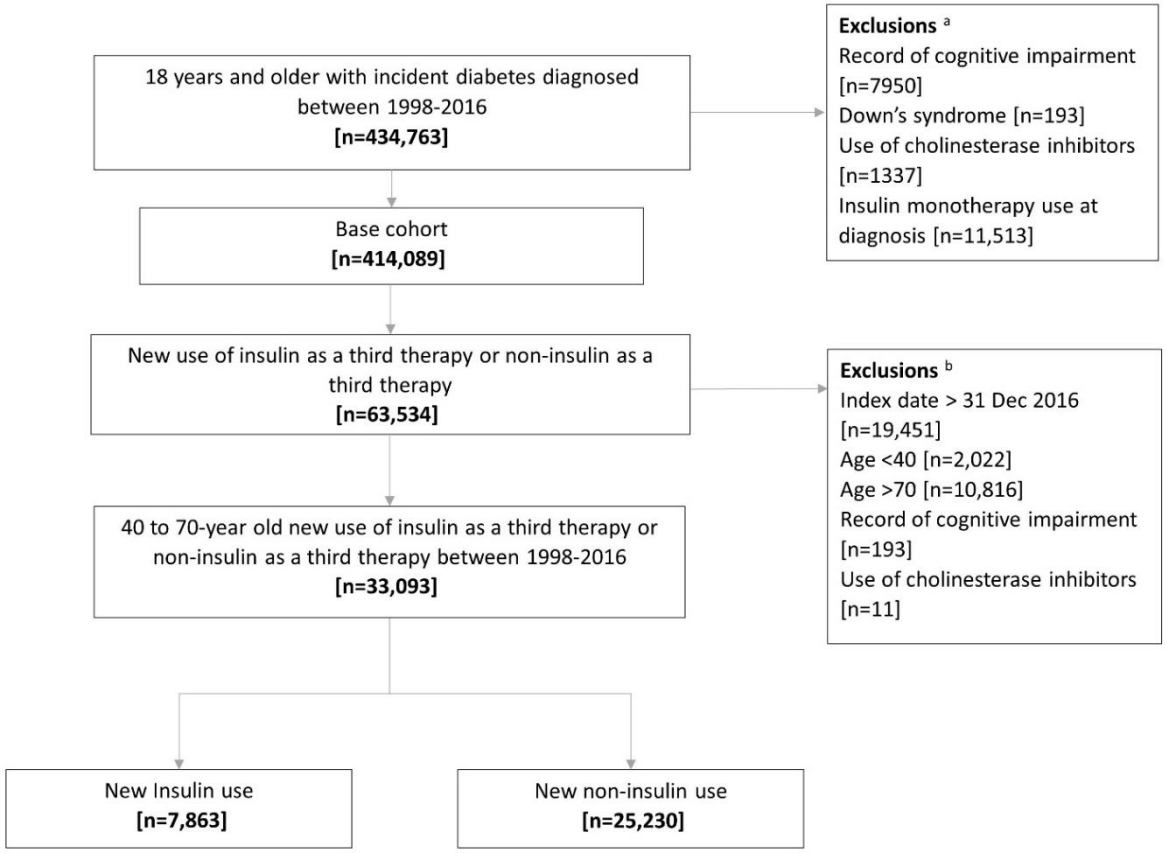


Figure 4.2: Weighted hazard ratios (95% confidence intervals) from sensitivity analyses of the association between insulin use and the incidence of dementia. Third line indicates the initiation of insulin or a non-insulin class after receiving two non-insulin antihyperglycemic classes. Fourth line indicates the initiation of insulin or a non-insulin class after receiving three non-insulin antihyperglycemic classes.

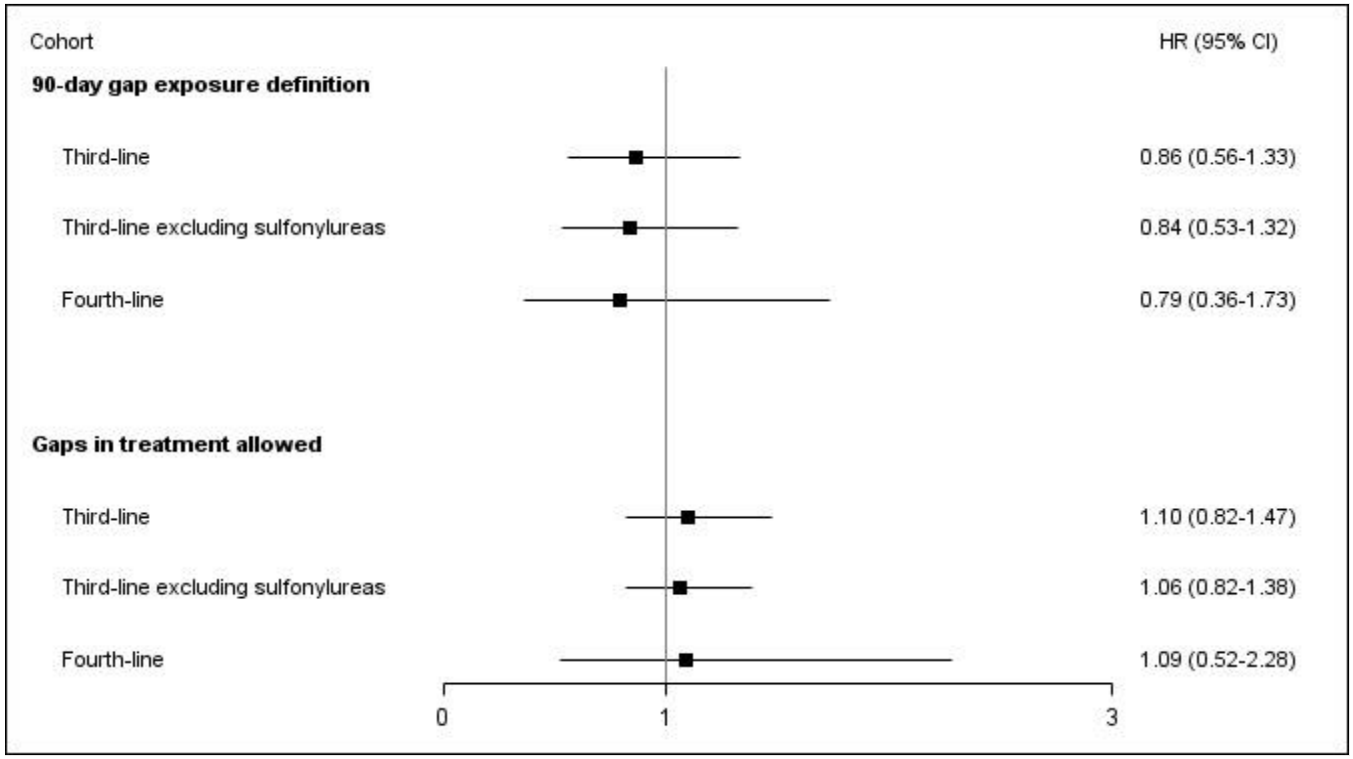


Figure 5.1A: Simple mediation diagram; a, b, and c' are crude regression coefficients with P values.

A

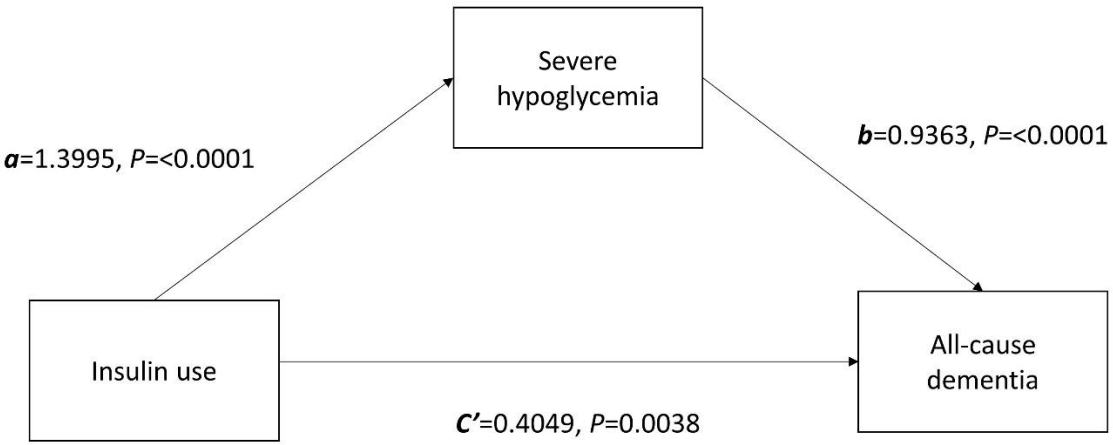
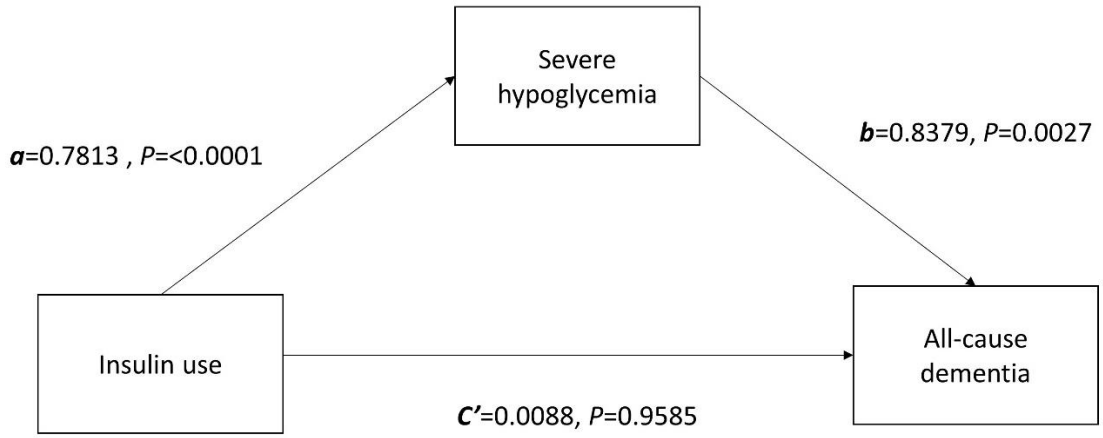


Figure 5.1B: Simple mediation diagram; a, b, and c' are weighted regression coefficients with P values.

B



Appendix A

Supplementary tables

Supplementary Table S2.1: Diagnostic codes used to capture hypoglycemia and dementia

Condition	ICD 9 code	ICD 10 codes
Hypoglycemia	251.0 Hypoglycemic coma 251.1 Other specified hypoglycemia 251.2 Hypoglycemia, unspecified	E16.0 Drug-induced hypoglycemia E16.1 Other hypoglycaemia E16.2 Hypoglycaemia, unspecified
Dementia	290.0 Senile dementia, uncomplicated 290.1 Presenile dementia 290.2 Senile dementia with delusional or depressive features 290.3 Senile dementia with delirium 290.4 Vascular dementia 331.0 Alzheimer disease 331.1 Frontotemporal dementia 331.5 Idiopathic normal pressure hydrocephalus 331.82 Dementia with lewy bodies	F00 Dementia in Alzheimer disease F01 Vascular dementia F02 Dementia in other diseases classified elsewhere F03 Unspecified dementia G30 Alzheimer's disease

Supplementary Table S3.1: Diagnostic codes used to capture hypoglycemia and dementia

Condition	ICD 9 code	ICD 10 codes
Hypoglycemia (primary definition)	251.0 Hypoglycemic coma 251.1 Other specified hypoglycemia 251.2 Hypoglycemia, unspecified	E16.0 Drug-induced hypoglycemia E16.1 Other hypoglycaemia E16.2 Hypoglycaemia, unspecified
Hypoglycemia (broad definition)	251.0 Hypoglycemic coma 251.1 Other specified hypoglycemia 251.2 Hypoglycemia, unspecified	E15 Non-diabetic hypoglycemia E11.63 Type 2 diabetes with hypoglycemia E13.63 Other specified diabetes with hypoglycemia E14.63 Unspecified diabetes with hypoglycemia E16.0 Drug-induced hypoglycemia E16.1 Other hypoglycaemia E16.2 Hypoglycaemia, unspecified
Dementia	290.0 Senile dementia, uncomplicated 290.1 Presenile dementia 290.2 Senile dementia with delusional or depressive features	F00 Dementia in Alzheimer disease F01 Vascular dementia F02 Dementia in other diseases classified elsewhere F03 Unspecified dementia G30 Alzheimer's disease

	290.3 Senile dementia with delirium 290.4 Vascular dementia 331.0 Alzheimer disease 331.1 Frontotemporal dementia 331.5 Idiopathic normal pressure hydrocephalus 331.82 Dementia with lewy bodies	

Appendix B
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Author List: Wajd Alkabbani, Colleen J Maxwell, Ruth Ann Marrie, Suzanne L Tyas, Iliana C Lega, John-Michael Gamble

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