Long-acting insulin analogues and the risk of diabetic retinopathy among patients with type 2 diabetes

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ABSTRACT

Type 2 diabetes (T2D) is chronic metabolic condition characterized by elevated blood sugar levels. As a result of the obesity epidemic, sedentary lifestyles, and poor dietary habits, its prevalence has grown substantially over the last several decades. Basal insulins, including long-acting insulin analogues and Neutral Protamine Hagedorn (NPH) insulin, are among the few effective therapeutic options available to manage the glycemia of patients with advanced, longstanding T2D or of patients with T2D and advanced chronic kidney disease. While long-acting insulin analogues are effective for glycemic control, concerns about a possible link between their use and the risk of diabetic retinopathy (DR) exist. These concerns first surfaced during the registration trials for glargine, one of the most widely used long-acting insulin analogues. However, little real-world evidence regarding this potential drug safety issue exists. Our objective was to determine whether the use of long-acting insulin analogues, compared to the use of NPH insulin, was associated with an increased risk of DR incidence among patients with T2D.

We conducted a retrospective, population-based cohort study of adults with T2D initiating either NPH insulin or a long-acting analogue insulin (glargine, detemir, degludec). The study was conducted using data from the Clinical Practice Research Datalink (CPRD) Aurum, a UK database that contains the electronic health records of 738 general practitioner practices, which were linked to Hospital Episode Statistics Admitted Patient Care and Office of National Statistics death registration data. The date of first prescription for any long-acting analogue insulin or NPH insulin defined study cohort entry. Exposure was defined using an as-treated approach in which patients were followed until an event or censoring due to drug discontinuation (defined by a treatment gap >60 days), insulin switch, death, departure from the CPRD, or end of the study period, whichever occurred first. The primary outcome was incident DR, and secondary outcomes were proliferative and non-proliferative DR. We estimated hazard ratios (HR) and 95% confidence intervals (CI) of incident DR for long-acting insulin analogues versus NPH insulin using Cox proportional hazards models with inverse probability of treatment weighting (IPTW) using propensity scores. Molecule-specific associations were examined in secondary analyses.

There were 66,280 users of insulin analogues and 66,173 users of NPH insulin. The groups were well balanced following IPTW. The median durations of follow-up time were 182 and 179 days,

respectively. In the primary analysis, the incidence rate of DR was 101.7 per 1000 person-years (95% CI, 98.7-104.8) for insulin analogues and 93.20 (95% CI, 90.03-96.46) per 1000 person-years for NPH insulin. The adjusted HR for DR with insulin analogues vs NPH insulin was 1.04 (95% CI, 0.99-1.09). The adjusted HRs were 0.84 (95% CI, 0.66-1.07) for proliferative DR and 1.02 (95% CI, 0.97-1.08) for non-proliferative DR. The molecule-specific analysis revealed no increased risks of incident DR for glargine (adjusted HR 1.04, 95% CI 0.99-1.09) or detemir (adjusted HR 1.01, 95% CI 0.93-1.09). The adjusted HR for degludec was 0.97 (95% CI, 0.63-1.50).

Compared with NPH insulin, long-acting insulin analogues were not associated with an increased risk of incident DR. Similar findings were observed in molecule-specific analyses. This thesis provides important reassurance regarding the safety of long-acting insulin analogues with respect to incident DR among patients with T2D.

RÉSUMÉ

Le diabète de type 2 (DT2) est une condition métabolique chronique caractérisée par un taux sanguin de sucre élevé. À cause de l'épidémie d'obésité, de modes de vie sédentaires et de mauvaises habitudes alimentaires, sa prévalence a grandement augmenté au courant des dernières décennies. Les insulines basales, incluant les insulines analogues de longue action et l'insuline *Neutral Protamine Hagedorn* (NPH), sont parmi les rares options thérapeutiques de gestion de la glycémie disponibles pour les patients présentant une forme avancée de DT2 ou les patients avec DT2 ayant une insuffisance rénale chronique avancée. Bien que les insulines analogues de longue action soient efficaces pour le contrôle glycémique, des inquiétudes subsistent sur un lien possible entre les insulines analogues de longue action et la rétinopathie diabétique (RD). Ces inquiétudes sont d'abord apparues lors des essais cliniques d'enregistrement pour glargine, l'une des insulines analogues de longue action les plus utilisées au monde. Toutefois, peu d'évidence tiré du monde réel existe concernant ce potentiel problème de sûreté pharmacologique. L'objectif de notre mémoire était de déterminer si l'utilisation des insulines analogues de longue action est associée avec un risque accru de RD chez les patients avec DT2.

Nous avons créé une étude de cohorte rétrospective basée sur la population portant sur des adultes avec DT2 initiant soit l'insuline NPH, soit une insuline analogue de longue action (glargine, détémir ou dégludec). L'étude a fait usage de données provenant du *Clinical Practice Research Datalink* (CPRD) Aurum, une base de données britannique contenant les dossiers médicaux électroniques de 738 pratiques médicales généralistes, qui ont été relié aux données enregistrées du *Hospital Episode Statistics Admitted Patient care*, et aux données sur la mortalité de l'*Office of National Statistics*. La date de la première prescription pour une insuline analogue de longue action ou pour l'insuline NPH définissait l'entrée dans la cohorte. L'exposition était définit par une approche 'tel que traité', dans laquelle les patients étaient suivis jusqu'à ce que se produise soit l'observation d'un événement, soit la censure due à l'arrêt de l'insuline (définit par un saut de traitement de plus de 60 jours), au changement vers l'autre type d'insuline, la sortie du CPRD, ou la fin de la période d'étude. L'issue primaire était l'incidence de la RD, et les issues secondaires étaient la RD proliférative et la RD non-proliférative. Nous avons estimé des rapports de risque (RR) et des intervalles de confiance (IC) à 95% pour l'incidence de la RD pour les insulines analogues de longue action versus l'insuline NPH à l'aide de modèles de risque proportionnel de

type Cox avec la pondération du traitement selon la probabilité inverse (PTPI) et des scores de propensité. Les associations pour molécules spécifiques d'insulines analogues ont été déterminées dans les analyses secondaires.

Il y avait 66 280 utilisateurs des insulines analogues et 66 173 utilisateurs de l'insuline NPH. Les temps médians de suivi étaient de 182 et 179 jours, respectivement. Les cohortes étaient équilibrées après la PTPI. Dans l'analyse primaire, le taux d'incidence de la RD était de 101,70 par 1000 personne-années (intervalle de confiance (IC) à 95%, 98,71-104,76) pour les insulines analogues et de 93,20 (IC 95% 90,02-96,46) par 1000 personne-années pour l'insuline NPH. Le RR ajusté pour la RD avec les insulines analogues vs l'insuline NPH était de 1,04 (IC 95% 0,99-1,09). Le RR ajusté était de 0,84 (IC 95%, 90,03-96,46) pour la RD proliférative et de 1,02 (IC 95%, 0,97-1,08) pour la RD non-proliférative. L'analyse pour les molécules spécifiques n'a pas révélé d'augmentation du risque d'incidence de RD ni pour glargine (RR ajusté 1,04, IC 95% 0.99-1.09), ni pour détémir (RR ajusté 1,01, IC 95% 0,93-1,09). Le RR ajusté de dégludec était de 0,97 (IC 95% 0,63-1,50).

Comparées à l'utilisation de l'insuline NPH, les insulines analogues longue action n'ont pas de risque accru d'incidence de la RD chez les patients avec DT2. Des résultats similaires ont été observés dans les analyses pour les molécules spécifiques. Cette thèse apporte de la réassurance concernant la sécurité des insulines analogues longue action en lien avec l'incidence de la RD chez les patients avec DT2.

CONTRIBUTIONS OF AUTHORS

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I developed the main thesis question and the protocol, which was submitted to the Independent Scientific Advisory Committee (ISAC) of the Clinical Practice Research Datalink (CPRD). I defined variables and contributed to the study design and statistical analysis. Finally, I wrote the thesis.

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Dr. Filion supervised the study and thesis. He provided precious advice on and contributed to study design, protocol preparation, statistical analysis, and result interpretation. He reviewed the protocol and thesis for important intellectual content.

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Mr Filliter conducted the data management and analyses. He also provided insight into results interpretation and reviewed the manuscript for important intellectual content.

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ABBREVIATIONS

ADA	American Diabetes Association
APC	Admitted Patient Care
BMI	Body Mass Index
BNF	British National Formulary
CAD	Coronary artery disease
CKD	Chronic kidney disease
CPRD	Clinical Practice Research Datalink
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl peptidase-4
DR	Diabetic retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
GLP1-RA	Glucagon-like peptide-1 receptor agonist
HbA1c	Hemoglobin A1c
HES	Hospital Episode Statistics
HIV	Human immunodeficiency virus
IGF-1	Insulin-like Growth Factor-1
IPTW	Inverse probability of treatment weighting
IQR	Interquartile range
ITT	Intention-to-treat
MI	Myocardial infarction
MODY	Monogenic diabetes of the young
mTOR	mechanistic target of rapamycin
NPDR	Non-proliferative diabetic retinopathy
ONS	Office for National Statistics
OR	Odds ratio
PDR	Proliferative diabetic retinopathy

PS	Propensity score
RCT	Randomized controlled trial
RR	Risk Ratio
SD	Standard deviation
SGLT2	Sodium glucose transport protein 2
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TZD	Thiazolidinedione
UK	United Kingdom
VEGF	Vascular Endothelial Growth Factor

CHAPTER 1: BACKGROUND

1.1 Type 2 diabetes

1.1.1 Definition of type 2 diabetes

Diabetes is a disease of glucose metabolism caused by defects in insulin secretion, insulin action, or both, leading to chronic hyperglycemia (i.e., elevated blood glucose levels) (1). Insulin is a peptide hormone secreted by the beta-cells of the pancreas that promotes entry of glucose into the cells of the body by attaching itself to its membrane receptor (2, 3). Diabetes is classified by Diabetes Canada and the American Association of Diabetes (ADA) into 4 groups: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes, and diabetes caused by various other specific causes (1, 4).

T1D is caused by autoimmune destruction of the pancreatic beta-cells, leading to a complete absence of insulin secretion (1, 4). T2D, representing approximately 90% of patients with diabetes, is a polygenic disease caused by a mix of insulin resistance at the cellular level, increased production of glucose by the liver, and non-autoimmune, slowly progressive pancreatic beta-cell destruction that leads to insulin secretion deficiency (1, 4, 5). The relative importance of each of those pathophysiological mechanisms varies from person to person and with time as T2D progresses (1, 6). Gestational diabetes usually manifests itself in the second or third trimester of pregnancy and is predominantly caused by insulin resistance driven by anti-insulin action hormones secreted by the placenta (1, 4, 7). Finally, diabetes caused by various other specific causes includes a heterogenous group of diseases that include diabetes as one of their clinical manifestations (1, 4). This group includes, but is not limited to, cystic fibrosis-related diabetes, monogenic diabetes of the young (MODY), diabetes induced by medications, hemochromatosis and Cushing's syndrome, and diabetes secondary to chronic pancreatitis (i.e., extensive disease of the exocrine pancreas) (1, 4). T1D, T2D, and diabetes with specific causes can all lead to end-organ damage (1), which I describe in **Sections 1.1.5** and **1.1.6**. This thesis examines the use of insulin analogues among patients with T2D. Consequently, the rest of this thesis will focus on T2D.

1.1.2 Epidemiology of type 2 diabetes

T2D is a growing epidemiological concern worldwide, due in part to the global obesity epidemic and the increased life expectancy in many countries (8). According to the International Federation of Diabetes, the prevalence of diabetes has more than tripled in the last 20 years, reaching 463 million people worldwide in 2019 (8). This represents 9.3% of the global population, and this number is expected to rise to 578 million by 2030. This trend is especially worrisome in the context of an increasing prevalence of T2D among young adults (8, 9).

In Canada, the prevalence of diabetes across all age groups was 8.1% in 2013-2014 (10). However, the prevalence varies greatly by age, reaching 25.4% of men and 19.1% of women aged 65-69 years (10). The overall incidence of diabetes is 7.6 per 1000 among Canadian adults (10). Data from the Public Health Agency of Canada also indicate that while the age-standardized prevalence of diagnosed diabetes increased from 5.6% to 7.8% between 2003-2004 and 2013-2014, the incidence rate fluctuated over time (10). Thus, while Canada is not immune to the global trend of increased incidence rates of diabetes, its increasing prevalence is also due to the increasing life expectancy of people living with diabetes. In the UK, the source population of this thesis, the prevalence of T2D rose from 3.2% to 5.3% between 2004 and 2014, while overall mortality rates declined for both individuals living with T2D and those without diabetes (9).

1.1.3 Risk factors for type 2 diabetes

T2D is a multifactorial disease involving both genetic and environmental factors. The risk factors for T2D are summarized in **Table 1.1** and are discussed in more detail below.

Some (but not all) patients have a genetic predisposition to developing T2D. It is a polygenic disease: some genetic variants seem to predispose individuals to impaired insulin secretion, while others lead to the development of obesity, ectopic fat deposition, or insulin resistance.(5) A combination of many T2D-promoting single-nucleotide polymorphisms with environmental factors substantially increases the risk of developing T2D (5).

Non-white ethnicity is a multifactorial determinant of T2D, where genetic factors, differences in ectopic fat deposition between ethnic groups, environmental risk factors, and access to health care all interact to create large disparities in T2D incidence, prevalence, and outcomes between racial/ethnic groups (5, 11, 12). For example, Black Caribbean and South Asian people living in the UK have a T2D prevalence that is approximately twice that of the general UK

population, while Asian Indians living in Western countries have a prevalence that is approximately four times that of Asian Indians living in India (11). These examples are a testament to the multifactorial nature of ethnicity as a risk factor for developing T2D and its complications.

Gestational diabetes can lead to changes in insulin sensitivity and alter beta-cell function, increasing a woman's long-term risk of developing T2D up to 10-fold (13-16). In addition, established T2D during pregnancy can increase the offspring's risk of future T2D through epigenetic changes (i.e., altered patterns of DNA methylation) caused by very early in-utero exposure to hyperglycemia (17, 18). Maternal obesity and excessive gestational weight gain are associated with intrauterine overnutrition of the fetus, leading to an increased risk of both obesity and T2D in the offspring (18-20). Interestingly, excessive maternal caloric restriction during pregnancy, such as that observed during recent famines, is also associated with increased incidence of T2D in the offspring (21-23).

There are many modifiable risk factors for T2D. Obesity is traditionally defined in epidemiological studies using the World Health Organization (WHO) criteria using body mass index (BMI): obesity corresponds to a BMI $\geq$ 30 kg/m^2 and overweight to a BMI $\geq$ 25 kg/m^2. Obesity, however, is a complex modifiable risk factor; ectopic fat distribution in the liver, pancreas, muscles, and around the heart (i.e., visceral fat) is more strongly associated with an increased incidence of T2D than a high BMI (24, 25). Specific dietary patterns (e.g., consumption of sugar-sweetened beverages) are independently associated with T2D (26). On the other hand, consumption of nuts, plant-based diets, and the Mediterranean diet are associated with a decreased risk of T2D (26, 27). A sedentary lifestyle is independently associated with T2D, while increased physical activity levels are protective, despite their small effect on weight loss (24, 28). Sleep duration has a U-shaped, dose-response relationship with incident T2D, with the lowest risk of T2D associated with 7 to 8 hours of sleep per day (29). In addition, sleep disruption can alter insulin action and hormonal hunger signals, and obstructive sleep apnea, which fragments sleep, is an independent risk factor for T2D (5).

The gut microbiome is the mass of bacteria residing in our digestive tract, most of which are commensal (neutral on health) or beneficial to us (5). The gut microbiome contributes to the barrier function of the intestine and the breakdown of certain indigestible food components, interacts with the immune system, and metabolizes various fatty acids and amino acids; all these tasks have a downstream effect on weight gain and on an individual's level of insulin resistance

and thus, their risk of developing T2D (5, 30, 31). Finally, **Table 1** summarizes the various medications associated with an increased risk of developing T2D.

Table 1.1 Risk factors for T2D

Risk factor category	Risk factor	Strength of risk factor
Genetic factors	Family history of T2D	
Age		
Ethnic group	Native American	2-fold vs non-Hispanic White Americans (5)
	African and Caribbean descent (in Western countries)	Up to 12-fold vs indigenous Africans (11)
	South Asian (immigrants in Western countries)	Up to 4-fold vs indigenous Asian Indians (11)
	Hispanic American (5)	
Epigenetics and fetal exposure	Maternal diabetes (17, 18)	
	Intrauterine undernutrition (21-23)	
	Intrauterine overnutrition (18-20)	
	Maternal obesity (19, 20)	
Personal history	Gestational diabetes (13-16)	Risk up to 10-fold (13-16)
Modifiable risk factors	Obesity (BMI $\geq$ 30 kg/m^2) or overweight (BMI $\geq$ 25 kg/m^2) (5)	

	Diet (26, 27)
	Sedentary lifestyle (24, 28)
	Altered sleep patterns (5)
	Chronic stress (32, 33)
	Smoking (34, 35)
Gut microbiome alterations (36)	
Medications (5, 37)	Glucocorticoids
	Atypical antipsychotics
	Calcineurin inhibitors (tacrolimus, cyclosporine)
	mTOR inhibitors (sirolimus, everolimus)
	Phosphoinositide-3-kinase inhibitors
	Statins
	Niacin
	Thiazides
	Beta-blockers
	HIV antiretroviral therapy (protease inhibitors)

Adapted from Kahn et al, 2020 (5)
Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; mTOR, mechanistic target of rapamycin.

1.1.4 Identification of type 2 diabetes

The ADA and Diabetes Canada propose 4 ways of screening for diabetes. The first is measuring the percentage of glycated hemoglobin (called the HbA1c or A1c), an indirect measure of the average blood glucose over the previous 3 months (1, 4). A1c screening test is considered positive for a diagnosis of diabetes if the result is 6.5% or above. The second is measuring a fasting blood glucose level (positive if 7.0 mmol/L and above). The third is measuring the blood glucose level at random (positive if 11.1 mmol/L and above and if the patient presents symptoms typical of hyperglycemia). The fourth is performing an oral glucose tolerance test (OGTT) with 75 g of glucose and measuring the blood glucose level 2 hours post ingestion (positive if blood glucose of 11.1 mmol/L and above).(1, 4) Once a screening test is positive, the same test or another test is usually repeated to confirm the diagnosis, except if the initial test was a random blood glucose with a clear history of symptomatic hyperglycemia (1, 4). Symptoms of hyperglycemia usually appear at a threshold of 10 to 12 mmol/L, a level at which the kidneys are no longer able to reabsorb the glucose that it filters, leading to glycosuria (glucose in the urine)(38). This, in turn, leads to polyuria (urinating frequently and in large quantities), polydipsia (frequent thirst), dehydration, polyphagia (increased food intake from intense hunger), weight loss, and fatigue (38). The diagnosis of diabetes type (1, 2, gestational, other) can be more complex and requires a combination of auto-antibodies blood tests and a clinical assessment that considers the presence or absence of various risk factors for T2D (1).

1.1.5 Diabetes complications

Diagnosing and treating diabetes is important not only to avoid symptoms of hyperglycemia but also to prevent the development of the complications of diabetes. The complications of diabetes are categorized as either macrovascular or microvascular. Macrovascular complications include coronary artery disease (CAD), cerebrovascular accidents and peripheral vascular disease; they are caused by both the intracellular effects of hyperglycemia and the insulin resistance that underlies T2D (39). Patients with diabetes are at much higher risk of developing cardiovascular disease (CVD) than individuals without diabetes: the former become high-risk for CVD (i.e., a 10-year risk of having a CVD event of 20% or more) almost 15 years earlier than the latter (40). In a UK cohort of 1.9 million patients, there was a strong association

between the presence of T2D and the occurrence of various CVD events, notably with peripheral arterial disease (adjusted hazard ratio (HR): 2.98, 95% confidence interval (CI) 2.76-3.22), ischemic stroke (adjusted HR: 1.72, 95% CI 1.52-1.95), stable angina (adjusted HR: 1.62, 95% CI 1.49-1.77), non-fatal myocardial infarction (adjusted HR: 1.54, 95% CI 1.42-1.67), and heart failure (adjusted HR: 1.56, 95% CI 1.45-1.69) (41). Intensive glycemic control among T2D patients has been shown to decrease the risk of macrovascular complications (42-47). Intensive glycemic control is defined and discussed in more detail in **Section 1.4**.

1.1.6 Microvascular complications of diabetes

Microvascular complications include retinopathy, nephropathy, and neuropathy and are caused by chronic hyperglycemia (39). Diabetic retinopathy (DR) is one of the main ocular complications of diabetes along with cataracts (discussed in detail in **Section 1.3**). Diabetic nephropathy is a form of kidney damage caused specifically by diabetes, characterized by the progression (of variable length) from microalbuminuria (small albumin leakage into the urine, up to 30 mg/day or 2.0 mg/mmol) to macroalbuminuria (300 mg/day or 20.0 mg/mmol) and chronic kidney disease (CKD) with a decreased glomerular filtration rate (i.e., decreased kidney function) (48). It is a leading cause of CKD in Canada and industrialized countries (48). Prevalent DR is a predictor of diabetic nephropathy (49). Diabetic neuropathy is diabetes-induced damage of the peripheral nervous system; the most common form is distal symmetric polyneuropathy, where tingling, numbness, and sometimes neuropathic pain affect the limbs' extremities first ('stocking and glove' distribution) and progress in a centripetal or proximal fashion (50). Diabetic neuropathy affects more than half of all patients living with diabetes (50). It is a well-recognized risk factor for foot ulcer and lower extremity amputations (51).

The intracellular mechanisms of hyperglycemia in microvascular complications are complex, but studies in both humans and animal models show a strong correlation between duration and extent of hyperglycemia and the rate of progression and degree of damage of DR, nephropathy, and neuropathy (39, 46, 47). In microvascular complications, hyperglycemia leads to an increase in intracellular glucose concentration in the endothelial cells that line the interior of the small arterial vessels of the eyes, kidneys, and nerves, making them dysfunctional (39). On the other hand, intensive glycemic control among T2D patients has been shown to decrease the risks

of retinopathy and nephropathy, as well as microvascular complications as a composite outcome (42-47).

1.1.7 Glycemic control among patients with type 2 diabetes

The management of T2D has two main goals: preventing symptomatic hyperglycemia and the long-term complications of chronic hyperglycemia. Glycemic control, which is necessary for both goals, is achieved with lifestyle changes (i.e., changes in diet, increased physical activity, and weight loss of at least 5-10% of body weight for overweight and obese patients), use of non-insulin antihyperglycemic medications, and insulin therapy (52, 53). Typically, patients with T2D will first initiate lifestyle changes alone or in combination with oral antihyperglycemic medications (52, 53). A list of the commonly used classes of antihyperglycemic medications and their characteristics is presented in **Table 1.2**.

A subset of patients with T2D eventually require insulin therapy to manage their disease: when antihyperglycemic medications are not enough or are no longer effective in reaching glycemic targets or when CKD has made the use of most oral medications either restricted in dosage or contraindicated (52). According to NHS data, 12.5% of patients diagnosed with T2D require insulin (54). Insulin treatments will be discussed in more detail in the next section. **Figure 1.1** describes the typical management algorithm for T2D in Canada.

Independent of the type of treatment used for glycemic control, patients with T2D and their physicians must monitor glycemic levels to assess the therapy's effectiveness. For physicians, this requires measuring periodically HbA1c level. For most patients with T2D, the target HbA1c is < 7.0%, which is approximately equivalent to a mean blood glucose of 8.6 mmol/L over 3 months (55). To achieve this target, patients must follow their management plan and measure their capillary blood glucose with a glucometer, aiming for an fasting blood glucose between 4.0 and 7.0 mmol/L and 2-hour post-prandial blood glucose between 5.0 and 10.0 mmol/L (55). The pharmacotherapy or insulin regimen is typically augmented after 3 to 6 months if the target is not reached (53).

Table 1.2 Antihyperglycemic agents used in T2D and their characteristics.

Class and mechanism of action	Drug	Cost*	A1C lowering†	Weight†	Cautions	Other therapeutic considerations
Biguanide: Enhances insulin sensitivity in liver and peripheral tissues by activation of AMP-activated protein kinase	Metformin Metformin extended-release	$ $$	Approx. 1.0%††	Neutral	Use lower dose if eGFR <60ml/min/1.73m^2 Do not initiate if eGFR is <30ml/min/1.73m^2 GI side effects	Hold during acute illnesses associated with risk for dehydration or procedures associated with high risk of acute kidney injury Provide education regarding sick day management (Appendix 8: 2018 CPG) Can reduce vitamin B_{12} absorption
Incretin: Increases glucose dependent insulin release, slows gastric emptying, inhibits glucagon release	**GLP1-RA** Short-acting Exenatide Lixisenatide Longer-acting Dulaglutide Exenatide extended-release Liraglutide Semaglutide	$$$$	0.6-1.4%	Loss of 1.1- 4.4 kg	GI side effects Monitor retinopathy (especially if pre-existing retinopathy) because of risk of progression with rapid drops in A1C Contraindicated with personal/family history of medullary thyroid cancer or multiple endocrine neoplasia syndrome type 2 Caution with a history of pancreatitis or pancreatic cancer	Less A1C reduction with short-acting agents No proven CV benefit with lixisenatide or short-acting exenatide
	DPP4i Alogliptin Linagliptin Saxagliptin Sitagliptin	$$$	0.5-0.7%	Neutral	Risk of heart failure with saxagliptin Caution with a history of pancreatitis or pancreatic cancer	Rare cases of pancreatitis Rare cases of severe joint pain
SGLT2i: Reduces glucose reabsorption by the kidney	Canagliflozin Dapagliflozin Empagliflozin *Ertugliflozin****	$$$	0.5-0.7%	Loss of 2-3 kg	Genital mycotic infections Urinary tract infections Hypotension Rare cases of diabetic ketoacidosis (which may occur without hyperglycemia) Caution required when combined with low carbohydrate eating patterns or with suspected insulin deficiency Good foot care always recommended – particularly in those with high-risk feet (loss of protective sensation, previous foot ulcer or amputation) Dapagliflozin contraindicated with bladder cancer	Less glycemic efficacy at lower GFR Do not initiate if eGFR is <30ml/min/1.73m^2 Hold prior to major surgery or during serious illness or infections Hold during acute illnesses associated with risk for dehydration or procedures associated with high risk of acute kidney injury Provide education regarding sick day management (Appendix 8: 2018 CPG) Small reduction in eGFR (<20%) expected when initiated Only CV safety shown for ertugliflozin
Alpha-glucosidase inhibitor: Inhibits pancreatic α-amylase and intestinal α-glucosidase	Acarbose	$$	0.7-0.8%†††	Neutral	GI side effects common	Requires 3 times daily dosing

Class and mechanism of action	Drug	Cost*	A1C lowering†	Weight†	Cautions	Other therapeutic considerations
Insulin: Activates insulin receptors to regulate metabolism of carbohydrate, fat and protein	**Bolus (prandial) Insulins** Rapid-acting analogues Aspart Aspart (faster-acting) Glulisine Lispro U-100 Lispro U-200 Short-acting Regular **Basal Insulins** Intermediate-acting NPH Long-acting analogues Degludec U-100 Degludec U-200 Detemir Glargine U-100 Glargine U-300 **Premixed Insulins** Premixed regular-NPH Biphasic insulin aspart Lispro/lispro protamine suspension **Other** Concentrated U-500 regular	$-$$$$ (depending on agent and dose)	0.9-1.2% or more	Gain Gain of 1.0-2.0 kg Gain of 2.0-3.5 kg Gain	Education required regarding • blood glucose monitoring • preventing, detecting and treating hypoglycemia Numerous formulations and delivery systems • increases complexity and risk for errors	Potentially greatest A1C reduction and (theoretically) no maximum dose Dose escalation may be limited by hypoglycemia Numerous formulations and delivery systems • allows for regimen flexibility Recommended in individuals taking >200 units basal insulin per day by 4 or more injections** Used 2 or 3 times daily instead of basal insulin
	Insulin/GLP1 fixed-ratio combinations Degludec/liraglutide Glargine/lixisenatide	$$$-$$$$		Neutral		Can mitigate weight gain seen with initiation or intensification of basal insulin Maximum dose of insulin 50 units for degludec and liraglutide or 60 units for glargine and lixisenatide
Insulin secretagogue: Activates sulfonylurea receptor on β-cell to stimulate endogenous insulin secretion	**Sulfonylureas** Gliclazide Gliclazide-modified release Glimepiride Glyburide	$	0.6-1.2%	Gain of 1.2-3.2 kg	Higher risk of hypoglycemia with glyburide Risk of hypoglycemia increased with fasting or with eGFR <60ml/min/1.73m^2 Provide education regarding sick day management (Appendix 8: 2018 CPG)	Glycemic control is relatively rapid but may not be durable Gliclazide preferred over glyburide due to lower risk of hypoglycemia Glimepiride showed CV safety similar to DPP4 (linagliptin) in CAROLINA trial

Class and mechanism of action	Drug	Cost*	A1C lowering†	Weight†	Cautions	Other therapeutic considerations
	Meglitinides Repaglinide	$$	0.7-1.1%	Gain of 1.4-3.3 kg	Repaglinide contraindicated when coadministered with clopidogrel or with gemfibrozil	Useful to reduce postprandial hyperglycemia Requires dosing with each meal (e.g. 3 times daily) Lower risk for hypoglycemia than sulfonylureas in renal impairment
Thiazolidinedione: Enhances peripheral and hepatic insulin sensitivity by activation of peroxisome proliferator activated receptor-gamma receptors	Pioglitazone Rosiglitazone	$$$	0.7-0.9%	Gain of 2.0-2.5 kg	Greater weight gain in some individuals May induce edema and/or congestive heart failure	Durable glycemic control Rare occurrence of macular edema • Higher occurrence of fractures • Pioglitazone not to be used with bladder cancer • Uncertainty about CV safety with rosiglitazone, suggestion of increased risk of MI

Agents are listed in alphabetical order.

* Estimated costs based on recommended daily doses (from DiPiro Pharmacotherapy: A Pathophysiologic Approach, 11th edition) and reviewing provincial formulary costs of generic agents (if available) from AB and ON. Where costs differed between provinces, the higher cost was used. $ = less than 50¢ per day, $$ = 50¢ to $1 per day, $$$ = $1 to $4 per day and $$$$ = >$4 per day.

† Values are the min and max point estimates from 3 meta or network metanalyses (26,27,47). It does not represent the range of responses in treated populations. Large variations between individuals in degree of weight gain can be seen with insulin and thiazolidinediones.

†† Glycated hemoglobin (A1C) lowering vs placebo, Sherifali D, Nerenberg K, Pullenayegum E, Cheng JE, Gerstein HC. *Diabetes Care* 2010;33:1859–64.

††† Based on data from 2 trials in <100 patients.

** Hood RC, Arakaki RF, Wysham C, Li YG, Settles JA, Jackson JA. Two treatment approaches for human regular U-500 insulin in patients with type 2 diabetes not achieving adequate glycemic control on high-dose U-100 insulin therapy with or without oral agents: A randomized, titration-to-target clinical trial. *Endocr Pract* 2015;21:782–93.

*** VERTIS (cardiovascular [CV] outcome trial for ertugliflozin) presented at American Diabetes Association (ADA) June 2020 showed noninferiority for MACE. Manuscript not published at time of writing.

Approx. , approximately; *CPG* , clinical practice guidelines; *DPP4i* , dipeptidyl peptidase-4 inhibitors; *eGFR* , estimated glomerular filtration rate; *GI*, gastrointestinal; *GLP1-RA* , glucagon-like peptide-1 receptor agonists; *MI*, myocardial infarction; *SGLT2i* , sodium-glucose cotransporter 2 inhibitors.

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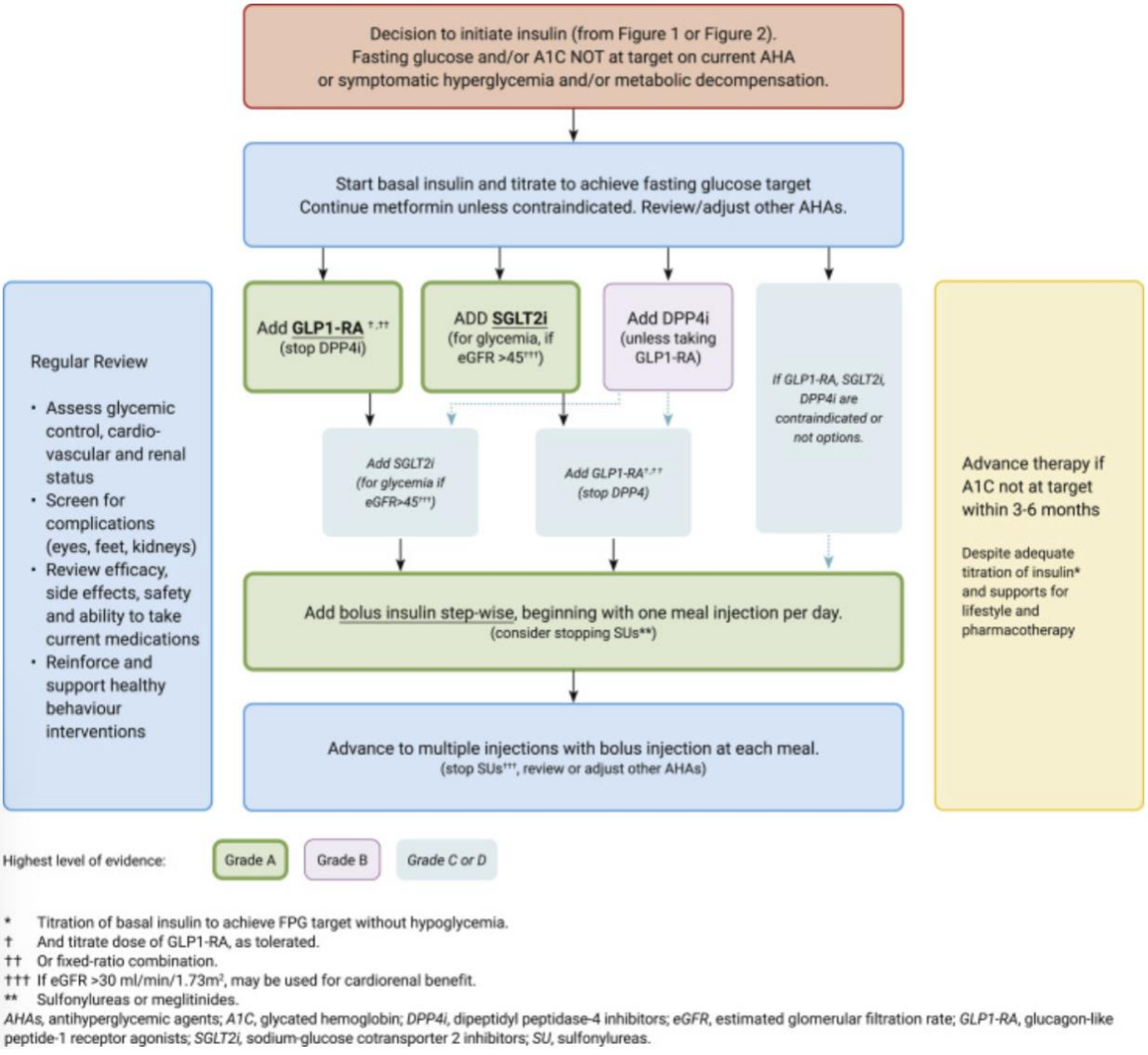
Figure 1.1 Management of T2D as per the Diabetes Canada guidelines: Pharmacologic Glycemic Management of T2D in Adults, 2020 Update.



* Titration of basal insulin to achieve FPG target without hypoglycemia.
† And titrate dose of GLP1-RA, as tolerated.
†† Or fixed-ratio combination.
††† If eGFR >30 ml/min/1.73m², may be used for cardiorenal benefit.
** Sulfonylureas or meglitinides.
AHAs, antihyperglycemic agents; *A1C*, glycated hemoglobin; *DPP4i*, dipeptidyl peptidase-4 inhibitors; *eGFR*, estimated glomerular filtration rate; *GLP1-RA*, glucagon-like peptide-1 receptor agonists; *SGLT2i*, sodium-glucose cotransporter 2 inhibitors; *SU*, sulfonylureas.

Reproduced with permission from Diabetes Canada 2020 Guidelines Update (56).

1.2 Insulin therapy

Exogenous insulin is one of the therapeutic options that can be used to control glycemia among patients with T2D. Insulin is a small protein hormone secreted by pancreatic beta cells that binds to its receptor on the cells of the liver, peripheral muscles, and adipose tissue (5). This binding activates intracellular signaling pathways, leading to the expression of the GLUT4 receptor on cells and increased glucose uptake, as well as increased glycolysis (glucose molecule breakdown) (5). The signaling pathways also lead (depending on the cell type) to the stimulation of lipogenesis (formation of adipose tissue) and protein synthesis and to the inhibition of glycogenolysis (breakdown of glycogen into glucose in the liver and muscles), lipolysis (breakdown of adipose tissue into free fatty acids), and protein catabolism (5).

As briefly discussed above, insulin is mostly used among patients with long-standing T2D that no longer responds to oral antihyperglycemic agents alone due to insufficient endogenous insulin secretion. It is also used in patients who develop contraindications to antihyperglycemic agents (most often severe CKD) (52). In those patients, insulin is one of the few remaining options that can be used effectively and safely to lower blood glucose levels (53). As shown in **Figure 1**, it can also be used transiently (for a few weeks or a few months) to treat patients with symptomatic hyperglycemia and metabolic decompensation (weight loss from chronic glycosuria and protein catabolism from lack of insulin) (5, 52, 53). The main side effects of insulin are hypoglycemia and weight gain (52).

Among patients with T2D, the first insulin introduced as part of the therapeutic regimen is usually a basal insulin, i.e., either an intermediate-acting (Neutral Protamine Hagedorn [NPH]) or long-acting insulin (such as the insulin analogues) (57). Basal insulin is usually given at bedtime and helps to control fasting blood glucose levels. If the addition of basal insulin to oral agents is insufficient to achieve glycemic control, short- or rapid-acting insulin can then be added to control post-prandial blood glucose levels (58). The different types of insulin are summarized in **Table 1.2**.

In its natural form, the insulin molecule is a protein composed of 51 amino acids arranged in 2 chains bound by disulfide bridges (59). Insulin was discovered in 1921 by Banting and Best in Toronto by injecting pancreatic extract into depancreatized dogs (60). Two years later, porcine and bovine sources of insulin were commercialized for human use; human insulin (created in a

laboratory) would only come decades later (60). Today, insulin is created by recombinant DNA techniques: the human or a modified human proinsulin gene is inserted into either a strain of Escherichia coli or Saccharomyces cerevisiae to synthetize proinsulin, which can then be used to form either short-acting or intermediate actin human insulin or to form rapid-acting or long-acting insulin analogues (59, 61).

1.2.1 Neutral Protamine Hagedorn insulin

NPH insulin is a human insulin that has been modified by the addition of protamine, which delays NPH insulin's absorption, onset of action, peak of action, and duration compared to unmodified human insulin (59, 61). NPH insulin comes in a neutral pH suspension (59). Despite these modifications, its pharmacokinetics can be problematic: it has a peak of action 5 to 8 hours after injection, which can lead to hypoglycemia, including nocturnal hypoglycemia when it is injected in the evening (62, 63). The action of NPH insulin is also variable due to various factors affecting its absorption in subcutaneous tissue and the risk of inadequate resuspension prior to injection (62). Its onset of action is 2 to 5 hours after injection, and its maximum duration varies from 12 to 18 hours, with larger doses having more delayed peaks of action and longer durations of action (59, 61). Regular human insulin, a short-acting human insulin modified by the addition of zinc to make it more stable, has a slower onset (61). It is in this context that insulin analogues, both rapid- and long-acting ones, were created and finally marketed almost 20 years ago (62).

1.2.2 Long-acting insulin analogues

Long-acting insulin analogues are created by the recombinant DNA techniques described above and then by modifying some of the amino acids of the insulin molecule (61). The glargine molecule, for example, is created by attaching 2 arginine amino acids to carboxyl terminal of one the insulin's chain and by substituting a glycine for an asparagine amino acid in a specific position of the insulin molecule (59). This new configuration makes glargine soluble in the low pH suspension in which it is dispensed and makes it precipitate into a slowly dissolving crystalline depot after its subcutaneous injection (59).

Glargine U100 (i.e., concentration of 100 units/ml) was first patented in 1988, and it was approved by the US Food and Drug Administration (FDA) for once a day injections among patients

with diabetes in 2000 (64). Other long-acting insulin analogues include detemir and degludec, first marketed in the UK in 2004 and 2013, respectively.

The long-acting basal insulin analogues have a more predictable pharmacokinetic profile than NPH and longer durations of action: 20 hours for detemir, 24 hours for glargine, and > 24 hours for both glargine U300 (i.e., concentration of 300 units/ml) and degludec. They also have minimal peaks of action (62, 65). Detemir can have a mild peak of action 7 to 14 hours post injection at higher doses, but this peak is much flatter than that of NPH (65). Glargine U100 has an even flatter peak of action 4 to 12 hours post injection (66). Glargine U300 and degludec have no discernable peaks and are often called ultra-long-acting insulin analogues due to their very long durations of action (65).

The efficacy and safety profiles of insulin analogues have been studied extensively in clinical trials. These previous studies have shown that insulin analogues have a lower risk of nocturnal hypoglycemia and possibly lead to less weight gain than NPH, while not providing a clear advantage in terms of glycemic control (62, 65, 67-70). However, safety outcomes concerning macrovascular complications have not been systematically assessed as primary outcomes in large trials, since insulins are exempt from the FDA's requirement for new T2D pharmacotherapies of demonstrating cardiovascular safety through dedicated cardiovascular outcome trials (71). Similarly, randomized controlled trials (RCTs) of insulins dedicated to microvascular outcomes are rare. In a systematic review (described in detail below) comparing the efficacy and safety of long-acting insulin analogues to NPH insulin, information on outcomes such as death from any cause or diabetic complications was "insufficient or lacking in almost all included trials" (72). The ORIGIN trial published in 2012 randomized over 12,000 patients aged 50 years or older with CVD risk factors plus either impaired fasting blood glucose, impaired glucose tolerance, or T2D to either glargine or "standard of care" (67). A total of 11% of patients in the standard of care group used insulin of any type. Both groups were treated to a target of reaching a normal fasting blood glucose. The co-primary outcomes were a composite endpoint of nonfatal myocardial infarction, nonfatal stroke, and death from CVD and a composite endpoint that included these events and coronary revascularization or hospitalization for heart failure. Secondary outcomes included microvascular events (not defined in detail), limb or digit amputation, incident cancer (any type), and death of any cause (67). No differences in the

cumulative incidence of any of these primary or secondary outcomes were observed over a median follow-up duration of 6.2 years (67).

The RCT DEVOTE, published in 2017, was a double-blind, treat-to-target trial assessing the CVD safety of degludec versus glargine U100 among patients with T2D (69). At baseline, the 7637 patients had a mean age of 65 years and a mean diabetes duration of over 16 years. They had poor glycemic control (mean A1c of 8.4 +/- 1.7%), had a high prevalence of comorbidities (e.g., 85.2% with established CVD or CKD), and 83.9% were already using insulin (69). The primary outcome was the first occurrence of a major adverse cardiovascular event (MACE, a composite endpoint that included death from CVD, non-fatal myocardial infarction, and non-fatal stroke), for which degludec was found to be non-inferior over 2 years of follow-up. Microvascular complications were not included as a secondary outcome, nor were they mentioned as a potential adverse event in the Supplementary Material (69).

Studies concerning DR and insulin analogues are described in detail below.

1.3 Diabetic retinopathy

DR is one of the main ocular complications of diabetes and a potentially sight-threatening microvascular complication of diabetes. The prevalence of DR in patients with newly-diagnosed T2D is estimated at 19.0% (95% CI 18.3-19.7%) in the UK, according to the most recent primary care data (73). This prevalence of DR soon after T2D diagnosis has fallen substantially since it was first measured in 1998 in the UKPDS study, where it was reported to be 37% (74). The prevalence of DR increases substantially as diabetes progresses; more than half of patients with T2D are diagnosed with DR after 20 to 25 years of disease history (75). Although the age-standardized prevalence of sight-threatening DR was estimated to be only 6.9% (95% CI 5.8-7.0) in a recent meta-analysis (76), due to the high prevalence of T2D itself, DR is a major cause of blindness in the developed world. Blindness of any cause is estimated to be 25 times more common among patients with T2D than among those without diabetes (77, 78). Rates of progression of DR to more advanced forms of the disease (e.g., from non-proliferative to proliferative DR) are decreasing in the developed world due to improvements in glycemic, medical, and ophthalmologic management (75, 78).

1.3.1 Diabetic retinopathy classification

DR is classified as either proliferative (PDR) or non-proliferative DR (NPDR), and NPDR can progress to PDR (75, 78). NPDR is usually characterized on fundoscopic exam by retinal hemorrhages, microaneurysms (small outpouchings in the retinal small vessels due to loss of retinal pericytes), cotton-wool spots (caused by microinfarcts of retinal nerve fiber layer), increased vascular permeability, venous and intraretinal microvascular abnormalities, and vascular closure (78). PDR is characterized by proliferation of new, but often fragile small vessels in the retina, including on the optic disc. This neovascularization can break down and cause hemorrhages (78). If these hemorrhages are extensive enough or implicate the optic disc, they can lead to vision loss (78). The prevalence of PDR among patients with T2D is less than 5% (75). Finally, diabetic macular edema is a DR event that can happen at any stage of this microvascular complication and is characterized by retinal thickening and sometimes hard exudates (accumulated lipid in the retina from increased permeability of the vessels) in the vicinity of the fovea (78). Diabetic macular edema that involves the center of the macula is more likely to cause vision loss (78, 79).

DR severity is typically measured with the well-validated Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale, using results from seven-field stereoscopic fundus photographs (80).

1.3.2 Risk factors for diabetic retinopathy

As with T2D itself, we can classify the many risk factors for developing DR as either non-modifiable or modifiable. Risk factors enumerated below refer to the risk of incident DR, unless specified as a risk factor for progression. Duration of diabetes itself, family history of DR, and genetic predisposition are all non-modifiable risk factors for incident DR (77). Being pregnant, on the other hand, is a risk factor of DR progression, both among patients with T1D and T2D (81, 82). Modifiable risk factors include poor glycemic control, high triglyceride levels, high LDL or non-HDL cholesterol levels, hypertension, smoking, and anemia (77, 83). Some RCTs and observational studies of glucagon-like peptide-1 receptor agonists (GLP1-RAs, a class of incretin-based drugs used as antihyperglycemic agents for diabetes) have suggested that they may increase the risk of DR progression, likely through a rapid correction of hyperglycemia, but results of other studies on this association have produced mixed results (84-86). Studies on rat models found that GLP1-RAs may protect against DR through its activation of a specific molecular pathway (87, 88).

1.3.3 Pathophysiology of diabetic retinopathy

In DR, hyperglycemia increases blood flow and intracapillary pressure in the retina; this, coupled with the endothelial cell dysfunction, leads to deterioration of the blood-retina barrier and fluorescein leakage (78). The hyperglycemia-induced small vessels hypertension and microvascular permeability also stimulate expression of genes that will in turn increase the production of growth factors, cytokines, and extracellular matrix components (78). This matrix accumulates in the small vessel walls and leads to their occlusion and to the destruction of cells essential to retinal function, such as Müller cells, ganglion cells, and pericytes (78). These lead to retinal ischemia, which stimulates the production of angiogenic growth factors that will promote the development of fragile new vessels and vessel permeability (78).

Insulin-like Growth Factor-1 (IGF1) and Vascular Endothelial Growth Factor (VEGF) are among the growth factors that are produced in this process (89-92). Paradoxically, the growth factor activity of IGF-1 and VEGF is also essential to develop and maintain the integrity of the initially normal retinal vasculature (93, 94). The insulin deficiency that occurs in diabetes leads to decreased hepatic synthesis of IFG-1 and an increase in its binding protein (95); this decreased bioavailability of IGF-1 contributes to the loss of pericytes, the initial process of DR (96, 97). However, the eventual retinal ischemia described above upregulates IGF-1 and VEGF pathways, a response that, in this abnormal milieu, leads to microaneurysms and to the weak, permeable neo-vasculature characteristic of established DR (98-102). Increased intra-ocular concentrations of IGF-1 also trigger the breakdown of the blood-retinal barrier (103). Finally, IGF-1 upregulates VEGF expression itself (104). There is accumulating evidence that some IGF-1 polymorphisms may be associated with the risk of developing DR (91, 105, 106), highlighting inter-individual variability of DR presentation.

1.3.4 Diabetic retinopathy and hyperglycemic memory

While intensive glycemic control can prevent or slow the progression of DR, microvascular damage can sometimes persist or progress even after hyperglycemia has been adequately treated. This phenomenon has been termed hyperglycemic memory or metabolic memory (78, 107). Hyperglycemia, even when transient, creates an inflammatory milieu at the intracellular level and increases reactive oxygen species levels that induce epigenetic changes that can last years after the

hyperglycemia has been corrected, increasing the risk of incident microvascular complications (78, 107).

The concept of metabolic memory has been used to explain the slower progression of microvascular complications among patients with T1D who were previously treated intensively (target A1c of 6.05% or less, compared to those previously treated to conventional targets) in the Epidemiology of Diabetes Interventions and Complications (EDIC) study (108). This study was a 4-year follow-up study of the Diabetes Control and Complications Trial (DCCT), the difference in A1c between both arms narrowed substantially during follow-up (108). In the DCCT study, patients in the intensive treatment arm received 3 or more injections of insulin daily and were treated to target an A1c of 6.05% or less, while patients in the conventional treatment arm were treated with one or two injections of insulin daily, with the aim of avoiding symptomatic hyperglycemia, ketonuria, and hypoglycemia (46). The intensive and conventional arms achieved mean A1c levels of 7.2 and 9.1%, respectively, over a mean follow-up duration of 6.5 years (range 3-9 years). The DCCT trial found that the incident DR and progression of DR among T1D patients randomized to intensive therapy was lower than for patients randomized with conventional therapy (46, 109). The odds ratio (OR) for early worsening of DR at either the 6- month or 12-month visit was (2.06, 95% CI 1.42-2.99) (110). At the end of the DCCT study, patients in the conventional arm were offered intensive therapy. The observational add-on study (EDIC) found that, after 4 additional years of follow-up, the difference in A1c between the groups narrowed (median A1c of 8.2% and 7.9% [$p<0.001$], respectively)(108). However, the decreased risk of DR progression persisted in the formerly intensive therapy arm compared to the conventional arm, with an adjusted OR of 0.28 (95% CI 0.19-0.41) adjusted for the level of DR at the end of DCCT (108). Although the annual incidence was similar in both arms due to improved control and risk in the former conventional arm over time, the overall risk of DR progression was still lower in the formerly intensive therapy arm 18 years into follow up of the EDIC study (111). In similar trials involving patients with T2D, metabolic memory has been referred to as the legacy effect (112, 113).

Similar to the DCCT, the multicenter UK Prospective Diabetes Study (UKPDS) randomized 3867 patients newly diagnosed with T2D to either intensive therapy (with a sulfonylurea or insulin) treating to a target of fasting blood glucose below 6.0 mmol/L, or to conventional therapy (diet only initially), treating to a target of fasting blood glucose below 15.0 mmol/L (47). (A third, smaller group of patients with overweight were randomized to metformin.)

The intensive group achieved a median A1c of 7.0% over 10 years, and the conventional group achieved a median A1c of 7.9% over 10 years. The UKPDS found that intensive therapy was associated with a lower risk of microvascular complications compared to conventional therapy (Risk Ratio (RR) 0.75, 95% CI 0.6-0.93), including risk of retinopathy requiring photocoagulation specifically (RR 0.71, 95% CI 0.52-0.96) (47). The 10-year follow-up study of UKPDS patients found that the patients previously randomized to the intensive therapy arm maintained an RR for microvascular complications of 0.76 (95% CI 0.64-0.89) for the former sulfonylurea/insulin group (112).

Finally, a continued reduction in the risk of progression of DR was also found in the 8-year follow-up of the ACCORD Eye Study trial (114). This trial was conducted in patients with more established diabetes, who were randomized eight years earlier to either intensive or conventional glycemic targets, leading to mean A1c levels of 6.4% and 7.7% respectively, after a median follow-up duration of 3.7 years (114). Thereafter, the difference in A1c narrowed between both groups until finally, 8 years after randomization, the difference disappeared between groups (7.8% and 7.9% respectively) (114).

1.4 Intensive glycemic control and early diabetic retinopathy

While intensive glycemic control can prevent incident or progression of DR (115), rapid progression of pre-existing DR with initiation of intensive glycemic control is a well-documented phenomenon among patients with T1D and T2D (116). Trials that followed patients with T1D or T2D who were randomized to either intensive or conventional glycemic control treatment with regular ophthalmic examinations found that there were signals of worsening (i.e., progression) of DR in the first 6 or 12 months, especially among those with a high baseline A1c and a rapid decrease in the first 6 months in the intensive arms of those studies (46, 84, 110, 117-119) (Table 1.3). These trials included insulin therapy and GLP1-RAs in their treatment arms. Of note, in the SUSTAIN trial, data suggest that the difference in DR complications is thought to be due to important glycemic improvement in the first 4 months of the trial rather than the drug itself (84, 86). As mentioned earlier, however, the intensive arms of these multicenter RCTs benefited from decreased risks of incident, long-term microvascular complications due to metabolic memory.

Observational data also support the notion that rapid glycemic improvement can lead to DR progression, whether it is among patients with T1D (120) or T2D (121, 122).

Table 1.3 RCTs demonstrating early worsening of DR with improved glycemic control.

Trial	Patient population	Design	Follow up	DR outcomes
Kroc Collaborative Study (117, 119) 1988	T2D n = 64	CSII vs unchanged conventional injections	Treatment period: 8 months, follow up period post-treatment: 24 months	Change in mean DR level: At 8 months: 4.0 points in CSII arm vs 1.6 points in conventional arm (significant difference when adjusted with baseline level of DR as covariate) From 8 to 24 months: -1.8 +/- 0.9 points in CSII arm vs 2.3 +/- 1.3 points in conventional arm From 0 to 24 months: 2.3 +/- 1.4 points in CSII arm vs 4.5 +/- 1.4 points in conventional arm
DCCT[a] (46, 110) 1993	T1D n = 1441	Intensive insulin therapy aiming for normal A1c vs conventional insulin therapy aiming for absence of symptoms	Mean follow up: 6.5 years	OR for early worsening of DR at the 6- and/or 12-month visit: 2.06 (95% CI 1.42-2.99)
LEADER[b] (123)	T2D n = 9340	Liraglutide 1.8mg once a day vs placebo	Median follow up: 3.8 years	Mean difference in A1c between liraglutide and placebo arms at 36 months:

2016				-0.40% (95% CI: -0.45 to -0.34) HR for DR= 1.15 (95% CI: 0.87-1.52; p = 0.3)
SUSTAIN 6[c] (84) 2016	T2D n= 3297	Semaglutide (0.5mg or 1.0mg once a week) vs placebo	Treatment period: 104 weeks, follow up period post-treatment: 5 weeks	DR complications HR 1.76, 95% CI 1.11-2.78, p = 0.02)

Adapted from Bain et al, 2019 (116).
Abbreviations: CSII = continuous subcutaneous insulin infusion
[a] DCCT: Diabetes Control and Complications Trial
[b] LEADER: Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results
[c] SUSTAIN 6: Trial to Evaluate Cardiovascular and Other Long-Term Outcomes With Semaglutide in Subjects With Type 2 Diabetes

1.5 Insulins and diabetic retinopathy

It is unclear whether insulin itself has the pathophysiological potential to cause incident DR and DR progession independently of rapid and profound glycemic improvement. Many authors have sought a causal link between insulin use and DR, and subsequently between insulin analogues and DR via either insulin receptor stimulation or IGF-1 receptor stimulation. Indeed, IGF-1 and insulin receptor stimulation have been found to have mitogenic activity on human breast cancer cells (124-126). There is also some small-scale observational data that show that higher serum levels of IGF1 are associated with a higher risk of DR progression while on insulin therapy (127), but results of other observational studies have not supported this finding (128). More recently, a prospective study has demonstrated that insulin therapy is associated with a transient reduction in retinal vessel density within three months after initiation among patients with T2D (with either NPDR or absence of DR at baseline) (129). This effect was not seen in the oral hypoglycemiant-treated control group, suggesting it was caused by a mechanism other than rapid glycemic decrease (129).

There are conflicting data as to whether insulin analogues can lead to incident DR or its progression. In vivo and in vitro studies showed that some insulin analogues have a strong affinity to IGF-1 receptor (130-132). Of the insulin analogues, glargine and aspart (a short-acting analogue) have the highest IGF-1 affinity and mitogenic potency on cell lines, while those of detemir are lower than that of human insulin (133). However, it has not been shown that this increased affinity leads to increased mitogenicity outside of tumor cell lines and below supraphysiological concentrations (130, 134-137). The potential for mitogenicity of insulin analogues is complex, as it integrates the time these molecules might spend attached to the IGF-1 receptor, the dissociation rate, and the degree of activation of intracellular pathways (58). In humans, the addition of either NPH or glargine to metformin monotherapy among poorly controlled patients with T2D decreases IGF-1 bioactivity to the same degree (117 +/- 10 pmol/L vs 116 +/- 9 pmol/L for NPH and glargine, respectively) (138). However, insulin analogues could potentially lead to DR independently of their affinity for the IGF-1 receptor: a more recent study found that administration of a short-acting insulin analogue (AspB10) increased mammary tumor growth and insulin receptor phosphorylation independent of IGF-1 stimulation (139). Of note, only insulin analogues (both

rapid- and long-acting) were used for the exposure group of the prospective study described above that demonstrated early, transient reductions in retinal vessel density among T2D patients initialing insulin (129).

There is still a gap in our knowledge regarding the association between different kinds of insulins and the risk of diabetic complications. There are many studies, including many RCTs, comparing basal insulin analogues to NPH for efficacy and safety outcomes (65-67, 72, 140-143), but few had the size or duration required to study their effects on microvascular complications and most did not include these complications as secondary outcomes. A few studies, however, did address the potential risk of insulin analogues or NPH insulin on DR (66, 72, 144-146). These studies are discussed in detail in **Chapter 2** of this thesis.

1.6 Thesis objectives

This thesis contains one primary objective and four secondary objectives.

1.6.1 Primary objective

The primary objective of this retrospective, cohort study is to determine if the current use of long- or ultra-long-acting insulin analogues (glargine, detemir, or degludec) is associated with the risk of incident DR among patients with T2D, compared to the current use of intermediate acting NPH insulin.

1.6.2 Secondary objectives

1. To determine if the current use of long- or ultra-long-acting insulin analogues, relative to current use of NPH insulin, is associated with an increased risk of 1) non-proliferative, 2) proliferative DR and 3) unspecified type of DR among patients with T2D.
2. To determine if the association between the current use of long- or ultra-long-acting insulin analogues and the risk of incident DR, compared to current use of NPH, varies by age, sex, duration of diabetes, and prior history of other microvascular or macrovascular complications among patients with T2D.
3. To determine if the association between the current use of long- or ultra-long-acting insulin analogues and the risk of incident diabetic retinopathy, compared to the current use of NPH,

varies by type of analogue insulin (glargine, detemir, or degludec) among patients with T2D.

4. To determine if the association between the use of long- or ultra-long-acting insulin analogues and the risk of incident diabetic retinopathy, compared to NPH, varies by duration of use of insulin type among patients with T2D.

1.7 Thesis overview

Chapter 1 presented prerequisite knowledge of the present thesis on T2D, insulin, and DR, as well as the thesis objectives. Chapter 2 provides a literature review of long-acting insulin analogues and NPH insulin among patients with T2D and their risk of incident DR. Chapter 3 describes the methods of the database study in detail. Chapter 4 presents the manuscript describing the original research study comparing long-acting insulin analogues versus NPH insulin and the risk of incident DR. Chapter 5 discusses the implications of this thesis. Finally, Chapter 6 provides overall conclusions.

CHAPTER 2: LITERATURE REVIEW

This chapter describes the available literature on the use of NPH insulin and long-acting insulin analogues in non-pregnant, non-hospitalized adults with T2D and the risk of DR as either a primary or secondary outcome. Notable studies conducted among patients with T1D that have examined this outcome have been added, as the general pathophysiology of DR is the same in both types of diabetes and because the literature for some specific long-acting insulin analogues among patients with T2D is sparse. Of note, most studies discussed examined progression of DR rather than incident DR as an outcome, and often measured progression using the EDTRS severity scale. The latter includes absence of DR as one of its steps, and thus DR progression can be understood as combining both incident DR and progression of pre-existing DR in cohort studies using this scale.

2.1 Systematic reviews and meta-analyses

To my knowledge, there have been no systematic reviews or meta-analysis published to date that assessed insulin analogues and NPH and the risk of DR as a primary outcome. However, a 2007 Cochrane systematic review (without meta-analysis) comparing long-acting analogues (except degludec, which was not yet marketed) to NPH insulin among patients with T2D did report a composite secondary outcome of "late complications", which included blindness and worsening DR (140). It described the results of the FDA reports of 2 registration RCTs (147, 148). This Cochrane review was updated in November 2020 to include degludec, although the authors did not find any trials comparing the effects of degludec to those of NPH, just as they did not find trials comparing the effects of glargine U300 to those of NPH with respect to DR (72). The updated Cochrane review also included a random-effects meta-analysis of included trials. Although the systematic review included 24 RCTs (16 with glargine and 8 with detemir), only 5 RCTs (1947 participants) comparing glargine versus NPH reported DR progression as an outcome and were included in the meta-analysis (72). When data were pooled across trials, the RR of glargine vs NPH for DR progression was 1.03 (95% CI 0.60-1.77), with moderate to substantial heterogeneity (I^2 = 54%) (72). Only 2 studies (972 participants) compared detemir versus NPH for DR

progression. When data were pooled across these trials, the RR was 1.50 (95% CI 0.68-3.32), with low heterogeneity (I^2 = 0%) (72).

2.2 Randomized controlled trials

2.2.1 Glargine vs NPH: registration trials

Concerns about a possible association between insulin analogues and DR surfaced during the registration trials for glargine (145). Indeed, these studies showed an increased progression of DR among patients randomized to glargine compared to those randomized to NPH (145, 147).

The Rosenstock (2001) registration trial was an open label, multicenter trial that randomized 518 patients with T2D previously on NPH insulin to receive either glargine once daily or NPH once or twice daily for 28 weeks (147). The aim of the trial was to evaluate the efficacy and safety of glargine, with its primary outcome being change in A1c. At baseline, patients were similar in both arms: they were approximately 59 years old, had a 13 to 14-year history of diabetes, had a baseline A1c of 8.6 +/- 1.2 % for glargine and 8.5 +/- 1.2% for NPH, and had a mean BMI of 30 kg/m^2 (147). There was a small imbalance in retinopathy at baseline, with a greater prevalence of DR among patients randomized to NPH (56.8%) than among those randomized to glargine (47.9%) (147). During the study, doses were adjusted to normalize the fasting blood glucose levels, and both treatment arms reached a median total insulin daily dose of 0.75 IU/kg (147), an unremarkable amount for patients with T2D. Both arms had modest improvements in A1c, with mean changes of -0.41 +/- 0.1% for glargine and -0.59 +/- 0.1% for NPH (147). However, patients in the glargine insulin arm had higher rates of three steps or more of DR progression at 28 weeks (7.5% vs 2.7%, RR: 2.75, 95% CI 1.10-6.91) (72, 145). This difference was observed despite the presence of the small initial imbalance in DR at baseline that favored glargine.

The Massi (2003) registration trial was also a multicenter, open label, randomized trial evaluating the efficacy and safety of glargine compared to those of NPH insulin (148). In this trial, 570 patients with T2D were randomized to receive either glargine or NPH insulin for 52 weeks (148). The two groups were well balanced, with a mean age of 59 years, a mean BMI of 29 kg/m^2, a history of diabetes of 10 years, a baseline A1c 9.0 +/ 1.2% in the glargine group and 8.9 +/- 1.1% in the NPH group, and baseline prevalences of DR of 18.0% in the glargine group and 16.0% in

the NPH group (148). Both groups achieved similar, modest decreases in A1c after 52 weeks (-0.46% and -0.36%, respectively, p = 0.4) (148). Although only found in the FDA reports and not reported in the published article, rates of clinically significant macular edema were numerically higher among patients randomized to glargine (11.2%) than among patients randomized to NPH (6.5%) at 52 weeks (140, 145). This difference was more pronounced in the subgroup of insulin-naïve patients (14% for glargine vs 4% for NPH), but completely reversed among patients already pre-treated with insulin (1.9% vs 12.7%, respectively) (140). Overall, the data concerning macular edema are sparse, and results are inconclusive. In this same trial, there was less progression of DR by at least 3 stages among patients randomized to glargine (11/187) than among those randomized to NPH (15/165) (RR for DR progression: 0.65, 95% CI: 0.31-1.37).

Overall, these two registration trials produced inconsistent results. Of note, the other glargine registration trials did not report DR data and follow-up was too short to assess long-term DR risk (140, 145).

The results of a small number of registration trials conducted among patients with T1D also warrant some brief discussion, as T2D and T1D have a common pathophysiologic process towards DR development. A homologous registration trial comparing glargine to NPH among patients with T1D (with lispro as the bolus insulin used in both arms) found an increased risk of retinal events (2.9% vs 2.3%) and in retinal vascular disorder events (1.9% vs 1.0%) among patients randomized to glargine versus NPH during the 16-week follow-up duration (149). Only one other RCT comparing glargine to NPH among patients with T1D assessed retinopathy progression between the two groups, finding no differences after 28 weeks of follow-up in the development of severe DR, clinically significant macular edema, or a three-step progression on the Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy scale, but the data were not shown (150).

2.2.2 Glargine vs NPH: more recent RCTs

The efficacy and safety of glargine and NPH have also been compared in subsequent RCTs, presented here in chronological order. Published in 2006, the LANMET trial, a multicenter, open label, parallel group RCT, compared the combination of glargine and metformin vs the combination of NPH and metformin among 110 patients with T2D (151). The patients were randomized to receive either combination for 36 weeks and had to self-titrate their insulin at home

(151). The characteristics of randomized patients were well balanced at baseline (151). There were no data presented on baseline prevalence of DR, however those with DR at baseline necessitating treatment in the 3 months prior to or during the study were excluded. The groups achieved comparable A1c levels at week 36 (7.14% +/- 0.12 and 7.16% +/- 0.14, respectively). No difference was observed in the risk of DR progression between groups (relative risk: 1.00, 95% CI: 0.28-3.54) (72). However, these results should be interpreted with caution, as there are very few events, resulting in imprecise treatment effects, and the presence of baseline, silent DR is unknown.

Only one published RCT compared DR progression between a long-acting analogue (glargine) and NPH as a primary outcome (66). This open-label, multicenter non-inferiority trial conducted in North America followed patients with T2D randomized to either NPH twice-daily (n = 509) or glargine once daily (n= 515) for 5 years (66). The primary outcome of the trial was the percentage of patients developing 3 or more step progression in the ETDRS score. Although most baseline patient characteristics were well balanced between groups, there was slightly more DR (15.6% vs 12.1%) and a slightly higher severity of DR at baseline among patients randomized to glargine than among those randomized to NPH. There was a smaller decrease in A1c with glargine than with NPH (-0.55, 95% CI -0.49 to -0.61% versus -0.76%, 95% CI -0.70 to -0.82%; difference: 0.21, 95% CI 0.14-0.28%). However, the clinical significance of this difference is unclear. The study found no difference in DR progression between the two groups (glargine vs NPH: 12.5% vs 14.6%, difference: -2.1%, 95% CI: -6.29, 2.09) (66). The RR was 0.86 (95% CI 0.63-1.18) (72). Results were similar in the per protocol analysis (14.2% vs 15.7%; difference: -1.5%, -7.02, 3.06) (66). Major advantages of this RCT were its long follow-up duration (which is necessary for an outcome such as DR progression), and the reporting of baseline data for DR prevalence and severity.

Finally, in 2019, Betônico et al. published a 2-way crossover open label trial comparing the glycemic response to glargine vs NPH insulin among patients with T2D and CKD stage 3 and 4 (146). They randomized 34 patients to either glargine U100 or NPH insulin for 24 weeks, at which point they were switched to the other basal insulin. After 24 weeks, the glargine group decreased their mean A1c level from 8.86 +/- 1.4 % to 7.95 +/- 1.1% while the NPH group increased their mean A1c level from 8.21 +/- 1.3% to 8.44 +/- 1.3% (146). Although the data were

not included in the main published article, the 2020 Cochrane review revealed that there were no reported events of DR progression during this trial (72).

2.2.3 Detemir vs NPH

No trials were found that compared detemir to NPH with DR as a primary outcome among patients with T2D. Only one RCT compared detemir to NPH insulin and reported some data on DR. Another trial (NN304-1337) was mentioned in the 2020 Cochrane review, but the details of this study could not be found.

The Haak (2005) study was a multicenter, open label trial that randomized 505 patients with T2D to either detemir or NPH insulin (152). Randomized patients were similar in both groups, with a mean age of 60 years old, a mean duration of diabetes history of 12.9 +/ 7.4 years for detemir users and 13.7 +/- 8 years for NPH users, and an A1c of 7.9% +/- 1.3% for detemir users and 7.8 +/- 1.3% for NPH users. There were no data on baseline prevalence of DR, but presence of baseline proliferative DR was an exclusion criterion (152). After 26 weeks of treatment, the mean A1c level was 7.6 +/- 0.1% in the detemir group and 7.5 +/- 0.1% in the NPH group (152). The DR progression events were not reported in the published article, but the 2007 Cochrane review mentioned that those data were "available through personal communication with the author" (140). The 2020 Cochrane review reported an elevated risk of DR progression with detemir versus NPH (RR: 2.24, 95% CI: 0.65-7.70) (72), although results were inconclusive due to spare data and wide 95% CIs. The duration of the Haak trial was also short, further limiting the possibility of drawing definitive conclusions from these data. The 2020 Cochrane review also reported DR progression data for a trial named NN304-1337 (72). This open-label, parallel design trial was conducted in the United States and Puerto Rico, with A1c change as the primary outcome and fundoscopy results as a secondary outcome. They excluded patients with proliferative DR at baseline (72). There were 11 DR progression events in the detemir group (309 patients) and 5 events in the NPH group (158 patients) (RR: 1.12, 95% CI: 0.40-3.18) (72). Other details of NN304-1337 are unknown.

Two parallel group trials conducted among patients with T1D reported safety results concerning DR. The first one is a multinational, open-label parallel group trial published in 2004 that followed patients with T1D randomized to either detemir or NPH as a basal insulin in their basal-bolus regimen for up to a year (n= 461 patients were enrolled in the study and n=252 completed the 12-month follow-up; human soluble insulin was the mealtime insulin in both groups)

(153). The authors reported that, at 12 months, two patients in the NPH group had clinically significant abnormal fundoscopies compared to none in the detemir group (153). The occurrence of "retinal disorders" also differed between groups: 8.2% in the NPH and 5.2% in the detemir group (153). In contrast, the occurrence of more broadly defined "vision disorders" was similar between groups (11.2% vs 11.0%, respectively) (153). The second trial is a multinational, open-label parallel group trial investigating the efficacy and safety of detemir (n=331) vs NPH (n=166) as a basal insulin among patients with T1D (154). In both groups, aspart was used as the bolus insulin at meals (154). During the 24-month follow-up, no differences were observed with regard to fundoscopy or fundus photography results between the two groups (154). The DR event rate was very low: there was only one event of retinal detachment, which was in a patient in the detemir arm. This event was considered an adverse event possibly or probably related to the trial drug and led to withdrawal of the patient from the trial (154).

2.2.4 Degludec vs NPH

Unfortunately, there are no trials comparing the DR risk of degludec to NPH head-to-head. One multinational, open-label RCT comparing degludec (n=555) to glargine (n=278) among insulin-naïve T2D patients found no differences between both arms in fundoscopy or fundus photography results after 26 weeks of follow up (155).

2.3 Observational studies

A retrospective cohort study of patients with T2D in Taiwan assessed the risk of developing sight-threatening DR in matched cohorts of glargine, detemir and NPH insulin (144). The study matched patients with T2D aged at least 20 years who initiated either glargine (n= 9141), detemir (n= 1413), or NPH (n = 36,185) between 2004 and 2006 on propensity scores (PS). Patients were also matched on their use of antidiabetic drugs, statins and antihypertensive drugs, their health resource utilization, year of insulin initiation (2004, 2005 or 2006), and their physician characteristics including specialty. After matching on propensity score, the adjusted incidence rate ratios for the development of sight-threatening DR were 0.93 (95% CI 0.8-1.1) for glargine vs NPH and 1.10 (95% CI 0.8-1.4) for detemir vs NPH (144). The authors also conducted an unmatched analysis that used the entire cohort and adjusted for PS and other potential confounders.

This analysis found no difference for HRs in the main analysis: HR 0.94 (95% CI 0.86-1.04) for glargine vs NPH and HR 1.10 (95% CI 0.88-1.38) for detemir vs NPH. However, again using the unmatched, full cohorts, glargine was associated with a reduced risk in both the intention-to-treat analysis (HR 0.70, 95% 0.63-0.77) and time-varying analyses (HR 0.64, 95% CI 0.58-0.71) compared to NPH (144). The sensitivity analyses, which excluded patients with exposure to any insulin in the 12 months preceding the cohort entry found no increased or decreased risk for the insulin analogues.

This observational study had several strengths. These strengths include its large sample size and propensity score matching to minimize potential confounding. However, it has some potential limitations. First, the mean follow-up time was relatively short, ranging from 363.9 (standard deviation (SD) = 173.0) days for detemir initiators to 548.8 (SD = 310.9) days for the NPH initiators matched with glargine initiators (144). This study was also limited by the lack of information for certain potential confounders, such as baseline presence of DR or degree of severity, diabetes duration, blood pressure at baseline (144), and use of fenofibrate, a dyslipidemia drug known to reduce DR progression in T2D patients (156). There was also no information provided on potential changes in A1c over time.

The surprising results of a protective adjusted HR for glargine in the unmatched cohort in both the intention-to-treat and time-varying use analyses likely reflects residual confounding caused by differences in statistical analyses. Unlike the main unmatched analysis, these analyses lacked adjustments for diabetes-related complications, antidiabetic medications, and antihypertensive medications, all of which could be associated with the outcome.

Another recent retrospective observational study compared the effects of long-acting insulin analogue (mostly glargine) vs NPH on a composite of long-term outcomes using Veterans Health Administration and Medicare administrative data from 2000 to 2010 (157). The study did not find increased risks between long-acting insulin analogues and NPH for its primary outcomes, which were mortality and hospitalization for any of 13 “ambulatory care-sensitive conditions” that included cardiovascular and diabetic-specific events (157). HR for the latter outcome was 1.05 (95% CI 0.95-1.16) (157). DR was not reported as a separate outcome.

Finally, a multicenter observational study compared the incidence of microvascular complications as a secondary composite outcome between patients with T2D using either glargine or NPH as a basal insulin supporting oral hypoglycemic therapy (57). Patients were included if

they were adults with T2D duration of 10 years or less, had an A1c at baseline of at least 7.5%, and were on a mix of oral antihyperglycemic drugs and a basal insulin during the previous 3-6 months. Major exclusion criteria were a history of a cardiovascular event and the use of a GLP1-RA (57). The co-primary outcome was the number of patients achieving a fasting blood glucose of 6.7 mmol/L or less at the final visit without the occurrence of symptomatic hypoglycemia during follow-up and the number of patients achieving an A1c of 7.0% or less at the final visit without occurrence of symptomatic hypoglycemia. Of the 2629 patients enrolled, 1931 met the inclusion criteria: 1614 were receiving glargine and 303 were receiving NPH. The analytical cohort included 285 patients in each group, matched on PS. At baseline, the total cohort of glargine and NPH patients were quite similar even before matching, with a mean age of 64 years old, a mean length of diabetes history of 5 years, and a minority of patients with DR at baseline (9.4% of glargine patients and 10.2% NPH patients) (57). They also had similar baseline prevalences of other diabetic complications (e.g., micro- and macroalbuminuria, neuropathy, diabetic foot) and mostly similar use of various oral antihyperglycemic drugs. After matching, 10.2% of the 285 glargine patients and 10.5% of the 285 NPH patients had DR at baseline (57). Certain baseline characteristics were recorded again at 12 weeks (second documentation) and then at 24 weeks (final documentation), including A1c, current antidiabetic treatment, along with occurrence of new micro- and macro-vascular complications and insulin adjustments (57). The observation period for each patient ended at 24 weeks, with the final documentation, or at the time of switch to a different basal insulin, if done prior to the final documentation (57). Of note, for the matched cohorts, 54.4% of the patients in the NPH group switched to glargine insulin between the first and last visit (final documentation), while only 1.4% of the patients in the glargine group did so towards an NPH insulin regimen (57). Duration of the initial basal insulin treatment was 25.9 +/- 4.9 weeks for glargine and 18.2 +/- 7.8 weeks for NPH (57). Insulin switching and duration of initial basal insulin treatment were similar for the non-matched cohorts.

In the pre-matched study cohort, 3 of the 1614 patients on glargine and none of the 303 patients on NPH developed DR; no patients developed DR in any of the matched cohorts (57). Strengths of this study are that all patients are equally relatively new to basal insulin at baseline and PS matching. They also considered some important confounders often missing in observational studies, including BMI, last A1c recorded at baseline and last A1c before initiation of the basal insulin. However, the overall follow-up period (24 weeks) was short. Similarly, the

reduced period of observation for patients initially treated with NPH is also major limitation of this study: with a mean observation period of 18 weeks, there is even less time for the patients exposed to NPH to develop an event of DR. Furthermore, the article does not report the data on baseline level of glycemic control. It does report a marginally greater decrease in A1c in the glargine (-1.2 +/- 1.1 %) than NPH insulin group (-0.7 +/- 0.9%) (57). Baseline A1c and degree of A1c correction are particularly important here since a rapid glycemic correction in a poorly controlled patient with T2D is a risk factor for progression of DR (116). Thus, the longer mean observation period and greater decrease in A1c in the glargine group could have biased the DR results in favor of NPH. Finally, the outcome of "retinopathy" is not well defined (i.e., incidence vs progression of DR vs either), further limiting the interpretation of these data.

2.4 Literature review summary

This review of the literature underscores the modest literature available on the risk of DR among T2D patients using long-acting insulin analogues versus NPH insulin. As detailed above, only one RCT compared a long-acting insulin analogue (glargine) to NPH insulin with DR as a primary outcome; its results reassuringly showed that 5 years of glargine did not increase the risk of DR progression (66).. This trial was conducted specifically because of the concerns surrounding a potential increased risk of DR with glargine compared to NPH following the completion of the registration trials (145). Few RCTs reported DR as a secondary outcome, even when mixed with nephropathy and neuropathy into a composite outcome of microvascular complications. It is worth noting that patients included in RCTs are inherently different from those seen in a 'real-world' setting, where the patient population is more varied (with patients often having more comorbidities) and clinical monitoring is not as intensive as in a dedicated clinical trial (158). Only one observational study comparing long-acting analogues to NPH had DR as a primary outcome (144), but it predates the advent of degludec. Indeed, the bulk of the available literature involves glargine. There are very few studies assessing DR incidence or progression among detemir users compared to NPH users. Furthermore, there are no trials comparing degludec to NPH. Finally, while some studies had a non-trivial prevalence of DR at baseline (when specified) to drive DR progression events, others had a small sample size and very few events to measure, limiting interpretation. This literature review accentuates the need for more real-world evidence regarding the risk of incident

DR as a pharmacological safety issue among T2D patients using insulin analogues. The use of incident DR instead of the broader outcome of DR progression would avoid confounding by rapid decreases in A1c due to insulin therapy initiation, a known risk factor for pre-existing DR progression but not clearly established for incident DR (86). All T2D studies discussed in this literature review are summarized in **Table 2.1** below.

Table 2.1 Summary of literature on the risk of DR with long-acting insulin analogues versus NPH insulin among patients with T2D.

Study	Design	Exposure (vs NPH)	Population	Follow up duration	DR outcomes
Rosenstock 2001	Open-label RCT	Glargine	n = 518	28 weeks	DR progression RR = 2.75 (95% CI 1.10-6.91)
Massi 2003	Open-label RCT	Glargine	n = 570	52 weeks	DR progression RR = 0.65 (95% CI: 0.31-1.37)
LANMET[a] 2006	Open-label, randomized trial	Glargine	n = 110	36 weeks	DR progression RR: 1.00 (95% CI: 0.28-3.54)
Rosenstock 2009	RCT	Glargine	n = 1024	5 years	DR progression RR: 0.86 (95% CI 0.63-1.18)
Betonico 2019	2-way cross-over, open-label trial	Glargine	n = 34 CKD stage 3 and 4	24 weeks	DR progression No events reported
Lin 2014	Retrospective cohort study	Glargine, Detemir	glargine n= 9141 detemir n= 1413 NPH n = 36,185	Glargine initiators 523.5 (SD 320.6) days vs NPH initiators 548.8 (SD 310.9) days Detemir initiators 363.9 (SD = 173.0) days vs NPH	Sight-threatening DR Glargine matched cohort IRR 0.93 (95% CI 0.8-1.1) Detemir matched cohort IRR 1.10 (95% CI 0.8-1.4)

				initiators 431.4 (196.1) days	Glargine unmatched, ITT analysis HR 0.70 (95% 0.63-0.77) Glargine unmatched, time-varying use analysis HR 0.64 (95% CI 0.58-0.71)
Prentice 2015	Retrospective cohort study	Glargine (99% of long-acting analogues' cohort)	n = 142,940	Unknown, minimum 12 months outcome period Cohort entry between 2001 and 2009	Ambulatory care sensitive conditions HR 1.05 (95% CI 0.95-1.16)
Feisselmann 2015	Retrospective cohort study	Glargine	n = 1931 full cohort n = 570 matched cohort	24 weeks	No DR events in matched cohort 3 DR events among full cohort, all in glargine users
Haak 2005	Open-label RCT	Detemir	n = 505	26 weeks	DR progression RR: 2.24 (95% CI: 0.65-7.70)
NN304-1337	Open-label RCT	Detemir	n = 467		DR progression RR 1.12 (95% CI: 0.40-3.18)

Abbreviations : DR, diabetic retinopathy; IRR, incidence rate ratio; ITT, intention-to-treat; RR, risk ratio; RCT, randomized controlled trial; SD, standard deviation

[a] LANMET: Insulin glargine or NPH combined with metformin in type 2 diabetes: the LANMET study (Yki-Järvinen et al)

CHAPTER 3: METHODS

3.1 Clinical Practice Research Datalink

Our study used linked data from the Clinical Practice Research Datalink (CPRD) Aurum, Hospital Episode Statistics (HES) Admitted Patient Care (APC), and Office of National Statistics (ONS) death registration data. The CPRD is one of the world's largest databases of longitudinal, anonymized medical records from general practitioners (159). There are two versions of the CPRD: Gold and Aurum. Data collection for CPRD has been ongoing since 1987 (when the database was named General Practice Research Database). Data are collected on a monthly basis from the electronic health records of participating primary care practices all around the UK (England, Wales, Scotland and Northern Ireland), and about 93.5% of all patients from participating practices are eligible for linkage to other National Health Services (NHS) data holdings such as HES. (159, 160). Linkage for CPRD Aurum is restricted to patients who (i) were registered at a participating English practice prior to the transfer of identifiers to the trusted third party for matching; (ii) had a valid identifier for linkage (either NHS number or postcode); and (iii) had not opted out or dissented from the CPRD or the linkage scheme. This thesis uses data from CPRD Aurum, and we will refer to it as CPRD hereafter.

Data collected in the CPRD include demographic details, patients' diagnoses, various symptoms and signs that have been recorded in the electronic chart, medications prescribed by the general practitioner, and ancillary tests performed and their results (159). Available linkage includes that to hospitalization data with the HES and mortality data with the ONS (159). HES contains all relevant data on inpatient, emergency and outpatient hospital admission in England, including date of admission, date of discharge and primary and secondary diagnoses made during the hospitalization stay (161, 162). The data is collected while the patient is in hospital and processed by NHS to create a coded database. Of note, HES APC, a subset of HES, only collects inpatient admission data (162). The ONS is a governmental agency and registers all deaths in the UK, along with the deceased's age, sex, cause of death and geographical area. The information is then coded by the ONS and is used to create mortality datasets.

CPRD specifically harnesses data from primary care practices that use the EMIS Web ® electronic medical record software (the most frequently used EMR software by primary care practices in the UK) (163). CPRD includes 19 million patients in total (of whom about 7 million were alive as of September 2018) from 738 primary care practices. Of note, over 98% of the general English population is assigned to a general practitioner, who are considered the gatekeeper of the NHS in the UK and provide the care for most patients with T2D (163). CPRD started its longitudinal data collection in 1987; as of September 2018, median follow-up time was 4.2 years (interquartile range: 1.5-11.4) for all patients ever included and 9.1 years (interquartile range: 3.3-20.1) for living, currently participating patients (163). Finally, we must note that patients can opt out of the CPRD, although this is relatively uncommon; as of September 2018, 2.7% of the English primary care registered patients had done so for CPRD Aurum (163). CPRD, which covers approximately 13% of the English population, is representative of the English population for age, sex, geographical spread, and socioeconomical deprivation (163).

CPRD uses a combination of coding systems to render the data accessible, which are collected as coded elements of the medical record. The medical history observations such as diagnoses and symptoms are coded using SNOMED CT (UK Edition), Read Version 2, and local EMIS Web ® codes (163), and are cross-mapped to the CRPD Aurum Code Browser's medical term dictionary (164). SNOMED CT is a standardized terminology, used by UK general practitioners since April 2018, to codify their clinical observations. The use of SNOMED codes allows different primary care practices to use a shared, standardized terminology where all related terms and synonyms for a given medical observation are connected together in a relationship tree (165). Read codes, on the other hand, form a hierarchical coding system where each level of codes becomes more specific as more letters and numbers are added (e.g., F represents diseases of the nervous system and F12z, Parkinson's disease) (165). The Read coding system is easy to use, but limited by its tendency to have duplicate codes for a same medical observation (165).

Medications prescribed by general practitioners recorded in the EMIS Web ® electronic medical record are coded using the Dictionary of Medicines and Devices (dm + d) in CPRD Aurum (163). Each product code is associated with a corresponding British National Formulary (BNF) chapter, and with a unique corresponding CPRD product code. The latter is limited by the fact that over-the-counter medications and medications ordered by specialists are not recorded in the electronic medical record, nor is information on dispensing and adherence.

The validity of diagnoses and prescription information recorded into the CPRD Aurum have been assessed previously. In 2020, Jick et al assessed the accuracy and completeness of diagnostic information in the CPRD by comparing the proportion of pulmonary embolism diagnoses found in a random sample of 50,000 patients in the database with the proportion found using HES data for the same sample (161). The authors found that 76.8% of patients with an anticoagulated pulmonary embolism recorded in this CPRD AURUM sample were also recorded in HES (161). Completeness was comparable, with up to 79.1% of pulmonary embolism events (depending on definition) found in HES also present in the CPRD AURUM sample. As for accuracy, very few patients were missing from CPRD or present in CPRD but not in HES due to potential outcome misclassification. Of note, HES APC cannot be considered a true gold-standard data source, as it does not record medical events in non-admitted emergency department visits or that do not result in hospitalization (161).

A similar comparative study by Persson et al published in 2020 assessed the validity of CPRD for 3 comorbidity diagnostic codes, including 2 that are particularly relevant for the present thesis: T2D and hyperlipidemia (164). The study assessed whether patients with T2D, hyperlipidemia, or iron deficiency or unspecified anemia diagnostic codes also had laboratory test results, treatment, or supporting diagnostic codes in support of the diagnosis. The study was conducted on a random sample of 50,000 patients, all 20 years of age or above with at least one recorded laboratory test result of any type. In this sample, a total of 4,412 patients were found to have a T2D diagnostic code. Of these patients, 88% were found to have at least one concordant diabetes-related blood test result (such as an A1c or blood glucose above the upper limit of normal); 82% had at least one prescription for a diabetes medication; and 99% had at least one or more supporting clinical codes (such as codes referring to a diabetes-related microvascular complication screening test) (164). Accuracy of hyperlipidemia diagnoses was similarly high, up to 93% depending on the type of supporting codes (164). However, completeness of data was high only for T2D (94-98%), while many patients with CPRD-recorded abnormal lipid profiles did not have a corresponding hyperlipidemia diagnostic code (completeness 51-59% for hyperlipidemia). The authors suggest that while the general practitioners have medical and financial incentives to correctly record all T2D cases, they do not for hyperlipidemia and iron deficiency, which may instead be recorded in the free text of clinical notes, which are unavailable to database users (164). While there are no studies reporting the validity of DR diagnostic codes and completeness of DR

data in CPRD, the remunerated incentive program UK Quality and Outcomes Framework, put in place from 2004 to 2014 by the NHS, required that all patients with diabetes be screened annually for DR and that this screening be recorded by general practices (166). We expect a high degree of data completeness in this time period.

3.2 Definition of study population

The date of first prescription of any long-acting insulin analogue (glargine, detemir, or degludec) or NPH insulin between September 2002 and December 2019 defined entry into the study cohort (with a minimum look-back period of 365 days). With insulin analogues first becoming available in the UK in September 2002, this study includes the entire period of availability of insulin analogues. Inclusion was restricted to patients who were linkable to HES inpatient data and ONS vital statistics. The exclusion criteria were: 1) age < 18 years at cohort entry, 2) a previous diagnosis of type 1 diabetes any time before cohort entry, 3) a history of DR or retinopathy of any cause any time before or at cohort entry (to detect de novo DR only and to avoid confounding by etiology, respectively), 4) use of imatinib, acitretin, nicotinic acid, rituximab, taxanes, interferon, zilovudine, rifabutine, or fingolimod (drugs known to cause or associated with retinopathy)(167) in the year before cohort entry, 5) less than 365 days of recorded CPRD history at cohort entry, 6) prescription of both NPH and a long-acting insulin analogue at cohort entry, 7) a history of gestational diabetes in the year before or at cohort entry, and 8) the absence of a T2D diagnostic code before or at cohort entry. In molecule-specific analyses, the study period was restricted to the periods of availability of each long-acting insulin analogue to avoid violating the positivity assumption. In all analyses, patients were followed until the occurrence of a study outcome (defined below) or censoring due to death of any cause (recorded in CPRD, HES, or ONS), end of CPRD practice registration, end of HES data coverage, the last date of data collection in CPRD, or end of the study period (December 31, 2019), whichever occurred first.

3.3 Exposure assessment

In our primary analysis, we used an ‘as-treated’, time-fixed exposure definition in which exposure was determined by the insulin prescribed at cohort entry. We hypothethize that the risk

of incident DR is constant over time with long-acting insulin analogues. Using recorded prescriptions of insulin, we classified patients into one of two mutually-exclusive groups: A) current use of a long-acting insulin analogue (glargine, detemir, or degludec), with or without use of other antidiabetic drugs and without a prescription for NPH insulin (the exposure of interest); or B) current use of NPH insulin, with or without use of other antidiabetic drugs and without a prescription for long-acting insulin analogues (the reference group). In the as-treated exposure definition, discontinuation of the cohort entry defining insulin type, defined as a prescription gap of 60 days or more between consecutive prescriptions, or treatment crossover (defined as initiation of a long-acting insulin analogue (in the reference group) or NPH (in the exposure of interest group)) resulted in censoring. While there was a theoretical risk of informative censoring, supplementary data analyses did not find any differential censoring (see **chapter 4**).

NPH insulin was used as comparator because it is clinically relevant: it was the immediate predecessor to the new, long-acting analogue insulins, but is still widely used as it is cheaper. NPH insulin is used for the same clinical purpose as the long-acting insulin analogues, i.e. mainly as a basal insulin (see Chapter 1), and it is often the active drug comparator in RCTs for new long-acting insulin analogues to this day. By using a comparator used at the same point in the management of T2D, we have avoided time-lag bias (168) and we have minimized potential confounding by indication by other variables.

3.4 Outcomes

The primary endpoint was incident DR (including both non-proliferative and proliferative), defined using the CPRD and HES. Secondary endpoints were: 1) incident non-proliferative DR; 2) incident proliferative DR and 3) incident unspecified type of DR (as some codes did not specify the type of DR). DR was defined by the presence of ICD-10 code H36.0 for HES-defined events, with the date of hospital admission defining the event date, and using relevant SNOMED codes in the CPRD, with the date of diagnosis defining the event date (see appendix 1).

The outcomes pertain to *incident* DR rather than to progression of DR, the latter being a much more common outcome in previous studies and trials in this area. Focusing on incident DR has the advantage of avoiding likely confounding by rapid decreases in A1c, which may occur with insulin therapy and are a known risk factor for DR progression but not for incident DR (see

Chapter 1). The outcome is clinically relevant for patients with T2D and their health care providers, as the rationale for maintaining good, long-term glycemic control is to prevent microvascular and macrovascular complications.

3.5 Covariates

The following covariates, identified from the literature as variables related either to diabetes duration and severity or to the occurrence of DR itself, were measured at cohort entry: age, sex, ethnic origin, BMI, smoking status (ever, never, missing; measured in the last 5 years), most recent A1c in the year before and including cohort entry, systolic and diastolic blood pressure, duration of diabetes, presence of hypertension, presence of cardiovascular disease (coronary artery disease, heart failure, stroke, or peripheral vascular disease), presence of diabetic neuropathy, presence of CKD from diabetic nephropathy, presence of CKD from any cause, presence of dialysis, baseline eGFR category (≥ 90; 60-89; 45-59; 30-44; 15-29, < 15 ml/min/1.73 m^2, missing), prescribed anti-diabetic drugs in the year before and including cohort entry, relevant non-diabetic drugs prescribed in the year before and including cohort entry and hospitalizations in the year before cohort entry. We also adjusted for use of fenofibrate in the previous year as it is protective against retinopathy (169, 170) and for the following risk factors for retinopathy, all measured any time before cohort entry: history of human immunodeficiency virus (HIV) and history of bariatric surgery. Bariatric surgery may be protective for incident DR, while there is conflicting data regarding its association with progression of DR (171, 172). The rapid improvement in hyperglycemia post-operatively appears to lead to early worsening of DR among patients with known, advanced DR at baseline (173). Comorbidities were assessed any time prior to cohort entry, while medications, blood pressure, and laboratory test results were assessed in the year before cohort entry. The details of all covariates included are presented in **Table 3.1**. Continuous variables were modelled flexibly through the use of restricted cubic splines. Covariates with missing data were included through the use of multiple imputation.

Table 3.1 Covariates measured at cohort entry for the retrospective cohort study of long-acting insulin analogues vs NPH insulin and the risk of incident diabetic retinopathy among patients with type 2 diabetes

Covariate		Functional form	Units	Look-back period
Age				
	Mean age	mean (SD), median (IQR)	years	N/A
	Groups: 18-35; 36-45; 46-55; 56-65; 66-75; 76-85; > 85 years of age	n (%)	patients	N/A
Sex				
	males, females	n (%)	patients	Any time
Ethnic origin				
	white, other, unknown	n (%)	patients	Any time
Year of cohort entry				
	Groups: 2002-2007; 2008-2013; 2014-2019	n (%)	patients	N/A
Duration of diabetes (groups)				
	Mean duration	mean (SD), median (IQR)		
	<1 year, 1 to <5 years, 5 to < 10 years, ≥ 10 years	n (%)	patients	Any time
BMI				
	Mean BMI	mean (SD)	kg/m^2	5 years
	<30, ≥30, unknown	n (%)	patients	5 years
Smoking status				
	Never, ever, unknown	n (%)	patients	5 years
A1c				
	Mean A1c	mean (SD)	%	1 year
	≤ 7, 7.1-8, ≥ 8, unknown	n (%)	patients	1 year
Blood pressure				
	Systolic (SBP), diastolic (DBP)	Mean (SD)	mmHg	1 year
	DBP < 90 and SBP < 140 DBP ≥ 90 or SBP ≥ 140 Unknown	n (%)	patients	1 year

Hypertension		n (%)	patients	Any time
Cardiovascular disease[a]				
	MI, CAD, HF, Cerebrovascular disease, Stroke, Peripheral vascular disease	n (%)	patients	Any time
Diabetic neuropathy		n (%)	patients	Any time
Chronic kidney disease				
	Chronic kidney disease from diabetic nephropathy	n (%)	patients	Any time
	CKD any cause	n (%)	patients	Any time
	Dialysis	n (%)	patients	Any time
Baseline eGFR				
	Mean eGFR	Mean (SD)	ml/min	1 year
	≥ 90; 60-89; 45-59; 30-44; 15-29, < 15 ml/min/1.73 m^2, unknown	n (%)	patients	1 year
HIV		n (%)	patients	Any time
Bariatric surgery		n (%)	patients	Any time
Antidiabetic drugs[b]				
	Metformin, Sulfonylureas + meglitinides, DPP4 inhibitors, GLP1-RAs, SGLT2 inhibitors, TZDs	n (%)	patients	1 year
Fenofibrate				1 year
Statins				1 year
Blood thinners[b]				
	Aspirin, Warfarin, Direct anti-coagulants	n (%)		1 year
Antihypertensive drugs[b]				
	ACEI, ARB, beta-blockers, CCB, thiazides, loop diuretics, potassium sparing diuretics, other diuretics	n (%)		1 year
Hospitalizations				
	Mean number of hospitalizations	Mean (SD), median (IQR)		1 year
	0, 1-2, 3 or more hospitalizations	n (%)		1 year

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blockers; CKD, chronic kidney disease; DBP, diastolic blood pressure; DPP4, Dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP1-RA, Glucagon-like peptide-1 receptor agonists; HF, heart failure; IQR, interquartile range; MI, myocardial infarction; SBP, systolic blood pressure; SD, standard deviation; SGLT-2, sodium glucose cotransporter-2; TZD, thiazolidinediones

[a] All comorbidities mentioned are individual covariates

[b] All medication classes mentioned are individual covariates

3.6 Statistical analyses

3.6.1 Descriptive statistics

Discrete data are presented as counts and percentages, and continuous data as means and standard deviation. For continuous variables with skewed distributions, medians and inter-quartile ranges were used. Absolute values of standardized differences were estimated to assess covariate balance, with absolute values of 10% or more considered to be important (174). We estimated incidence rates and their 95% CIs for diabetic retinopathy overall and by exposure group based on the Poisson distribution.

3.6.2 Primary analysis

In our primary analysis, we estimated a propensity score (PS) using a logistic model that included the pre-defined covariates described above. To estimate the HRs and its corresponding 95% CIs for DR for long-acting insulin analogues vs NPH insulin, we constructed Cox proportional hazards models as our outcome model with a time axis of follow-up duration, with inverse probability of treatment weighting (IPTW) using the PS to minimize potential confounding (175). Our PS estimates the probability of receiving long-acting insulin analogues at cohort entry based on the baseline characteristics (the pre-defined covariates, see **Table 3.1**), and this PS was used to estimate weights used for IPTW. The re-weighting then led to the creation of a pseudo-population, where the two insulin groups were exchangeable, under the assumption that the measured confounders used to create the PS are sufficient to create exchangeability. The probability of having received one insulin type (analogue vs NPH) over another is thus 50% for this pseudo-population. Of note, individual observations with PS values close to either 1 or 0 had low probabilities of receiving one of the treatments of interest, suggesting little clinical equipoise for patients with their characteristics. For such patients, IPTW can give them extreme, excessive weights. Truncation of IPTW weights greater than 10 was planned, but not needed as no extreme weights were observed.

3.6.3 Secondary analyses

We conducted 5 pre-specified secondary analyses. First, we repeated our primary analyses for the individual endpoints of 1) non-proliferative DR; 2) proliferative DR and 3) unspecified

type of DR. Second, we repeated the primary analysis stratified by prior history of diabetic nephropathy (none vs present (diagnostic code or eGFR of 59 ml/ml/1.73m^2 or less)) and by prior history of macrovascular complications (defined as any history of coronary artery disease, heart failure, myocardial infarction, cerebrovascular disease, stroke, or peripheral vascular disease). Third, we repeated the primary analysis stratified by duration of diabetes (0-5, >5-10, >10 years). Fourth, we repeated the primary analysis by type of analogue insulin (glargine, detemir, and degludec). Finally, we stratified by duration of use of insulin type (0-1, >1-2, >2-5, and >5 years). While A1c is a relevant determinant of DR risk, we did not include a stratification according to baseline A1c. We expected our cohort to be mostly poorly controlled on average and thus to have a relatively small sample size of patients with A1c under 8%.

3.6.4 Sensitivity analyses

Seven sensitivity analyses were conducted. First, treatment discontinuation was defined by gaps of 30 days and 90 days between consecutive prescription. Second, we excluded patients with a history of using GLP1-RAs and censored upon their use to avoid confounding due to increased rates of DR complications requiring ophthalmological interventions from rapid A1c correction with some GLP1-RAs, as described above in Chapter 1. Third, we used an 'intention-to-treat' exposure definition in which exposure was defined by the cohort entry insulin and patients were followed for a maximum of one year. Patients were not censored upon discontinuation or crossover in this analysis. The maximum follow-up time was 1 year to avoid exposure misclassification that is inherent to intention-to-treat analyses. Fourth, we restricted our outcome definition to CPRD-defined events. Fifth, we repeated the analysis including only the patients who had at least one DR screening exam in the year before or on cohort entry (to avoid misclassification of prevalent as incident DR). Sixth, we excluded patients with a very high A1c at baseline (i.e., 9.0% or above). As for the GLP1-RAs, this is again a proxy to exclude patients who developed DR from a drastic and rapid decrease in glycemia (116). Seventh, we excluded patients who were pregnant at the time of cohort entry as pregnancy is a risk factor for acute worsening of DR in women with T2D (176).

3.6.5 Missing data

From previous studies using CPRD, some degree of missingness was expected for our non-binary data, such as for HbA1C (~12%) (177), systolic blood pressure (1.2%) (177), diastolic blood pressure (2.6%) (177), smoking status (~9%) (178), and BMI (~8%) (178). Other variables with anticipated missing data were eGFR and ethnicity. We used multiple imputation by chained equations to impute missing data. Multiple imputation by chained equation are flexible and allow imputation of missing data from both continuous and categorical variables (179). An underlying assumption of this missing data approach is that data were missing at random (180). The multiple datasets created with multiple imputation also have the advantage of retaining the uncertainty of the imputed value, rather than treating it as a known, true value (180, 181).

We created imputation models to impute the missing values for blood pressure, smoking status, BMI, eGFR, and ethnicity. These models include all the following auxiliary variables: all the covariates measured before and on cohort entry (see **Table 1.1**), a time-to-event variable of the outcome, a binary indicator variable of the outcome (i.e., presence or absence of incident DR), variable-specific predictors, and past and future values of the missing variable. Variable-specific predictors were comorbidities and medications strongly associated with the variables of blood pressure, smoking status, BMI, eGFR and ethnicity. For the missing variables of A1c, blood pressure, BMI, eGFR, and smoking status, we used values measured in the year prior to the assessment window and in the first year of follow-up time to identify values for the imputation model. These values were used in the imputation model along with the other auxiliary variables to impute the missing value.

Five imputations were performed, generating five datasets. Imputed data from those datasets were combined (using Rubin's rules) to create the final missing variable values, presented with a mean of the 5 datasets. We did not include interactions between the auxiliary variables. For the continuous variables of age and diabetes duration, we used restricted cubic splines, which allowed us to avoid making assumptions regarding a linear relationship between variables.

3.6.6 Ethics

The protocol of this study (20_178R) has been approved by the CPRD's Independent Scientific Advisory Committee (ISAC) and by the CIUSSS West-Central Montreal Research Ethics Board (Montréal, Canada).

CHAPTER 4: ORIGINAL RESEARCH STUDY

4.1 Preface to original research study

The importance of assessing the impact of antihyperglycemic medications and insulins on diabetes-related outcomes (such as macrovascular complications) is increasingly recognized by regulatory agencies, guideline writing committees, researchers, clinicians, and patients. However, our literature review revealed that few studies have assessed the risk of DR among long-acting insulin analogue users. This clinically relevant endpoint has been most often reported as a secondary outcome, and it has rarely been broken out from other microvascular complications in this literature. For this reason, we designed and conducted a population-based, retrospective cohort study to address this knowledge gap.

4.2 Original research study

Long-acting insulin analogues and the risk of diabetic retinopathy among patients with type 2 diabetes

Short title: Long-acting insulin analogues and diabetic retinopathy

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4.1.1 Abstract

Background: Concerns regarding a possible association between long-acting insulin analogues and diabetic retinopathy (DR) first surfaced during the registration trials for insulin glargine. Our objective was to determine whether the use of long-acting insulin analogues was associated with an increased risk of incident DR among patients with type 2 diabetes (T2D).

Methods: Using data from the Clinical Practice Research Datalink Aurum, this retrospective, population-based cohort study included patients with T2D who initiated a long-acting insulin analogue (glargine, detemir, degludec) or Neutral Protamine Hagedorn (NPH) insulin. The primary outcome was incident DR. We used Cox proportional hazards models with inverse probability of treatment weighting to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for incident DR with insulin analogues vs NPH insulin.

Results: There were 66,280 new users of long-acting insulin analogues and 66,173 new users of NPH insulin. In the primary analysis, the incidence rate of DR was 101.7 per 1000 person-years (95% CI, 98.7-104.8) for insulin analogues and 93.2 (95% CI, 90.0-96.5) per 1000 person-years for NPH insulin. Compared with the current use of NPH insulin, insulin analogues were not associated with the risk of incident DR (HR 1.04, 95% CI, 0.99-1.09). The adjusted HRs were 0.84 (95% CI, 0.66-1.07) for proliferative DR and 1.02 (95% CI, 0.97-1.08) for non-proliferative DR.

Discussion: Compared with NPH insulin, long-acting insulin analogues were not associated with an increased risk of incident DR. This finding provides important reassurance regarding the safety of long-acting insulin analogues with respect to incident DR.

4.1.2 Introduction

Concerns about a possible link between insulin analogues and diabetic retinopathy (DR) first surfaced during the registration trials for the long-acting insulin analogue glargine. The first open label, randomized registration trial (2001) found that patients randomized to glargine had higher rates of DR progression at 28 weeks than those randomized to Neutral Protamine Hagedorn (NPH) (risk ratio [RR] 2.75; 95% confidence interval [CI] 1.10-6.91) (72, 145). A subsequent registration trial (2003) showed inconsistent results, with higher rates of clinically significant macular edema, but less progression of DR among patients randomized to glargine (140, 145). In response, an RCT was conducted in 2009 with progression of DR as a primary outcome that compared the risk with glargine versus NPH insulin(66). It showed no increased risk of DR progression after 5 years of follow-up. Available data regarding incident DR or DR progression for the long-acting insulin analogues determir and degludec are even more limited. DR was assessed as a secondary outcome in one of detemir's registration trial; while no difference was observed; data for DR were very sparse (72). Finally, very few observational studies assessed the safety of long-acting insulin analogues with respect to DR. Such studies are essential to assess the generalizability of those RCT results to the real-world setting.

There is a strong pathophysiological rationale supporting a potential increased risk of incident DR and DR progression with insulin analogues. The upregulation of IGF-1 is a well described component of the pathophysiology of DR (98, 101, 103), and in vitro studies demonstrated that glargine has a much higher IGF-1 receptor affinity and mitogenic potency on cell lines than human insulin (130-132). While it has not been shown for other long-acting analogues and it remains to be seen if this increased affinity leads to increased mitogenicity outside of tumor cell lines (130, 134-137), insulin analogues could potentially lead to DR independently of their affinity for the IGF-1 receptor (139).

Given heterogeneous evidence of the DR risk of insulin glargine, the limited available information regarding the other long-acting insulin analogues, and the pathophysiological rationale supporting an increased risk, the objective of our present study was to determine if long-acting insulin analogues were associated with an increased risk of incident DR compared to NPH insulin among patients with type 2 diabetes (T2D).

4.1.3 Methods

4.1.3.1 Clinical Practice Research Datalink

We conducted a retrospective, population-based cohort study using data from the Clinical Practice Research Datalink (CPRD) Aurum, linked to Hospital Episode Statistics (HES) Admitted Patient Care (APC) hospitalization data, and Office of National Statistics (ONS) death registration data. The CPRD has been described in detail previously (159, 160). Briefly, it is one of the world's largest databases of longitudinal, anonymized medical records from general practitioners (159). CPRD data include demographic details, patients' diagnoses, various symptoms and signs that have been recorded in the electronic chart, the patients' medications prescribed by the general practitioner, and ancillary tests performed and their results (159). It includes over 19 million patients (163). Of note, over 98% of the general English population is assigned to a general practitioner, who is considered the gatekeeper of the National Health Service (NHS) in the UK and provides care for most patients with T2D (163). CPRD Aurum covers approximately 13% of the English population and is representative of the English population for age, sex, geographical spread, and socioeconomical deprivation (163).

CPRD Aurum uses a combination of coding systems to render accessible the data, which are collected as coded elements of the medical record. The medical history observations such as diagnoses and symptoms are coded with SNOMED CT (UK Edition), Read Version 2, and local EMIS Web ® codes (163), and are cross-mapped to the CRPD Aurum Code Browser's medical term dictionary (164). Medications prescribed by general practitioners recorded in the EMIS Web ® electronic medical record are coded using the Dictionary of Medicines and Devices (dm + d) in CPRD Aurum (163). The validity of diagnoses and prescription information recorded into the CPRD Aurum have been validated previously and are of high quality (161, 164).

HES APC data contain all relevant data on inpatient admissions in England, including date of admission, date of discharge and primary and secondary diagnoses made during the hospitalization stay (161, 162). The ONS registers all deaths in the UK, along with the deceased's age, sex, cause of death and geographical area. Diagnostic data in HES APC and ONS are recorded using International Classification of Disease (ICD) 10 codes.

The protocol of this study (20_178R) has been approved by the CPRD's Independent Scientific Advisory Committee (ISAC) and by the CIUSSS West-Central Montreal Research Ethics Board (Montréal, Canada). The study protocol was made available to journal reviewers.

4.1.3.2 Study population

Our study population included patients with T2D who initiated a long-acting insulin analogue (glargine, detemir, or degludec) or NPH insulin between September 2002 (when insulin analogues first became available in the UK) and December 2019. The date of their first prescription of any long-acting insulin analogue or NPH insulin defined entry into the study cohort. Inclusion was restricted to patients who were linkable to HES APC and ONS vital statistics data. Exclusion criteria were: 1) age < 18 years at cohort entry, 2) a recorded history of type 1 diabetes any time before cohort entry, 3) a history of DR or retinopathy of any cause any time before or at cohort entry, 4) use of imatinib, acitretin, nicotinic acid, rituximab, taxanes, interferon, zilovudine, rifabutine, or fingolimod (drugs known to cause or associated with retinopathy)(167) in the year before cohort entry, 5) less than 365 days of recorded CPRD history at cohort entry (to determine new use and assess comorbidities), 6) prescription of both NPH and a long acting insulin analogue on the day of cohort entry, 7) a history of gestational diabetes in the year before or at cohort entry, and 8) the absence of a T2D diagnostic code before or at cohort entry. In molecule-specific analyses, the study period was restricted to the periods of availability of each long-acting insulin analogue to avoid violating the positivity assumption. In all analyses, patients were followed until the occurrence of a study outcome (defined below) or censoring due to death of any cause (recorded in CPRD, HES, or ONS), end of CPRD practice registration, end of HES data coverage, the last date of data collection in CPRD, or end of the study period (December 31, 2019), whichever occurred first.

4.1.3.3 Exposure

In our primary analysis, we used an 'as-treated' exposure definition. In this time-fixed definition, exposure was determined by the insulin prescribed at cohort entry. Using recorded prescriptions of insulin, we classified patients into one of two mutually-exclusive treatment groups: A) current use of a long-acting insulin analogue (glargine, detemir, or degludec) without a

prescription for NPH insulin (the exposure of interest); or B) current use of NPH insulin without a prescription for long-acting insulin analogues (the reference group). In the as-treated exposure definition, discontinuation of the cohort entry defining insulin (defined as a gap of 60 days or more between consecutive prescriptions) or treatment crossover (defined by the initiation of the other type of insulin) resulted in censoring.

NPH insulin was used as comparator because it is a clinically relevant comparator: it was the immediate predecessor to the new, long-acting analogue insulins, but is still widely used as it is less costly. NPH insulin is used for the same purposes as long-acting insulin analogues (i.e., mainly as a basal insulin) at the same point in the management of T2D. Consequently, the use of NPH insulin as the comparator avoided time-lag bias (168) and reduced potential confounding by indication and by other variables.

4.1.3.4 Outcome

The primary endpoint was incident DR (including both non-proliferative and proliferative), defined using relevant diagnostic codes recorded in the CPRD or HES. Secondary endpoints were: 1) incident non-proliferative DR, 2) incident proliferative DR, and 3) unspecified type of DR. DR was defined by the presence of ICD-10 code H36.0 for HES-defined events, with the date of hospital admission defining the event date, and by relevant SNOMED codes in the CPRD, with the date of diagnosis defining the event date.

4.1.3.5 Covariates

The following covariates, identified from the literature as variables related to diabetes duration and severity, were measured at cohort entry: age, sex, ethnic origin, body mass index (BMI), smoking status (ever, never, missing; measured in the last 5 years), most recent hemoglobin A1c, systolic and diastolic blood pressure, duration of diabetes, presence of hypertension, presence of cardiovascular disease (coronary artery disease, heart failure, stroke, or peripheral vascular disease), presence of diabetic neuropathy, presence of CKD from diabetic nephropathy, presence of CKD from any cause, presence of dialysis, baseline eGFR category ($\geq$ 90, 60-89, 45-59, 30-44, 15-29, < 15 ml/min/1.73 m^2, missing), prescribed anti-diabetic drugs in the year before cohort entry, relevant non-diabetic drugs prescribed in the year before cohort entry, and hospitalizations in the year before cohort entry. We also adjusted for use of fenofibrate in the previous year as it is

a protective drug for retinopathy (169, 170) and for history of human immunodeficiency virus (HIV) and history of bariatric surgery, two risk factors for retinopathy. Comorbidities were assessed any time prior to cohort entry, while medications, blood pressure, and laboratory test results were assessed in the year before cohort entry. Continuous variables of age and diabetes duration were modelled flexibly using restricted cubic splines.

4.1.3.6 Statistical analyses

Discrete data are presented as counts and percentages, and continuous data as means and standard deviation. For continuous variables with skewed distributions, medians and inter-quartile ranges were used. Absolute values of standardized differences were estimated to assess covariate balance, with absolute values of 10% or more considered to be important (174). We estimated incidence rates and 95% CIs for DR overall and by exposure group using the Poisson distribution.

In our primary analysis, we estimated a PS using a logistic model that included the pre-defined covariates described above as independent variables and treatment as the dependent variable. To estimate the HRs and its corresponding 95% CIs for incident DR for long-acting insulin analogues vs NPH insulin, we constructed Cox proportional hazards models with time to incident DR as our outcome model with inverse probability of treatment weighting (IPTW) by propensity score and a time axis of follow-up duration (175). IPTW weights greater than 10 were truncated. We used multiple imputation by chained equations to impute missing data, imputing 5 datasets and combining results using Rubin's rules.

We conducted 5 pre-specified secondary analyses. First, we repeated our primary analyses for the individual endpoints of 1) non-proliferative DR; 2) proliferative DR, and 3) unspecified DR. Second, we repeated the primary analysis stratified by prior history of diabetic nephropathy (none vs present, defined as presence of diagnostic code or eGFR of 59 ml/ml/1.73m^2 or less) and by prior history of macrovascular complications (defined as any history of coronary artery disease, heart failure, stroke, or peripheral vascular disease). Third, we repeated the primary analysis stratified by duration of diabetes (0-5, >5-10, >10 years). Fourth, we repeated the primary analysis with exposure subclassified by type of analogue insulin (glargine, detemir, and degludec). Finally, we stratified by duration of use of insulin type (0-1, >1-2, >2-5, and >5 years).

Seven sensitivity analyses were also conducted. These analyses are described in the **Supplementary Methods**.

4.1.4 Results

Our cohort included a total of 66,244 patients with T2D who initiated a long-acting insulin analogue or NPH insulin (**Figure 4.1)**. Mean follow up time was 430.1 days (standard deviation 609.2 days) and the median was 180 days (IQR 88-486 days). The baseline characteristics of the included patients are presented in **Table 4.1** and **Supplementary Table 4.5**. Patients were relatively well balanced between treatment groups before weighting. However, differences were present in year of cohort entry, which were likely due to differences in the timing of market entry of the long-acting insulin analogues. While diabetes duration and mean A1c were similar between groups, an A1c above 8% was more prevalent among patients using long-acting insulin analogues than among those using NPH (74.6% vs 67.2%). Some differences were also present in use of oral antihyperglycemic drugs in the previous year, the prevalence of myocardial infarction, and hospital admissions in the previous year. Following imputation and weighting, no patient characteristics had a standardized difference > 0.1.

Table 4.2 presents the results of our primary analysis. The incidence rate of DR was 101.7 per 1000 person-years (95% CI, 98.7-104.8) for insulin analogues and 93.2 (95% CI, 90.0-96.5) per 1000 person-years for NPH insulin. Compared with the current use of NPH insulin, the current use of long-acting insulin analogues was not associated with an increased risk of incident DR (adjusted HR 1.04, 95% CI, 0.99-1.09). **Table 4.2** also presents the secondary analyses for proliferative, non-proliferative, and unspecified type of DR. Compared with NPH insulin, long-acting insulin analogues were not associated with the risks of proliferative (adjusted HR 0.84, 95% CI 0.66-1.07) or non-proliferative DR (adjusted HR 1.02, 95% CI, 0.97-1.08). However, long-acting insulin analogues were associated with a modestly increased risk of unspecified type of DR (adjusted HR 1.10, 95% CI 1.02-1.18). Molecule-specific analyses did not identify differences in the risk of incident DR compared with NPH insulin (**Table 4.3)**.

Table 4.4 presents the risk of incident DR with long-acting insulin analogue vs NPH insulin among clinically important subgroups. Long-acting insulin analogues were associated with a reduced risk of incident DR compared with NPH insulin among users who had been on insulin for more than 5 years (adjusted HR 0.82, 95% CI 0.69-0.97), while no difference was observed among

those using insulin for < 1 year or 1-5 years. Similarly, no differences were observed across subgroups defined by duration of diabetes, the presence of macrovascular complications, or the presence of diabetic nephropathy, with all estimated HRs between 1.02 and 1.12 and overlapping 95% CIs.

The results of sensitivity analyses are reported in **Table 4.6**. These sensitivity analyses produced results that were consistent with those of our primary analysis.

4.1.5 Discussion

Our study was designed to compare the risk of incident DR with the current use of long-acting insulin analogues to that with the current use of NPH insulin among patients with T2D. We found that, compared with NPH insulin, long-acting insulin analogues were not associated with an increased risk of incident DR. Similar findings were observed for proliferative and non-proliferative DR, in molecule-specific analyses, and across clinically important subgroups. We did observe a modestly increased risk of incident DR of unspecified type with long-acting insulin analogues (adjusted HR 1.10, 95% CI 1.02-1.18) and a decreased risk with long-acting insulin analogues among users who have been on insulin for more than 5 years (adjusted HR 0.82, 95% CI 0.69-0.97). Results were consistent across several sensitivity analyses, with estimated HRs ranging from 1.01 to 1.05, with overlapping 95% CIs, suggesting that results are robust to study assumptions.

Our results are reassuring regarding the risk of long-acting insulin analogues with respect to incident DR and suggest that patients should be able to benefit from the use of these drugs without concerns about an increased risk of DR. While we did observe a 10% increased risk of DR of unspecified type, there is no biological rationale to support an increased risk of this subtype but not of proliferative or non-proliferative DR, suggesting that this is likely a chance finding. Furthermore, given the magnitude of the estimated increased risk, it is unlikely to be clinically significant. We also observed a decreased risk of incident DR with long-acting insulin analogues among patients who had been on insulin for at least 5 years. This observed protective association may be explained by a depletion of susceptible patients over time, but this finding requires confirmation in subsequent studies.

In terms of implications for future research, we need more well-powered, 'real world' observational studies examining the risk of long-acting insulin analogues with respect to DR

progression. In addition, while we found no evidence of increased risk of incident DR for each specific molecule, we suggest a similar study to ours be repeated once more data have been accumulated for degludec or the use of databases from multiple jurisdictions to obtain more precise estimates of the molecule-specific incident DR risk. Observational studies with longer follow-up time and different analytical approaches for confounding mitigation would also help consolidate our findings. These analytical approaches could include the use of inverse probability of censoring weights or other approaches to address potential selection bias resulting from informative censoring. Finally, there remains a need to assess the real-world effects of long-acting insulin analogues with respect to other microvascular complications. As for policy implications, diabetes management guideline writing committees and drug plan managers for third party payers may want to consider our findings, which reduce the uncertainty surrounding the risk of incident DR and could inform their assessments of long-acting insulin analogues, which balance medications costs with their impacts on clinical outcomes including complications and quality of life.

The very first clinical studies to suggest a link between the long-acting insulin analogues and DR risk were the randomized controlled registration trials for glargine. In the Rosenstock (2001) registration trial, patients in the glargine insulin arm had higher rates of three steps or more of DR progression (RR of 2.75, 95% CI 1.10-6.91), despite the presence of a small initial imbalance in DR at baseline that favored glargine. (72, 145). The Massi (2003) registration trial showed more incident macular edema among patients randomized to glargine (11.2%) than among those randomized to NPH (6.5%) at 52 weeks, but the former had a protective RR for DR progression of 0.65 (95% CI: 0.31-1.37) (72). Overall, these two registration trials produced inconsistent results. Furthermore, they had relatively short follow-up durations (28 and 52 weeks, respectively). Of note, the other glargine registration trials did not report on DR, and the follow-up was too short to assess long-term DR risk (140, 145). The only published RCT that has compared DR progression between a long-acting analogue (glargine) and NPH as a primary outcome was an open-label, multicenter non-inferiority trial that followed patients with T2D randomized to either NPH twice-daily (n = 509) or glargine once daily (n= 515) for 5 years (66). The study found no difference in DR progression between the two groups (RR of 0.86, 95% CI, 0.63-1.18 for glargine)(72). Two other recent experimental studies (one RCT, and one cross-over study) comparing glargine and NPH found no increased risk of DR progression, but the latter was a secondary outcome and data was sparse (146, 151). The follow-up durations were short (36

weeks or less), and one of them (Betônico 2019) did not report any DR progression event (146). Two RCTs compared detemir and NPH for efficacy and safety, finding no increased risk of DR progression as a secondary outcome (72). However, follow-up was short (26 weeks). A detemir registration trial (2005) reported a signal for an elevated risk of DR progression with detemir versus NPH (RR: 2.24, 95% CI: 0.65-7.70), but results were inconclusive due to spare data and wide 95% CIs (72). Of note, to our knowledge no RCT has compared degludec to NPH with DR incidence or progression as an outcome. Our study brings novelty in this regard.

Only one retrospective cohort study of patients with T2D assessed the risk of DR among patients long-acting insulin analogues vs NPH insulin as a primary outcome (144). The study matched patients with T2D who initiated either glargine (n= 9141), detemir (n= 1413), or NPH (n = 36,185) on propensity scores (PS) and other relevant characteristics (144). The adjusted incidence rate ratios for the development of sight-threatening DR were 0.93 (95% CI 0.8-1.1) for glargine vs NPH and 1.10, 95% CI (0.8-1.4) for detemir vs NPH (144). Using the full, unmatched cohort, glargine was associated with a reduced risk in both the intention-to-treat analysis (HR 0.70, 95% 0.63-0.77) and the unmatched, time-varying use analysis (HR 0.64, 95% CI 0.58-0.71) (144). Differences in statistical analyses most likely caused residual confounding and resulting protective HRs in the unmatched cohort. Other major limitations of this study were its use of sight-threatening DR only as an outcome and the absence of information on baseline presence of DR or degree of severity, diabetes duration, and blood pressure (144). The latter two were controlled for in our study, and patients with prevalent retinopathy of any type at cohort entry were excluded by design.

Our study has several strengths. First, we minimized confounding by indication and by other variables through our use of an active comparator, rigorous statistical approaches including the use of IPTW, and the availability of detailed clinical data not typically found in administrative databases. The use of an active comparator used at the same point in the management of T2D also avoided time lag bias (168). Second, we examined incident DR (instead of the more commonly used progression of DR), a clinically relevant endpoint associated with substantial morbidity, decreased quality of life, and important treatment costs that has been understudied in the literature. Finally, the CPRD has been validated extensively (164) and provided the sample size required to estimate precise treatment effects and to examine molecule-specific risks.

Our study also has some potential limitations. First, it is observational and thus may be affected by confounding. However, as discussed above, we used rigorous methods to limit its potential

effects. Second, the CPRD does not record information regarding patient adherence or capture prescriptions from specialists. Thus, exposure misclassification is possible. This bias would likely be non-differential misclassification, and thus bias the HR towards the null hypothesis. Outcome misclassification is also possible, as retinopathy from another cause (e.g., HIV, hypertension) may be misdiagnosed as DR (or vice versa). However, we expect this misclassification to be non-differential and minimal with the well validated diagnostic medical codes of the CPRD (164). We also excluded patients using medications known to cause retinopathy, minimizing the risk of outcome misclassification. Fourth, DR is often asymptomatic until it is advanced and is often only identified during screening, which is conducted every 1 to 2 years (182). Consequently, misclassification of the event date is possible, and some events may have only been diagnosed after censoring. For this reason, we also conducted an intention-to-treat analysis, which produced results that were consistent with those of our primary analysis. On the other hand, DR screening was not measured in our primary analysis, but a sensitivity analysis was conducted that excluded patients without a screening test in the year prior to cohort entry to minimize potential detection bias. Sixth, our analyses of degludec produced imprecise estimates given its later entry to the market. Seventh, the follow-up duration in our study was relatively modest (median of 180 days, IQR 88-486), which may have contributed to the null findings. Additional studies with longer follow-up duration may be needed to assess longer term DR risks. Finally, our lack of information on insulin dosing and on short-acting insulins is also a limitation.

4.1.6 Conclusions

Compared with the current use of NPH insulin, the current use of long-acting insulin analogues is not associated with an increased risk of incident DR among patients with T2D. Similar findings were observed for proliferative and non-proliferative DR and in molecule-specific analyses. These results provide important reassurance for physicians and patients with T2D regarding the risk of incident DR of these commonly used drugs.

4.1.7 Acknowledgements

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4.1.8 Disclosures

RWP has received consulting fees from Biogen, Boehringer Ingelheim, Merck, Nant Pharma, and Pfizer for work unrelated to this project,

4.1.9 Authors' contributions

SL developed the study protocol under the supervision of KBF, created variables definitions, and contributed to the study design and statistical analysis. SL wrote the manuscript. CF conducted the data management and statistical analyses. All authors contributed to the study design, interpretation of data, critically reviewed the manuscript for important intellectual content, and approved the final version of the manuscript. KBF supervised this study and is the guarantor.

4.2 Tables and figures

Table 4.1 Baseline characteristics of patients with T2D who initiated a long-acting insulin analogue or NPH insulin in the UK, before and after inverse probability of treatment weighting and multiple imputation.

Characteristic	Full cohort, pre imputation and weighting			Full cohort, post imputation and post weighting		
	Long-acting insulin analogue	NPH insulin	Standardized mean difference	Long-acting insulin analogue	NPH insulin	Standardized mean difference
	N/mean (%/SD)	N/mean (%/SD)		N/mean (%/SD)	N/mean (%/SD)	
Patients	36,060	30,184		66,280	66,173	
Age (years), mean (SD)	61.1 (14.6)	62.2 (14.7)	0.07	61.6 (20.0)	61.6 (21.8)	0.00
Females n (%)	16,406 (45.5)	14,476 (48.0)	0.05	30,959 (46.7)	30,878 (46.7)	0.00
Ethnicity, n (%)						
White	26,063 (72.3)	21,775 (72.1)	0.00	50,906 (76.8)	50,842 (76.8)	0.00
Other	7,611 (21.1)	6,640 (22.0)	0.02	15,373 (23.2)	15,330 (23.2)	0.00
Unknown	2,386 (6.6)	1,769 (5.9)	0.03	- -	- -	-
Year of cohort entry, n (%)						
2002-2007	11,078 (30.7)	9,821 (32.5)	0.04	20,529 (31.0)	20,516 (31.0)	0.00
2008-2013	13,077 (36.3)	7,197 (23.8)	0.27	20,273 (30.6)	20,237 (30.6)	0.00
2014-2019	11,905 (33)	13,166 (43.6)	0.22	25,478 (38.4)	25,420 (38.4)	0,00
Diabetes duration (years)[a]						
Mean (SD)	9.0 (11.7)	8.9 (11.6)	0.00	9.0 (15.9)	9.0 (17.0)	0.00
Median (IQR)	7.2 (3.8 - 11.4)	7.1 (3.4 - 11.6)		7.1 (3.6 - 11.5)	7.1 (3.6 - 11.5)	
BMI (kg/m^2), mean (SD)[b]	31.1 (7.2)	31.1 (7.0	0.01	30.6 (10.1)	30.6 (10.7)	0.00
Smoking						
Never	108,21 (30.0)	8,319 (27.6)	0.05	20,487 (30.9)	20,432 (30.9)	0.00
Ever	23,744 (65.8)	19,881 (65.9)	0.00	45,793 (69.1)	45,741 (69.1)	0.00

Unknown	1,495 (4.1)	1,984 (6.6)	0.11	- -	- -	-
A1c						
Mean (SD)	9.8 (2.1)	9.8 (2.3)	0.04	9.8 (2.9)	9.8 (3.3)	0.00
≤ 7	2,197 (6.1)	2,833 (9.4)	0,12	5,188 (7.8)	6,667 (10.1)	0.08
7.1-8	4,081 (11.3)	3,251 (10.8)	0,02	8,268 (12.5)	7,752 (11.7)	0.02
> 8	26,892 (74.6)	20,292 (67.2)	0,16	52,823 (79.7)	51,754 (78.2)	0.04
Unknown	2,890 (8.0)	3,808 (12.6)	0,15	- -	- -	-
Systolic blood pressure, mean (SD)[b]	132.4 (16.7)	132.1 (17.3)	0.01	132.1 (22.19)	132.2 (25.4)	0.00
Diastolic blood pressure, mean (SD)[b]	76.8 (10.2)	76.3 (10.2)	0.05	76.6 (13.9)	76.6 (15.1)	0.00
Comorbidities, n (%)						
Heart failure	3,713 (10.3)	3,778 (12.5)	0.07	7,503 (11.3)	7,505 (11.3)	0.00
Myocardial infarct	3,338 (9.3)	3,913 (13.0)	0.12	7,165 (10.8)	7,187 (10.9)	0.00
CAD	9,662 (26.8)	9,246 (30.6)	0.08	18,806 (28.4)	18,806 (28.4)	0.00
Stroke	2,558 (7.1)	2,514 (8.3)	0.05	5,089 (7.7)	5,092 (7.7)	0.00
CeVD	3,856 (10.7)	3,672 (12.2)	0.05	7,538 (11.4)	7,538 (11.4)	0.00
PVD	2,747 (7.6)	2,605 (8.6)	0.04	5,338 (8.1)	5,352 (8.1)	0.00
Neuropathy	1,617 (4.5)	1,244 (4.1)	0.02	2,880 (4.3)	2,889 (4.4)	0.00
Dyslipidemia	17,972 (49.8)	14,351 (47.5)	0.05	32,321 (48.8)	32,218 (48.7)	0.00
CKD	6,033 (16.7)	5,023 (16.6)	0.00	11,126 (16.8)	11,155 (16.9)	0.00
CKD-DN	2,999 (8.3)	2,284 (7.6)	0.03	5,280 (8.0)	5,266 (8.0)	0.00
Dialysis	252 (0.7)	241 (0.8)	0.01	487 (0.7)	487 (0.7)	0.00
HIV	41 (0.1)	32 (0.1)	0.00	71 (0.1)	69 (0.1)	0.00
Hypertension	22,709 (63.0)	19,160 (63.5)	0.01	41,856 (63.2)	41,817 (63.2)	0.00
Bariatric surgery	102 (0.3)	97 (0.3)	0.01	203 (0.3)	200 (0.3)	0.00
Antihyperglycemic drugs n (%)						
Metformin	27,866 (77.3)	21,758 (72.1)	0.12	49,621 (74.9)	49,480 (74.8)	0,00
Sulfonylureas and meglitinides	24,815 (68.8)	19,611 (65.0)	0.08	44,449 (67.1)	44,381 (67.1)	0,00
DPP4 inhibitors	7,250 (20.1)	6,463 (21.4)	0.03	13,858 (20.9)	13,823 (20.9)	0,00

SGLT2 inhibitors	1,703 (4.7)	1,566 (5.2)	0.02	3,324 (5)	3,324 (5)	0,00
GLP1-RA	3,290 (9.1)	2,668 (8.8)	0.01	6,050 (9.1)	6,090 (9.2)	0,00
Thiazolidinediones	12,667 (35.1)	8,475 (28.1)	0.15	21,210 (32.0)	21,129 (31.9)	0,00

Abbreviations: CAD, coronary artery disease; CeVD, Cerebrovascular disease; CKD-D, CKD due to diabetic nephropathy; DPP4i, Dipeptidyl peptidase-4 inhibitors; GLP1-RA, Glucagon-like peptide-1 receptor agonists; IQR, interquartile range; PVD, peripheral vascular disease; SD, standard deviation; SGLT2i, Sodium glucose cotransporter -2 inhibitors

[a] Duration of type 2 diabetes, defined as time since first diagnostic HbA1c, diagnostic code, or initiation of antihyperglycemic medication

[b] Percentage of missing data presented in supplementary material, along with categorical presentation of the data

Table 4.2 Association between current use of long-acting insulin analogues vs NPH insulin and the risk of incident diabetic retinopathy among patients with type 2 diabetes, overall and by diabetic retinopathy subtype.

	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted	Adjusted*
					HR (95% CI)	HR (95% CI)
DR						
Long-acting insulin analogues	36,060	4,374	43,008.5	101.7 (98.7-104.8)	1.09 (1.04-1.14)	1.04 (0.99-1.09)
NPH insulin	30,182	3,261	34,987.5	93.2 (90.0-96.5)	1.00 (Reference)	1.00 (Reference)
PDR						
Long-acting insulin analogues	36,060	149	54,084.4	2.8 (2.3-3.2)	0.87 (0.68-1.10)	0.84 (0.66-1.07)
NPH insulin	30,182	128	41,639.2	3.1 (2.6-3.7)	1.00 (Reference)	1.00 (Reference)
NPDR						
Long-acting insulin analogues	36,060	3,305	72,486.4	72.5 (70.0-75.0)	1.07 (1.02-1.13)	1.02 (0.97-1.08)
NPH insulin	30,182	2,469	36,642.6	67.4 (64.8-70.1)	1.00 (Reference)	1.00 (Reference)
Unspecified DR						
Long-acting insulin analogues	36,060	2,044	48,909.1	41.8 (39.1-43.6)	1.15 (1.07-1.23)	1.10 (1.02-1.18)
NPH insulin	30,182	1,414	38,814.6	36.4 (34.6-38.4)	1.00 (Reference)	1.00 (Reference)

Abbreviations: CI, confidence interval; DR, diabetic retinopathy; HR, Hazard ratio; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

* The following baseline characteristics were included in the propensity score model used for inverse probability of treatment weighting: age,sex, year of cohort entry, duration of diabetes, BMI, smoking status, A1c level, blood pressure level, eGFR category, comorbidities, antidiabetic drugs, other drug classes, number of hospitalizations in the year before cohort entry

Table 4.3 Association between current use of molecule-specific long-acting insulin analogues vs NPH insulin and the risk of overall incident diabetic retinopathy among patients with type 2 diabetes.

	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Glargine	26,330	3,176	31,254.1	101.6 (98.1-105.2)	1.09 (1.04-1.15)	1.04 (0.99-1.09)
NPH insulin	30,179	3,261	34,984.4	93.2 (90.0-96.5)	1.00 (Reference)	1.00 (Reference)
Detemir	9,151	1,156	11,213.6	103.1 (97.2-109.2)	1.10 (1.03-1.18)	1.01 (0.93-1.09)
NPH insulin	26,394	2,758	29,553.2	93.3 (89.9-96.9)	1.00 (Reference)	1.00 (Reference)
Degludec	578	42	540.6	77.7 (56.0-105.0)	0.93 (0.69-1.27)	0.97 (0.63-1.50)
NPH insulin	14,695	1,237	14,773.1	83.7 (79.1-88.5)	1.00 (Reference)	1.00 (Reference)

Abbreviations: CI, confidence interval; HR, Hazard ratio

* The following baseline characteristics were included in the propensity score model used for inverse probability of treatment weighting: age, sex, year of cohort entry, duration of diabetes, BMI, smoking status, A1c level, blood pressure level, eGFR category, comorbidities, antidiabetic drugs, other drug classes, number of hospitalizations in the year before cohort entry

Table 4.4 Association between current use of long-acting insulin analogues vs NPH insulin and the risk of overall incident diabetic retinopathy among patients with type 2 diabetes, according to diabetes complications and duration, and to duration of insulin use.

Subgroups	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Macrovascular disease						
Present						
NPH insulin	12,586	1,308	15,122.9	86.5 (81.9-91.3)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	13,346	1,476	15,305.8	96.4 (91.6-101.5)	1.11 (1.03-1.20)	1.08 (0.998-1.16)
Absent						
NPH insulin	17,587	1,953	19,856.7	98.4 (94.0-102.8)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	22,712	2,897	27,697.9	104.6 (100.8-108.5)	1.06 (1.01-1.13)	1.02 (0.96-1.08)
Diabetic nephropathy						
Present						
NPH insulin	12,406	1,305	13,935.8	93.6 (88.6-98.9)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	12,984	1,436	14,182.7	101.3 (96.1-106.6)	1.08 (1.00-1.16)	1.04 (0.96-1.12)
Absent						
NPH insulin	17,768	1,956	21,038.6	93.0 (88.9-97.2)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	23,072	2,938	28,816.4	102.0 (98.3-105.7)	1.10 (1.03-1.16)	1.04 (0.98-1.11)
Duration of T2D[a]						
0-5 years						
NPH insulin	10,864	817	11,263.2	72.5 (67.7-77.7)	1.00 (Reference)	1.00 (Reference)

Subgroups	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Long-acting insulin analogues	12,193	1,109	13,815.6	80.3 (75.6-85.1)	1.11 (1.01-1.21)	1.06 (0.96-1.16)
5-10 years						
NPH insulin	9,308	1,044	11,964.3	87.3 (82.0-92.7)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	12,196	1,587	16,076.0	98.7 (93.9-103.7)	1.13 (1.05-1.22)	1.06 (0.98-1.15)
> 10 years						
NPH insulin	9,997	1,257	11,989.1	104.8 (99.1-110.8)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	11,647	1,524	13,339.5	114.2 (108.6-120.1)	1.09 (1.01-1.18)	1.03 (0.96-1.11)
Duration of insulin use						
≥ 1 year						
NPH insulin	30,182	1,657	17,475.8	94.8 (90.3-99.5)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	36,060	2,148	21,018.1	102.2 (97.9-106.6)	1.08 (1.01-1.15)	1.01 (0.95-1.08)
1 to ≤ 2 years						
NPH insulin	9,389	604	6,988.2	86.4 (79.7-93.6)	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	11,265	832	8,320.6	100.0 (93.3-107.0)	1.16 (1.04-1.28)	1.12 (1.00-1.25)
> 2-5 years						
NPH insulin	5,176	729	8,167.7	89,25438	1.00 (Reference)	1.00 (Reference)
Long-acting insulin analogues	6,123	1,022	9,797.3	104,3145	1.17 (1.06-1.29)	1.12 (1.01-1.23)
> 5 years						
NPH insulin	1,188	271	2,349.9	115,3241	1.00 (Reference)	1.00 (Reference)

Subgroups	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Long-acting insulin analogues	1,555	356	3,695.3	96,33854	0.84 (0.72-0.98)	0.82 (0.69-0.97)

Abbreviations: CI, confidence interval; HR, Hazard ratio; T2D, Type 2 diabetes

* The following baseline characteristics were included in the propensity score model used for inverse probability of treatment weighting: age, sex, year of cohort entry, duration of diabetes, BMI, smoking status, A1c level, blood pressure level, eGFR category, comorbidities, antidiabetic drugs, other drug classes, number of hospitalizations in the year before cohort entry

[a] Duration of type 2 diabetes, defined as time since first diagnostic HbA1c, diagnostic code, or initiation of antihyperglycemic medication

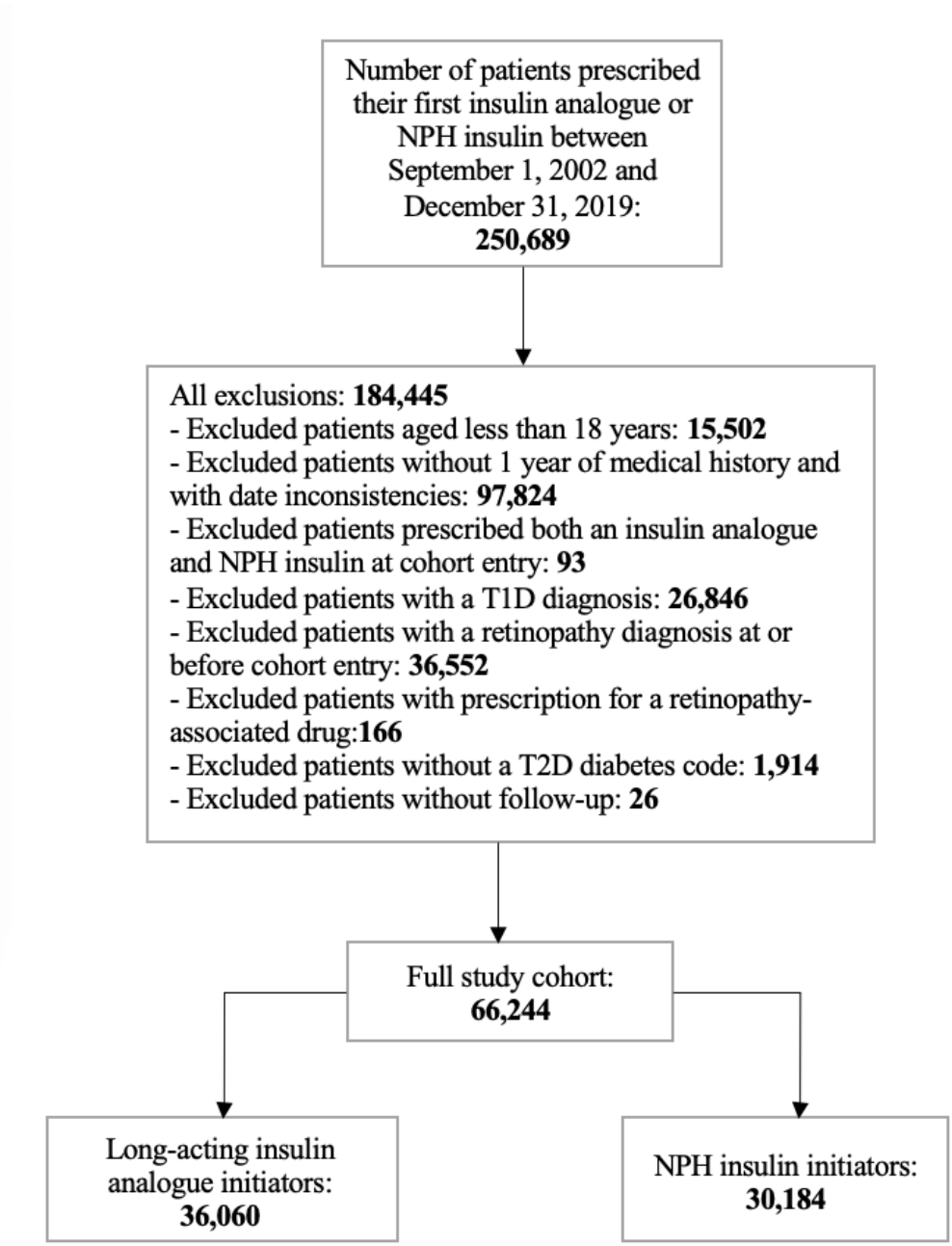
4.2.2 Figure legends

FIGURE 1.1 MANAGEMENT OF T2D AS PER THE DIABETES CANADA GUIDELINES: PHARMACOLOGIC GLYCEMIC MANAGEMENT OF T2D IN ADULTS, 2020 UPDATE..... 12

FIGURE 4.1 FLOWCHART OF THE RETROSPECTIVE COHORT STUDY LONG-ACTING INSULIN ANALOGUES VS NPH INSULIN AND THE RISK OF OVERALL INCIDENT DIABETIC RETINOPATHY AMONG PATIENTS WITH T2D. 74

4.2.3 Figures

Figure 4.1 Flowchart of the retrospective cohort study long-acting insulin analogues vs NPH insulin and the risk of overall incident diabetic retinopathy among patients with T2D.



4.3 Supplementary material

4.3.1 Supplementary Methods: sensitivity analyses

We conducted seven sensitivity analyses were conducted. First, treatment discontinuation was defined by gaps of 30 days and 90 days between consecutive prescription. Second, we excluded patients with a history of using GLP1-RAs, and censored upon their use, to avoid confounding due to increased rates of DR complications requiring ophthalmological interventions from rapid A1c correction with some GLP1-RAs (116). Third, we used an 'intention-to-treat' exposure definition in which exposure was defined by the cohort entry insulin and patients were followed for a maximum of one year. Patients were not censored upon discontinuation or crossover in this analysis. The maximum follow-up time was 1 year to avoid exposure misclassification that is inherent to intention-to-treat analyses. Fourth, we restricted our outcome definition to CPRD-defined events. Fifth, we repeated the analysis including only the patients who had at least one DR screening exam in the year before cohort entry (to avoid misclassification of prevalent as incident DR). Sixth, we excluded patients with a very high A1c at baseline (i.e., 9.0% or above). As for the GLP1-RAs, this is again a proxy to exclude patients who developed DR from a drastic and rapid decrease in glycemia. Finally, we excluded patients who were pregnant at the time of cohort entry, since pregnancy is a known risk factor for acute worsening of DR (176).

4.3.2 Supplementary tables and figures

Table 4.5 Additional baseline characteristics of patients with T2D who initiated a long-acting insulin analogue or NPH insulin in the UK, before and after inverse probability of treatment weighting and multiple imputation.

Characteristic	Full cohort, pre imputation and weighting			Full cohort, post imputation and post weighting		
	Long-acting insulin analogue	NPH insulin	Standardized mean difference	Long-acting insulin analogue	NPH insulin	Standardized mean difference
	N/mean (%/SD)	N/mean (%/SD)		N/mean (%/SD)	N/mean (%/SD)	
Patients	36,060	30,184		66,280	66,172	
Age group, n (%)						
18-35 years	1,674 (4.6)	1,577 (5.2)	0.03	3,277 (4.9)	3,358 (5.1)	0.01
36-45 years	3,696 (10.2)	2,759 (9.1)	0.04	6,397 (9.7)	6,545 (9.9)	0.01
46-55 years	7,388 (20.5)	4,954 (16.4)	0.11	12,503 (18.9)	12,095 (18.3)	0.02
56-65 years	8,832 (24.5)	7,253 (24)	0.01	16,015 (24.2)	16,048 (24.3)	0.00
66-75 years	7,964 (22.1)	7,735 (25.6)	0.08	15,706 (23.7)	15,586 (23.6)	0.00
76-85 years	5,208 (14.4)	4,861 (16.1)	0.05	10,046 (15.2)	10,143 (15.3)	0.00
>85 years	1,298 (3.6)	1,045 (3.5)	0.01	2,336 (3.5)	2,398 (3.6)	0.01
Diabetes duration group[a], n (%)						
<1 year	2,889 (8.0)	3,050 (10.1)	0.07	5,912 (8.9)	5,897 (8.9)	0.00
1-4.9 years	9,304 (25.8)	7,814 (25.9)	0.00	17,034 (25.7)	17,153 (25.9)	0.01
5-10 years	12,201 (33.8)	9,317 (30.9)	0.06	21,581 (32.6)	21,387 (32.3)	0.01

> 10 years	11,666 (32.4)	3,050 (10.1)	0.02	21,753 (32.8)	21,736 (32.8)	0.00
BMI						
< 30 kg/m^2	16,651 (46.2)	13,533 (44.8)	0.03	33,610 (50.7)	33,334 (50.4)	0.01
≥ 30 kg/m^2	17,717 (49.1)	14,554 (48.2)	0.02	32,670 (49.3)	32,839 (49.6)	0.01
Unknown	1,692 (4.7)	2,097 (6.9)	0.10	0 (0.0)	0 (0.0)	0.00
Blood Pressure Level						
DBP < 90 and SBP < 140	22,243 (61.7)	18,427 (61)	0.01	43,607 (65.8)	43,432 (65.6)	0.0
DBP ≥ 90 or SBP ≥ 140	11,794 (32.7)	9,583 (31.7)	0.02	22,673 (34.2)	22,742 (34.4)	0.00
Unknown	2,023 (5.6)	2,174 (7.2)	0.07	-	-	0.00
eGFR (ml/min)						
Mean (SD)	77.8 (25.5)	75.9 (26.3)	0.07	77.6 (35.0)	77.5 (38.7)	0.00
<15	147 (0.4)	171 (0.6)	0.02	353 (0.5)	376 (0.6)	0.00
15-29	1,119 (3.1)	1,059 (3.5)	0.02	2,254 (3.4)	2,348 (3.5)	0.01
30-44	2,726 (7.6)	2,548 (8.4)	0.03	5,522 (8.3)	5,803 (8.8)	0.02
45-59	3,871 (10.7)	3,405 (11.3)	0.02	7,915 (11.9)	7,976 (12.1)	0.00
60-89	12,535 (34.8)	9,683 (32.1)	0.06	25,238 (38.1)	24,268 (36.7)	0.03
≥90	12,654 (35.1)	9,616 (31.9)	0.07	24,998 (37.7)	25,403 (38.4)	0.01
Unknown	3,008 (8.3)	3,702 (12.3)	0.13	0 (0.0)	0 (0.0)	0.00

Number of Anti-hyperglycemic drugs prescribed in the year before cohort entry						
Mean (SD)	2.2 (1.2)	2.0 (1.3)	0.12	2.1 (1.7)	2.1 (1.9)	0.00
Median (IQR)	2 (1 – 3)	2 (1 – 3)		2 (1 – 3)	2 (1 – 3)	
0	3,610 (10.0)	4,319 (14.3)	0.13	7,917 (11.9)	7,936 (12.0)	0.00
1	6,395 (17.7)	6,183 (20.5)	0.07	12,563 (19.0)	12,523 (18.9)	0.00
2	12,843 (35.6)	9,754 (32.3)	0.07	22,551 (34.0)	22,540 (34.1)	0.00
3+	13,212 (36.6)	9,928 (32.9)	0.08	23,249 (35.1)	23,174 (35.0)	0.00
Other medications, n (%)						
Antiplatelets	14,811 (41.1)	11,947 (39.6)	0.03	26,583 (40.1)	26,468 (40.0)	0.00
DOAC	556 (1.5)	615 (2.0)	0.04	1,185 (1.8)	1,183 (1.8)	0,00
Warfarin	2,131 (5.9)	2,016 (6.7)	0.03	4,141 (6.2)	4,135 (6.2)	0,00
ACEI	15,915 (44.1)	13,224 (43.8)	0.01	29,029 (43.8)	28,977 (43.8)	0,00
ARBs	9,982 (27.7)	8,324 (27.6)	0.00	18,302 (27.6)	18,253 (27.6)	0,00
Beta-blockers	9,533 (26.40)	9,032 (29.9)	0.08	18,519 (27.9)	18,496 (28.0)	0,00
CCB	9,579 (26.6)	8,445 (28.0)	0.03	17,960 (27.1)	17,916 (27.1)	0,00
Thiazides	6,135 (17)	5,000 (16.6)	0.01	11,089 (16.7)	11,059 (16.7)	0,00
Loop diuretics	6,613 (18.3)	6,194 (20.5)	0.06	12,774 (19.3)	12,797 (19.3)	0,00
K-sparing diuretics	2,154 (6.0)	2,190 (7.3)	0.05	4,358 (6.6)	4,368 (6.6)	0,00
Other diuretics	22 (0.1)	13 (0)	0.01	35 (0.1)	37 (0.1)	0,00

Statins	25,222 (69.9)	19,641 (65.1)	0.10	44,777 (67.6)	44,694 (67.5)	0,00
Fenofibrate	663 (1.8)	432 (1.4)	0.03	1,104 (1.7)	1,109 (1.7)	0,00
Number of hospitalization in the year before cohort entry						
Mean (SD)	1.0 (3.4)	1.3 (4.2)	0.08	1.1 (5)	1.2 (5.7)	0.00
Median (IQR)	0 (0 – 1)	1 (0 – 1)		0 (0 – 1)	0 (0 – 1)	
0	21,028 (58.3)	14,920 (49.4)	0.18	35,912 (54.2)	35,805 (54.1)	0.00
1-2	11,474 (31.8)	11,144 (36.9)	0.11	22,628 (34.1)	22,632 (34.2)	0.00
3+	3,558 (9.9)	4,120 (13.6)	0.12	7,739 (11.7)	7,737 (11.7)	0.00

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; eGFR, estimated glomerular filtration rate; CCB, calcium channel blockers; DBP, diastolic blood pressure; DOAC, direct oral anti-coagulant; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation.

[a] Duration of type 2 diabetes, defined as time since first diagnostic HbA1c, diagnostic code, or initiation of antihyperglycemic medication

Table 4.6 Sensitivity analyses of association between current use of long-acting insulin analogues vs NPH insulin and the risk of overall incident diabetic retinopathy among patients with type 2 diabetes.

	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Grace period: 30 days[a]						
Long-acting insulin analogues	36,060	2,138	20,673.4	103.4 (99.1-107.9)	1.11 (1.05-1.19)	1.05 (0.99-1.12)
NPH	30,182	1,715	18,509.1	92.7 (88.3-97,2)	1.00 (Reference)	1.00 (Reference)
Grace period: 90 days[a]						
Long-acting insulin analogues	36,060	6,141	60,632.9	101.3 (98.8-103.9)	1.09 (1.05-1.13)	1.05 (1.004-1.09)
NPH	30,182	4,354	46,835.5	93.0 (90.2-95.8)	1.00 (Reference)	1.00 (Reference)
Intention-to-treat [b]						
Long-acting insulin analogues	36,060	3,077	31,364.9	98.1 (94.7-101.6)	1.08 (1.03-1.14)	1.01 (0.95-1.06)
NPH	30,182	2,347	25,934.9	90.5 (86.9-94.2)	1.00 (Reference)	1.00 (Reference)
GLP1-RAs exluded[c]						
Long-acting insulin analogues	32,770	3,696	31,098.6	118.8 (115.0-122.7)	1.11 (1.05-1.16)	1.05 (1.001-1.11)
NPH	27,511	2,784	25,685.7	108.4 (104.4-112.5)	1.00 (Reference)	1.00 (Reference)
A1c ≥ 9% excluded[d]						
Long-acting insulin analogues	13,556	1,452	15,691.4	92.5 (87.8-97.4)	1.10 (1.01-1.19)	1.04 (0.95-1.13)
NPH	11,831	1,010	11,997.6	84.2 (79.1-89.5)	1.00 (Reference)	1.00 (Reference)
DR restricted to CPRD events[e]						

	No. of patients	No. of events	Person-years	Incidence rate, per 1,000 person-years (95% CI)	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
Long-acting insulin analogues	36,060	4,224	43,252.2	97.7 (94.7-100.7)	1.05 (1.05-1.16)	1.05 (0.997-1.10)
NPH	30,182	3,119	35,230.7	88.5 (85.5-91.7)	1.00 (Reference)	1.00 (Reference)
Restricted to patients screened for DR [f]						
Long-acting insulin analogues	16,530	2,034	21,036.1	96.7 (92.5-101.0)	1.10 (1.02-1.17)	1.02 (0.95-1.10)
NPH	12,444	1,396	15,796.3	88.4 (83.8-93.1)	1.00 (Reference)	1.00 (Reference)
Pregnant women excluded[g]						
Long-acting insulin analogues	35,224	4,169	42,718.9	97.6 (94.7-100.6)	1.11 (1.06-1.16)	1.05 (1.002-1.10)
NPH	28,547	3,042	34,513.1	88.1 (85.0-91.3)	1.00 (Reference)	1.00 (Reference)

Abbreviations: CI, confidence interval; GLP1-RA, Glucagon-like peptide-1 receptor agonists; HR, Hazard ratio

* The following baseline characteristics were included in the propensity score model used for inverse probability of treatment weighting: age, sex, year of cohort entry, duration of diabetes, BMI, smoking status, A1c level, blood pressure level, eGFR category, comorbidities, antidiabetic drugs, other drug classes, number of hospitalizations in the year before cohort entry

[a] Grace periods of 30 and 90 days, respectively, for insulin re-prescription

[b] Sensitivity analysis with intention-to-treat approach

[c] Sensitivity analysis in which GLP1-RA users are excluded

[d] Sensitivity analysis in which patients with A1c 9% or above at baseline are excluded

[e] Sensitivity analysis in which outcome definition is restricted to CPRD-defined events

[f] Sensitivity analysis in which only patients with DR screening in the year prior to cohort entry are included

[g] Sensitivity analysis in which pregnant women were excluded

4.4 APPENDICES

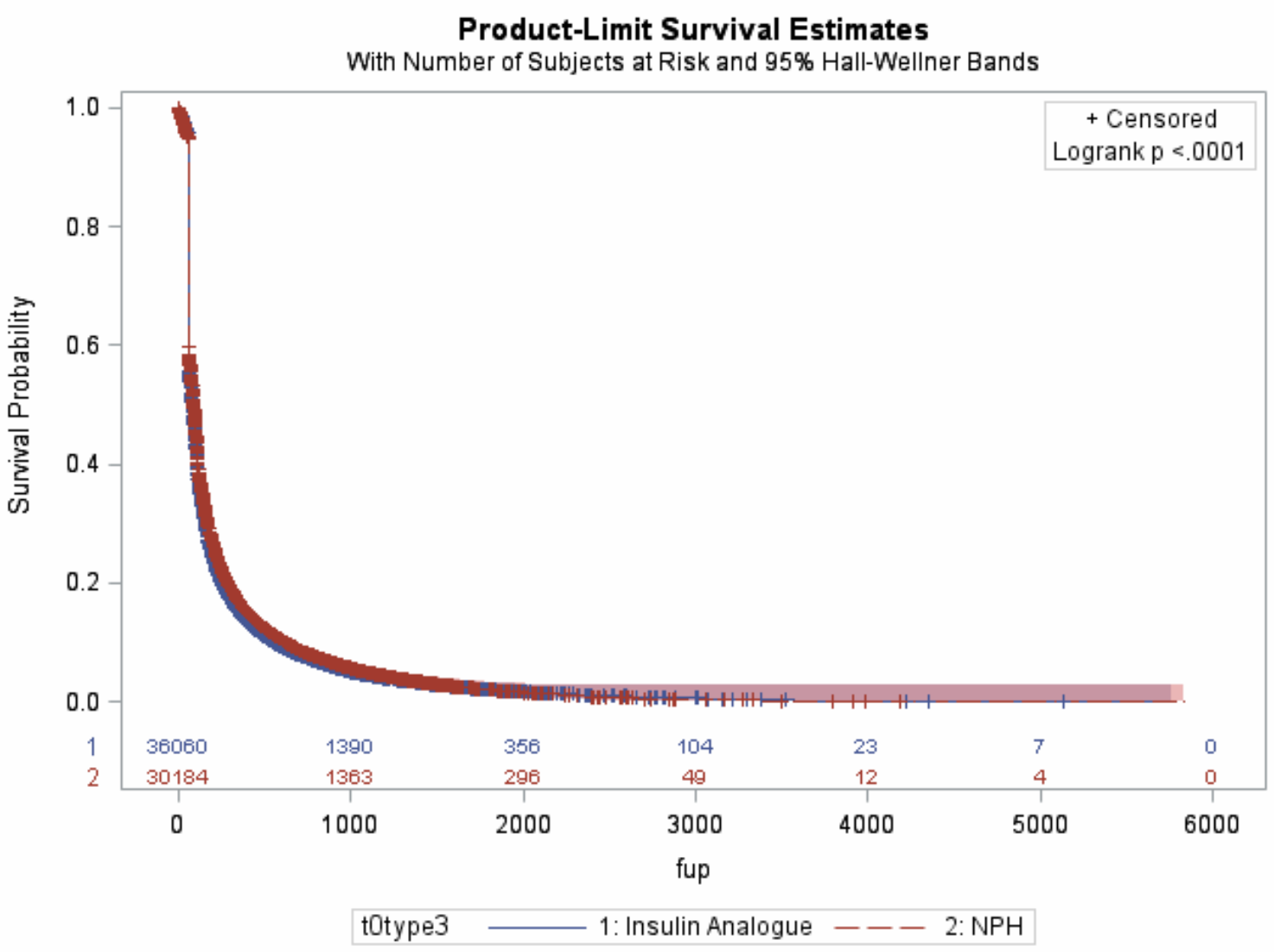
Appendix 1. SNOMED codes and their term descriptions for the components of the DR outcome: NPDR, PDR and unspecified DR.

NPDR		PDR		Unspecified DR	
SNOMED codes	Term	SNOMED codes	Term	SNOMED codes	Term
1785332013	Background diabetic retinopathy	98476015	Proliferative diabetic retinopathy	9093013	Diabetic retinopathy
2159973010	O/E - right eye background diabetic retinopathy	2474726011	Pan retinal photocoagulation for diabetes	347657010	Diabetic maculopathy
2159974016	O/E - left eye background diabetic retinopathy	975261000006113	O/E - left eye stable treated prolif diabetic retinopathy	2159979014	O/E - right eye diabetic maculopathy
297754014	Preproliferative diabetic retinopathy	733161000000116	Impaired vision due to diabetic retinopathy	1484887015	O/E - diabetic maculopathy present both eyes
2159975015	O/E - right eye preproliferative diabetic retinopathy	2159977011	O/E - right eye proliferative diabetic retinopathy	297758012	Diabetic retinopathy NOS
2159976019	O/E - left eye preproliferative diabetic retinopathy	2159978018	O/E - left eye proliferative diabetic retinopathy	914151000006112	Type 2 diabetes mellitus with retinopathy
1484867016	Non proliferative diabetic retinopathy	975251000006111	O/E - right eye stable treated prolif diabetic retinopathy	169731000006118	Retinal abnormality - diabetes related
1785332013	Background diabetic retinopathy	2549896013	O/E - sight threatening diabetic retinopathy	297755010	Advanced diabetic maculopathy
857031000006113	Left non-proliferative diabetic retinopathy	1785163015	High risk proliferative diabetic retinopathy	771411000006117	Insulin dependent diabetes mellitus with retinopathy
857051000006118	Left preproliferative diabetic retinopathy	841011000006112	High risk non proliferative diabetic retinopathy	938321000006116	Type 2 diabetes mellitus with exudative maculopathy

857971000006110	Right non-proliferative diabetic retinopathy	98476015	Proliferative diabetic retinopathy	455408014	Advanced diabetic retinal disease
857981000006113	Right preproliferative diabetic retinopathy	1785163015	High risk proliferative diabetic retinopathy	641581000006115	Non-insulin-dependent diabetes mellitus with retinopathy
841011000006112	High risk non proliferative diabetic retinopathy	856611000006114	Laser treated diabetic retinopathy	914161000006114	Type II diabetes mellitus with retinopathy
297754014	Preproliferative diabetic retinopathy	856631000006115	Left laser treated diabetic retinopathy	84991000006112	Type II diabetes mellitus with retinopathy
2159973010	O/E - right eye background diabetic retinopathy	857421000006119	Right laser treated diabetic retinopathy	84621000006117	Type 2 diabetes mellitus with retinopathy
2159974016	O/E - left eye background diabetic retinopathy	856621000006118	Left proliferative diabetic retinopathy	913651000006115	Insulin dependent diabetes mellitus with retinopathy
2159975015	O/E - right eye preproliferative diabetic retinopathy	733161000000116	Impaired vision due to diabetic retinopathy	297758012	Diabetic retinopathy NOS
2159976019	O/E - left eye preproliferative diabetic retinopathy	857411000006110	Right proliferative diabetic retinopathy	84621000006117	Type 2 diabetes mellitus with retinopathy
		2159977011	O/E - right eye proliferative diabetic retinopathy	84991000006112	Type II diabetes mellitus with retinopathy
		975261000006113	O/E - left eye stable treated prolif diabetic retinopathy	641581000006115	Non-insulin-dependent diabetes mellitus with retinopathy
		975251000006111	O/E - right eye stable treated prolif diabetic retinopathy	914151000006112	Type 2 diabetes mellitus with retinopathy
		2549896013	O/E - sight threatening diabetic retinopathy	914161000006114	Type II diabetes mellitus with retinopathy

	771411000006117	Insulin dependent diabetes mellitus with retinopathy
	913651000006115	Insulin dependent diabetes mellitus with retinopathy
	9093013	Diabetic retinopathy

Appendix 2. Kaplan-Meyer curves for long-acting insulin analogue and NPH use during follow up time of the retrospective cohort study



CHAPTER 5: DISCUSSION

5.1 Summary

This thesis investigated the risk of incident DR among adults with T2D using long-acting insulin analogues versus NPH insulin. The rationale behind this thesis was to address the concerns about a possible link between long-acting insulin analogues and DR that surfaced during the registration trials for glargine. The first registration trial, published in 2001, revealed that patients randomized to glargine had higher rates of DR progression on the ETDRS severity scale at 28 weeks than those randomized to NPH (RR 2.75, 95% CI 1.10-6.91) (72, 145). Other registration trials for glargine had either inconsistent results, did not report DR data, or had follow-up durations that were too short to assess long-term DR risk (140, 145). One registration trial (2005) reported a signal for an elevated risk of DR progression with detemir versus NPH (RR: 2.24, 95% CI: 0.65-7.70), but results were inconclusive due to spare data and wide 95% CIs (72). Only one RCT (Rosenstock, 2009) was subsequently designed to assess the risk of DR with long-acting insulin analogues, comparing glargine to NPH insulin with progression of DR as a primary outcome (66). This non-inferiority trial showed no increased risk of DR progression after 5 years of follow-up. While reassuring, glargine was the only long-acting insulin analogue tested, and the results of such RCTs are not typically generalizable to the real-world setting. To date, only one observational cohort study compared the risk of DR among patients using long-acting analogues versus NPH (144), but it predates the advent of degludec, had incident sight-threatening DR as its primary outcome, and many several other limitations. It is important to examine the risk of DR for long-acting insulin analogues given their increasing use among patients with T2D and the morbidity, reduced quality of life, and costs associated with incident DR.

To address this important knowledge gap, we conducted a retrospective, population-based cohort study using data extracted from the CPRD. The CPRD is a large database of longitudinal, anonymized medical records from general practitioners in the UK (159). With its large sample size, long observation period and detailed clinical data, it is well suited for this research question. We found that, compared with NPH insulin, long-acting insulin analogues were not associated with an increased risk of incident DR. We observed similarly reassuring findings for proliferative and

non-proliferative DR, in molecule-specific analyses, and across clinically important subgroups (such as duration of diabetes, duration of insulin use and associated diabetic complications). Our observations of a slightly increased risk of incident DR of unspecified type with long-acting insulin analogues (adjusted HR 1.10, 95% CI 1.02-1.18) and a decreased risk with long-acting insulin analogues among users who have been on insulin for more than 5 years (adjusted HR 0.82, 95% CI 0.69-0.97) are discussed further below. Our seven sensitivity analyses demonstrated consistent results, with estimated HRs ranging from 1.01 to 1.05, suggesting that the main analyses' results are robust to study assumptions.

The originality of our study was to assess overall incident DR as our primary outcome and use all long- and ultra-long-acting insulin analogues as exposures of interest, compared to NPH. Unlike previous studies, patients with pre-existing DR were excluded to better assess the role of long-acting insulin analogues on the early developments of DR and to limit the confounding of rapid glycemic improvement. While the latter is a known risk factor for progression of pre-existing DR, it has not been demonstrated for incident DR (86). The inclusion of degludec and its comparison to NPH is novel as well. Indeed, no RCTs were found that compared degludec to NPH with respect to this clinically important outcome. The use of an observational, retrospective cohort study to address this knowledge gap is appropriate as it allowed us to estimate precise treatment effects. While it would be interesting to conduct additional this approach has allowed us to generate real-world evidence in a timely manner while awaiting the conduct of any such trials and provides knowledge users with the evidence needed to inform their decision making now.

5.2 Thesis implications

We found no evidence of increased incident DR in patients with T2D using long-acting insulin analogues compared to NPH. This suggests that patients, including high-risk ones like those of our cohort, should be able to benefit from the use of these insulins without concerns about an increased risk of incident DR. Indeed, the total, post-weighting cohort of this study could be considered high risk due to a relatively elevated age (mean of 61.6 years), longer duration of diabetes (mean of 9 years), and the presence of comorbidities comorbidities (28.4% have CAD and 63.2% had hypertension, notably). This is expected for a group of patients with T2D using basal insulin, and thus our study and its findings are generalizable to a real-world, vulnerable population.

Concerning the secondary outcomes, a small increased estimated risk was found for incident unspecified DR that was of unclear clinical importance. However, there is no logical physiological explanation to support a truly increased risk of incident unspecified DR, but not of incident proliferative or non-proliferative DR. This result is likely a chance finding. Another remarkable observation was the decreased risk of incident DR with long-acting insulin analogues among patients who had been on their molecule-specific (or NPH) insulin for at least 5 years, which may be explained by a depletion of susceptible patients over time. It is unclear why this depletion would be differential in our cohort of new long-acting insulin analogue users compared to that of our new NPH insulin users. This finding would require confirmation in future studies, adequately designed to avoid prevalent user bias.

5.3 Implications for future studies

This thesis also has implications for future studies. First, we need additional pharmacoepidemiological studies with larger databases (or multiple databases) to more definitively assess the molecule-specific risk of incident DR in long-acting insulin analogues (in particular degludec, which entered the market later than the other ones), and longer follow-up time to better assess longer term risks of incident DR. Fortunately, as degludec becomes more popular and accessible, more users and more events will be recorded into the CPRD and other large-scale databases. Second, a retrospective cohort study similar to ours in design could be envisioned for the short-acting insulin analogues, since experimental data showed that aspart, a widely used short-acting analogue, was among the insulins with the highest affinity for the IGF-1 receptor and mitogenic potency on cell lines, along with glargine (131). Third, future studies should use analytical approaches such as inverse probability of censoring weighting (IPCW) to explore further the potential effects of censoring upon drug discontinuation. Indeed, the lack of IPCW is one of our study's limitations. However, we did generate Kaplan-Meyer curves (see Appendix 2) to assess if censoring was differential and informative. Censoring due to discontinuation was more frequent for NPH than insulin analogues in the first ~1000 days after cohort entry, but at this point in follow up time, only a few thousand patients were left in the cohort to experience an event. We also mitigated the impact of lack of IPCW by conducting an intention-to-treat sensitivity analysis, which did not show an increased risk of DR for insulin analogues. Fourth, future studies should

develop different analysis methods to better assess the independent impact of insulin analogue molecules versus that of rapid glycemic improvement on the development or progression of DR. Such methods could include stratification by baseline A1c levels and comparing time-dependent effects of A1c change and degree of change to time-dependent effects of insulin therapy. Fifth, further studies should be conducted to assess the risk of other microvascular complications of diabetes among long-acting insulin analogue users. Our literature review revealed that there were few studies that used microvascular complications as primary outcomes.

Finally, our study has some policy implications for diabetes management guideline committees and drug plan managers. They may want to consider our findings, which can reduce the uncertainty surrounding the use of long-acting insulin analogues. Given their higher cost, the impact of long-acting insulin analogues on complications of diabetes warrant consideration when determining their pharmacoeconomic profile. We hope our results will also encourage a larger discussion among health care providers' associations and patients' advocacy groups about the need for safety RCTs and observational studies for various diabetes medications and insulins with microvascular complications as primary outcomes, similar to the cardiovascular safety trials that must be conducted for all new antihyperglycemic medications (71).

CHAPTER 6: CONCLUSIONS

Many RCTs and observational studies have assessed the safety of long-acting insulin analogues, but few have specifically examined their risk of incident DR or DR progression among patients with T2D. Experimental studies have suggested a plausible pathophysiological association between DR development and insulin analogues' affinity with the IGF-1 receptor, although it is unclear that this increased affinity is sufficient to result in the development of DR. Glargine's registration trials, however, suggested an increased risk of DR progression among glargine users compared to NPH users, and this prompted researchers to conduct an RCT comparing glargine to NPH among patients with T2D to assess DR progression over 5 years (66). This study did not identify an increased risk of DR progression with glargine. However, little information is available regarding incident DR, and no previous studies have examined the risk of DR with the other long-acting insulin analogues other than one underpowered registration trial of detemir and one retrospective cohort study with many limitations. Furthermore, the results of RCTs are often not generalizable to a real-world setting. Consequently, we generated real-world evidence regarding the safety of long-acting insulin analogues by conducting a retrospective, population-based cohort study that examined the risk of incident DR with long-acting insulin analogues versus NPH insulin among patients with T2D. We found that, compared with NPH insulin, long-acting insulin analogues were not associated with an increased risk of incident DR. Similar findings were observed for proliferative and non-proliferative DR and by molecule. Our study provides reassuring real-world results concerning DR risk and use of long-acting insulin analogues, which are widely used among patients with longstanding or complicated forms of T2D.

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