Observational studies in a diabetic population: The impact of glycemic control on the risk of fractures and venous thromboembolism

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The Treachery of Images – painted 1929 by René Margritte, Belgian surrealist artist

«There is no education without research and no research without education»

Paulo Freire

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«An organization, no matter how well designed, is only as good as the people who live and work in it.»

Dee Hock

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Summary

Diabetes Mellitus (DM) is an increasingly prevalent chronic condition affecting around 450 million patients worldwide in 2017. It is estimated to cause around 5 million deaths per year. The main types of diabetes are type 1 (T1DM) and type 2 diabetes mellitus (T2DM). Both types of DM are associated with an increased risk for several diseases and health problems, including fractures and venous thromboembolisms (VTEs). While some diseases and health problems have been directly and causally linked to diabetes, the association between DM and other diseases is unclear. This is, amongst others, the case for the association between DM and fractures, as wells as between DM and VTE. Since DM is a disease characterized by hyperglycemia over prolonged periods of time, the level of glycemic control is a key aspect of DM treatment. With its proper control, the onset of several complications can be delayed or prevented. This raises the question of whether good glycemic control could also impact the association between DM and diseases with an unclear direct causal link to DM, such as fractures and VTEs.

Thus, this thesis aimed to contribute to the general understanding of how the level of glycemic control in patients with T1DM and T2DM may affect DM complications, more specifically the risk for fractures and VTE. To shed light onto this matter, we conducted three exemplary studies aiming at answering three different research questions. The studies were performed in specifically defined study populations with data taken from the Clinical Practice Research Datalink (CPRD) GOLD.

In *study I*, we comprehensively assessed the association between the level of glycemic control and the risk of low-trauma fractures in patients with T1DM and T2DM. Patients with newly diagnosed T1DM and T2DM between 1995 to 2015 were included in this 1:4 matched case-control study and we used conditional logistic regression to perform the analyses. The study included over 3’200 patients with T1DM and over 44’000 patients with T2DM and allowed us to have a detailed insight into the characteristics of the DM population in the UK. We found the median duration between the onset of T1DM or T2DM and the low-trauma fracture event to be 4.5 years, meaning that they were identical for both types of DM. The T1DM population included 46% female patients, the T2DM population, however, included over 71% female patients. While the risk of fracture was increased in patient with T1DM with mean hemoglobin A1c (HbA1c) level of > 8.0% (aOR 1.39, 95% CI 1,06-1.83) when compared to those with T1DM and mean HbA1c levels ≤7.0%, we saw no such effect in patients with T2DM. In other words, the level of glycemic control had no impact on the risk of fracture in the T2DM population. Accordingly, we found that the effect of glycemic control on the risk of low-trauma fracture differs between patients with T1DM and T2DM. This shows that more studies assessing the impact of glycemic control in patients T1DM, but especially in those with T2DM, are necessary to explore this difference in detail.

Building on our previous results, in *study II* we deepened the understanding of the association between the level of glycemic control, the use of antidiabetic medication, and the risk of low-trauma fractures in patients with T2DM. We included patients with newly diagnosed T2DM from 1995 to 2017 into our 1:4 matched case-control study. Our exposures of interest were glycemic control (measured through HbA1c levels) and the antidiabetic medication schemes. We identified almost 9000 cases and were able to assess the association between glycemic control and the risk of low-trauma fracture in patients receiving the following medication schemes: No drug treatment (only behavioral and dietary recommendations), metformin monotherapy (initial drug treatment), metformin plus either DPP4-inhibitors, glitazones, or sulfonylureas (first intensification of drug treatment), and metformin plus 2 drugs out of DPP4-inhibitors, glitazones, or sulfonylureas as well as any treatment regimen containing insulin (second intensification treatment). Like in our previous study, most patients included in the study were female (70%). We found that patients with current use of metformin and HbA1c levels <7.0% and between 7.0-8.0% had a reduced risk of fractures (aOR 0.89, 95% CI 0.83-0.96 and 0.81, 95% CI 0.73-0.90, respectively) compared to patients receiving no drug treatment. However, we did not find an association between good glycemic control and the risk of low-trauma fractures in patients receiving other antidiabetic treatment schemes when compared to medically untreated patients. This study highlighted the need for a better understanding of the interaction between the different involved characteristics of DM and its treatment and their association to the risk of low-trauma fractures.

In study III we directed our focus toward the association between glycemic control (measured as HbA1c levels) and the risk of unprovoked VTE in patients with T2DM. We assessed this in a 1:4 matched, nested case-control study within a cohort of patients with newly diagnosed T2DM between 1995 and 2019. We identified 2'653 VTE cases, out of which 53.1% were female. To ensure our cases had an unprovoked VTE, we excluded patients with a history of VTE (at any time prior to the diagnosis of T2DM), a code for surgery, immobilization, trauma, paralysis and paresis, or a code for the use of hormone replacement therapy or the contraceptive pill within 3 months prior to the VTE. We also excluded patients with a code for pregnancy of puerperium within 12 months prior to the VTE. We assessed the level of glycemic control using 7 categories: ≤6.5%, >6.5-7.0% (reference group), >7.0-7.5%, >7.5-8.0%, >8.0-9.0%, >9.0%, and no HbA1c measurement. We found no association between the last HbA1c measurement and the risk of VTE in patients with T2DM. However, in an analysis taking only the HbA1c value within 90 days prior to the VTE into account, women with HbA1c levels >7.0% had a 36-55% increased relative risk of VTE when compared to women with HbA1c levels >6.5-7-0%. With this result, our study raised the suspicion that female T2DM with HbA1c levels >7.0% may have a slightly higher risk for unprovoked VTE when compared to women with HbA1c levels >6.5-7.0%. We observed no similar effect of glycemic control on the risk of VTE in men.

In summary, the studies presented in this thesis contribute to the understanding of the impact of glycemic control on two important health conditions that are more common in the diabetic population than in those without diabetes: low-trauma fractures and VTE. While this thesis provided answers to research questions related to the association between glycemic control and the risk of fractures and VTE, the need for further research in DM and its complication is obvious, especially given that the number of patients with DM worldwide is steadily growing.

«Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.»

Louis Pasteur

Abbreviations

aOR	Adjusted Odds Ratio
ADA	American Diabetes Association
ADE	Adverse Drug Event
ATP	Adenosine triphosphate
BCDSP	Boston Collaborative Drug Surveillance Program
BMD	Bone Mineral Density
BMI	Body Mass Index
CCK	Cholecystokinin
CI	Confidence Interval
CPRD	Clinical Practice Research Datalink
DM	Diabetes Mellitus
ES	Endocrine Society
EMA	European Medicines Agency
EU	European Union
FDA	US Food and Drug Administration
FPG	Fasting plasma glucose
GAD65	Glutamic acid decarboxylase 65 kD
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like petide-1
GLUT	Glucose transport protein
GP	General practitioner
HbA1c	Glycated hemoglobin A1c
HES	Hospital Episode Statistics
HLA	Human leukocyte antigen
IAA	Insulin-associated Antibody
IA-2	Tyrosine phosphatase insulinoma-associated 2
ICD	International Classification of Diseases
IDDM	Insulin-dependent diabetes mellitus
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IQR	Interquartile range

ISAC	Independent Scientific Advisory Committee
IV	Intravenous
JDRF	Juvenile Diabetes Research Foundation
LADA	Latent autoimmune diabetes in adults
MHRA	Medicines and Healthcare Products Regulatory Agency
MODY	Maturity onset diabetes of the young
NADH	Nicotinamide adenine dinucleotide
NHS	National Health Service
OGTT	Oral glucose tolerance test
OR	Odds Ratio
OTC	Over-the-counter (drugs)
RCT	Randomized Control Trial
RG	Reference group
RR	Relative Risk
RWD	Real World Data
RWE	Real World Evidence
SAS	Statistical analysis software
SD	Standard deviation
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UK	United Kingdom
US	United States
VTE	Venous thromboembolism
WHO	World Health Organization

Chapter 1

Introduction to Diabetes Mellitus

1 Introduction to Diabetes Mellitus

Diabetes mellitus (DM) has affected mankind for hundreds of years. Descriptions of the polyuric state, the main symptom of DM, appear as early as 1550 BC in ancient Egypt.[1, 2] The term “diabetes” itself, however, only appeared in the second century AD. It stems from the Ionian Greek and means “siphon” or “to pass through”, which was a generic description of how the body acted as a conduit for the excess fluid the patients drank and caused increased urine output. The addition of “mellitus”, however, was only added in 1809 by the surgeon John Rollo, even though others had documented the sweet and honey-like taste of the urine of patients with polyuric state much earlier. [1-3] Already during the fifth and sixth centuries AD, Hindu physicians described two forms of DM. One occurred in older, overweight and physically inactive people, while the other occurred in young and slender people, who died soon after the onset of the disease. This empiric notion led to the modern classification of DM into diabetes mellitus type 1 (T1DM) and diabetes mellitus type 2 (T2DM).[1-3] There are also other types of DM, e.g. monogenic diabetes such as neonatal diabetes and maturity-onset diabetes of the young (MODY), latent autoimmune diabetes in adults, gestational diabetes, diseases of the exocrine pancreas, drug or disease induced diabetes. Prevalence of these rare types of DM are not well known and vary from 1% up to 10%, depending on definitions and ethnicities evaluated in the studies. T1DM and T2DM are the most common types of DM.[3-9] From this point forward, the term DM will be used in this dissertation referring to T1DM and T2DM.

DM is a metabolic disorder characterized by a disturbance of the glucose metabolism, where plasma glucose concentrations are elevated over prolonged periods of time (chronic hyperglycemia).[10] The reason for the elevated plasma glucose concentrations may either be due to defects in insulin secretion, or to decreased insulin action, or both.[2] Insulin is the main hormone responsible for the glucose homeostasis. It is produced in the β cells located in the pancreatic islets (also known as islets of Langerhans). Deficiencies in the secretion or activity of insulin lead to an imbalance that causes disturbances in the metabolism of carbohydrates, fats, and proteins.[2, 10-14] These metabolic disturbances are responsible for the development of the disease. In long-term, these disturbances can lead to dysfunction, damage, and finally to failure of various organs. Most commonly affected organs are the kidneys and the eyes, as well as the heart and the skin (see also chapter 1.4). Patients suffering from DM require continuous medical care with multifactorial treatment approaches, ongoing education regarding the disease, as well as support and training in self-management to reduce the risk of short and long-term complications.[2, 10, 11, 13-15]

Glucose represents the primary source of energy in humans and is the main stimulator of insulin release. [10, 11, 14, 16] Insulin acts as a stimulator of glucose metabolism in a direct manner by activating glucose transport, glycolysis, and glycogen synthesis, and in an indirectly manner by inhibiting lipolysis,

lipid oxidation, and protein degradation.[17] Since glucose is required for the normal functioning of the brain and bodily functions, the process of glucose metabolization is tightly regulated though a complex orchestration of hormone activity. When no nutrients are ingested, an endogenous production of glucose (gluconeogenesis) takes place in the liver and kidney, and insulin is secreted at a low, basal level.[10, 11, 14, 18] However, upon nutrient intake, glucose is absorbed from the gastrointestinal system and metabolized to produce energy, to be converted to amino acids, proteins, and keto-acids, or to be stored as glycogen.[10-12, 14]

The ingested and absorbed glucose enters the cells by facilitated diffusion through glucose transport proteins (GLUTs). These GLUTs are located in several organs (including the intestines, the liver, and the islet β cells in the pancreas) and while some GLUTS are insulin mediated, others are not. Once the glucose has been transported into the β cells by the GLUTs, the glucose is phosphorylated. The phosphorylation acts as a glucose sensor and couples the insulin secretion to the present glucose concentration. [19] Insulin release then occurs in two phases: A first, transient phase with rapidly rising insulin levels almost immediately after ingestion, and a following second phase of insulin release over a prolonged period of time. Glucose absorption and the rise of its concentration in the plasma also triggers the signal to suppress the production of endogenous glucose production, stimulates glucose uptake, and increases the secretion of insulin from the β cells. The mechanism of insulin action, in summary, consists in the suppression of glucose output from the liver by inhibiting glycogen breakdown as well as inhibiting gluconeogenesis. [10-12, 14]

The response of the β cells to glucose concentration, however, can be modified through several hormones and neurotransmitters, so that insulin secretion is amplified or inhibited. Therefore, the resulting amount of secreted insulin is determined by the direct stimulation of the β cells through elevated glucose and nutrient levels, and the relative input of non-nutrient dependent potentiators or inhibitors of insulin secretion.[20] In case of ingested glucose, insulin release is increased through the incretin effect, which is mediated by gut-derived hormones, the main ones being glucagon-like petide-1 (GLP-1), gastric inhibitory polypeptide (GIP), and cholecystokinin (CCK). This effect is not present in the response to intravenous glucose. [10-12, 14]

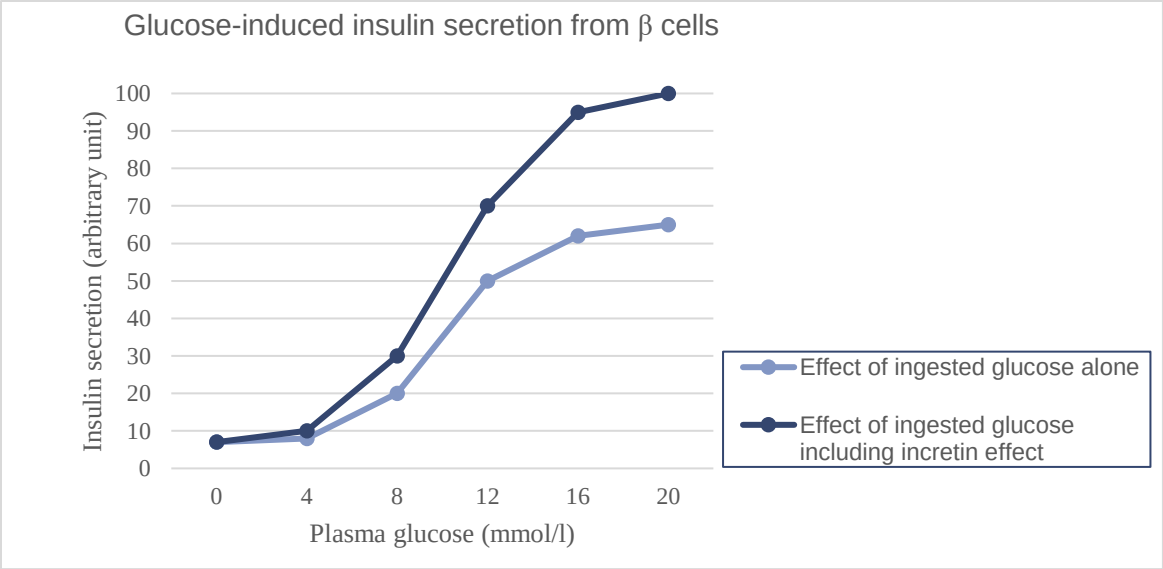

Figure 1: Glucose-induced insulin secretion from β cells with and without incretin effect [20]

Once glucose levels begin to fall, the amount of phosphorylated glucose drops and the β cells thereby detect this change, which causes them to diminish or stop insulin secretion in order to prevent hypoglycemia. This leads to decreasing insulin levels and the return to basal insulin secretion levels.[14, 20]

Hypoglycemia (low plasma glucose levels), is usually caused by drugs used in the treatment of DM, including insulin and oral antihyperglycemic drugs. Low plasma glucose levels cause a surge in autonomic activity, where the body tries to mobilize energy reserves.[10, 11, 21] The body responds to hypoglycemia by decreasing insulin secretion, increasing secretion of glucose counter-regulatory hormones such as glucagon and epinephrine, as well as by intensifying sympathoadrenal response.[11] Finally, when these measures fail to raise plasma glucose levels or if the body is unable to uphold an appropriate response due to e.g. DM, hypoglycemia may lead to cognitive dysfunction, seizures, or coma. Diagnosis of hypoglycemia requires verification of low plasma glucose levels and the immediate treatment consists in the intake of glucose.[10, 11, 21]

The islets of Langerhans constitute around 2-3% of the volume of the pancreas. As the endocrine region of the pancreas, the islets of Langerhans contain several endocrine cell subsets which produce several hormones.[14, 20] In addition to β cells, the pancreatic islets also contain α, δ, PP cells, as well as ghrelin-positive (ε cells) and two other islet cell types.[20, 22] The most frequent cells in the pancreatic islets are the β cells, which produce insulin and account for around 50% of the islet cells.[23, 24] The δ, PP cells, and the ε cells (including the other two endocrine pancreatic cells) only appear in small numbers. The δ and PP cells produce the hormones somatostatin and pancreatic polypeptide, the ghrelin-positive and other islet cell types produce ghrelin, serotonin, gastrin, and small granules of unknown content. [20, 22]

The α cells constitute around 40% or the pancreatic islet cells and are responsible for the production and secretion of glucagon, the antagonist of insulin.[24] Glucagon secretion is promoted by low glucose levels in the blood and is inhibited by high glucose levels. By consequence, high levels of insulin, GLP-1 and other enteric peptides also inhibit glucagon secretion.[22] Glucagon triggers hepatic gluconeogenesis and activates glycogenolysis, thus this hormone counteracts impending or existing hypoglycemia. Glycogen is the storage form of glucose found mainly in muscle and liver tissues. Glycogenesis, the synthesis of glycogen from glucose molecules, is activated by insulin in response to high glucose levels. Glycogenolysis, on the other hand, converts glycogen back to glucose, while glycolysis breaks down glucose into pyruvic acid to release adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH), and accordingly represents the process of obtaining energy from glucose molecules. The free energy released in this process is used to form the high-energy molecules ATP and reduced NADH.[14, 23-26]

The body also secretes glucagon in times of stress, hunger, or physical exertion, triggering the mobilization of needed glucose resources. Accordingly, glucose homeostasis is mainly achieved by the balance and the counteracting mechanisms of insulin and glucagon.[14, 25]

In patients with DM and elevated, poorly controlled glucose levels, who may sporadically even suffer from diabetic complications such as severe insulin deficiency or diabetic ketoacidosis, plasma levels of glucagon are noticeably elevated.[11, 18] Especially in patients with T2DM, these DM complications based on poorly controlled plasma glucose levels lead to an increased rate of hepatic gluconeogenesis.[27] On the long run, this effect contributes to glucose intolerance.[25] Increased glucagon levels also lead to increased ketogenesis, a process where ketone bodies are produced by breaking down fatty acids to supply energy to several organs under conditions of metabolic stress (such as fasting, caloric restriction, or sleep) when only fatty acids are readily available as a source of energy.[12]

The buildup of ketone bodies, however, can lead to diabetic ketoacidosis. Diabetic ketoacidosis is a life-threatening condition, in which the body can no longer maintain the pH of the blood within physiological ranges. Due to the accumulation of ketone bodies, the pH of the blood drops. Typically, patients present with high blood sugar levels and ketoacids in the blood or urine.[28, 29]

1.1 Diagnosis of diabetes mellitus and glucose measurements

In healthy individuals, blood glucose levels are maintained within narrow and low physiological limits of around 5 mmol/L. Glucose levels lower than this limit do not trigger glucose-induced insulin secretion, while extracellular glucose levels between 5-15 mmol/L progressively elevate insulin secretion, with levels of around 8 mmol/l leading to half-maximal stimulation.[10, 11, 14, 30]

Since tight limits of glucose levels are closely controlled in the healthy body, DM can be detected or monitored through several ways and with various methods that assess these mentioned glucose levels, which cannot be upheld in patients suffering from DM. Patients with DM may present with different symptoms, which can sometimes be ambiguous and patients can appear to have low individual risk for DM showing no known risk factors. The detection of DM as well as its classification is of great importance to timely initiate appropriate treatment and prevent complications and progression.[31, 32]

Typical symptoms for DM, both T1DM and T2DM, are polydipsia (excessive thirst), polyuria, and ketoacidosis. Since the skin is one of the most commonly affected organs, patients may suffer from skin infections, sepsis and pruritus. Especially in T1DM, weight loss may also be present, even though patients present with polyphagia (excessive hunger). As clear as the symptoms may be, the diagnosis of DM must always be confirmed by an unequivocal blood glucose measurement.[2, 33]

Methods for detection and monitoring of DM are: [31]

- elevated fasting plasma glucose (FPG) levels, assessed with the impaired fasting glucose (IFG) method
- elevated plasma glucose levels after the intake of oral glucose (impaired glucose tolerance, IGT), assessed with the oral glucose tolerance test (OGTT) method, or
- increased glycated hemoglobin A1c levels (HbA1c), assessed through a blood sample.[31]

The World Health Organization (WHO) together with and the International Diabetes Federation (IDF) have created a guideline for the definition and diagnosis of DM and intermediate hyperglycemia (impaired glucose tolerance and impaired fasting glucose), in which standardized limits and test methods are presented.[5, 31] Since DM is defined by certain measurable limits, its early stage may not reach the threshold for a diagnosis. This early stage below the defined thresholds for disease diagnosis is known as ‘prediabetes’ or ‘intermediate hyperglycemia’. The WHO favors the term ‘intermediate hyperglycemia’.[31, 34, 35]

Intermediate hyperglycemia is also characterized by elevated blood sugar levels (see table 1 for details on blood glucose limits) and there is a reported chance of progression to T2DM of about 1:3.[34] The odds of developing T2DM increase over time in patients with intermediate hyperglycemia.[31, 34] Like in DM, there are different presentations of intermediate hyperglycemia (e.g. IGT and IFG). Intermediate hyperglycemia and DM are diagnosed with the same test methods, except with different limits. Recommendations for diagnosis of DM and intermediate hyperglycemia from other associations, such as the American Diabetes Association (ADA), may slightly differ or result in different individual classification of glucose tolerance status.[31]

Even though the opinion of different scientific associations and DM experts regarding the limits for diagnostic criteria may not be identical, the criteria for DM defined by the WHO encompass a population with a materially increased risk for premature mortality as well as microvascular and cardiovascular complications.[31] Each detection method and their determined WHO limits for detection will be presented in detail in the following paragraphs.

Detecting elevated levels of FPG is a commonly used method for the detection of DM and intermediate hyperglycemia. The IFG test is performed after a fasting period of at least 8 hours. During this time, no caloric intake must take place. FPG levels of ≥7.0 mmol/l (126 mg/dl) are considered to be a diagnostic criterion for DM. [31, 34]

For the OGTT test, 75 g of glucose are dissolved in water and taken orally. After 2 hours, venous plasma glucose levels are measured.[31] For children, 1.75 g/ kg bodyweight and a maximum of 75 g of glucose are given orally.[36] DM is considered to be present if OGTT levels are ≥11.1 mmol/l (200 mg/dl).[31] In a systematic review, the annualized relative risk of progression to DM in patients with IGT increased 6-fold when compared to people with normal glucose tolerance. In the same systematic review, the risk of DM progression in patients with IFG and IGT even increased up to 12-fold compared to people with normoglycemia.[31, 37]

HbA1c values represent the mean plasma glucose levels over the previous 8-12 weeks. The measurement requires no preparation, such as fasting or the ingestion of glucose, and has, therefore, become the gold standard for the assessment of glycemic control in patients with DM.[31] While patients with HbA1c values ≥6.5% (48 mmol/mol) could be considered to have DM, the WHO does currently not recommend HbA1c measurements for the detection of DM or intermediate hyperglycemia.[31, 35] This is due to the fact that several factors (such as anemia, abnormalities of hemoglobin, or uremia) influence HbA1c levels. Additionally, consistency in HbA1c measurements is not given.[31] However, the ADA includes elevated HbA1c levels as a mean to diagnose DM and intermediate hyperglycemia.[35, 36]

IGT is considered to be present in case of FPG values from <7.0 mmol/l (126 mg/dl) and 2-hour plasma glucose levels between ≥7.8 and <11.1 mmol/l (140 mg/dl and 200 mg/dl). IFG is considered to be present in case of FPG values from 6.1-6.9 mmol/l and 2-hour plasma glucose values of <7.8 mmol/l (140 mg/dl). While IGT and IFG are both states of intermediate hyperglycemia, they are not clinical entities, but rather risk factors for future DM and/or adverse outcomes.[31]

The International Diabetes Federation (IDF) also considers a random plasma glucose measurement of >11.1 mmol/mol (>200 mg/dl) in the presence of symptoms of hyperglycemia as a diagnosis criterion for DM.[38]

All limits for the diagnosis of intermediate hyperglycemia and DM are summarized below in table 1. Since the limits vary depending on the blood sample, the table shows the individual values by sample type (i.e., whole blood or plasma).

Table 1: Limits for the detection of intermediate hyperglycemia and DM [24, 26]

Disease status*	Test method*	Glucose concentration for diagnosis [mmol/l (mg/dl)]			
		Whole blood*		Plasma***	
		Venous	Capillary	Venous	Capillary
IGT	FPG	<6.1 (<110) **and**	<6.1 (<110) **and**	<7.0 (<126) **and**	<7.0 (<126) **and**
	OGTT	≥6.7-<10.0 (≥120-<180)	≥7.8-<11.1 (≥140-<200)	≥7.8-<11.1 (≥140-<200)	≥8.9-<12.2 (≥160-<220)
IFG	FPG	≥5.6-<6.1 (≥100-110) **and**	≥5.6-<6.1 (≥100-110) **and**	≥6.1-6.9 (≥110-125) **and**	≥6.1-6.9 (≥110-125) **and**
	OGTT	<6.7 (<120)	<7.8 (<140)	<7.8 (<140)	<8.9 (<160)
DM	FPG	≥6.1 (≥110) **and/or**	≥6.1 (≥110) **and/or**	≥7.0 (≥126) **and/or**	≥7.0 (≥126) **and/or**
	OGTT	≥10.0 (≥180)	≥11.1 (≥200)	≥11.1 (≥200)	≥12.2 (≥220)

* Legend:
DM: Diabetes Mellitus, FPG: Fasting Plasma Glucose, IFG: Impaired Fasting Glucose, IGT: Impaired Glucose Tolerance, OGTT: Oral Glucose Tolerance Test

** Values for measurements in whole blood (venous or capillary) and in blood plasma vary. Therefore, limits for measurements made with venous or capillary whole blood are different to the values displayed in this table.

While the presence of glycosuria (excretion of glucose into the urine) is an indicator of DM, it is not a recommended method to detect DM, since patients with high renal thresholds or mild hyperglycemia may be missed. Accordingly, this detection method would lead to an underestimation of the prevalence of DM.[33]

Even though T1DM (in which usually a total lack of insulin is present due to autoimmune β cell destruction) and T2DM (with a disturbance of insulin secretion and/or a resistance of the peripheral tissues to the effects of insulin leading to insulin resistance) have different disease pathways, classification of DM type may be challenging at presentation.[5, 10] However, the specific diagnosis may become clearer over time or with the progression of the disease.[5] Misdiagnosis is especially common in patients with rare types of DM and in those with untypical age at disease onset due to a considerable overlap between phenotypes of T1DM and TDM2.[5, 39, 40]

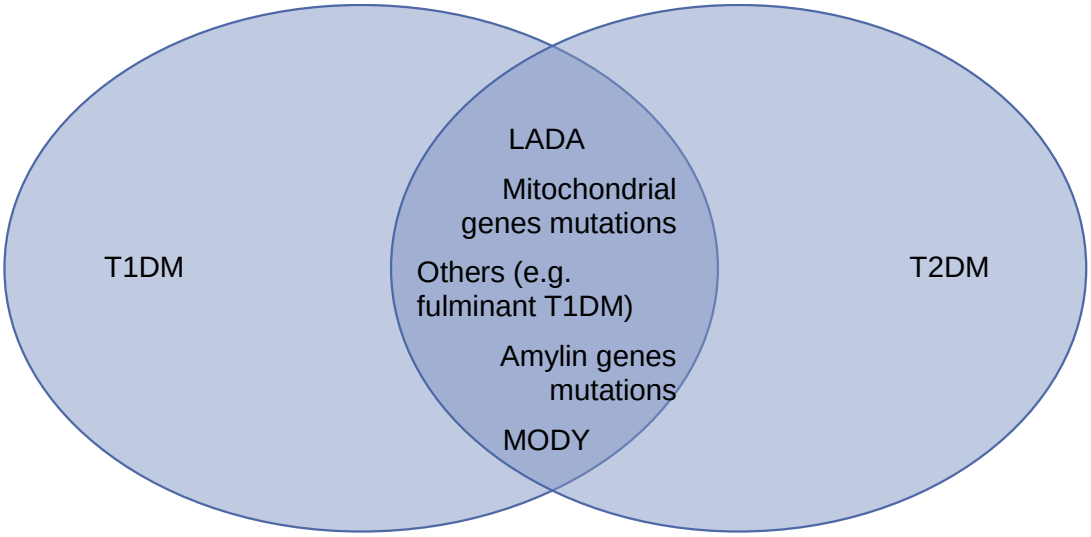

Figure 2: Overlap in phenotypes of T1DM and T2DM [39]
Legend: *MODY: Maturity Onset Diabetes of the Young, LADA: late autoimmune diabetes in adults (see chapter 1.2)*

In the following two chapters, T1DM and T2DM will be described in more detail.

1.2 Diabetes type 1 (T1DM)

T1DM is a multifactorial, chronic metabolic disorder, which comprises around 5-10% of all patients who have DM. Patients with T1DM suffer from pancreatic islet β-cell destruction, eventually leading to an absolute insulin deficiency. Accordingly, insulin is substantial for the survival of T1DM patients.[33, 41, 42] It is estimated that in 2019 around 1.1 million people aged 0-19 years lived with T1DM.[38] T1DM is the most common form of DM among children and adolescents of European origin and one of the most common chronic diseases of childhood, adolescence and early adulthood worldwide. A great variability exists in disease prevalence and incidence around the world. While T1DM is most prevalent in individuals of European descent, individuals from Asia and Oceania are the least affected. [19, 33, 41-43]

Regarding new T1DM cases, the IDF estimates that almost 130’000 children and adolescents under the age of 20 years developed T1DM in 2019.[38] However, global incidence rates are rising, making T1DM ever more common, and the overall annual increase is estimated to be approximately 3%.[38, 41] This increased incidence of T1DM is especially pronounced in young European children less than 5 years of age.[44] Incidence rates vary wildly across countries, ranging from <5 to >40 per 100’000 person years (in children aged 0-14 years). Incidence rates in countries with low prevalence show a steeper increase than incidence rates in high-prevalence countries.[38]

Considering the age of disease onset, incidence rates are the highest around puberty (12-14 years) and decrease after the age of 15 years.[41] Since girls have an earlier onset of puberty, the pubertal peak in the incidence of T1DM in females occurs about 1-2 years prior to the peak in males.[42] Incidence rates

already begin to rise abruptly at around 9 months of age and stabilize between the ages of 20-30 years.[41, 45] Similar patterns of age at DM diagnose are often seen throughout the world independently of the incidence rates.[41] Interestingly, incidence rates often increase or decrease to match the incidence rates of the new geographical location if individuals relocate. This is a strong indicator for a causative role of environmental factors in the development of T1DM.[41]

While on average females and males have about the same risk of developing T1DM worldwide, distinctive regional patterns are observed. In regions with a low incidence of T1DM, females have a higher risk of developing T1DM, whereas males have a higher risk in regions with a high incidence.[41] Furthermore, more males than females are diagnosed with T1DM between the ages of 15 to 40 years.[32] Since T1DM is an autoimmune disease, it is untypical to see equal sex-representation, as most autoimmune diseases have a strong female excess.[41]

For T1DM, as well as for T2DM, disease onset is difficult to pinpoint and is preceded by a presymptomatic period. For this presymptomatic period in T1DM, the ADA, the Juvenile Diabetes Research Foundation (JDRF), and the Endocrine Society (ES) suggest a subdivision into 3 stages:[46]

- Stage 1: Patients have no symptoms and normal glucose tolerance. However, β-cell autoimmunity is present (≥2 autoantibodies detected).
- Stage 2: β-cell autoimmunity AND dysglycemia are present, patients remain asymptomatic.
- Stage 3: Onset of symptomatic disease, where a significant loss of β-cells is present.[46]

While patients in stage 2 show no symptoms, the stage is already considered a disease state. The objective of this subdivision of presymptomatic stages is to create a common understanding and terminology of disease evolution, which supports an advance in research efforts.[46]

First symptoms of T1DM usually appear during childhood and adolescence with the onset of the disease. Typical symptoms are polydipsia, polyphagia, weight loss, and nocturnal enuresis. Unspecific symptoms, such as blurred vision, muscle cramps, skin pyogenic infections, and fatigue may also develop.[2] Diabetic ketoacidosis occurs in around 40% of children who develop DM and present acutely with polyuria.[47] Usually, early disease symptoms, such as polyuria and polydipsia, have already developed but were not recognized in those patients presenting with diabetic ketoacidosis. Patients with T1DM are rarely overweight at the time of consultation and diagnosis, as opposed to patients with T2DM.[19] However, higher BMIs are associated with an earlier onset of T1DM, especially in patients with a reduced beta-cell function.[41] Sadly, due to the higher prevalence of excessive body weight and obesity in modern society, insulin resistance (which precedes T1DM and favors the progression of islet autoimmunity) affects around 20% of young T1DM patients.[46]

Many characteristics (incl. genetics, epigenetic, metabolic and environmental factors) contribute to the development of T1DM. Individuals with a positive family history or certain immunological or metabolic

markers have an elevated risk for T1DM. The more markers an individual has, the higher the risk of developing T1DM. However, only 15% of all newly diagnosed T1DM cases have a family member with T1DM.[41, 42]

Why some individuals with a genetic susceptibility develop T1DM and others do not is still unknown. Several factors (including diet during childhood, viral infections as well as the lack of exposure to certain infections, psychological stress, and other environmental factors) and the combination thereof will eventually determine, whether the disease develops or not. For example, the decreasing number of infections in Europe is inversely proportional to the increasing number of autoimmune diseases.[41, 42]

Prenatal risk factors, such as family history are also relevant factors. Individuals with a diabetic sibling have a 15 times higher risk of becoming T1DM patients themselves when compared to the general population. However, even monozygotic twins only have concordance rates ranging from 30-65% for the development of the disease.[42] Maternal risk factors (e.g. infections during the pregnancy) and postnatal risk factors, including environmental factors, play a big role and must generally be present, for the disease to develop.[41, 42] For example, β-cells have been shown to become infected, damaged, and killed through induced apoptosis by enteroviruses, leading to inflammation of the β-cells and severely impaired insulin secretion. This stress reaction of β-cells caused by the virus may trigger autoimmunity through the presentation of self-molecules during the inflammation or through molecular mimicry, and may change and trigger harmful autoimmune mechanisms and genetic predispositions. [46] Even the season of birth as well as dietary habits leading to changes in gut microbiota can modify the risk of developing T1DM. Studies also indicate a connection between vitamin D deficiency and the development of T1DM.[41, 42] So far, however, no single risk factor or combination of risk factors could be defined as a definite trigger for T1DM.[41, 42]

The pathway that triggers the destruction of β-cells also remains unclear. It is clear that once a critical mass of β-cells has been destroyed, the insulin deficiency begins and leads to a total dependence on exogenous insulin. In many patients, the β-cell loss is eventually almost complete.[41, 42]

The β-cell destruction can be auto-immune mediated or idiopathic, leading to two categories within T1DM:[33]

- Immune-mediated DM: This subtype of T1DM was previously also known as insulin-dependent DM (IDDM), juvenile-onset DM.[33] Sometimes it is also referred to as type 1a DM.[42] It results from a cellular-mediated autoimmune destruction of the pancreatic islet β-cells. While peak incidence of immune-mediated DM occurs during childhood and adolescence, the rate of destruction is variable and disease onset may vary accordingly. When the destruction progresses slowly and disease onset is delayed, the term latent autoimmune diabetes in adults (LADA) is

sometimes used. In individuals with immune-mediated DM, markers of immune destruction (incl. islet cell antibodies, insulin autoantibodies) are present in 85-90% of the cases.[33]

- Idiopathic DM: This rare subtype is sometimes also called type 1b DM. For this subtype of T1DM, the etiology is unknown.[42] Patients have no evidence of autoimmune β-cell destruction, but suffer instead from a permanent insulinopenia (insulin deficiency) and are prone to ketoacidosis.[33] This type of DM most commonly affects individuals of Asian and African ethnicity and has strong hereditary features.[42] Since very little is known about idiopathic DM, [42] further elaborations in this thesis will focus on immune mediated T1DM unless otherwise specified.

Metabolic changes affecting the profiles of amino acids, lipids, and fatty acids precede the start of islet autoimmunity. However, the first markers of autoimmunity to appear are usually insulin-associated antibodies (IAAs), followed by glutamic acid decarboxylase 65 kDA (GAD65) and tyrosine phosphatase insulinoma-associated 2 (IA-2) antibodies. These are all antibodies from the IgG1 subclass. The destruction of the β-cells themselves, however, is primarily mediated by T-cells. Other immune cells, such as B lymphocytes, monocytes, macrophages, and natural killer cells also infiltrate the islets of Langerhans.[42, 46]

Even though the pathophysiological mechanism of β-cells destruction and the development of T1DM are not well understood, many of the known primary risk factors are of genetic origin. Autoantibodies to insulin, the enzyme of glutamate decarboxylase (GAD) and the protein tyrosine phosphatase-like protein IA-2/ICA512 are especially predictive for T1DM.[45] However, the hyper-expression of human leukocyte antigen (HLA) and their histocompatibility antigens, especially the haplotypes HLA-DR3-DQ2 and HLA-DR4-DQ8 are known to be risk promoting.[41, 46] Hyper-expression of HLA antigens is known to be present in the pancreas of T1DM patients and highlights a chronic inflammatory state. It may also be induced by viral infections.[46] Even though variants in the HLA system elevate the risk for the development of T1DM, most carriers do not develop the disease. This is also true for other autoimmunity markers, not only HLA haplotypes.[42] There is also evidence that defects in the function of regulatory T-lymphocytes and imbalances with proinflammatory Th17 T-lymphocytes in the pancreatic lymph nodes can lead to impaired immune regulation.[46] This indicates that while autoimmunity is a prerequisite for the onset of T1DM, it is not the defining characteristic.[19] Many of the genes predisposing increased reactivity and impaired regulation of the immune system are not disease-specific and elevate the risk of several autoimmune disorders.[46]

A characteristic feature of T1DM are inflammatory processes. They hinder proper β-cell functioning and may cause β-cell death. Inflammation processes often occur due to a combination of genetic and environmental factors. A typical inflammation process in T1DM patients is the infiltration of Langerhans islets by lymphocytes. This infiltration and inflammation represents an autoimmune action

directed at the β-cells and is called insulitis. These autoimmune attacks usually occur in insulin-producing islets. Once the β-cells have been destroyed, the infiltrate clears out. The composition of infiltrating lymphocytes is assumed to influence the severity and progression of the disease, e.g. B-lymphocytes seem to accelerate disease progression. However, also autoreactive memory T-lymphocytes are found in T1DM patients, but not in healthy subjects. Even though insulitis is a typical feature of T1DM, only around 10-30% of the islets are usually affected at any point in time and not all patients necessarily experience insulitis. The presence of insulitis is inversely correlated with DM duration, but it is not correlated with the age of the patients. These findings substantiate the chronic character of the disease as well as its progression over time.[46]

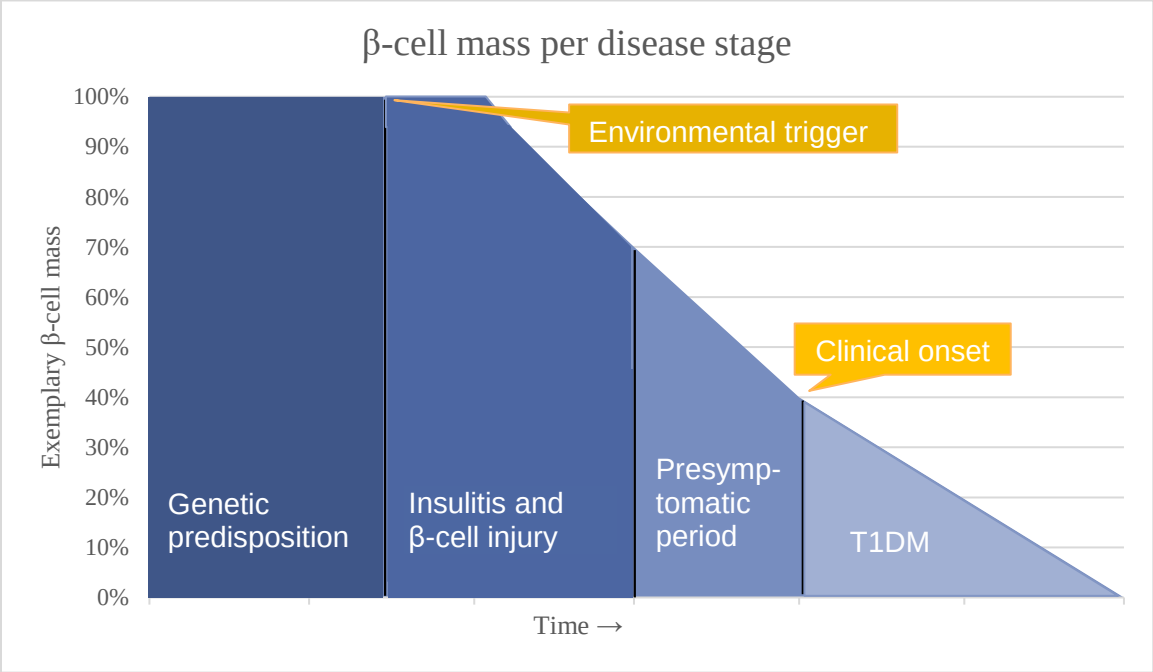

Figure 3: Progression of T1DM from a presymptomatic period to clinical disease onset of T1DM starting with individuals at a genetic risk, who over time develop multiple autoantibodies, lose first phase insulin response and become glucose intolerant [42, 43]

While β cell destruction progresses at a different speed in different patients (from months to years), younger patients usually suffer from a faster development of the disease than older patients. Autoantibodies to insulin, or the enzyme of glutamate decarboxylase 65 kDa (GAD65), or the protein tyrosine phosphatase-like protein IA-2/ICA512) are especially predictive for T1DM. Other identified autoantibodies include islet specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), the cation efflux transporter ZnT8. The more antibodies are found, the higher the risk for the development and progression of T1DM. Many other genes, alleles, and other genetic mechanism have been identified as risk-promoting for the development of T1DM. However, since the interaction of these genes as well

as the pathway of the development of T1DM is unknown, no further known genetic factors will be highlighted in this thesis.[33, 46]

Overall, the combination of environmental and genetic factors resulting in inflammation and metabolic changes seem to be important factors in the pathogenesis of T1DM. These changes cause dysregulations of the immune system affecting the innate and adaptive system, which then can trigger insulitis and islet autoimmunity, and cause chronic stress in β-cells leading to impaired function and cell death. The inflammatory processes also cause stress to the endoplasmic reticulum, which impairs β-cell function, triggers islet autoimmune responses and the release of cytokines, and activates the unfolded protein response. The fact that β-cells can persist after diagnosis for years and that their destruction is incomplete support the notion that T1DM is a chronic autoimmune disease.[46]

The development of the disease can be subdivided in several stages, which may or may not all occur to individual patients. The first stage of T1DM is the start of the autoimmune destruction of β-cells, which then moves on to a preclinical stage with insulitis and progressive loss of β-cells and their function, to the onset of clinical disease, with a transient remission phase, followed by the establishment of T1DM. The start of the disease is marked by the loss of β-cell function and the complete dependence on exogenous insulin, which for most patients occurs within 1-3 years after diagnosis. Once the disease is established, complications may develop. These secondary complications may develop over time, especially in patients with poorly controlled DM.[42]

1.3 Diabetes type 2 (T2DM)

T2DM is a heterogeneous, serious, and chronic condition. It is the most common type of DM, accounting for around 90% of DM cases worldwide,[33, 38] and also one of the most common metabolic disorders worldwide.[13] Accordingly, T2DM has become an important global health priority in recent decades.[48]

T2DM is characterized by elevated blood glucose, caused by disturbances in insulin secretion and insulin resistance in the peripheral tissues. This leaves the patient unable to produce any or enough insulin, or incapable to effectively utilize the available insulin.[13, 33, 38] Usually patients are affected by both deficiencies and either may be predominant. These disturbances in the insulin metabolism and insulin response lead to high levels of blood glucose (hyperglycemia), a clinical indicator of T2DM.[33, 38] Insulin resistance does not develop even in the presence of hyperglycemia or glucose intolerance, as long as sufficient insulin is secreted by the β-cells. However, mostly β-cell dysfunction will arise over time and worsen after onset of T2DM. Other factors also contribute to the gradual loss of β-cells, but the main factors are the direct consequences of the altered metabolic environment due to T2DM itself.[49]

Worldwide, 400 million people had diagnosed or undiagnosed T2DM in 2019. Its prevalence has been rising for decades. If current trends continue, projections suggest that by the year 2045 around 630 million people will have diagnosed or undiagnosed T2DM.[38] This statement is made under the assumption that proportion of cases with T1DM and T2DM remains unchanged.

The rise in T2DM prevalence is mostly caused by the aging of the population, as well as by the economic development and progressive urbanization. Better treatment options for diabetic patients result in extended life expectancy, which leads to a higher disease prevalence. Economic improvement and live in urban rather than rural areas are associated with sedentary lifestyles and increased consumption of high-caloric diets, resulting in growing number of obese individuals.[38]

While the prevalence of T2DM is mostly rising due to the ageing of the population, more and more children and young people are affected by T2DM. The driving factor for this is the increasing prevalence of obesity of children and young people. Still, the risk of retrieving T2DM increases with age. The lowest DM prevalence is seen among adults aged 20-24 year and the highest in adults aged 75-79 years. Women aged 20-79 years are estimated to have a slightly lower prevalence than men of the same age.[38]

Prevalence of DM is increasing the most where economies are moving from low- to middle-income status. The worldwide highest prevalence of DM are found in Marshall Islands, Kiribati, and Sudan. In absolute number, China, India, and the United States are at the top of the list with 116.4 million, 77.0 million, and 31.0 million of DM patients in 2019.[38]

Even though DM prevalence is on the rise worldwide, trends in DM incidence show a more differentiated picture. Between 2006 and 2014, the incidence of DM was stable in 27% of reported populations, grew in 36%, and declined in 36%. In previous years, DM incidence had shown increasing trends. Trends in incidence, just like trends in prevalence, can be reasonably attributed to T2DM, even though the type of DM is usually not indicated in studies analyzing these trends. A limitation to be considered is that data on trends in DM incidence originate almost entirely from high-income countries and only apply to diagnosed DM. The reason for the decrease in DM incidence in certain high-income populations is yet unknown.[38]

T2DM is most commonly seen in older, obese adults.[13, 38] However, the number of children and younger adults with T2DM is increasing due to rising levels of obesity, sedentary lifestyles and high-caloric diets.[38] The most relevant risk factor for the development of T2DM is excessive body fat.[48] Often, the excess body fat is predominantly present in the abdominal region.[13, 38] Also male sex is a risk factor for T2DM, where the excess body fat in the abdominal region is also most often observed.[50] Almost all people affected by T2DM, as well as predisposed individuals, suffer from insulin resistance.

[48] Insulin resistance often comes along with the metabolic syndrome. The metabolic syndrome describes the combined presence of visceral obesity, dyslipidemia, hypertension, and dysglycemia in an individual.[50]

Symptoms of T1DM and T2DM can be similar, although those of T2DM are often less severe in comparison. T2DM patients may even be free of any symptoms. This fact, along with the possibly slow transition from insulin resistance to T2DM, makes determining the exact time of onset of the disease nearly impossible. Therefore, as many as ⅓ to ½ of the people affected by T2DM are estimated to be undiagnosed. This is also the reason why T2DM complications such as retinopathy or lower-limb ulcer may already be present at the time of diagnosis.[38]

The disease pathway of T2DM is not completely understood. However, a strong link with overweight and obesity, increasing age, as well as ethnicity and family history exists.[38] Mainly, the lipid metabolism has been linked with the disease pathway of T2DM, including factors such as the plasma concentration of non-esterified or free fatty acids, serum triacylglyceride-to-serum high density lipoprotein, and ectopic fat in the skeletal muscle or in the liver.[50] Other pathophysiological factors are inflammation (including higher levels of circulation proinfloammatory markers, e.g. C-reactive protein), altered secretion patterns of adipokines (mainly cytokines), immune dysregulation, and abnormalities in gut microbiota.[13, 50] Similarly to T1DM, a combination of multi-gene predisposition and environmental triggers is responsible for the onset of T2DM, while family history and genes associated with T2DM alone do not allow for a prediction of T2DM.[38, 50]

To better understand the pathological pathway, we must understand that both, the synthesis and release of insulin, as well as the insulin response in the tissues, are tightly regulated processes and must match the metabolic demand. Once metabolic imbalances start occurring, so do the pathogenic mechanisms of T2DM.[13] Patients, who are affected by T2DM later in their life, initially develop insulin resistance. Insulin resistance is characterized by the inability of the body's cells to respond adequately to insulin, rendering the hormone ineffective. As a coping mechanism, pancreatic β-cells increase the production of insulin.[38] Over time, constant excess production of insulin and eventually an inability of pancreatic β-cells to keep up demand may result. This leads to a progression of the disease and a state, where glucose homeostasis can no longer be maintained and hyperglycemia ensues.[13, 38]

The BMI is frequently used to assess the proportion of body fat in individuals. It is a ration between the body weight in kilograms and the squared height in meters, expressed in unit of measurement kg/m^2. Common BMI classifications for adults are:[48]

- Under weight (<18.5 kg/m^2)
- Normal weight (18.5-24.9 kg/m^2)

- Overweight (25.0-29.9 kg/m²)
- Obese (≥30 kg/m²)

BMIs over 25.0 kg/m² are associated with a stepwise increase of the risk of T2DM, especially in case of abdominal obesity or higher waist circumference/wait-to-hip ratio. Longer durations of overweight or obesity have been found to increase the risk of T2DM by 14 % for every additional 2 years.[48] However, the Women's Health Initiative compared women who consumed a low-fat diet (around 24% of dietary energy as fat) to those that consumed a standard US diet (around 35% of dietary energy as fat) and found no difference in the incidence of treated T2DM. The study suggests that weight loss itself may be the driving factor for a reduced risk of DM, rather than the composition of the diet.[48, 51] Also, the type of consumed fats seems to be more relevant than the total amount of fat intake.[48]

The same appears to be true regarding the carbohydrate intake and the risk of DM: the exact type of carbohydrate (differentiated through the glycemic index or glycemic load and their impact on the physiological response) may be more relevant than the total amount of carbohydrate intake. Other dietary factors such as higher intake of heme-iron, magnesium, Vitamin D, red or processed meats, dairy products, nuts, vegetables, and fruit, may also have an impact on the risk of T2DM, but data are often inconclusive due to the wide range of nutrient intake and the differences in their preparation.[48]

Genetic variations are estimated to influence modifiable risk factors for T2DM based on different individual responses to environmental risk factors. Over 250 loci linked to several forms of T2DM and obesity have already been identified through fine mapping.[48] However, most genetic variants associated with T2DM seem to have limited size effects. The great majority of gene variants detected so far seem to affect insulin secretion, only few variants affect insulin resistance.[49] While recognizing genetic risk factors may not improve prediction of T2DM, better comprehension of these factors and their influence on environmental risk factors may help prevent and treat T2DM in the future.[48]

Other genetic factors, such as genetic predisposition based on first-degree relatives with T2DM, seem to be qualitatively more relevant than genetic variations.[49] Children of mothers with T2DM have a higher risk of elevated birth weight, childhood overweight, IGT in early adulthood and, accordingly, of developing T2DM themselves.[48]

Physical inactivity, a consequence of the modern, sedentary lifestyle, is a known risk for T2DM. The WHO assumes that physical inactivity is responsible for as much as 7% of the total global burden of T2DM. Several studies show that different types of physical activity, ranging from regular walking to vigorous sport and muscle-strengthening activity, reduce the risk of T2DM significantly.[48]

Obesity, with the associated insulin resistance, are known to go hand in hand with dyslipidemia, higher concentrations of circulation leptin, and chronic inflammation.[49] The adipose tissue again promotes insulin resistance through several inflammatory mechanisms. These include the increase of free fatty acid release into the blood and the dysregulation of adipokines, for example leptin.[13] It is estimated that abnormalities in the insulin-stimulated glucose transport through the glucose transporter 4 (GLUT-4) are the main reason for the insulin resistance in the muscles of T2DM patients.

Insulin resistance by itself is not only an early abnormality and precursor of T2DM, which the body compensates with elevated β-cell function until the insulin-glucose feedback loops fails, but it also correlates with an increasing risk of cardiovascular disease and its outcomes.[50] A large amount of complications have been directly and indirectly linked to diabetes, such as diabetic retinopathy, diabetic neuropathy, diabetic kidney disease, peripheral vascular disease, cerebrovascular disease, foot ulcers, erectile dysfunction, gastrointestinal functions (e.g. dysphagia, heartburn, nausea and vomiting), non-alcoholic fatty liver disease, skin disorders, bone and rheumatic disorders, among others.[52, 53]

1.4 Long-term complications and comorbidities of T1DM and T2DM

The main long-term complications resulting from chronic diabetes stem from tissue damage, leading to microvascular disease (microangiopathy) and macrovascular disease (macroangiopathy). These two are the most common and well-documented long-term complications of prolonged exposure to hyperglycemia.[54, 55]

Microvascular complications affect the capillaries and arterioles, damaging the retina, the kidneys, and the nerves causing retino-, nephron-, and neuropathy. Whereas macrovascular complications affect the bigger blood vessels and result in cardiovascular disease.[54, 56, 57]

Both, T1DM and T2DM, have been linked to microvascular disease.[54, 55] There is strong evidence for the relation of duration and severity of hyperglycemia and the development of microvascular disease. It has also been shown in several studies that with longer duration of DM, patients with poor glycemic control had a higher prevalence of retinopathy, nephropathy, and neuropathy when compared to patients with good glycemic control. While good glycemic control is associated with a decreased risk of development and early progression of microvascular disease , no consistent impact of glucose control was found on established or advanced microvascular disease.[54]

Just like in microvascular disease, hyperglycemia seems to contribute to macrovascular disease. However, while hyperglycemia and DM are the main risk factors for microvascular diseases (retinopathy, nephropathy, and neuropathy), macrovascular disease is multifactorial. Therefore, the impact of glycemic control on the risk of developing complications resulting from macrovascular disease has not been conclusively proven. Even though evidence is lacking that good glycemic control

lowers the risk of developing macrovascular disease complications, tight glycemic control may prevent macrovascular disease with its buildup of fat and blood clots in large blood vessels from developing in the first place.[54]

What has been shown convincingly, however, is that long-term hyperglycemia can cause tissue complications. These are mostly, but not exclusively, microvascular and macrovascular complications.[54] While all cells of hyperglycemic patients are exposed to increased glucose levels, certain cells seem to be especially vulnerable to this condition. This is the case for cells that are unable to downregulate the glucose transport into the cell, leading to intracellular hyperglycemia.[55] The complications, therefore, occur in cells and tissues, where glucose transport can no longer be limited due to hyperglycemia.[54]

The known pathological pathway of hyperglycemia and DM complications may include:[54]

- Upregulation of various metabolic processes leading to vascular complications,
- Activation of the polyol pathway leading to accumulation of intracellular glucose and glucose-derived substances
- Accumulation of advanced glycation endproducts
- Overactivity of protein kinase C, which leads to increased vascular permeability and blood flow
- Activation of the hexosamine pathway leading to upregulated activity of many genes
- Increase of oxidative stress due to mitochondrial superoxide overproduction as well as all of the above mentioned mechanisms
- Other factors including endoplasmatic reticulum stress and elevated angiotensin II production.

Accordingly, the most important pillar in the treatment of T1DM and T2DM is the management of blood glucose.[58-60] The management of blood glucose is mainly addressed through dietary measures, increased physical activity, and medication, as well as patient education on the afore mentioned topics.[58, 59] A list of drugs used in DM can be found in table 2.

It is essential for patients with DM to define adequate glycemic targets to manage their disease, so that the risk of long-term complications can be reduced. Since long-term hyperglycemia plays a major role in inducing chronic complications in patients with DM, the maintenance of adequate glycemic targets is also key in preventing or ameliorating the progression of micro- and macrovascular complications.[55]

Aside from micro- and macrovascular complications, patients with DM have been shown to be at higher risk for several other conditions such as psychological problems (e.g. anxiety, depression, behavioral and conduct disorders, diabetes distress), eating disorders, thyroid disease, coeliac disease, dental complications, elevated risk of fractures, as well as elevated risk of thromboembolisms.[61-64] It is not always known how and why certain comorbidities and conditions are more common in patients with

DM than in the general population, as seen in the example of the risk of fractures and of thromboembolism in patients with DM (see chapters 1.5 and 1.6).[62-64]

These two conditions, that are more common in patients with DM compared with the general population, will be described in the next chapters, as they are the focus of the analyses conducted in this thesis.

Table 2: Drugs used in diabetes

ATC drug category	Class of API	API
ATC code A10A Insulins and analogues	Insulins and analogues for injection, fast-acting	Insulin (human, beef, pork, lispro, aspart, glulisine)
	Insulins and analogues for injection, intermediate-acting	
	Insulins and analogues for injection, intermediate- or long-acting combined with fast-acting	
	Insulins and analogues for injection, long-acting	
	Insulins and analogues for inhalation	
ATC code A10B Blood Glucose Lowering Drugs, excl. Insulins	Biguanides	Phenformin, metformin, buformin
	Sulfonylureas	Glibenclamide, chlorpropamide, tolbutamide, glibornuride, tolazamide, carbutamide, glipizide, gliquidone, gliclazide, metahexamide, glisoxepide, glimepiride, acetohexamide
	Sulfonamides (heterocyclic)	Glymidine
	Combinations of oral blood glucose lowering drugs	
	Alpha glucosidase inhibitors	Acarbose, miglitol, voglibose
	Thiazolidinediones	Troglitazone, rosiglitazone, pioglitazone, lobeglitazone
	Dipeptidyl peptidase 4 (DPP-4) inhibitors	Sitagliptin, vildagliptin, saxagliptin, alogliptin, linagliptin, gemigliptin, evogliptin, teneligliptin, sitagliptin and simvastatin, gemigliptin and rosuvastatin
	Glucagon-like peptide-1 (GLP-1) analogues	Exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, semaglutide, beinaglutide
	Sodium-glucose co-transporter 2 (SGLT2) inhibitors	Dapagliflozin, canagliflozin, empagliflozin, ertugliflozin, ipragliflozin, sotagliflozin, luseogliflozin
	Other blood glucose lowering drugs, excl. insulins	Guar gum, repaglinide, nateglinide, pramlintide, benfluorex, mitiglinide, imeglimin
ATC code A10X Other drugs used in diabetes	Aldose reductase inhibitors	Tolrestat

1.5 Fractures in patients with diabetes type 1 and 2

T1DM as well as T2DM are associated with an elevated risk of bone fractures. Several studies show that this elevated risk affects various fracture sites, such as vertebral, hip, and all non-vertebral fractures. However, while the risk of fracture is increased and affected fracture sites are similar in both types of DM, the pathways seem to differ: Compared to the general population, the bone mineral density is lower in patients with T1DM, on the other hand, it is higher in patients with T2DM.[65]

Also, while the risk of fractures is elevated for both types of DM when compared to non-diabetic patients, the risk increase is more pronounced in patients with T1DM than in patients with T2DM.[66] When the risk of fractures in T1DM was compared to non-diabetic controls, several meta-analyses found the risk of hip fracture to be 4- to 7-times higher. A meta-analysis from 2018 calculated a pooled RR for hip fractures of 4.40 (95% I 2.58-7.50) and a pooled RR of 1.88 (95% CI 1.52-2.32) for all fractures in patients with T1DM when compared to non-diabetic controls.[67] In patients with T2DM, different meta analyses found RRs or ORs ranging from 1.16-2.03 for the risk of fracture when compared to non-diabetic patients, with stronger evidence for an increased risk of hip fracture and weaker evidence for an increased risk in wrist, spine and foot fractures.[66]

Patients with T1DM have significantly lower bone mineral density (BMD) than control subjects without T1DM, especially if they suffer from comorbidities such as microvascular complications. Even though lower BMD is a risk factor for the increased risk of fracture in patients with T1DM, it is not enough to explain the disproportionate increase of this risk. This suggests that other factors than BMD may also contribute to the elevated risk of fracture. One of the contributing risk factors could be the change in microarchitecture of the bone and bone strength.[65] Patients with T1DM are affected by changes in bone macro- and microstructure, which lead to thinner cortices and trabecular bone. These deficiencies in the bone architecture are more pronounced if microvascular complications are present. Other factors contributing to bone frailty in patients with T1DM include the accumulation of advanced glycation endproducts, detrimental alterations of the mineral phase because of reduced bone turnover, and blockage of vascular channels in the bone due to mineralized tissue.[68] The pathogenesis of this mentioned elevated risk of fracture is not conclusively understood and most likely multifactorial.[65]

Furthermore, falls are more common in patients with T1DM than in diabetes-free controls for several reasons, including insulin treatment, microvascular complications (e.g. retinopathy and peripheral neuropathy), and hypoglycemia.[68]

For individuals with T2DM, a number of factors seems to contribute to the increased risk of fracture. These factors include, but are not limited to:[66]

- Obesity: Obesity, or an elevated BMI, is positively associated with increased BMD. Obesity is likely to impact the risk of fractures through several factors, e.g. impact of falls, immobility,

inflammation, vitamin D insufficiency, and adverse effects of adipose tissue on bone remodeling.[66]

- Increased risk of falls: Patients with T2DM are more prone to falls than individuals without T2DM. An example of a DM-specific risk factor for falls is hypoglycemia. Diabetic comorbidities (e.g. visual impairment due to retinopathy or cataracts, peripheral neuropathy, cardiac arrhythmias due to cardiovascular disease) also contribute to the higher risk of falls in patients with T2DM.[66]
- Sarcopenia: Sarcopenia affects patients with T2DM more often than those without T2DM. Its prevalence also increases with age and with prolonged duration of DM, which often go hand in hand.[66]
- Co-morbidities associated with DM: Several comorbidities are associated with a higher risk for fractures independently of DM-status. However, many of them are more common in the T2DM population (such as heart disease, stroke, renal dysfunction, and cognitive impairment), putting patients with T2DM at a higher risk for fractures.[66]
- Altered glucose metabolism: Increased levels of insulin (leading to insulin resistance and hyperinsulinemia) have been shown to have effects on the bone and contribute to elevated BMD. There is also some evidence that these increased levels of insulin may affect bone remodeling via direct effects on osteoblasts, osteoclasts and osteocytes, since these cells all express insulin receptors. An additional effect of high insulin levels on the bone include the accumulation of advanced glycation end products in the bone matrix, which may inhibit bone formation.[66]
- Vitamin D insufficiency: Vitamin D plays a vital role in the development of T2DM as well as in glycemic control, as vitamin D receptors are expressed in the β-cells. Patients with T2DM have a higher prevalence of vitamin D insufficiency than individuals without T2DM.[66]
- Antidiabetic drugs: Some antidiabetic medication have been shown to have an impact on bone health and the risk of fracture through direct (medication-induced) or indirect (e.g. higher risk of hypoglycemia and therefore higher risk of falls) effects.[66]

Thus, details on the pathophysiology of bone remodeling and alterations in the bone matrix in patients with T2DM remain unclear.[66]

Since the prevalence of osteoporosis increases with older age, the number of patients with DM worldwide is increasing, and life-expectancy of patients with DM is significantly improving due to several medical advances, the burden of fractures in patients with DM is bound to increase as well. Both diseases have high associated costs to health systems all over the world. Therefore, the topic of fractures in DM patients is of growing concern and research on ways of attenuating this burden are highly sought-after.[65]

1.6 Venous thromboembolism in patients with diabetes type 2

Venous thromboses are predominantly caused by blood stasis or decelerated blood flow of venous blood to the right heart. This occurs most often in case of right heart failure, immobilization, or chronic venous insufficiency. The enlargement of the veins (such as in venous lakes or varicose veins), as well as insufficient or calcified venous valves, cause a reduction of the flow velocity and a disturbance of laminar flow. These factors contribute significantly to the development of venous thrombosis. In addition, dehydration with elevated hematocrit levels or congenital disorders of the coagulation system with hypercoagulability can considerably increase the risk of thrombosis. The embolus itself can be made up of own or foreign tissue/material, which is carried along with the bloodstream, until it becomes lodged in a blood vessel with a smaller diameter than the embolus. This results in a partial or complete vessel occlusion. [69]

The term venous thromboembolism (VTE) comprises the conditions of deep vein thrombosis (DVT) and its complication pulmonary embolism (PE).[70] VTE is the third most common cardiovascular manifestation worldwide after myocardial infarction and stroke. Its incidence increases sharply after the age of 60 years in men and women alike, while most of the increase is due to PE. [71]

The incidence of VTE has risen over the last decades, however, exact numbers are difficult to determine.[71, 72] Part of the increase may be due to the higher sensitivity of diagnostic imaging and better detection of small pulmonary emboli. However, there are several other factors contributing to the increased incidence of VTE, including increasing numbers of patients with permanent pacemakers, internal cardiac defibrillators, and indwelling central venous catheters, as well as rising numbers of obese individuals.[71] Apart from the factors contributing to the increased incidence of the disease, there are several major risk factors for incident VTE.[70] These include hospitalization, active cancer, inherited risk factors (e.g. inherited thrombophilia), neurological diseases with leg paresis, nursing-home confinement, and trauma or fracture, among others. VTE occurring due to the presence of the mentioned factors are considered to be provoked VTEs. [70, 71] Still, the avoidance of these major risk factors is insufficient to prevent VTEs and the occurrence of unprovoked VTE seems to be fairly consistent.[70]

DM is considered a risk factor for VTE. However, it is unclear whether it should be considered an independent risk factor, or whether the increased risk is due to more frequent hospitalizations, confinement to nursing homes, or other comorbidities more common in patients with DM than in the general population.[73] A study analyzing the risk and severity of unprovoked VTE with clustering cardiovascular risk factors for atherosclerosis found that patients with DM had a higher risk of unprovoked VTE than diabetes-free patients, since DM itself represents a cardiovascular risk factor. Other considered cardiovascular risk factors in the mentioned study were age, active smoking, obesity, high blood pressure, dyslipidemia, and family history of coronary heart disease. The same study also

found high blood pressure (which is often present in patients with T2DM) and other cardiovascular risk factors to increase the probability for unprovoked VTE, especially when present simultaneously.[74]

VTE has a significant economic burden for initial hospital encounters as well as for the post-hospital discharge period.[72] Additionally, it is a common disorder and the risk of recurrent VTE is exacerbated once patients have suffered from a VTE. Post-thrombotic syndrome and chronic pulmonary hypertension are two other long-term complications of PE.[75] Accordingly, preventing incident as well as recurrent VTE and its long-term complications is critical.[75, 76]

«There is not a discovery in science, however revolutionary, however sparkling with insight, that does not arise out of what went before.»

Isaac Asimov

Chapter 2

Aims of the thesis

2 Aims of the thesis

With the continuously increasing global burden of DM, scientifically sound suggestions and solutions on how to reduce the risk of direct and indirect complications of DM are highly sought after.[58, 59, 61, 76] Proper glycemic control has proven to be a valuable measure for several complications, such as cardiovascular disease, diabetic eye disease, and chronic kidney disease.[38] Therefore, the focus of this thesis lies on the impact of the level of glycemic control on the risk of fracture in patients with T1DM and T2DM, and the risk of VTE in patients with T2DM. These associations have not been well analyzed so far and the CPRD provides a valuable data source to investigate these study questions.

Association between glycemic control and risk of fracture in diabetic patients: a nested case-control study | The objective of this study was to evaluate the association between glycemic control and the risk of low-trauma fractures in patients with T1DM and T2DM. To analyze this association, glycemic control and its impact on the risk of fracture was assessed through HbA1c levels, which were categorized into 4 categories for T1DM patients (good control ≤7.0%, medium control >7.0-8.0%, poor control >8.0, and unknown HbA1c level) and into 7 categories for T2DM patients (very good control ≤6.5%, good control > 6.5-7.0%, medium control >7.0-7.5%, poor control >7.5-8.0%, very poor control >8.0-9.05, unsatisfactory control >9.0%, and unknown HbA1c level).

Antidiabetic treatment, level of glycemic control, and risk of fracture in type 2 diabetes: a nested, case-control study | This study focused on the association between glycemic control and the risk of low-trauma fracture in patients with newly diagnosed T2DM, taking the effect of the prescribed antidiabetic medication into consideration. To analyze this association, the level of glycemic control as well as the antidiabetic drug regimen was taken into account by creating 2 different classifications of HbA1c categories:

- For comparisons between users of the same medication regimen, we defined 4 HbA1c categories plus 1 category for missing measurements. We chose HbA1c levels from greater than 6.5% to 7.5% as the reference group.
- For comparisons considering the timing and duration of drug intake, we defined 3 HbA1c categories plus 1 category for missing measurements and used patients without prescribed antidiabetic drugs as the reference group.

Association between glycemic control and risk of venous thromboembolism in diabetic patients: a nested case-control study | In this study our objective was to analyze the association between glycemic control and the risk of unprovoked (idiopathic), first-time VTE in men and women with newly diagnosed T2DM. The exposure of interest in this study was glycemic control after the onset of T2DM defined by HbA1c levels. We assessed HbA1c levels in 7 categories: ≤ 6.5%, > 6.5–7.0% (reference group), > 7.0–7.5%, > 7.5–8.0%, > 8.0–9.0%, > 9.0%, and no HbA1c measurement.

Chapter 3

Methodology: Case-Control studies

3 Methodology: Case-control studies

In pharmacoepidemiology, there are two main types of study designs, namely cohort and case-control studies.[77, 78] While in cohort studies the exposure status of individuals is determined at the starting time of the study and then subsequent association between the exposure and the outcome(s) are analyzed, in case-control studies the direction of the study is inversed: The outcome is determined at the starting time of the study and the antecedent association between the outcome and the exposure(s) are analyzed. Accordingly, the case-control and cohort study designs differ essentially by the direction of inference. Cohort studies look forward in time from the exposure to possible outcomes, and case-control studies look back in time from an outcome back to present exposures in the past.[77]

Both, case-control and cohort studies are considered longitudinal studies, because the obtained information relates to different points in time. The implied reasoning in this setup is that the causal action of an exposure comes before the subsequent development of the outcome, giving an indication of a causal action. However, another used type of study design is the cross-sectional study, where all the obtained information stems from the same point in time. Accordingly, cross-sectional studies can only provide a snapshot of the characteristics of the population at a specific point in time, giving information on disease prevalence. Information on disease incidence, as well as risk or rate calculations cannot be performed with cross-sectional data, since data across a time period is required for that purpose.[78]

The difference between the two study types is depicted in Figure 4: [79]

			Case-Control Studies ↓	
			Outcome	
			Present (cases)	Absent (controls)
Cohort studies →	Exposure (risk factor)	Present (exposed)	A	B
		Absent (unexposed)	C	D

Figure 4: Perspectives of cohort and case-control study designs

All the studies performed in scope of this PhD thesis used a case-control design. The design was chosen due to its suitability to answer the study questions at hand in rare, multifactorial events/diseases. This chapter will, therefore, focus on the design and methodology of case-control studies, its determining factors, and highlight the strengths and weaknesses of the design, in order to better understand this methodology.

In case-control studies, cases with a disease or condition are compared to controls without the disease or condition of interest. To do so, one or more exposures are defined and the occurrence of the outcome of interest is statistically analyzed in cases and controls.[79]

In Figure 5 the case-control design is illustrated.

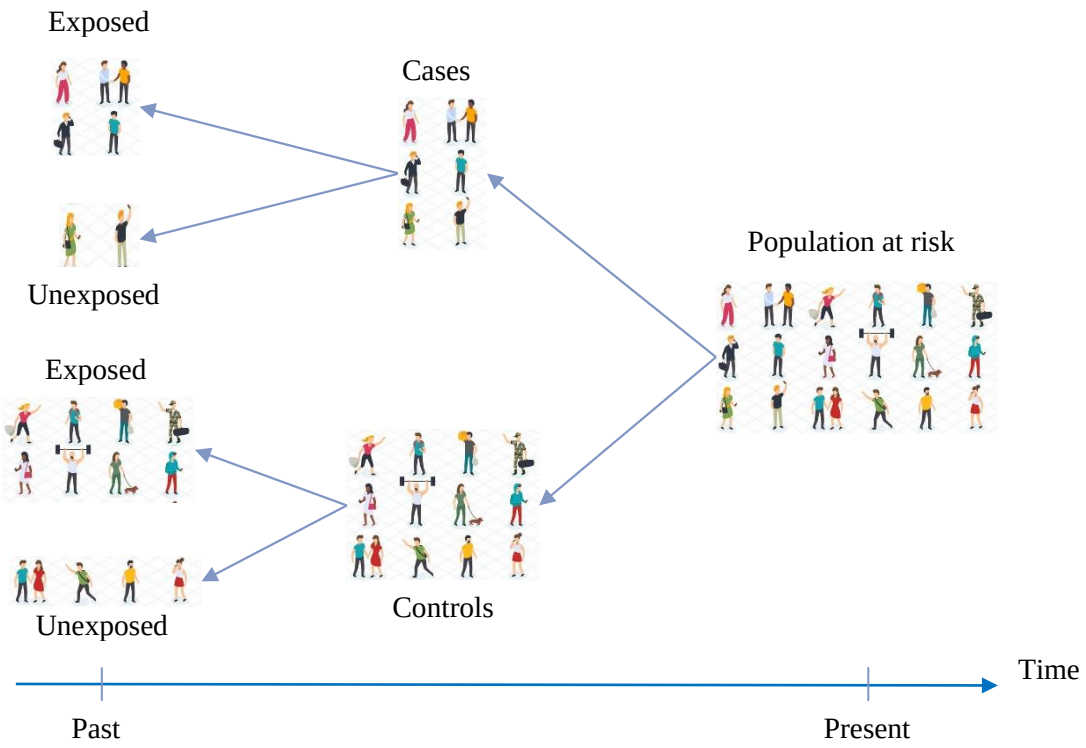

Figure 5: Case-control study design

The information on exposures is mostly obtained retrospectively, for example by abstracting medical records and using health care databases, or through questionnaires and interviews. Since the information used for the case-control studies are often not specifically collected for the purpose of the study, these studies are susceptible to limitations in the validity due to the retrospectively obtained exposure information. For example, if information is obtained through questionnaires or interviews, cases and controls may remember past exposures differently. Also, the selection of appropriate controls can be a complex endeavor which, if not performed in a proper manner, can lead to selection bias and erroneous conclusions in consequence.[79] However, case-control studies can also be conducted with prospective data, since the retrospective direction of inference is relevant for the study design and not the period of data collection.[77]

Strengths of case-control studies include the possibility to study multiple possible causes of a single disease, because different exposures can be compared between cases and controls. The design is also useful to study relatively rare diseases or outcomes that take a long time to develop, since the cases are

collected at the start of the study. Additionally, the number of cases required to study the specific disease are smaller than in other designs, such as in cohort studies.[79] Also, no long periods of follow-up are required due to the often used retrospective study design, meaning that researcher do not have to wait for longer periods of time to obtain the required information for the study.[77] For these reasons, the case-control study design has proven to be of great value and is regarded as a powerful tool in pharmacoepidemiology.[79]

In case-control studies, the measure of association is usually presented as an odds ratio (OR), unless the case and control sampling probabilities are known.[77] The odds of an event represent the ratio between the probability of said event and the probability of the same event not occurring (number of cases divided by the number of controls), therefore, the OR compares the ratio of an outcome between the odds of exposed and unexposed subjects. An OR >1 signifies that an exposure more often leads to the outcome under investigation (most often an unfavorable outcome), whereas the outcome is less likely to manifest under a specific exposure if the ORs smaller than 1 (mostly indicating a protective effect). The odds of an event should not be misinterpreted as the risk of an event. The risk represents the probability of an individual to suffer a specific outcome. The resulting statistical measure, known as the relative risk or risk ratio (RR), compares the risk of unexposed and exposed subjects.[80]

In rare diseases, meaning that the risk of disease is low in the exposed as well as the unexposed group, risks and odds are similar. This is the case because the number of outcome-free subjects does not differ much from the number of study subjects. Accordingly, the OR approximates the RR in rare diseases.[80]

3.1 Types of case-control studies

Since controls for case-control studies can be selected in numerous ways, different implications may arise from this sampling, leading to varying approaches for the design, analysis, and interpretation of the study. Three basic types of sampling for controls in case-controls studies are distinguished: [78]

- Density-based sampling
- Cumulative sampling
- Case-cohort sampling [78]

Controls in density sampling are selected in a such a way that the distribution of person-time in the source population with respect to exposure is represented. In cumulative sampling, the focus lies on sampling after a period of risk for the source population, which is supposed to be concluded at the time of study conduction. An example for cumulative sampling could be a case-control study on the effect of vaccination against influenza, which is conducted after the end of influenza season. Controls would be defined as those who did not retrieve influenza during the season. A third type of control sampling is performed in case-cohort studies. Here, the controls are selected out of the entire source population,

independently of whether or not they later become cases. Accordingly, a certain proportion of the controls may also be selected as cases at a certain point in time.[78]

3.2 Nested case-control study

Case-control studies can be conducted as nested case-control studies, instead of only as case-control studies (without the study population being nested within a cohort).[77, 81] In case-control studies that are not nested, cases and controls are selected from the general eligible population. However, when conducting a nested case-control study, a cohort of subjects with a certain characteristic is defined (e.g. patients newly diagnosed with T2DM). These individuals are followed during a predefined time period.[81] The cases and controls are then selected from within this cohort and the analysis is performed as a case-control study: a fixed amount of controls are randomly and individually matched to a case from the cohort.[77, 81]

Nested case-controls studies are favorable in situations where a large cohort of study participants is followed for many years. This is often the case in pharmacoepidemiological studies that use administrative data. In the nested case-control design, a single study individual can be eligible as a control for more than one case. Furthermore, a case may be sampled as a control for an earlier case. These two possibilities lead to unbiased estimations of the relative risk parameter.[77]

3.3 Matching

Case-control studies can be matched or unmatched. In matched case-control studies, the cases and controls are matched individually or group-wise based on key characteristics of the study population (e.g. age, sex, or socioeconomic status). In unmatched case-control studies, cases and controls are not matched on any characteristics. However, especially individual matching is regarded as an important factor to ensure the comparability of case and control(s) on characteristics that represent important risk factors for the outcome.[77]

Individual matching is done by selecting (a) control(s) that most closely match the case on predefined characteristics, such as sex, age, or socioeconomic status. For group-wise matching, also known as stratum or frequency matching, cases are divided into mutually exclusive strata. Then, controls are assigned at random out of an eligible pool of individuals without the outcome in each stratum. This is done at a fixed ratio to the number of cases. For individual as well as for group-wise matching, discrete variables must usually have and exact match, while for continuous variables the match must lie within a stratum or a predefined range. The characteristics may be prioritized, for example matching on sex may be selected as the most important criterion and, therefore, may occur before matching on criteria defined as less critical. For matching characteristics defined as less critical, the closeness of matching may be relaxed in order to find suitable controls.[77]

In the studies presented in this thesis, matching was done on main characteristics that included sex, age, and DM-duration. While no relaxation is required for sex, it is required for age (±3 years) and DM-duration (±365 days).

3.4 Interaction and effect modification

The term 'interaction' is used to express cumulating effects of interacting variables in statistical models, which influence the occurrence of an outcome.[78, 82] In other words, the joint causal effect of two (or more) exposures differs from their expected combined effect.[82] The term 'effect modification' is commonly used in epidemiology and is present, if there is a variation in the effect measure for the outcome due to the effect of one explanatory variable on the level or value of another explanatory variable.[82, 83] This means that the causal effect of a specific exposure differs across levels of a second exposure.[82] Neither interaction nor effect modification must be eliminated from studies, as opposed to bias, since both are part of causal reality and should be elucidated instead.[82]

In one of the studies presented in this thesis (see chapter 6), we tested for effect modification by obesity status of the patients in the association between level of HbA1c and risk of VTE.

3.5 Bias

A bias is a systematic, nonrandom error that leads to distorted study results. Accordingly, the study results will be distorted and will not reflect the true value of an analyzed association.[77, 83] Several biases have been described, however, they are mostly classified into three main types: confounding, selection, and information bias.[77] While some forms of bias can be mathematically and statistically corrected, other forms cannot.[83] These three main types of bias will be explained in more details in the next few subchapters.

3.5.1 Confounding

Confounding can be defined as a confusion of effect, where the confounding variable correlates (positively or negatively) with the exposure as well as with the outcome.[83] In other words, confounding refers to underlying relationships of exposure and outcome with the confounding variable(s) in the source population. Therefore, it is as much related to the exposure as it is an independent risk factor for the outcome. An example a confounding variable is age, since not only the rates of most diseases increase with its advance, but so does the cumulative exposure to almost all and any factor. Accordingly, the risk of an analyzed outcome would appear to be positively associate with the cumulation of any given exposure even though the outcome may not be associated with the exposure.[77]

Confounding can be mathematically and methodically accounted for, if recognized by the researcher(s).[83] There are three methods of addressing and eliminating confounding: [77, 78]

- Matching (on confounding variables)
- Restriction (e.g. exclusion of study subjects so that the study population is left with only one single value of a confounding variable, for example only female patients, or stratification)
- Randomization (can be used only in experiments)

Additionally, statistical adjustment can be used during the analysis of studies to limit confounding.[77] Even though much can be done to avoid confounding, the possibility of residual confounding in observational studies cannot be excluded, as no method or technique can eliminate all confounders, including unknown ones.[77] Confounding can only be avoided in randomized trials as long as the randomized groups are sufficiently balanced on all potential (observed and unobserved) confounding variables.[83]

3.5.2 Selection bias

Selection bias occurs due an erroneous, non-randomized method of selection of the study population, where study subjects are preferentially sampled to have particular characteristics. In case of selection bias, the relationship between the exposure and the outcome in study subjects who are included in the study is substantially different from those subjects who should have been eligible and chosen for the study. Randomization is the most simple and effective way of elimination selection bias.[83]

3.5.3 Information bias

Information bias, also known as misclassification bias, produces a systematic error in the information about the exposure and/or the outcome due to the collection of data on the subjects included in a study.[77, 83] This error can be nondifferential or differential. Recall bias is an example of an information bias, which can occur in retrospective case-control studies, if information relies on memory of study participants or interviews with study participants.[83]

3.6 Random error

Random error occurs due to a variation in the accuracy or precision of the data used to conduct the study. It may be due to natural or periodic fluctuations, applied sampling technique, measurement tools or scales, or any other reason that could influence or modify data collection. Random error has no direction of impact on the study results, meaning that it can positively or negatively distort the study outcome.[83] However, random error can be reduced with increasing study and sample size, while systematic error cannot be reduced with this method.[78]

3.7 Statistical analysis

Two primary purposes of statistical models in epidemiology are to make predictions (prediction models) and to control for confounding (causal models). This can be accomplished by using regression models, which are the most important statistical techniques for epidemiologic analysis.[78] The most common regressions models are linear regression (used for continuous outcomes), logistic regression (used for binary outcomes), Cox regression (for time-to-event data), and Poisson regression (for frequencies and rates). However, logistic regression the most commonly used model in epidemiological research. All regression models rely on different types of multivariable analyses.[78, 84]

Regression models allow for the investigation of the effect of one or more explanatory variables (e.g., risk factors, exposures, patient characteristics) on a response variable (e.g. a specific disease, mortality, clinical endpoint). When only one explanatory variable is used, the model is called 'simple regression', when multiple explanatory variables are applied, the model is called 'multiple regression', 'multifactorial regression', or 'multivariate regression'. Regression models represent a universal tool as they can be applied to assess data in all study designs used in epidemiological research.[84]

All studies conducted in scope of this thesis were matched case-control studies. Therefore, a short excursion on an important extension for the logistic regression model in matched case-control studies is presented is this paragraph. For the statistical analysis of matched case-control studies, conditional logistic regression is typically used.[84] This results from the need to address the matching of cases and controls in the analysis so that no distortion of the association between the matching variables and the outcome occur.[85] This is achieved by considering strata of cases and controls which are homogeneous for the matching variables but are otherwise not distinguished in the logistic regression.[86]

Due to the reasons provided above, we used conditional logistic regression to assess the association between levels of glycemic control and several outcomes in the studies presented in this thesis.

«If you think research is expensive, try disease!»

Mary Lasker

Chapter 4

Study I: Study on glycemic control and the risk of fracture

4 Study I

4.1 Association between glycemic control and risk of fracture in diabetic patient: A nested case-control study

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4.1.1 Abstract

Context

Diabetes mellitus (DM) is associated with an increased risk of fractures. However, the impact of glycemic control on the risk of fracture is not well understood.

Objective

To evaluate the association between the level of glycemic control and the risk of low-trauma fractures in patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus.

Design

Nested case-control analysis

Setting

UK-based Clinical Practice Research Datalink

Patients or other participants

The study population consisted of patients whose T1DM or T2DM was newly diagnosed between 1995 and 2015. Cases were patients with a low-trauma fracture after DM onset. We matched 4 controls to each case on age, sex, general practice, fracture date, and diabetes type and duration.

Statistical analysis

Conditional logistic regression analyses adjusted for covariates including BMI, smoking, diabetes complications and medications.

Results

The study population consisted of 3,329 T1DM and 44,275 T2DM patients. Median duration between diabetes onset and fracture date was 4.5 years for both, T1DM and T2DM. The risk of fracture was increased in T1DM patients with mean HbA1c >8.0% (aOR 1.39, 95% CI 1.06-1.83) compared to T1DM patients with mean HbA1c values ≤7.0%. There was no such effect in T2DM patients. Independently of glycemic control, the risk of fracture was elevated in patients with T2DM and current use of rosiglitazone and pioglitazone.

Conclusions

The impact of glycemic control on the risk of low-trauma fracture differs between T1DM and T2DM patients. Poor glycemic control increased the risk of fracture in T1DM but not in T2DM patients.

4.1.2 Introduction

Diabetes mellitus (DM) is associated with an increased risk of fragility fractures. In particular, the risk of hip fractures is increased about 6-fold in subjects with type 1 diabetes (T1DM), and 2- to 3-fold in patients with type 2 diabetes (T2DM).[87]

The pathophysiological mechanisms that contribute to skeletal fragility differ across the two diabetes types.[88] Differences in age at disease onset, insulin availability (insulin deficiency vs insulin resistance), and influence of antidiabetic drugs lead to altered skeletal fragility. In patients diagnosed with T1DM during adolescence and early adulthood, deficiencies in insulin and insulin-like growth factor 1 (IGF-1) seem to impair osteoblast function leading to lower bone mass, smaller bone size, and alterations in bone microstructure.[88-91]

In contrast, patients with T2DM, who usually suffer from obesity-related insulin resistance and hyperinsulinemia, present with normal to increased bone mass and preserved or even increased trabecular bone volume, but with increased cortical porosity. This pattern is found particularly in patients with fractures and microvascular complications.[89, 92]

In DM patients (T1DM and T2DM) with advanced disease, glucotoxicity, chronic inflammation, and microvascular changes are thought to be critical factors in accelerating bone aging and progression of diabetic bone disease.[93, 94] In addition, non-skeletal factors [95, 96] such as chronic diabetic complications, comorbidities, and drug effects [97, 98] may increase the risk of falls [92, 99] and thus the overall risk of fracture.

It remains unclear to what extent glycemic control has an impact on the risk of fracture. Some studies have reported an association between poor glycemic control and increased risk of fractures [100-103] or falls,[104] whereas others did not.[87, 105-107] In contrast, good glycemic control was also associated with an increased risk of fracture [99, 105] or falls [108] in some studies. Although several studies have examined the impact of glycemic control on the risk of fractures by analyzing HbA1c levels (a measure of the average glycemia over a period of about 12 weeks), results remain inconsistent due to methodological heterogeneities. Only a few of these studies included a substantial number of patients [100-102] and analyzed the impact of glycemic control not only based on a single HbA1c measurement,[100, 108, 109] but on mean HbA1c levels during a longer follow-up time;[100] the latter may more accurately reflect the degree of glycemic control. Additionally, only Forsén et al. analyzed the association between glycemic control and risk of fracture separately for T1DM and T2DM patients.[87]

Therefore, we conducted a study to evaluate the association between the degree of glycemic control and the risk of non-vertebral low-trauma fractures in patients with newly diagnosed T1DM and T2DM.

4.1.3 Methods

Study design and data source

We conducted a nested case-control analysis within a cohort of patients with incident T1DM or T2DM using data from the UK-based primary care database called Clinical Practice Research Datalink (CPRD). The study period encompassed 21 years between 1995 and 2015.

The CPRD is a governmental, nonprofit research service and a joint venture from the Medicines and Healthcare Regulatory Agency (MHRA) and the National Institute for Health Research (NIHR).[110] This large database of anonymized medical records was established in 1987 and covers medical records for over 11.3 million general practice patients from 674 practices in the UK.[110] The patients are representative of the UK general population in terms of age, sex, and ethnicity.[111] The general practitioners (GPs) are trained to record medical information including medical diagnoses, referrals to specialists and secondary care settings, prescriptions, diagnostic testing, lifestyle information, and demographic data using standard software and standard coding systems.[110] The MHRA checks the raw data before release and performs quality control checks. The CPRD is widely used internationally for studies in pharmacoepidemiology and disease epidemiology, including bone fractures.[112, 113] The CPRD has proven to be of high quality.[110, 114, 115]

The study protocol was approved by the Independent Scientific Advisory Committee (ISAC) for MHRA database research (protocol number 17_061R), and the protocol was made available to the journal reviewers.

Study population

We selected patients with an incident diagnosis of T1DM or T2DM between January 1, 1995, and December 31, 2015. T2DM patients were required to have a minimum of 3 years of recorded history in the database prior to the first recorded diabetes code to ensure that we only included incident T2DM cases. For T1DM patients, we only asked for 1 year of recorded history since these patients are usually much younger upon disease onset and may not have a long medical history.

We identified patients with DM based on specific codes for diabetes and by new use of antidiabetic drugs (oral antidiabetics or insulin). We defined the study entry date as the date of the first recorded diabetes code.

We classified patients who had no specific DM codes indicating the DM type according to age of DM onset and the prescribed antidiabetic drugs:

- Patients with DM onset before the age of 30 years who received insulin were classified as T1DM patients

- Patients who received oral antidiabetic drugs (OADs) with or without insulin were classified as having T2DM
- Patients whose first DM record was after the age of 30 and who received only insulin remained unclassified.

We did not consider laboratory tests for the classification of diabetes, but only GP-recorded disease codes, as well as the age and the medication of the patients as mentioned above. We excluded all unclassified patients from the study. We further excluded patients with a diagnosis of cancer (except non-melanoma skin cancer), alcoholism, or HIV at any time in the patient record because these patients usually have many comorbidities and receive many drugs, which could lead to substantial bias and confounding.

Case definition

The cases were patients with a recording of a low-trauma fracture (e.g., non-vertebral fractures of the proximal and distal upper and lower extremities, ribs and thorax, hip and foot) during the study period (i.e., after their incident DM diagnosis). We excluded fractures of the shoulder blade or cranium, since these fractures are not considered low-trauma fractures. We identified fracture cases by specific codes and assigned the date of the fracture diagnosis as the 'index date'. We used risk set sampling to identify controls from among the DM patient study population who did not experience a fracture between DM onset and the index date of their matched case.

We matched cases to controls 1:4 on age (+/- 3 years), sex, general practice, index date (control present in the database on the index date), DM type, and DM duration (+/- 365 days). We assessed DM duration by counting the days between the first recorded DM code and the index date.

Exposure definition

The exposure of interest in this study was glycemic control after DM onset defined by HbA1c levels and expressed as a categorical variable (**Table 3**). The categories were wider for the patients with T1DM because the patient numbers were smaller and did not accommodate as many levels.

Table 3: Categories for HbA1c levels in patients with T1DM and T2DM

T1DM			**T2DM**		
Definition	DCCT-HbA1c [%]	IFCC-HbA1c [mmol/mol]	Definition	DCCT-HbA1c [%]	IFCC-HbA1c [mmol/mol]
Good control*	≤7.0	≤53	Very good control	≤6.5	≤48
			Good control	>6.5-7.0	>48-53
Medium control*	>7.0-8.0	>53-64	Medium control	>7.0-7.5	>53-59
			Poor control	>7.5-8.0	>59-64
Poor control*	>8.0	>64	Very poor control	>8.0-9.0	>64-75
			Unsatisfactory control	>9.0	>75
Unknown*	NA**	NA**	Unknown	NA**	NA**

* Categories for the patients with T1DM were wider owing to smaller patient numbers
** Not applicable due to missing data

We analyzed available HbA1c measurements throughout the study period at several points in time: initial HbA1c level, mean HbA1c level over the 3 years before the index date, and last HbA1c level before the index date. Only the data for the mean HbA1c levels over 3 years were presented since results were similar for all HbA1c measurements. Missing values were presented in a separate category.

Statistical analysis

We used conditional logistic regression to assess the association between HbA1c values and the risk of low-trauma fractures, expressed as odds ratios (ORs) with 95% confidence intervals (CI). We assessed a variety of comorbidities and comedications (recorded at any time in the patient record before the index date) for confounding, including those associated with the risk of fracture. For antidiabetic drugs, we assessed the risk of fractures in current users defined as patients with a prescription for the respective drug recorded ≤60 days prior to the index date. Additionally, we assessed the association between the patients' number of GP visits within one year prior to the index date and the risk of fracture.

We adjusted the analyses of T1DM patients for BMI (as categorical variable, **Table 4**), smoking (current, past, non-smokers, and unknown), previous fractures, chronic renal failure, previous falls, decreased vision (all yes/no), and use of bisphosphonates, calcium and supplements, and metformin. We adjusted the analyses of T2DM (**Table 5**) for the same covariates plus use of insulin, rosiglitazone, and pioglitazone, but not for chronic renal failure or decreased vision.

Table 4: Patient characteristics and covariates in fracture cases and controls T1DM

	Diabetes mellitus type 1			
	Cases T1DM n (%)	**Controls T1DM n (%)**	**T1DM Crude OR (95% CI)**	**T1DM Adjusted OR* (95% CI)**
Age at fracture (years)				
<20	254 (37.8)	1021 (38.4)	NA	NA
20-29	91 (13.5)	353 (13.3)	NA	NA
30-39	56 (8.3)	231 (8.7)	NA	NA
40-49	82 (12.2)	329 (12.4)	NA	NA
50+	189 (28.1)	723 (27.2)	NA	NA
Sex				
Male	363 (54.0)	1438 (54.1)	NA	NA
Female	309 (46.0)	1219 (45.9)	NA	NA
BMI (kg/m^2)				
<18.5	19 (2.8)	40 (1.5)	1.78 (1.10-2.87)	1.66 (1.02-2.69)
18.5 to <25.0	206 (30.7)	743 (28.0)	1 (reference)	1 (reference)
25.0 to <30.0	146 (21.7)	603 (22.7)	0.84 (0.67-1.05)	0.86 (0.68-1.08)
30.0 to <35.0	53 (7.9)	306 (11.5)	0.58 (0.42-0.80)	0.62 (0.44-0.87)
35.0 to <40.0	22 (3.3)	74 (2.8)	1.01 (0.65-1.56)	1.03 (0.66-1.62)
≥40.0	11 (1.6)	49 (1.84	0.75 (0.40-1.41)	0.81 (0.41-1.60)
Unknown	215 (32.0)	842 (31.7)	1.10 (0.76-1.59)	1.15 (0.79-1.67)
Smoking status				
Non-smoker	291 (43.3)	1153 (43.4)	1 (reference)	1 (reference)
Current smoker	106 (15.8)	382 (14.4)	1.12 (0.89-1.41)	1.05 (0.82-1.33)
Ex-smoker	123 (18.3)	497 (18.7)	0.98 (0.78-1.24)	0.98 (0.77-1.24)
Unknown	152 (22.6)	625 (23.5)	0.91 (0.69-1.21)	0.91 (0.68-1.23)
Comorbidities				
Previous fracture	121 (18.0)	307 (11.6)	1.76 (1.43-2.16)	1.67 (1.35-2.07)
Ischemic heart disease	49 (7.3)	136 (5.1)	1.52 (1.09-2.12)	1.50 (1.05-2.14)
Chronic renal failure	25 (3.7)	42 (1.6)	2.45 (1.61-3.74)	2.24 (1.47-3.42)
Diabetic retinopathy	225 (33.5)	756 (28.5)	1.36 (1.12-1.65)	1.29 (1.06-1.57)
Previous falls	90 (13.4)	197 (7.4)	1.97 (1.55-2.51)	1.73 (1.35-2.22)
Decreased vision	37 (6.8)	104 (3.9)	1.44 (1.02-2.04)	1.24 (0.86-1.78)
Co-medication				
Insulin	647 (96.3)	2541 (95.6)	1.24 (0.79-1.95)	1.05 (0.66-1.69)
Pioglitazone	7 (1.0)	13 (0.5)	2.13 (0.83-5.44)	2.83 (1.05-7.61)
Rosiglitazone	4 (0.6)	30 (1.1)	0.53 (0.19-1.51)	0.60 (0.20-1.77)
Metformin	99 (14.7)	436 (16.4)	0.82 (0.63-1.07)	0.87 (0.67-1.12)
Bisphosphonates	25 (3.7)	41 (1.5)	2.65 (1.54-4.56)	1.84 (1.09-3.09)
Calcium & supplements	56 (8.3)	121 (4.6)	2.04 (1.43-2.92)	1.39 (0.98-1.98)

*Adjustment for T1DM: BMI, smoking, previous fractures, chronic renal failure, previous falls, decreased vision (all yes/no), and use of bisphosphonates, calcium & supplements, and metformin.

Table 5: Patient characteristics and covariates in fracture cases and controls T2DM

	Diabetes mellitus type 2			
	Cases T2DM	**Controls T2DM n (%)**	**T2DM Crude OR (95% CI)**	**Adjusted T2DM OR* (95% CI)**
Age at fracture (years)				
<60	1635 (18.7)	6510 (18.4)	NA	NA
60-69	1791 (20.2)	7245 (20.5)	NA	NA
70-79	2576 (29.1)	10307 (29.1)	NA	NA
80-89	2342 (26.4)	9349 (26.4)	NA	NA
90+	515 (5.8)	2005 (5.7)	NA	NA
Sex				
Male	2575 (29.1)	10288 (29.1)	NA	NA
Female	6284 (70.9)	25128 (71.0)	NA	NA
BMI (kg/m^2)				
<18.5	149 (1.7)	346 (1.0)	1.54 (1.30-1.83)	1.41 (1.18-1.68)
18.5 to <25.0	1843 (20.8)	6369 (18.0)	1 (reference)	1 (reference)
25.0 to <30.0	2889 (32.6)	11688 (33.0)	0.84 (0.79-0.89)	0.85 (0.80-0.90)
30.0 to <35.0	2127 (24.0)	8994 (25.4)	0.79 (0.74-0.84)	0.79 (0.74-0.85)
35.0 to <40.0	1004 (11.3)	4405 (12.4)	0.75 (0.70-0.82)	0.75 (0.69-0.82)
≥40.0	620 (7.0)	2743 (7.8)	0.74 (0.67-0.81)	0.72 (0.65-0.80)
Unknown	228 (2.6)	872 (2.5)	0.90 (0.78-1.05)	0.90 (0.77-1.06)
Smoking status				
Non-smoker	3678 (41.5)	16042 (45.3)	1 (reference)	1 (reference)
Current smoker	1137 (12.8)	3739 (10.6)	1.34 (1.26-1.44)	1.30 (1.21-1.40)
Ex-smoker	3937 (44.4)	15207 (42.9)	1.14 (1.09-1.20)	1.15 (1.10-1.20)
Unknown	107 (1.2)	428 (1.2)	1.07 (0.88-1.31)	1.07 (0.86-1.33)
Comorbidities				
Previous fracture	1910 (21.6)	4879 (13.8)	1.75 (1.66-1.84)	1.63 (1.55-1.72)
Ischemic heart disease	2002 (22.6)	7762 (27.9)	1.04 (0.99-1.10)	1.00 (0.95-1.05)
Chronic renal failure	565 (6.4)	1994 (5.6)	1.16 (1.06-1.27)	1.15 (1.05-1.26)
Diabetic retinopathy	2278 (25.7)	8601 (24.3)	1.11 (1.05-1.17)	1.12 (1.06-1.18)
Previous falls	2621 (29.6)	6849 (19.3)	1.86 (1.77-1.96)	1.75 (1.67-1.84)
Decreased vision	885 (10.0)	3008 (8.5)	1.20 (1.12-1.29)	1.10 (1.02-1.18)
Co-medication				
Insulin	1002 (11.3)	3531 (10.0)	1.17 (1.08-1.26)	1.10 (1.02-1.18)
Pioglitazone	694 (7.8)	2267 (6.4)	1.28 (1.16-1.41)	1.36 (1.25-1.49)
Rosiglitazone	501 (5.7)	1653 (4.7)	1.24 (1.12-1.39)	1.32 (1.20-1.46)
Metformin	5366 (60.6)	21758 (61.4)	1.00 (0.95-1.05)	0.96 (0.91-1.01)
Bisphosphonates	1076 (12.2)	2834 (8.0)	1.64 (1.51-1.77)	1.30 (1.20-1.41)
Calcium & supplements	1579 (17.8)	4670 (13.2)	1.48 (1.39-1.58)	1.15 (1.07-1.23)
Hormone replacement therapy	1739 (19.6)	6550 (18.5)	1.03 (1.03-1.18)	1.03 (0.96-1.09)

*Adjustment for T2DM: BMI, smoking, previous fractures, previous falls, and use of bisphosphonates, calcium & supplements, metformin, insulin, rosiglitazone, and pioglitazone.

No analyzed covariates changed the model by 10% or more, but we included several covariates in the model based on established risk factors for fractures and statistically significant univariate ORs. We conducted all analyses using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA).

4.1.4 Results

We identified 9,531 patients with a low-trauma fracture and 38,073 subjects with no fractures (**Figure 7**). The types and numbers of nonvertebral low-trauma fractures have been summarized in **Table 6**. The patient characteristics, selected comorbidities, and drug exposure of the study population are listed in **Table 4** and **5**. The risk of fractures associated with the different mean HbA1c values (mean for previous 3 years) is presented in **Table 7**.

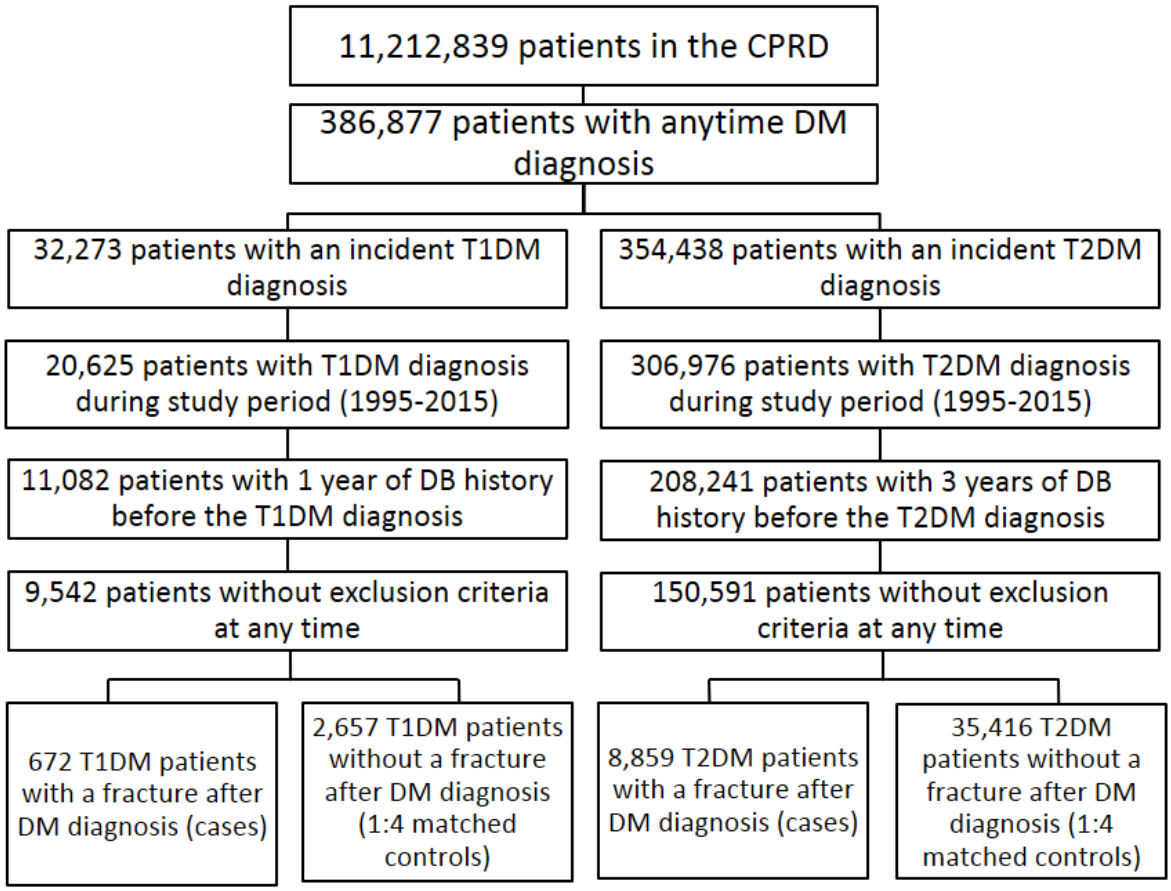

Figure 6: Flowchart showing selection of study population

Table 6: Frequencies of non-vertebral low-trauma fractures by DM type

Low-Trauma Fracture	T1DM n (%)	T2DM n (%)
Proximal upper extremity (humerus, elbow)	142 (23.5%)	1908 (20.6%)
Distal upper extremity (ulna, radius)	87 (14.2%)	1084 (11.9%)
Ribs	23 (5.1%)	428 (4.6%)
Hip	6 (0.8%)	458 (5.9%)
Femur, patella	52 (9.2%)	1797 (22.6%)
Distal lower extremity (tibia, fibula)	93 (18.6%)	1229 (13.3%)
Foot	54 (10.9%)	550 (5.9%)
Unspecified	109 (18.6%)	1405 (15.6%)

Table 7: Risk of fractures associated with HbA1c levels (3-year mean prior to the index date)

Mean HbA1c	Patients with T1DM			
	Cases n (%)	Controls n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)
≤7%	61 (9.1)	284 (10.7)	1 (reference)	1 (reference)
>7-8%	121 (18.0)	594 (22.4)	0.95 (0.70-1.30)	0.99 (0.72-1.35)
>8%	407 (60.6)	1421 (53.5)	1.37 (1.04-1.79)	1.39 (1.06-1.83)
No Recording	83 (12.4)	358 (13.5)	1.07 (0.74-1.52)	1.09 (0.76-1.56)
Mean HbA1c	**Patients with T2DM**			
	Cases n (%)	**Controls n (%)**	**Crude OR (95% CI)**	**Adjusted OR** (95% CI)**
≤6.5%	2634 (29.7)	10208 (28.8)	1.05 (0.98-1.11)	1.02 (0.96-1.09)
>6.5-7%	1615 (18.2)	6521 (18.4)	1 (reference)	1 (reference)
>7-7.5%	1386 (15.7)	6228 (17.6)	0.90 (0.84-0.97)	0.89 (0.83-0.96)
>7.5-8%	826 (9.3)	3385 (9.6)	0.99 (0.91-1.07)	0.96 (0.88-1.05)
>8-9%	1009 (11.4)	3964 (11.2)	1.03 (0.95-1.12)	0.99 (0.91-1.08)
>9%	879 (9.9)	3229 (9.1)	1.11 (1.02-1.21)	1.03 (0.94-1.13)
No Recording	510 (5.8)	1881 (5.3)	1.13 (1.01-1.26)	1.12 (1.00-1.26)

* Adjustment for BMI, smoking, previous fractures, chronic renal failure, previous falls, decreased vision (all yes vs no), and use of bisphosphonates, calcium and supplements, and metformin.

** Adjustment for BMI, smoking, previous fractures, previous falls, and use of bisphosphonates, calcium and supplements, metformin, insulin, rosiglitazone, and pioglitazone.

The patients with T2DM had a greater number of recorded HbA1c measurements and better glycemic control compared with the patients with T1DM. In addition, 11.6% of the T1DM cases and 11.9% of the T1DM controls had no reported HbA1c measurements during the 3 years before the index date. This was only the case for 5.6% of the T2DM cases and 4.9% of the T2DM controls. The mean 3-year HbA1c level was 8.7% for the patients with T1DM and 7.3% for all patients with T2DM. However, the patients with T2DM with a prescription for oral antidiabetic drugs or insulin had greater mean HbA1c levels than the patients with medically untreated T2DM. The results, stratified by sex, age, DM duration, and type of DM, were similar to the results from the unstratified analyses and, therefore, were not included.

Type 1 diabetes

Of the 32,273 individuals with incident T1DM, we identified 672 patients with a recorded fracture after the DM diagnosis and 2657 matched DM controls. Overall, the median age at the index date of the patients with T1DM (cases and controls) was 28 years (quartile 1, 14; quartile 3, 52 years), and the mean BMI (last available value before the index date) was 26.5 ± 5.5 kg/m2. The median interval between the DM diagnosis and fracture was 4.5 years (quartile 1, 2.0; quartile 3, 8.0 years), and 46% of the patients were female. During the study period, the patients with T1DM had a mean of nine recorded HbA1c measurements.

Although the risk of fracture was not increased in the patients with T1DM with moderate glycemic control (3-year mean HbA1c level, >7% to 8%; adjusted OR, 0.99; 95% CI, 0.72 to 1.35), compared with the patients with T1DM and good glycemic control, the risk of fracture for the patients with T1DM and poor glycemic control was slightly increased (3-year mean HbA1c level, >8.0%; adjusted OR, 1.39; 95% CI, 1.06 to 1.83; **Table 7**).

In patients with recorded comorbidities associated with the micro- and macrovascular complications of DM, such as diabetic retinopathy (adjusted OR, 1.29; 95% CI, 1.06 to 1.57) and chronic renal failure (adjusted OR, 2.24; 95% CI, 1.47 to 3.42), the risk of fracture was also increased compared with patients without the respective comorbidity (**Table 4**). The number of GP visits was not associated with the risk of fracture.

Type 2 diabetes

Of the 354,438 individuals with T2DM, we identified 8859 patients with a fracture and 35,416 matched controls. The median age of the patients with T2DM (cases and controls) was 71.7 years (quartile 1, 63; quartile 3, 82), and mean BMI (last available measurement before the index date) was 30.2 ± 6.5 kg/m2. The median interval between the DM diagnosis and the first fracture was 4.5 years (quartile 1, 2.0; quartile 3, 7.9), and 71% of the patients were female. During the study period, the patients with T2DM had a mean of 11 recorded HbA1c measurements before the index date.

Glycemic control was not associated with the risk of fracture in the patients with T2DM with an HbA1c level >6.5% to 7.0% compared with those with T2DM and other HbA1c levels (**Table7**). The micro- and macrovascular complications of DM were not clearly associated with the risk of fracture in this patient group.

In analyses stratified for DM treatment, we observed an increased risk of fracture among patients with T2DM and current (last prescription <60 days before the index date) use of pioglitazone (OR, 1.36; 95% CI, 1.25 to 1.49) and rosiglitazone (OR, 1.32; 95% CI, 1.20 to 1.46) compared with nonusers. This effect was independent of glycemic control (**Table 3**).

Increasing numbers of GP visits were associated with an increased risk of fracture (adj. ORs for 21-30 GP visits 1.22, CI 95% 1.14-1.31 and for over 30 visits 1.58, CI 95% 1.48-1.69) compared to patients with 20 or less GP visits in the last year prior to the index date (data not shown).

4.1.5 Discussion

Our results suggest that the effect of glycemic control on the risk of non-vertebral low-trauma fractures differs between T1DM and T2DM patients. While poor glycemic control (HbA1c levels >8%) was associated with a slightly increased risk of fracture (OR 1.39, 95% CI 1.06-1.83) in patients with T1DM

compared to T1DM patients with good glycemic control (HbA1c levels ≤7.0%), we did not observe such an association in patients with T2DM.

We observed an association between comorbidities related to micro- and macrovascular complications, such as diabetic retinopathy and ischemic heart disease, and risk of fracture in T1DM patients. In T2DM, the risk of fracture was elevated with current use of pioglitazone and rosiglitazone independently of glycemic control but not in patients with vascular complications. In T1DM as well as T2DM patients, the first fracture after DM onset occurred relatively early in the course of the disease (after a mean of 4.5 years).

To date, only a few small studies [87, 116] have assessed the impact of glycemic control on the risk of fracture in T1DM. Although the Trondelag Health Survey by Forsén et al. reported a trend between glycemic control and the risk of fracture,[87] the association was not statistically significant. This was probably primarily due to the small number of patients with T1DM (only 2.9% [n=54] of all DM patients included). Heap et al. [109] demonstrated that glycemic control was associated with whole-body bone mineral content in adolescents with T1DM, but these authors did not assess the risk of fracture as an outcome in their study. Neumann et al. [116] showed that poor glycemic control was associated with an increased risk of fracture in T1DM (OR for reported clinical fracture associated with 1-SD increase in median HbA1c was 1.92, CI 95% 1.09-2.75). However, the study was limited by its cross-sectional design and by the fact that it only included 122 T1DM patients with HbA1c measurements. The study by Conway et al. [100] which found an increased risk of fracture in association with both poor (HbA1c 8-9% and >9%) and good (<6.5%) glycemic control compared to HbA1c levels of 7-7.9%, had a minimum of two HbA1c measurements per patients and a longer duration of follow up. However, they had not distinguished between T1DM and T2DM.[100]

In contrast, we found the risk of incident fracture to be slightly increased in T1DM patients (adj. OR 1.39, CI 95% 1.06-1.83) with poor glycemic control (3-year mean HbA1c >8.0%) compared to T1DM patients with HbA1c levels ≤7.0%, in a large cohort of 3,329 patients with T1DM and a mean of nine HbA1c measurements per patient. However, there was no such association in patients with T2DM.

Our findings can be explained by the different pathophysiological mechanisms contributing to skeletal fragility in each DM type. During puberty, approximately 50-60% of peak bone mass is accrued.[117] Insulin as anabolic hormone is thought to have a stimulatory effect on osteoblast function. Therefore, insulin deficiency in T1DM may cause a reduction in osteoblast cell numbers,[118] resulting in impaired peak bone mass. Furthermore, specifically in the first few years after disease onset, there is rapid bone loss in T1DM, stabilizing thereafter to a steady state in conjunction with disease control. This could be due to a decline in insulin secretion, or to inadequate control of diabetes itself.[91, 93] Hyperglycemia also seems to play an important role in the pathophysiology of poor bone quality. It appears that hyperglycemia impairs bone mineral acquisition [119] and is associated with a decreased bone mineral

content,[109] impaired vitamin D and calcium metabolism,[120] reduced osteoblast differentiation,[121] and an increased rate of osteoblast apoptosis.[122]

Although some studies did report an association between glycemic control and the risk of fracture in patients with T2DM, we and several others [87, 105, 123] have failed to confirm this association. Poor glycemic control (HbA1c ≥8%) was associated with increased hospitalization rate due to fractures compared to HbA1c levels <8% in the Atherosclerosis Risk in Communities Study.[102] In the Rotterdam Study, patients with HbA1c greater than 7.5% had an increased risk of fracture relative to those with a lower HbA1c level (HR 1.62, 95% CI 1.09-2.40) [103]. Finally, Li et al. observed an increased risk of fracture associated with HbA1c levels from 9-10% and ≥10% compared to HbA1c levels of 6-7% (HR 1.24, 95% CI 1.02–1.49 and 1.32, 95% CI 1.09–1.58, respectively), in a large geriatric T2DM population.[101]

Of all T2DM patients in our study population, 9,081 (21%) were poorly controlled with HbA1c levels >8%, while the largest proportion (n=20,978, 47%) presented with good HbA1c control (<7%). In the study of Li et al.,[101] 43% of T2DM patients had HbA1c levels >8%. The study by Schneider et al. [102] included only 1,195 T2DM patients, but 51.6% of them had HbA1c >8%. In contrast to these studies,[101-103] which were small and based on only one or two HbA1c measurements per person, we were able to analyze the impact of long-term glycemic control by using a mean of 11 HbA1c measurements per person in our T2DM population. Additionally, we had access to the medical information on a large cohort of 44,275 T2DM patients and to additional information to adjust for risk factors such as BMI, smoking, comedications, and diabetes-related complications.

One possible explanation for the null effect of glycemic control in T2DM patients could be the beneficial effect of insulin resistance in early disease.[88] Patients with T2DM have superior trabecular indices due to obesity-related insulin resistance and hyperinsulinism in the initial years of DM compared to healthy controls.[90] Circulating insulin is considered to stimulate osteoblastogenesis and to enhance bone formation.[124] Thus, patients with T2DM usually present with a higher bone mass [92, 125] compared to healthy controls.

Independently of glycemic control, several previous epidemiological studies have demonstrated an increased risk of fracture in patients with T2DM.[126, 127] While we did not evaluate the risk of fracture in T2DM patients overall compared to patients with no diabetes, our findings support the notion that the risk of fracture in patients with T2DM might be related to risk factors independent of glycemic control. T2DM is part of a chronic metabolic disorder and is associated with a range of cardiovascular comorbidities.[104] Microangiopathy is thought to be a critical factor in the progression of diabetic bone disease, inducing accelerated bone loss [93, 94] and increasing the risk of falls and fractures.[128] Lee et al. recently showed that a significant portion of this risk is explained by diabetes-related comorbidities.[128]

We identified many T1DM and T2DM patients with diabetes-related complications despite the relatively short disease duration; median disease duration before fracture was only 4.5 years. The potential effect of disease duration itself on the risk of fracture was not the focus of our study. In fact, we adjusted for diabetes duration by matching in order to separate the effect of glycemic control from a potential effect of disease duration.

The current use of rosiglitazone and pioglitazone was associated with an increased risk of fracture in our study independent of glycemic control. Preclinical [129] and clinical [130] studies indicated that thiazolidinediones adversely affect bone metabolism, resulting in reduced osteoblastic bone formation and accelerated bone loss, and thus may increase fracture risk. Furthermore, their current use was associated with an approximately 2- to 3-fold increased risk of hip and non-vertebral osteoporotic fractures.[131]

The present findings should be interpreted within the context of the study strengths and limitations. The strengths of our study were a) the large observational nested case control design within a cohort of newly diagnosed DM patients, b) the fact that our data were based on a large and validated primary-care database and that data were recorded prospectively thus avoiding recall bias, and c) that we analyzed the impact of glycemic control on the risk of fracture based on a mean of 9 and 11 HbA1c measurements for T1DM and T2DM, respectively. Furthermore, we were able to assess the risk of fracture separately for patients with T1DM and T2DM.

However, several limitations should be considered. Our study population included a high proportion of T2DM patients with good glycemic control who may have been healthier than T2DM populations analyzed in other studies. Nevertheless, our T2DM population included over 30,000 medically treated T2DM patients including many with poor glycemic control. Therefore, we expect our results to be applicable to other T2DM patients with poor glycemic control. Furthermore, fractures are associated with a wide range of comorbidities and with the use of many drugs. While we adjusted for a variety of diseases and drugs, we cannot rule out that some residual confounding may be present in our analyses.

Some misclassification of patients into T1DM and T2DM may have occurred for patients with a nonspecific DM code. Since diagnoses of DM (positive predictive value of >98%) and fractures were well recorded (positive predictive value of around 90% for hip and vertebral fractures) and validated in the CPRD, minimal misclassification is likely.[132] However, it is possible that we missed some fracture cases. This possible misclassification would likely have been non-differential and would not have materially changed the results. Also, the cause for the fracture is mostly unknown. We therefore are not aware if fractures could have been caused by diabetic emergencies, such as hypo- or hyperglycemia. These episodes are presumably rather poorly reported in the CPRD. For this reason, we did not assess the impact of diabetic emergencies on the risk of fracture. However, this association was not the focus

of the study, since HbA1c levels and fractures are recorded and were analyzed independently of the reason for the fracture.

Additionally, the time of disease onset was uncertain since T2DM can remain undiagnosed for many years, possibly leading to the inclusion of some prevalent (instead of incident) T2DM cases. This was already shown in the UK Prospective Diabetes study, where a prevalence of diabetes tissue damage was shown by the time of the diabetes diagnosis as a hint of a preexisting diabetes.[133] Therefore, we might have underestimated the time until fracture (after DM onset) in our T2DM study population, which would potentially affect our matching on DM duration. However, this misclassification is unlikely to have been differential, and we therefore did not expect a major influence on our findings.

In conclusion, the impact of glycemic control on the risk of non-vertebral low-trauma fractures differed in T1DM and T2DM patients with short-term disease. While poor glycemic control elevated the risk of fracture in T1DM patients, we observed no such association in patients with T2DM. This might be attributed to a protective effect of insulin resistance in early disease.

4.1.6 Abbreviations:

BMI	Body mass index
CI	Confidence Interval
CPRD	Clinical Practice Research Datalink
DCCT	Diabetes Control and Complications Trial
DB	Database
DM	Diabetes mellitus
GP	General practitioner
IFCC	International Federation of Clinical Chemistry
HbA1c	Hemoglobin A1c
HR	Hazard ratio
MHRA	Medicines and Health Care Regulatory Agency
n	Number
NA	not applicable
OR	Odds Ratio
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

4.1.7 Acknowledgments

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> «Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.»
>
> *Marie Curie*

Chapter 5

Study II: Study on antidiabetic treatment, level of glycemic control, and risk of fracture

5 Study II

5.1 Antidiabetic treatment, level of glycemic control, and risk of fracture in diabetes type 2: A nested case-control study

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5.1.1 Abstract

Context

Patients with type 2 diabetes mellitus (T2DM) have an increased risk of low-trauma fractures. However, the effect of antidiabetic medication in relation to glycemic control on the risk of fracture is poorly understood.

Objective

This work aimed to evaluate the association between the level of glycemic control, use of antidiabetic medication, and risk of low-trauma fractures in patients with newly diagnosed T2DM.

Methods

We conducted a nested case-control analysis among individuals registered in the Clinical Practice Research Datalink. The base population consisted of patients with newly diagnosed T2DM from 1995 to 2017. Cases were patients with a low-trauma fracture after T2DM diagnosis. We matched 4 controls to each case. Exposures of interest were glycemic control (last glycated hemoglobin [HbA1c] level before fracture) and type of diabetes treatment. We conducted conditional logistic regression analyses adjusted for several confounders.

Results

We identified 8809 cases and 35 219 controls. Patients with current metformin use and HbA1c levels of less than 7.0% and between 7.0-8.0% had a reduced risk of fractures (adjusted odds ratio 0.89; 95% CI, 0.83-0.96 and 0.81; 95% CI, 0.73-0.90, respectively) compared with untreated patients. However, in patients receiving metformin plus 1 or 2 other antidiabetic drugs, or insulin (alone or in addition to other antidiabetic medication), the level of glycemic control was not associated with the risk of fracture compared with untreated patients.

Conclusions

While patients with good or medium glycemic control receiving current metformin monotherapy had a lower risk of fracture compared with untreated patients, glycemic control in patients receiving treatment other than metformin was not associated with risk of fracture.

5.1.2 Introduction

Diabetes mellitus (DM) is a chronic condition with a high global prevalence, affecting some 450 million (8.8%) patients worldwide and causing approximately 5 million deaths per year.[134] In the UK, 4.7 million patients (7.0%) were living with diagnosed or undiagnosed DM in 2019.[135] DM type 1 (T1DM) and type 2 (T2DM) are characterized by hyperglycemia due to insulin deficiency and insulin resistance, respectively, and both have been linked to an increased risk of fragility fractures compared with non-diabetic individuals.[88-91, 136, 137] T2DM is more prevalent and accounts for 90% of all diabetes patients.[135] The risk of hip fracture has been reported to be elevated around 2- to 3-fold in T2DM patients.[87, 138] Therefore, the impact of T2DM and related fractures on public health is significant.[135]

While the association between T2DM and an elevated risk of fracture has been consistently shown in several studies,[87, 88, 90, 92, 139] the impact of glycemic control on the risk of fracture in T2DM patients is less clear. Suggested mechanisms in patients with T2DM and elevated HbA1c levels include altered body composition, accumulation of advanced glycation end products in bones, impaired bone healing, and lower bioavailability of insulin-like growth factor 1.[100] In the Rotterdam study, T2DM patients with HbA1c-levels ≥7.5% had an increased risk of fracture compared with non-diabetic patients (47-62% higher risk of fracture). The authors stated that "This association did not seem to be influenced by potential confounders or arising from diabetes complications (extra-skeletal risk factors), such as risk of falling at baseline or decline in renal function, nor by the use of systemic corticosteroids or diuretics".[103] In contrast, authors of a study from Singapore including 932 fracture cases observed that T2DM patients with HbA1c levels <6% and from 6.1-7.0% had an increased risk of fracture compared with those with HbA1c levels above 8% (OR 3.01, 95% CI 2.01-4.51 and OR 2.34, 95% CI 1.71-3.22, respectively). Cases and matched controls included similar proportions of insulin and sulfonylurea users. Controls were more likely to be metformin and/or acarbose users.[105] A study from the US including over 10'000 geriatric patients with T1DM and T2DM yielded no significant difference in the risk of fracture in patients with HbA1c levels of <6.5% (HR 0.89, 95% CI 0.75-1.06), 6.5-6.9% (HR 0.92, 95% CI 0.76-1.12), 8-8.9% (HR 1.35, 95% CI 1.09-1.66), and ≥9% (HR 1.21, 95% CI 0.97-1.52) compared with patients with HbA1c levels of 7-7.9%.[100] In a Taiwanese cohort study including 20'025 patients with T2DM, however, the risk of fracture was increased in patients with HbA1c levels of 9%–10% (HR 1.24, 95% CI 1.02-1.49) and ≥ 10.0% (HR 1.32, 95% CI 1.09-1.58) compared with patients with HbA1c levels of 6–7%.[101] A study from Rome including 92 postmenopausal women (19 T2DM patients and 73 nondiabetic controls) affected by osteoarthritis, who were scheduled for elective hip replacement surgery, suggests that pathways of bone formation in T2DM may be impaired despite good glycemic control.[140] Previously, our group reported a positive association between the level of glycemic control and the risk of fracture in T1DM patients, but not in T2DM patients.[62]

While different study designs may, to a certain extent, explain the discordant findings, another possible explanation for these discrepant findings could be differences in antidiabetic medications taken by T2DM patients. This aspect has not been extensively considered in previous studies. Furthermore, the medication scheme can also serve as an indicator of the patient's disease severity, which may impact the risk of fracture and the level of glycemic control (measured as HbA1c levels). Therefore, an analysis by medication type could provide insights into the impact of glycemic control on the risk of fracture in T2DM patients. To our knowledge none of the previous studies analyzed the level of glycemic control according to different antidiabetic medication schemes and its joint impact on the risk of fracture.

The objective of this study was therefore to analyze the association between glycemic control and the risk of fracture in patients with T2DM, taking the diabetes medication into account.

5.1.3 Methods

Study design and data source

We used the UK-based primary care Clinical Practice Research Datalink (CPRD) GOLD to conduct a nested case-control analysis within a cohort of patients with newly diagnosed T2DM. The study period started on 01. January 1995 and ended on 31. December 2017.

CPRD GOLD is a governmental, non-profit database containing anonymized medical records of over 11.3 million patients from more than 600 general practices in the UK. CPRD GOLD patients represent approximately 6.9% of the UK population. The database is a collaborative project between the National Institute for Health Research (NIHR) and the Medicines and Healthcare Regulatory Agency (MHRA) established in 1987. Participating general practitioners (GPs) are trained on recording medical information using standard software and coding systems. The recorded information includes medical diagnoses, referrals to specialists and secondary care settings, prescriptions, diagnostic testing, lifestyle information (such as BMI and smoking), and demographic data.[110] Patients of the CPRD are representative of the UK general population with respect to age, sex, and ethnicity.[111] Additionally, the raw data entered by the GPs undergoes quality control checks by the MHRA before release.[112, 113] This contributes to the proven validity and high quality of the data.[110, 114, 115] The validity for the diagnoses of T2DM and fractures has been shown previously.[112, 113, 132, 141]

The study protocol was approved by the Independent Scientific Advisory Committee (ISAC) for MHRA database research (protocol number 17_061RAR), and the protocol was made available to the journal reviewers.

Study population

We selected patients of any age with a newly diagnosed diagnosis of T2DM between January 1, 1995, and December 31, 2017. To ensure that we only included newly diagnosed cases, patients were required to have a minimum of 3 years of history in the database prior to the first recorded diabetes diagnosis code. We identified patients based on specific codes for T2DM. We defined the first recorded T2DM code as the study entry date.

From the study population we excluded patients who experienced one or more fractures after age 18 and before the study entry date.[142] We further excluded patients with a diagnosis of cancer (except non-melanoma skin cancer), alcoholism, or HIV at any time in the patient record because such patients usually have many comorbidities and medications, which could lead to substantial bias and confounding. See **Figure 7** for details on numbers of individuals excluded for each of the criteria mentioned above.

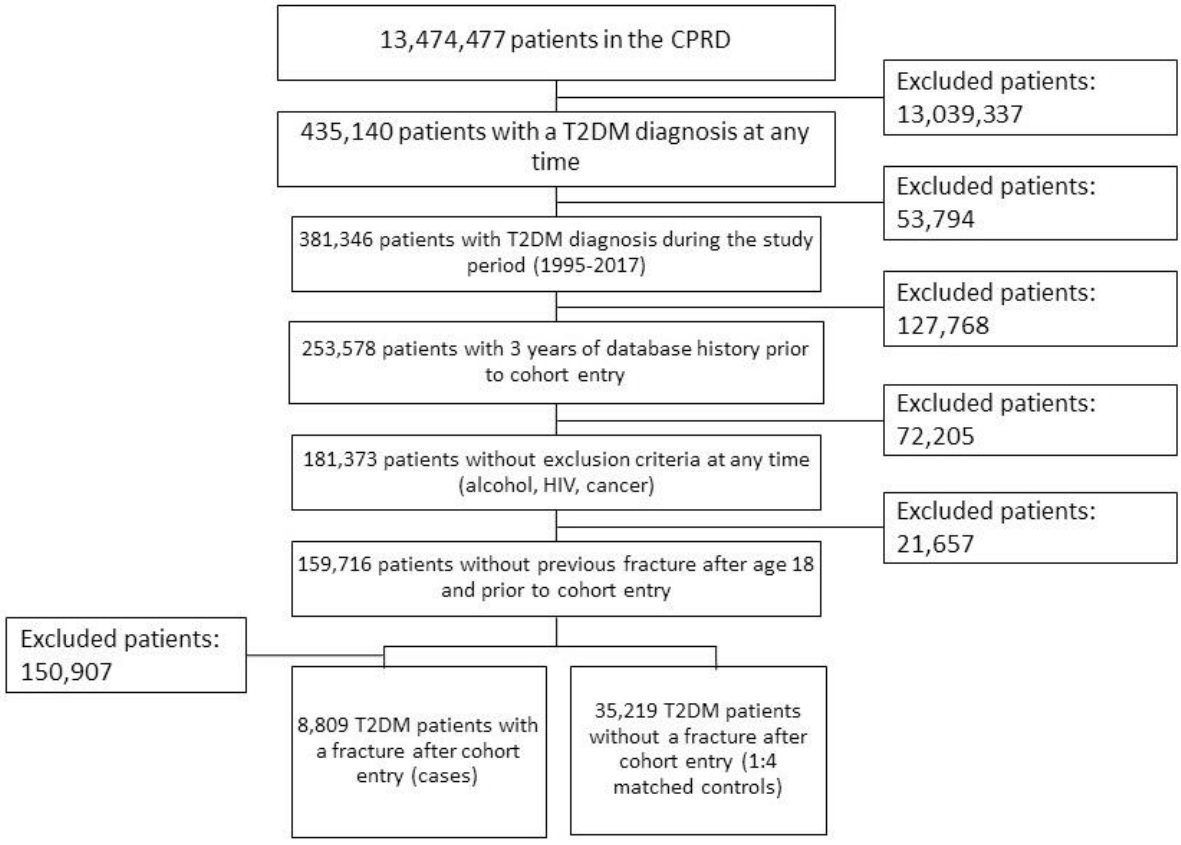

Figure 7: Selection of the study population

Case definition

A specialized clinician (JV) looked at the READ codes for fractures in the database and divided them into “low-trauma fracture” and “high-trauma fracture” based on the site of the fracture or evidence

included in the codes (e.g., the READ code 'Open fracture vault of skull with intracranial injury' was assigned to the category of high trauma fractures).

We defined cases as patients with a READ code for low-trauma fracture (non-vertebral fractures of the proximal and distal upper and lower extremities, ribs and thorax, hip and foot) after study entry date. We excluded patients with indication of high-trauma fractures such as fractures of the shoulder blade, cranium or clavicle, as well as open fractures or fractures of multiple bones. Since diabetes is known to be associated with an increased risk of peripheral fragility fractures at skeletal sites, we excluded patients with recorded vertebral fractures.[87, 138] A study from the US also confirms that the risk of vertebral fractures is not affected by T2DM in elderly men when compared to diabetes-free individuals.[143]

The date of the fracture diagnosis will subsequently be called the 'index date'. A diagram illustrating the timeline of our study can be found in **Figure 8**. We used risk set sampling to identify controls from our cohort of T2DM patients who did not experience a fracture between T2DM onset and the index date of their matched case. We matched four controls to each case on age (+/- 3 years), sex, general practice, index date (same index date as the case and the control had to be present in the database on the index date), and T2DM duration (+/- 365 days, assessed by counting the days between the first recorded T2DM code and the index date).

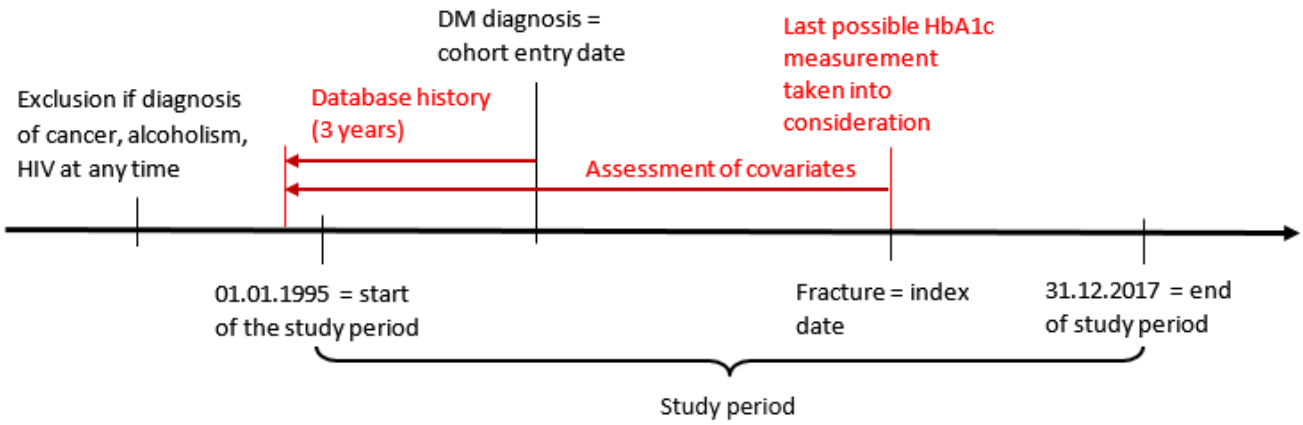

Figure 8: Graphical representation of the timeline of the study

Exposure definition

The exposure of interest in this study was glycemic control in patients combined with information on type of antidiabetic medication (metformin, glitazones, sulfonylureas (SU), dipeptidyl peptidase-4-inhibitors (DPP4-I), SGLT2-inhibitors (SGLT2-I), glucagon-like peptide-1 agonists (GLP1-A), and glinides). We further assessed the association of different T2DM treatment schemes and fracture according to timing of the prescription (past or current drug use related to the fracture date, see definition below) and the number of prescriptions received.

We used the last available HbA1c level before the index date as a categorical variable to define glycemic control. We assigned patients with missing HbA1c values to a separate category.

In order to assess the risk of fracture associated with the use of antidiabetic drugs and drug combinations reflecting the real world setting, we defined exposure according to the antidiabetic medication schemes recommended by the NICE guidelines during the study period 1995-2017.[144] The guidelines mainly describe 4 types of diabetes treatment:

- Behavioral and dietary recommendations (no drug treatment)
- Initial drug treatment with metformin as monotherapy (unless metformin is contraindicated or not tolerated)
- First intensification of drug treatment (metformin + either DPP4-I, glitazones, or sulfonylurea)
- Second intensification of drug treatment with either 3 non-insulin blood glucose lowering therapies (triple therapy with metformin + any two out of DPP4-I, glitazones, or sulfonylurea) or any treatment scheme containing insulin.

We also calculated median T2DM duration for cases and controls as well as the mean of the last HbA1c measurements by antidiabetic medication scheme.

We used 2 different classifications of HbA1c categories including different HbA1c levels to assess the impact of HbA1c levels and of T2DM treatment on the risk of fracture:

- For comparisons between users of the same medication scheme, we defined four HbA1c categories plus one category for missing measurements. We chose HbA1c levels from >6.5-7.5% as the reference group.
- For comparisons considering the timing and duration of drug intake, we defined only three HbA1c categories plus one category for missing measurements in order to have enough power for each comparison. In these analyses, which compared all medication schemes described by the NICE guidelines,[144] we chose “non-use of antidiabetic drugs” as reference.

We classified the HbA1c levels differently for the two comparisons defined above (i.e., HbA1c levels ≤6.5%, >6.5-7.5%, >7.5-8.5, and >8.5 in **Tables 10** and HbA1c levels ≤7.0%, >7.0-8.0%, and >8.0% in **Tables 11**).

Statistical analysis

We used conditional logistic regression to assess the association between HbA1c values according to diabetes medication and the risk of low-trauma fractures, expressed as odds ratios (ORs) or adjusted ORs (aORs) with 95% confidence intervals (CI). We assessed a variety of comorbidities and comedications (recorded at any time in the patient record before the index date) for confounding based on previous clinical knowledge. As a result, we adjusted our analyses for the following potential

confounders: BMI (last available value before the index date as categorical variable),[62, 145, 146] smoking status (last available information before the index date),[147-150] recorded diagnosis of previous fractures after the age of 18 years and prior to the diabetes diagnosis,[151-153], recorded diagnosis of previous falls (as a marker of frailty),[154-156] and recorded prescription for bisphosphonates (ever-use).[157-159] Independently of the chosen adjustments, the unadjusted and adjusted ORs remained similar throughout our analyses.

For users of antidiabetic drugs and drug combinations, we assessed the risk of fractures separately for patients with different last mean HbA1c levels in the following exposure groups:

- Current and past users: patients with a prescription for the respective drug recorded ≤60 days prior to the index date and >60 days, respectively
- Duration of drug exposure: 1-9, 10-19, and ≥20 prescriptions (i.e., the number of prescriptions of the last prescribed drug or drug combination).

Thus, we looked at all drugs prescribed before the index date including those that people had switched to or from. For current and past use we evaluated the prescriptions within the respective timeframes. We defined 60 days as the cut-off for past and current use based on the most commonly prescribed pack sizes for diabetes medication (30 days) plus a grace period of another month.

We conducted all analyses using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA).

5.1.4 Results

We identified 8’809 T2DM patients with a low-trauma fracture from a cohort of 159’716 patients with newly diagnosed T2DM who fulfilled all study inclusion criteria. We matched them to 35’219 control patients (**Figure 7**).

The fractures in the cases of our population were located on the following sites:

- Humerus and elbow (n_{tot}=1968, n_{female}=1460, n_{male}=508)
- Femur/patella (n_{tot}=1748, n_{female}=1219, n_{male}=529)
- General and unspecified fractures (n_{tot}=1473, n_{female}=1036, n_{male}=437)
- Distal lower extremities (n_{tot}=1265, n_{female}=813, n_{male}=452)
- Distal upper extremities including wrist (n_{tot}=916, n_{female}=714, n_{male}=202)
- Foot and ankle fracture (n_{tot}=553, n_{female}=378, n_{male}=175)
- Ribs and thorax (n_{tot}=427, n_{female}=187, n_{male}=240)
- Hip fracture (n_{tot}==419, n_{female}=326, n_{male}=93)
- Else (n_{tot}=40, n_{female}=26, n_{male}=14)

The mean age of patients was 71.1 years (SD 13.4 years) at the index date and approximately 70% were female. **Table 8** provides the distributions and ORs for basic characteristics of cases and their matched controls at the index date including patient demographics, comorbidities and basic information on exposure to antidiabetic medication.

«I have not failed. I've just found 10'000 ways that won't work.»

Thomas Edison

Table 8: Characteristics of fracture cases and their matched controls at the index date and univariate and adjusted odds ratios for risk of fracture

Patient characteristics	T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
Study population	8,122	32,470	NA	NA
Age at fracture (years)				
<60	1771 (20.1)	7046 (20.0)	NA	NA
60-69	1803 (20.5)	7302 (20.7)	NA	NA
70-79	2537 (28.8)	10131 (28.8)	NA	NA
80-89	2231 (25.3)	8906 (25.3)	NA	NA
90+	467 (5.3)	1834 (5.2)	NA	NA
Sex				
Male	2650 (30.1)	10597 (30.1)	NA	NA
Female	6159 (69.9)	24622 (69.9)	NA	NA
BMI (kg/m^2)				
<18.5	154 (1.8)	276 (0.8)	1.98 (1.67-2.36)	1.90 (1.60-2.26)
18.5 to <25.0	1798 (20.4)	6276 (17.8)	1 (reference)	1 (reference)
25.0 to <30.0	2874 (32.6)	11515 (32.7)	0.86 (0.81-0.91)	0.87 (0.82-0.92)
30.0 to <35.0	2118 (24.0)	9073 (25.8)	0.79 (0.74-0.85)	0.79 (0.74-0.85)
35.0 to <40.0	1024 (11.6)	4362 (12.4)	0.79 (0.73-0.85)	0.79 (0.73-0.86)
≥40.0	645 (7.3)	2911 (8.3)	0.73 (0.67-0.80)	0.73 (0.66-0.80)
Unknown	196 (2.2)	806 (2.3)	0.84 (0.72-0.98)	0.89 (0.76-1.05)
Last HbA1c measurement (%)				
≤6.5%	3131 (35.5)	11993 (34.1)	1.10 (1.05-1.16)	1.07 (1.02-1.13)
>6.5->7.5%	2929 (33.3)	12338 (35.0)	1 (reference)	1 (reference)
>7.5->8.5%	1141 (13.0)	4692 (13.3)	1.02 (0.96-1.10)	1.03 (0.96-1.11)
>8.5%	1206 (13.7)	4744 (13.5)	1.07 (1.00-1.15)	1.06 (0.99-1.14)
No recorded HbA1c level	402 (4.6)	1452 (4.1)	1.22 (1.08-1.37)	1.26 (1.11-1.43)
Comorbidities (Reference: no diagnosis)				
Hypertension	5824 (66.1)	23759 (67.5)	0.94 (0.89-0.98)	0.98 (0.94-1.03)
Hyperlipidemia	2418 (27.5)	9710 (27.6)	0.99 (0.95-1.04)	0.98 (0.94-1.03)
Congestive heart failure	748 (8.5)	2443 (6.9)	1.26 (1.17-1.36)	1.23 (1.13-1.33)
Chronic renal failure	597 (6.8)	2080 (5.9)	1.17 (1.08-1.28)	1.17 (1.08-1.28)
Ischemic heart disease	1875 (21.3)	7201 (20.5)	1.06 (1.00-1.11)	1.03 (0.97-1.08)
Osteoporosis	531 (6.0)	1441 (4.1)	1.53 (1.40-1.68)	1.11 (0.99-1.24)
Previous falls	2379 (27.0)	6155 (17.5)	1.85 (1.76-1.95)	1.83 (1.74-1.92)
Previous fractures (after 18 years)	150 (1.7)	422 (1.2)	1.44 (1.22-1.71)	1.44 (1.22-1.70)
Comedication (Reference: never use)				
Bisphosphonates	910 (10.3)	2406 (6.8)	1.61 (1.50-1.73)	1.50 (1.39-1.61)
Systemic glucocorticoids	2620 (29.7)	8755 (24.9)	1.29 (1.23-1.35)	1.18 (1.12-1.24)
Hormone replacement therapy	1753 (19.9)	6743 (19.2)	1.07 (1.00-1.13)	1.01 (0.95-1.07)
Antidiabetic medication (Reference: non-use)				
Metformin	5692 (64.6)	22925 (65.1)	0.98 (0.93-1.02)	1.00 (0.95-1.05)
First escalation of treatment	1721 (19.5)	6852 (19.5)	0.97 (0.90-1.04)	0.98 (0.91-1.05)
Second escalation of treatment	1718 (19.5)	5968 (16.9)	1.11 (1.03-1.21)	1.12 (1.03-1.21)
Glitazones	1282 (14.6)	4349 (12.4)	1.26 (1.18-1.34)	1.29 (1.21-1.38)
DPP4-I	602 (6.8)	2562 (7.3)	0.92 (0.85-1.01)	0.94 (0.86-1.03)
GLP1	189 (2.2)	715 (2.0)	1.07 (0.91-1.25)	1.10 (0.94-1.29)
SGLT2	69 (0.8)	291 (0.8)	0.94 (0.74-1.21)	0.94 (0.73-1.21)
Glinides	128 (1.5)	510 (1.5)	1.00 (0.84-1.20)	0.98 (0.82-1.17)
Sulfonylureas	3727 (42.3)	14173 (40.2)	1.11 (1.06-1.16)	1.09 (1.04-1.14)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, and use of bisphosphonates

Mean last HbA1c level before the index date was 7.2% (SD 3.6%) for both, cases and controls. Median last HbA1c level was slightly lower with 6.8% (Q1=6.3%, Q3=7.7%) for cases and 6.9% (Q1=6.3, Q3=7.7%) for controls. Median time between T2DM diagnosis and first fracture was 4.9 years (Q1=2.2, Q3=8.7 years). The last HbA1c measurement took place 119 days (Q1 56 days, Q3 214 days) before the index date for cases and 114 days (Q1 53 days, Q3 207 days) for controls. During the study period, patients with T2DM had a mean of 12.2 and 12.1 recorded HbA1c measurements before the index date for cases and controls, respectively. Only 5.7% of the T2DM cases and 5.2% of the controls had no recorded HbA1c measurements during the study period.

Independently of their HbA1c levels, patients exposed to glitazones (aOR 1.29, 95% CI 1.21-1.38) or to a combination of metformin and glitazones (aOR 1.34, 95% CI 1.19-1.51) had a slight increased risk of fracture compared with never-users of those specific drugs. No other antidiabetic drugs in our analysis were materially associated with the risk of fracture, or too little data was available to provide meaningful results, especially for newer drugs (such as GLP1-A and SGLT2-I).

Table 9 shows mean values of the last HbA1c levels recorded before the index date by medication scheme according to the NICE guidelines, and **Table 10** shows the median T2DM-duration in days.

Table 9: Last HbA1c levels (mean) prior to index date by medication scheme [135]

Antidiabetic treatment	T2DM patients with fractures		T2DM patients without fractures	
	n (%)	HbA1c-values [% (SD)]	n (%)	HbA1c-values [% (SD)]
No antidiabetic drugs	2090 (24.9)	6.4 (2.9)	8578 (25.4)	6.5 (3.0)
Metformin	1939 (23.1)	7.0 (3.3)	8803 (26.1)	7.0 (3.3)
First intensification	1691 (20.1)	7.4 (3.5)	6744 (20.0)	7.5 (3.5)
Second intensification	1693 (20.1)	8.1 (4.0)	5914 (17.5)	8.1 (3.9)
Other combinations	994 (11.8)	7.5 (3.6)	3728 (11.0)	7.6 (3.5)
Sum	8407*		33’767*	

* Around 5% of the patients did not have a HbA1c measurement recorded during the study period.

Table 10: T2DM-duration (mean) prior to index date by medication scheme [135]

T2DM-duration	T2DM patients with fractures		T2DM patients without fractures	
	n (%)	Median T2DM duration [days (Q_1, Q_3)]	n (%)	Median T2DM duration [days (Q_1, Q_3)]
No antidiabetic drugs	2324 (26.4)	1029 (401, 2015)	9462 (26.9)	1034 (426, 2043)
Metformin	2000 (22.7)	1386 (665, 2530)	8986 (25.5)	1439 (673, 2517)
First intensification	1721 (19.5)	2241 (1257, 3415)	6852 (19.5)	2266 (1275, 3459)
Second intensification	1718 (19.5)	3035 (1830, 4270)	5968 (16.9)	3191 (1950, 4359)
Other combinations	1046 (11.9)	2143 (973, 3576)	3951 (11.2)	2174 (1063, 3551)
Sum	8809		35219	

Mean last HbA1c levels for patients with T2DM and use of oral antidiabetic drugs varied widely: Patients without T2DM medication had the lowest last HbA1c levels, while HbA1c levels increased as patients progressed from the first choice of treatment (metformin), to succeeding intensification of treatment. This correlated with the T2DM duration, meaning that patients with less T2DM medication had a shorter T2DM duration and vice versa.

Tables 11, **12**, and **13** show the risk of fracture associated with different last HbA1c values by exposure to different antidiabetic medication (according to the NICE guidelines [144]) in ever versus never-users. ORs are adjusted for BMI, smoking, previous fractures (after age 18), previous falls, and use of bisphosphonates.

Compared with metformin users with HbA1c levels >6.5-7.5%, only those with no recorded HbA1c levels or those receiving other antidiabetic medication than metformin had a 30% or more increased risk of fracture. All others were consistent with the null (**Table 11**).

Table 11: Association of use of metformin (ever use) per HbA1c level and the risk of fracture

Treatment	Last HbA1c value before the fracture [%]	T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
Non-exposed	No antidiabetic drug	2324 (26.4)	9462 (26.9)	1.17 (1.08-1.26)	1.14 (1.05-1.24)
Metformin	≤6.5%	752 (8.5)	3217 (9.1)	1.11 (1.00-1.22)	1.07 (0.97-1.18)
	>6.5-7.5%	819 (9.3)	3882 (11.0)	1 (reference)	1 (reference)
	>7.5-8.5%	201 (2.3)	1021 (2.9)	0.94 (0.80-1.09)	0.96 (0.82-1.12)
	>8.5%	167 (1.9)	683 (1.9)	1.16 (0.98-1.37)	1.16 (0.98-1.37)
	No recorded HbA1c level	61 (0.7)	183 (0.5)	1.61 (1.23-2.12)	1.65 (1.25-2.17)
Other exposure	Other antidiabetic drugs	4485 (50.9)	16771 (47.6)	1.31 (1.21-1.41)	1.27 (1.18-1.37)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, and use of bisphosphonates

In the analysis of patients with first intensification of treatment (**Table 12**), only patients with intensive glycemic control (≤6.5%) had a 30% increased risk of fracture (aOR 1.28, 95% CI 1.13-1.44) compared with those with HbA1c levels >6.5-7.5%.

«The first principle is that you must not fool yourself and you are the easiest person to fool.»

Richard Feyman

Table 12: Association of use of first intensification of treatment (ever use) per HbA1c level and the risk of fracture

Treatment	Last HbA1c value before the fracture [%]	T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
Non-exposed	No antidiabetic drug	2324 (26.4)	9462 (26.9)	1.05 (0.96-1.15)	1.04 (0.95-1.14)
First intensification[2]	≤6.5%	469 (5.3)	1562 (4.4)	1.29 (1.14-1.45)	1.28 (1.13-1.44)
	>6.5-7.5%	639 (7.3)	2736 (7.8)	1 (reference)	1 (reference)
	>7.5-8.5%	316 (3.6)	1309 (3.7)	1.04 (0.90-1.18)	1.03 (0.90-1.19)
	>8.5%	267 (3.0)	1137 (3.2)	1.00 (0.87-1.16)	1.01 (0.87-1.17)
	No recorded HbA1c level	30 (0.3)	108 (0.3)	1.18 (0.81-1.73)	1.15 (0.79-1.67)
Other exposure	Other antidiabetic drugs	4764 (54.1)	18905 (53.7)	1.08 (0.99-1.17)	1.07 (0.98-1.16)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, and use of bisphosphonates

[2] First intensification of drug treatment is defined by the use of metformin + either DPP4-I, glitazones, or sulfonylurea (according to NICE)

Similar to our results for patients with a prescription for a first intensification treatment, patients receiving second intensification of treatment (**Table 13**) and HbA1c levels ≤6.5% also had an increased risk of fracture compared with patients with HbA1c levels >6.5-7.5%: aOR 1.33 (95% CI 1.14-1.53).

Table 13: Association of use of second intensification of treatment (ever use) per HbA1c level and the risk of fracture

Treatment	Last HbA1c value before the fracture [%]	T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
Non-exposed	No antidiabetic drug	2324 (26.4)	9462 (26.9)	0.88 (0.80-0.98)	0.88 (0.79-0.97)
Second intensification[2]	≤6.5%	311 (3.5)	842 (2.4)	1.37 (1.18-1.58)	1.33 (1.14-1.53)
	>6.5-7.5%	468 (5.3)	1742 (5.0)	1 (reference)	1 (reference)
	>7.5-8.5%	371 (4.2)	1335 (3.8)	1.03 (0.90-1.18)	1.04 (0.90-1.19)
	>8.5%	543 (6.2)	1995 (5.7)	1.02 (0.90-1.15)	0.98 (0.87-1.12)
	No recorded HbA1c level	25 (0.3)	54 (0.2)	1.69 (1.11-2.57)	1.75 (1.15-2.68)
Other exposure	Other antidiabetic drugs	4767 (54.1)	19789 (56.2)	0.88 (0.80-0.97)	0.88 (0.79-0.97)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, and use of bisphosphonates

[2] Second intensification of drug treatment is defined by the use of either 3 non-insulin blood glucose lowering therapies (triple therapy with metformin + any two out of DPP4-I, glitazones, or sulfonylurea or any treatment scheme containing insulin (according to NICE)

In those patients with a second intensification of treatment, also the group with no HbA1c levels had an increased risk of fracture compared with those with HbA1c levels >6.5-7.5%; OR 1.75 (95% CI 1.15-2.68). Patients with HbA1c levels of >7.5-8.5% and >8.5%, who obtained first or second intensification treatment, did not show an increased risk of fracture compared to patients with HbA1c levels >6.5-7.5% receiving first or second intensification treatment, respectively.

There were no material differences in risk for patients receiving antidiabetic treatments other than those specified in the NICE guidelines, when comparing patients with HbA1c levels >6.5-7.5% to patients with other HbA1c levels and those without any antidiabetic treatment (data not shown).

Tables 14, **15**, and **16** show the results of the analyses restricted to current use of the respective treatment at the index date. We also analyzed past use of the respective drugs on the risk of fracture, but the analyses yielded no material differences in effect (data not shown).

Table 14: Current use of metformin and the risk of fracture by HbA1c levels

Medication scheme		T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
No antidiabetic drug use		2324 (26.4)	9462 (26.9)	1 (reference)	1 (reference)
Current use of metformin	**HbA1c ≤7.0%**	980 (11.1)	4481 (12.7)	0.89 (0.82-0.96)	0.89 (0.83-0.96)
	HbA1c >7.0%-≤8.0%	387 (4.4)	2014 (5.7)	0.78 (0.70-0.87)	0.81 (0.73-0.90)
	HbA1c >8.0%	184 (2.1)	821 (2.3)	0.91 (0.79-1.06)	0.95 (0.81-1.10)
	no HbA1c value	52 (0.6)	142 (0.4)	1.52 (1.14-2.02)	1.60 (1.19-2.15)
All other antidiabetic drug use		4485 (50.9)	16771 (47.6)	1.12 (1.06-1.19)	1.11 (1.05-1.18)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, use of bisphosphonates, and stratified by last HbA1c, and antidiabetic medication

Current users (last prescription ≤60 days prior to the index date) of metformin (who never received other antidiabetic medication) with HbA1c levels ≤7.0% had a decreased risk of fractures compared with non-users of any antidiabetic medication (**Table 14**, aOR 0.89; 95% CI 0.83-0.96). The reduction in risk was marginally stronger for patients with HbA1c levels of 7.0 to 8.0% (aOR 0.81; CI 95% 0.73-0.90). However, patients currently exposed to metformin with HbA1c levels >8.0% had a risk of fracture similar to patients not using any antidiabetic drugs (aOR 0.95; CI 95% 0.81-1.10).

Table 15: Current use of first intensification of treatment and the risk of fracture by HbA1c levels

Medication scheme		T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
No antidiabetic drug use		2324 (26.4)	9462 (26.9)	1 (reference)	1 (reference)
Current use of first intensification treatment	**HbA1c ≤7.0%**	540 (6.1)	2044 (5.8)	1.08 (0.98-1.19)	1.10 (1.00-1.21)
	HbA1c >7.0%-≤8.0%	369 (4.2)	1692 (4.8)	0.89 (0.80-1.00)	0.90 (0.80-1.01)
	HbA1c >8.0%	291 (3.3)	1274 (3.6)	0.93 (0.83-1.06)	0.96 (0.84-1.08)
	no HbA1c value	22 (0.3)	760.2)	1.18 (0.77-1.81)	1.18 (0.77-1.80)
All other antidiabetic drug use		4764 (54.1)	18905 (53.7)	1.03 (0.98-1.09)	1.03 (0.98-1.09)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, use of bisphosphonates, and stratified by last HbA1c, and antidiabetic medication

In patients with current use of first intensification of treatment (**Table 15**) and HbA1c levels >7.0-8.0%, there is a suggestion of a decreased risk of fracture compared with non-use of antidiabetic drugs (aOR 0.90; CI 95% 0.80-1.01). There was no increased risk of fracture in those with HbA1c levels ≤7.0) compared with non-use of antidiabetic drugs.

Patients receiving current second intensification of treatment had an elevated risk of fracture in every HbA1c category compared with untreated patients (**Table 16**).

Table 16: Current use of second intensification of treatment and the risk of fracture by HbA1c levels

Medication scheme		T2DM patients with fractures [n (%)]	T2DM patients without fractures [n (%)]	Unadjusted [OR (95% CI)]	Adjusted[1] [OR (95% CI)]
No antidiabetic drug use		2324 (26.4)	9462 (26.9)	1 (reference)	1 (reference)
Current use of second intensification treatment	**HbA1c ≤7.0%**	379 (4.3)	1208 (3.4)	1.32 (1.18-1.48)	1.32 (1.18-1.49)
	HbA1c >7.0%-≤8.0%	403 (4.6)	1389 (3.9)	1.23 (1.10-1.37)	1.26 (1.13-1.41)
	HbA1c >8.0%	566 (6.4)	2092 (5.9)	1.14 (1.04-1.26)	1.13 (1.02-1.25)
	no HbA1c value	19 (0.2)	36 (0.1)	2.19 (1.36-3.51)	2.26 (1.39-3.66)
All other antidiabetic drug use		4767(54.1)	19789 (56.2)	0.99 (0.94-1.05)	1.00 (0.95-1.05)

[1] Adjusted for BMI, smoking, previous fractures (after the age of 18 years), previous falls, use of bisphosphonates, and stratified by last HbA1c, and antidiabetic medication

When the number of prescriptions as well as the timing of the last prescription of metformin were taken into account, the beneficial effect of HbA1c levels between >7.0-8.0% and >8%, compared with patients not receiving any antidiabetic medication was only present in current users of metformin with 10-19 and 20 or more prescriptions (HbA1c >7.0-8.0% aOR 0.82, 95% CI 0.70-0.95 and HbA1c >8.0% aOR 0.77, 95% CI 0.61-0.98 for 10-19 prescriptions; HbA1c >7.0-8.0% aOR 0.92, 95% CI 0.84-1.01 and HbA1c >8.0% aOR 0.80, 95% CI 0.69-0.92 for 20 or more prescriptions).

5.1.5 Discussion

The results of our large study based on primary care data from the UK suggest a beneficial effect of HbA1c levels <8.0% in current users of metformin monotherapy on the risk of fracture compared with non-use of any antidiabetic medication (HbA1c levels ≤7.0: aOR 0.89, 95% CI 0.83-0.96; HbA1c >7.0-8.0%: aOR 0.81, 95% CI 0.73-0.90). Only those current metformin users without HbA1c measurements had a higher fracture risk than T2DM patient not receiving any antidiabetics.

Past use of metformin was not associated with a changed risk of fracture in any HbA1c category compared with non-use of antidiabetic drugs. This could be an indication that metformin as a substance has a beneficial effect on the bone. Metformin is considered the first-line therapeutic agent for the treatment of T2DM.[144] Preclinical studies indicate that metformin has an anabolic effect on the bone and stimulates the differentiation and mineralization of osteoblasts.[160-162] Furthermore, results from some epidemiological studies suggest that metformin use, irrespective of glycemic control, is associated with a lower incidence of fractures in patients with T2DM.[127, 162-164] However, other studies have not confirmed this protective effect.[131, 165, 166] In our study, a significant protective effect of metformin was restricted to current use in combination with HbA1c levels <8.0%. Thus, it seems that patients with current metformin therapy and good glycemic control benefit the most with regards to their risk of fracture. Although, on average, non-users of any antidiabetic medication had the shortest disease

duration, non-users still had a minimally increased risk of fracture (aOR 1.14, 95% CI 1.05-1.24) compared with metformin users with HbA1c levels >6.5-7.5%. Metformin was usually the initial treatment received in this population and, consequently, metformin users had longer mean disease duration than non-users of any antidiabetic medication and a shorter mean diabetes duration compared with patients receiving first or second intensification of treatment. Since we matched fracture cases and controls on their T2DM disease duration, we eliminated the effect of duration on the risk estimate. This adds evidence to the hypothesis that there is a beneficial effect of metformin on the risk of fracture, as long as the blood glucose levels are >6.5-7.5% or >7.5-8.5%.

Patients with prescriptions for any antidiabetic treatment scheme but no recorded HbA1c measurement frequently had the highest risk of fractures compared with patients with HbA1c levels >6.5-7.5% receiving the same treatment. This could be a proxy for lack of patient-doctor interaction and thus less controlled disease. In our analysis including timing of exposure in patients without any HbA1c measurements, we observed an increased risk of fracture in patients receiving any current treatment scheme. This effect was most pronounced in patients receiving current second intensification of treatment.

Second intensification of treatment was defined as prescriptions for three drugs (metformin plus two of DPP4i, SU or glitazone), or for insulin alone or in addition to other medication. HbA1c levels ≤6.5% in patients receiving second intensification of treatment at any time (ever-users) were associated with an increased risk of fracture compared with ever-users of second intensification of treatment with HbA1c levels >6.5-7.5% (**Table 13**). A possible explanation for this finding may be that patients with HbA1c levels ≤6.5% are at higher risk of hypoglycemia,[167] which could in turn elevate the risk of fractures due to falls.[105, 107, 108]

In patients with current second intensification of treatment, all categories of glycemic control were associated with an increased risk of fracture compared with patients receiving no antidiabetic treatment (**Table 16**). It is possible that second intensification of treatment represents a more severe disease stage often including diabetic microvascular complications,[107, 168] particularly diabetic neuropathy and cardiovascular comorbidities which potentially increase the risk of falls [128] and thus the risk of fractures.

Use of other antidiabetic drugs or treatment schemes than those recommended by NICE either did not have a material effect on the risk of fracture in our analysis, or too little data were available to provide conclusive results. This was particularly true for newer drugs, such as GLP1-receptor agonists and SGLT2-inhibitors, which were used less in the UK during our study period from 1995 to 2017 compared with metformin, glitazones, or sulfonylureas (see **Table 8**), and which were only included in the 2015 NICE guidelines under certain circumstances.

Furthermore, for some antidiabetic drug combinations, there were too few patients exposed per HbA1c group, to calculate meaningful ORs.

The present findings should be interpreted within the context of the study strengths and limitations. The strengths of our study were the large observational nested case-control design within a cohort of patients with newly diagnosed T2DM. Our data come from a well validated primary care database that contains prospectively and routinely collected data, which avoids recall bias. DM diagnoses are well validated within the CPRD with a positive predictive value of 98.6% (92.2-100.0%).[132] For the analyses in this study we only used the last HbA1c measurement before the index date. However, on average 12 HbA1c measurements per patients were recorded in the database during the study period. The median time between the index date and the last HbA1c measurement was 119 days for cases and 114 for controls. This shows that the recorded HbA1c measurements provide a reliable source for our analyses on the effect of glycemic control on the risk of fracture.

Our study population included a high proportion of patients with T2DM with good glycemic control who might have been healthier than the T2DM populations analyzed in other studies. Nevertheless, our T2DM population included over 32,000 patients with medically treated T2DM, including many with HbA1c levels >8.0%. Therefore, we expect our results to be applicable to those of other populations with T2DM and HbA1c levels >8.0%. Our study population includes more women than men, even though men are slightly more often affected by T2DM than women.[134] This may be due to the fact that women are at higher risk for fractures than men.[169]

Though fractures are well recorded and have been validated in the CPRD (positive predictive value ~90% for hip and vertebral fractures),[132] it is possible that we missed some unrecorded fracture cases. This possible misclassification would likely be non-differential and would not have materially changed the results. Additionally, we did not analyze the cause of the fracture. Thus, we do not know whether some fractures were caused by diabetic emergencies, such as hypo- or hyperglycemia. This level of detailed information is not well captured in the CPRD. Furthermore, T2DM is a disease with uncertain onset and can remain undiagnosed for many years, possibly leading to the inclusion of some prevalent (instead of newly diagnosed) T2DM cases. This was previously shown in the UK Prospective Diabetes study, in which a high prevalence of T2DM tissue damage was shown by the time of the T2DM diagnosis as an indication of pre-existing T2DM.[133] Therefore, we may have underestimated the time until fracture (after T2DM onset) in our study population, which could have potentially affected our matching on T2DM duration. However, this misclassification is unlikely to have been differential, and we do not expect that it had a major influence on our findings.

In conclusion, our study suggests that patients with HbA1c levels <8.0% who were currently exposed to metformin monotherapy had a lower risk of fracture compared with patients not currently exposed to an antidiabetic drug. Our data confirm that metformin is safe in terms of skeletal changes and

predominantly excerpts its beneficial effect on the risk of fracture in an early and well-controlled disease stage. In contrast, the risk of fracture in patients receiving intensified treatment was not or only slightly associated with glycemic control. Those receiving intensified T2DM treatment are at a slight increased risk of fracture independent of glycemic control. This could be due to a more severe disease stage with microvascular complications increasing the risk of falls.

5.1.6 Abbreviations

aOR	adjusted Odds Ration
BMI	body mass index
CPRD	Clinical Practice Research Datalink
DM	diabetes mellitus
DPP4-I	Dipeptidyl peptidase-4 inhibitor
GLP-1–A	Glucagon-like peptide-1 agonist
HbA1c	glycated hemoglobin
HR	hazard ratio
MHRA	Medicines and Healthcare Regulatory Agency
OR	odds ratio
SGLT2-I	SGLT2 inhibitor
SU	sulfonylurea
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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«The good thing about science is that it's true whether or not you believe in it.»

Neil Degrasse Tyson

Chapter 6

Study III: Glycemic control and risk of venous thromboembolism

6 Study III

6.1 Association between glycemic control and risk of venous thromboembolism in diabetic patients: A nested case-control study

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6.1.1 Abstract

Background

Previous studies suggested an elevated risk of venous thromboembolism (VTE) among patients with type 2 diabetes mellitus (T2DM), with a possible sex difference. The impact of glycemic control on the risk of VTE is unclear. Our objective was to analyze the association between glycemic control and the risk of unprovoked (idiopathic) VTE in men and women with T2DM.

Methods

We conducted a nested case-control analysis (1:4 matching) within a cohort of patients with incident T2DM between 1995-2019 using data from the CPRD GOLD. We excluded patients with known risk factors for VTE prior to onset of DM. Cases were T2DM patients with an unprovoked treated VTE. The exposure of interest was glycemic control measured as HbA1c levels. We conducted conditional logistic regression analyses adjusted for several confounders.

Results

We identified 2'653 VTE cases and 10'612 controls (53.1% females). We found no association between the HbA1c level and the risk of VTE in our analyses. However, when the most recent HbA1c value was recorded within 90 days before the index date, women with HbA1c levels >7.0% had a 36-55% increased relative risk of VTE when compared to women with HbA1c >6.5-7.0%.

Conclusions

Our study raises the possibility that female T2DM patients with HbA1c levels >7% may have a slightly higher risk for unprovoked VTE compared to women with HbA1c level >6.5-7.0%. This increase may not be causal and may reflect differences in life style or other characteristics. We observed no effect of glycemic control on the risk of VTE in men.

«The good thing about science is that it's true whether or not you believe in it.»

Neil Degrasse Tyson

6.1.2 Introduction

Diabetes mellitus (DM) is a chronic disease with a high global prevalence, affecting some 450 million (8.8%) patients worldwide and causing approximately 5 million deaths per year.[134] In the UK, 4.7 million patients (7.0%) had diagnosed or undiagnosed DM in 2019.[135] Because the majority (90%) of the cases are DM type 2 (T2DM) [135], T2DM and its complications are of great importance for the health system. [134] T2DM is characterized by hyperglycemia due to insulin deficiency and insulin resistance, and it is linked to an increased risk for several cardiovascular diseases.[170, 171]

While it has been shown that T2DM patients have a higher risk for arterial thrombosis, the association between T2DM and the risk of venous thromboembolism (VTE) has been studied less. VTE, a medical condition in which a thrombus forms in the venous system, can manifest as deep vein thrombosis (DVT) or as pulmonary embolism (PE), if the thrombus travels to the pulmonary arteries.[73, 172, 173] VTE is associated with a high mortality.[72, 172] Its prevention and management is a priority for the NHS, the National Health Service of the UK.[174] Unprovoked VTE [172] occurs at an incidence of 62.1 per 100’000 person years.[175] However, especially at older ages (>60 years), men have an approximately 20%–25% higher incidence rate of VTE than women.[173, 176] The term unprovoked is used in accordance with the definition provided by the NICE (National Institute for Health and Care Excellence) guideline on PE and DVT, meaning that - similar to the term idiopathic - no recent known major risk factors were present prior to the VTE.[172]

Published findings regarding DM as an independent risk factor for VTE are not consistent [73, 177] However, it is well established that VTE occurs more than twice as often in patients with DM than in DM-free individuals.[178, 179] Studies also show that men are at a higher risk for T2DM and VTE than women when both diseases are considered individually, while women are at higher risk of VTE once other comorbidities (such as DM, cardiovascular disease, and atherosclerosis) are involved.[177, 180, 181]

Since the degree of hyperglycemia is crucial in the development of DM-related complications,[182, 183] the question arises whether there is also an association between hyperglycemia and the risk of VTE.

To date, recent studies assessing the impact of glycemic control on the risk of VTE in male and female patients with DM yielded conflicting results. While some authors found a statistically significant association between the level of glycemic control and the risk of VTE,[184, 185] others did not.[186] In a population-based cohort study from Norway, the risk of VTE increased by 5% per one standard deviation increase in HbA1c. However, in this study, there were no HbA1c measurements available at a time point close to the VTE event.[186] None of the published studies analyzed the impact of glycemic

control on the risk of VTE stratified by sex. However, the sex of the patient could not only have an impact on the development and progression of the disease itself,[180, 187-189] but also on the association of glycemic control and risk of VTE.

A hypothesized pathway for an increased risk of VTE in patients with DM is that hyperglycemia contributes to elevated coagulation factors and impaired fibrinolysis.[171, 178, 190] A single unifying mechanism of DM complications might be hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain, which activates several damaging pathways.[191] The activation of these pathways causes additional intracellular oxidative stress, abnormalities of the gene expression of glomerular cells, hyperglycemia-induced cardiomyocyte dysfunction, and an increase of the enzyme GFAT (glutamine fructose-6 phosphate amidotransferase), resulting in a variety of effects on gene expression and advanced glycation end product formation.[191]

The objective of the present study was therefore to analyze the association between glycemic control and the risk of unprovoked VTE in patients with T2DM overall, as well as separately for men and women.

6.1.3 Methods

Study design and data source

We conducted a nested case-control analysis within a cohort of patients with incident T2DM between 01. January 1995 and 31. December 2019 in the UK-based primary care Clinical Practice Research Datalink (CPRD) GOLD.

CPRD GOLD contains anonymized medical records of over 11.3 million patients from more than 600 general practices in the UK. It is a governmental, non-profit database; the enrolled patients account for approximately 6.9% of the UK population. Patients within CPRD GOLD are representative of the UK general population with respect to age, sex, and ethnicity.[111] The database was established in 1987 and is a collaborative project between the National Institute for Health Research (NIHR) and the Medicines and Healthcare Regulatory Agency (MHRA). The information in the database comes from participating general practitioners (GPs), who are trained on recording medical information using standard software and coding systems. Medical diagnoses, referrals to specialists and secondary care settings, prescriptions, diagnostic testing, lifestyle information, and demographic data are all part of the recorded information.[110] Many validation studies have been performed that demonstrate the high quality of CPRD GOLD data.[110, 114, 115] The validity of the diagnoses of T2DM and VTE has been shown previously.[112, 113, 132]

Study population

In order to ensure that we only included incident DM cases in the study population, patients had to have a minimum of 3 years of DM-free history in the database prior to onset. We identified patients based on specific codes for T2DM. We also included patients with an unspecific code for DM (e.g. general code for "diabetes") if they were older than 30 years at diagnosis and received an oral antidiabetic drug (OAD). Independently of age, if DM patients never received insulin, we classified them as T2DM patients. We used the onset of DM as the study entry date, defined as the date of the first recorded DM code or the date of the first prescription for a DM medication. If the prescription occurred more than 365 days prior to the first recording of a DM diagnosis code, we excluded the patient.

We excluded patients with a diagnosis of cancer (except non-melanoma skin cancer), alcoholism, or HIV at any time in the patient record to avoid substantial bias and confounding.

We excluded patients with a history of VTE (at any time prior to the diagnosis of T2DM), or a code for surgery, immobilization, trauma, paralysis and paresis, or use of HRT or the contraceptive pill within 3 months prior to the index date. We further excluded patients with a code for pregnancy or puerperium within 12 months prior to the index date.

Case and control definition

We defined cases as patients with a first-time recording of VTE during the study period, who received at least one prescription for an antithrombotic drug within 7 days prior until 90 days after the VTE,[172, 192, 193] including vitamin K antagonists, heparins, direct factor Xa inhibitors, direct thrombin inhibitors, fibrinolytic enzymes, or the synthetic penta-saccharide factor Xa inhibitor fondaparinux. The index date for each case was the date of the first recorded VTE. Since we excluded patients with known risk factors for a VTE prior to the outcome, we regard the VTE cases included in this study as having an unprovoked or idiopathic VTE.[172]

We used risk set sampling to match each case to 4 controls from the study population, i.e. patients who did not experience a VTE between the onset of DM and the index date of their matched case. We matched controls to cases on age (+/- 3 years), sex, general practice, index date (same index date as the case, and the control had to be present in the database on the index date), and T2DM duration (+/- 365 days assessed by counting the days between the study entry date and the index date).

Exposure definition

The exposure of interest in this study was glycemic control after the onset of DM defined by HbA1c levels. We used the last recorded HbA1c value before the index date for our analyses. We assessed HbA1c levels in 7 categories: ≤6.5% (≤48 mmol/mol), >6.5-7.0% (>48-53 mmol/mol, reference group), >7.0-7.5% (>53-58 mmol/mol), >7.5-8.0% (>58-64 mmol/mol), >8.0-9.0% (>64-75 mmol/mol), >9.0% (>75 mmol/mol), and no HbA1c measurement. Results for patients with missing values were presented in a separate category.

Statistical analysis

We used conditional logistic regression to assess the association between levels of glycemic control (expressed as HbA1c levels) with HbA1c levels of >6.5-7.0% (>48-53 mmol/mol) as the reference group and the risk of VTE, expressed as odds ratios (ORs) or adjusted ORs (aORs) with 95% confidence intervals (CI). We also assessed the association between HbA1c level and the risk of VTE according to the patients' number of GP visits during the study period. Lastly, we conducted analyses in men and women separately.

We adjusted for the following comorbidities and co-medications (recorded at any time in the patient record before the index date) in the final model based on previous clinical knowledge: BMI (categorical variable), smoking status (current, past, non-smokers, and unknown), CVD (including congestive heart failure, ischemic heart disease, myocardial infarction, hypertension, stroke), osteoarthritis, use of insulin, bisphosphonates, systemic corticosteroids, low-dose acetylsalicylic acid, and current (last prescription within 30 before the index date) or past (last prescription >30 days prior to index date) use of metformin or sulfonylureas. We additionally tested for effect modification by obesity status (non-obese versus obese, defined as BMI levels <30 and ≥30) of the association between level of HbA1c and risk of VTE.

In sensitivity analyses, we 1) restricted the sample to patients whose last HbA1c measurement was recorded within less than 90 days prior to the index date, 2) analyzed the risk of VTE separately for patients with a previous CVD diagnosis, and 3) conducted separate analyses of the risk of VTE by HbA1c levels for patient groups of different T2DM durations.

We conducted analyses using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA).

6.1.4 Results

Within a cohort of 231'439 patients with incident T2DM who fulfilled all study inclusion and exclusion criteria, we identified 2'653 T2DM patients with an incident VTE diagnosis and 10'612 matched control patients (**Figure 9**).

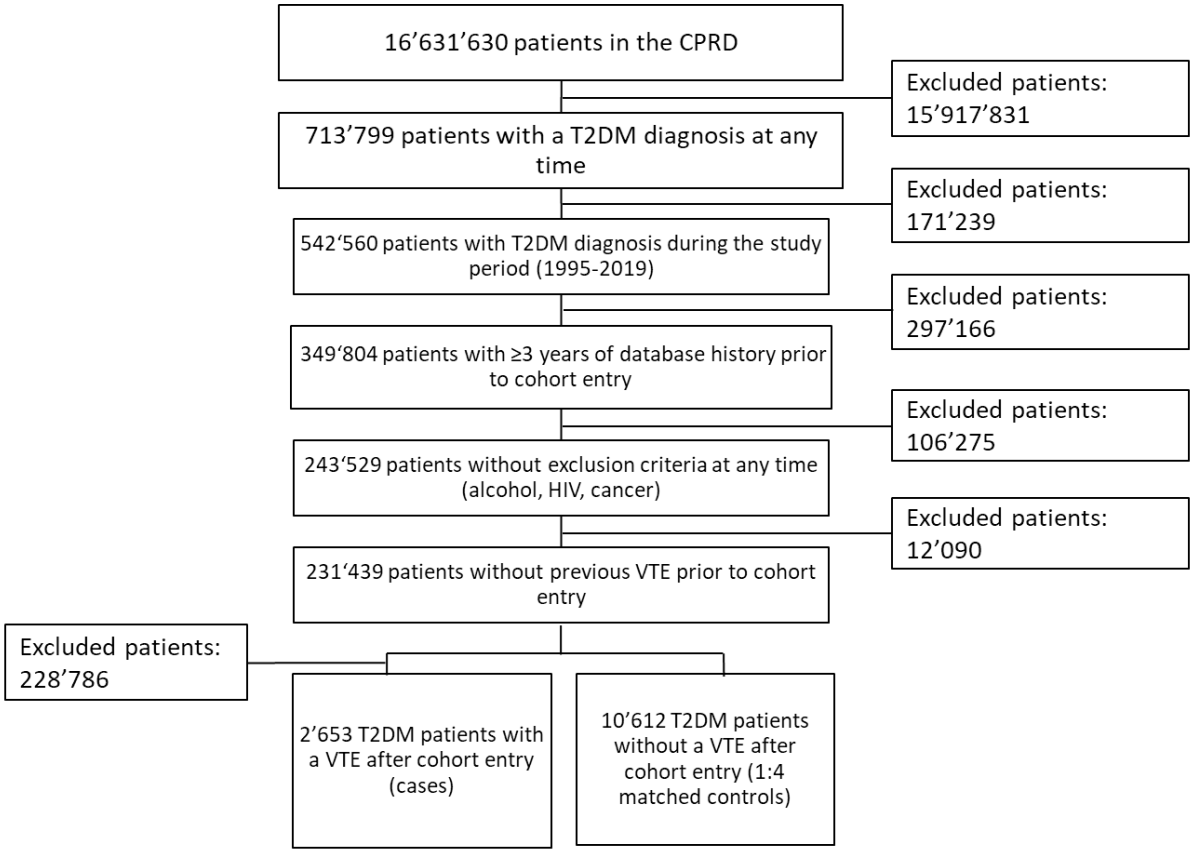

Figure 9: Selection of the study population

Cases and controls were similar with respect to age and time from most recent HbA1C value to index date. We observed a mean of 12.5 HbA1c measurements per case and 12.3 HbA1c measurements per control during the study period. The median time between the index date and the last HbA1c measurement was 117 days for cases and 116 for controls.

Patients exposed to insulin (aOR 1.63, 95% CI 1.38-1.92) had an increased risk of VTE compared to never-users of insulin, independently of HbA1c levels. However, cases and controls who had at least one prescription for insulin also had longer mean T2DM duration than non-users of these drugs (approximately 4.2 years longer). We found no effect modification by BMI on the association between HbA1c level and the risk of VTE.

Table 17 provides information on the basic characteristics of cases and their matched controls at the index date.

Table 17: Characteristics of the included cases and controls

Characteristics	Number of cases (%)	Number of controls (%)	Unadjusted ORs (95% CI)	Adjusted ORs* (95% CI)
Age (years)				
<60	526 (19.8)	2112 (19.9)	NA	NA
60-69	570 (21.5)	2283 (21.5)	NA	NA
70-79	872 (32.9)	3500 (33.0)	NA	NA
80+	685 (25.8)	2717 (25.6)	NA	NA
Sex				
Male	1245 (46.9)	4980 (46.9)	NA	NA
Female	1408 (53.1)	5632 (53.1)	NA	NA
BMI (kg/m^2)				
<18.5	23 (0.9)	81 (0.8)	1.42 (0.93-2.18)	1.30 (0.82-2.07)
18.5 to <25.0	340 (12.8)	1779 (16.8)	1 (reference)	1 (reference)
25.0 to <30.0	770 (29.0)	3705 (34.9)	1.12 (0.99-1.28)	1.15 (1.01-1.31)
30.0 to <35.0	742 (28.0)	2823 (26.6)	1.47 (1.28-1.68)	1.46 (1.28-1.67)
35.0 to <40.0	371 (14.0)	1208 (11.4)	1.80 (1.54-2.11)	1.72 (1.47-2.02)
≥40.0	343 (12.9)	749 (7.1)	2.82 (2.39-3.34)	2.65 (2.24-3.15)
Unknown	64 (2.4)	267 (2.5)	1.26 (0.96-1.67)	1.30 (0.95-1.79)
Smoking status				
Non-smoker	1001 (37.7)	4301 (40.5)	1 (reference)	1 (reference)
Current smoker	304 (11.5)	1257 (11.9)	1.04 (0.91-1.19)	1.00 (0.87-1.14)
Ex-smoker	1312 (49.5)	4908 (46.3)	1.16 (1.07-1.27)	1.05 (0.96-1.15)
Unknown	36 (1.4)	146 (1.4)	1.04 (0.74-1.47)	1.04 (0.71-1.54)
No. of HbA1c measurements in the medical history before the index date				
1-4	946 (35.7)	4006 (37.8)	1 (reference)	1 (reference)
5-9	1087 (41.0)	4375 (41.2)	1.06 (0.96-1.19)	1.06 (0.95-1.19)
10 or more	365 (13.8)	1343 (12.7)	1.19 (1.02-1.40)	1.06 (0.90-1.26)
No Recording	255 (9.6)	886 (8.4)	1.26 (1.06-1.50)	1.30 (1.08-1.55)
Comorbidities				
Inflammatory bowel disease	107 (4.0)	204 (1.9)	2.17 (1.76-2.66)	1.82 (1.47-2.25)
Chronic renal failure	223 (8.4)	626 (5.9)	1.51 (1.31-1.75)	1.26 (1.08-1.46)
Diabetic retinopathy	835 (31.5)	3090 (29.1)	1.17 (1.07-1.29)	1.20 (1.08-1.32)
Asthma	564 (21.3)	1718 (16.2)	1.41 (1.29-1.55)	1.04 (0.93-1.15)
Congestive heart failure (CHF)	315 (11.9)	712 (6.7)	1.93 (1.71-2.19)	1.53 (1.34-1.76)
Ischemic heart disease (IHD)	690 (26.0)	2189 (20.6)	1.38 (1.26-1.51)	1.21 (1.08-1.35)
Myocardial infarction (MI)	293 (11.0)	996 (9.4)	1.21 (1.07-1.37)	0.93 (0.80-1.07)
Stroke	358 (13.5)	1220 (11.5)	1.21 (1.08-1.35)	1.19 (1.05-1.34)
Arterial hypertension	1697 (60.4)	6866 (64.7)	0.97 (0.89-1.05)	0.93 (0.85-1.01)
Peripheral arterial disease	139 (5.2)	340 (3.2)	1.68 (1.41-2.01)	1.52 (1.26-1.84)
Osteoarthritis	985 (37.1)	3057 (28.8)	1.52 (1.40-1.65)	1.37 (1.25-1.50)
Rheumatoid arthritis	94 (3.5)	226 (2.1)	1.69 (1.37-2.08)	1.29 (1.03-1.62)
Hyperlipidemia	657 (24.8)	2640 (24.9)	0.99 (0.91-1.09)	0.91 (0.83-1.00)
Cardiovascular disease	2033 (76.6)	7916 (74.6)	1.14 (1.03-1.25)	1.05 (0.90-1.24)
Co-medication **				
Insulin	345 (13.0)	820 (7.7)	1.93 (1.67-2.23)	1.63 (1.38-1.92)
Glitazones	367 (13.8)	1227 (11.6)	1.28 (1.12-1.47)	1.09 (0.94-1.26)
Sulfonylurea	1009 (38.0)	3651 (34.4)	1.22 (1.10-1.34)	1.12 (0.99-1.26)
Metformin	1621 (61.1)	6537 (61.6)	0.97 (0.88-1.07)	0.64 (0.41-1.01)
GLP1	81 (3.1)	240 (2.3)	1.44 (1.09-1.90)	0.94 (0.70-1.27)
DPP4	224 (8.4)	926 (8.7)	0.96 (0.81-1.13)	0.84 (0.71-1.00)
SGLT2	43 (1.6)	170 (1.6)	1.01 (0.71-1.46)	0.99 (0.68-1.44)
All oral antidiabetics	1814 (68.4)	7142 (67.3)	1.06 (0.96-1.17)	1.17 (0.95-1.43)
Statins	1941 (73.2)	8077 (76.1)	0.81 (0.72-0.91)	0.74 (0.66-0.84)
Bisphosphonates	340 (12.8)	850 (8.0)	1.79 (1.55-2.06)	1.49 (1.28-1.74)
Contraceptive pill	53 (2.0)	263 (2.5)	0.72 (0.50-1.03)	0.77 (0.53-1.12)
Hormone replacement therapy	392 (14.8)	1626 (15.3)	0.94 (0.82-1.09)	0.89 (0.77-1.03)
Corticosteroids (systemic)	975 (36.8)	2612 (24.6)	1.84 (1.67-2.02)	1.55 (1.40-1.71)
Coronary vasodilators	888 (33.5)	2797 (26.4)	1.43 (1.30-1.57)	1.22 (1.07-1.39)
Low dose acetylsalicylic acid	1556 (58.7)	5991 (56.5)	1.11 (1.01-1.22)	0.95 (0.86-1.06)
Loop diuretics	1152 (43.4)	2875 (27.1)	2.24 (2.04-2.46)	1.69 (1.52-1.88)
All diuretics	1854 (69.9)	6323 (59.6)	1.73 (1.56-1.91)	1.52 (1.36-1.70)

* Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas

** Use of other medication possible

We found no elevated relative risk for VTE in patients with the last HbA1c measurement >7.0% (>53 mmol/mol) compared to the reference group of patients with HbA1c levels >6.5-7.0% (>48-53 mmol/mol). The ORs for the various HbA1c categories are displayed in **Table 18**. There was no consistent linear increase in the risk of developing VTE with increasing HbA1c levels. Patients with missing HbA1c measurements had the highest risk of VTE (aOR 1.56, 95% CI 1.29-1.88) when compared to patients with last HbA1c measurements of >6.5-7.0% (>48-53 mmol/mol) before the index date. Around one thirdof the cases with no HbA1c measurements (8.4% in total) had little GP contact (0-14 GP visits: 34.7% (n=77), 15-29 visits: 18.5% (n=41), and 30+ visits: 46.9% (n=104). We provide a separate table summarizing the characteristics of those patients without any HbA1c measurements as a Supplement.

When we stratified our analyses by sex (**Table 18**), we observed a slightly higher risk of VTE in women with HbA1c levels >8.0% (>64 mmol/mol) compared to the reference group of women with HbA1c levels >6.5-7.0% (>48-53 mmol/mol). There was no association of HbA1c levels with risk of VTE in men.

«I see they found out the universe is 80 million years older than we thought. It's also been lying about its weight.»

Bill Maher

Table 18: Risk of VTE by HbA1c level

HbA1c-level	Number of cases (%)	Number of controls (%)	Unadjusted ORs (95% CI)	Adjusted ORs* (95% CI)
HbA1c-Values (last measurement before the index date) and risk of VTE				
≤6.5% (≤48 mmol/mol)	843 (31.8)	3809 (35.9)	0.98 (0.87-1.10)	0.98 (0.87-1.10)
>6.5-7.0% (>48-53 mmol/mol)	441 (16.6)	1963 (18.5)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	397 (15.0)	1522 (14.3)	1.17 (1.02-1.34)	1.13 (0.98-1.30)
>7.5-8.0% (>58-64 mmol/mol)	208 (7.8)	839 (7.9)	1.13 (0.95-1.33)	1.09 (0.92-1.29)
>8.0-9.0% (>64-75 mmol/mol)	253 (9.5)	834 (7.9)	1.38 (1.18-1.61)	1.30 (1.10-1.52)
>9.0% (>75 mmol/mol))	289 (10.9)	929 (8.8)	1.45 (1.24-1.69)	1.18 (1.00-1.40)
No Recording	222 (8.4)	716 (6.8)	1.54 (1.28-1.85)	1.56 (1.29-1.88)
HbA1c-Values (last measurement before the index date) and risk of VTE in women				
≤6.5% (≤48 mmol/mol)	468 (33.2)	2106 (37.4)	1.04 (0.89-1.22)	1.05 (0.89-1.23)
>6.5-7.0% (>48-53 mmol/mol)	220 (15.6)	1035 (18.4)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	211 (15.0)	809 (14.4)	1.24 (1.03-1.50)	1.16 (0.95-1.41)
>7.5-8.0% (>58-64 mmol/mol)	102 (7.2)	422 (7.5)	1.16 (0.92-1.47)	1.15 (0.90-1.48)
>8.0-9.0% (>64-75 mmol/mol)	122 (8.7)	411 (7.3)	1.43 (1.15-1.79)	1.29 (1.02-1.63)
>9.0% (>75 mmol/mol))	154 (10.9)	456 (8.10)	1.68 (1.36-2.09)	1.36 (1.07-1.72)
No Recording	131 (9.3)	393 (7.0)	1.81 (1.42-2.31)	1.87 (1.46-2.40)
HbA1c-Values (last measurement before the index date) and risk of VTE in men				
≤6.5% (≤48 mmol/mol)	375 (30.1)	1703 (34.2)	0.92 (0.78-1.09)	0.91 (0.77-1.08)
>6.5-7.0% (>48-53 mmol/mol)	221 (17.8)	928 (18.6)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	186 (14.9)	713 (14.3)	1.10 (0.91-1.34)	1.10 (0.90-1.34)
>7.5-8.0% (>58-64 mmol/mol)	106 (8.5)	417 (8.4)	1.09 (0.86-1.37)	1.03 (0.81-1.30)
>8.0-9.0% (>64-75 mmol/mol)	131 (10.5)	423 (8.5)	1.33 (1.07-1.65)	1.29 (1.03-1.62)
>9.0% (>75 mmol/mol))	135 (10.8)	473 (9.5)	1.24 (0.99-1.55)	1.03 (0.81-1.30)
No Recording	91 (7.3)	323 (6.5)	1.26 (0.95-1.66)	1.24 (0.93-1.66)

*Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas.

Among patients with preexisting CVD (**Table 19**), individuals with HbA1c levels >7.0% (>53 mmol/mol) had a similar risk of VTE compared to patients with HbA1c levels between >6.5-7.0% (>48-53 mmol/mol). There was a slightly higher risk of VTE with increased HbA1c levels in women with CVD, but not in men.

«Nothing shocks me. I'm a scientist.»

Harrison Ford as *"Indiana Jones"*

Table 19: Risk of VTE according to HbA1c in patients with CVD

Characteristics	Number of cases (%)	Number of controls (%)	Unadjusted ORs (95% CI)	Adjusted ORs* (95% CI)
CVD and risk of VTE				
≤6.5%	670 (33.0)	2998 (37.9)	0.99 (0.87-1.13)	1.01 (0.89-1.15)
>6.5-7.0%	352 (17.3)	1524 (19.3)	1 (reference)	1 (reference)
>7.0-7.5%	307 (15.1)	1142 (14.4)	1.19 (1.02-1.39)	1.14 (0.97-1.34)
>7.5-8.0%	163 (8.0)	610 (7.7)	1.18 (0.98-1.43)	1.13 (0.93-1.38)
>8.0-9.0%	193 (9.5)	568 (7.2)	1.49 (1.24-1.78)	1.39 (1.16-1.68)
>9.0%	201 (9.9)	624 (7.9)	1.47 (1.22-1.77)	1.16 (0.95-1.42)
No Recording	147 (7.2)	450 (5.7)	1.55 (1.25-1.94)	1.56 (1.24-1.96)
HbA1c-Values (last measurement before the index date) and risk of VTE in women				
≤6.5%	377 (34.4)	1689 (39.2)	1.04 (0.87-1.23)	1.07 (0.90-1.28)
>6.5-7.0%	177 (16.2)	818 (19.0)	1 (reference)	1 (reference)
>7.0-7.5%	166 (15.2)	618 (14.4)	1.29 (1.04-1.59)	1.21 (0.96-1.51)
>7.5-8.0%	87 (7.9)	317 (7.4)	1.30 (1.00-1.69)	1.26 (0.95-1.66)
>8.0-9.0%	93 (8.5)	285 (6.6)	1.39 (1.08-1.80)	1.30 (0.99-1.70)
>9.0%	107 (9.8)	318 (7.4)	1.64 (1.28-2.12)	1.27 (0.97-1.68)
No Recording	89 (8.1)	260 (6.0)	1.80 (1.35-2.41)	1.83 (1.35-2.46)
HbA1c-Values (last measurement before the index date) and risk of VTE in men				
≤6.5%	293 (31.3)	1309 (36.3)	0.94 (0.78-1.14)	0.94 (0.78-1.13)
>6.5-7.0%	175 (18.7)	706 (19.6)	1 (reference)	1 (reference)
>7.0-7.5%	141 (15.1)	524 (14.5)	1.09 (0.87-1.37)	1.06 (0.84-1.34)
>7.5-8.0%	76 (8.1)	293 (8.1)	1.07 (0.82-1.41)	0.98 (0.74-1.31)
>8.0-9.0%	100 (10.7)	283 (7.8)	1.59 (1.23-2.05)	1.50 (1.15-1.96)
>9.0%	94 (10.0)	306 (8.5)	1.30 (0.99-1.72)	1.06 (0.79-1.41)
No Recording	58 (6.2)	190 (5.3)	1.26 (0.90-1.78)	1.26 (0.88-1.81)

*Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas.

Also in an analysis restricted to patients with a last HbA1c measurement within 90 days prior to the index date (**Table 20**), we only found a slight association between HbA1c levels >7.0% (>53 mmol/mol) and risk of VTE in women. The risk of VTE among women with a 90-day HbA1c level above 7.0% (>53 mmol/mol) increased around 36-55% as compared to those with HbA1c levels >6.5-7.0% (>48-53 mmol/mol).

«Aerodynamically, the bumble bee shouldn't be able to fly, but the bumble bee doesn't know it so it goes on flying anyway.»

Mary Kay Ash

Table 20: Risk of VTE according to HbA1c levels measured within 90 days prior to the index date (i.d.)

HbA1c level <90 days prior to i.d. overall	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5%	278 (22.9)	1345 (28.6)	1.00 (0.81-1.23)	1.04 (0.84-1.30)
>6.5-7.0%	161 (13.3)	789 (16.8)	1 (reference)	1 (reference)
>7.0-7.5%	172 (14.2)	616 (13.1)	1.40 (1.10-1.78)	1.44 (1.11-1.87)
>7.5-8.0%	108 (8.9)	380 (8.1)	1.43 (1.10-1.88)	1.46 (1.09-1.94)
>8.0-9.0%	132 (10.9)	394 (8.4)	1.69 (1.30-2.19)	1.64 (1.23-2.17)
>9.0%	140 (11.5)	460 (9.8)	1.50 (1.17-1.92)	1.32 (1.01-1.73)
No Recording**	222 (18.3)	716 (15.2)	1.75 (1.36-2.23)	1.78 (1.38-2.31)
HbA1c level <90 days prior to i.d. in women	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5%	155 (11.0)	746 (13.3)	0.99 (0.74-1.34)	1.03 (0.75-1.40)
>6.5-7.0%	82 (5.8)	417 (7.4)	1 (reference)	1 (reference)
>7.0-7.5%	99 (7.0)	335 (6.0)	1.47 (1.05-2.06)	1.55 (1.08-2.24)
>7.5-8.0%	51 (3.6)	192 (3.4)	1.36 (0.92-2.01)	1.43 (0.93-2.18)
>8.0-9.0%	66 (4.7)	208 (3.7)	1.50 (1.04-2.15)	1.36 (0.91-2.03)
>9.0%	79 (5.6)	239 (4.2)	1.61 (1.13-2.29)	1.47 (0.99-2.17)
No Recording**	876 (62.2)	3495 (62.1)	1.94 (1.39-2.71)	1.98 (1.40-2.81)
HbA1c level <90 days prior to i.d. in men	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5%	125 (10.0)	614 (12.3)	1.00 (0.75-1.34)	1.04 (0.76-1.43)
>6.5-7.0%	81 (6.5)	379 (7.6)	1 (reference)	1 (reference)
>7.0-7.5%	76 (6.1)	292 (5.9)	1.32 (0.93-1.86)	1.35 (0.91-1.99)
>7.5-8.0%	58 (4.7)	191 (3.8)	1.50 (1.04-2.17)	1.48 (0.99-2.22)
>8.0-9.0%	67 (5.4)	186 (3.7)	1.93 (1.33-2.80)	1.94 (1.28-2.96)
>9.0%	62 (5.0)	226 (4.5)	1.37 (0.96-1.95)	1.23 (0.83-1.82)
No Recording**	776 (62.3)	3092 (62.1)	1.50 (1.04-2.17)	1.56 (1.04-2.34)

*Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas.

**Included are patients with missing HbA1c measurements as well as those with a last HbA1c level recorded >90 days prior to the index date

We found no association between the risk of VTE by HbA1c level in the group of patients with a T2DM duration of more than 5 years (**Table 21**). However, among patients with shorter T2DM duration (0-5 years), those with HbA1c levels >7.0% (>53 mmol/mol) had slightly higher aORs for VTE when compared to T2DM patients with HbA1c levels of >6.5-7.0% (HbA1c >7.0-7.5%: aOR 1.20, 95% CI 0.97-1.49; HbA1c >7.5-8.0%: aOR 1.29, 95% CI 1.00-1.67; HbA1c >8.0-9.0%: aOR 1.44, 95% CI 1.13-1.83; HbA1c >9.0%: aOR 1.39, 95% CI 1.07-1.79).

«Science is wonderfully equipped to answer the question 'How?' but it gets terribly confused when you ask the question 'Why?'»

Erwin Chargaff

Table 21: Risk of VTE in patients with different T2DM durations

Last HbA1c: 0-5 y since T2DM diagnosis	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5% (≤48 mmol/mol)	446 (33.7)	2042 (38.6)	1.02 (0.86-1.20)	1.06 (0.89-1.25)
>6.5-7.0% (>48-53 mmol/mol)	210 (15.9)	990 (18.7)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	172 (13.0)	651 (12.3)	1.24 (1.01-1.51)	1.20 (0.97-1.49)
>7.5-8.0% (>58-64 mmol/mol)	86 (6.5)	326 (6.2)	1.30 (1.01-1.68)	1.29 (1.00-1.67)
>8.0-9.0% (>64-75 mmol/mol)	104 (7.9)	336 (6.4)	1.51 (1.20-1.91)	1.44 (1.13-1.83)
>9.0% (>75 mmol/mol))	114 (8.6)	348 (6.6)	1.61 (1.27-2.03)	1.39 (1.07-1.79)
No Recording	191 (14.4)	596 (11.3)	1.75 (1.40-2.19)	1.78 (1.41-2.24)
Last HbA1c: 5-10 y since T2DM diagnosis	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5% (≤48 mmol/mol)	260 (32.1)	1168 (35.8)	0.84 (0.69-1.02)	0.83 (0.68-1.01)
>6.5-7.0% (>48-53 mmol/mol)	161 (19.9)	618 (19.0)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	138 (17.1)	525 (16.1)	1.00 (0.80-1.27)	0.97 (0.76-1.23)
>7.5-8.0% (>58-64 mmol/mol)	58 (7.2)	284 (8.7)	0.78 (0.58-1.05)	0.73 (0.53-1.01)
>8.0-9.0% (>64-75 mmol/mol)	80 (9.9)	270 (8.3)	1.19 (0.90-1.57)	1.11 (0.83-1.48)
>9.0% (>75 mmol/mol))	85 (10.5)	306 (9.4)	1.07 (0.81-1.40)	0.87 (0.65-1.16)
No Recording	27 (3.3)	88 (2.7)	1.24 (0.81-1.91)	1.46 (0.93-2.31)
Last HbA1c: >10 y since T2DM diagnosis	**Number of cases (%)**	**Number of controls (%)**	**Unadjusted ORs (95% CI)**	**Adjusted ORs* (95% CI)**
≤6.5% (≤48 mmol/mol)	137 (26.3)	599 (29.0)	1.16 (0.87-1.55)	1.10 (0.81-1.48)
>6.5-7.0% (>48-53 mmol/mol)	70 (13.4)	355 (17.2)	1 (reference)	1 (reference)
>7.0-7.5% (>53-58 mmol/mol)	87 (16.7)	346 (16.8)	1.29 (0.95-1.76)	1.20 (0.87-1.64)
>7.5-8.0% (>58-64 mmol/mol)	64 (12.3)	229 (11.1)	1.42 (1.00-2.02)	1.36 (0.94-1.96)
>8.0-9.0% (>64-75 mmol/mol)	69 (13.2)	228 (11.1)	1.53 (1.10-2.14)	1.40 (0.98-1.99)
>9.0% (>75 mmol/mol))	90 (17.3)	275 (13.3)	1.74 (1.26-2.40)	1.33 (0.92-1.91)
No Recording	4 (0.8)	32 (1.6)	0.56 (0.23-1.37)	0.58 (0.22-1.52)

*Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas.

6.1.5 Discussion

In this large case-control study based on primary care data from the UK, patients with HbA1c >7.0% (>53 mmol/mol) did not have an increased risk of unprovoked VTE compared to patients with HbA1c >6.5-7.0% (>48-53 mmol/mol). In the subset of female patients, we found a suggestion of a slightly increased risk of VTE in women with HbA1c >8.0% (for example HbA1c >-8.0-9.0%: aOR 1.29, 95% CI 1.02-1.63) when compared to those with HbA1c >6.5-7.0% (>48-53 mmol/mol). This increase was slightly more pronounced if we only considered patients with HbA1c measurements taken within 90 days prior to the index date. Overall, however, the association in women was weak, and there was no trend of increasing risk of VTE in association with increasing HbA1c values. We did not observe an increased risk of VTE in men at any level of glycemic control.

The weak association between elevated HbA1c levels and risk of VTE in women, but not in men, may be explained by the fact that pre-diabetic and diabetic women are more affected by chronically elevated cardiovascular risk factors, and their health declines faster when compared to men. [187, 194, 195].

Since T2DM is a disease with uncertain onset, which can remain undiagnosed for many years, this difference in risk factor levels between men and women is relevant. Several studies, including a comprehensive meta-analysis, suggest that the presence of diabetes eliminates the biological female advantage that is often used to explain the lower absolute rates of coronary heart disease (CHD) and stroke in women compared to men.[187, 196, 197] The authors of this meta-analysis estimate that the relative risk for CHD is 44% greater in women with diabetes than in similarly affected men.[197] In general, our study population included more women than men, even though men are more often affected by T2DM and by VTE, when the diseases are observed independently of each other. The T2DM cohort for our study also included more men than women prior to the identification of the VTE cases (50.8% vs 49.2%). Several studies provide an explanation for this imbalance in the rates of affected females and males by showing that adverse changes in metabolic and vascular risk factor profiles are greater in women than in men. These changes occur in diabetic individuals as well as earlier in pre-diabetic individuals.[180, 187-189]

Patients with CVD and HbA1c levels >7.0% (>53 mmol/mol) did not have an increased risk of VTE when compared to those with HbA1c levels between >6.5-7.0% (>48-53 mmol/mol), though women with CVD and HbA1c level >7% (>53 mmol/mol) had a slightly elevated risk for VTE, while men with CVD did not. This result emphasizes the general importance of proper glycemic control in women suffering from both, CVD and T2DM.

In our study, patients with no recorded HbA1c measurements had a higher risk of VTE compared to patients with HbA1c >6.5-7.0% (>48-53 mmol/mol) throughout our analyses. This could be a proxy for a lack of patient-doctor interaction and poor treatment adherence, which could lead not only to a higher risk for VTE (as suggested in this study), but potentially to other complications caused by improper management of T2DM. This assumption is reinforced by the results shown in the supplementary table, where patients without HbA1c measurements had much lower numbers for diagnosis of comorbidities, as well as for a corresponding prescription, when compared to patients who had at least 1 HbA1c measurement.

The present findings should be interpreted within the context of the strengths and limitations of an observational study. A delayed diagnosis of T2DM may have led to the inclusion of some prevalent (instead of incident) T2DM cases in our cohort. Additionally, the UK Prospective Diabetes study found that a high prevalence of DM tissue damage was already present by the time the DM diagnosis was made, which is an indication of pre-existing DM.[133] Therefore, we may have underestimated the time until VTE events (after the recorded DM diagnosis) in our study population, which could have potentially affected our matching on DM duration. However, this misclassification is unlikely to have been differential by HbA1c level, and we do not expect that it had a major influence on our findings.

Though VTE events are well recorded and have previously been validated in the CPRD (positive predictive value 88.2% [82.3-92.6%] for VTE),[132] it is possible that we missed some unrecorded VTEs. This possible misclassification would likely be non-differential and would not materially change the results.

We considered BMI, previously diagnosed CVD, use of statins, as well as well as other factors that may increase the risk of VTE (see **Table 17**) in our analyses. However, we were not in the position to include data on diet, waist circumference, or physical activity, since this data is not available in the CPRD.

The strengths of our study include the large study sample and the observational nested case-control design within a cohort of patients with newly diagnosed T2DM. Our data come from a well validated primary care database that contains prospectively and routinely collected data, which avoids recall bias. Even though we only used the last HbA1c measurement before the index date, HbA1c measurements are regularly performed in the diabetic population, and median time between the index date and the last HbA1c measurement was short. This shows that the recorded HbA1c measurements provide a reliable and timely source for our analyses on the effect of glycemic control on the risk of VTE.

Our study population included a high proportion of patients with T2DM with HbA1c ≤7% (>53 mmol/mol) who may have been healthier than the T2DM populations analyzed in other studies. Nevertheless, our population consisted of over 19’480 patients with T2DM, many of whom had HbA1c levels >7% (>53 mmol/mol). Therefore, we expect our results to be generalizable to those of other populations with T2DM and HbA1c levels >7% (>53 mmol/mol).

In conclusion, our study provides evidence that HbA1c levels >7% (>53 mmol/mol) are not associated with a materially increased risk for unprovoked VTE overall. There was a suggestion of a slightly increased VTE risk in women, which may be real or may reflect differences in lifestyle or other patient characteristics.

6.1.6 Conclusion

Our study raises the possibility that female T2DM patients with HbA1c levels >7% may have a slightly higher risk for unprovoked VTE compared to women with HbA1c level >6.5-7.0%. This increase may not be causal and may reflect differences in life style or other characteristics. We observed no effect of glycemic control on the risk of VTE in men.

6.1.7 List of abbreviations

aOR	Adjusted Odds Ratio
BMI	Body Mass Index
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CI	Confidence Interval
CPRD	Clinical Practice Research Datalink
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DVT	Deep Vein Thrombosis
GFAT	Glutamine Fructose-6 Phosphate Amidotransferase
GP	General Practitioner
HbA1c	Glycated Hemoglobin A1c
HRT	Hormone Replacement Therapy
i.d.	Index Date
IHD	Ischemic Heart Disease
ISAC	Independent Scientific Advisory Committee
MHRA	Medicines And Healthcare Products Regulatory Agency
MI	Myocardial infarction
NHS	National Health Services
NIHR	National Institute For Health Research
OAD	Oral Antidiabetic Drug
OR	Odds Ratio
PE	Pulmonary Embolism
UK	United Kingdom
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
VTE	Venous Thromboembolism

«We especially need imagination in science. It is not all mathematics, nor all logic, but it is somewhat beauty and poetry.»

Maria Mitchell

6.1.8 Supplementary information

Supplementary Table 22: Characteristics of the included cases and controls without HbA1c-measurement

Characteristics	Number of cases (%)	Number of controls (%)	Unadjusted ORs (95% CI)	Adjusted ORs* (95% CI)
Age (years)				
<60	29 (27.9)	90 (24.7)	NA	NA
60-69	24 (23.1)	85 (23.4)	NA	NA
70-79	28 (26.9)	98 (26.9)	NA	NA
80+	23 (22.1)	91 (25.0)	NA	NA
Male	63 (60.6)	197 (54.1)	NA	NA
Female	41 (39.4)	167 (45.9)	NA	NA
BMI (kg/m^2)				
<18.5	3 (2.9)	4 (1.1)	--	--
18.5 to <25.0	15 (14.4)	87 (23.9)	1 (reference)	1 (reference)
25.0 to <30.0	30 (28.9)	103 (28.3)	0.82 (0.34-1.96)	0.35 (0.08-1.49)
30.0 to <35.0	15 (14.4)	66 (18.1)	0.98 (0.36-2.69)	0.16 (0.02-1.02)
35.0 to <40.0	14 (13.5)	27 (7.4)	1.85 (0.36-2.69)	1.35 (0.26-6.91)
≥40.0	11 (10.6)	12 (3.3)	4.34 (0.87-22.03)	3.54 (0.54-23.43)
Unknown	16 (15.4)	65 (17.9)	1.49 (0.43-5.12)	0.24 (0.03-1.74)
Smoking status				
Non-smoker	44 (42.3)	159 (43.7)	1 (reference)	1 (reference)
Current smoker	16 (15.4)	47 (12.9)	0.97 (0.42-2.24)	0.86 (0.25-2.94)
Ex-smoker	33 (31.7)	105 (28.9)	1.03 (0.54-1.95)	0.85 (0.19-3.78)
Unknown	11 (10.6)	53 (14.6)	1.74 (0.70-4.37)	1.18 (0.13-10.56)
Comorbidities				
Inflammatory bowel disease	3 (2.9)	10 (2.8)	--	--
Chronic renal failure	5 (4.8)	7 (1.9)	--	--
Diabetic retinopathy	7 (6.7)	16 (4.4)	1.00 (0.25-4.00)	0.60 (0.08-4.62)
Asthma	16 (15.4)	51 (14.0)	2.00 (0.67-5.99)	1.11 (0.20-6.05)
Congestive heart failure (CHF)	12 (11.5)	19 (5.2)	1.56 (0.53-4.63)	0.50 (0.11-2.20)
Ischemic heart disease (IHD)	19 (18.3)	66 (18.1)	3.15 (0.26-7.88)	74.78 (1.64-3416.0)
Myocardial infarction (MI)	9 (8.7)	28 (7.7)	1.86 (0.62-5.56)	0.10 (0.01-1.40)
Stroke	12 (11.5)	33 (9.1)	0.93 (0.35-2.49)	4.32 (0.35-53.91)
Arterial hypertension	45 (43.3)	172 (47.3)	1.95 (1.01-3.77)	3.14 (0.95-10.44)
Peripheral arterial disease	3 (2.9)	7 (1.9)	--	--
Osteoarthritis	31 (29.8)	91 (25.0)	1.23 (0.64-2.35)	3.01 (1.26-7.19)
Rheumatoid arthritis	4 (3.9)	6 (1.7)	3.24 (0.48-21.86)	0.91 (0.07-11.08)
Hyperlipidemia	16 (15.4)	45 (12.4)	3.31 (0.94-11.72)	4.52 (0.24-86.34)
Cardiovascular disease	61 (58.7)	219 (60.2)	1.87 (0.95-3.72)	0.09 (0.01-0.94)
Co-medication **				
Insulin	7 (6.7)	9 (2.5)	3.00 (0.31-28.84)	18.91 (0.69-516.8)
Glitazones	0 (0.0)	0 (0.0)	--	--
Sulfonylurea	24 (23.1)	61 (16.8)	1.64 (0.61-4.40)	2.48 (0.41-14.94)
Metformin	16 (15.4)	64 (17.6)	0.80 (0.29-2.23)	0.19 (0.03-1.47)
GLP1	0 (0.0)	0 (0.0)	--	--
DPP4	99 (7.5)	470 (8.9)	0.90 (0.69-1.17)	0.74 (0.55-0.98)
SGLT2	0 (0.0)	0 (0.0)	--	--
All oral antidiabetics	32 (30.8)	105 (28.9)	1.16 (0.50-2.69)	0.04 (0.00-9.41)
Statins	28 (26.9)	104 (28.6)	2.14 (0.62-7.41)	6.38 (0.50-81.58)
Bisphosphonates	4 (3.9)	24 (6.6)	0.25 (0.03-2.24)	0.06 (0.00-1.79)
Contraceptive pill	1 (1.0)	11 (3.0)	--	--
Hormone replacement therapy	10 (9.6)	38 (10.4)	0.15 (0.02-1.21)	0.05 (0.00-1.40)
Corticosteroids (systemic)	29 (27.9)	60 (16.5)	2.56 (0.86-7.60)	8.52 (0.88-82.95)
Coronary vasodilators	21 (20.2)	62 (17.0)	1.26 (0.45-3.56)	--
Low dose acetylsalicylic acid	36 (34.6)	104 (28.6)	1.53 (0.65-3.59)	0.38 (0.04-3.49)
Loop diuretics	26 (25.0)	69 (19.0)	0.77 (0.23-2.64)	0.21 (0.02-1.88)
All diuretics	61 (58.7)	168 (46.2)	2.30 (0.81-6.53)	1.66 (0.29-9.61)

* Adjusted for BMI (categorical), smoking (categorical), CHF, IHD, MI, stroke, hypertension, osteoarthritis, and use of insulin, bisphosphonate, systemic corticosteroids, low-dose acetylsalicylic acid, current and past use of metformin, and current and past use of sulfonylureas

** Use of other medication possible

6.1.9 Declarations

Ethics approval and consent to participate

The study protocol was approved by the Independent Scientific Advisory Committee (ISAC) for MHRA database research (protocol number 20_033) prior to the initiation of the study. The study protocol was made available to the journal reviewers.

Consent for publication

All of the authors have approved the contents of this paper and have agreed to the Cardiovascular Diabetology's submission policies.

Availability of data and materials

The data that support the findings of this study are available from the CPRD but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of CPRD.

Competing interests

We have no conflict of interest to declare, financial or otherwise.

Funding

Not applicable

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Chapter 7

Discussion and outlook

7 Discussion and outlook

7.1 Discussion

With DM as a growing public health concern across the world due to its increasing burden to financial and health systems, as well as society in general, measures to contain the pandemic and its complications are highly sought-after.[58, 59, 134]

There is abundant evidence that good glycemic control prevents or delays many DM-related short- and long-term complications, including CVD and diabetic nephropathy or retinopathy. Accordingly, diabetes associations around the world provide guidelines and targets for glycemic control, such as the American Diabetes Association, the NICE guidelines, or the International Diabetes Federation.[38, 58, 59, 61, 198] However, the impact of glycemic control on specific diabetes complications such as fractures and VTE is still unclear.[100-103, 105-107, 140, 184-186]

This thesis aimed to contribute to the general understanding of how glycemic control is associated with two DM-related outcomes (fractures and venous thromboembolisms) by means of three case-control studies. A thorough discussion of the results and limitations of each individual study is presented in the discussion section of the respective studies in chapters 4.1.6, 5.1.5, and 6.1.5/6.1.6. The most intriguing findings as well as more general methodological aspects and their relevance are highlighted and discussed in the following subchapters.

7.1.1 The CPRD

All studies conducted within the framework of this thesis used data originating from the CPRD. The CPRD is a governmental and non-profit UK-based primary care database that resulted from joint venture between the MHRA and the NIHR. This database contains anonymized medical records of over 16 million active patients from over 2’000 practices across the UK and was established in 1987.[110, 199]

The big size of the database allows for the detailed investigation of epidemiological associations with a high level of statistical precision.[110] It also enables the conduction of studies on rare exposures and outcomes with incidence rates of less than 10 per 100 000 person-years with enough statistical power.[200] Along with the big size of the database, the CPRD also covers a broad time range. The median overall follow-up duration of patients is 5.1 years (IQR 1.8-11.1 years), which enables researchers to address questions regarding diseases with long latency, as well as to study long-term outcomes.[110]

The CPRD has many other strengths. For once, it includes information on medical diagnoses, symptoms, laboratory test results, some lifestyle factors (e.g., smoking status, alcohol consumption), and other patient characteristics (e.g., BMI).[110] Even more data can be obtained through linkage to other

governmental datasets such as hospital data (i.e., HES data), national cancer registration data, and national mortality records. This enables the study of research questions relying on these additional data. More than 50% of the patients are eligible for data linkage.[110, 111]

Secondly, many data validation studies have been conducted on the CPRD and its data has shown to be of great quality.[111] The data provide high positive predictive values of many diagnoses and, where evaluated, largely similar incidence numbers compared with other UK data sources.[110] Lastly, the patient data on the CPRD patients are representative of the UK general population in terms of age, sex, and ethnicity, and they cover around 7% of the UK population. This suggests that study results obtained with CPRD data are generalizable and comparable to the general UK population.[111]

Valid and highly predictive data on diagnoses is vital to carry out accurate analytical studies. The additionally available data on patient characteristics can further be used to achieve comparability between groups by minimizing bias and confounding.[110]

The CPRD has been and still is widely used for studies in pharmacoepidemiology and disease epidemiology around the world. The studies cover a wide range of health-related research questions including descriptive and analytical pharmacoepidemiology, health services research, health economics, comparative effectiveness research, classical risk factor epidemiology, prognosis research, assessments of temporal trends in disease incidence, and even RCTs.[110, 201]

However, like any database, the CPRD also has some weaknesses that need to be considered when working with its data. One of the weaknesses of the CPRD data is tied the nature of primary care data. Since the data are entered into the database by GPs during routine examinations and not specifically for research purposes, data quality may vary. As a result, comprehensive data quality checks are inadmissible before conducting a study.[202]

Another main drawback of CPRD data is tied to missing data. Although GPs are expected to record information holistically, some information stemming from specialists and inpatient events may not or not fully be captured in the electronic patient records. These events are often received in hard copy and must be entered manually into the database in the respective GP practice. Examples of such events could be discharge letters from hospitals, feedback from specialists, and laboratory test results. Due to the additional time required for this data entry, some practices may decide to only enter information they think may potentially affect the future care of the patient. Also, the recording of lifestyle factors, while done for many patients, is not collected for all patients and may accordingly be missing. In addition to missing data, some variation between GPs in regard to coding diagnoses may be present.[110, 200]

Given the afore mentioned reasons, the inconsistency in completeness of data across patients and throughout different time periods of the patient record must be taken into account when conducting a study. For instance, cholesterol levels may be recorded more frequently in individuals with pre-existing

hyperlipidemia or associated risk factors. By restricting the study population to patients with complete data, biased estimates and results may ensue due to the missing data not being missing at random. This also makes imputation approaches difficult, due to the complex patterns and interpretation of the missing data.[110]

Another risk of missing or incomplete primary care data emerges in the absence of a 'READ Code', a clinical classification system including over 96 000 codes. In that case, the patients may be regarded as not having the disease based on the lack of a specific READ-Code, which could be a wrong assumption and would, accordingly, carry the risk of misclassification.[203] However, the impact of such a possible misclassification may strongly differ between diseases.[204]

Since multiple codes on exposures and diagnoses exist and this data is entered into the CPRD manually in every practice, READ-Code lists and identifying algorithms must be elaborated for every study. Due to a lack of standardization for the definitions of exposures and diagnoses, variations leading to inconsistent results between studies using the same data may occur.[110]

Lastly, some patient data may only be recorded very infrequently or not all. Examples include OTC (freely purchasable without prescription) and illicit drug use. Also, some patient groups are not recorded in primary care records. These include prisoners, private patients, individuals dwelling in certain residential homes, and home-less people.[110, 200]

7.1.2 Association between glycemic control and risk of fracture in diabetic patient: A nested case-control study

The objective of this study was to evaluate the association between glycemic control and the risk of low-trauma fractures in patients with type 1 DM (T1DM) and type 2 DM (T2DM). Key aspects of this study are listed below:

- To our knowledge, we were the first to comprehensively evaluate the association between the level of glycemic control (assessed with several and timely HbA1c measurements) and the risk of fractures in patients with T1DM and T2DM individually.
- The effect of glycemic control on the risk of low-trauma fracture differs between patients with T1DM and T2DM. When compared to the general T1DM or T2DM population, respectively, the impact of glycemic control on the risk of low-trauma fracture was bigger on patients with T1DM than in those with T2DM.
- Poor glycemic control (3-year mean HbA1c >8.0%) increased the risk of fractures in patients with T1DM but not in those with T2DM.

This nested case-control study encompassed a time period of 21 years and included almost 9'700 cases (~700 with T1DM and ~9000 with T2DM) with a low-trauma fracture. With this big study, we were able to generate novel data related to the risk of fracture that are relevant for the clinical treatment, and emphasize the importance of proper glycemic control, especially in patients with T1DM.

Generalizability and comparability

In this study, methodological techniques such as stratification and individual matching were used to increase generalizability to the underlying UK population and to increase comparability between cases and controls, respectively.

To prevent confounding, we matched cases to controls with great care and only on major confounding factors. These included age (+/- 3 years), sex, general practice, index date (control being present in the database on the index date), DM type, and DM duration (+/- 365 days).

Age, sex, and geographic location are commonly used matching factors.[78] In our study, matching on the general practice takes the place of matching on geographic location, since patients must usually visit GP practices that are within their living are. Alternatively, they may register at a GPs office that's more convenient, for example closer to their work or their children's school.[205]

We also matched on index date. Matching on this factor is important for two reasons:

- Firstly, to avoid immortal time bias,[206] meaning that the outcome under study could not have occurred to a control if said patients is, for example, no longer registered at the GP office or has died before the matched case reached the index date.
- Secondly, because treatment options as well as disease knowledge change over time. For this reason, it is important to compare cases and controls from similar time periods in order to avoid selection bias and increase internal validity of our study.[207] Otherwise, cases and controls may not be comparable.

Matching on DM type was of paramount importance for this study, since the objective was to analyze the impact of the level of glycemic control separately in patients with T1DM and T2DM. The matching on DM duration, on the other hand was done in order to separate the effect of glycemic control from a potential effect of disease duration. Since diabetes complications tend to develop over time, a longer disease duration represents a risk factor for outcomes such as low-trauma fractures.[5, 38, 208] Matching on DM duration accordingly eliminates this confounding factor.

The percentage of patients with T1DM or T2DM achieving good and medium levels of glycemic control (HbA1c levels <7% and <8.0%, respectively) we found in our study were largely in line with studies performed in high and low- to middle income countries. This may seem surprising, given that more treatment options and facilities are available in high-income countries. However, it is an indicator that

the challenge of attaining good glycemic control is universal.[209, 210] While the percentage of patients by level of glycemic control may be similar in high- versus low- to middle-income countries, conclusions regarding generalizability of UK-data to other countries, whether low- or high-income, may still be difficult and are unsupported, given that availability of medical treatment and of treatment centers may vary.

Association and causation

While we found poor glycemic control with HbA1c levels >8% in patients with T1DM to be associated with a slightly increased risk of fracture when compared to T1DM patients with good glycemic control (HbA1c levels ≤7%), we did not see a similar effect in patients with T2DM. A possible explanation may lie in the different pathophysiological mechanisms contributing to skeletal fragility in each DM type. However, another valid complementary hypothesis could be that T2DM patients are possibly more heterogeneous due to their advanced age, frequently present overweight and/or additional comorbidities, as well as a wider range of treatment options, starting with behavioral and dietary recommendations to complex medical treatments with several drugs including insulin.

Due to this hypothesis, we decided to conduct an exhaustive research on the risk of fracture in T2DM patients based on their antidiabetic treatment and the level of glycemic control.

7.1.3 Antidiabetic Treatment, Level of Glycemic Control, and Risk of Fracture in Type 2 Diabetes: A nested case-control study

As mentioned above, this study was inspired by our previously conducted study on the association of glycemic control and the risk of fractures. The aim of this second study was to evaluate the association between the level of glycemic control, use of antidiabetic medication, and risk of low-trauma fractures in patients with newly diagnosed T2DM. To our knowledge no previously published study analyzed the level of glycemic control according to different antidiabetic medication regimens and its joint impact on the risk of fracture.

Some of the main aspects and conclusions of the study are listed below:

- We considered several HbA1c categories and different antidiabetic medication schemes to assess the association between medication, glycemic control and the risk of fractures in T2DM patients.
- The antidiabetic medication schemes were based on the NICE-guideline recommendations and included **a)** behavioral and dietary recommendations (without drug treatment), **b)** initial drug treatment with metformin as monotherapy, **c)** fist intensification of drug treatment (metformin plus either DPP4-Is, glitazones, or SUs), and **d)** second intensification of drug treatment with

either 3 noninsulin blood glucose-lowering therapies or any treatment regimen containing insulin.

- The study included analyses on current and past users of antidiabetic drugs and drug combination, and on different durations of drug exposure.
- Our study suggests a beneficial effect of HbA1c levels <8.0% in current users of metformin monotherapy on the risk of fracture compared with nonuse of any antidiabetic medication (HbA1c levels ≤7.0: aOR 0.89; 95% CI, 0.83-0.96; HbA1c >7.0%-8.0%: aOR 0.81; 95% CI, 0.73-0.90). Thus, patients with current metformin therapy and good glycemic control could benefit the most with regards to their risk of fracture.
- While current use of metformin was associated with a changed risk of fracture in any HbA1c category compared with nonuse of antidiabetic drugs, past use of metformin was not. This may be an indication of a beneficial effect of metformin on the bones.

Our study included almost 9000 cases with a low-trauma fracture and encompassed a time period of 23 years.

Personalized medicine

With the exposure not only being the level of glycemic control, but also the type of antidiabetic medication scheme, this study provided insight on the risk of fracture based on actual, clinically measurable and visible characteristics.

With the focus of future treatments shifting toward personalized medicine, identifying successful prevention measures and treatments for individuals based on their characteristics is becoming more and more important. The idea behind this concept is that neither time nor money should be wasted on interventions based on trial and error. Instead, patients should receive interventions tailored to their needs which are based on their characteristics (e.g. genetics, molecular profiling, medical imaging, lifestyle data) and accordingly work best for them as individuals.[211]

By being able to define the benefits of good glycemic control on the risk of low-trauma fractures specifically to identifiable substrata of the T2DM population, we are able to provide helpful additional information for clinicians and health practitioners regarding the treatment and treatment advice for these patients. Results like ours, including patient-specific characteristics that are easily identified in the clinical setting (e.g. sex, DM-type, medication scheme, DM duration, comorbidities), could also provide a better or supplemental incentive for patients to achieve good glycemic control. This, of course, has been shown to be beneficial for not only low-trauma fractures, but many other DM complications.[5, 38, 208]

Generalizability and comparability

Also in this study we used methodological techniques such as stratification and individual matching to increase generalizability to the underlying UK population and to increase comparability between cases and controls, respectively.

We matched cases to controls on the same major confounding factors than in our previous study, apart from DM type. Matching on DM type was not required, since only T2DM patients were included in the study. Due to the similarity of the study setup, the main confounding factors remained the same. Correspondingly, so have the rationale and arguments for the choice of confounding variables.

7.1.4 Association between glycemic control and risk of venous thromboembolism in diabetic patients: A nested case-control study

Our objective of this study was to analyze the association between glycemic control (assessed as HbA1c levels) and the risk of unprovoked (idiopathic) VTE separately for men and women with T2DM. To our knowledge, no published study analyzed the impact of glycemic control on the risk of VTE stratified by sex.

A summary of the most important aspects of this study is given here:

- We used the last recorded HbA1c value before the index date for our analyses and assessed HbA1c levels in 7 categories ($\leq$ 6.5%, > 6.5-7.0%, > 7.0-7.5%, > 7.5-8.0%, > 8.0-9.0%, > 9.0%, and no HbA1c measurement).
- We conducted three sensitivity analyses where we **a)** restricted the study population to patients whose last HbA1c measurement was recorded within less than 90 days prior to the index date, **b)** analyzed the risk of VTE separately for patients with a previous CVD diagnosis, and **c)** conducted separate analyses of the risk of VTE by HbA1c levels for patient groups of different T2DM durations.
- We did not find patients with HbA1c > 7.0% to have an increased risk of unprovoked VTE compared to patients with HbA1c > 6.5-7.0%. However, in the subset of female patients we found a slightly increased risk of VTE in women with HbA1c > 8.0% (for example HbA1c > 8.0–9.0%: aOR 1.29, 95% CI 1.02–1.63) when compared to those with HbA1c > 6.5-7.0%.
- Overall, patients with missing HbA1c measurements had the highest risk of VTE (aOR 1.56, 95% CI 1.29-1.88) when compared to patients with last HbA1c levels > 6.5-7.0% before the index date.

This nested case-control study encompassed a time period of 25 years and included over 2’500 cases who suffered a VTE and received subsequent antithrombotic treatment.

Personalized medicine

In order for personalized medicine to become reality, the sex of the patient must, of course, also be considered. This has been neglected over long periods of time, leading to unequal opportunities for women and men to achieve their full health potential. Based on biological as well as social differences, both genders face different health risk, experience different responses from health systems, and have varying health-seeking behavior. Accordingly, their health outcomes differ. While in many societies women have less access to health information, care, services, and resources, risk-taking behavior causing neglect of their own health is promoted in men.[212]

With this study, we have contributed to the limited knowledge and helped gather sex-specific data on the association between the level of glycemic control and the risk of VTE. Our study showed that sex-specific differences exist on one hand in the risk for VTE in T2DM patients, on the other also in the impact of glycemic control on said risk.

Generalizability and comparability

As for the previous studies, we used methodological techniques such as stratification and individual matching to increase generalizability to the underlying UK population and to increase comparability between cases and controls, respectively. However, in this study we also conducted sensitivity analyses.

Sensitivity analyses can be used to assess the impact of unmeasured confounders on causal associations. The presence of unmeasured confounding is possible in every non-randomized study. And while their presence cannot be excluded, their potential impact can be estimated through sensitivity analyses.[213]

The sensitivity analyses conducted during our study on the association of glycemic control and the risk of VTE suggest that our results are robust and that unmeasured confounding was not present or at least negligible. Consistent results generated from the study population and from the sensitivity analyses are an indicator for external validity.[214] This, again, increases the generalizability and comparability of the results from the study with the general UK population.

7.1.5 Strengths and limitations of the pharmacoepidemiological database research

All studies conducted within the scope of this thesis were based on pharmacoepidemiological database research. While randomized clinical trials (RCTs) continue to be the gold standard for evidence in clinical medicine, database research is a powerful tool and comes with many benefits. Also, RCTs have their very own limitations. This leads to an increased interest in using real-world data (RWD) to generate real-world-evidence (RWE). RWD is provided in databases and several study designs can be used to analyze such RWE.[215-217]

When databases are big and of good quality, they offer cost-effective means to consistent long-term follow-up of patients and their health issues and medications. [215-217] The bigger databases are; the more generalizable results may be generated. With RCTs, on the other hand, generalizability is a major concern, as study participants are often few and have no/less comorbidities compared to those found in the RWD. Therefore, RWD is well suited for post-marketing drug surveillance, including the detection of safety concerns and quantification of side effects. Furthermore, RWD are able to provide required information to tackle public health topics. This is one of the reasons why the use of RWD was recently integrated into the regulatory decision-making by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA).[215, 216]

Other benefits of database research with its RWD include the possibility to evaluate drug prescribing, utilization patterns, and adherence.[215] RWE, as the name suggests, provides answers from the 'real world', i.e., the clinical reality encountered by health practitioners on a daily basis. This type of data cannot be gathered through RCTs. Therefore, RCTs cannot replace database research, just as database research cannot replace RCTs. Both types of research are complimentary, and can, when combined, benefit patients worldwide greatly by leading to the most precise and detailed answers to research questions.

7.1.6 Overarching conclusions

This thesis contributed to the general understanding of the association between the level of glycemic control in patients with T1DM or T2DM and the risk of fractures. For patients with T2DM, an additional study was conducted. Therein, we analyzed the risk of fractures not only based on the level of glycemic control, but also based on the antidiabetic medication scheme. Further, the thesis contributed to a deeper insight into the association between the level of glycemic control and the risk of VTE.

While achieving low mean HbA1c levels is important for many known diabetes complications,[5, 38, 208] our studies suggest that it may not be equally important for all DM patients (e.g. patients with T1DM versus patients with T2DM) or in all analyzed comorbidities of DM. However, since DM has many complications and tends to come along with many comorbidities (especially over time), a statement regarding which patients should focus on good glycemic control, at what disease stage, and by which means, is impossible to make based solely on our study results.

Accordingly, the general recommendations regarding the maintenance of good glycemic control should always be considered, even though it is not yet known which patients will benefit the most.

7.2 Outlook

In the frame of this thesis, I conducted three exemplary studies. Each of these studies revealed novel and unreported findings, and extended previous knowledge based on limited existing evidence. The

research questions varied from study to study, however, the focus of all of them was on the impact of glycemic control on the risk of different outcomes and/or under different conditions. The following paragraphs summarize individual needs for future research and give a more general perspective on the content of this thesis.

Study I, *Association Between Glycemic Control and Risk of Fracture in Diabetic Patients: A Nested Case-Control Study*, demonstrated how the level of glycemic control could impact the risk of low-trauma fractures in patients with T1DM or T2DM. Additional research on the most common reasons for low-trauma fractures in T1DM and whether they are associated with glycemic levels could be helpful, to prevent falls and fractures in patients with T1DM prospectively. The results also highlighted the need for a more granular analysis of this association in patients with T2DM, since this patient group appeared to be more heterogeneous and, accordingly, results were not applicable or generalizable to its entirety.

In study II, *Antidiabetic Treatment, Level of Glycemic Control, and Risk of Fracture in Type 2 Diabetes: A Nested Case-Control Study*, we found that different disease stages of T2DM had a varying impact on the association between the level of glycemic control, the prescribed medication scheme, and the risk of low-trauma fractures. Future research should focus on deepening the understanding between the interaction of antidiabetic medication, the level of glycemic control, and their impact on bone structure and fragility. Since T2DM, as well as the propensity for fractures, is multifactorial and T2DM patients often suffer from several comorbidities and receive several medications, multidimensional research could help in obtaining a better understanding of the interaction between the different involved factors and how they impact the risk of fracture. With new antidiabetic medications and medication regimens reaching the market, a focus on changing medication schemes and their impact on the risk of fracture based on the level of glycemic control should be kept in view.

Concerning our study III, *Association between glycemic control and risk of venous thromboembolism in diabetic patients: a nested case–control study*, additional data on the differing pathophysiology of male and female T2DM patients, who are diagnosed with a VTE would be of major interest. With more knowledge on the pathophysiology, a better understanding of the sex-specific impact of glycemic control on the risk of VTE may ensue. Possible additional sex-specific risk factors for VTE in the diabetic population should be another focus of future research. Regarding the association between glycemic control and the risk of VTE, a shift towards improved personalized medicine with increased probability of the detection of at-risk patients in the clinical setting could benefit patients and prevent VTEs as well as stress on the public health system.

The need for further research in DM has long been recognized, especially with the steadily growing number of patients with DM around the world.[38, 134] This thesis contributed to the understanding of the role of glycemic control in some of the many comorbidities of DM. It is an important reminder that rigorous research is still much required in this area. Observational research, along with other types of

research, is essential to further improve general understanding of DM, its many complications and comorbidities, clinical practice, as well as to further health policies and management of DM.

«Science never solves a problem without creating ten more.»

George Bernard Shaw

Chapter 8

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