Diabetic retinopathy screening in Denmark

Validation, attendance and complications in relation to systemic treatment

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PhD Thesis

Diabetic retinopathy screening in Denmark

Validation, attendance and complications in relation to systemic treatment.

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Contents

1 Acknowledgements

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2 Ethical approvals and Financial Disclosures

The papers included in this thesis was part of the Ocular And Systemic complications In diabetic retinopathy Study (OASIS), which is embedded in the Danish Excellence Centre in Ophthalmic Epidemiology (DECODE-EYE)(1). All studies were performed in accordance with the tenets of the Declaration of Helsinki and all necessary permissions related to data management were obtained under OASIS and DECODE-EYE. The datasets created and analyzed for the Papers included in the thesis are available from the Danish Health Data Authority and Statistics Denmark, but restrictions apply to the availability of the data as they are not publicly available and were used under license from Open Patient data Explorative Network (OPEN), the Danish Health Data Authority and Statistics Denmark.

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3 List of publications

Paper I
Inter-grader reliability in the Danish screening program for diabetic retinopathy
Thykjær, A. S., Andersen, N., Andresen, J., Bek, T., Heegaard, S., Hajari, J, Laugesen, C. S., Möller, S., Pedersen, F. N., Rosengaard, L., Schielke, K. C., Kawasaki, R., Højlund, K., Peto, T., Rubin, K.H., Stokholm, L., Grauslund, J.
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Paper II
Attendance in a national screening program for diabetic retinopathy – a population-based study of 205,970 patients
Thykjær, A. S., Andersen, N., Bek, T., Heegaard, S., Hajari, J, Laugesen, C. S., Möller, S., Pedersen, F. N., Rosengaard, L., Schielke, K. C., Kawasaki, R., Højlund, K., Rubin, K.H., Stokholm, L., Grauslund, J.
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Paper III
Long-term development of diabetic retinopathy in individuals with type 1 diabetes, in response to the use of continuous subcutaneous insulin injections: a national cohort study
Thykjær, A. S., Andersen, N., Andresen, J., Bek, T., Heegaard, S., Hajari, J, Laugesen, C. S., Möller, S., Pedersen, F. N., Rosengaard, L., Schielke, K. C., Kawasaki, R., Højlund, K., Stokholm, L., Grauslund, J.
Manuscript in preparation.

Paper IV
Bariatric surgery in individuals with type 2 diabetes is not associated with short or long-term risk of diabetic retinopathy progression: results from a nationwide cohort study.
Thykjær, A. S., Andersen, N., Andresen, J., Bek, T., Heegaard, S., Hajari, J, Laugesen, C. S., Möller, S., Pedersen, F. N., Rosengaard, L., Schielke, K. C., Kawasaki, R., Højlund, K., Stokholm, L., Grauslund, J.
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4 List of abbreviations and acronyms

AC	Agreement coefficient
ATC	Anatomical Therapeutic Chemical Classification System
BMI	Body mass index
CCI	Charlson Comorbidity Index
CI	Confidence Interval
CPR	Civil Personal Registration (number)
CSII	Continuous Subcutaneous Insulin Infusion
DECODE-EYE	Danish Excellence Centre in Ophthalmic Epidemiology
DiaBase	Danish Registry of Diabetic Retinopathy
DKK	Danish kroner
DR	Diabetic retinopathy
GLP-1	Glucagon like peptide 1
HbA1c	Glycated hemoglobin
HR	Hazard ratio
ICD	International Classification of Diseases
ICDR	International Clinical Diabetic Retinopathy severity scale
IQR	Interquartile range
ISCED	International Standard Classification of Education
MDI	Multiple daily injections
NPDR	Non proliferative diabetic retinopathy
NPU	Nomenclature for Properties and Units
OASIS	Ocular And Systemic complications In diabetic retinopathy Study
OPEN	Open Patient data Explorative Network
OR	Odds ratio
PABAK	Prevalence adjusted, bias adjusted kappa
PDR	Proliferative diabetic retinopathy
RRR	Relative risk ratio
SGLT-2	Selective sodium glucose co transporter 2
VEGF	Vascular endothelial growth factor
VIOLA	VIrtual Ocular Learning plAtform
κ	Kappa

5 Summary

5.1 English

Background and aims

The global occurrence of diabetes is increasing and has been described as having pandemic proportions. The prevalence is estimated to reach 643 million affected individuals in 2030(2). In Denmark a similar tendency is observed and the Danish Diabetes Association estimates that 467,000 Danish citizens will be affected by 2030(3). Diabetic retinopathy (DR) is the most frequent complication to diabetes and a significant concern as it can lead to severe vision loss or blindness(4). In Denmark it affects approximately 24% of individuals with diabetes(5). To ensure proper management and early detection of DR, Denmark has had a nationwide screening program for DR since 2013. Individuals with diabetes are offered to attend screening either at a practicing ophthalmologist or at a hospital-based screening unit. Results from screening are reported to the national clinical quality database the Danish Registry of Diabetic Retinopathy (DiaBase)(6). In this thesis, we have examined different aspects of the Danish screening program for DR including the quality of the contents of DiaBase (Substudy A, Paper I), the attendance in the screening program (Substudy A Paper II) as well as the potential relation between systemic interventions and DR development (Substudy B).

Methods

We performed a clinical reliability study to examine the inter-rater agreeability in the Danish screening program for DR, to establish the validity of DR levels given at screening and represented in DiaBase (Substudy A, Paper I). The analysis was done by re-grading a nationwide sample of retinal images, representing randomly selected eyes of individuals who attended DR screening at both practicing ophthalmologists and hospitals. In addition to images, we collected information on primary graded DR level, screening facility and geographical

screening region. All information except for the images themselves were blinded until re-graded. We utilized prevalence- and bias-adjusted kappa (PABAK) and Gwet's agreement coefficient (AC) to determine agreement between primary and secondary grader. The remaining sub-studies were prospective register-based matched cohort studies, in which we utilized the Danish nationwide registers, of which DiaBase defined our cohorts with data from 2013-2022 (2018 for Substudy A, Paper II). We characterized the individuals attending DR screening in Denmark, determined by their attendance patterns (Substudy A, Paper II). We categorized individuals as either attending, delayed or one-time attending and used a multinomial regression model to analyze risk factors for varying degrees of non-attendance reported as odds ratios (OR) as well as a Cox regression model to examine the risk of DR progression associated with delayed screening reported as hazard ratios (HR) both with 95% confidence intervals. Finally, we examined systemic interventions and their potential relation to worsening of DR in individuals attending the Danish screening program (Sub study B). We examined continuous subcutaneous insulin infusion (CSII) in individuals with type 1 diabetes and bariatric surgery in individuals with type 2 diabetes with logistic regression and Cox regression models resulting in OR and HR respectively, with 95% confidence intervals.

Results

To establish the validity of DR levels available in DiaBase, we collected images representing 230 individuals (458 eyes). Overall agreement amongst primary and secondary grader were 93% (k=0.83). When stratified by screening facility agreement was 96% (k=0.89) and 90%(k=0.76) for practicing ophthalmologists and hospitals respectively. A sub-analysis allowing one-step-difference in grading resulted in an agreement of 95.5% (k=0.93).

When examining attendance in the Danish screening program, we concluded that 53.0% followed the program as intended whereas 35.5% had one or more delayed screenings and 11.5% only attended screening once, despite the recommendation to continue screenings. Individuals who had any delay in screenings were more than twice as likely to get a clinically significant progression of DR and individuals who had three or more delayed appointments had almost 13 times higher risk of clinically significant progression.

When examining systemic treatments in relation to DR, we examined both treatments used for individuals affected by type 1 (CSII) and type 2 diabetes (bariatric surgery). We identified 674 individuals with type 1 diabetes, who had received CSII treatment from 2013-2022 after their first screening in DiaBase. Our cohort were primarily young and female, and cases had comparable glycemic stability (HbA1c), other comorbidities and diabetes duration compared to controls. We found no difference in risk of DR worsening (HR 1.05 [95%CI 0.91;1.22], p=0.48, in individuals treated with CSII.
We identified 553 individuals with type 2 diabetes who underwent bariatric surgery after first screening in DiaBase. The cohort was primarily young and female and cases had more comorbidities, shorter diabetes duration as well as more frequent use of antidiabetic and antihypertensive medication compared to individuals using MDI. We found no increased risk of DR worsening in individuals who underwent bariatric surgery, neither short- nor long-term (OR 0.41 [CI 95% 0.13-1.33] p=0.14 and OR 0.71 [CI 95% 0.34-1.46] p=0.35 respectively) compared to individuals who did not.

Conclusions

The Danish screening program for DR can be regarded as effective in aiding prevention of sight-threatening progression in DR as long as individuals attend screenings as recommended, as delays in screenings are associated with increased risk of progression. The data collected from screening are of high validity and it can be trusted that screening ophthalmologists grade DR levels to with a high degree of correctness. This ensures that individuals are referred for timely treatment when needed, as well as attest to the high quality of data in DiaBase, which can be used confidently for register-based studies. Systemic treatments in both type 1 (insulin pumps) and type 2 diabetes (bariatric surgery) were found to be safe in regards to DR, with no increased risk of worsening. However, pre-surgical glycemic control was relatively good, in both groups which must be taken into account, when interpreting the results and for the sake of reproducibility in different geographical populations.

5.2 Danish

Baggrund og formål

Den stigende globale forekomst af diabetes er blevet beskrevet som en pandemi, og det skønnes, at 643 millioner mennesker vil være berørt i 2030(2). I Danmark er tendensen den samme, og Diabetesforeningen estimerer, at 467.000 danske borgere vil have diagnosen i 2030(7). Diabetisk retinopati (DR) er den hyppigste komplikation til diabetes og en alvorlig bekymring, grundet risikoen for synstab og blindhed. I Danmark påvirker DR 24% af alle patienter med diabetes(5). For at sikre en tidlig påvisning og korrekt behandling af DR har Danmark siden 2013 haft et landsdækkende screeningsprogram. Patienter med diabetes tilbydes at deltage i øjenscreening enten hos deres praktiserende øjenlæge eller ved en hospitalsbaseret screeningsenhed. Resultaterne fra screeningen indrapporteres til den Landsdækkende kliniske kvalitetsdatabase for screening af DR (DiaBase)(6). I denne afhandling har vi undersøgt forskellige aspekter af det danske screeningsprogram for DR, herunder enigheden blandt graderende læger i screeningsprogrammet og dermed kvaliteten af indholdet i DiaBase (Delstudie A, Artikel I), deltagelsen af screeningsprogrammet (Delstudie A, Artikel II) samt den potentielle sammenhæng mellem systemiske interventioner og udviklingen af DR (Delstudie B).

Metoder

Vi udførte en klinisk undersøgelse af overensstemmelsen mellem bedømmere i det danske screeningsprogram for DR for at fastslå validiteten af de DR-grader, der gives ved screening og indrapporteres til DiaBase (Delstudie A, Artikel I). Analysen blev udført ved at re-gradere et landsdækkende udvalg af retinale billeder, der repræsenterede tilfældigt udvalgte øjne fra patienter, der deltog i DR-screening hos både praktiserende øjenlæger og på hospitaler. Foruden billederne indsamlede vi oplysninger om primær DR grad, screeningsfacilitet og geografisk screeningsregion. Alle oplysninger foruden selve billederne var blændet for re-graderende læge, indtil sekundær gradering var færdig. Vi anvendte prævalens justeret og bias justeret kappa (PABAK) og Gwets enigheds koefficient (Gwets AC) til at bestemme overensstemmelsen mellem primære og

sekundære graderende læge. De resterende delstudier (Delstudie A, Artikel II og Delstudie B) var prospektive registerbaserede matchede kohortestudier, hvor vi anvendte de danske landsdækkende registre. DiaBase definerede vores kohorte med data fra 2013-2022 (2018 for Delstudie A, Artikel II). Vi karakteriserede patienterne, der deltog i DR screening i Danmark, baseret på deres deltagelsesmønstre (Delstudie A, Artikel II). Vi kategoriserede patienterne som enten deltagende, forsinkede eller engangsdeltagende og anvendte en multinomial regressionsmodel til at analysere risikofaktorer for forskellige grader af manglende deltagelse, rapporteret som odds ratio (OR), samt en Cox-regressionsmodel til at undersøge risikoen for DR progression forbundet med forsinket screening, rapporteret som hazard ratio (HR), begge med 95% konfidensintervaller. Endelig undersøgte vi systemiske interventioner og deres potentielle sammenhæng med forværring af DR hos patienter, der deltog i det danske screeningsprogram (Delstudie B). Vi undersøgte insulinpumper hos patienter med type 1 diabetes og bariatrisk kirurgi hos patienter med type 2 diabetes ved hjælp af logistiske- og Cox regressionsmodeller, hvilket resulterede i henholdsvis OR og HR med 95% konfidensintervaller.

Resultater

For at fastslå validiteten af de tilgængelige DR grader i DiaBase indsamlede vi billeder repræsenterende 230 patienter (458 øjne). Samlet enighed mellem primær og sekundær graderer var 93% (k=0,83). Når resultaterne blev opdelt efter screeningsfacilitet, var enigheden 96% (k=0,89) og 90% (k=0,76) for henholdsvis praktiserende øjenlæger og hospitaler. En sub-analyse med tilladt ét-trins variation i graderingen resulterede i en enighed på 95,5% (k=0,93).
Vores undersøgelse af deltagelsen i det danske screeningsprogram, konkluderede at 53,0% fulgte programmet som planlagt, mens 35,5% havde én eller flere forsinkede screeninger, og at 11,5% kun deltog i programmet én gang, på trods af anbefalingen om at fortsætte screeningforløbet.
Personer, der havde en forsinkelse i løbet af deres screeningsforløb, var i mere end dobbelt så høj risiko for en klinisk signifikant progression af DR, og personer der havde tre eller flere forsinkede screeninger, havde næsten 13 gange højere risiko for klinisk signifikant progression.

Vi undersøgte systemiske behandlinger brugt hos både personer med type 1 (insulin pumper) og type 2 (bariatrisk kirurgi) diabetes og deres potentielle relation til DR udvikling. Vi identificerede 674 personer med type 1-diabetes, der havde modtaget en insulinpumpe fra 2013-2022 efter deres første screening i DiaBase. Vores kohorte var primært yngre og kvinder, og havde sammenlignelig glykæmisk status (HbA1c), grad af komorbiditeter og diabetesvarighed i forhold til kontroller ved indeksdato. Vi fandt ingen statistisk signifikant forskel i risikoen for forværring af DR (HR 1,05 [95% CI 0,91;1,22], p=0,5).
Vi identificerede 553 personer med type 2 diabetes, der gennemgik bariatrisk kirurgi efter den første screening i DiaBase. Kohorten var primært yngre og kvinder, og cases havde flere komorbiditeter, kortere diabetesvarighed samt hyppigere brug af antidiabetisk og antihypertensiv medicin sammenlignet med kontroller. Vi fandt ingen øget risiko for forværring af DR hos personer, der fik foretaget bariatrisk kirurgi, hverken på kort eller lang sigt (OR 0,41 [95% CI 0,13-1,33], p=0,14 og OR 0,71 [95% CI 0,34-1,46], p=0,35) sammenlignet med kontroller.

Konklusion

Det danske screeningsprogram for DR er effektivt til at hjælpe med forebyggelse af synstruende progression i DR, så længe patienterne deltager som anbefalet, da forsinkelser i screeninger er forbundet med øget risiko for progression. Data fra screening er af høj validitet, og det kan betros, at screenende øjenlæger oftest graderer DR korrekt. Dette sikrer, at patienterne henvises til rettidig behandling ved behov, og vidner samtidig om den høje kvalitet af data i DiaBase, som kan bruges til register-baserede studier. Systemiske behandlinger ved både type 1-diabetes (insulinpumper) og type 2-diabetes (bariatrisk kirurgi) blev fundet at være sikre i forhold til DR, uden øget risiko for forværring. Det er dog vigtigt at bemærke, at det glykæmiske niveau før operationen var relativt godt i begge grupper, hvilket skal tages i betragtning ved fortolkningen af resultaterne og med henblik på reproducerbarhed i andre populationer.

6 Background

The background section of this thesis offers an overview of the clinical entities, and the methodological components represented by the Danish registers, and provides insights into both the medical and epidemiological dimensions of the research.

6.1 Diabetes Mellitus

The increasing global prevalence of diabetes mellitus has been described as having pandemic proportions and is estimated to reach 643 million affected individuals in 2030(2). In Denmark the same tendency is present and the Danish Diabetes Association estimates that 467,000 Danish citizens will be affected by 2030(3). Diabetes mellitus is by definition characterized by an abnormal carbohydrate metabolism, with hyperglycemia(8). The disease can be etiologically classified as either type 1 or type 2 diabetes(9), characterized by impairment of insulin production and peripheral insulin sensitivity respectively. Several subtypes have been identified(10), but focus in this thesis will be on the two main types. Type 1 diabetes is caused by the autoimmune destruction of insulin producing pancreatic beta cells and is most often diagnosed in childhood or early adulthood(11). It is representative of 5-10% of all diabetes cases. Type 2 diabetes is caused by a progressive loss of insulin secretion from the pancreatic beta cells, due to insulin resistance, which in turn results in a relative insulin deficiency(12). Hyperglycemia is the common denominator for both types of diabetes and it can present with symptoms such as increased thirst, polyuria, weight-loss and blurred vision, but it can also be asymptomatic, and thus diagnosed during other routine testing(13). Diagnosis is confirmed either with two random blood glucose measurements of 11.1 mmol/L combined with relevant symptoms of hyperglycemia or a singular glycated hemoglobin (HbA1c) measurement of ≥48 mmol/mol in asymptomatic individuals(14).

Diabetes is associated with several severe complications of both macro- (cardiovascular disease and stroke) and microvascular (retinopathy, nephropathy and

neuropathy) nature(15, 16). In addition diabetes and its complications is known to have a mental and emotional impact on affected individuals and studies show that a diabetes diagnosis can impact quality of life, and is associated with an increased prevalence of depression(17). Diabetes is estimated to be the condition related to highest health care costs and in Denmark alone the cost amounts to upwards of 87 million DKK daily(3).

6.2 Diabetic Retinopathy

Diabetic retinopathy (DR) is the most prevalent microvascular complication of diabetes and a feared cause of blindness, despite of declining prevalence, due to improved detection and management of diabetes(18). It affects the retina; the back, innermost layer of the eye, which is a neuro-epithelial layer that serves important functions in visual perception(19). Pathophysiology of DR is complicated, but the mechanisms of chronic hyperglycemia combined with other risk factors (e.g. hypertension) are believed to instigate a cascade of biochemical events, including inflammation, hypoxia and growth factor responses, which ultimately causes retinal vascular endothelial dysfunction, increased vascular permeability and retinal neovascularization(20-23). The most significant risk factors for developing DR are duration of diabetes, high and/or unstable HbA1c, hypertension, dyslipidemia, high body mass index (BMI) and smoking(24-26). Essentially the retina will, due to abovementioned causes, show several clinical signs of DR, that can be assessed using various grading scales(27, 28). The focus, in this thesis, will be on the International Clinical Diabetic Retinopathy severity (ICDR) scale (Figure 1) (29). The ICDR scale is a clinically usable scale ranging from 0-4 (0 = no DR, 1 = mild non-proliferative DR [NPDR], 2 = moderate NPDR, 3 = severe NPDR and 4 = proliferative DR [PDR]) representing increasing severity of DR. The ICDR scale also includes grading of diabetic macular edema (DME), which will not be covered or assessed in this thesis. DR levels can fluctuate according to glycemic stability and can both progress or regress, however once a patient has been diagnosed with PDR, regression is no longer possible and the DR is considered sight threatening. The first objectively visible signs of DR is visible microaneurysms, hereafter other microvascular lesions such as intraretinal hemorrhages as well as structural venous abnormalities,

nerve-fiber ischemia (cotton wool spots) and lipid deposits (hard exudates) can be seen. Neovascularization characterizes the latest, proliferative stage of the disease, potentially causing pre-retinal- and vitreous hemorrhages as well as tractional retinal detachment due to progressive fibrosis. Non-proliferative disease is primarily treated via optimization of modifiable risk factors like smoking and dietary habits with vitamin supplements as well as systemic treatment, including good glycemic stability, improved lipid status and strict hypertension management, which are crucial to halt further progression and improve retinal health(26, 30, 31). Once neovascularizations are present (DR level 4), they can be treated by retinal photocoagulation, intra-vitreal vascular endothelial growth factor inhibitor (anti-VEGF) injections and/or vitrectomy(32-34). In relation to rapid improvement of blood glucose levels a risk of transient worsening of DR are well known(35).

The exact cause of the phenomenon is debated, and might correlate to both the size of HbA1c decline, the fluctuation of glucose levels, hypertension and diabetes duration due to auto regulatory mechanisms(35-37). The retinal autoregulation includes the retinal vessels ability to regulate their diameter to maintain a relatively constant blood flow and oxygen supply(38). In individuals with diabetes, with high and unstable glycemic levels, this process becomes impaired. In the case of sudden improvement of blood glucose levels the auto-regulatory mechanisms, which have been compensating for high glucose levels in individuals with diabetes, might not adapt to the improved glucose-levels, causing he retina to experience a phase of relative hypoxia. This might trigger the retina to release vascular growth factors, thus causing neovascularizations.

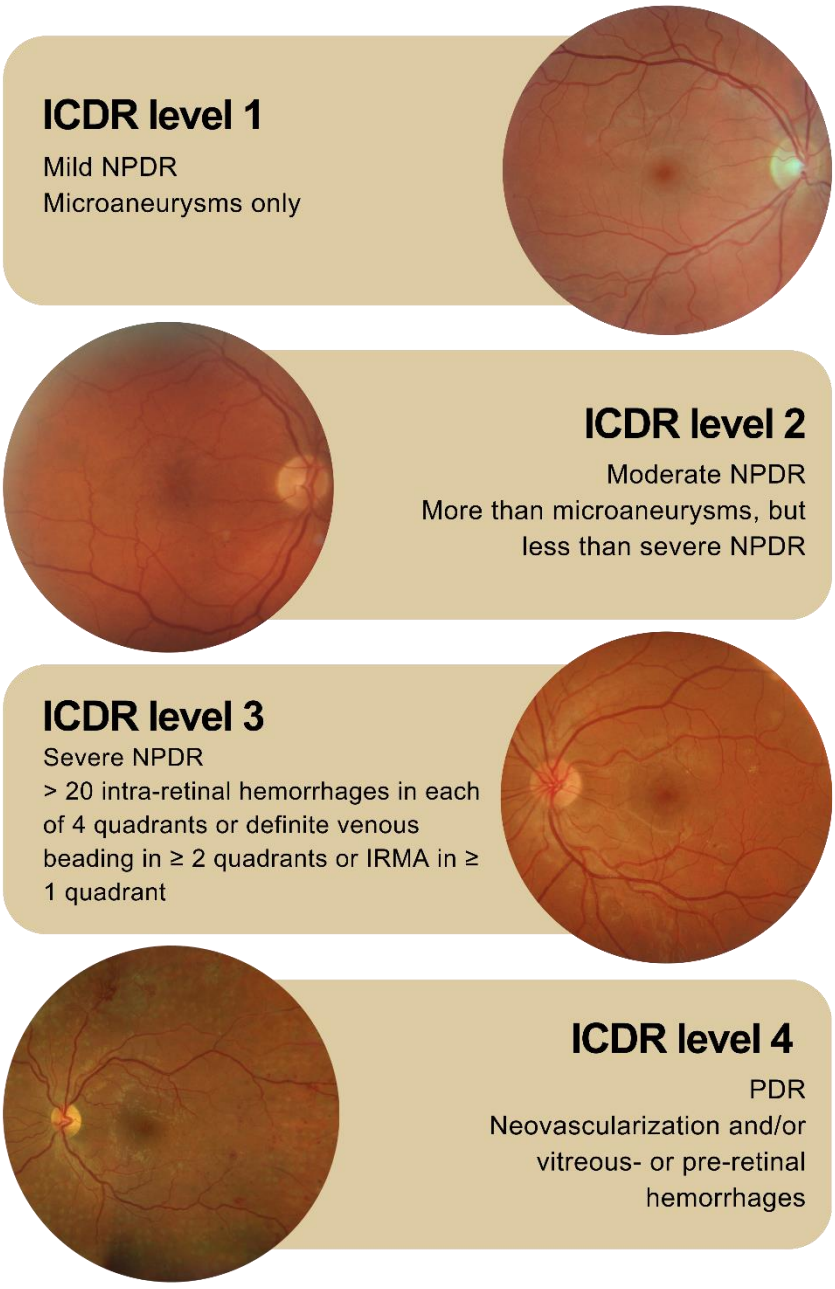

Figure 1 – ICDR grading scale with retinal fundus images, representing each level of DR.
ICDR = International Clinical Diabetic Retinopathy severity scale, IRMA = intraretinal macrovascular abnormalities, DR = diabetic retinopathy, NPDR = non-proliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy.
Images from Steno Diabetes Centre Odense. Figure created using Canva.

6.2.1 The Danish screening program for DR

A successful screening program in healthcare is characterized by clear target populations, evidence-based guidelines, accessibility, accurate tests, well-trained healthcare professionals and comprehensive treatment protocols(39). Such programs play a crucial role in early detection and management of health conditions, leading to improved health outcomes and cost-effectiveness(40). The decreasing tendency of sight-threatening DR and DR related vision loss, especially in high income countries, exemplifies the significance of an effective screening program(4). By identifying the condition early through screenings, ophthalmologists can implement timely interventions and treatments to manage the progression of DR effectively. This can help preserve vision and prevent severe complications that could arise if the condition goes undetected and untreated(18). DR screening has been implemented in many developed nations, including Denmark(39). The Danish screening program for DR is a national initiative that has been in effect since 2013. The setup of the screening program, including invitation for screening, recommended screening intervals, equipment requirements and recommendations regarding referrals for treatment are defined by national clinical guidelines (Table 1)(41). Individuals with type 1 diabetes are recommended to participate in screening beginning five years after their initial diagnosis of diabetes, whereas individuals with type 2 diabetes are recommended to attend screenings immediately after diagnosis. Screening can be attended at either a practicing ophthalmologist or at a hospital-based screening facility. Individuals with type 1 diabetes and complicated type 2 diabetes are primarily screened at hospital-based settings, whereas the majority of individuals with type 2 diabetes are screened at practicing ophthalmologists. Screening intervals are determined on an individualized basis, as recommended by screening ophthalmologist depending on the patient's DR status as well as all-round health including, but not limited to, glycemic stability. Regardless of screening facility, type of diabetes and number of screenings, attendance is tax-funded and visits cost-free to the attending individuals. At screening visits, the screening physician evaluates the patient's retina, by a minimum of two retinal fundus photographs, sometimes supplemented by indirect ophthalmoscopy. Grading of the potential DR lesions

are done referencing the ICDR scale. Lastly, the screening physician is obligated to report the results to The Danish Registry of Diabetic Retinopathy (DiaBase) along with recommended next date of screening.

Examination	Digital retinal fundus photography, with specific technical requirements, through dilated pupil. Widefield imaging can be done though un-dilated pupil. A minimum of two images must be obtained (one macula- and one optic disc centered), covering 70-80 degrees of the retina horizontally and 45 degrees vertically. In patients with blurry refractive media, indirect ophthalmoscopy must be performed. If there are any signs of clinically significant macular edema or a clinically significant vision loss (≥2 lines on the Snellen chart) macular OCT must be performed
Screening initiation and intervals	Type 1 diabetes: recommended to initiate screening five years after diagnosis (no earlier than age 12). Type 2 diabetes: recommended to initiate screening at the time of diagnosis. It is recommended to use flexible and individualized screening intervals, ranging from 3-48 months, depending on patients general diabetes regulation (glycemic stability and hypertensive control).
Specific recommendations for select groups of patients	Pregnant individuals: screening in early pregnancy, during late second trimester as well as 3-6 months postpartum. A third screening in the third trimester is recommended in the case of severe DR changes, fast progression of DR or in patients with high risk of DR. Individuals undergoing bariatric surgery: eye screening should be performed within 12 months prior to surgery date. This screening will determine the individualized risk assessment for each patient. Individuals with dysregulated diabetes: information on diabetes duration, HbA1c and hypertension should be accessible to screening ophthalmologist. Screening intervals should be adapted accordingly. Vulnerable individuals: it should be of particular focus that individuals with socio-economic challenges and patients with other ethnic descent than Danish are attending the screening program.

Table 1 – Overview of the examination requirements, screening initiation and intervals as well as specific recommendations for select patients in the Danish screening program for DR
OCT = optical coherence tomography, DR = diabetic retinopathy, HbA1c = glycated hemoglobin.
Recommendations modified from full guidelines available at https://dansk-oftalmologisk-selskab.dk/wp-content/uploads/2021/05/National-retningslinje-for-screening-af-diabetisk-retinopati.pdf

6.3 Systemic Intervention

In this thesis, we aimed to gain insight into systemic interventions that demonstrate potential in improving the prognosis of DR, by addressing underlying pathophysiology and improve glycemic stability. The scope of this investigation encompasses both patients with type 1 and type 2 diabetes, exploring interventions primarily utilized for each respective group.

6.3.1 Continuous Subcutaneous Insulin Infusion

Continuous Subcutaneous Insulin Infusion (CSII) is a mode of insulin treatment primarily utilized in individuals with type 1 diabetes and commonly referred to as 'insulin pumps'(42). CSII has been used to treat type 1 diabetes since the late 1970s(43). It utilizes continuous infusion of rapid-acting insulin through a subcutaneous canula connected to a cartridge, ensuring a stable flow of insulin, known as basal rate, as well as prandial boluses, which the individual wearing the pump can adjust according to dietary intake(44). Some pumps have an integrated continuous glucose monitoring system, that provides real-time glucose measurements, enabling the pump to automatically adjust the insulin delivery. CSII treatment is mainly offered to individuals with type 1 diabetes, especially children. In Denmark there are national clinical guidelines for the allocation of CSII which includes, but are not limited to, HbA1c > 53, problems identifying hypoglycemia and/or issues with hyperglycemia(45). Possible complications related to CSII use can both be injection-site related (infection and/or blockage) or pump-related (technical malfunction), and can potentially lead to both hyper- or hypoglycemia, depending on the specific issue(46).

6.3.2 Bariatric surgery

Bariatric surgery is an intervention to treat obesity and improve individuals overall metabolic profile, that has been used, in some form, since the 1950s(47). There are different variations of the procedure, which can be divided into malabsorptive (jejunoileal bypass and biliopancreatic diversion), restrictive (banded gastroplasty or adjustable gastric banding) or a combination of the two (Roux-

en-Y-bypass)(48). All types of surgery limits the absorption of calories and nutrients as well as adjusts the neuro-hormonal response that regulates the feeling of hunger(49). When performed in individuals with type 2 diabetes it has been known to vastly improve overall health, and sometimes even result in diabetes remission(50, 51). The post-surgical decline in HbA1c is often instantaneous and quite sizable(52). Complications to bariatric surgery includes issues related to the surgery itself (infection, bleeding, deep vein thrombosis), but the changes to anatomical features can also result in gastrointestinal issues (obstruction, ulcers or strictures), dumping syndrome and nutritional deficits(48). In Denmark strict pre-surgical guidelines have been implemented to ensure the best possible surgical outcomes and includes pre-surgical weight loss, biochemical measurements and optimization, patient guidance and lifestyle changes(53).

6.4 The Danish National Health Registers

Conducting research in Denmark we had the privilege of accessing the national Danish health registers (Figure 2) which is a powerful tool for population-based studies. The registers are vast databases that contain information on the entire Danish population, covering demographics, health, education and employment on an individualized level(54). The information in the registers are highly representative of the Danish population due to their comprehensive and well-structured nature(55). Data in the various registers can be linked with each other, but also with external sources such as smaller research databases. Information in the registers are subject to strict data protection and regulations, and ethical guidelines must be upheld by researchers. In the studies presented in this thesis we utilized the following registers:

The Danish Registry of Diabetic Retinopathy (DiaBase) is a national clinical quality database that holds information from the Danish screening program for DR. Information on level of DR, screening dates, screening facility, geographical region of screening and examination type are all recorded in the register, which

has existed since 2013. As of May 2022 the register holds data from 263,660 individuals with a total of 1,018,143 screening visits. Approximately 100,000 new screening visits are added to the database annually(6).

The Danish National Patient Registry was established in 1977 and holds information regarding all in- and outpatient visits in the Danish hospital-based healthcare system. This includes International Classification of Disease (ICD) diagnostic- (primary and secondary) and procedure-codes(56), and exact hospital as well as department where the codes were registered(57).

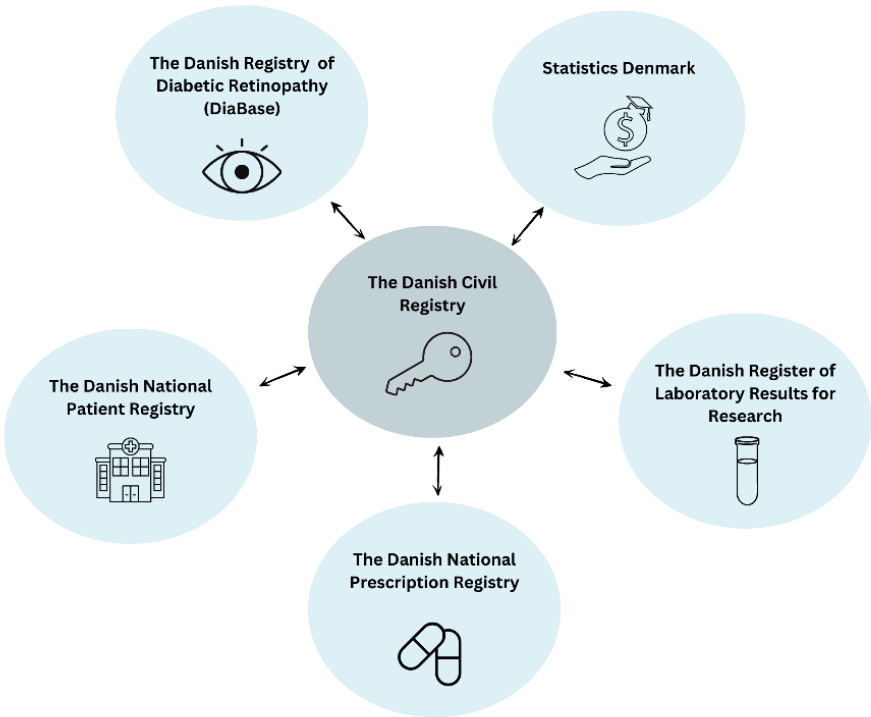

Figure 2 – Illustration of the National Danish Health Registers
Visual representation of the registries utilized in Paper II, III and IV.
Figure created using Canva.com

The Danish National Prescription Registry holds information on all over the counter, prescribed and redeemed medications in Denmark, dating back to 1994. It carries details such as Anatomical Therapeutic Chemical Classification System (ATC) codes, package sizes, date of prescription and collection as well as information on prescribing practice or hospital department(58).

The Danish Register of Laboratory Results for Research has since 2013 collected data on biochemical measurements from general practitioners and hospitals in Denmark. There are no formal requirements or obligation to report to the register. The register holds information on date and time of sampling, Nomenclature for properties and units (NPU) codes, name of biomarker, identification code for responsible laboratory and requisitioner as well as the result of the test including upper and lower reference limits(59).

Statistics Denmark provides unique socio-economic data including income, educational level given as International Standard Classification of Education (ISCED)(60), affiliation to the labor-marked, profession and ethnic heritage(61). Statistics Denmark are able to provide a vast selection of data, from many different registers, to be used for research under the authorization of Statistics Denmark(62).

The Danish Civil Registry can be used to link all abovementioned registers, due to the individual 10 digit identification number (CPR number) given to all Danish citizens. The register also holds information on date of birth, sex, vital-, migration- and marital status. Data dates back to 1968(63).

7 Motivation

The field of medicine is often driven by the pursuit of improving patient care, advancing knowledge of treatments, and making a significant impact on public health. My motivations to complete this thesis were no different and underscores a belief of the importance of comprehensive diabetes care and effective prevention strategies to lessen the personal and societal burdens of this condition. When examining different aspects of the Danish screening program including quality, attendance and systemic interventions, this thesis seeks to uncover potential issues that affects comprehensive DR management. This is not only to the benefit of the patients, by aiding in disease understanding and uncovering potential burdens to tackle, but also an asset to medical professionals, and the overall healthcare system, as it provides new knowledge that can enhance DR management and assist in clinical decision-making.

8 Objectives

The aim of this thesis was to provide a thorough examination of different aspects of the Danish screening program for DR ranging from reliability in screening results to assessment of attendance amongst individuals attending the screening program (Substudy A)(Table 2). Additionally the thesis investigates systematic treatments in individuals with type 1 and type 2 diabetes (Substudy B) (Table 2).

Detailed objectives related to each paper was to:

Substudy A: evaluate the inter-grader reliability in the Danish screening program for DR, by re-grading images from screening facilities demonstrating a nationwide representation of examinations and thus determine the accuracy of graded DR levels in the Danish screening program and stored in DiaBase. Furthermore we aimed to characterize individuals attending the Danish screening program for DR according to attendance patterns, as well as evaluate the potential consequences of non-attendance on DR development.

Substudy B: evaluate the effects (incidence, progression and need for ocular intervention) of CSII treatment and bariatric surgery on DR development in individuals with type 1 and type 2 diabetes respectively.

	Paper I	Paper II	Paper III	Paper IV
Study design	Clinical reliability study	Register-based cohort study	Register-based matched cohort study	Register-based matched cohort study
Data sources	DiaBase and RedCap database with retinal fundus images	DiaBase, Statistics Denmark*, The Danish Civil Registry, The Danish National Patient Register and The Danish National Prescription Registry	DiaBase, The Danish Civil Registry, The Danish National Patient Register, The Danish National Prescription Registry and the Danish Registry for Laboratory Results for Research	DiaBase, The Danish Civil Registry, The Danish National Patient Register, The Danish National Prescription Registry and the Danish Registry for Laboratory Results for Research
Study population	230 individuals (458 eyes)	205,970 individuals	674 individuals with CSII and 2006 individuals with MDI. All with type 1 diabetes.	553 individuals with bariatric surgery 2677 individuals without bariatric surgery. All with type 2 diabetes.
Duration of follow-up/Inclusion period	February - March 2020	January 2013 - December 2018	January 2013 - May 2022	January 2013 - May 2022
Outcome	Agreement between graders	Patterns of attendance. Consequences of delayed attendance on DR development	DR worsening (incidence of DR and progression of DR [(≥2 step progression]) or improvement (≥2 step regression) at last screening. Need of ocular intervention (vitrectomy, anti-VEGF injection, focal- or panretinal photocoagulation) during follow-up. At eye level.	DR worsening (incidence of DR and progression of DR [(≥2 step progression]) or improvement (≥2 step regression) at 6 and 36 months. Need of ocular intervention (vitrectomy, anti-VEGF injection, focal- or panretinal photocoagulation). At eye level.
Statistical methods	PABAK and Gwet's AC	Multinomial logistic regression and Cox regression resulting in RRR	Cox regression resulting in HR	Multiple logistic regression and Cox regression resulting in OR and HR
Confounders	-	Age, sex, marital status, diabetes type, CCI, DR level, screening facility, geographical screening region, income, education length occupation and ethnic heritage.	Age, sex and marital status	Age, sex, CCI, duration of diabetes, HbA1c, use of metformin, antihypertensive medication, GLP-1 analogues and SGLT-2 inhibitors.
Inclusion criteria	Images graded from February 3rd-March 3rd in ophthalmological practices and hospital-based screening facilities	≥2 screening episodes from 2013-2018. Above 18 years of age.	Cases: Individuals with type 1 diabetes AND ≥2 screening episodes from 2013-2022 AND registered with ≥2 CSII (BBHF02) codes AND/OR relevant CSII medication from 2013-2022 Controls: Individuals with type 1 diabetes and ≥2 screening episodes from 2013-2022 with no registrations of CSII prior to 2013. Matching: 1:3 by age (± one year), sex and level of DR at index date.	Cases: Individuals with type 2 diabetes AND ≥2 screening episodes from 2013-2022 AND registered with bariatric surgery (KJDF*) code from 2013-2022. Bariatric surgery must be within one year of nearest screening date. Controls: Individuals with type 2 diabetes and ≥2 screening episodes from 2013-2022 with no registrations of bariatric surgery prior to 2013. Matching: 1:5 by age, sex and level of DR at index date.

Table 2 – Study overview of Papers (I-IV) included in thesis

AC = agreement coefficient, CCI = Charlson Comorbidity Index, CSII = continuous subcutaneous insulin infusion, MDI = multiple daily injections, DR = diabetic retinopathy, DiaBase = The Danish Registry of Diabetic Retinopathy, HbA1c = glycated hemoglobin, PABAK = prevalence adjusted, bias adjusted kappa. GLP-1 = Glucagon like peptide 1, SGLT-2 = Selective sodium glucose co transporter 2, VEGF = vascular endothelial growth factor. Diagnostic- and treatment codes in ICD format.
Please note that not all endpoints examined and reported in Paper I-IV are reported in this thesis.
*Registers used from Statistics Denmark includes income-, labour marked-, education- and ethnic heritage registers.

9 Substudy A – Paper I and II

9.1 Paper I – Reliability in the Danish Screening Program for DR

9.1.1 Methods

9.1.1.1 Study design and population

We performed a clinical reliability study to examine the inter-grader agreement in the Danish screening program for DR by regrading retinal fundus images supplied by screening facilities across all five geographical regions of Denmark (Capital Region of Denmark, Central Denmark Region, North Denmark Region, Region Zealand and Region of Southern Denmark). Retinal images, originally graded in the screening program, was uploaded anonymously by the primary screening ophthalmologist to an encrypted RedCap database (Vanderbilt University 2021, Nashville, Tennessee, USA) managed by Open Patient data Explorative Network (OPEN), Odense. Alongside the images the primary graders gave information on primary grading, type of screening facility, geographical region and image modality. All information relating to the images, except the images themselves, were blinded to the secondary grader until the secondary grading was complete. Primary graders were ophthalmologists in practice or at hospital-based screening facilities, some with certification in DR grading through a virtual training platform (VIOLA) (64). Secondary grader was certified in DR grading at the Centre for Public Health, Royal Victoria Hospital Site, Queens University, Belfast, Northern Ireland, United Kingdom, as well as virtually through VIOLA. All images were graded and re-graded using the ICDR scale.

9.1.1.2 Statistical analysis

The sample size was determined using a z-test, revealing the requirement of 316 eyes to achieve a power of 0.80 at a significance level (α) of 0.05. Agreement analyzes resulting in prevalence adjusted, bias adjusted kappa (PABAK, κ) and Gwet's agreement coefficient (AC) were performed to assess the inter-grader agreement, between primary grading ophthalmologist, and secondary, certified grader(65). Both κ and AC are expressed as a value between 0 (no agreement) and 1 (complete agreement, beyond chance). Both analyzes were weighted and took the ordinal nature of DR levels into account, when estimating coefficients; mismatched gradings were penalized according to the number of levels between primary and secondary graders results, in a linear fashion (complete match = 1.0, one level of = 0.75 etc.). Additional characteristics supplied by the primary graders, were evaluated and compared using Chi-square tests where p-values <0.05 were considered statistically significant.

9.1.2 Results

Retinal images from a total of 230 individuals (458 eyes) were included in the study. Images were supplied from all five Danish geographical regions and from both practicing ophthalmologists (52.6%) and hospital-based screening facilities (47.4%). Primary graded levels of DR varied significantly (p-value < 0.001) according to screening facility as the share of images graded as "no DR" was markedly larger at practicing ophthalmologists compared to hospital-based screening facilities. Hospital-based screening facilities reported more than nine times as many PDR cases compared to practicing ophthalmologists (Table 3).

	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Not classifiable
Practicing ophthalmologists	219 (90.9)	12 (5.0)	6 (2.5)	0 (0.0)	2 (0.8)	2 (0.8)
Hospitals	87 (40.1)	44 (20.3)	54 (24.9)	6 (2.8)	23 (10.6)	3 (1.4)

Table 3 - Number of eyes included according to primary screening facility
Given in counts (%). DR = diabetic retinopathy, NPDR = non-proliferative DR, PDR = proliferative DR. Reprinted from Paper I.

Distribution of DR level gradings did not vary according to image modalities or geographical regions. When primary gradings were compared to secondary gradings exact percentual agreement was observed in 78.6% of all eyes. 80.0% of all disagreement cases were underestimations of DR level by primary grader, compared to secondary grader (Table 4). Primary and secondary graders were mostly in agreement when grading images with no DR (84.1%) and PDR (68.6%). Least agreement was observed when grading severe NPDR (11.1%). When statistical agreement analyzes were performed an overall agreement of κ 0.83/AC 0.88 (93%, p<0.001) was concluded between primary and secondary grader.

		Secondary grader					
		No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Not classifiable
Primary grader	No DR	**265 (84.1)**	33	6	2	0	0
	Mild NPDR	8	**23 (23.9)**	17	3	5	0
	Moderate NPDR	1	7	**42 (49.4)**	6	3	1
	Severe NPDR	0	0	2	**2 (11.1)**	2	0
	PDR	0	0	0	1	**24 (68.6)**	0
	Not classifiable	0	0	0	0	0	**5 (83.3)**

Table 4 – Schematic, exact agreement of diabetic retinopathy gradings between primary and secondary graders based on retinal fundus photographs
Given in numbers (%). DR = diabetic retinopathy, NPDR = non-proliferative DR and PDR = proliferative DR.
Reprinted and modified from Paper I.

When stratified by screening facility agreement was κ 0.89/AC 0.95 (96%, p<0.001) and κ 0.76/AC 0.78 (90%, p<0.001) for practicing ophthalmologists and hospitals versus secondary grader respectively. We performed a sub-analysis where a one-step grading difference was allowed, between primary and secondary grader. This resulted in an overall agreement of κ 0.93/AC 0.96 (95.5%, p<0.001).

9.2 Paper II – Non-attendance in the Danish Screening program for DR

9.2.1 Methods

9.2.1.1 Data Sources

DiaBase served as the principal data source for this study, providing a broad range of data, including both proposed and actual screening dates, graded DR levels recorded during screening visits, the geographical regions where screenings took place, and the corresponding screening facilities. To enhance the richness of the dataset, supplementary information was obtained from various Danish registers. Specifically, the Danish National Patient Registry supplied diagnostic codes required to calculate the Charlson Comorbidity Index for participants. Additionally, this register facilitated the determination of the specific type of diabetes on an individualized level, by utilizing diagnostic codes in conjunction with data from The Danish National Prescription Registry, which contained information on prescribed medications. From Statistics Denmark we extracted socio-economic information on household income, educational level, occupation and ethnic background. From The Danish Civil Registry we extracted CPR number which was used to link the information from all registries. This register also supplied basic information on birth year, sex, vital-, migration- and marital status.

9.2.1.2 Study Design and Population

The study was a register-based longitudinal study where our cohort was defined by the population in DiaBase, and comprised of all individuals above 18 years of age who attended DR screening at least once, from 2013-2018. Index date was determined as first date of screening, and to assess the different attendance patterns we examined delays in screening according to dates of screening suggested by screening ophthalmologist.
The study population was categorized into three groups based on attendance patterns and delays. A delay was determined as an exceedance of >33% of the proposed screening date, referencing the definition used in the National Danish

screening guidelines(41). Individuals who attended all screenings without any delays were classified as timely attendees. Individuals who experienced any delays exceeding 33% during follow-up were classified as delayed attendees. Some individuals only participated in the screening program once, despite being recommended subsequent screenings. These individuals were categorized as one-time attendees. We conducted a multinomial logistic regression analysis that generated relative risk ratios (RRR) with 95% confidence intervals (CI), allowing us to quantify the associations between different characteristics and the likelihood of exhibiting the specific attendance patterns. To assess the consequences of delayed attendance, we examined clinically relevant progression of DR (progression to severe NPDR or PDR) using a multivariable Cox regression model resulting in hazard ratios (HR) with 95% CI.

9.2.2 Results

We analyzed data from 591,136 individual screening visits made by 205,970 individuals. Majority of included individuals were male (56.6%), had a median age of 65 years (IQR 55;73) and 89.1% were of Danish descent. Preexisting DR was present in 16.5% of the population at index date and during follow-up, rates of timely-, delayed- and one-time attendance were 53.0%, 35.5% and 11.5% respectively. Individuals with timely attendance were more often male, married, and of Danish descent, they had less preexisting DR, were screened at practicing ophthalmologists and had type 2 diabetes (Table 5). A multinomial regression analysis (Table 6) showed that individuals who were women, younger than 40 years of age, married or widowed/divorced, diagnosed with type 1 diabetes and had preexisting DR were at higher risk of being delayed. Individuals in higher risk of one-time attendance were men, younger than 40 years of age, who were widowed/divorced, diagnosed with type 2 diabetes, had more advanced levels of DR, a lower income as well as shorter duration of education, higher rates of unemployment and was of other ethnic heritage than Danish.

	All N = 205,970	Timely N = 109,135	Delayed N = 73,242	One-time N = 23,593	P value
Sex, % male	116,534 (56.6)	62,567 (57.3)	40,610 (55.4)	13,357 (56.6)	< 0.001
Age, years (IQR)	66 (55;73)	66 (56;73)	65 (54;73)	66 (54;74)	< 0.001
Marital status					< 0.001
Never married	30,904 (15.0)	16,035 (14.7)	11,050 (15.1)	3,819 (16.2)	
Married	118,764 (57.7)	63,833 (58.5)	42,718 (58.3)	12,213 (51.8)	
Widowed or divorced	56,302 (27.3)	29,267 (26.8)	19,474 (26.6)	7,561 (32.0)	
Diabetes type, n (%)					< 0.001
Type 1 diabetes	16,999 (8.3)	7,492 (6.9)	8,375 (11.4)	1,132 (4.8)	
Type 2 diabetes	153,238 (74.4)	85,786 (78.6)	48,791 (66.6)	18,661 (79.1)	
Unknown	35,733 (17.3)	15,857 (14.5)	16,076 (21.9)	3,800 (16.1)	
DR level (ICDR), n (%)[a]					< 0.001
No DR	171,633 (83.3)	95,507 (87.5)	55,464 (75.7)	20,662 (87.6)	
Mild NPDR	20,964 (10.2)	9,009 (8.3)	10,157 (13.9)	1,798 (7.6)	
Moderate NPDR	6,551 (3.2)	2,405 (2.2)	3,583 (4.9)	563 (2.4)	
Severe NPDR	1,153 (0.6)	327 (0.3)	687 (0.9)	139 (0.6)	
PDR	5,165 (2.5)	1,727 (1.6)	3,007 (4.1)	431 (1.8)	
CCI, n (%)[b]					< 0.001
Low	148,615 (72.2)	79,792 (73.1)	51,920 (70.9)	16,903 (71.6)	
Moderate low	27,728 (13.5)	12,984 (11.9)	11,798 (16.1)	2,946 (12.5)	
Moderate high	18,721 (9.1)	10,252 (9.4)	6,137 (8.4)	2,332 (9.9)	
High	10,906 (5.3)	6,107 (5.6)	3,387 (4.6)	1,412 (6.0)	
Screening facility, n (%)					< 0.001
Private practice	161,418 (78.4)	89,210 (81.7)	53,241 (72.7)	18,967 (80.4)	
Hospital	44,552 (21.6)	19,925 (18.3)	20,001 (27.3)	4,626 (19.6)	
Region of screening, n (%)					< 0.001
Capital Region of Denmark	53,303 (25.9)	24,363 (22.3)	20,908 (28.5)	8,032 (34.0)	
Region Zealand	33,299 (16.2)	17,531 (16.1)	11,332 (15.5)	4,436 (18.8)	
Central Denmark Region	41,499 (20.1)	24,581 (22.5)	12,733 (17.4)	4,185 (17.7)	
North Denmark Region	22,248 (10.8)	9,945 (9.1)	97,61 (13.3)	2,542 (10.8)	
Region of Southern Denmark	55,575 (27.0)	32,690 (30.0)	18,488 (25.2)	4,397 (18.6)	
Income (household net worth), n (%)					< 0.001
Low	50,484 (24.5)	23,942 (21.9)	19,704 (26.9)	6,838 (29.0)	
Moderate low	50,310 (24.4)	26,383 (24.2)	18,140 (24.8)	5,787 (24.5)	
Moderate high	50,953 (24.7)	27,857 (25.5)	17,640 (24.1)	5,456 (23.1)	
High	52,660 (25.6)	29,491 (27.0)	17,711 (24.2)	5,458 (23.1)	
Education, n (%)					< 0.001
Lower secondary	77,796 (37.8)	40,620 (37.2)	27,676 (37.8)	9,500 (40.3)	
Upper secondary	85,012 (41.3)	45,902 (42.1)	29,880 (40.8)	9,230 (39.1)	
Post-secondary	36,122 (17.5)	19,103 (17.5)	13,232 (18.1)	3,787 (16.1)	
Occupation, n (%)					< 0.001
Employed or employer	58,533 (28.4)	31,016 (28.4)	21,332 (29.1)	6,185 (26.2)	
Student or other	5,179 (2.5)	2,571 (2.4)	1,953 (2.7)	655 (2.8)	
Early retirement	28,404 (13.8)	14,608 (13.4)	10,526 (14.4)	3,270 (13.9)	
Retirement	101,135 (49.1)	54,663 (50.1)	34,882 (47.6)	11,590 (49.1)	
Unemployed	12,715 (6.2)	6,274 (5.7)	4,549 (6.2)	1,892 (8.0)	
Ethnic background, n (%)					< 0.001
Danish heritage	183,476 (89.1)	98,237 (90.0)	65,072 (88.8)	20,167 (85.5)	
Other heritage	22,457 (10.9)	10,882 (10.0)	8,160 (11.1)	3,415 (14.5)	

Table 5 – Characteristics of individuals at index date, according to attendance group (timely attendees, delayed attendees and one-time attendees)

Results given counts (%) or medians (IQR). [A]Classification of DR given by the ICDR Scale. [B]Excluding diabetes. CCI = Charlson Comorbidity Index, DR = diabetic retinopathy, NPDR = non proliferative DR, PDR = proliferative DR, IQR = interquartile range, ICDR = International Clinical DR severity scale. *Reprinted and modified from Paper II.*

	Delayed attendees			One-time attendees		
	n (%)	RRR (95%CI)	P-value	n (%)	RRR (95%CI)	P-value
Sex						
Female	32,632 (44.6)	ref		10,236 (43.4)	ref	
Male	40,610 (55.4)	0.94 (0.92;0.96)	<0.001	13,357 (56.6)	1.04 (1.01;1.08)	0.0083
Age, years						
18-39	5,823 (8.0)	ref		1,538 (6.5)	ref	
40-59	21,027 (28.7)	0.79 (0.75;0.83)	<0.001	7,145 (30.3)	0.70 (0.65;0.75)	<0.001
60-79	40,223 (54.9)	0.76 (0.72;0.81)	<0.001	11,865 (50.3)	0.53 (0.49;0.58)	<0.001
+80	6,169 (8.4)	0.78 (0.73;0.84)	<0.001	3,045 (12.9)	0.83 (0.75;0.92)	<0.001
Marital status						
Never married	11,050 (15.1)	ref		3,819 (16.2)	ref	
Married	42,718 (58.3)	1.19 (1.15;1.23)	<0.001	12,213 (51.8)	0.85 (0.81;0.89)	<0.001
Widowed or divorced	19,474 (26.6)	1.14 (1.10;1.18)	<0.001	7,561 (32.0)	1.09 (1.03;1.15)	0.0014
Diabetes type, n (%)						
Type 1 diabetes	8,375 (11.4)	ref		1,132 (4.8)	ref	
Type 2 diabetes	48,791 (66.6)	0.67 (0.64;0.70)	<0.001	18,661 (79.1)	1.47 (1.36;1.59)	<0.001
Unknown	16,076 (21.9)	0.99 (0.95;1.04)	0.7542	3,800 (16.1)	1.53 (1.41;1.66)	<0.001
DR level (ICDR), n (%)[a]						
No DR	55,464 (75.7)	ref		20,662 (87.6)	ref	
Mild NPDR	10,157 (13.9)	1.68 (1.63;1.74)	<0.001	1,798 (7.6)	0.95 (0.90;1.01)	0.0959
Moderate NPDR	3,583 (4.9)	2.27 (2.14;2.40)	<0.001	563 (2.4)	1.18 (1.06;1.30)	0.0017
Severe NPDR	687 (0.9)	3.14 (2.72;3.62)	<0.001	139 (0.6)	2.07 (1.67;2.57)	<0.001
PDR	3,007 (4.1)	2.44 (2.29;2.61)	<0.001	431 (1.8)	1.26 (1.13;1.42)	<0.001
CCI, n (%)[b]						
Low	51,920 (70.9)	ref		16,903 (71.6)	ref	
Moderate low	11,798 (16.1)	1.08 (1.04;1.11)	<0.001	2,946 (12.5)	1.04 (0.99;1.09)	0.1280
Moderate high	6,137 (8.4)	0.87 (0.84;0.90)	<0.001	2,332 (9.9)	1.07 (1.02;1.13)	0.0068
High	3,387 (4.6)	0.74 (0.71;0.78)	<0.001	1,412 (6.0)	1.09 (1.02;1.16)	0.0090
Screening facility, n (%)						
Practicing ophthalmologist	53,241 (72.7)	ref		18,967 (80.4)	ref	
Hospital	20,001 (27.3)	1.07 (1.04;1.10)	<0.001	4,626 (19.6)	0.92 (0.88;0.96)	<0.001
Region of screening, n (%)						
Capital Region of Denmark	20,908 (28.6)	1.30 (1.26;1.34)	<0.001	8,032 (34.0)	1.31 (1.26;1.37)	<0.001
Region Zealand	11,332 (15.5)	ref		4,436 (18.8)	ref	
Central Denmark Region	12,733 (17.4)	0.77 (0.74;0.79)	<0.001	4,185 (17.7)	0.68 (0.65;0.72)	<0.001
North Denmark Region	9,761 (13.3)	1.52 (1.46;1.58)	<0.001	2,542 (10.8)	1.01 (0.96;1.08)	0.6338
Region of Southern Denmark	18,488 (25.2)	0.84 (0.81;0.87)	<0.001	4,397 (18.6)	0.51 (0.49;0.54)	<0.001
Income, n (%)						
Low	19,704 (26.9)	1.19 (1.16;1.23)	<0.001	6,838 (29.0)	1.18 (1.13;1.24)	<0.001
Moderate low	18,140 (24.8)	ref		5,787 (24.6)	ref	
Moderate high	17,640 (24.1)	0.91 (0.89;0.94)	<0.001	5,456 (23.2)	0.92 (0.88;0.96)	<0.001
High	17,711 (24.2)	0.84 (0.82;0.87)	<0.001	5,458 (23.2)	0.85 (0.81;0.89)	<0.001
Education, n (%)						
Lower secondary	27,676(39.1)	0.97 (0.94;1.00)	0.0254	9,500 (42.2)	1.11 (1.06;1.16)	<0.001
Upper secondary	29,880(42.2)	0.94 (0.91;0.96)	<0.001	9,230 (41.0)	0.97 (0.93;1.02)	0.2127
Post-secondary	13,232(18.7)	ref		3,787 (16.8)	ref	
Occupation, n (%)						
Employed or employer	21,332 (29.1)	1.22 (1.14;1.31)	<0.001	6,185 (26.2)	0.93 (0.84;1.03)	0.1658
Student or other	1,953 (2.7)	ref		655 (2.8)	ref	
Early retirement	10,526 (14.4)	1.23 (1.14;1.33)	<0.001	3,270 (13.9)	0.85 (0.76;0.95)	0.0043
Retirement	34,882 (47.6)	1.33 (1.23;1.44)	<0.001	11,590 (49.1)	0.87 (0.78;0.98)	0.0209
Unemployed	4,549 (6.2)	1.08 (0.99;1.16)	0.0680	1,892 (8.0)	1.03 (0.92;1.16)	0.5775
Ethnic background, n (%)						
Danish heritage	65,072 (88.9)	ref		20167 (85.5)	ref	
Other heritage	8,160 (11.1)	0.98 (0.94;1.01)	0.2181	3415 (14.5)	1.20 (1.14;1.26)	<0.001

Table 6 - Multinomial regression showing the risk of delayed and one-time attendance according to exposure variables

Data are given as counts (%), medians (IQR) and relative risk ratios (95% CI). Reference group is patients with timely attendance. [A]Classification of DR given by the ICDR scale. [B]Excluding diabetes. Model adjusted for all statistically significant differences in covariates between pre-defined attendance groups in Table 5. CCI = Charlson Comorbidity Index, DR = diabetic retinopathy, NPDR = non proliferative DR, PDR = proliferative DR, IQR = interquartile range, ICDR = International Clinical DR severity scale, RRR = relative risk ratio, CI = confidence interval.

Table reprinted, and modified, from Paper II.

Our Cox regression analysis, revealed that individuals with any significant delay (>33%) at screening appointments, had twice the risk (2.28 [95% CI 1.97;2.64], p<0.001) of developing severe NPDR or PDR during follow-up (Figure 3). The risk of progression increased according to number of appointments with delayed attendance and showed that individuals with delays in 1, 2 or 3+ appointments had two, six and almost 13 times (2.27 [95%CI 1.93;2.68], 6.25 [95% CI 4.96;7.88] and 12.84 [95% CI 9.21;17.88], < 0.001) higher risk of progression to severe NPDR or PDR, compared to individuals who attended their appointments at recommended screening intervals.

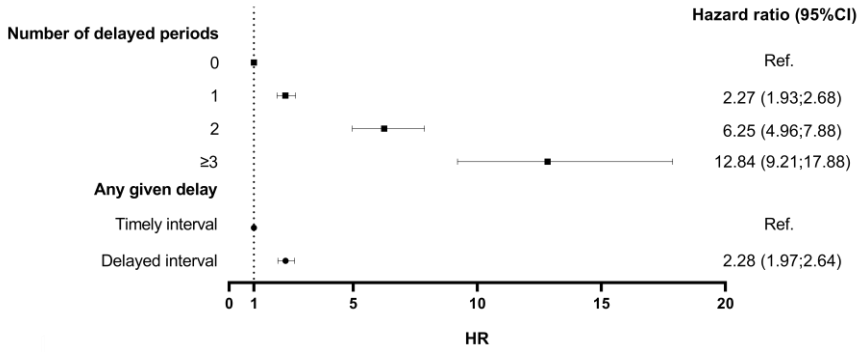

Figure 3 – Forrest plot illustrating risk of progression to severe NPDR or PDR according to delays
Model adjusted for all statistically significant differences in covariates amongst pre-defined attendance groups in Table 5. Delayed attendance was defined as >33% delay at screenings. HR = hazard ratio, CI = confidence interval, NPDR = non proliferative DR, PDR = proliferative DR
Modified from Paper II using GraphPad Prism 9.

9.3 Summary

In Substudy A we found a high level of inter-grader agreement in the Danish screening program for DR. Images from 458 eyes, representing the entire country geographically, were re-graded and an overall agreement of κ 0.83/AC 0.88 (93%, p<0.001) was found. In addition we concluded that 47.0% of individuals who attended DR screening at least once, did not attend the screening program as intended from 2013-2018, thus following the recommended screening intervals given by their ophthalmologist. Our results showed that any delay in screenings was associated with a more than two times higher risk of progression to severe NPDR or PDR. The risk of progression increased in accordance with number of delays, and delays in three or more planned screenings increased the risk almost 13 times.

10 Substudy B – Paper III and IV

10.1 Methods

10.1.1 Data Sources

Substudy B utilized data from five national Danish registers; DiaBase which defined the cohorts and provided information on individuals screened in the Danish screening program for DR from 2013-2022, including screening dates and level of DR graded at each screening visit. The Danish National Patient Registry provided information on all in- and out-patient diagnostic and treatment codes give to individuals in our cohort, and thus assisted in both the definition of our diabetes classification, as well as the definition of systemic interventions (bariatric surgery and CSII) in our cohort. From The Danish National Prescription Registry we extracted information on prescribed and redeemed medications (insulin, non-insulin glucose lowering-, lipid lowering- and antihypertensive medication), which also aided our diabetes classification.
The Danish Register of Laboratory Results for Research provided biochemical measurements of HbA1c, lipids and kidney function. Lastly we utilized the Danish Civil Registry, to provide basis information on date of birth, sex, marital-, migration- and vital status as well as link data from all registries together by CPR number.

10.1.2 Study Design

We performed prospective register-based matched cohort studies focusing on the potential consequences of CSII and bariatric surgery on DR, in individuals with type 1 and type 2 diabetes respectively.

Paper III

When examining CSII we matched individuals with type 1 diabetes, who had a minimum of two registrations with CSII treatment (BBHF02*) or was prescribed insulin used exclusively for CSII treatment between 2013-2022 with individuals who used multiple daily injections (MDI) of insulin. Index date was the screening visit leading up to the first registration of either treatment or medication. We examined two main outcomes; the first was the worsening of DR at the last recorded screening visit. Worsening was defined as either the incident occurrence of DR or progression by two or more steps in either eye. Secondly, we investigated the need for ocular interventions (focal or panretinal photocoagulation, vitrectomy, or anti-VEGF injections) during the follow-up period. Furthermore, as an additional outcome, we examined the changes in biochemical measurements (plasma creatinine, albumin/creatinine ratio in urine, estimated glomerular filtration rate, low density lipoprotein cholesterol, high density lipoprotein cholesterol, total cholesterol and triacylglyceroles) from the index date and throughout the three following years. HbA1c was assessed during the full follow-up period.

Paper IV

When examining bariatric surgery we matched individuals with type 2 diabetes, who had a registration of any form of bariatric surgery (KJDF*) between 2013-2022 with individuals who never had a registration of such intervention. Our main outcomes were DR worsening and need for ocular intervention as previously defined, however assessed at fixed time points at screening visits closest to six and 36 months post-surgery expressed as odds ratios (OR) with 95% CI. Biochemical measurements were assessed exactly as previously described.

10.2 Results

10.2.1 Paper III – CSII and DR

From 2013-2022 we identified 674 individuals who initiated CSII treatment amongst the 22,530 individuals with type 1 diabetes in DiaBase (Figure 4), making the prevalence of CSII use 3.0%. The prevalence of all patients receiving

CSII treatment from 2013-2022, regardless of our inclusion criteria, was 22.8%. Cases were matched with 2006 controls comparable in age, sex and DR level at index date.

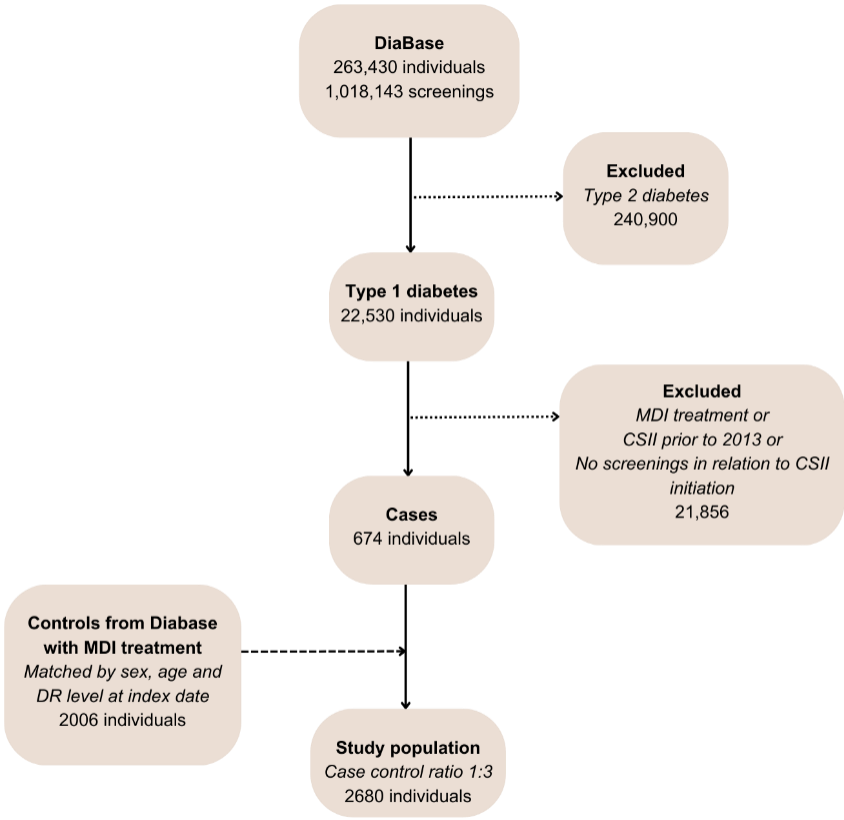

Figure 4 – Flowchart depicting selection of study population, with individuals with CSII (cases) and with MDI (controls)
DiaBase = The Danish Registry of Diabetic Retinopathy, CSII = Continuous subcutaneous insulin infusion. MDI = Multiple daily injections, DR = Diabetic retinopathy.
Figure created using Canva.

The majority of the cohort was female (53.4%), with a median age of 36 years (interquartile range [IQR] 27-47) (Table 7). At the index date, 38.5% of the included individuals had preexisting DR; 24.9% had mild, 8.5% moderate, 0.6% severe NPDR, and 4.5% had PDR. Cases were more likely to be married compared to controls (45.9% versus 38.3%). There were no significant differences between cases and controls in terms of comorbidities (CCI score), duration of diabetes, or glycemic status at the index date (Table 7).

		CSII		
	Total	**Yes (cases)**	**No (controls)**	**P-value**
	N=2,680	N=674	N=2,006	
Age, years (IQR)	36 (27-47)	36 (27-47)	36 (27-47)	0.93
Sex, % Female	1,432 (53.43%)	361 (53.56%)	1,071 (53.39%)	0.94
Duration of diabetes, year (IQR)	11.41 (5.42-17.55)	11.75 (5.02-17.52)	11.28 (5.48-17.56)	0.70
Marital status, n (%)				0.002
Never married	1,348 (50.30%)	311 (46.14%)	1,037 (51.69%)	
Married or living together	1,077 (40.19%)	309 (45.85%)	768 (38.29%)	
Divorced or widow	255 (9.51%)	54 (8.01%)	201 (10.02%)	
CCI score, n (%)[A]				0.43
0 (low)	2,087 (77.87%)	517 (76.71%)	1,570 (78.27%)	
1 (moderate low)	484 (18.06%)	134 (19.88%)	350 (17.45%)	
2 (moderate high)	82 (3.06%)	17 (2.52%)	65 (3.24%)	
>=3 (high)	27 (1.01%)	6 (0.89%)	21 (1.05%)	
Level of DR, n (%)[B]				0.85
No DR	1,648 (61.49%)	412 (61.13%)	1,236 (61.62%)	
Mild NPDR	667 (24.89%)	167 (24.78%)	500 (24.93%)	
Moderate NPDR	229 (8.54%)	58 (8.61%)	171 (8.52%)	
Severe NPDR	16 (0.60%)	6 (0.89%)	10 (0.50%)	
PDR	120 (4.48%)	31 (4.60%)	89 (4.44%)	
HbA1c (IQR)	62 (53-73)	63 (55-72)	61 (52-73)	0.23

Table 7 - Characteristics of individuals with CSII (cases) and with MDI (controls) at index date

Results given as counts (%) or medians (IQR). [A]Excluding diabetes. [B]Classification of DR given by the ICDR Scale. CCI = Charlson Comorbidity Index, DR = diabetic retinopathy, NPDR = non proliferative DR, PDR = proliferative DR, CSII = continuous subcutaneous insulin infusion, MDI = multiple daily injections, IQR = interquartile range, ICDR = International Clinical DR severity scale, HbA1c = glycated hemoglobin

Reprinted and modified from Paper III.

A decline in HbA1c of 5 mmol/mol (0.4% points) was observed after the initiation of CSII treatment, in the case population (Figure 5). HbA1c remained steady and high in the control population through the course of follow-up.

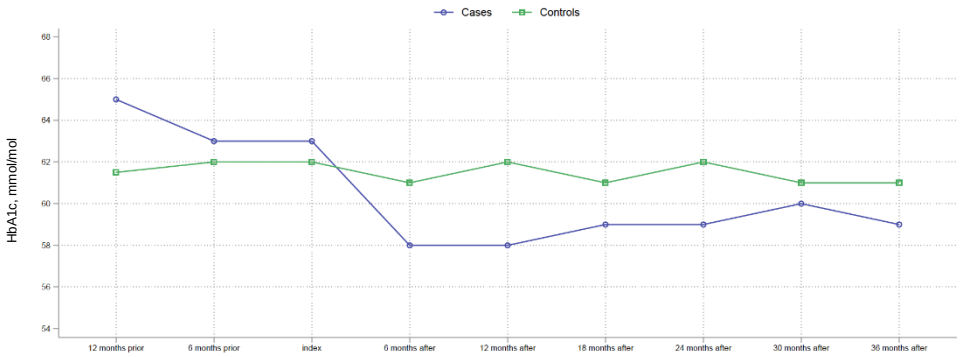

Figure 5 – HbA1c levels in individuals with CSII (cases) and with MDI (controls) at fixed time points (with 6 months increments)
Index date = time of first registration of CSII treatment. CSII = Continuous subcutaneous insulin infusion, MDI = Multiple daily injections, HbA1c = glycated hemoglobin.
Figure reprinted from Paper III.

Using a Cox regression model adjusted for sex, age, and marital status, we found no significant difference in the risk of DR worsening between cases and controls at the last individual screening visit registered in DiaBase (HR 1.05 [95%CI 0.91;1.22], p=0.49) (Figure 6). The incidence of ocular interventions (anti VEGF injections, photocoagulation and/or vitrectomy) at or before the last screening did not differ significantly between cases and controls when combined (HR 1.28 [95%CI 0.87;1.90], p=0.22).

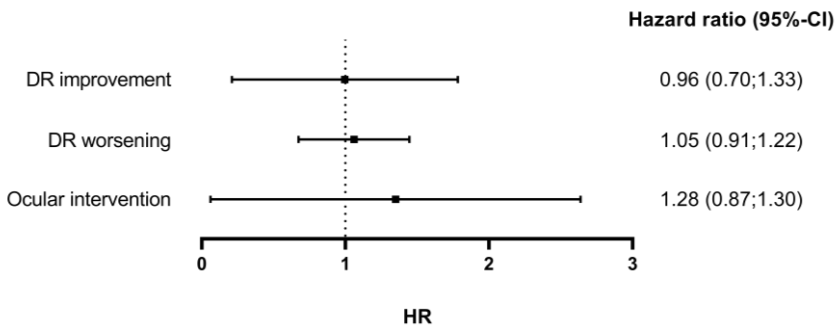

Figure 6 – Forrest plot illustrating risk of DR worsening, DR improvement and ocular intervention in individuals with CSII (cases) compared to individuals with MDI (controls) on or before last screening date
Adjusted for sex, age and marital status. HR = hazard ratio, CI = confidence interval, DR = diabetic retinopathy.
Figure is created in GraphPad Prism 9, using data from Table 2, Paper III.

10.2.2 Paper IV – Bariatric surgery and DR

Among the 238,967 individuals with type 2 diabetes registered in DiaBase, we identified 553 cases who underwent bariatric surgery during the follow-up period resulting in a prevalence of 0.2% from 2013-2022. When disregarding our inclusion criteria, the total prevalence of patients receiving bariatric surgery from 2013-2022 was 1.05%. We matched these cases with 2,677 non-bariatric controls (Figure 7).

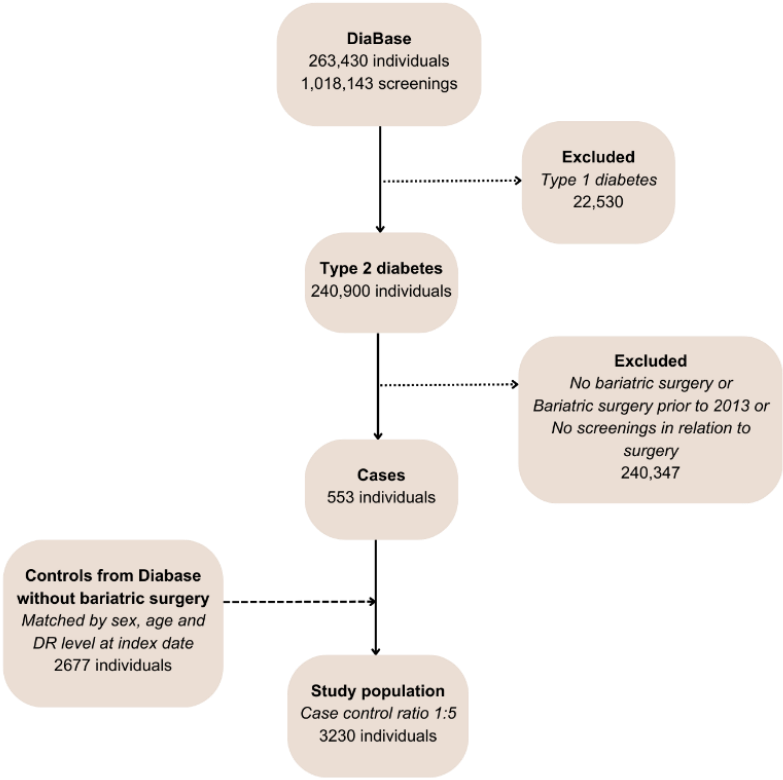

Figure 7 – Flowchart depicting selection of study population, with individuals with (cases) and without (controls) bariatric surgery in Paper IV
DiaBase = The Danish Registry of Diabetic Retinopathy, DR = Diabetic retinopathy.
Figure creating using Canva.

The individuals included in the study were predominantly female (62.9%) and had a median age of 49 years (IQR 42-55). Compared to the controls, the cases had a higher CCI score, with a higher proportion in the moderate low (16.3%

versus 12.8%) and moderate high (8.9% versus 5.5%) categories (p<0.01). The cases also had a shorter duration of diabetes (5.1 versus 6.2 years, p<0.01), better glycemic stability (48.0 versus 53.0 mmol/mol, p<0.01), and a higher frequency of use of metformin (82.1% versus 70.3%, <0.01), antihypertensive medications (73.4% versus 56.6%, <0.01), GLP-1 analogues (49.5% versus 21.1%, p<0.01), and SGLT-2 inhibitors (17.7% versus 14.4%, p=0.04) at index date (Table 8).

	All	**Bariatric surgery**		
	n = 3230	Yes (cases) n = 553	No (controls) n = 2677	P value
Sex, % female	2,032 (62.9%)	348 (62.9%)	1,684 (62.9%)	0.99
Age, years (IQR)	49 (42-55)	49 (42-55)	49 (42-55)	0.50
Duration of diabetes, years (IQR)	6.09 (2.4-11.1)	5.10 (1.9-9.9)	6.22 (2.57-11.35)	<0.001
Marital status, n (%)				0.55
Never married	981 (30.4%)	163 (29.5%)	818 (30.6%)	
Married	1,732 (53.6%)	293 (52.9%)	1,439 (53.8%)	
Widowed or divorced	517 (16.0%)	97 (17.5%)	420 (15.7%)	
CCI score, n (%)[A]				0.002
0 (low)	2,536 (78.5%)	406 (73.4%)	2,130 (79.6%)	
1 (moderate low)	432 (13.4%)	90 (16.3%)	342 (12.8%)	
2 (moderate high)	195 (6.0%)	48 (8.7%)	147 (5.5%)	
≥3 (high)	67 (2.1%)	9 (1.6%)	58 (2.2%)	
Level of DR, n (%)[B]				0.40
No DR	2,878 (89.1%)	487 (88.1%)	2,391 (89.3%)	
Mild NPDR	209 (6.5%)	36 (6.5%)	173 (6.5%)	
Moderate NPDR	101 (3.1%)	18 (3.3%)	83 (3.1%)	
Severe NPDR	22 (0.7%)	6 (1.1%)	16 (0.6%)	
PDR	20 (0.6%)	6 (1.1%)	14 (0.5%)	
BMI, n (%)				
Class I obesity (BMI 30-34.9)	12 (2.2%)	12 (2.2%)	-	-
Class II obesity (BMI 35-39.9)	119 (21.6%)	119 (21.6%)	-	-
Class III obesity (BMI 40-55+)	195 (35.3%)	195 (35.3%)	-	-
Undefined overweight	226 (40.9%)	226 (40.9%)	-	-
HbA1c, median [IQR]*	52 mmol/mol (45-62)	48 mmol/mol (42-55)	53 mmol/mol (46-63)	<0.001
Pharmacological treatment*				
Glucose-lowering medication				
GLP-1 analogues	838 (25.9%)	274 (49.6%)	564 (21.1%)	<0.001
SGLT-2 inhibitors	483 (14.9%)	98 (17.7%)	385 (14.4%)	0.045
Metformin	2,337 (72.4%)	454 (82.1%)	1,883 (70.3%)	<0.001
Insulin	939 (29.1%)	156 (28.2%)	783 (29.3%)	0.62
Antihypertensive medication n (%)	1,920 (59.4%)	406 (73.4%)	1,514 (56.6%)	<0.001
Cholesterol lowering medication n (%)	2,019 (62.5%)	354 (64.0%)	1,665 (62.2%)	0.42

Table 8 - Characteristics of individuals with type 2 diabetes with (cases) and without (controls) bariatric surgery at index date

Results given as counts (%) or medians (IQR). [A]Excluding diabetes. [B]Classification of DR given by the ICDR Scale. BMI was only available for cases, as it is not measured routinely for patients not undergoing bariatric surgery. CCI = Charlson Comorbidity Index, DR = diabetic retinopathy, NPDR = non proliferative DR, PDR = proliferative DR, BMI = body mass index, HbA1c = glycated hemoglobin, IQR = interquartile range, GLP-1 = Glucagon Like Peptide 1, SGLT-2 = Selective Sodium Glucose co Transporter 2. *Closest measurement/registration prior to index date (within 1 year).

Table reprinted and modified from Paper IV.

However, there were no significant differences between the cases and controls regarding marital status or the use of insulin or cholesterol-lowering medications. A HbA1c decline of 13 mmol/mol (1.2% points) was observed from 6 months pre-surgery until 6 months post-surgery in cases (Figure 8). Glycemic status remained stable after that.

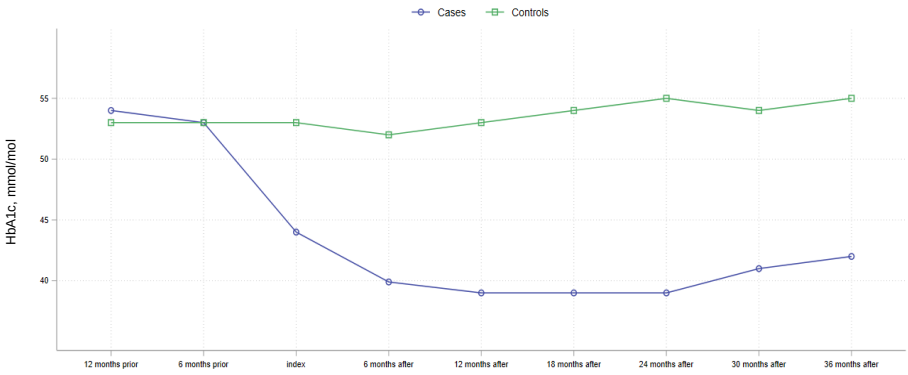

Figure 8 – HbA1c levels in individuals with (cases) and without (controls) bariatric surgery at fixed time points (with 6 months increments)
Given in in mmol/mol. Index date = time of bariatric surgery, HbA1c = glycated hemoglobin.
Figure reprinted from Paper IV.

In a fully adjusted multiple regression analysis with multiple imputations for missing HbA1c values (9.4% and 13.7% of cases and controls, respectively), the odds of short-term and long-term worsening were OR 0.41 (95% CI 0.13-1.33, p=0.14) and OR 0.71 (95% CI 0.34-1.46, p=0.35), respectively (Figure 9). We registered too few instances of need for ocular intervention short- or long-term to analyze. A post-hoc analysis stratified by preexisting DR at index date, found no increased odds of DR worsening at any point in either group.

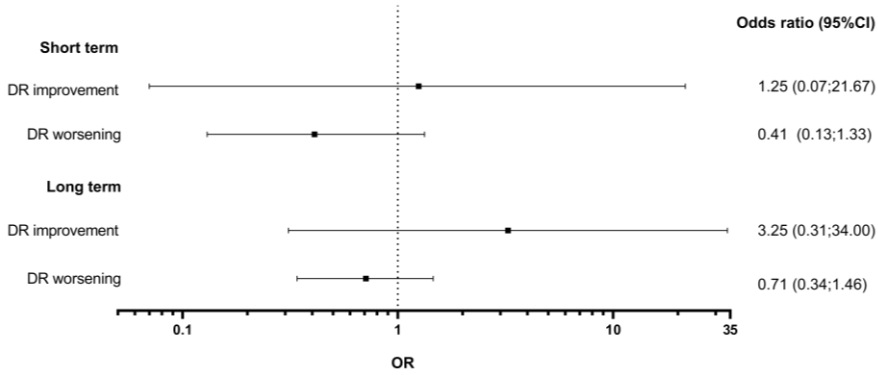

Figure 9 – Forrest plot illustrating short- and long term odds of DR worsening and improvement in individuals with (cases) compared to individuals without (controls) bariatric surgery
Short-term = 6 months ± three months. Long-term = 36 months ± nine months.
CI = confidence interval, DR = diabetic retinopathy, OR = odds ratio.
Please note that x-axis is portrayed as logarithmic, to better illustrate confidence intervals.
Figure created in GraphPad Prism 9, with data from Table 3, Paper IV.

10.3 Summary

In Substudy B we found a prevalence of CSII and bariatric surgery of 3.0 and 0.2% respectively in our study populations. No increased risk of DR worsening, nor need for ocular intervention, related to systemic treatments, was observed in individuals with type 1 (CSII) or type 2 (bariatric surgery) diabetes compared to controls.

11 Discussion

11.1 Substudy A

In this study, we evaluated different aspects of the Danish screening program for DR; we confirmed a high inter-grader agreement and validated the levels of DR reported to DiaBase. We concluded that only half of the individuals screened for DR, attended at timely intervals, the rest were either delayed or did not re-attend at all. We also found a markedly increased risk of DR proportionate to the amounts of missed screenings, in individuals who did not attend as recommended. The high inter-grader agreement in the screening program is difficult to directly compare to other, similar studies done in the field of inter-rater agreement in DR grading, due to the fact that the majority of literature establishes agreement amongst different sub-groups of medical professionals (e.g. general practitioners, retinal specialists or nurses)(66, 67), whereas our study compared ophthalmologists grading DR on a regular basis, to an ophthalmologist in training, who had received similar grading training, and certification. It must also be noted that agreement statistics are notoriously difficult to compare in general, due to both varying calculation and interpretation methods(68).
When an in depth examination of images that were graded differently by primary and secondary grader was performed, it was established that most disagreements were underestimations by primary grader, compared to secondary grader. The tendency of under-grading has been observed previously(69). This might be due to the different examination settings available to primary and secondary grader, including differences in equipment, time available per image as well as the opportunity to perform an indirect ophthalmoscopy or other clarifying examinations. The majority of discrepancies in gradings were within a single DR level, indicating that the gradings are still very relevant for clinical practice, with high reliability and usability.
Our study was strengthened by the nationwide setting, enabling us to re-grade images from the whole country, thus ultimately having results representative of the Danish screening program as a whole, in regards to screening facility and

geography. To minimize the risk of selection bias, we applied no specific selection criteria, and primary graders were asked to simply upload all images captured on their days of screening in the inclusion period. The utilization of different markers of agreeability (Gwet's AC and PABAK) both showing very high agreement, further strengthened the results. Limitations of the study include issues related to the data collection, including a low number of images with severe NPDR, thus results related to the grading of these images should be considered more carefully. Furthermore, the anonymity of the primary screening ophthalmologists compromised the knowledge of the exact number of primary graders included in the study. However, high variability in geographical and facility-wise distribution was still established. Overall, our analyzes confirmed high inter-grader agreement, meaning that the vast majority of DR levels graded in the screening program are correct, and that DR changes are diagnosed in a timely manner for referrals. However, correct diagnosis and timely referral depends, first and foremost, on attendance in the screening program. Our study showed, that of the population registered in DiaBase, 47.0% did not attend screening as recommended, meaning either late for screenings or only attended once. This result must be regarded in a larger setting, remembering that some individuals with diabetes has never attended DR screening, and thus are not represented in DiaBase or this study. A study from 2022 examining individuals in Denmark with type 2 diabetes, found that 26.8% never attended DR screening during the follow-up period (2013-2018)(70). These individuals are unaccounted for in our study, and their DR status is unknown. Considering the fact that more than one in four with type 2 diabetes never attends DR screening, it highlights the severity of the issue with non-attendance in the Danish screening program for DR. The discovery of increased risk of DR progression, with increasing number of delayed screening intervals was in accordance with an English study from 2021, showing similar results with delays resulting in increased risk of incident referable DR of 20%(71). Ensuring a high participation rate in DR screening programs is a challenging task, influenced by various factors that can be broadly categorized into patient-related and system-specific aspects(72, 73). Patient-related factors include awareness of the disease and its complications, logistical challenges, personal mental resources, and other systemic comorbidities. On the other hand, system-specific factors encompass issues like

miscommunication as well as technical and logistical challenges. Unfortunately, we were unable to examine these potential barriers directly due to the register-based design of this study. Therefore, we cannot determine the specific causes for non-attendance in the Danish screening program for DR, nor can we propose potential solutions. Our results do however indicate a need for further research, to establish causality of non-attendance in a Danish setting, in addition to the correlation found in our study.

11.2 Substudy B

We examined both CSII and bariatric surgery in individuals with type 1 and type 2 diabetes respectively. Our findings confirmed that both treatments are safe in regards to DR development, with no increased risk of incident or progressive disease. This is in agreement with previous research which have shown that utilizing CSII instead of MDI can potentially halt or even enhance the outlook for the development of DR, by stabilizing or lowering blood glucose levels(74-76). Previous research on bariatric surgery and DR, observed similar results(77, 78). As expected we observed both a decrease in, and a stabilization of, HbA1c after initiation of treatments in our case populations. We focused on long-term effect of CSII, whereas we examined both short- and long-term effects of bariatric surgery, as the rapid metabolic alterations have been known to cause a transient, early worsening of DR(79). This effect is not as well described after the initiation of CSII in newer studies, where the comparison group is most often individuals using MDI(80). However the risk of transient worsening is apparent in older studies when initiating CSII or MDI, in comparison to more conservative, and less intensive insulin administration regimens(81). It must also be regarded that the pathophysiological differences amongst the groups of individuals (type 1 versus type 2 diabetes) as well as the direct effects of bariatric surgery, which includes metabolic changes with rapid fluctuations in blood glucose as well as hormonal and dietary changes are more profound compared to the ones observed when transitioning from MDI to CSII. Despite a substantial percentual decrease in HbA1c levels, we found no increased risk of either temporary or permanent worsening of DR in our population and when stratified by preexisting DR and BMI (Paper IV) at index date, the results remained the same. Both

groups did however have reasonably well managed blood glucose levels before starting their respective treatments, which could be a significant factor to consider when interpreting these findings. Previous research lacks consensus regarding the cause of DR worsening following systemic interventions. One perspective suggests that the progression in DR is attributed to a significant decrease in HbA1c levels, from a high value, in this case pre-surgical(36). Conversely, other sources suggests that this is not the case(82). The relatively well regulated values of HbA1c in our study, even before initiation or change of systemic interventions might be due to the strict pre-surgical recommendations in regards to bariatric surgery, as well as the guidelines for CSII treatment. Prior to bariatric surgery individuals wishing to undergo surgery must follow pre-surgical guidelines(53) intended to promote better glycemic stability, pre-surgical weight loss and mental preparedness, to ensure optimal results of surgery. Individuals who are offered CSII treatment will not necessarily be the individuals having the highest HbA1c values, but rather individuals with issues related to hyper/hypoglycemic episodes(45). Our population reflects this, and that must be taken into account when considering the reproducibility of the results in other populations than Denmark.

11.3 Methodological and epidemiological considerations

Register-based studies provide a myriad of opportunities, enabling research on subjects that would be ethically and logistically challenging to examine in clinical settings. In Denmark, we are particularly privileged to be able to study subjects on a national level, due to the array of national health registries with high completeness, some of which are described earlier in this thesis. In specific instances where high completeness were not the case in our study, we utilized multiple imputations for missing data, to further increase the internal validity of the results.

The large sample sizes enabled us to enhance statistical power and ensure that the results are generalizable. We had access to a large assortment of covariates, to be assessed as well as adjusted for, including socioeconomic factors,

which were highly relevant for the characterization of attendance groups. Unfortunately, the registers used did not provide information on several life-style choices, which could have been beneficial, including smoking status, alcohol consumption and dietary choices. The measurement of height and weight was not readily available in the Danish registers either. To address this limitation, we created a variable using diagnostic codes for weight to categorize individuals into different groups of overweight. The case population had ample registrations, thanks to the pre-surgical guidelines for bariatric surgery, which include BMI requirements. However, only a small proportion of the control population (6%) had recorded measurements, making subgroup analysis possible only for cases. It is important to note that the registrations available for the control population were prone to a significant risk of bias, as measurements are likely to have been taken mainly from pregnant women or individuals who were particularly under- or overweight. Consequently, while BMI served as an interactive factor in the context of bariatric surgery, adjusting for it in the regression analysis would have been ideal, however due to the non-random nature of the missing data this was not feasible.

We utilized the registers for longitudinal analyzes, with extensive follow-up times, which helped us establish patterns in screening attendance, as well as examine long-term outcomes of systemic interventions. This population-based approach potentially helps reduce selection bias and enhances the extern validity of the results. However, as our cohorts, in all three register-based papers, were defined by DiaBase; a screening database, we must acknowledge the limitations associated with this, especially in regards to selection bias. As previously noted, not everyone with a diagnosis of diabetes attends the Danish screening program for DR, and a screening population can never be completely representative of the Danish population as a whole, or even of all individuals with diabetes. As attendance in the screening program is voluntary we can only speculate as to how this potential self-selection bias affects our results. It must be considered that individuals who have the ability to attend screening, and keep attending must have a minimum of personal resources available. Especially when considering the fact that they have a chronic disease, that requires ongoing management and monitoring, which impacts daily routines and require

significant adjustments to diet, exercise, and medication. Furthermore individuals might choose to attend, or not attend, screening based on a personal, cultural or health-related basis.

When conducting studies with an epidemiological approach, another potential pitfall is misclassification. This was especially apparent in this study, when classifying individuals based on their diabetes type, using a combination of ICD and ATC codes. To enhance our approach, we adjusted our strategy after completing Substudy A. We first identified individuals with type 1 diabetes based on criteria related to continuous use of insulin. Then, we presumed that the remaining population had type 2 diabetes, thereby eliminating the category of 'unknown diabetes' present in Paper II, which is justifiable as all attending patients must be assumed to have diabetes. It is worth considering that our code-based classification system does not account for sub-types of diabetes, and therefore, the division into type 1 and type 2 diabetes is a rough division. While we made improvements to the classification process as the study progressed, it is important to note that there is no infallible method to guarantee complete accuracy in classification. Potential misclassification could, in the case of our studies, result in the fact that individuals characterized and analyzed as one particular diabetes type, in reality is diagnosed with the other, leading to skewed conclusions being draught.

12 Conclusions

Diabetes and its complications can have profound and life-changing effects on affected individuals. The chronic nature of the disease necessitates ongoing management and monitoring, impacting daily routines and requiring significant adjustments to diet, exercise, and medication and in some cases, interventions such as bariatric surgery or the use of CSII. Complications arising from diabetes, such as DR, can lead to long-term disability and reduced quality of life, and it is important that interventions in diabetes management are not inadvertently worsening complications related to the disease. Our findings demonstrate the high quality of the Danish screening program for DR, with accurate gradings of DR levels. This not only validates the reliability of the DR levels given in the screening program, enabling appropriate referrals at the necessary time points, but also confirms the suitability of the data in DiaBase for register-based studies. Moreover, the screening program proves to be effective in early detection of DR, aiding in reducing the incidence of sight-threatening DR progression when followed appropriately. We observed a significantly higher risk of DR progression in individuals who deviated from the recommended screening program, emphasizing the importance of timely attendance for effective management of DR.

In our extensive nationwide matched cohort studies, we made the important observation that neither CSII in individuals with type 1 diabetes nor bariatric surgery in individuals with type 2 diabetes were linked to higher rates of incident or progressive DR. Additionally, we did not find evidence suggesting an increased need for ocular intervention either.

13 Perspectives

These findings contribute significantly to our understanding of the quality of DiaBase, the adherence patterns to the Danish screening program for DR, as well as the impact of systemic interventions on the development and progression of DR. Further research and interventions can build upon these findings to optimize the screening program and develop effective strategies for managing DR from a systemic viewpoint. It will be important for future research to focus on establishing why the attendance in the Danish screening program is not followed as intended by a larger number of individuals with diabetes. Qualitative research could offer a unique perspective allowing us to explore the nuances and intricacies of non-attendance. Through methods such as interviews, focus groups and surveys we can engage directly with patients, physicians, and other stakeholders involved in the screening program. By actively listening to their narratives, we can gain a comprehensive understanding of the barriers, fears, misconceptions, and practical challenges that contribute to non-attendance. The insights gained from qualitative research will be crucial in developing targeted strategies and interventions to improve screening attendance. By identifying the specific causes and addressing them at both the physician and patient levels, we can implement tailored initiatives that are more effective in encouraging participation. Our research on systemic interventions and DR aids in the ongoing efforts to enhance the management and prevention of DR, ultimately improving visual health outcomes for individuals with diabetes. The positive results of our study, suggests that CSII could be considered as a viable option for a broader range of individuals, without the fear of potential negative effects on DR. In accordance, our results also encourage the continued use of bariatric surgery, in individuals with diabetes. However, future research might target other less invasive options such as GLP-1 analogues, as an alternative to surgery.

14 Tables and Figures

Tables

Figures

15 References

1. Grauslund J, Stokholm L, Ohm Kyvik K, Dornonville de la Cour M, Kessel L, Hass Rubin K. Interactions between ocular and systemic disease using national register-based data in the Danish Excellence Centre in Ophthalmic Epidemiology (DECODE-EYE): study perspective. Acta Ophthalmol. 2020;98(6):573-8.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.
3. The Danish Diabetes Association: Diabetes in numbers 2023 [Available from: https://www.diabetestal.nu/.
4. Teo ZL, Tham YC, Yu M, Chee ML, Rim TH, Cheung N, et al. Global Prevalence of Diabetic Retinopathy and Projection of Burden through 2045: Systematic Review and Meta-analysis. Ophthalmology. 2021;128(11):1580-91.
5. Danish Clinical Quality Database of Screening for diabetic retinopathy. Annual Report 2022. The Danish Clinical Quality Program, National Clinical Registries, 2023. : The Danish Registry of Diabetic Retinopathy.
6. Andersen N, Hjortdal JO, Schielke KC, Bek T, Grauslund J, Laugesen CS, et al. The Danish Registry of Diabetic Retinopathy. Clin Epidemiol. 2016;8:613-9.
7. Diabetes foreningen [Available from: https://diabetes.dk/forskning/viden-om-diabetes/diabetes-i-danmark.
8. Silvio E. Inzucchi BL. Clinical presentation, diagnosis, and initial evaluation of diabetes mellitus in adults. UpToDate: UpToDate; 2023.
9. Redondo MJ, Hagopian WA, Oram R, Steck AK, Vehik K, Weedon M, et al. The clinical consequences of heterogeneity within and between different diabetes types. Diabetologia. 2020;63(10):2040-8.
10. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes. 2017;66(2):241-55.
11. Gillespie KM. Type 1 diabetes: pathogenesis and prevention. Cmaj. 2006;175(2):165-70.
12. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(17).

13. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes-2023. Diabetes Care. 2023;46(Suppl 1):S19-s40.
14. World Health Organization Use of glycated hemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. Abbreviated report, WHO guidelines, approved by the Guidelines Review Committee. 2011.
15. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88-98.
16. Genuth S, Ismail-Beigi F. Clinical implications of the ACCORD trial. J Clin Endocrinol Metab. 2012;97(1):41-8.
17. Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord. 2012;142 Suppl:S8-21.
18. Vujosevic S, Aldington SJ, Silva P, Hernández C, Scanlon P, Peto T, et al. Screening for diabetic retinopathy: new perspectives and challenges. Lancet Diabetes Endocrinol. 2020;8(4):337-47.
19. American Academy of Ophthalmology Basic and Clinical Science Course (BCSC). Retina and Vitreous2016-2017. p. 31-43.
20. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010;376(9735):124-36.
21. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis? Eye. 2009;23(7):1496-508.
22. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, et al. Diabetic Retinopathy: Seeing Beyond Glucose-Induced Microvascular Disease. Diabetes. 2006;55(9):2401-11.
23. Arden GB, Sivaprasad S. The pathogenesis of early retinal changes of diabetic retinopathy. Documenta Ophthalmologica. 2012;124(1):15-26.
24. Lin KY, Hsih WH, Lin YB, Wen CY, Chang TJ. Update in the epidemiology, risk factors, screening, and treatment of diabetic retinopathy. J Diabetes Investig. 2021;12(8):1322-5.
25. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35(3):556-64.
26. The Diabetes Control and Complications Trial (DCCT) The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. New England Journal of Medicine. 1993;329(14):977-86.
27. Solomon Sharon D, Goldberg Morton F. ETDRS Grading of Diabetic Retinopathy: Still the Gold Standard? Ophthalmic Research. 2019;62(4):190-5.

28. Early Treatment Diabetic Retinopathy Study Research Group Grading Diabetic Retinopathy from Stereoscopic Color Fundus Photographs - An Extension of the Modified Airlie House Classification: ETDRS Report Number 10. Ophthalmology. 2020;127(4s):S99-s119.
29. Wilkinson CP, Ferris FL, 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677-82.
30. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. Jama. 2007;298(8):902-16.
31. Wong TY, Sun J, Kawasaki R, Ruamviboonsuk P, Gupta N, Lansingh VC, et al. Guidelines on Diabetic Eye Care: The International Council of Ophthalmology Recommendations for Screening, Follow-up, Referral, and Treatment Based on Resource Settings. Ophthalmology. 2018;125(10):1608-22.
32. The Diabetic Retinopathy Study Research Group Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. . Ophthalmology. 1981;88(7):583-600.
33. Diabetic Retinopathy Vitrectomy Study Group Two-year course of visual acuity in severe proliferative diabetic retinopathy with conventional management. DRVS report #1. Ophthalmology. 1985;92(4):492-502.
34. Sun JK, Glassman AR, Beaulieu WT, Stockdale CR, Bressler NM, Flaxel C, et al. Rationale and Application of the Protocol S Anti-Vascular Endothelial Growth Factor Algorithm for Proliferative Diabetic Retinopathy. Ophthalmology. 2019;126(1):87-95.
35. Bain SC, Klufas MA, Ho A, Matthews DR. Worsening of diabetic retinopathy with rapid improvement in systemic glucose control: A review. Diabetes Obes Metab. 2019;21(3):454-66.
36. Murphy R, Jiang Y, Booth M, Babor R, MacCormick A, Hammodat H, et al. Progression of diabetic retinopathy after bariatric surgery. Diabet Med. 2015;32(9):1212-20.
37. Early Treatment Diabetic Retinopathy Study Research Group Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial. Arch Ophthalmol. 1998;116(7):874-86.
38. O'Hare M, Esquiva G, McGahon MK, Hombrebueno JMR, Augustine J, Canning P, et al. Loss of TRPV2-mediated blood flow autoregulation recapitulates diabetic retinopathy in rats. JCI Insight. 2022;7(18).
39. Lanzetta P, Sarao V, Scanlon PH, Barratt J, Porta M, Bandello F, et al. Fundamental principles of an effective diabetic retinopathy screening program. Acta Diabetol. 2020;57(7):785-98.
40. WHO Screening programmes: a short guide. Increase effectiveness, maximize benefits and minimize harm. Copenhagen: WHO Regional Office for Europe; 2020. Licence: CC BY-NC-SA 3.0 IGO.

41. Grauslund J, Andersen N, Andresen J, Flesner P, Haamann P, Heegaard S, et al. Evidence-based Danish guidelines for screening of diabetic retinopathy. Acta Ophthalmol. 2018;96(8):763-9.
42. Virk SA, Donaghue KC, Wong TY, Craig ME. Interventions for Diabetic Retinopathy in Type 1 Diabetes: Systematic Review and Meta-Analysis. Am J Ophthalmol. 2015;160(5):1055-64.e4.
43. Skyler JS. Continuous subcutaneous insulin infusion--an historical perspective. Diabetes Technol Ther. 2010;12 Suppl 1:S5-9.
44. Dovc K, Battelino T. Evolution of Diabetes Technology. Endocrinol Metab Clin North Am. 2020;49(1):1-18.
45. National Clinical Guidelines for the use of insulin pumps in Denmark [press release]. The Danish National Board of Health 2012.
46. Nimri R, Nir J, Phillip M. Insulin Pump Therapy. Am J Ther. 2020;27(1):e30-e41.
47. Phillips BT, Shikora SA. The history of metabolic and bariatric surgery: Development of standards for patient safety and efficacy. Metabolism. 2018;79:97-107.
48. Arterburn DE, Telem DA, Kushner RF, Courcoulas AP. Benefits and Risks of Bariatric Surgery in Adults: A Review. Jama. 2020;324(9):879-87.
49. Colquitt JL, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. Cochrane Database Syst Rev. 2014;2014(8):Cd003641.
50. Ilyas S, Al-Refai R, Maharjan R, Diaz Bustamante L, Ghattas KN, Khan S. Bariatric Surgery and Type 2 Diabetes Mellitus: Assessing Factors Leading to Remission. A Systematic Review. Cureus. 2020;12(8):e9973.
51. Affinati AH, Esfandiari NH, Oral EA, Kraftson AT. Bariatric Surgery in the Treatment of Type 2 Diabetes. Curr Diab Rep. 2019;19(12):156.
52. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, et al. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. N Engl J Med. 2017;376(7):641-51.
53. National Danish Treatment Guidelines for bariatric surgery: Danish Society of Endocrinology; [Available from: https://endocrinology.dk/nbv/andre-endokrinologiske-sygdomme/fedmekirurgi/.
54. Thygesen LC, Ersbøll AK. When the entire population is the sample: strengths and limitations in register-based epidemiology.
55. Erlangsen A, Fedyszyn I. Danish nationwide registers for public health and health-related research. Scand J Public Health. 2015;43(4):333-9.
56. NOMESCO Classification og Surgical Procedures: Nordic Centre for Classifications in Health Care; 2011 [Available from: https://nhwstat.org/publications/ncsp-classification-surgical-procedures.
57. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol. 2015;7:449-90.

58. Pottegård A, Schmidt SAJ, Wallach-Kildemoes H, Sørensen HT, Hallas J, Schmidt M. Data Resource Profile: The Danish National Prescription Registry. Int J Epidemiol. 2017;46(3):798-f.
59. Arendt JFH, Hansen AT, Ladefoged SA, Sørensen HT, Pedersen L, Adelborg K. Existing Data Sources in Clinical Epidemiology: Laboratory Information System Databases in Denmark. Clin Epidemiol. 2020;12:469-75.
60. UNESCO International Standard Classification of Education, ISCED 2011. UNESCO Institute for Statistics. 2011.
61. Møller B, Solbjerghøj L, Kraus F, Schmaus G. Official statistics in Denmark: Socio economic microdata for research. 2001.
62. Statistics Denmark Register overview and descriptions: Statistics Denmark; 2023 [Available from: https://www.dst.dk/extranet/forskningvariabellister/Oversigt%20over%20registre.html.
63. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol. 2014;29(8):541-9.
64. Andersen JKH, Hubel MS, Savarimuthu TR, Rasmussen ML, Sørensen SLB, Grauslund J. A digital online platform for education and certification of diabetic retinopathy health care professionals in the Region of Southern Denmark. Acta Ophthalmol. 2022;100(5):589-95.
65. Gwet KL. Handbook of Inter-Rater Reliability, 5th Edition. 5th ed2021. p. 28-69.
66. Cunha LP, Figueiredo EA, Araújo HP, Costa-Cunha LVF, Costa CF, Neto JMC, et al. Non-Mydriatic Fundus Retinography in Screening for Diabetic Retinopathy: Agreement Between Family Physicians, General Ophthalmologists, and a Retinal Specialist. Front Endocrinol (Lausanne). 2018;9:251.
67. Islam FMA. Accuracy and reliability of retinal photo grading for diabetic retinopathy: Remote graders from a developing country and standard retinal photo grader in Australia. PLoS One. 2017;12(6):e0179310.
68. Xie Q. Agree or Disagree? A Demonstration of An Alternative Statistic to Cohen's Kappa for Measuring the Extent and Reliability of Agreement between Observers. National Center for Education Statistics. 2013.
69. Sallam A, Scanlon PH, Stratton IM, Jones V, Martin CN, Brelen M, et al. Agreement and reasons for disagreement between photographic and hospital biomicroscopy grading of diabetic retinopathy. Diabet Med. 2011;28(6):741-6.
70. Petersen GB, Byberg S, Vistisen D, Fangel MV, Vorum H, Joensen LE, et al. Factors Associated With Nonattendance in a Nationwide Screening Program for Diabetic Retinopathy: A Register-Based Cohort Study. Diabetes Care. 2022;45(2):303-10.

71. Lawrenson JG, Bourmpaki E, Bunce C, Stratton IM, Gardner P, Anderson J, et al. Trends in diabetic retinopathy screening attendance and associations with vision impairment attributable to diabetes in a large nationwide cohort. Diabet Med. 2021;38(4):e14425.
72. Strutton R, Du Chemin A, Stratton IM, Forster AS. System-level and patient-level explanations for non-attendance at diabetic retinopathy screening in Sutton and Merton (London, UK): a qualitative analysis of a service evaluation. BMJ Open. 2016;6(5):e010952.
73. Graham-Rowe E, Lorencatto F, Lawrenson JG, Burr JM, Grimshaw JM, Ivers NM, et al. Barriers to and enablers of diabetic retinopathy screening attendance: a systematic review of published and grey literature. Diabet Med. 2018;35(10):1308-19.
74. Reid LJ, Gibb FW, Colhoun H, Wild SH, Strachan MWJ, Madill K, et al. Continuous subcutaneous insulin infusion therapy is associated with reduced retinopathy progression compared with multiple daily injections of insulin. Diabetologia. 2021;64(8):1725-36.
75. de Oliveira Loureiro T, Cardoso JN, Lopes C, Carreira AR, Rodrigues-Barros S, Vide-Escada A, et al. The effect of insulin pump therapy in retinal vasculature in type 1 diabetic patients. Eur J Ophthalmol. 2021;31(6):3142-8.
76. Klefter ON, Hommel E, Munch IC, Nørgaard K, Madsbad S, Larsen M. Retinal characteristics during 1 year of insulin pump therapy in type 1 diabetes: a prospective, controlled, observational study. Acta Ophthalmol. 2016;94(6):540-7.
77. Merlotti C, Ceriani V, Morabito A, Pontiroli AE. Bariatric surgery and diabetic retinopathy: a systematic review and meta-analysis of controlled clinical studies. Obes Rev. 2017;18(3):309-16.
78. Kim YJ, Kim BH, Choi BM, Sun HJ, Lee SJ, Choi KS. Bariatric surgery is associated with less progression of diabetic retinopathy: A systematic review and meta-analysis. Surg Obes Relat Dis. 2017;13(2):352-60.
79. Yu CW, Park LJ, Pinto A, Ma ON, Lee Y, Gupta R, et al. The Impact of Bariatric Surgery on Diabetic Retinopathy: A Systematic Review and Meta-Analysis. Am J Ophthalmol. 2021;225:117-27.
80. Holfort SK, Nørgaard K, Jackson GR, Hommel E, Madsbad S, Munch IC, et al. Retinal function in relation to improved glycaemic control in type 1 diabetes. Diabetologia. 2011;54(7):1853-61.
81. Brinchmann-Hansen O, Dahl-Jørgensen K, Hanssen KF, Sandvik L. The response of diabetic retinopathy to 41 months of multiple insulin injections, insulin pumps, and conventional insulin therapy. Arch Ophthalmol. 1988;106(9):1242-6.
82. Kim YJ, Seo du R, Kim MJ, Lee SJ, Hur KY, Choi KS. Clinical course of diabetic retinopathy in korean type 2 diabetes after bariatric surgery: A Pilot Study. Retina. 2015;35(5):935-43.

PAPER I

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ORIGINAL ARTICLE

Inter-grader reliability in the Danish screening programme for diabetic retinopathy

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Abstract

Purpose: The Danish Registry of Diabetic Retinopathy includes information from >200 000 patients who attends diabetic retinopathy (DR) screening in Denmark. Screening of patients with uncomplicated type 2 diabetes is often performed by practicing ophthalmologists, while patients with type 1 and complicated type 2 diabetes attends screening at hospitals. We performed a clinical reliability study of retinal images from Danish screening facilities to explore the inter-grader agreement between the primary screening ophthalmologist and a blinded, certified grader.

Methods: Invitations to participate were sent to screening facilities across Denmark. The primary grader uploaded fundus photographs with information on estimated level of DR (International Clinical Diabetic Retinopathy scale as 0 [no DR], 1–3 [mild, moderate or severe nonproliferative DR {NPDR}], or 4 [proliferative DR {PDR}]), region of screening, image style, and screening facility. Images were then regraded by a blinded, certified, secondary grader. Weighted kappa analysis was performed to evaluate agreement.

Results: Fundus photographs from 230 patients (458 eyes) were received from practicing ophthalmologists (52.6%) and hospital-based grading centres (47.4%) from all Danish regions. Reported levels of DR by the primary graders were 66.8%, 12.2%, 13.1%, 1.3% and 5.5% for DR levels 0–4. The overall agreement between primary and secondary graders was 93% (κ=0.83). Based on screening facility agreement was 96% (κ=0.89) and 90% (κ=0.76) for practicing ophthalmologists and hospital-based graders.

Conclusion: In this nationwide study, we observed a high overall inter-grader agreement and based on this, it is reasonable to assume that reported DR gradings in the screening programme in Denmark, accurately reflect the truth.

KEYWORDS
diabetic retinopathy, interrater agreement, reliability, screening

1 | INTRODUCTION

Diabetic eye screening is an important and trusted method of examination offering a safety net for patients with diabetes in order to secure regular examinations and timely referrals for relevant treatment. Few studies have been done in the field of inter-grader agreeability when examining images and grading retinal disease and

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more specifically DR. In a study aiming to evaluate the inter-grader reliability between ophthalmologists, Rêgo et al. (2021) reported a modest agreement of kappa (κ) 0.49–0.54 between a retinal specialist versus a general ophthalmologist, and called their results 'concerning from a clinical perspective'. Other studies found slightly higher values for agreement ranging from κ=0.55 (Scott et al., 2008) and κ=0.61 (Thapa et al., 2020) to κ=0.65 (Cunha et al., 2018) and κ=0.74 (Cunha et al., 2018). The studies sought to examine the inter-rater variability among health and eye care professionals, primarily to examine the agreement across different profession groups. Interrater reliability in general is a difficult subject to examine due to the well-known statistical uncertainties, and direct comparison between kappa statistics is problematic due to the many different types of formulas for calculation, but also the different interpretation methods (Xie, 2013).

The Danish Registry of Diabetic Retinopathy (DiaBase) is a national database that holds information on patients with diabetes that have attended diabetic eye screenings in Denmark (Andersen et al., 2016). The database is used for quality insurance, monitoring of the screening programme and for research purposes. As all DR levels from DR screenings in Denmark are reported to DiaBase by practicing and hospital-based ophthalmologists nationwide, an inter-grader analysis of images graded by the same ophthalmologists could act as a pseudo-marker for the reliability of this parameter in DiaBase, as no images are uploaded to this database directly. The inter-grader agreeability has not been examined in the Danish screening programme for DR, and thus the reliability of DR levels in DiaBase are yet to be determined. This is important not only to ensure correct screening intervals and timely referrals for treatment, but also because data in DiaBase are used for several large register based studies (Grauslund et al., 2020).

In this study, we aimed to examine the DR levels registered in DiaBase by regrading images, graded by practicing and hospital-based ophthalmologists, in patients who attended DR screenings and thus are registered in DiaBase, thereby estimating the inter-grader agreeability within the Danish screening programme and the reliability of DiaBase.

2 | PATIENTS AND METHODS

We conducted a clinical reliability study using retinal fundus photographs (images) from patients who attended the national screening programme for DR. Completely anonymous images, used for screening, were collected from practicing ophthalmologists and hospital-based screening units. Primary graders were asked to send in the exact image that they used for grading, and in the same form (i.e. mosaic as mosaic, not individual images). The same images were then regraded, in generic image viewing software, by a certified grader blinded to the primary grading.

DiaBase is a national clinical quality database where registration of data from all DR screenings in Denmark has been mandatory since 2013. The database holds information on more than 200 000 patients and includes information regarding levels of DR, screening dates (actual and recommended), other eye-related diseases and surgeries and screening facilities (practicing ophthalmologists and hospitals; Andersen et al., 2016). Images in the Danish screening programme are graded according to the International Clinical Diabetic Retinopathy Severity Scale (ICDR, 0=no DR, 1=mild non proliferative DR [NPDR], 2=moderate NPDR, 3=severe NPDR and 4=proliferative DR [PDR]), which was also used in this study (Grauslund et al., 2018). The primary graders were all medical doctors specialized in ophthalmology and practicing either at individual clinics or at public hospitals. All graders are familiar with the Danish National Guidelines provided by the Danish Ophthalmological Society, and additional free virtual training resources are available to all graders in Denmark. The secondary grader was also a medical doctor in the field of ophthalmology, certified in DR grading of retinal images at the Ophthalmic Reading Centre, Centre for Public Health, Royal Victoria Hospital Site, Queens University, Belfast, Northern Ireland and had also completed a virtual training platform for education and certification of DR; Virtual ocular learning platform (VIOLA; Andersen et al., 2022) at Odense University Hospital, Denmark.

We received retinal images of 257 patients, but 27 of these were excluded due to failed uploads (repeated or no images). Hence, we included 230 patients (458 eyes) in this study.

2.1 | Inclusion criteria and data collection

A digital, invitational letter was sent to all practicing ophthalmologists (n=160) and screening hospital departments (n=5) representing all Danish geographical regions. Fundus images from a specified period (03 February–03 March 2020) were requested, and no selection was to be made in regard to patient age, sex, diabetes type or level of DR. Data were collected continuously from the invitation letter which was issued from August 2020 to October 2021. Reminders were issued continuously. All images that primary graders used to make their grading, were requested. Only the images themselves were available to the second grader in the grading process, all information regarding primary grader, including the primary graded level of DR, were blinded. Both images and primary grader were not personally identifiable to the secondary grader at any point. Images were uploaded to an encrypted RedCap database (Vanderbilt University 2021, Nashville, Tennessee, USA, managed by Open Patient data Explorative Network [OPEN], Odense, Denmark). The uploader also provided information on screening facility (practicing ophthalmologist or hospital), region (Capital Region of Denmark, Central Denmark Region, North Denmark Region, Region Zealand and Region of Southern Denmark), level of DR (no, mild, moderate, severe and proliferative DR [0, 1, 2, 3 or 4] or not classifiable [due to undefinable, retinal features]) and image modality (mosaic, two fields, widefield or other).

TABLE 1 Number of diabetic retinopathy gradings distributed based on Danish geographical region, from where they were reported.

	Capital region of Denmark	Central Denmark region	North Denmark region	Region Zealand	Region of Southern Denmark	Total
No DR	75 (24.4)[a]	59 (19.2)	16 (5.2)	30 (9.8)	127 (41.4)	307 (100.0)
	(70.8)[b]	(67.1)	(47.1)	(49.2)	(75.2)	(67.0)
Mild NPDR	8 (14.3)	14 (25.0)	9 (16.1)	9 (16.1)	16 (28.6)	56 (100.0)
	(7.6)	(15.9)	(26.5)	(14.8)	(9.5)	(12.2)
Moderate NPDR	14 (23.3)	8 (13.3)	5 (8.3)	13 (21.7)	20 (33.3)	60 (100.0)
	(13.2)	(9.1)	(14.7)	(21.3)	(11.8)	(13.1)
Severe NPDR	1 (16.7)	2 (33.3)	2 (33.3)	0 (0.0)	1 (16.7)	6 (100.0)
	(0.9)	(2.3)	(5.9)	(0.0)	(0.6)	(1.3)
PDR	6 (24.0)	4 (16.0)	2 (8.0)	9 (36.0)	4 (16.0)	25 (100.0)
	(5.7)	(4.6)	(5.9)	(14.8)	(2.4)	(5.5)
Not classifiable	2 (50.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (25.0)	4 (100.0)
	(1.89)	(1.1)	(0.0)	(0.0)	(0.6)	(0.9)
Total	106 (23.1)	88 (19.2)	34 (7.4)	61 (13.3)	169 (36.9)	458 (100.0)
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)

Note: Given in numbers (%). Diabetic retinopathy (DR), non-proliferative DR (NPDR) and proliferative DR (PDR).

[a]Row percentage shows what percentage of all received images of a certain degree that came from which specific geographical region.

[b]Column percentage shows how all received images from a specific geographical region was distributed according to the different DR levels.

2.2 | Statistical analysis

Sample size was established using *z*-test, and an estimated total of 316 eyes was needed to ensure a power of 0.80 with a significance level (α) of 0.05. Weighted agreement analyses were performed to assess inter-grader agreement between the primary and secondary graders; results are reported as both PABAK (prevalence-adjusted, bias-adjusted kappa) and Gwet's AC (Gwet, 2021). The coefficient (κ) gives the agreement between two or more measurements; from no agreement ($\kappa=0$) to complete agreement ($\kappa=1$).The weighted kappa takes into consideration the ordinal nature of DR levels, rating a difference in gradings to be more severe the further graders were from agreement. Mismatched gradings were penalized in a linear fashion, subtracting equal points for agreements along with each level of disagreement, meaning that a complete match = 1, one level off = 0.75, two levels off = 0.5 etc. Images deemed not classifiable by either primary or secondary grader were excluded from both agreement analyses ($n=10$). Chi-square (χ^2) tests were performed to evaluate baseline characteristics of data. *p*-values lower than 0.05 were considered statistically significant. All data analyses were performed using STATA 17 (StataCorp, 2021).

3 | RESULTS

All five Danish regions; Capital Region of Denmark, Central Denmark Region, North Denmark Region, Region Zealand and Region of Southern Denmark were represented and accounted for 23.1%, 19.2%, 7.4%, 13.3% and 36.9% of the included patients respectively (Table 1). Images were received from both practicing ophthalmologists (52.6%) and hospital-based screening units (47.4%), and image modalities were distributed between two fields, mosaic, widefield and other with 30.4%, 33.6%, 3.5% and 32.5% respectively. Reported levels of DR were 66.8%, 12.2%, 13.1%, 1.3% and 5.5% for no, mild, moderate, severe NPDR and proliferative DR as graded by primary graders. The remaining images were deemed ungradable (1.1%; Table 2). A significant difference ($p<0.001$) in the reported levels of DR between the screening facilities was observed (Table 3); images from practicing ophthalmologists showed a distribution of 90.9%, 5.0%, 2.5%, 0.0% and 0.8%, whereas images from hospital-based ophthalmologists showed a distribution of 40.1%, 20.3%, 24.9%, 2.8% and 10.6% for no, mild, moderate, severe and proliferative DR. Of the received images, 0.8% and 1.4% were deemed as not classifiable by practicing ophthalmologists and hospital-based graders respectively. No significant difference was found in DR level gradings according to image modalities or geographical regions.

TABLE 2 Distribution of diabetic retinopathy gradings according to primary and secondary graders.

	Primary grader	Secondary grader
No Dr	306 (66.8)	274 (59.8)
Mild NPDR	56 (12.2)	63 (13.7)
Moderate NPDR	60 (13.1)	67 (14.6)
Severe NPDR	6 (1.3)	14 (3.1)
PDR	25 (5.46)	34 (7.4)
Not classifiable	5 (1.1)	6 (1.3)
Any DR	147 (32.1)	178 (38.9)

Note: Given in numbers (%). Diabetic retinopathy (DR), non-proliferative DR (NPDR) and proliferative DR (PDR).

When graded by the secondary grader, the distribution of no, mild, moderate, severe NPDR and PDR followed the same pattern as primary gradings; 59.8%, 13.7%, 14.6%, 3.1% and 7.4% (Table 2). Exact agreement was observed in 78.6% of all graded eyes. Agreement

TABLE 3 Number of eyes included according to primary screening facility.

	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Not classifiable
Practicing ophthalmologists	219 (90.9)	12 (5.0)	6 (2.5)	0 (0.0)	2 (0.8)	2 (0.8)
Hospitals	87 (40.1)	44 (20.3)	54 (24.9)	6 (2.8)	23 (10.6)	3 (1.4)

Note: Given in numbers (%). Diabetic retinopathy (DR), non-proliferative DR (NPDR) and proliferative DR (PDR).

TABLE 4 Schematic, exact agreement of diabetic retinopathy gradings between primary and secondary graders based on retinal fundus photographs.

	Secondary grader					
Primary grader	**No DR**	**Mild NPDR**	**Moderate NPDR**	**Severe NPDR**	**PDR**	**Not classifiable**
No DR	**265 (84.1)**	33	6	2	0	0
Mild NPDR	8	**23 (23.9)**	17	3	5	0
Moderate NPDR	1	7	**42 (49.4)**	6	3	1
Severe NPDR	0	0	2	**2 (11.1)**	2	0
PDR	0	0	0	1	**24 (68.6)**	0
Not classifiable	0	0	0	0	0	**5 (83.3)**

Note: Given in numbers (%). Diabetic retinopathy (DR), non-proliferative DR (NPDR) and proliferative DR (PDR).
The bold express agreement amongst primary and secondary graders.

within DR levels was 84.1%, 23.9%, 49.4%, 11.1%, 68.6% and 83.3% for no, mild, moderate, severe NPDR, PDR and ungradable eyes (Table 4). In 96 cases of disagreements, 80.0% were underestimations of DR level by primary grader. This was most prevalent in DR levels 2–4, where primary grader underestimated DR in 34.2%, 78.6% and 29.4% of images respectively.

When analysed using a weighted kappa analysis, overall agreement between primary and secondary graders was 93% (PABAK κ=0.83, p<0.001, Gwet AC κ=0.88, p<0.001). When sub-analysed for screening facility, the results were 96% (PABAK κ=0.89, p<0.001, Gwet AC κ=0.95, p<0.001) for practicing ophthalmologists and 90% (PABAK κ=0.76, p<0.001, Gwet AC κ=0.78, p<0.001) for hospitals. When allowing a one-step difference between DR grading levels, an agreement of 95.5% was seen (PABAK κ=0.93, p<0.001, Gwet AC κ=0.96, p<0.001). When interpreted according to weighted kappa categories and Gwet's AC, the inter-grader agreement was high, in both cases.

4 | DISCUSSION

In the Danish screening programme for DR, we found a high degree of inter-grader agreement between primary graders from practicing ophthalmologists and hospital-based units compared to a secondary, certified grader. Whereas most of the literature regarding the field of inter-grader agreement in DR screening assesses the agreement between, and within, different subgroups of medical personnel (ophthalmologists [general, retinal specialists] nurses, general practitioners), at a single site, we examined a broad selection of practice and hospital-based ophthalmologists, from all over Denmark, presenting a nationwide perspective. The agreement found was evident both in the PABAK and Gwet's AC coefficients as well as in the sheer percentage agreement. Gwet's AC is not related to bias due to its definition of agreement by chance. Other well-known issues with marginal distribution and similar ratings, which is penalized in Cohens kappa, are not in the formulas for Gwet's AC. The considerable distribution difference between levels of DR, when examining screening facilities is most likely explained by the different patient clientele; we especially found a large difference in reported degrees of PDR, which was expected as the majority of these patients are screened at hospital-based settings. This is representative of the distribution seen within the screening programme in general; the screened population are mostly divided between otherwise healthy patients with uncomplicated type 2 diabetes, who are screened at practicing ophthalmologists and patients with type 1 diabetes, as well as complicated type 2 diabetes who are screened at hospital-based settings (Danish Diabetes Database National Yearly Report, 2020/2021). Variation in gradings between primary and secondary graders could be due to equipment-related differences (monitor brightness, sharpness and quality) and available time to examine images. The possibility to do an ophthalmoscopic fundus exam, in cases of doubt, was only available to some primary graders and never to secondary grader, which also might affect the final grading decision. The study showed that the most difficult gradings, represented by the levels with least agreement, were mild, moderate and severe NPDR—thus patients without disease (no DR) and treatment-requiring disease (PDR) had the highest agreement between primary and secondary graders. However out of the 34 images that secondary grader identified as PDR, 10 were identified as either mild, moderate or severe DR by primary grader. When investigaing this disagreement by re-examining graded images, the reason was in part due to primary grader not classifying eyes without active PDR, but with previous panretinal laser treatment,

as PDR—this is however not correct according to the ICDR scale and Danish screening guidelines. Other instances includes difficulty distinguishing intraretinal microvasculature abnormalities (IRMA) from neovascularizations elsewhere (NVE). The vast majority of differences in gradings lay within one level of DR (95.5%), encouraging the hypothesis that gradings are trustworthy, and even in cases of disagreement, gradings are still clinically relevant, of high reliability and useable for research purposes.

Even so, though relatively few grading disagreements were present, a general tendency of underestimation by primary graders is an important, and potentially severe, issue to address, as it might represent a need for more extensive education and certification among graders. Such a certification could ensure that fewer underestimates were made, and that patients are screened at correct intervals as well as referred for timely treatment.

The agreement across graders in the screening programme in Denmark might be largely contributed to the already established education of upcoming ophthalmologists which is consistent across geographical regions, as well as the structured and nationally applied guidelines by the Danish Ophthalmological Society (Grauslund et al., 2018). It might also be attributed, in part, to the ICDR scale used, as this is not based on the graders ability to locate single lesions, but rather evaluate the retina all together. In a study from Denmark, published in 2003, the graders were asked to identify specific retinal lesions and inter-grader agreeability was significantly lower (Larsen et al., 2003). For the sake of testing the reliability of the screening programme for DR in Denmark, the ICDR scale must however be regarded as the most relevant measurement tool, as the most clinically significant consequence is timely referral and treatment, and thus an agreement on overall DR level, not individual lesions.

Further initiatives to strengthen DR gradings, by educational resources such as VIOLA (Andersen et al., 2022), is available to all Danish ophthalmologists screening for DR, and ensures continued skill development, maintenance of high grading quality following up-to-date guidelines. More than 150 ophthalmologists and ophthalmologists in training have completed the programme so far, and even more are utilizing it as a training resource.

This study presents several strengths, which includes the nationwide data from both practicing and hospital-based ophthalmologists, as well as the certification of the secondary grader. The high reliability of DR classification among the various graders in the Danish national screening programme could also have potential to be used for training of artificial intelligence-based algorithms for DR grading. Such algorithms would often require access to tens of thousands of annotated retinal images, but the high agreement among observers in this study is likely to provide a sufficient ground truth grading for algorithm training, as it has also been demonstrated by Potapenko, Kristensen, et al. (2022); Potapenko, Thiesson, et al. (2022). in neovascular age-related macular degeneration.

This study also had some limitations; a small number of images with severe NPDR and PDR were graded in this study, thus indicating a larger degree of uncertainty within these results. Due to the anonymization of primary graders, it was not possible to report the exact number of graders, only to determine the width of geographical range as well as type of screening facility. A number of received images were unfortunately excluded from analyses due to failure during the uploading process, which might have skewed the distribution of DR levels from primary graders. Data collection was done during a limited period in 2020, and whether this is representative to gradings made across the years 2013–2022 is not clear.

In conclusion, we observed a high degree of inter-grader reliability within the Danish screening programme for DR when examining the gradings of DR performed by primary screening ophthalmologists and a secondary certified grader in this nationwide study using different statistic tools for agreement measurement. We believe the results support the reliability of the levels of DR, available in DiaBase, being correct to a broad extend and speaks of the high quality of the Danish screening programme for DR. However, there might still be room for improvement in this established system. The results also has the added benefit of ensuring that the data stored in DiaBase is reliable, and able to be used for register-based research.

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ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki. Consent to participate was obtained from all participating ophthalmologists who participated anonymously and provided anonymized fundus photographs.

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REFERENCES

Andersen, J.K.H., Hubel, M.S., Savarimuthu, T.R., Rasmussen, M.L., Sørensen, S.L.B. & Grauslund, J. (2022) A digital online platform for education and certification of diabetic retinopathy health care professionals in the region of southern Denmark. *Acta Ophthalmologica*, 100, 589–595.

Andersen, N., Hjortdal, J.O., Schielke, K.C., Bek, T., Grauslund, J., Laugesen, C.S. et al. (2016) The Danish registry of diabetic retinopathy. *Clinical Epidemiology*, 8, 613–619.

Cunha, L.P., Figueiredo, E.A., Araújo, H.P., Costa-Cunha, L.V.F., Costa, C.F., Neto, J.M.C. et al. (2018) Non-mydriatic fundus retinography in screening for diabetic retinopathy: agreement between family physicians, general ophthalmologists, and a retinal specialist. *Frontiers in Endocrinology*, 9, 251.

Dansk Diabetes Database: National årsrapport (2020/2021): [Danish Diabetes Database: National Annual Report 2020/2021]. Newest report available from: https://www.sundhed.dk/sundhedsfaglig/kvalitet/kliniske-kvalitetsdatabaser/kroniske-sygdomme/diabetes/.Danish

Grauslund, J., Andersen, N., Andresen, J., Flesner, P., Haamann, P., Heegaard, S. et al. (2018) Evidence-based Danish guidelines for screening of diabetic retinopathy. *Acta Ophthalmologica*, 96, 763–769.

Grauslund, J., Stokholm, L., Ohm Kyvik, K., Dornonville de la Cour, M., Kessel, L. & Hass Rubin, K. (2020) Interactions between ocular and systemic disease using national register-based data in the Danish excellence Centre in Ophthalmic Epidemiology (DECODE-EYE): study perspective. *Acta Ophthalmologica*, 98, 573–578.

Gwet, K.L. (2021) *Handbook of inter-rater reliability*, 5th edition, Analysis of Categorical Ratings. USA: Publisher Services. pp. 28–69.

Larsen, M., Godt, J., Larsen, N., Lund-Andersen, H., Sjølie, A.K., Agardh, E. et al. (2003) Automated detection of fundus photographic red lesions in diabetic retinopathy. *Investigative Ophthalmology & Visual Science*, 44, 761–766.

Potapenko, I., Kristensen, M., Thiesson, B., Ilginis, T., Lykke Sørensen, T., Nouri Hajari, J. et al. (2022) Detection of oedema on optical coherence tomography images using deep learning model trained on noisy clinical data. *Acta Ophthalmologica*, 100, 103–110.

Potapenko, I., Thiesson, B., Kristensen, M., Hajari, J.N., Ilginis, T., Fuchs, J. et al. (2022) Automated artificial intelligence-based system for clinical follow-up of patients with age-related macular degeneration. *Acta Ophthalmologica*, 100, 927–936.

Rêgo, S., Dutra-Medeiros, M., Bacelar-Silva, G.M., Borges, T., Soares, F. & Monteiro-Soares, M. (2021) Reliability of classification by ophthalmologists with telescreening fundus images for diabetic retinopathy and image quality. *Journal of Diabetes Science and Technology*, 15, 710–712.

Scott, I.U., Bressler, N.M., Bressler, S.B., Browning, D.J., Chan, C.K., Danis, R.P. et al. (2008) Agreement between clinician and reading center gradings of diabetic retinopathy severity level at baseline in a phase 2 study of intravitreal bevacizumab for diabetic macular edema. *Retina*, 28, 36–40.

StataCorp. 2021. *Stata Statistical Software: Release 17. College Station*, TX: StataCorp LLC.

Thapa, R., Bajimaya, S., Pradhan, E., Sharma, S., Kshetri, B. & Paudyal, G. (2020) Agreement on grading retinal findings of patients with diabetes using fundus photographs by allied medical personnel when compared to an ophthalmologist at a diabetic retinopathy screening program in Nepal. *Clinical Ophthalmology*, 14, 2731–2737.

Xie, Q. (2013) *Agree or disagree? A demonstration of an alternative statistic to Cohen's kappa for measuring the extent and reliability of agreement between observers*. MacroSys, LLC: The Federal Committee on Statistical Methodology (FCSM), pp. 1–12.

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PAPER II

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ORIGINAL ARTICLE

Attendance in a national screening program for diabetic retinopathy: a population-based study of 205,970 patients

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Abstract

Aims A nationwide diabetic retinopathy (DR) screening program has been established in Denmark since 2013. We aimed to perform an evaluation of adherence to DR screenings and to examine whether non-adherence was correlated to DR progression.

Methods The population consisted of a register-based cohort, who participated in the screening program from 2013 to 2018. We analyzed age, gender, marital status, DR level (International Clinical DR severity scale, none, mild-, moderate-, severe non-proliferative DR (NPDR) and proliferative DR (PDR)), comorbidities and socioeconomic factors. The attendance pattern of patients was grouped as either timely (no delays > 33%), delayed (delays > 33%) or one-time attendance (unexplained).

Results We included 205,970 patients with 591,136 screenings. Rates of timely, delayed and one-time attendance were 53.0%, 35.5% and 11.5%, respectively. DR level at baseline was associated with delays (mild-, moderate-, severe NPDR and PDR) and one-time attendance (moderate-, severe NPDR and PDR) with relative risk ratios (RRR) of 1.68, 2.27, 3.14, 2.44 and 1.18, 2.07, 1.26, respectively ($P < 0.05$). Delays at previous screenings were associated with progression to severe NPDR or PDR (hazard ratio (HR) 2.27, 6.25 and 12.84 for 1, 2 and 3+ delays, respectively). Any given delay doubled the risk of progression (HR 2.28).

Conclusions In a national cohort of 205,970 patients, almost half of the patients attended DR screening later than scheduled or dropped out after first screening episode. This was, in particular, true for patients with any levels of DR at baseline. DR progression in patients with delayed attendance, increased with the number of missed appointments.

Keywords Complications · Diabetes · Diabetic retinopathy · Attendance · Progression · Screening

This article belongs to the topical collection Eye Complications of Diabetes, managed by Giuseppe Querques.

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Introduction

Diabetic retinopathy (DR) is a frequent complication of diabetes, and sight-threatening DR is among the leading causes of preventable blindness in the working-age population [1]. According to the International Diabetes Federation, the global prevalence of diabetes is 10.5% equivalent to 536.6 million people [2] and amongst patients with diabetes the prevalence of DR is approximately 30% [1]. DR, especially at more severe levels, can have vast physical and emotional consequences for the affected patients, and management of the disease requires many resources from healthcare systems [3]. Diabetic eye screening is a crucial part of disease management for all patients with diabetes. In Denmark, screening is recommended immediately after diagnosis of type 2 diabetes and within five years of diagnosis of type 1 diabetes (at age 12 at the earliest) and lifelong screening is recommended [4]. Non-attendance or delay of scheduled screenings might result in new and potential sight-threatening DR changes, that are not discovered timely, hence delaying proper treatment [5]. Incidence of DR can rise significantly in association with delay of screenings [6]. Still the cause for non- and delayed attendance seems to be multifaceted and optimal attendance might be dependent on both incentives and obstacles being prioritized [7, 8]. No studies have, to our knowledge, examined attendance patterns and the potential health consequences in a population-based cohort.

Denmark has a national tax-funded screening program for DR. It is recommended that patients attend screening at either a practicing ophthalmologist or a hospital-based screening facility. Financial reimbursement is provided regardless of screening site and patients with proliferative DR (PDR) or diabetic macular edema are referred for treatment at the public hospital departments of ophthalmology. Denmark is divided geographically into five regions; the Capital Region of Denmark, Central Denmark Region, North Denmark Region, Region Zealand and Region of Southern Denmark [9]. The regions are responsible for the Danish hospitals and the health services provided by practicing physicians, including practicing ophthalmologists. The capital of Denmark, Copenhagen, is located in the Capital Region of Denmark. Screening is done by either retinal fundus photographs alone or by a combination of photographs and clinical examination. Individualized intervals are planned according to national guidelines [4] and defined by the level of DR as well as glycemic control.

In this study, we aimed to utilize the Danish registers to examine attendance patterns in the Danish nationwide DR screening program, to characterize timely, delayed and one-time attending patients, as well as explore the effects of delayed attendance on DR progression.

Methods and materials

Participants

In this retrospective nationwide cohort study, our population was defined by the data in The Danish Registry of Diabetic Retinopathy (DiaBase), which contains data of all patients who had attended DR screening at least once, from January 2, 2013, to December 30, 2018 [10]. We included data from all 591,136 screening visits by 205,970 patients (Table 1), above 18 years of age.

Data Sources

We utilized the Danish national registers where all data can be linked on an individualized level. This includes entire medical records, socioeconomic data and prescription medication usage.

Diabase, which defined our population, contains data reported by the screening ophthalmologist, and the database has approximately 100,000 additions annually [11]. From DiaBase, we extracted reported and planned screening dates, DR level according to the International Clinical DR severity scale (ICDR scale, no DR = 0, mild non-proliferative DR (NPDR) = 1, moderate NPDR = 2, severe NPDR = 3 or PDR = 4), screening facility (hospital or practicing ophthalmologists) and geographical region of screening (Capital Region of Denmark, Central Denmark Region, North Denmark Region, Region Zealand and Region of Southern Denmark).

In addition to DiaBase, we utilized the following registers:

The Danish Civil Registry (1968) was used to link data across registries using an individual identification number (CPR number) given to all citizens in Denmark [12]. We extracted date of birth, sex (female or male), status (alive, institutionalized, living in Greenland, living abroad, missing or dead) and marital status (never married, married or divorced/widowed). The Danish National Patient Register (1976) contains information on all patients treated at Danish hospitals. This includes the specific department, diagnoses according to the International Classification of Disease (ICD) version ten codes, surgical procedures, treatments and other procedures [13]. The Danish National Prescription Registry (1994) is a unique pharmacological register and one of the largest of its kind worldwide [14]. The registry contains information on all collected prescriptions of medicine nationwide, connected to CPR number. This includes information on the Anatomical Therapeutic Chemical (ATC) classification of the medication as well as detailed information on all prescriptions. The Danish National Patient Register and The Danish National Prescription Registry

Table 1 Characteristics of patients at baseline, according to attendance group

	All, $n=205,970$	Timely attendance, $n=109,135$	Delayed attendance, $n=73,242$	One-time attendance, $n=23,593$	*P* value
Sex, % Male	116,534 (56.6)	62,567 (57.3)	40,610 (55.4)	13,357 (56.6)	< 0.001
Age, Years (IQR)	66 (55;73)	66 (56;73)	65 (54;73)	66 (54;74)	< 0.001
Marital status					< 0.001
Never married	30,904 (15.0)	16,035 (14.7)	11,050 (15.1)	3819 (16.2)	
Married	118,764 (57.7)	63,833 (58.5)	42,718 (58.3)	12,213 (51.8)	
Widowed or divorced	56,302 (27.3)	29,267 (26.8)	19,474 (26.6)	7561 (32.0)	
Diabetes type, *N* (%)					< 0.001
Type 1 diabetes	16,999 (8.3)	7492 (6.9)	8375 (11.4)	1132 (4.8)	
Type 2 diabetes	153,238 (74.4)	85,786 (78.6)	48,791 (66.6)	18,661 (79.1)	
Unknown	35,733 (17.3)	15,857 (14.5)	16,076 (21.9)	3800 (16.1)	
DR level (ICDR), *N* (%)[A]					< 0.001
No DR	171,633 (83.3)	95,507 (87.5)	55,464 (75.7)	20,662 (87.6)	
Mild NPDR	20,964 (10.2)	9009 (8.3)	10,157 (13.9)	1798 (7.6)	
Moderate NPDR	6551 (3.2)	2405 (2.2)	3583 (4.9)	563 (2.4)	
Severe NPDR	1153 (0.6)	327 (0.3)	687 (0.9)	139 (0.6)	
PDR	5165 (2.5)	1727 (1.6)	3007 (4.1)	431 (1.8)	
Charlson comorbidity index score, *N* (%)[B]					< 0.001
Low	148,615 (72.2)	79,792 (73.1)	51,920 (70.9)	16,903 (71.6)	
Moderate low	27,728 (13.5)	12,984 (11.9)	11,798 (16.1)	2946 (12.5)	
Moderate high	18,721 (9.1)	10,252 (9.4)	6137 (8.4)	2332 (9.9)	
High	10,906 (5.3)	6107 (5.6)	3387 (4.6)	1412 (6.0)	
Screening facility, *N* (%)					< 0.001
Private practice	161,418 (78.4)	89,210 (81.7)	53,241 (72.7)	18,967 (80.4)	
Hospital	44,552 (21.6)	19,925 (18.3)	20,001 (27.3)	4626 (19.6)	
Region of screening, *N* (%)					< 0.001
Capital region of Denmark	53,303 (25.9)	24,363 (22.3)	20,908 (28.5)	8032 (34.0)	
Region Zealand	33,299 (16.2)	17,531 (16.1)	11,332 (15.5)	4436 (18.8)	
Central Denmark region	41,499 (20.1)	24,581 (22.5)	12,733 (17.4)	4185 (17.7)	
North Denmark Region	22,248 (10.8)	9945 (9.1)	9761 (13.3)	2542 (10.8)	
Region of Southern Denmark	55,575 (27.0)	32,690 (30.0)	18,488 (25.2)	4397 (18.6)	
Socioeconomic status					
Income (household net worth), *N* (%)					< 0.001
Low	50,484 (24.5)	23,942 (21.9)	19,704 (26.9)	6838 (29.0)	
Moderate low	50,310 (24.4)	26,383 (24.2)	18,140 (24.8)	5787 (24.5)	
Moderate high	50,953 (24.7)	27,857 (25.5)	17,640 (24.1)	5456 (23.1)	
High	52,660 (25.6)	29,491 (27.0)	17,711 (24.2)	5458 (23.1)	
Education, *N* (%)					< 0.001
Lower secondary	77,796 (37.8)	40,620 (37.2)	27,676 (37.8)	9500 (40.3)	
Upper secondary	85,012 (41.3)	45,902 (42.1)	29,880 (40.8)	9230 (39.1)	
Post-secondary	36,122 (17.5)	19,103 (17.5)	13,232 (18.1)	3787 (16.1)	
Occupation, *N* (%)					< 0.001
Employed or employer	58,533 (28.4)	31,016 (28.4)	21,332 (29.1)	6185 (26.2)	
Student or other	5179 (2.5)	2571 (2.4)	1953 (2.7)	655 (2.8)	
Early retirement	28,404 (13.8)	14,608 (13.4)	10,526 (14.4)	3270 (13.9)	
Retirement	101,135 (49.1)	54,663 (50.1)	34,882 (47.6)	11,590 (49.1)	
Unemployed	12,715 (6.2)	6274 (5.7)	4549 (6.2)	1892 (8.0)	
Ethnic background, *N* (%)					< 0.001
Danish heritage	183,476 (89.1)	98,237 (90.0)	65,072 (88.8)	20,167 (85.5)	
Other heritage	22,457 (10.9)	10,882 (10.0)	8160 (11.1)	3415 (14.5)	

Results given as number (%) or median (IQR). [A]Classification of DR given by the International Clinical Diabetic Retinopathy Severity Scale [30], [B]Excluding diabetes

were utilized for categorizing diabetes type (type 1, type 2 or unknown) [15] as well as to categorize patients' comorbidities according to the Charlson Comorbidity Index score (CCI, 1 = low, 2 = moderate low, 3 = moderate high or 4 = high) [16]. Furthermore, socioeconomic data were acquired from Statistics Denmark [17]; we extracted information on equivalent household income (low, moderate low, moderate high and high), highest achieved level of education (lower secondary, upper secondary and post-secondary) in accordance with the International Standard Classification of Education (ISCED) [18], affiliation to the labor market (employed, student, unemployed, early retirement or retirement) [19] and ethnicity (Danish or other).

Quantitative variables

The index date was defined as the first screening date, and delay was calculated according to the next recommended screening interval, as given by the screening physician. Patients were classified as having timely attendance if they were never delayed > 33% and did not miss any screenings during follow-up. Patients were classified as delayed if the actual date of the next screening was registered beyond 33% of the intended interval, e.g., a patient with a recommended interval of 90 days, would therefore be classified as delayed if the next screening date was more than 30 days after the planned screening date (Fig. 1).

One-time attendance was defined as a patient only participating in screening once, with no follow-up appointments, without apparent reason. Patients with a scheduled next screening date beyond the observation period or who were referred for treatment (for DR or other eye-related illness) and therefore exited the screening program, as well as patients, who disappeared or died before their next screening, were censored at exit date and, thus, only included in the analyses in the periods where they could be clearly classified. DR progression was defined as a worsening in DR to either severe NPDR or PDR in either eye.

Statistical methods

Descriptive data on the population were reported in numerical format with percentages for all variables except age, which was reported in median and interquartile range. Statistical significance was calculated using the Chi^2 test. Using a multinomial logistic regression model with relative risk ratio (RRR) calculations, we compared the characteristics of patients with delayed and one-time attendance to patients with timely attendance depending on various exposure variables. The model included a crude, semi-adjusted (age and gender) and fully adjusted multivariable analysis, adjusted for all statistically significant exposure variables from Table 1 (age, gender, marital status, diabetes type, DR level, modified CCI score (excluding diabetes), screening facility, geographical region of screening, income, education length, occupation and ethnic descent). A multivariable Cox regression model with hazard ratios (HRs) was performed to examine a potential risk of progression in DR level that could be associated with delayed screening intervals. Time-varying analyses were utilized to examine each individual screening period. A period was defined as the time from one screening to next screening and could be timely or delayed. Risk time only included delayed periods, and time splitting at missed screening visits was utilized to define delayed periods from timely periods. A patient stopped contributing with risk time, when they attending a screening again, but could contribute again later on, if another > 33% delay occurred (Fig. 1). All analyses were done in Stata 17 (StataCorp, College Station, Texas, USA), and P-values < 0.05 and confidence intervals (CIs) not including 1.0 were considered statistically significant.

Results

Descriptive data

The population (n = 205,970) consisted of 56.6% males, had a median age of 65.7 years (55;73), and 89.1% were of Danish lineage (Table 1, Fig. 2). Baseline prevalence of DR was 16.5% (10.2%, 3.2%, 0.6% and 2.5% for levels 1–4, respectively). Rates of timely attendance, delayed attendance and one-time attendance in the population was 53.0%, 35.5% and 11.5%, respectively. Compared to patients with timely attendance, delayed attendance and one-time attendance were more often observed in females (42.7% vs. 44.6% and

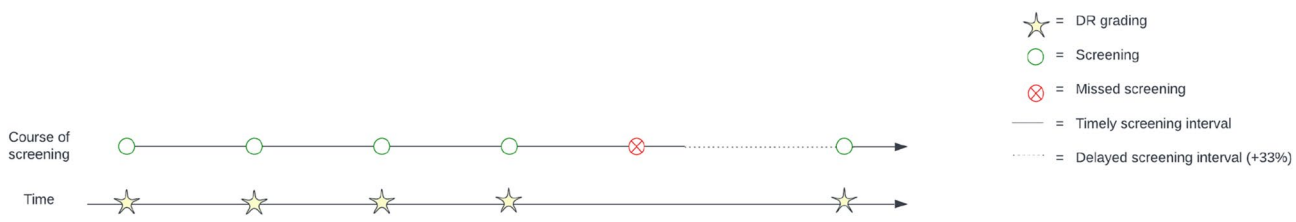

Fig. 1 Timeline illustration of intervals and screening visits with DR gradings, indicating risk time as delayed screening interval

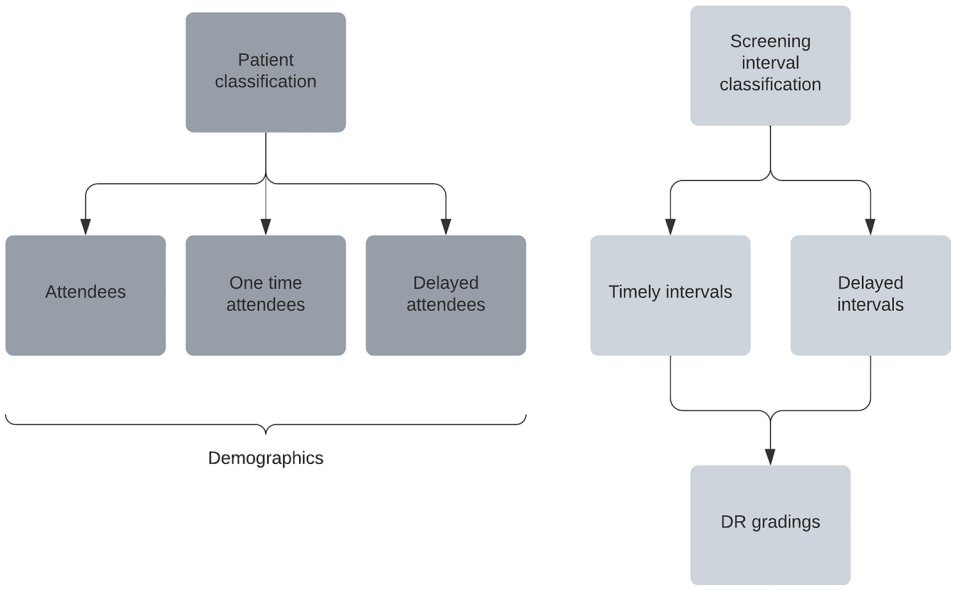

Fig. 2 Flowchart with key elements of study design

43.4%), non-married patients (14.7% vs. 15.1% and 16.2%) and patients of other ethnic descent than Danish (10.0% vs. 11.1% and 14.5%). Furthermore, compared to patients with timely attendance, patients with delayed attendance had a higher prevalence of DR (12.5% vs. 24.3%), more often type 1 diabetes (6.9% vs. 11.4%) and were screened more frequently at hospitals (18.3% vs. 27.3%). Patients with one-time attendance were more comparable to patients with timely attendance in all three parameters (12.4%, 4.8% and 19.6%). Patients from all five Danish regions were represented, but with varying degrees of adherence. The Central Denmark Region had the highest percentage of attendance, and the North Denmark Region had the lowest (59.2% vs. 44.7%). The North Denmark Region had the highest number of patients with delayed attendance, while the Central Denmark Region had the lowest, within their screened populations (43.9% vs. 30.7%). The highest number of patients with one-time attendance was found in the Capital Region of Denmark and the lowest in the Region of Southern Denmark (15.1% vs. 7.9%). Compared to patients with timely attendance, delayed and one-time attendance were more often observed in patients with lower income (26.9% and 29.0% vs. 21.9%), lower educational level (37.8% and 40.3% vs. 37.2%) and a higher rate of unemployment (6.2% and 8.0% vs. 5.7%).

Main results

Delayed attendance

The multivariable multinomial logistic regression (Table 2) showed that patients with delayed attendance were less likely to be of male gender (RRR 0.94 (95% CI 0.92–0.96)), be older in age (40–59 years (RRR 0.79 (95% CI 0.75;0.85)), 60–79 years (0.76 (95% CI 0.72;0.81)), 80+ years (0.78 (95% CI 0.73;0.84)) and have type 2 diabetes (0.67 (95% CI 0.64;0.70)) compared to patients attending screening at recommended intervals. Having delayed attendance was associated with being either divorced/widowed or married (RRR 1.14 (95% CI 1.10;1.18) and 1.19 (95% CI 1.15;1.23)), having DR level 1–4 (RRR 1.68 (95% CI 1.63;1.74), 2.27 (95% CI 2.14;2.40), 3.14 (95% CI 2.72;3.62), 2.44 (95% CI 2.29;2.61)), a CCI score of 1 (RRR 1.08 (95% CI 1.04;1.11)) and being screened at a hospital based facility (RRR 1.07 (95% CI 1.04;1.10)) in either the Capital Region of Denmark of Denmark (RRR 1.31 (95% CI 1.28;1.35)) or the North Denmark Region (RRR 1.52 (95% CI 1.46;1.58)). Socioeconomically, delayed attendance was mainly associated with having a low income (RRR 1.19 (95% CI 1.16;1.23)), but also being employed (RRR 1.22 (95% CI 1.14;1.31)), in retirement (RRR 1.33 (95% CI 1.23;1.44)) or in early retirement (RRR 1.23 (95% CI 1.14;1.33)).

One-time attendance

One-time attendance was associated with being male (RRR 1.04 (95% CI 1.01;1.08)), divorced or widowed (RRR 1.09 (95% CI 1.03;1.15)), having type 2 diabetes (RRR 1.47 (95% CI 1.36;1.59)), DR level 2–4 ((RRR 1.18 (95% CI 1.06;1.30), 2.07 (95% CI 1.67;2.57), 1.26 (95% CI 1.13;1.42)) or CCI scores of 2 or 3 ((RRR 1.07 (95% CI 1.02;1.13), 1.09 (95% CI 1.02;1.16)) compared to patients attending screening at recommended intervals (Table 2). One-time attending patients were more likely to be screened in the Capital Region of Denmark of Denmark (RRR 1.31 (95% CI 1.26;1.37)). One-time attendance was

Table 2 Multinomial regression showing the risk of delayed and one-time attendance according to exposure variables, reported in relative risk ratios (RRR) with 95% confidence intervals (CI)

	Delayed attendees					One-time attendees				
	n (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value	*n* (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value
Sex										
Male	40,610 (55.4)	0.93 (0.91;0.94)	0.92 (0.90;0.94)	0.94 (0.92;0.96)	0.0000	13,357 (56.6)	0.97 (0.94;1.00)	0.97 (0.94;1.00)	1.04 (1.01;1.08)	0.0083
Female	32,632 (44.6)	Ref	Ref	Ref		10,236 (43.4)	Ref	Ref	Ref	
Age, Years										
18–39	5823 (8.0)	Ref	Ref	Ref		1538 (6.5)	Ref	Ref	Ref	
40–59	21,027 (28.7)	0.69 (0.66;0.72)	0.69 (0.66;0.72)	0.79 (0.75;0.83)	0.0000	7145 (30.3)	0.89 (0.83;0.94)	0.89 (0.83;0.94)	0.70 (0.65;0.75)	<0.001
60–79	40,223 (54.9)	0.63 (0.61;0.66)	0.64 (0.61;0.66)	0.76 (0.72;0.81)	0.0000	11,865 (50.3)	0.71 (0.67;0.75)	0.71 (0.67;0.75)	0.53 (0.49;0.58)	<0.001
80+	6169 (8.4)	0.62 (0.59;0.65)	0.62 (0.59;0.65)	0.78 (0.73;0.84)	0.0000	3045 (12.9)	1.16 (1.08;1.24)	1.16 (1.08;1.24)	0.83 (0.75;0.92)	<0.001
Marital Status										
Never married	11,050 (15.1)	Ref	Ref	Ref		3819 (16.2)	Ref	Ref	Ref	
Married	42,718 (58.3)	0.97 (0.95;1.00)	1.08 (1.05;1.11)	1.19 (1.15;1.23)	0.0000	12,213 (51.8)	0.80 (0.77;0.84)	0.82 (0.78;0.85)	0.85 (0.81;0.89)	<0.001
Widowed or divorced	19,474 (26.6)	0.97 (0.94;1.00)	1.11 (1.07;1.15)	1.14 (1.10;1.18)	0.0000	7561 (32.0)	1.08 (1.04;1.13)	1.12 (1.06;1.17)	1.09 (1.03;1.15)	0.0014
Diabetes type, *N* (%)										
Type 1 diabetes	8375 (11.4)	Ref	Ref	Ref		1132 (4.8)	Ref	Ref	Ref	
Type 2 diabetes	48,791 (66.6)	0.51 (0.49;0.53)	0.53 (0.51;0.55)	0.67 (0.64;0.70)	0.0000	18,661 (79.1)	1.44 (1.35;1.54)	1.55 (1.44;1.66)	1.47 (1.36;1.59)	<0.001
Unknown	16,076 (21.9)	0.91 (0.87;0.94)	0.94 (0.91;0.98)	0.99 (0.95;1.04)	0.7542	3800 (16.1)	1.59 (1.48;1.70)	1.69 (1.57;1.82)	1.53 (1.41;1.66)	<0.001
DR level (ICDR), *N* (%)[A]										
No DR	55,464 (75.7)	Ref	Ref	Ref		20,662 (87.6)	Ref	Ref	Ref	
Mild NPDR	10,157 (13.9)	1.94 (1.88;2.00)	1.92 (1.86;1.98)	1.68 (1.63;1.74)	0.0000	1798 (7.6)	0.92 (0.88;0.97)	0.92 (0.87;0.97)	0.95 (0.90;1.01)	0.0959
Moderate NPDR	3583 (4.9)	2.57 (2.43;2.70)	2.54 (2.41;2.68)	2.27 (2.14;2.40)	0.0000	563 (2.4)	1.08 (0.99;1.19)	1.08 (0.99;1.19)	1.18 (1.06;1.30)	0.0017
Severe NPDR	687 (0.9)	3.62 (3.17;4.13)	3.51 (3.07;4.00)	3.14 (2.72;3.62)	0.0000	139 (0.6)	1.96 (1.61;2.40)	1.96 (1.61;2.39)	2.07 (1.67;2.57)	<0.001
PDR	3007 (4.1)	3.00 (2.82;3.18)	2.96 (2.79;3.14)	2.44 (2.29;2.61)	0.0000	431 (1.8)	1.15 (1.04;1.28)	1.15 (1.04;1.28)	1.26 (1.13;1.42)	<0.001
Charlson comorbidity index score, *N* (%)[B]										
Low	51,920 (70.9)	Ref	Ref	Ref		16,903 (71.6)	Ref	Ref	Ref	
Moderate low	11,798 (16.1)	1.40 (1.36;1.44)	1.41 (1.37;1.45)	1.08 (1.04;1.11)	0.0000	2946 (12.5)	1.07 (1.03;1.12)	1.07 (1.03;1.12)	1.04 (0.99;1.09)	0.1280
Moderate high	6137 (8.4)	0.92 (0.89;0.95)	0.96 (0.93;1.00)	0.87 (0.84;0.90)	0.0000	2332 (9.9)	1.07 (1.02;1.13)	1.08 (1.03;1.14)	1.07 (1.02;1.13)	0.0068
High	3387 (4.6)	0.85 (0.82;0.89)	0.91 (0.87;0.95)	0.74 (0.71;0.78)	0.0000	1412 (6.0)	1.09 (1.03;1.16)	1.10 (1.04;1.17)	1.09 (1.02;1.16)	0.0090
Screening facility, *N* (%)										
Practicing Ophthalmologist	53,241 (72.7)	Ref	Ref	Ref		18,967 (80.4)	Ref	Ref	Ref	

Table 2 (continued)

	Delayed attendees					One-time attendees				
	n (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value	*n* (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value
Hospital	20,001 (27.3)	1.68 (1.64;1.72)	1.65 (1.61;1.69)	1.07 (1.04;1.10)	0.0000	4626 (19.6)	1.09 (1.05;1.13)	1.09 (1.05;1.14)	0.92 (0.88;0.96)	< 0.001
Region of screening, *N* (%)										
Capital region of Denmark	20,908 (28.6)	1.33 (1.29;1.37)	1.31 (1.28;1.35)	1.30 (1.26;1.34)	0.0000	8032 (34.0)	1.30 (1.25;1.36)	1.30 (1.25;1.36)	1.31 (1.26;1.37)	< 0.001
Region Zealand	11,332 (15.5)	Ref	Ref	Ref		4436 (18.8)	Ref	Ref	Ref	
Central Denmark region	12,733 (17.4)	0.80 (0.78;0.83)	0.79 (0.77;0.82)	0.77 (0.74;0.79)	0.0000	4185 (17.7)	0.67 (0.64;0.70)	0.67 (0.64;0.70)	0.68 (0.65;0.72)	< 0.001
North Denmark region	9761 (13.3)	1.52 (1.46;1.57)	1.52 (1.46;1.57)	1.52 (1.46;1.58)	0.0000	2542 (10.8)	1.01 (0.96;1.07)	1.01 (0.96;1.07)	1.01 (0.96;1.08)	0.6338
Region of Southern Denmark	18,488 (25.2)	0.87 (0.85;0.90)	0.87 (0.85;0.90)	0.84 (0.81;0.87)	0.0000	4397 (18.6)	0.53 (0.51;0.56)	0.53 (0.51;0.56)	0.51 (0.49;0.54)	< 0.001
socioeconomic status										
income (Household net worth), *N* (%)										
Low	19,704 (26.9)	1.20 (1.17;1.23)	1.19 (1.16;1.22)	1.19 (1.16;1.23)	0.0000	6838 (29.0)	1.30 (1.25;1.35)	1.30 (1.25;1.35)	1.18 (1.13;1.24)	< 0.001
Moderate low	18,140 (24.8)	Ref	Ref	Ref		5787 (24.6)	Ref	Ref	Ref	
Moderate high	17,640 (24.1)	0.92 (0.90;0.95)	0.93 (0.90;0.95)	0.91 (0.89;0.94)	0.0000	5456 (23.2)	0.89 (0.86;0.93)	0.89 (0.86;0.93)	0.92 (0.88;0.96)	< 0.001
High	17,711 (24.2)	0.87 (0.85;0.90)	0.88 (0.86;0.90)	0.84 (0.82;0.87)	0.0000	5458 (23.2)	0.84 (0.81;0.88)	0.84 (0.81;0.88)	0.85 (0.81;0.89)	< 0.001
Education, *N* (%)										
Lower secondary	27,676 (39.1)	0.98 (0.96;1.01)	1.01 (0.98;1.04)	0.97 (0.94;1.00)	0.0254	9500 (42.2)	1.18 (1.13;1.23)	1.19 (1.14;1.24)	1.11 (1.06;1.16)	< 0.001
Upper secondary	29,880 (42.2)	0.94 (0.92;0.97)	0.95 (0.93;0.98)	0.94 (0.91;0.96)	0.0000	9230 (41.0)	1.01 (0.97;1.06)	1.02 (0.97;1.06)	0.97 (0.93;1.02)	0.2127
Post-secondary	13,232 (18.7)	Ref	Ref	Ref		3787 (16.8)	Ref	Ref	Ref	
OCCUPATION, *N* (%)										
Employed or employer	21,332 (29.1)	0.91 (0.85;0.96)	1.10 (1.03;1.17)	1.22 (1.14;1.31)	0.0000	6185 (26.2)	0.78 (0.72;0.86)	0.76 (0.69;0.83)	0.93 (0.84;1.03)	0.1658
Student or other	1953 (2.7)	Ref	Ref	Ref		655 (2.8)	Ref	Ref	Ref	
Early retirement	10,526 (14.4)	0.95 (0.89;1.02)	1.10 (1.03;1.19)	1.23 (1.14;1.33)	0.0000	3270 (13.9)	1.18 (1.07;1.31)	1.16 (1.04;1.28)	0.85 (0.76;0.95)	0.0043
Retirement	34,882 (47.6)	0.95 (0.89;1.01)	1.20 (1.12;1.28)	1.33 (1.23;1.44)	0.0000	11,590 (49.1)	0.88 (0.80;0.97)	0.84 (0.76;0.93)	0.87 (0.78;0.98)	0.0209
Unemployed	4549 (6.2)	0.84 (0.79;0.89)	1.30 (1.21;1.40)	1.08 (0.99;1.16)	0.0680	1892 (8.0)	0.83 (0.76;0.91)	0.77 (0.69;0.85)	1.03 (0.92;1.16)	0.5775
Ethnic background, *N* (%)										
Danish heritage	65,072 (88.9)	Ref	Ref	Ref		20,167 (85.5)	Ref	Ref	Ref	

Table 2 (continued)

	Delayed attendees					One-time attendees				
	n (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value	*n* (%)	RRR crude (95%CI)	RRR semi adjusted (95%CI)	RRR fully adjusted (95%CI)	*P*-value
Other heritage	8160 (11.1)	1.13 (1.10;1.17)	1.08 (1.05;1.12)	0.98 (0.94;1.01)	0.2181	3415 (14.5)	1.53 (1.47;1.59)	1.53 (1.47;1.60)	1.20 (1.14;1.26)	< 0.001

Data are given as numbers (*n*, (%)) and relative risk ratios (95% CI). Reference group is patients with timely attendance. [A]Classification of DR given by the International Clinical Diabetic Retinopathy Severity Scale [30], [B]Excluding diabetes. Semi adjusted model adjusted for sex and age. Fully adjusted model adjusted for all statistically significant variables in Table 1

inversely associated with age (40–59 (RRR 0.70 (95% CI 0.65;0.75)), 60–79 (0.53 (95% CI 0.49;0.58)) and 80+ (0.83 (95% CI 0.75;0.92)). Socioeconomically one-time attendance was associated with a low income (RRR 1.18 (95% CI 1.13;1.24)), lower educational length (RRR 1.11 (95% CI 1.06;1.16)) and other ethnic heritage than Danish (RRR 1.20 (95% CI 1.14;1.26)).

Progression

Cox regression analysis (Table 3, Fig. 3) showed that any delay in screening resulted in double the risk of progression to severe NPDR or PDR (2.28 HR (95% CI 1.97;2.64). Patients with past delayed intervals were more likely to experience disease progression to severe NPDR or PDR during follow-up; the risk increased by the number of missed appointments so that patients with delays in 1, 2 or 3+ appointments had increased risks of HR 2.27 (95% CI 1.93;2.68), HR 6.25 (95% CI 4.96;7.88) and HR 12.84 (95% CI 9.21;17.88) for progression, compared to patients who attended screenings timely.

Discussion

This study is, to our knowledge, the most extensive study in the field of attendance to DR screening, utilizing 591,136 screening episodes by 205,970 patients with diabetes in a nationwide cohort. Our research showed that delayed attendance and one-time attendance of DR screenings were associated with younger age, divorce, lower income, screening in the Capital Region of Denmark, as well as higher levels of DR and competing illnesses. Progression to more advanced DR (severe NPDR and PDR) was seen more often in patients with delayed attendance, and the number of delays was correlated to a significantly increased risk of progression. This is in accordance with a study from England [6], in which the number of missed screenings were examined in a retrospective observational study of 62,067 patients in the North East London Diabetes Eye Screening Programme. A 20% increase in the incidence of referable DR was demonstrated in patients that missed ten or more consecutive appointments. We found that patients age 40 years and above were less likely to be delayed or have one-time attendance, compared to the 60–79 age group. Which is in agreement with previous studies from England [20–23], Ireland [24] and Scotland [25] thus confirming a trend across DR screening programs internationally. Delayed attendance was observed to be vastly increasing according to more severe DR levels at baseline compared to patients with no DR. Paradoxically, the patients who needed the timely screenings the most, were the ones who utilized it the least. This, in turn, could also be part of the explanation as to why their DR was in fact of a more

Table 3 Risk of progression to severe non-proliferative diabetic retinopathy (DR) or proliferative DR according to number of delays, given in hazard ratios (HR) and 95% confidence intervals (CI)

	Events	Risk time	Crude HR (CI 95%)	Semi adjusted HR (CI 95%)	Fully adjusted HR (CI 95%)	*P*-value
Number of delayed periods						
0	1015	324,108.39	Ref	Ref	Ref	
1	670	123,844.96	2.34 (1.97;2.79)	2.26 (1.90;2.68)	2.27 (1.93;2.68)	< 0.001
2	229	12,869.24	8.80 (6.89;11.24)	7.40 (5.82;9.41)	6.25 (4.96;7.88)	< 0.001
3+	75	1812.18	21.15 (15.13;29.57)	17.18 (12.29;24.03)	12.84 (9.21;17.88)	< 0.001
Any given delay						
Timely interval	1321	379,180.86	Ref	Ref	Ref	
Delayed interval	574	80,626.27	2.09 (1.81;2.42)	2.07 (1.79;2.39)	2.28 (1.97;2.64)	< 0.001

Data are given as numbers and hazard ratios (confidence interval). Semi adjusted model adjusted for sex and age. Fully adjusted model adjusted for all statistically significant variables in Table 1. [A]Progressions. [B]Risk time given in person-days per 1000

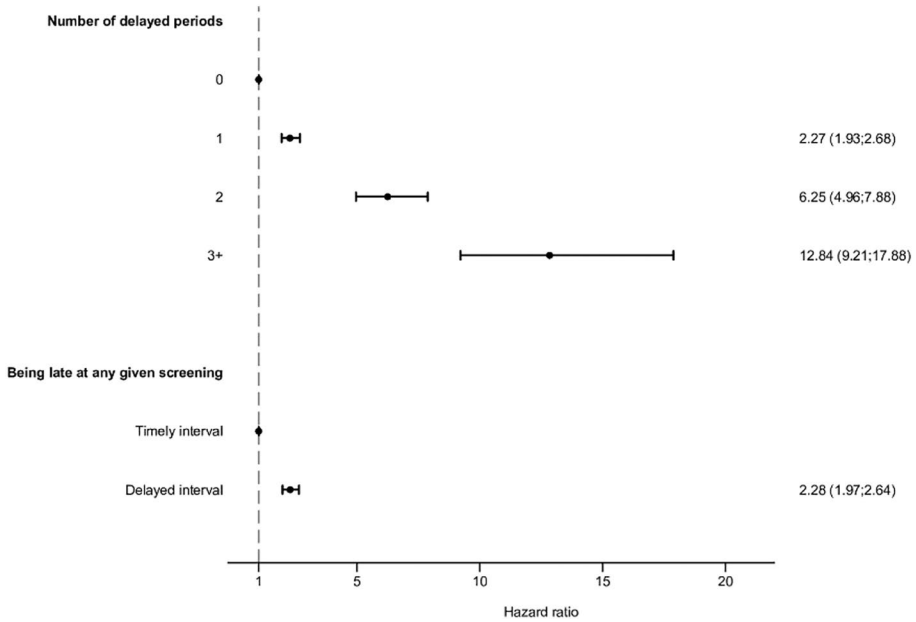

Fig. 3 Forest plot illustrating the findings of Table 3; the risk of progression to severe non-proliferative diabetic retinopathy (DR) or proliferative DR (PDR) according to number of delayed periods and at any given screening Data are given in hazard ratios (HRs) with confidence intervals (CI)

severe level. It should be noted that the groups of patients diagnosed with severe NPDR made up a small percentage of the cohort as a whole, and therefore, there might be a larger statistical uncertainty in the results for these patients. Several studies examining the incentives and barriers of patients to DR screening found that a great facilitator to attendance was the knowledge of the potential consequences of non-attendance on vision and DR progression [24, 26–28]. This could be a point of focus to ensure proper communication and dissemination of DR awareness from healthcare professionals to patients with diabetes—in Denmark, as well as internationally. This could also help combat the anxiety that might counterintuitively keep some patients from attending a screening, because the fear of a severe examination result or the possible societal stigma is too overwhelming. Patients who attended screenings at practicing ophthalmologists were more adherent to their given intervals than patients at hospitals. Because of the centralization of larger hospitals in Denmark, access to practicing ophthalmologists might be logistically easier and more accessible to patients, especially in rural areas. Distance to the screening facility has previously been shown as a barrier to screening [8]. To increase the convenience for patients, DR screenings can often be timed with other diabetes-related screenings including podiatry, cardiology and endocrinology appointments at most Danish hospitals. Attendance in the different geographical regions of Denmark varied; although we observed a greater non-adherence in the North Denmark Region, we also observed this in the Capital Region of Denmark of Denmark, where patients were more likely to have both delayed and one-time attendance. This could be due to the more diverse population composition in metropolitan areas, including younger

people, with lower incomes. Technical issues, partly due to the implementation of a new electronic medical record system, might also have affected the data received in DiaBase from hospitals in Region Zealand and the Capital Region of Denmark, introducing a potential bias. Patients with type 2 diabetes were more likely to only attend screening once compared to patients with type 1 diabetes. This could be due to the fact that type 2 diabetes often is discovered later in life, and perhaps in relation to other lifestyle-related illnesses; patients might, therefore, not be accustomed to the sudden burden of appointments this entails. One-time attendance could partly be explained by patients with prediabetes or who are undergoing a medical investigation to determine a potential diabetes diagnosis, that have been recommended a screening by their general practicing physician. We found a correlation between both delayed and one-time attendance and general comorbidity in regards to higher CCI scores across the regression analysis, indicating that patients who are suffering from competing illnesses might not have the surplus to also keep up screening at timely intervals, or at all. Socioeconomic deprivation in terms of low income and unemployment was seen as risk factor for delayed and one-time attendance. The risk of non-adherence was lower in patients with higher incomes, showcasing a potential distortion and inequality in health care access according to income. Several studies credit socioeconomic deprivation as the leading cause of non-attendance [25, 29], and even though an association in a Danish setting is apparent in regard to low income and non-Danish descent, it might not be as stark due to the generally flatter societal structure as well as the completely tax-funded healthcare system, where no out of pocket expenses are needed. Length of education did not significantly change the odds of delayed or one-time attendance, as seen in previous studies [7].

The inclusion of a large nationwide cohort with a considerable amount of screenings, and detailed, validated register information on an individualized level, is a clear strength of this study. The addition of socioeconomic data ensured the completeness of the characterization of the study population.

As our study focused on adherence to the screening program, and patients attending DR screening at least once, we did not address the issue of patients never attending screening, which might add another dimension. Due to the register-based nature of the study, the subjective reasons for non-adherence were not addressed. This would, however, be important for future reference, as an involvement of patients and a prioritization of their prerogative will be crucial in order to improve attendance.

In conclusion, our study of non-adherence successfully added information on a population basis using a national cohort of patients in the Danish screening program of DR in Denmark. We highlighted younger age, divorce, presence of DR, competing illnesses and low income as the characteristics of patients with delayed and one-time attendance in the Danish screening program and showed twice the risk of progression to severe NPDR and PDR in patients with delayed attendance.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analyses were performed by AST, LMS, LR and SM. The first draft of the manuscript was written by AST, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets created and analyzed during the current study are available from the Danish Health Data Authority and Statistics Denmark, but restrictions apply to the availability of these data, which were used under license from OPEN and Danish Health Data Authority and are not publicly available.

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was performed in accordance with the principles of the Declaration of Helsinki. No ethical approvals or informed consent agreements were required, as this is an observational register-based study.

References

1. Wong TY et al (2016) Diabetic retinopathy. Nat Rev Dis Primers 2:16012
2. Sun H et al (2022) IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract 183:109119
3. Fenwick EK et al (2011) The impact of diabetic retinopathy: understanding the patients' perspective. Br J Ophthalmol 95(6):774–782
4. Grauslund J et al (2018) Evidence-based Danish guidelines for screening of diabetic retinopathy. Acta Ophthalmol 96(8):763–769

5. Scanlon PH, Aldington SJ, Stratton IM (2014) Delay in diabetic retinopathy screening increases the rate of detection of referable diabetic retinopathy. Diabet Med 31(4):439–442
6. Virk R et al (2021) How is the risk of being diagnosed with referable diabetic retinopathy affected by failure to attend diabetes eye screening appointments? Eye (Lond) 35(2):477–483
7. Kashim RM, Newton P, Ojo O (2018) Diabetic retinopathy screening: a systematic review on patients' non-attendance. Int J Environ Res Public Health 15(1):157
8. Graham-Rowe E et al (2018) Barriers to and enablers of diabetic retinopathy screening attendance: a systematic review of published and grey literature. Diabet Med 35(10):1308–1319
9. Regional Denmark. Danske Regioner. https://www.regioner.dk/services/in-english/regional-denmark
10. Andersen N et al (2016) The Danish registry of diabetic retinopathy. Clin Epidemiol 8:613–619
11. DDD, National yearly report Danish Diabetes Database (DDD, The Danish Adult Diabetes Database, Danish Registry of Childhood and Adolescent Diabetes and The Danish Registry of Diabetic Retinopathy). 2020/2021
12. Schmidt M, Pedersen L, Sørensen HT (2014) The Danish civil registration system as a tool in epidemiology. Eur J Epidemiol 29(8):541–549
13. Schmidt M et al (2015) The Danish national patient registry: a review of content, data quality, and research potential. Clin Epidemiol 7:449–490
14. Kildemoes HW, Sørensen HT, Hallas J (2011) The Danish national prescription registry. Scand J Public Health 39(7 Suppl):38–41
15. Larsen MEC et al (2021) Diabetic retinopathy as a potential marker of Parkinson's disease: a register-based cohort study. Brain Commun 3(4):fcab262
16. Quan H et al (2011) Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol 173(6):676–682
17. Møller B et al (2001) Official statistics in Denmark: Socio economic microdata for research
18. UNESCO International Standard Classification of Education, ISCED 2011. UNESCO Institute for Statistics
19. Quitzau J, Pedersen U Documentations of statistics, labour and income. Statistics Denmark
20. Lawrenson JG et al (2021) Trends in diabetic retinopathy screening attendance and associations with vision impairment attributable to diabetes in a large nationwide cohort. Diabet Med 38(4):e14425
21. Sachdeva A et al (2012) Diabetic retinopathy screening: study to determine risk factors for non-attendance. Diabetes Prim Care 14(5):308
22. Gulliford MC et al (2010) Socio-economic and ethnic inequalities in diabetes retinal screening. Diabet Med 27(3):282–288
23. Millett C, Dodhia H (2006) Diabetes retinopathy screening: audit of equity in participation and selected outcomes in South East London. J Med Screen 13:152–155
24. Dervan E et al (2008) Factors that influence the patient uptake of diabetic retinopathy screening. Ir J Med Sci 177(4):303–308
25. Leese GP et al (2008) Screening uptake in a well-established diabetic retinopathy screening program: the role of geographical access and deprivation. Diabetes Care 31(11):2131–2135
26. Lake AJ et al (2017) What factors influence uptake of retinal screening among young adults with type 2 diabetes? A qualitative study informed by the theoretical domains framework. J Diabetes Complicat 31(6):997–1006
27. Hipwell AE et al (2014) Attitudes, access and anguish: a qualitative interview study of staff and patients' experiences of diabetic retinopathy screening. BMJ Open 4(12):e005498
28. Van Eijk KND et al Diabetic retinopathy screening in patients with diabetes mellitus in primary care: incentives and barriers to screening attendance. Diabetes Res Clin Pract
29. Waqar S et al (2012) Cost implications, deprivation and geodemographic segmentation analysis of non-attenders (DNA) in an established diabetic retinopathy screening programme. Diabetes Metab Syndr 6(4):199–202
30. Wilkinson CP et al (2003) Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 110(9):1677–1682

PAPER IV

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ORIGINAL ARTICLE

Bariatric surgery in individuals with type 2 diabetes is not associated with short or long-term risk of diabetic retinopathy progression: results from a nationwide cohort study

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Abstract

Aims Bariatric surgery is used to induce weight loss and glycemic stability in type 2 diabetes (T2D). It has been a concern that this may lead to early worsening of diabetic retinopathy (DR) due to a rapid decline in HbA1c. In this study, we evaluated the risk of short and long-term DR development and need for ocular intervention in an entire nation of individuals with T2D undergoing bariatric surgery.

Methods The study comprised a national, register-based cohort of individuals with T2D screened for DR. Cases were matched by age, sex and DR level at the date of surgery (index date) with non-bariatric controls. We extracted information on DR levels, in- and outpatient treatments, pharmaceutical prescriptions and laboratory values. We evaluated worsening of DR (incident and progressive DR) at follow-up (6 and 36 months).

Results Amongst 238,967 individuals with T2D, who attended diabetic eye screening, we identified 553 that underwent bariatric surgery (0.2%) and 2677 non-bariatric controls. Median age was 49 years, and 63% were female. Cases had more comorbidities, lower HbA1c as well as more frequent use of glucose-lowering and antihypertensive medication than controls at index date. In a fully adjusted logistic regression model, the risk of DR worsening for cases was not significantly different compared to controls, neither short-term (OR 0.41 [CI 95% 0.13; 1.33], $p=0.14$) nor long-term (OR 0.64 [CI 95% 0.33; 1.24], $p=0.18$).

Conclusions In this nationwide study, bariatric surgery did not associate with increased risk of short- or long-term DR worsening.

Keywords Bariatric surgery · Diabetes · Diabetic retinopathy · Epidemiology · Nationwide

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Abbreviations

BMI	Body mass index
CCI	Charlson comorbidity index
DR	Diabetic retinopathy
DiaBase	The Danish registry of diabetic retinopathy
GLP1	Glugacon like peptide 1
ICDR	International clinical diabetic retinopathy severity scale
MI	Multiple imputation
NPDR	Non proliferative DR
OPEN	Open patient data explorative network
PDR	Proliferative DR
SGLT2	Selective sodium glucose co transporter
TG	Triacylglycerol
uACR	Urine albumin/creatinine ratio

Introduction

The global continuously increasing prevalence of type 2 diabetes has been described as a pandemic, surpassing 462 million affected individuals in 2017 and estimated to be the ninth leading cause of mortality [1]. Diabetic retinopathy (DR) is the most frequent complication to diabetes and a prominent cause of blindness [2]. Many risk factors have proved significant in relation to the development of DR, including hyperglycemia, hypertension, hyperlipidemia and obesity (all modifiable) as well as duration of diabetes and pregnancy (both non-modifiable) [3]. Bariatric surgery is a well-established medical intervention in patients with type 2 diabetes and severe overweight. In addition to significant weight loss it drastically changes the metabolic profile of the patient [4], including improvement in lipids and insulin sensitivity, and sometimes leading to diabetes remission post operatively [5]. The effect of bariatric surgery on retinal microvasculature is not well established, and existing research is not in agreement on the potential effects on DR. A systematic review and meta-analysis done in 2014 found a tendency towards progression of DR, probably due to a rapid decline in HbA1c levels post-surgery, which decreased as much as 3.9% (18.6 mmol/mol) compared to pre-surgery measurements [6]. However, more recent systematic reviews and meta-analyzes from 2017, found a better prognosis of DR, with lower incident rates and less progression compared to patients who did not undergo bariatric surgery [7, 8]. To our knowledge, no larger, register-based studies have examined the need for ocular intervention (laser treatment, intravitreal anti VEGF injection or vitrectomy) after bariatric surgery, but an observational study from 2016 found no instances where surgical intervention was warranted amongst their cohort [9].

Research on the subject is still inconclusive, with smaller study populations and lacking information regarding regression as well as the potential interventional consequences of DR progression, affecting patients with type 2 diabetes undergoing bariatric surgery. Hence, in this study, we aimed to explore the effect of bariatric surgery on DR development, in an entire population of individuals with type 2 diabetes, during a 3 year follow-up period.

Methods

We performed a register-based matched cohort study utilizing the Danish registers. The cohort was identified in The Danish Registry of Diabetic Retinopathy (DiaBase), a national Danish clinical quality database, that holds information regarding all patients screened for DR in Denmark since 2013[10]. Data from various other national Danish registers were also included to enrich data; The Danish Civil Registration System [11] provided basic information on age, sex and civil status as well as enabled data linkage between registers due to the unique identification number (CPR number) given to all Danish inhabitants, The Danish National Patient Register [12] with diagnostic and treatment codes for in- and outpatient care, the Register of Laboratory Results for Research [13] with nationwide biochemical measurements and finally The Danish National Prescription Registry [14] that provided information on all prescribed and redeemed pharmaceuticals in Denmark.

The registers, utilized in this study, have been described in details by Grauslund et al. [15].

Participants

As cases, we included patients registered in DiaBase with type 2 diabetes, above the age of 18 at index date, that had undergone any form of bariatric surgery from 2013 to 2022. Index date was set as the date of bariatric surgery defined by the registration of a KJDF* (gastric bypass, gastric banding and gastric sleeve) ICD-10 surgical code. Patients registered with a KJDF* code prior to 2013, were excluded from the case population. The control group was selected amongst the remaining DiaBase population with type 2 diabetes, with no history of bariatric surgery, matched to cases by sex, age (year of birth) and level of DR at index date (Table 1). For screening specific outcomes, patients with fewer than 2 screening episodes, were excluded from both case and control groups.

Outcomes

Our main outcome was DR worsening (quantifiable at screening visits) in which incident (DR level 1–4 at

Table 1 Baseline characteristics for patients with type 2 diabetes with (cases) and without (controls) bariatric surgery at index date

	All	Bariatric surgery		*P* value
	n = 3230	Yes (cases) n = 553	No (controls) n = 2677	
Sex, % female	2032 (62.9%)	348 (62.9%)	1684 (62.9%)	0.99
Age, years (IQR)	49 (42–55)	49 (42–55)	49 (42–55)	0.50
Duration of diabetes, years (IQR)	6.09 (2.4–11.1)	5.10 (1.9–9.9)	6.22 (2.57–11.35)	< 0.001
Marital status, n (%)				0.55
Never married	981 (30.4%)	163 (29.5%)	818 (30.6%)	
Married	1732 (53.6%)	293 (52.9%)	1439 (53.8%)	
Widowed or divorced	517 (16.0%)	97 (17.5%)	420 (15.7%)	
CCI score, n (%)				0.002
0 (low)	2536 (78.5%)	406 (73.4%)	2130 (79.6%)	
1 (moderate low)	432 (13.4%)	90 (16.3%)	342 (12.8%)	
2 (moderate high)	195 (6.0%)	48 (8.7%)	147 (5.5%)	
≥ 3 (high)	67 (2.1%)	9 (1.6%)	58 (2.2%)	
Level of DR, n (%)*				0.40
0 (no DR)	2878 (89.1%)	487 (88.1%)	2391 (89.3%)	
1 (mild NPDR)	209 (6.5%)	36 (6.5%)	173 (6.5%)	
2 (moderate NPDR)	101 (3.1%)	18 (3.3%)	83 (3.1%)	
3 (severe NPDR)	22 (0.7%)	6 (1.1%)	16 (0.6%)	
4 (PDR)	20 (0.6%)	6 (1.1%)	14 (0.5%)	
BMI, n (%)				
Class I obesity (BMI 30–34.9)	12 (2.2%)	12 (2.2%)	NA	
Class II obesity (BMI 35–39.9)	119 (21.6%)	119 (21.6%)	NA	
Class III obesity (BMI 40–55 +)	195 (35.3%)	195 (35.3%)	NA	
Undefined overweight	226 (40.9%)	226 (40.9%)	NA	
HbA1c, median [IQR]*	6.9% (52 [45–62])	6.5% (48 [42–55])	7.0% (53 [46–63])	< 0.001
Pharmacological treatment*				
Glucose-lowering medication				
GLP-1	838 (25.9%)	274 (49.6%)	564 (21.1%)	< 0.001
SGLT-2	483 (14.9%)	98 (17.7%)	385 (14.4%)	0.045
Metformin	2337 (72.4%)	454 (82.1%)	1883 (70.3%)	< 0.001
Insulin	939 (29.1%)	156 (28.2%)	783 (29.3%)	0.62
Antihypertensive medication n (%)	1920 (59.4%)	406 (73.4%)	1514 (56.6%)	< 0.001
Cholesterol lowering medication n (%)	2019 (62.5%)	354 (64.0%)	1665 (62.2%)	0.42

Results are given in counts (n) or medians with percentages (%) or interquartile range (IQR). BMI was only available for cases, as it is not measured routinely for patients not undergoing bariatric surgery

Index date date of bariatric surgery, *CCI* Charlson comorbidity index, *DR* diabetic retinopathy, *NPDR* non proliferative DR, *PDR* proliferative DR, *BMI* body mass index, *HbA1c* glycated hemoglobin, *GLP-1* glucagon Like Peptide 1, *SGLT-2* selective sodium glucose co transporter 2

*Closest measurement/registration prior to index date (within 1 year)

follow-up) and progressive DR (≥ two-step progression or progression to PDR) were pooled and assessed at month 6 and month 36, as well as the need for post-surgical ocular intervention (panretinal or focal photocoagulation, intravitreal injections or vitrectomy) assed within 1 year and after 1 year of surgery. DR improvement (≥ two-step regression) during follow-up was also evaluated at month 6 and 36. Finally, we examined changes in pharmaceutical treatments as well as biochemical measurements amongst cases and controls during follow-up.

Covariates

From DiaBase, we extracted information on screening dates and level of DR at each screening (ICDR scale [16], 0–4 [0 = no DR, 1 = mild non proliferative DR (NPDR),

2 = moderate NPDR, 3 = severe NPDR and 4 = proliferative DR (PDR)]).

From The Danish Civil Registration System we used age, sex (female or male), and marital status (married/cohabitating, single or divorced).

The Danish National Patient Register provided information on surgical interventions (bariatric surgery [KJDF*], vitrectomy [(KCKD65 and DH334B) or (KCKD65 and DH431 and DH36*)], intravitreal anti VEGF injection [(KCKD05B) and not (DH34* or DH353*) within 6 months prior to injection], panretinal [KCKC15 and not DH34* within 6 months prior to injection] and focal photocoagulation [KCKC10 and DH36*]) as well as systemic illnesses used to calculate a modified (excluding diabetes) Charlson Comorbidity Index score (CCI score) 5 years prior to index date (0 [low], 1 [moderate low], 2 [moderate high] and ≥ 3 [high]). The register also provided the diagnostic codes for the classification of bodyweight (unspecified overweight [DE660, DE660A, DE668 and DE669], obesity grade 1 [DE660B], obesity grade 2 [DE660C] and obesity grade 3 [DE660E, D660F, D660G and D660H]), from which a marker of BMI was constructed.

From the Register of Laboratory Results for Research we extracted information on laboratory values for measurements of hemoglobin A1c (HbA1c [% (mmol/mol)]), plasma creatinine (P-crea [µmol/L]), albumin/creatinine ratio in urine (uACR [mg/g]), estimated glomerular filtration rate (eGFR [mL/minute/1.73 m^2]), low density lipoprotein cholesterol (LDL [mmol/L]), total cholesterol (mmol/L), high density lipoprotein cholesterol (HDL [mmol/L] and triacylglyceroles (TG [mmol/L]).

Finally, we utilized The Danish National Prescription Registry, from which information on prescribed and redeemed medications (antihypertensive-, antidiabetic- [GLP1 analogues, SGLT2 inhibitors, insulins and non-insulins] and lipid lowering medications) was used.

To differentiate between patients according to type of diabetes (type 1 and type 2 diabetes), we examined patients' diagnosis- and pharmaceutical codes from The Danish National Patient Register and The Danish National Prescription Registry and divided them using an endocrinologist recommended algorithm "Appendix".

Statistical methods

All data analyzes were performed with Stata 17.0 (StataCorp LLC., College Station, Texas, USA). Data are presented descriptively with medians and interquartile ranges (IQR) or counts and percentages. Statistical significance was defined as p-values < 0.05 and confidence intervals not including 1. In Table 1, Wilcoxon rank-sum and Pearson's chi-square test were used for continuous and categorical variables, respectively, to determine possible differences between cases and controls. To examine the relation of bariatric surgery and DR (worsening and improvement), semi-adjusted (age and sex) and fully adjusted (age, sex and all significant differences in Table 1) multiple logistic regression models resulting in odds ratios (OR) were used (Table 3). A Cox regression model resulting in hazard ratios (HR) with same adjustment steps was used for examining post-surgical ocular intervention. OR were calculated at fixed post-surgery timepoints to assess a potential transient worsening (month six ± 3 months), and also a more long-term effect (month 36 ± 9 months) using the screening date closest to these. HR for ocular intervention were calculated short-term (index date till month 12) and long-term (month 12 till end of follow-up). To utilize data from both eyes, clustered standard errors were applied to all regression models. Cases were matched to controls with replacements and we aimed for a case control ratio of 1:5, but as some cases were matched to fewer controls due to the demands of the matching criteria, a final ratio of 1:4.8 was obtained. In cases where missing data were present, and exceeded acceptable levels, multiple imputation (MI) was used when appropriate, determined by the type of missing data (missing completely at random, missing at random or missing not at random), which was evaluated both statistically and logically using preexisting, established knowledge of covariates and how they were obtained in clinical settings.

Results

Among 238,967 patients with type 2 diabetes attending the Danish screening program for DR from 2013 to 2022, we identified 553 cases who underwent bariatric surgery during follow-up and matched them to 2677 non-bariatric controls. Included individuals were primarily female (62.9%) and had a median age of 49 years (IQR 42–55 years), they had a higher CCI score (moderate low [16.3 vs. 12.8%] and moderate high [8.9 vs. 5.5%], $p < 0.01$), shorter duration of diabetes (5.1 vs. 6.2 years, $p < 0.01$), better glycemic stability (HbA1c 6.5% vs. 7.0% [48.0 vs. 53.0 mmol/l], $p < 0.01$) as well as more frequent use of metformin (82.1 vs. 70.3%, < 0.01), antihypertensive medications (73.4 vs. 56.6%, < 0.01), GLP-1 analogues (49.5 vs. 21.1%, $p < 0.01$) and SGLT-2 inhibitors (17.7 vs. 14.4%, $p = 0.04$) than controls at index date. They did not differ in regards to marital status, use of insulin or cholesterol lowering medications (Table 1).

DR worsening (incident DR and progressive DR pooled) at 6 and 36 months was seen in 2.9% and 5.2% of cases and 8.4% and 7.9% of controls (Table 2). Odds for short and long-term DR worsening after bariatric surgery were OR 0.32 (CI 95% 0.12–0.84, $p = 0.02$) and OR 0.68 (CI 95% 0.35–1.33, $p = 0.26$) in the semi adjusted model (Table 3).

Table 2 Post-surgical DR worsening and improvement events in cases (with bariatric surgery) and controls (without bariatric surgery) at 6 and 36 months

	Cases eligible for analysis (%)	Controls eligible for analysis (%)	6 months				36 months			
			Eligible cases with screening at 6 months (± 3 months)	Events, cases (%)	Eligible controls with screening at 6 months (± 3 months)	Events, controls (%)	Eligible cases with screening at 36 months (± 9 months)	Events, cases (%)	Eligible controls with screening at 36 months (± 9 months	Events, controls (%)
DR improvement	44 (22.1%)	155 (77.9%)	11 (25.0%)	< 5	76 (49.03%)	15 (19.7%)	9 (20.5%)	6 (66.7%)	68 (43.9%)	25 (36.8%)
DR worsening	1095 (17.1%)	5315 (82.9%)	274 (25.0%)	8 (2.9%)	404 (7.6%)	34 (8.4%)	249 (22.7%)	13 (5.2%)	2131 (40.1%)	169 (7.9%)

DR improvement was defined as 2 step improvement of DR. DR worsening was defined as incident, 2-step-progression or progression to proliferative DR (PDR). Criteria of eligibility for analysis of improvement was DR level 2 or 3 at baseline. Criteria for eligibility for analysis of DR worsening was DR level 0 at baseline and DR level 1–4 at follow-up (incident DR), DR level 0–2 at baseline and DR level 2–4 at follow-up (DR progression) or DR level 0–3 at baseline and DR level 4 at follow-up (progression to PDR)

DR diabetic retinopathy

In the fully adjusted model with MI for missing HbA1c values (9.4% and 13.7% of cases and controls respectively), results for short- and long-term worsening were OR 0.41 (CI 95% 0.13–1.33, $p = 0.14$) and OR 0.71 (CI 95% 0.34–1.46, $p = 0.35$) respectively (Table 3). We found no accounts of either short- or long-term ocular treatment needs post-surgery in our case population except for < 5 cases of intravitreal injections (too few events to statistically analyze). We performed a post-hoc analysis stratified by pre-existing DR at index date to examine whether this impacted the odds of DR development, however we found no increased odds of DR worsening at any point, in either group (Supplementary table 2).

We examined biochemical measurements (HbA1c, lipids and nephrology) as well as medication use (insulin, non-insulin glucose lowering, antihypertensive and lipid lowering medication) pre- and post-surgery (short- and long-term) to evaluate how bariatric surgery affected these parameters (Supplementary Table 1). In pre-surgical measurements, glycemic stability was best amongst cases and a significant drop in HbA1c was seen leading up to and directly following surgery (Fig. 1). Although a slight increase in HbA1c was seen in cases long-term compared to directly after surgery, the levels still remained lower than pre-surgical measurements, and were lower than control individuals levels at all times (6.5 vs. 7.0% [48.0 vs. 53.0 mmol/mol] $p < 0.001$, 5.8 vs. 6.9% [39.8 vs. 52.0 mmol/mol] $p < 0.001$ and 5.9 vs. 7.2% [41.0 vs. 55.0] $p < 0.001$) (Supplementary table 1). Plasma TG levels were reduced in cases after surgery, and stayed below the upper recommended limit for patients with diabetes, whereas TG levels in controls stayed high (1.98 vs. 1.80 mmol/l; $p < 0.001$, 1.29 vs. 1.81 mmol/l; $p < 0.001$ and 1.40 vs. 1.76 mmol/l; $p < 0.001$) throughout follow-up. Kidney function measured by glomerular filtration (eGFR), plasma creatinine and urine albumin/creatinine ratio (uACR) were within normal limits during the follow-up time, in both groups. Due to a detected interaction between bariatric surgery and BMI in regards to DR development, we also performed a stratified analysis according to BMI at 36 months post-surgery. This showed no difference in odds of DR worsening or ocular intervention, no matter the degree of overweight.

Discussion

In this nationwide study examining the risk of DR worsening amongst individuals with type 2 diabetes who underwent bariatric surgery, we did not find evidence indicating signs of post-surgical transient or long-term worsening of DR, nor an increased need for ophthalmological intervention. Regression analysis suggested lower or equal rates of DR

Table 3 Multiple regression analysis with short- and long-term odds of DR improvement and DR worsening in cases (with bariatric surgery) compared to controls (without bariatric surgery)

	Short-term				Long-term			
	Semi adjusted		Fully adjusted		Semi adjusted		Fully adjusted	
	Adjusted OR	*P* value	Adjusted OR	*P* value	Adjusted OR	*P* value	Adjusted OR	*P* value
DR improvement	1.53 (0.23; 10.19)	0.66	1.25 (0.07; 21.67)	0.88	3.44 (0.48; 24.41)	0.22	3.25 (0.31; 34.00)	0.33
DR worsening	0.33 (0.12; 0.86)	0.02	0.41 (0.13; 1.33)	0.14	0.64 (0.33; 1.24)	0.18	0.71 (0.34; 1.46)	0.35

Short-term = 6 months ± 3 months. Long-term = 36 months ± 9 months. Semi adjusted = sex and age adjusted. Fully adjusted = adjusted for age, sex and all significant factors in Table 1 with multiple imputations (MI) for HbA1c. DR improvement was defined as 2 step improvement of DR. DR worsening was defined as incident, 2-step-progression or progression to proliferative DR (PDR)

HbA1c glycated hemoglobin, *DR* diabetic retinopathy

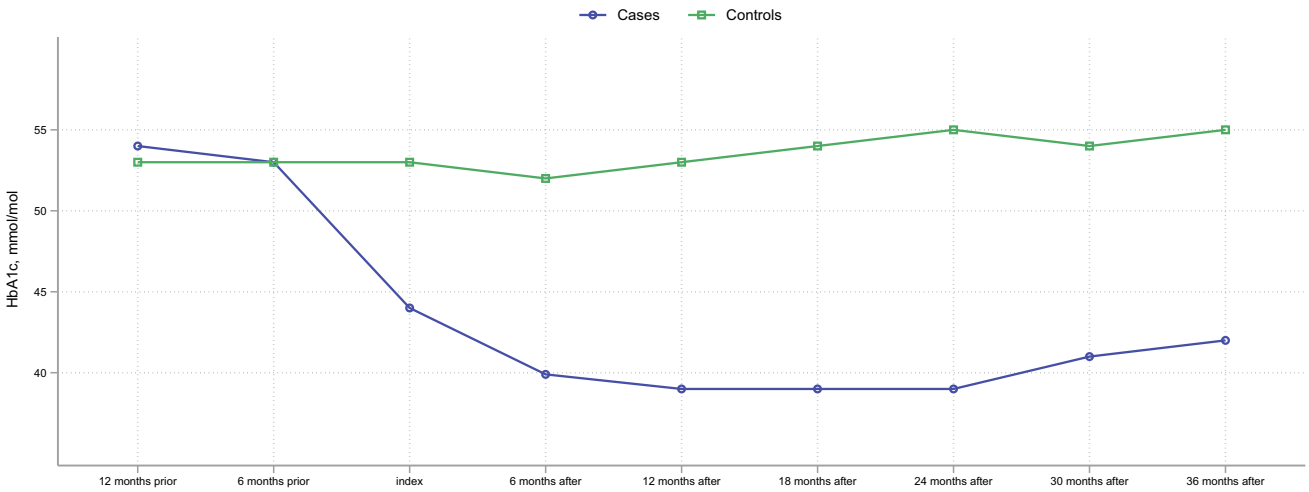

Fig. 1 Median HbA1c measurements in mmol/mol at fixed time points from 12 months prior to bariatric surgery until 36 months post-surgery for cases (with bariatric surgery) and at equal points for controls (without bariatric surgery)

worsening in individuals who underwent bariatric surgery compared to persons who did not.

This positive outcome aligns with several recent, smaller studies suggesting that bariatric surgery has no detrimental effect on the development of DR in patients with type 2 diabetes [7, 8]. Our nationwide data showed good baseline glycemic stability amongst our case population, as well as a pre-surgical decline in HbA1c followed by a further post-surgical decrease in HbA1c, which might explain the low rates of progression. Previous studies disagree on the role of pre-surgical HbA1c levels in regards to DR progression; with one study showing that a higher baseline HbA1c with a significant post-surgical drop increased the risk of DR progression [17], another study did not find an association [18]. The rates of DR worsening were also lower than seen amongst the general screening population of patients with type 2 diabetes in the Danish screening program [19] suggesting that patients eligible for bariatric surgery are following the pre-surgical guidelines promoting good pre-surgical glycemic stability, weightloss and lifestyle changes, amongst other initiatives, all intended to ensure optimal results of surgery [20]. Another reason for good glycemic stability, and generally acceptable biochemical measurements amongst our population as a whole, might be the effectiveness of the screening program itself alongside other healthcare appointments at the patients primary care physician; discovering tendencies towards worsening in DR or irregularities in systemic examinations and bloodwork, thus being able to induct initiatives to improve glycemic stability, and in turn halt further progression. Finally, our case population had a shorter diabetes duration than our control population which might also be in their favor, considering the known association between diabetes duration and DR development [21]. The low progression rates support the virtually non-existent need for ocular intervention post-surgery, where no differences were detected between cases and controls, as ocular intervention is tied to PDR and progression was seen in 2.9% and 8.4% of cases and controls, respectively. Nutritional deficits following malabsorptive bariatric surgery is

well known and vitamin D deficiency has been associated with DR worsening [22]. Lower levels of vitamin A could cause several ophthalmic issues such as nyctalopia, corneal and conjunctival xerosis leading to ulcerations might also be of concern [23], especially in patients with diabetes, with decreased corneal sensitivity. We were not able to conduct any sub-group analysis where cases were stratified by type of bariatric surgery, due to insufficient data on specific type's bariatric procedures. When stratified by DR level at index date no increased odds were seen in either group, which is in accordance with previous findings [8], however the overall small number of events in our study must be taken in to consideration. We found a significant postoperative drop in plasma TG amongst our case population, which is in accordance with previous studies [24–26].

Our study was strengthened by the vast amount of register-based data, linked throughout several registers, providing a representative sample of the majority of the Danish population with type 2 diabetes and with an established high level of completeness [14, 27]. The long-term follow-up time was also a strength, as post-surgical changes in DR potentially could be more long-term, due to the effect of post-prandial hypoglycemia seen in some patients after bariatric surgery [28].

We must also address some limitations to the study, starting with BMI measurements. BMI (or height and weight) is not routinely registered in any nationwide Danish registers, so a marker using ICD-10 codes had to be constructed, and measurements were only available for the case population. We did not have access to information on lifestyle factors such as smoking status, alcohol consumption and other dietary choices. Another limitation to consider is that our study relied on a screening database, which means that we did not have access to data from individuals with diabetes who never attended the DR screening program and the available data for screening specific outcomes are limited to the dates of screening.

In conclusion, this population-based study adds support to the claim that bariatric surgery is safe in regards to patients with type 2 diabetes. In our population of patients, with overall good glycemic stability, undergoing bariatric surgery, we did not observe increased odds of short- or long-term DR worsening or need for ophthalmic intervention, regardless of pre-existing DR at index date.

Appendix

Diabetes classification in the Danish registers developed by the Ocular and Systemic complications In diabetic retinopathy (OASIS) study group

In a generic, non-selected population (National Patient Registry).

Type 1 diabetes	Latest given diagnostic code must be DE10
	AND
	First prescription of A10A within a year of first DE10 diagnosis*
	AND
	Last prescription of A10A within a year of exit
	AND number of prescriptions ≥ number of years from first prescription to exit
Type 2 diabetes	Diagnostic code DE11
	AND
	≥ two A10B prescriptions**
	OR
	≥ two DE11 diagnostic codes
	OR
	≥ two prescriptions of A10A if age 40 + at prescription
	OR
	≥ two prescriptions of A10B if age 30 + at prescription
	Exclusions:
	Already grouped as type 1 diabetes
	OR/AND
	Female AND diagnostic code for PCOS (E282) AND no diagnostic code for diabetes type II (DE11)

In a population consisting exclusively of patients presumed to have diabetes (DiaBase).

Type 1 diabetes	Latest given diagnostic code in The National Patient Register = DE10*
	AND
	First prescription of A10A within a year of first DE10 diagnosis
	AND
	Last prescription of A10A within a year of exit
	AND number of prescriptions ≥ number of years from first prescription to exit
Type 2 diabetes	The remaining population

DiaBase Danish registry of diabetic retinopathy

*Since data from The Danish National Prescription Registry is available from 1995 and onward, patients with a diagnosis given before this year and prescriptions starting in 1995, could be excluded unnecessarily. In the case of diagnosis given before 1995, the first prescription must therefore be in 1995

**One prescription is allowed for patients who received their diagnosis during 2022

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Data availability The datasets analyzed in the current study are available from the Danish Health Data Authority, but restrictions apply to the availability of these data, which were used under license from OPEN and the Danish Health Data Authority and thus are not publicly available.

Declarations

Conflict of interest None of the authors have any relevant financial or non-financial involvement in relationships or activities benefitting of, or related to, the present study.

Informed consent No formal consent agreements were obtained, due to the observational, register-based design of the study.

References

1. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al KJ (2020) Epidemiology of type 2 diabetes—global burden of disease and forecasted trends. J Epidemiol Glob Health 10(1):107–111. https://doi.org/10.2991/jegh.k.191028.001
2. Heng LZ, Comyn O, Peto T et al (2013) Diabetic retinopathy: pathogenesis, clinical grading, management and future developments. Diabet Med 30(6):640–650. https://doi.org/10.1111/dme.12089
3. Milluzzo A, Maugeri A, Barchitta M, Sciacca L, Agodi A (2021) Epigenetic mechanisms in type 2 diabetes retinopathy: a systematic review. Int J Mol Sci. https://doi.org/10.3390/ijms221910502
4. Cornejo-Pareja I, Clemente-Postigo M, Tinahones FJ (2019) Metabolic and endocrine consequences of bariatric Surgery. Front Endocrinol (Lausanne) 10:626. https://doi.org/10.3389/fendo.2019.00626
5. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD et al (2014) Bariatric surgery versus intensive medical therapy for diabetes—3-year outcomes. N Engl J Med 370(21):2002–2013. https://doi.org/10.1056/NEJMoa1401329
6. Cheung D, Switzer NJ, Ehmann D, Rudnisky C, Shi X, Karmali S (2015) The impact of bariatric surgery on diabetic retinopathy: a systematic review and meta-analysis. Obes Surg 25(9):1604–1609. https://doi.org/10.1007/s11695-014-1539-9
7. Merlotti C, Ceriani V, Morabito A, Pontiroli AE (2017) Bariatric surgery and diabetic retinopathy: a systematic review and meta-analysis of controlled clinical studies. Obes Rev 18(3):309–316. https://doi.org/10.1111/obr.12490
8. Kim YJ, Kim BH, Choi BM, Sun HJ, Lee SJ, Choi KS (2017) Bariatric surgery is associated with less progression of diabetic retinopathy: a systematic review and meta-analysis. Surg Obes Relat Dis 13(2):352–360. https://doi.org/10.1016/j.soard.2016.10.002
9. Brynskov T, Laugesen CS, Svenningsen AL, Floyd AK, Sørensen TL (2016) Monitoring of diabetic retinopathy in relation to bariatric surgery: a prospective observational study. Obes Surg 26(6):1279–1286. https://doi.org/10.1007/s11695-015-1936-8
10. Andersen N, Hjortdal JO, Schielke KC, Bek T, Grauslund J, Laugesen CS et al (2016) The Danish registry of diabetic retinopathy. Clin Epidemiol 8:613–619. https://doi.org/10.2147/CLEP.S99507
11. Schmidt M, Pedersen L, Sørensen HT (2014) The Danish civil registration system as a tool in epidemiology. Eur J Epidemiol 29(8):541–549. https://doi.org/10.1007/s10654-014-9930-3
12. Lynge E, Sandegaard JL, Rebolj M (2011) The Danish national patient register. Scand J Public Health 39(7 Suppl):30–33. https://doi.org/10.1177/1403494811401482
13. Arendt JFH, Hansen AT, Ladefoged SA, Sørensen HT, Pedersen L, Adelborg K (2020) Existing data sources in clinical epidemiology: laboratory information system databases in Denmark. Clin Epidemiol 12:469–475. https://doi.org/10.2147/clep.S245060
14. Kildemoes HW, Sørensen HT, Hallas J (2011) The Danish national prescription registry. Scand J Public Health 39(7 Suppl):38–41. https://doi.org/10.1177/1403494810394717
15. Grauslund J, Stokholm L, Ohm Kyvik K, Dornonville de la Cour M, Kessel L, Hass RK (2020) Interactions between ocular and systemic disease using national register-based data in the Danish excellence centre in ophthalmic epidemiology (DECODE-EYE): study perspective. Acta Ophthalmol 98(6):573–578. https://doi.org/10.1111/aos.14415
16. Wilkinson CP, Ferris FL 3rd, Klein RE et al (2003) Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 110(9):1677–1682. https://doi.org/10.1016/s0161-6420(03)00475-5
17. Murphy R, Jiang Y, Booth M et al (2015) Progression of diabetic retinopathy after bariatric surgery. Diabet Med 32(9):1212–1220. https://doi.org/10.1111/dme.12727
18. Kim YJ, du Seo R, Kim MJ, Lee SJ, Hur KY, Choi KS (2015) Clinical course of diabetic retinopathy in Korean type 2 diabetes after bariatric surgery: a pilot study. Retina 35(5):935–943. https://doi.org/10.1097/iae.0000000000000412
19. Grauslund J, Pedersen FN, Andersen N et al (2023) Presence and development of diabetic retinopathy in 153 238 patients with type 2 diabetes in the Danish registry of diabetic retinopathy. Acta Ophthalmol 101(2):207–214. https://doi.org/10.1111/aos.15264
20. Endocrinology DSo National Danish treatment guidelines for bariatric surgery. https://endocrinology.dk/nbv/andre-endokrinologiske-sygdomme/fedmekirurgi/.
21. Yau JW, Rogers SL, Kawasaki R et al (2012) Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 35(3):556–564. https://doi.org/10.2337/dc11-1909

22. Chan HN, Zhang XJ, Ling XT et al (2022) Vitamin D and ocular diseases: a systematic review. Int J Mol Sci. https://doi.org/10.3390/ijms23084226
23. Guerreiro RA, Ribeiro R (2015) Ophthalmic complications of bariatric surgery. Obes Surg 25(1):167–173. https://doi.org/10.1007/s11695-014-1472-y
24. Zaki MKS, Al-Jefri OH, Kordi RE et al (2021) Correlation of bariatric surgery effect on lipid profile among obese patients. Cureus 13(9):e18118. https://doi.org/10.7759/cureus.18118
25. Lira NS, Macedo CES, Belo GM, Santa-Cruz F, Siqueira LT, Ferraz ÁAB (2018) Analysis of the lipid profile of patients submitted to sleeve gastrectomy and Roux-en-Y gastric bypass. Rev Col Bras Cir 45(6):e1967. https://doi.org/10.1590/0100-6991e-20181967
26. Heffron SP, Parikh A, Volodarskiy A et al (2016) Changes in lipid profile of obese patients following contemporary bariatric surgery: a meta-analysis. Am J Med 129(9):952–959. https://doi.org/10.1016/j.amjmed.2016.02.004
27. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT (2015) The Danish national patient registry: a review of content, data quality, and research potential. Clin Epidemiol 7:449–490. https://doi.org/10.2147/clep.S91125
28. Hsu CR, Chen YT, Sheu WH (2015) Glycemic variability and diabetes retinopathy: a missing link. J Diabetes Complicat 29(2):302–306. https://doi.org/10.1016/j.jdiacomp.2014.11.013

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