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TYPE 2 DIABETES AND BONE HEALTH

an epidemiological approach

Viggers, Rikke

DOI (link to publication from Publisher):
10.54337/aau561779793

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Link to publication from Aalborg University

Citation for published version (APA):
Viggers, R. (2023). *TYPE 2 DIABETES AND BONE HEALTH: an epidemiological approach*. Aalborg Universitetsforlag. https://doi.org/10.54337/aau561779793

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TYPE 2 DIABETES AND BONE HEALTH

– AN EPIDEMIOLOGICAL APPROACH

BY
RIKKE VIGGERS

DISSERTATION SUBMITTED 2023

AALBORG UNIVERSITY
DENMARK

TYPE 2 DIABETES AND BONE HEALTH

- an epidemiological approach

by

Rikke Viggers

AALBORG UNIVERSITY
DENMARK

Dissertation submitted 2023

Dissertation submitted: 2023.05.23

PhD supervisor: Peter Vestergaard,
Professor, DrMSc., MD, Aalborg University

Assistant PhD supervisors: Jakob Kau Starup-Linde Ph.D., MD
Aarhus University Hospital

Søren Gregersen, Ph.D., MD
Aarhus University Hospital

Rasmus Fuglsang-Nielsen, Ph.D., MD
Aarhus University Hospital

PhD committee: Clinical Professor Jette Kolding Kristensen (chair)
Aalborg University, Denmark

Professor Beata Lecka-Czernik
University of Toledo, Ohio, USA

Associate Professor Jens-Erik Beck Jensen
University of Copenhagen, Denmark

PhD Series: Faculty of Medicine, Aalborg University

Department: Department of Clinical Medicine

ISSN (online): 2246-1302
ISBN (online): 978-87-7573-704-8

Published by:
Aalborg University Press
Kroghstræde 3
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

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Printed in Denmark by Stibo Complete, 2023

CURRICULUM VITAE

Personal information

Full name: Rikke Vendelbo Viggers | **Birth year**: 1989

Nationality: Danish | **Citizenship:** Denmark, 9530 Støvring

Contact information: rikke.viggers@rn.dk | 0045 51520711

Education

01/2017: Medical doctor (MD), Aarhus University, Denmark.

Medical doctor authorization ID: 0C9Y6

Employment history

Academic employments

06/2023- Part-time (20%) Postdoc, Steno Diabetes Center North Denmark, Aalborg University Hospital, Denmark.

09/2019–05/2023: Ph.D. student, Steno Diabetes Center North Denmark, Aalborg University Hospital, Denmark. *Maternity leave from 2021.11.20 to 2022.09.01*.

09/2013-08/2014: Research year student, Department of Clinical Medicine, Endocrine Department and Medical Research Laboratory and Research Laboratory for Biochemical Pathology, Aarhus University Hospital.

Clinical employments

06/2023-: MD, Part-time (80%) residency in Endocrinology, Department of Endocrinology, Aalborg University Hospital, Denmark.

03/2019-08/2019: MD, residency in Endocrinology, Viborg Hospital, Denmark. *Leave of absence due to PhD-study and maternity leave from 01.09.2019-31.05.2023*

03/2018-02/2019: MD, first-year resident, Department of Clinical Medicine, Endocrinology, Aalborg University Hospital, Denmark.

03/2017-02/2018: MD, internship, Thisted Hospital, Denmark.

07/2016-09/2016: Locum doctor, Medical Center, Landssjúkrahúsið Tórshavn, Faroe Islands.

09/2014-12/2014: Locum doctor, Department of Urinary Tract Surgery, Holstebro Hospital, Denmark.

Scientific work and experience

Peer-reviewed accepted publications during the Ph.D. education (reversed chronological order).
For a full list, see ORCID: ttps://orcid.org/0000-0002-0742-9788.

Underlined, included in this thesis.
** These authors contributed equally to the manuscript and share the first authorship.*

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13. Starup-Linde J, **Viggers R**, Handberg A. Osteoglycin and Bone-a Systematic Review. Curr Osteoporos Rep. 2019 Oct;17(5):250-255. doi:10.1007/s11914-019-00523-z.

Other prominent and practical experience

2023: Referee reviewer, Diabetes Care, Submission ID DC23-0839.

2023: Review Editor, member of the editorial board of Frontiers in Endocrinology, the section of Clinical Diabetes.

2023: Referee reviewer, Nature Scientific Reports, Submission ID fb949ebb-94fb-4ffe-b562-d40cc2bfcbc8.

2023: Topic manager and co-editor of Frontiers in Clinical Diabetes. Research topic: “Diabetes and Bone Health”, https://www.frontiersin.org/research-topics/50479/diabetes-and-bone-health.

2023: Working group, National Guideline for Type 2 Diabetes, Danish Endocrine Society.

2023: Supervisor, Bachelor project, Medicine Industrial Specialization, Aalborg University.

2022: Referee reviewer, BMJ, Manuscript # BMJ-2022-073435.

2022: Teacher at the PhD-course “Clinical Research”, Topic “Randomized controlled trial and Power calculation”, Aalborg University.

2020-2022: Teacher, Medicine Industrial Specialization, Aalborg University, 150 hours.

2022: Podcast ”Medicin mod knoglebrud” in “Diabetesforskerne”, published by the Steno Diabetes Centers in Denmark, https://podcasts.apple.com/dk/podcast/medicin-mod-knoglebrud/id1566843707?i=1000552680406.

2021: Video profile “Forskerportræt” by Steno Diabetes Center North Denmark: https://video.rn.dk/sdcn-forskerportraet-rikke

2021: Working group, National Guideline for Monogenetic Diabetes, Danish Endocrine Society.

12/2020: Co-Referee reviewer on Nature Reviews Endocrinology (NREND-20-274V1) 2021 Nov;17(11):685-697. doi: 10.1038/s41574-021-00555-5.

2020 – onward: Investigator, “DiaBone2”, multicenter RCT, GCP-monitored.

2020-2021: Sub-investigator, “Paradighm”; Multicenter follow-up observational study by Shire, GCP-monitored.

2019 – 2021: Sub-investigator, “PaTH-forward”; Multicenter RCT by TransCon, GCP-monitored.

2020: A feature article in Danish Medical Journal, no. 2/2020 “ALOOH(A) vol. 2”.

2019: Working group, National Guideline for Hypokalemia, Danish Endocrine Society.

2016: A feature article in Danish Medical Journal, no. 24/2016 “ALOOHA”.

Professional activities

2020-2023: Board Member, Danish Endocrine Society (DES).

2020-2022: Chair of the board, Society of Young Danish Endocrinologists (FYEN).

2019-2022: Board member, Society of Young Danish Endocrinologists (FYEN).

International Experience

2020-2023: Collaboration with Steno Diabetes Center Aarhus, Denmark.

2020-2023: Collaboration with researchers in Holland (Maastricht), Meeting, 5 days.

2020: Stanford Health Care Hospital, U.S. California, Cardiovascular Medicine, Research lab. Observer, 2 weeks.

ENGLISH SUMMARY

Despite its importance, bone health is often neglected in diabetes care.

Type 2 diabetes mellitus and osteoporosis represent critical public health challenges globally. Both disorders are age-related, have substantial impacts on disability and mortality, and often develop simultaneously. Therefore, it is essential to implement protective strategies to prevent the development of type 2 diabetes and osteoporosis.

It is estimated that one in three women over age 50 will experience an osteoporotic fracture. Moreover, one in three post-menopausal women with osteoporosis have diabetes and one in three with type 2 diabetes have osteoporosis. Likewise, half of the elderly population with prediabetes or diabetes have compromised bone quality.

Bone metabolism is observed adynamic in individuals with type 2 diabetes and the risk of fractures related to osteoporosis is increased. In Denmark, the incidence of hip fractures is high, a fracture site related to high morbidity and mortality and estimated to be the most expensive. These facts underline the essential part of early detection of compromised bone health in individuals with type 2 diabetes.

Paradoxically, individuals with type 2 diabetes often exhibit normal or higher bone density compared to those without diabetes. Consequently, conditional techniques for detecting and diagnosing osteoporosis may not adequately identify or predict the risk of fractures associated with low bone quality.

Nevertheless, type 2 diabetes is not yet acknowledged as an independent risk factor for osteoporosis or fractures. Current guidelines for the management of type 2 diabetes and osteoporosis do not consider the frequent co-occurrence of these two conditions.

The dearth of focus and acceptance of low bone quality as a diabetes-related complication could well impede fracture prevention in type 2 diabetes. There is an imperative to grasp type 2 diabetes as a risk factor for fractures and change perspectives.

This Ph.D. thesis evaluates the relationship between type 2 diabetes and osteoporosis in the Danish population, covering aspects such as the types of first osteoporotic fractures, diagnostics, and treatment strategies.

The results indicate substantial bone health discrepancies among individuals with and without type 2 diabetes. Thus, it is essential to prioritize bone health as a crucial element in the management of type 2 diabetes. Moreover, the findings suggest the possibility of optimizing diagnostic and treatment strategies for both type 2 diabetes and osteoporosis and highlight the necessity for further investigation into the relationship between bone and glucose metabolism.

DANSK RESUME

Skeletale forandringer er ofte overset hos personer med diabetes, på trods af betydende konsekvenser.

Type 2 diabetes og osteoporose er to særskilte og betydelige kroniske folkesygdomme. De resulterer i globale sundhedsproblemer, heriblandt høj morbiditet og mortalitet og sundhedsøkonomiske konsekvenser. Type 2 diabetes og osteoporose er ofte til stede samtidig, særligt i den ældre del af befolkningen.

Det anslås, at hver tredje postmenopausale kvinde med osteoporose har diabetes og, at hver tredje person med diabetes har osteoporose eller forstadie dertil. Desuden er det estimeret, at hver tredje kvinde over 50 år vil opleve en fraktur relateret til osteoporose.

Knogleomsætningen er nedsat og anderledes og risikoen for frakturer er højere hos personer med type 2 diabetes. Danmark er kategoriseret som et land med høj risiko for hoftefraktur; en frakturtype som er vurderet til at være forbundet med den højeste mortalitet og de største samfundsmæssige omkostninger. Derfor er det afgørende med tidlig identifikation af kompromitteret knoglevæv hos personer med type 2 diabetes.

Til trods for en højere risiko for frakturer relateret til osteoporose, er knogledensiteten hos personer med type 2 diabetes oftest normal eller højere sammenlignet med personer uden diabetes. De nuværende teknikker, som benyttes til at vurdere knoglekvalitet og behandlingsindikation, er derfor utilstrækkelige i diagnosticeringen af osteoporose og prædiktion af frakturrisikoen hos personer med type 2 diabetes.

Frakturer er en velundersøgt komorbiditet til diabetes, men dette til trods, er det fortsat en udfordring for klinikerne at stille diagnosen osteoporose og forebygge frakturer hos personer med type 2 diabetes. Der findes endnu ikke en international konsensus omhandlende osteoporose hos personer med type 2 diabetes, og de nuværende retningslinjer for behandling af type 2 diabetes og osteoporose tager ikke højde for det faktum, at tilstandene ofte forekommer samtidig.

I denne afhandling undersøges sammenhængen mellem type 2 diabetes og osteoporose ved at se på forskellige i frakturtyper, diagnostik og behandling af osteoporose i den danske population.

Resultaterne i denne afhandling tilskynder fokus på skeletale forandringer hos personer med type 2 diabetes og, at dette bør indgå som en integreret og væsentlig del af sygdomshåndteringen. Desuden, at diagnostik og behandlingsstrategier kan og måske bør optimeres og slutteligt, at tilskynde yderligere undersøgelser af samspillet mellem knogle- og glukosemetabolismen.

ACKNOWLEDGMENTS

The factual people behind this piece of work are not justified by the cover of this thesis. It would not have been completed without the aid and support of unsung heroes who contributed and provided valuable assistance. I am deeply grateful to all who supported, encouraged, and helped me through an intermittently challenging and inspirational study time.

Thanks to Steno Diabetes Center North Denmark, Department of Endocrinology, Aalborg University Hospital, and Novo Nordisk Foundation for support and funding.

First and foremost, I wish to express my sincere appreciation to my main supervisor, Peter Vestergaard. Your knowledge, compassion, support, and high-speed responses are inspiring. Thank you for always encouraging curiosity.

To my co-supervisors Jakob Kau Starup-Linde, Søren Gregersen, and Rasmus Fuglsang-Nielsen: Thank you for your guidance, teaching, and trust. A special gratitude goes to Jakob, for your consideration, patience, and for always calling me in advance.

Thanks to all the fellow researchers and Ph.D.-students at Steno Diabetes Center Aalborg. I owe a special debt of gratitude to Zheer Kejlberg Al-Mashhadi, for bias assistance, outstanding English grammar teaching, and friendship.

Though not included in this thesis, my appreciation extends to the Aalborg “endo. lab.” staff who contributed to recruitment, blood draws, bone scans, and time coordination in an ongoing randomized clinical trial. A sincere gratitude goes to Ingelise Leegaard – everything is possible with you around.

Thanks to all the people I came across exploring other fields of internal medicine. This gave rise to, inter alia, a stay at Stanford University Hospital, U.S. learning insulin sensitivity testing, and fulfillment of research with the Department of Gastroenterology, Aalborg University Hospital.

Finally, my family deserves my deepest and most heartfelt gratitude. Thanks to Lærke, my best friend and life partner, for your never-ending support, fortitude, brilliant surgical suture at-home coaching, and continues snack provision. And thanks to Vester, our 1-year-old thesis cover artist, for endless joy.

I have learned and experienced more than I could have imagined, professionally and personally. And for that, I am deeply grateful.

Time is always ahead of you. Say yes, rush in, explore.

Rikke Vendelbo Viggers, May 2023.

PREFACE

Before you lies the Ph.D. thesis "Type 2 diabetes and bone health – an epidemiological approach".

The thesis is written to fulfill the graduation requirements of the Doctoral Schools at Aalborg University, The Danish Code of Conduct, and the Danish Ministerial Order on the Ph.D. Program. It is founded on research conducted at Steno Diabetes Center North Denmark, Aalborg University Hospital in the period of September 2019 to May 2023. It was supported by the Novo Nordisk Foundation (grant no. NNF18OC0052064).

I am a curious clinician. I find that our finest job is to conduct research and strategies that can answer a question most precisely, and so, the original Ph.D. plan contained two randomized controlled trials. Unfortunately, the pandemic of COVID-19 crossed our country in March 2020, just 1 month after all ethical requests were approved and the last method skills were obtained at Stanford University, California. Consequently, we were forced to lay down our biopsy needles, indefinitely.

It is always challenging, yet frustrating, to step outside the comfort zone. However, I did not only approach research differently than expected but also chose to write this thesis based on research methods that required skills and knowledge I did not yet have - or planned to obtain.

Hopefully, instead of answering one research question, you will find that this thesis generates hypotheses and plenty of other questions that need answers in the future. It was another path than expected but a fascinating and important one. I feel confident, that this is not my last writing in the world of epidemiology.

The thesis is based on the following 3 published manuscripts

1. **Discrepancies in type of first major osteoporotic fracture and anti-osteoporotic therapy in elderly people with type 2 diabetes mellitus: a retrospective Danish cohort study.**
   Viggers R, Starup-Linde J, Vestergaard P.
   Published 24 March 2023, doi.org/10.1016/j.bone.2023.116745 (1)
2. **The Efficacy of Alendronate Versus Denosumab on Major Osteoporotic Fracture Risk in Elderly Patients With Diabetes Mellitus: A Danish Retrospective Cohort Study.**
   Viggers R, Al-Mashhadi Z, Starup-Linde J, Vestergaard P.
   Published in Front Endocrinol. 2022 Jan 26;12:826997.
   doi:10.3389/fendo.2021.826997 (2)
3. **Alendronate Use and Risk of Type 2 Diabetes: A Nationwide Danish Nested Case-Control Study.**
   Viggers R, Al-Mashhadi Z, Starup-Linde J, Vestergaard P.
   Published in Front Endocrinol. 2021 Nov 19;12:771426.
   doi:10.3389/fendo.2021.771426 (3)

TABLE OF CONTENTS

Abbreviations **14**
List of Figures **15**
List of Tables **15**
Chapter 1. Objective **17**
1.1. Aims 17
1.2. Null hypotheses 17
Chapter 2. Introduction **19**
2.1. Osteoporosis 19
2.1.1. Definitory and diagnostic considerations 19
2.1.2. Alternative techniques for bone quality assessment 20
2.1.3. Occurrence 22
2.1.4. Clinical significance 24
2.1.5. Secondary osteoporosis 25
2.1.6. Management of osteoporosis 25
2.2. Type 2 diabetes 28
2.2.1. Definition and diagnosis 28
2.2.2. Occurrence and clinical significance 28
2.3. Osteoporosis in subjects with type 2 diabetes 30
2.3.1. Identification of bone quality in type 2 diabetes 30
2.3.2. Fractures in subjects with type 2 diabetes 31
2.4. The link between bone and glucose metabolism 32
2.4.1. The impact of glucose metabolism on bone 32
2.4.2. Bone modulation of glucose metabolism 34
2.4.3. Management of type 2 diabetes and its impact on bone 35
2.4.4. Management of osteoporosis and its impact on type 2 diabetes 39
2.5. Summary 40
Chapter 3. Methodology **43**
3.1. The National Danish registers 43
3.1.1. Data sources 43
3.1.2. Data management 44
3.2. Identification of subjects with type 2 diabetes 45
3.3. Exposure and outcome 45
3.4. Identification of confounding factors 46
3.5. Limitations and methodological considerations 47
3.5.1. Selection process 47

3.5.2. Confounding factors 48
3.5.3. Exposure and outcome 48
3.6. Statistical analyses 49
Chapter 4. Osteoporosis-related fractures in subjects with type 2 diabetes 51
4.1. Objective and hypothesis 51
4.2. Methodology 51
4.2.1. Study design, setting, and population 51
4.2.2. Exposure and outcome 52
4.2.3. Statistical analyses 52
4.3. Results 52
4.3.1. Baseline characteristics 52
4.3.2. Major osteoporotic fracture sites 54
4.4. Conclusion 58
Chapter 5. Diagnosis and treatment of osteoporosis in subjects with type 2 diabetes 59
5.1. Objective and hypothesis 59
5.2. Methodology 59
5.2.1. Exposure and outcome 59
5.2.2. Statistical analyses 59
5.3. Results 60
5.3.1. Baseline characteristics 60
5.3.2. Osteoporosis diagnosis after MOF 60
5.3.3. Anti-osteoporotic treatment after MOF 61
5.3.4. Mortality after MOF 64
5.4. Conclusion 65
Chapter 6. The efficacy of anti-osteoporotic treatment in subjects with type 2 diabetes 67
6.1. Study objective 67
6.2. Methodology 67
6.2.1. Study design and setting 67
6.2.2. Study population 67
6.2.3. Exposure and outcome 68
6.2.4. Statistical analysis 68
6.3. Results 68
6.3.1. Baseline characteristics 68
6.3.2. Risk of incident MOF 70
6.4. Conclusion 72
Chapter 7. Alendronate and the prospect of type 2 diabetes.....75
7.1. Study objective 75

7.2. Methodology 75
7.2.1. Study design and setting 75
7.2.2. Study population 75
7.2.3. Outcome and exposure 76
7.2.4. Statistical analyses 76
7.3. Results 76
7.3.1. Baseline characteristics 76
7.3.2. Characteristics of alendronate users 78
7.3.3. The odds of type 2 diabetes 78
7.4. Conclusion 80
Chapter 8. Discussion 83
8.1. Bone-specific interventions in type 2 diabetes management 83
8.1. Fracture types and osteoporosis in type 2 diabetes 84
8.1.1. Fracture type 84
8.1.2. Osteoporosis diagnosis and treatment 85
8.2. Anti-osteoporotic treatment efficacies in type 2 diabetes 87
8.3. Anti-osteoporotic therapies and glucose metabolism 89
Chapter 9. Conclusion 91
Chapter 10. Perspective 93
10.1. Type 2 diabetes as a risk factor 93
10.2. Management of type 2 diabetes 94
10.3. Management of osteoporosis 95
10.4. Epilogue 95
Literature list 97
Appendices 119

ABBREVIATIONS

ADA: American Diabetes Association

AGEs: Advanced glycation end products

BAP: Bone-specific alkaline phosphatase (bone formation marker)

BMC: Bone mineral content, g

BMD: Bone mineral density, g/cm2

BMI: Body mass index (weight (kg) divided by hight^2 (m))

CCI: Charlson comorbidity index

CI: Confidence interval

CT: Computerized tomography

CTX: C-terminal telopeptide of type 1 collagen (bone resorption marker)

DAG: Directed acyclic graph

DALYs: Disability-adjusted life years

DXA: Dual-Energy X-ray Absorptiometry

EASD: European Association for the Study of Diabetes

eGFR: Estimated glomerular filtration rate

FLS: Fracture Liaison Services

FRAX: The Fracture Risk Assessment Tool

GIP: Gastric inhibitory peptide

GLP-1: Glucagon-like peptide-1

HbA1c: Glycated hemoglobin A1c

HEC: Hyperinsulinemic euglycemic clamp

HR: Hazard Ratio

HRpQCT: High-Resolution Quantitative Computed Tomography

IOF: International Osteoporosis Foundation

IQR: Interquartile range

IR: Incidence rate

IST: Insulin suppression test

MOF: Major osteoporotic fracture

NTX: N-terminal cross-linked telopeptide of type 1 collagen (bone resorption marker)

OC: Osteocalcin (bone signaling/formation marker)

OGTT: Oral glucose tolerance test

OPG: Osteoprotegerin (bone signaling marker)

OR: Odds Ratio

P1NP: N-terminal propeptide of type 1 procollagen (bone formation marker)

RANKL: Receptor activator of nuclear factor kappa-β ligand (bone signaling marker)

RR: Relative risk ratio

RCT: Randomized controlled trial

SD: Standard deviation

TBS: Trabecular bone score (from DXA)

TRAP: Tartrate-resistant acid phosphatase (bone resorption marker)

WHO: The World Health Organization

LIST OF FIGURES

Figure 2.1, Osteoporosis in Denmark from 2000 to 2022. 23
Figure 2.2, Anti-osteoporotic drug dispenses in Denmark from 2000 to 2022. 27
Figure 2.3, Type 2 diabetes in Denmark from 2000 to 2021. 29
Figure 2.4, Glucose-lowering drug dispenses in Denmark from 2000 to 2022. 38
Figure 4.1, Type of the first MOF. 56
Figure 4.2, Type of first MOF by age. 57
Figure 5.1, Time to first treatment after the first MOF. 63
Figure 5.2, Mortality after the first MOF. 64
Figure 6.1, Kaplan-Meier failure curve of incident MOF. 72
Figure 7.1, Multiple adjusted ORs for type 2 diabetes. 80

LIST OF TABLES

Table 4.1, Baseline characteristics. 53
Table 4.2, Type of first MOF. 55
Table 5.1, Analyses of time to treatment. 62
Table 6.1, Baseline characteristics. 69
Table 6.2, Risk of MOF and stratifications. 71
Table 7.1, Baseline characteristics of cases and controls. 77
Table 7.2, ORs for type 2 diabetes: ever, effective, and compliant use. 79

CHAPTER 1. OBJECTIVE

"*Wisdom is the daughter of experience*"[1] was quoted by Leonardo Da Vinci more than 500 years ago and is the idiom that comes to mind when exploring the interplay between osteoporosis and type 2 diabetes.

1.1. AIMS

The objective of this thesis is to evaluate the interface between type 2 diabetes and bone health including osteoporotic fractures, diagnostics, and treatment strategies in the Danish population.

The aims are to

1. Provide an overview of the occurrence, diagnostic challenges, and management when osteoporosis and type 2 diabetes co-exist.
2. Evaluate the types of the first major osteoporotic fractures in subjects with type 2 diabetes compared to subjects without diabetes.
3. Assess diagnostics, anti-osteoporotic treatments, and mortality after the first major osteoporotic fracture in type 2 diabetes compared to subjects without diabetes.
4. Examine the efficiency of anti-osteoporotic treatments on osteoporotic fracture risk in subjects with type 2 diabetes.
5. Explore the proposed link between bone- and glucose metabolism by examining a potential relationship between alendronate and type 2 diabetes.

1.2. NULL HYPOTHESES

This thesis is restricted to epidemiological research. It is a counterpart to an ongoing randomized controlled trial (RCT) exploring the effects of alendronate on bone indices and insulin sensitivity in subjects with diabetes (the "DiaBone2" trial).

The null hypotheses are

1. The location of the first major osteoporotic fracture after diabetes diagnosis does not differ between subjects with type 2 diabetes and without diabetes.
2. A type 2 diabetes diagnosis does not impact the diagnosis or initiation of treatment against osteoporosis, or mortality after a MOF.
3. The risk of a new major osteoporotic fracture in subjects with type 2 diabetes does not differ between initiators of denosumab and alendronate.
4. The likelihood of developing type 2 diabetes is not altered by alendronate administration.

[1]The Notebooks of Leonardo Da Vinci (1478 – 1519).

CHAPTER 2. INTRODUCTION

Osteoporosis and type 2 diabetes are two major public health concerns and highly prevalent disorders associated with globally increased morbidity and mortality (4,5).

The aim of this thesis, Chapter 2 is to provide an overview of the occurrence, diagnostic challenges, and management when osteoporosis and type 2 diabetes co-exist.

Osteoporosis and type 2 diabetes will be introduced and evaluated separately and together with a focus on diagnostic adversities, prevalence, incidence, and treatment strategies in Denmark.

Please note that numbers presented in this thesis, Chapter 2 are obtained from freely available registers provided by *Sundhedsdatastyrelsen* (Statistics Denmark). Data are available as raw data and based on inventories updated in December 2021 or 2022 depending on the selected inventory (6) and presented from the year 2000. Data on the prevalence and incidence of osteoporosis and type 2 diabetes were obtained from the *Register for Udvalgte Kroniske Sygdomme* (RUKS)[2]. Data on drug dispenses were obtained from the register at *medstat.dk*[3]. The illustrations may differ from published manuscripts and presented figures in the following chapters based on data from Statistics Denmark with the latest data available on December 31, 2018. Consequently, the newest updated and available data are presented but some calculations in this chapter are based on 2018. All graphical illustrations are original and performed by the author of this thesis.

2.1. OSTEOPOROSIS

In the following, the definition, diagnostics, and management of osteoporosis will be introduced as this will connect the following discussion of bone health in subjects with type 2 diabetes.

2.1.1. DEFINITORY AND DIAGNOSTIC CONSIDERATIONS

Osteoporosis is a common bone disease in humans and was first defined by an international consensus in the early 1990s as:

"*A disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk*" (7,8).

The definition encircles the effects on bone mass and structure as well as the clinical manifestation (fracture) of an otherwise asymptomatic disorder. Following, The World Health Organization (WHO) defined the diagnostic criteria for osteoporosis as bone mineral density (BMD) equal to or more than 2.5 standard deviations (SD) below

[2]https://www.esundhed.dk/Emner/Operationer-og-diagnoser/Udvalgte-kroniske-sygdomme-og-svaere-psykiske-lidelser (Accessed December 2022).

[3] https://medstat.dk (Accessed March 2023).

the average healthy young adult (30 years old) Caucasian woman (9). The diagnosis was calculated and expressed as a T-score equal to or lower than -2.5.

Consequently, the diagnosis is defined by measured *"low bone mass"*, i.e., a two-dimensional areal value, BMD (g/cm^2), calculated by bone mineral content (BMC) measured in grams divided by the location area in cm^2, and not directly by identification of "*microarchitectural deterioration of bone tissue"*. The technique used to measure BMD is of high accuracy and has not changed since the definition of osteoporosis in 1994: it is still based on the dual-energy X-ray absorptiometry (DXA) introduced in 1987 with the capability to measure BMD at the lumbar spine, hip, or forearm to confirm the diagnosis (10). Thus, the measurement of BMD was and is used as a diagnostic tool to give information on disease status. However, BMD is also, and maybe more prominently, used as a prognostic tool to determine the probability of developing osteoporosis and related fractures and thus, the "*consequent increase in fracture risk".* Consequently, both the diagnostic and therapeutical threshold is implemented at T-score ≤ -2.5 (at hip or lumbar spine), however, the latter comes with a caveat in Denmark; one risk factor for fracture must be present as well as T-score ≤ -2.5.

The distribution of BMD in postmenopausal women is Gaussian at all ages but decreases progressively with aging as sex steroid levels decrease (9,11). The threshold of 2.5 SD was set to capture most patients with osteoporotic fractures and would identify 30% of all postmenopausal women as having osteoporosis which is approximately equivalent to the lifetime risk of fractures at the measured sites (lumbar spine, hip, and forearm) (9).

In many diseases, the clinical manifestation may occur without the most prominent risk factor present, e.g., stroke without high blood pressure or myocardial infarction without hypercholesterolemia. The same applies to osteoporosis. In 2004 a prospective study found that only 21% and 44% of all non-vertebral fractures in men and women, respectively, aged ≥55 years, occurred with T-score ≤ -2.5 (12). This underlines the importance of the diagnostic and prognostic considerations of BMD measurement as it might "just" be one important risk factor among many when evaluating the risk of a future fracture.

2.1.2. ALTERNATIVE TECHNIQUES FOR BONE QUALITY ASSESSMENT

Bone tissue is a composite, connective, and mineralized tissue, and the two most apparent structural features are cortical and trabecular bone (11). Uniquely, bone is undergoing constant regeneration throughout life through the remodeling process consisting of both bone formation and resorption (11). Thus, assessments of the different compounds of bone and the ability to modulate may indeed provide substantial information when evaluating the quality of the skeleton.

2.1.2.1 Bone structure

Cortical bone is the dense outer layer of bone and is located mainly in the bone shafts, e.g., in the femur, tibia, and radius (13). It is organized into structural units called osteons that consist of circular layers of mineralized matrix and makes the component stiff and resistant to bending (11). While cortical bone is a rather uniform

biomechanical compound, trabecular bone demonstrates variability in both strength and stiffness. Trabecular bone is found at the ends of long bones and in the interior of the vertebrae and ribs and provides flexibility and resilience (11). The trabecular connectivity contributes more to the biomechanical strength compared to the trabecular thickness or bone mineral density (13). Additionally, trabecular bone has a higher surface area to volume ratio compared to cortical bone, which makes it more metabolically active (13). Due to its structure and metabolic activity, trabecular bone is less brittle, more responsive to changes in mechanical loading, and capable to adapt and regulate remodeling (13). However, when bone turnover becomes adynamic, trabecular bone is also more susceptible to bone loss, e.g., as seen in osteoporosis (11).

In general, the DXA scanner is unable to distinguish between cortical and trabecular bone. However, it is possible to indirectly assess the trabecular compartment of the bone by the trabecular bone score (TBS). TBS is easily obtainable, and a high value reflects denser bone trabeculae, whereas a low TBS value is associated with a higher risk of osteoporotic fractures independently of BMD (14). Yet, TBS is not included in the diagnostics of osteoporosis or the assessment of fracture risk.

More advanced techniques have been constructed to evaluate the different compartments of bone. These modalities found that bone loss occurs with aging in both men and women but more rapidly in early menopause in women and to a greater extent in trabecular compared to cortical bone (11). Computed tomography-based finite element analysis (FEA) of a hip or spine provides an accurate evaluation of skeletal strength and may be used to predict the risk of fracture (15). The non-invasive imaging modality called high-resolution quantitative computed tomography (HRpQCT) is a newer technique to assess bone quality. HRpQCT enables 3D evaluation of the radial and tibial bone microarchitecture including both the outer cortical and inner trabecular structure as well as bone volume and strength (16). Another yet invasive, technique is microindentation of the tibia that enables in vivo evaluation of the cortical bone material strength (17). However, both techniques are restricted to the research setting of osteoporosis and fracture risk evaluation.

2.1.2.2 Bone turnover

A balanced bone remodeling process, i.e., bone resorption and formation, is paramount for optimal skeletal structure (18). There are three types of bone cells responsible for the remodeling process in constant communication with one another: osteocytes, responsible for signaling and intracellular communication; osteoblasts, responsible for bone formation; and osteoclasts, responsible for bone resorption (11). Roughly spoken, the balance between the activity of osteoblasts and osteoclasts determines the net rate of bone remodeling. If the activity of osteoblasts is greater than that of osteoclasts, there will be a net increase in bone mass, and inversely.

One of the key elements in the resorption of old bone and replacement with the synthesized bone matrix is the communication between osteoclasts and osteoblasts. This is mainly driven by the activation of osteoclasts by the interaction between the receptor activator of nuclear factor-κB (RANK) expressed on osteoclasts and the RANK ligand (RANKL) synthesized by osteoblasts (11). This activation is inhibited by the osteoblast-secreted soluble decoy receptor, osteoprotegerin (11).

Proteins and bone mineral components of the extracellular matrix, e.g., minerals, carbohydrates, and collagen, are released to the blood during bone turnover (11). Consequently, several circulating biomarkers of the bone remodeling process are identified. Two collagen products, C-terminal telopeptide of type 1 collagen (CTX) and N-terminal propeptide of type 1 procollagen (P1NP), have been suggested as appropriate markers to evaluate bone resorption and formation, respectively, in both clinical and research settings (19). However, the sample stability is affected by several factors including sample collection, analytical assays, fed and fasted states, anti-resorptive treatments, and the circadian cycle, all contributing to high variations (especially for CTX measurements) (19). Consequently, these do not stand alone in the evaluation of osteoporosis.

2.1.3. OCCURRENCE

Primary osteoporosis and corresponding fractures are increasingly common in women and men after age 50 and 65, respectively (10).

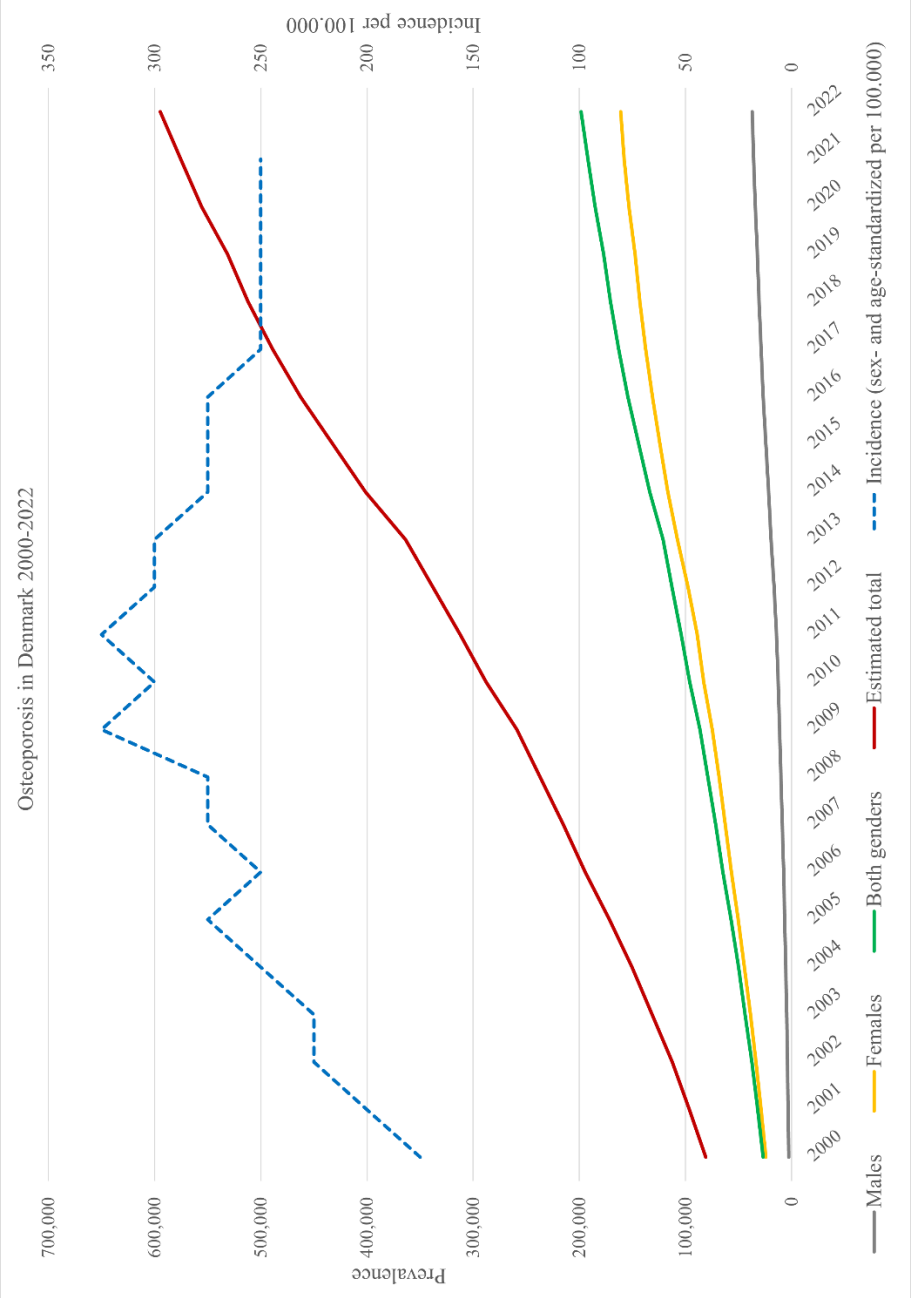
In 2010 it was estimated that 22 million women (21%) and 5.6 million (6%) men aged 50-84 were affected by osteoporosis worldwide (diagnostic criteria provided by the WHO assuming similar bone loss and mean femoral neck BMD after age 50 across the European countries with the highest population sizes) (20). It was further estimated that approximately 10% of all women aged 60, 20% of all women aged 70 and 80, and 60% of all women aged 90 are affected by osteoporosis (21,22).

In Denmark, osteoporosis is a disorder with increasing prevalence illustrated by Figure 2.1 which presents raw data obtained from *Sundhedsdatastyrelsen, Denmark* (6). It is estimated that the actual number of individuals with osteoporosis is 2-3 times the number identified (23) as illustrated by the red line. The total number of Danish citizens in 2018 was 5,8 million people (24) and the corresponding proxy of (diagnosed) prevalence was 3%. As illustrated by Figure 2.1, the prevalence of osteoporosis is highest among women and peaks at age 70 (Appendix A1). Correspondingly, the percentage of individuals above 50 years with osteoporosis in 2018 was 7.5% (12% for females and 3% for males)[4] and 14% of all individuals above 70 years (21% of females and 5% of males)[5] (6,24). The incidence of osteoporosis has been stable in Denmark during the last 5 years and is approximately 17,000 per year (6,23).

[4]Calculated by the number of total people in Denmark >50 years in last quarter of year 2018 (total 2,271,150; female 1,180,638; male 21,090,512) divided by same numbers of people with osteoporosis in 2018 > 50 years (total 169,725; female 140,650; male 29,075).

[5]Calculated by the number of total people in Denmark in year 2018 >70 years (total 810,962; female 447,083; male 363,879) divided by same numbers of people with osteoporosis in 2018 > 70 years (total 113,375; female 95,000; male 18,375).

Figure 2.1, *Osteoporosis in Denmark from 2000 to 2022.*
Estimated incidence (blue dashed line) per 100.000 persons (age and sex standardized) and prevalence in thousands (solid lines); Red line: estimated total number of individuals with osteoporosis (2 times the number of totals identified each year + the total number of identified); Green line: females and males identified with osteoporosis at all ages; Yellow line: females identified with osteoporosis at all ages; Grey line: males identified with osteoporosis at all ages.



2.1.4. CLINICAL SIGNIFICANCE

In Denmark, the diagnostic criteria for osteoporosis in postmenopausal women and men aged 50 is adapted from WHO and described in our national guidelines as a spine or hip T-score ≤ -2.5 *or* by a fragility fracture located at the spine or hip (25,26). Fragility fractures (or "osteoporotic" fractures) are the clinical manifestation of osteoporosis, defined by a mechanical force that would not normally result in a fracture, e.g., a fall from standing height or less (low impact), of which hip and spine historically has been considered the typical osteoporotic fracture sites (27). However, as osteoporosis is a systemic disorder all skeletal sites are affected (28).

Denmark is categorized as a country with a "very high" 10-year probability of hip fractures (29). All clinical and osteoporotic fractures are relevant, and some are major public health burdens globally and in Denmark with increased mortality (30). However, the first hip fracture is estimated as the most mortal and expensive in both men and women (30,31). Moreover, treatment directed against additional hip fractures is associated with improved survival (32). However, osteoporosis may be significantly underdiagnosed (33) resulting in challenging fracture prevention. In 2012, the International Osteoporosis Foundation (IOF) developed a global program called "Capture the Fracture" to support worldwide Fracture Liaison Services (FLS) (34). The main objective was (and is) to improve FLS programs across the world to prevent and reduce the risk of secondary fractures. FLS was further suggested in Denmark by *Sundhedsstyrelsen* in 2018 and is currently being implemented at hospitals across the country.

It is a well-known fact that fracture risk increases with decreased BMD (9). However, other factors may contribute to fracture risk as well, e.g., previous fracture, family history of fractures, body mass index (BMI), falls, use of corticosteroids, concomitant diseases, smoking, alcohol and maybe even factors that have amplified since the early 1990s, e.g., insulin resistance and type 2 diabetes mellitus. Thus, it has been suggested to develop algorithms for fracture risk assessment to be used when BMD testing is not available - or suitable (22).

The Fracture Risk Assessment Tool (FRAX) is a BMD-independent algorithm and freely available web-based implement developed and supported by WHO to estimate the 10-year probability of a hip or major osteoporotic fracture (MOF) defined by fractures at the hip, clinical spine, forearm, or proximal humerus (35). Several risk factors have been incorporated in the algorithm and FRAX has now been incorporated in clinical guidelines around the world without any consensus for an intervention threshold. However, a hip FRAX score >3% was recommended as a therapy threshold (36). The recommendation was based on an economic analysis from 2008, i.e., osteoporosis treatment was cost-effective when the 10-year hip fracture probability reached approximately 3% (37). The tool estimates the risk by taking the country of residence and a great number of risk factors into account, e.g., previous fractures, smoking, alcohol, and disorders strongly associated with osteoporosis; type 1 diabetes being one of them.

2.1.5. SECONDARY OSTEOPOROSIS

Secondary causes of osteoporosis are characterized by a group of heterogeneous disorders and medications that contribute to bone loss and fragility through various mechanisms independently of age or estrogen deficiency (15). Secondary bone loss is often more severe and associated with reduced bone quality that does not depend on changes in bone mass (15). Some risk factors for osteoporosis are modifiable, e.g., alcohol, smoking, low body weight, and adverse effects of several drugs, e.g., glitazones, androgen deprivation therapy, glucocorticoids, etc. Some are unmodifiable and several diseases are identified as contributors to secondary osteoporosis, including inflammatory disorders, e.g., arthritis, bowel-, and lung diseases, and endocrine disorders, e.g., hyperthyroidism, hypercortisolism, and growth hormone disturbances. Type 1 diabetes is acknowledged as a risk factor for fractures and was added to the list of secondary osteoporosis in the FRAX tool (22). However, in the nature of a secondary cause of osteoporosis, the impact of type 1 diabetes on the FRAX estimated fracture risk is independent of BMD but assumed to mediate the fracture risk as a result of low BMD. Thus, it only increases the estimated risk when a BMD value is not provided. Moreover, type 2 diabetes has not yet been recognized as an independent risk factor for or secondary cause of osteoporosis and consequently, is not incorporated in the risk assessment by FRAX.

2.1.6. MANAGEMENT OF OSTEOPOROSIS

Reduction in the likelihood of future fragility fractures is always the objective when treating a person with osteoporosis. In the following, a short notice will be outlined regarding the general management of uncomplicated primary postmenopausal osteoporosis.

2.1.6.1 Non-pharmacological treatment strategies

Bone tissue can adapt to whole-body metabolic changes including strain, weight, and energy demand (38). Nutritional recommendations in the management of osteoporosis and prevention of fractures are in general to ensure adequate energy and nutrient intake as low BMI is a risk factor for fractures (39). As calcium is the major component of bone matrix and mineralization process, prevention and treatment of osteoporosis includes calcium and vitamin D supplementation with recommendations to achieve an adequate intake of 1,000-1,200 mg calcium/day and 800-1,000 units (20-25 µg) vitamin D per day (40).

Obesity is associated with higher BMD and a lower risk of hip fractures in both postmenopausal women and men (41). Furthermore, weight loss may generate a long-term reduction of hip BMD regardless of weight regain (42,43). However, it seems that the BMD loss can be attenuated by combining diet-induced weight loss with exercise (44). Physical activity and exercise may indeed strengthen the tissues surrounding our skeleton, e.g., muscles and tendons, improve balance, and decrease fall risk. A detailed description of the impact of exercise on bone tissue can be achieved in the systematic review *The Impact of Exercise on Bone Health in Type 2 Diabetes Mellitus—a Systematic Review* (45). In short, bone tissue may benefit the most from a combination of resistance and weight-bearing exercise.

2.1.6.2 Pharmacological therapies

An imbalance between bone formation and resorption, in favor of the latter, is the main mechanism resulting in osteoporosis (11). Several anti-osteoporotic medications have been introduced during the last decades. The bisphosphonate, alendronate, and the antibody, denosumab, are the first choices in the general treatment of osteoporosis with high significance levels (25,46).

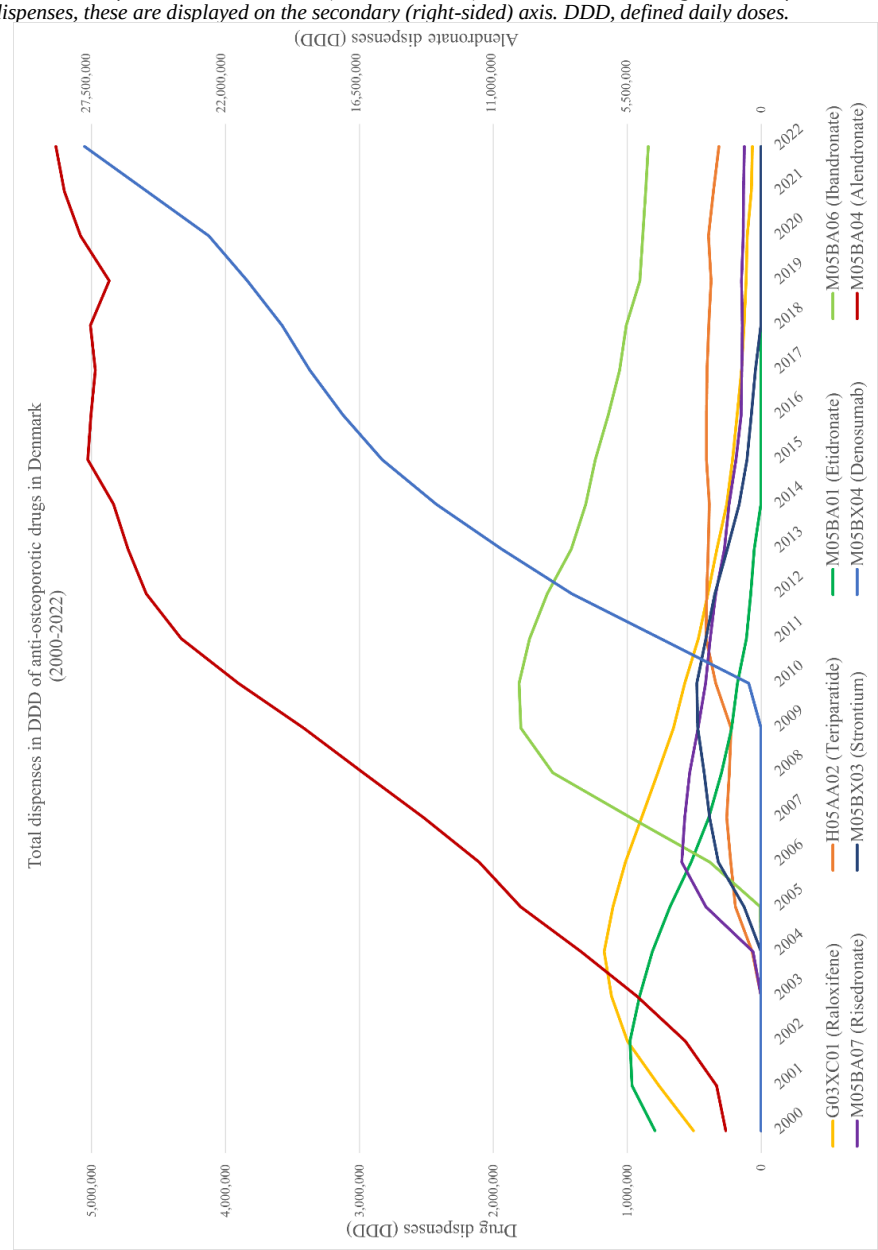
As illustrated by Figure 2.2, several anti-osteoporotic drugs are available in the treatment of osteoporosis. Figure 2.2 presents raw data freely available at *medstat.dk*. Alendronate is a once-weekly orally administrated bisphosphonate with few adverse events that sufficiently suppresses bone resorption by direct inhibition of osteoclast activity (47). Alendronate is a low-cost first-line treatment of osteoporosis in Denmark and globally (25,46) and is thus more widely used than other oral anti-resorptive agents. The Fracture Intervention Trial (FIT) from the late 1990s estimated the average BMD increase and fracture risk reduction of alendronate use compared to placebo among postmenopausal women with osteoporosis (defined by an existing vertebral fracture or femoral neck T-score ≤ -2.5 at baseline) (48). Alendronate use for 3-4 years increased BMD by approximately 5% and 6% in the total hip and lumbar spine, respectively, compared to placebo (49,50). Furthermore, alendronate reduced the risk of any clinical fracture by 30%, vertebral fractures by 48%, and hip fractures by 53% (48) after 3 years. In Denmark, alendronate is contraindicated when peptic ulcers or renal impairment is present (eGFR<30 or <35) (25,51). In some cases, another administration way than orally on an empty stomach may be beneficial.

Denosumab is most often the first choice of treatment when alendronate is not applicable. Denosumab is a monoclonal antibody to RANKL resulting in the inhibition of bone resorption (52). It is administrated subcutaneously every 6 months and became available for use in Denmark in 2010 whereas alendronate has been used for decades (53). The FREEDOM trial compared denosumab use for 3 years with placebo and reported a BMD increase of approximately 10% in the lumbar spine and 6% in the total hip as well as relative fracture risk reduction of 68% for vertebral fractures and 40% for hip fractures (52,54). It is suggested that denosumab increases BMD and suppresses bone turnover relatively more compared to alendronate in postmenopausal women (55). However, denosumab only seems to reduce the risk of vertebral fractures compared to other bisphosphonates (56).

Besides oral bisphosphonates and denosumab, other pharmacological therapies are available and used against osteoporosis. These include anabolic treatment with a parathyroid hormone analog, teriparatide, the intravenously administrated bisphosphonate, zoledronic acid, and the monoclonal antibody against sclerostin, romosozumab, the latter considered both forming and antiresorptive (25). A recent meta-regression evaluated the relative fracture rate reduction among users of different anti-osteoporotic medications (56). The analysis suggests that hip fracture prevention is mainly achieved by treatment with the antiresorptive agents alendronate, denosumab, and zoledronic acid. Au contraire, prevention of vertebral fractures was higher in those treated with denosumab and teriparatide compared to oral bisphosphonates, though the latter still show vertebral fracture prevention compared to placebo (56).

Collectively, the key elements in the pharmacological treatment of osteoporosis are to prevent fractures and depress bone turnover in favor of bone resorption as observed in both treatments with alendronate and denosumab in a dose-dependent manner (11,56).

***Figure 2.2**, Anti-osteoporotic drug dispenses in Denmark from 2000 to 2022.*
Data obtained from Statistics Denmark (www.medstat.dk). Note, that due to the high amount of alendronate dispenses, these are displayed on the secondary (right-sided) axis. DDD, defined daily doses.



2.2. TYPE 2 DIABETES

διαβαινω. Aretaeus of Cappadocia was the first to describe diabetes mellitus (323 BCE – 31 BCE) by the Greek word meaning "pass" or "go/run through" referring to *"the patients never stop making water"* (57).

2.2.1. DEFINITION AND DIAGNOSIS

Type 2 diabetes accounts for 90-95% of all diabetes cases and thus, is the most frequent type of diabetes (58). It is characterized by a chronic metabolic imbalance due to insulin resistance, and a non-autoimmune inadequate β-cell function, it is often accompanied by overweight and leads to serious complications that may affect multiple organs (58).

The diabetes diagnosis is based on international consensus by measuring blood plasma glucose levels ≥ 7.0 mmol/L after fasting, ≥ 11.1 mmol/L at any given moment or after an oral glucose tolerance test, or by the most used measurement of glycated hemoglobin A1c (HbA1c) ≥ 48 mmol/mol (58). Repeated measurements are required to confirm the diagnosis if diabetes-related symptoms are absent (59). The type 2 diabetes diagnosis differs from type 1 diabetes by older age (often above 40), absence of antibodies against pancreatic β-cells, and is often accompanied by overweight, insulin resistance, and initially normal or high levels of C-peptide; a marker of endogenous β-cell function (58). Thus, individuals with type 1 diabetes typically require prompt administration of insulin, whereas individuals with type 2 diabetes may be able to manage the disease by lifestyle interventions, other glucose-lowering drugs than insulin, and without hospitalization.

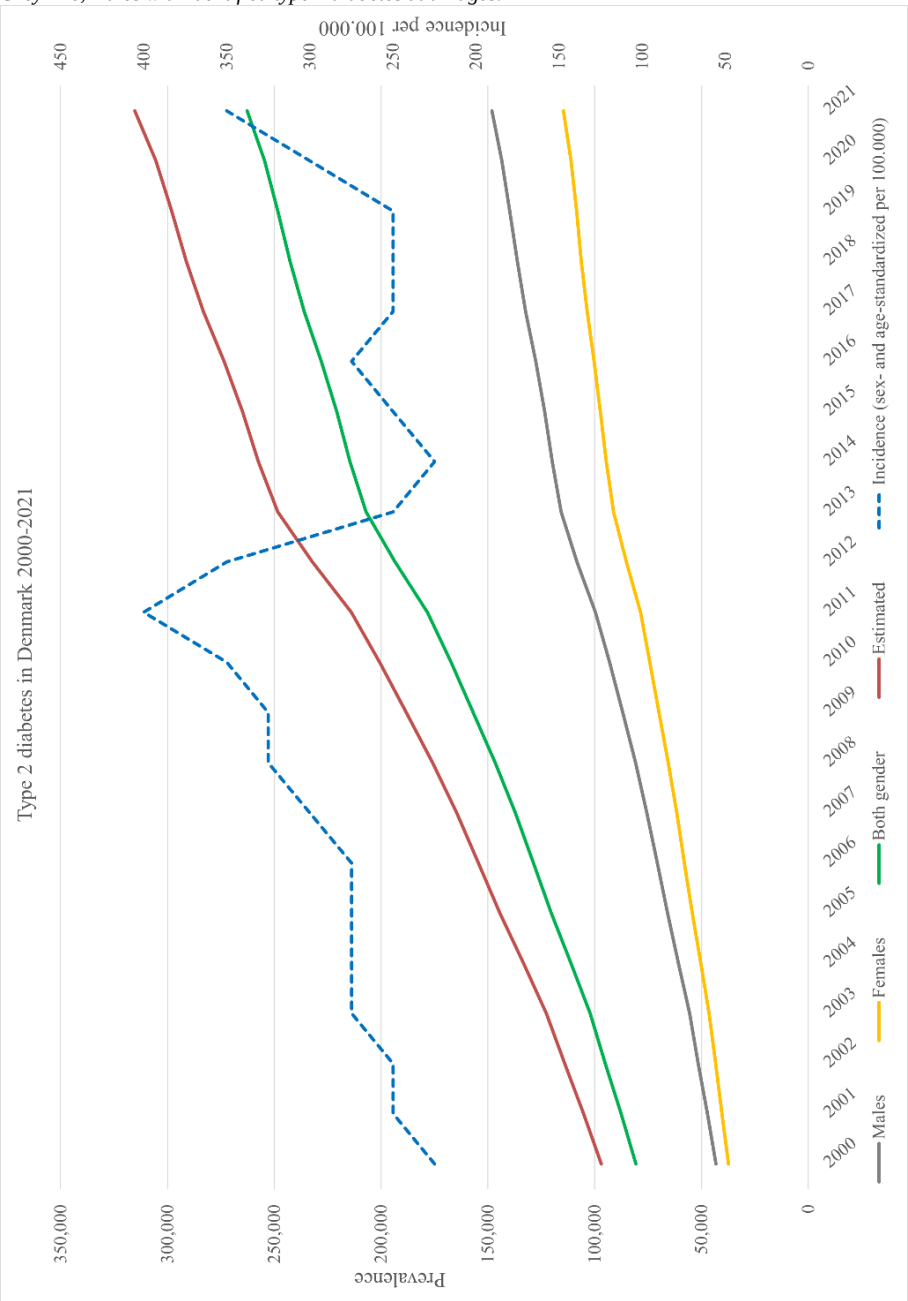
2.2.2. OCCURRENCE AND CLINICAL SIGNIFICANCE

In Denmark, type 2 diabetes is a highly frequent disorder illustrated by Figure 2.3 with data obtained from *Sundhedsdatastyrelsen*, *Denmark* (6)[6]. The number of Danish citizens diagnosed with type 2 diabetes in 2018 was 242,000 (6). If we add those who manage the disease by lifestyle intervention only, the number is approximately 300,000, and lastly, a significant number of undiagnosed individuals exist (approximately 32% of the diagnosed population, ≈77,000) (60,61). In total, approximately 377,000 individuals are estimated as having type 2 diabetes in Denmark in 2018, corresponding to a prevalence of 6,5% (61). Moreover, the number of individuals with pre-diabetes is estimated higher than the diagnosed type 2 diabetes population, which is approximately 300,000 Danish citizens (61).

As illustrated by Figure 2.3, diagnosed type 2 diabetes is most frequent among men. The incidence of type 2 diabetes in Denmark has been a U-formed slope during the last decade with the highest incidence in 2011 at 23,725 new diagnoses, to the lowest at 14,275 in 2014 and 23,225 in 2021 (6).

[6]Numbers were obtained from the National Danish registries provided online by Sundhedsdatastyrelsen (Register for Udvalgte Kroniske Sygdomme, RUKS) and graphically illustrated by the author of this thesis.

***Figure 2.3**, Type 2 diabetes in Denmark from 2000 to 2021.*
Estimated incidence (blue dashed line) per 100.000 persons (age and sex standardized) and prevalence in thousands (solid lines); Red line: estimated total number of individuals with identified type 2 diabetes (approximately 32% of the identified population + identified population); Green line, females and males at all ages with identified type 2 diabetes; Yellow line, females with identified type 2 diabetes at all ages; Grey line, males with identified type 2 diabetes at all ages.



The age distributions are illustrated in Appendix A2 showing the peak age for type 2 diabetes at approximately age 75 in the year 2022, as for osteoporosis, however, with a higher prevalence among females at younger ages (< age 45).

According to WHO, diabetes was the global 9th leading cause of death and the 8th leading cause of disability-adjusted life years (DALYs) in 2019 (62). Correspondingly, it was the 10th and 7th leading cause of death and DALYs in Denmark, respectively (62).

Historically, chronic diabetes-related comorbidities have been categorized into microvascular and macrovascular complications, covering retinopathy, nephropathy, and neuropathy and coronary artery disease, peripheral arterial disease, and stroke. There is strong evidence of an association between hyperglycemia and the development of vascular complications with the highest risk rate for microvascular disease (63,64). Moreover, lowering glycemia in patients with type 2 diabetes has been shown to decrease the risk of vascular complications (64). Lately, the choice of glucose-lowering therapy extends to include consideration regarding the risk or existence of vascular disease as several drugs have shown beneficial effects on the cardiovascular risk profile independently of glycemia (65).

For the management of type 2 diabetes, please see section 2.4.3.

2.3. OSTEOPOROSIS IN SUBJECTS WITH TYPE 2 DIABETES

WHO estimates that the average human lifespan has increased by approximately 6 years (from 66.8 to 73.4) during the last 2 decades and especially the Danish citizens are getting older with an average life expectancy of 81.3 years in 2019 (66). This underlines the importance of focusing on effective prevention and treatment strategies for chronic disorders related to aging, e.g., type 2 diabetes and osteoporosis, especially if they co-exist. As illustrated by Appendix A1, the prevalence and incidence of osteoporosis peaks at age 70-74 for both men and women, highest among women. In addition, the prevalence of type 2 diabetes is illustrated by Appendix A2 and peaks at age 70-74 for men and 75-79 for women. However, the incidence of type 2 diabetes peaks at age 55-59 for both men and women. Thus, incidence and prevalence increase with age in both diseases and peak after age 55, however, type 2 diabetes is diagnosed earlier in life compared to osteoporosis.

2.3.1. IDENTIFICATION OF BONE QUALITY IN TYPE 2 DIABETES

The relationship between body weight and bone size was acknowledged in the 17th century by Galileo (67). BMD is positively associated with BMI and evidence points toward an increased fracture risk with decreasing BMI (68). Though overweight has a favorable effect on BMD measured by DXA, the unmeasured "*microarchitectural deterioration of bone tissue"* might be affected negatively.

Individuals with type 2 diabetes are predominantly overweight and subsequently, a higher BMD is found in these subjects at all sites (69,70). However, after adjusting for BMI, BMD remains higher among individuals with type 2 diabetes compared to individuals without diabetes (69). Thus, BMD is likely overestimated in type 2

diabetes, yet approximately half of the population of elderly with prediabetes have a T-score below -1, and despite the higher hip BMD, a higher risk of hip fractures was reported (71).

It was recently estimated that approximately one in three women with type 2 diabetes above age 60 suffer from concomitant osteoporosis worldwide, though it is most likely underestimated as the definition of osteoporosis was based only on T-score ≤ -2.5 (72). Moreover, it is estimated that one in three women with osteoporosis has prediabetes (HbA1c 39–47 mmol/mol) (73).

In the previously published systematic review, *Bone Health in the Elderly with Type 2 Diabetes Mellitus–A Systematic Review*, current knowledge regarding the effects of type 2 diabetes on bone in the elderly population is presented (74). Concerning heterogeneity among the evaluated studies, the risk of osteoporotic fractures remains high despite a normal or elevated BMD (74). This limits the utility of the DXA scan in the diagnosis of osteoporosis and fracture risk assessment in the elderly with type 2 diabetes. In addition, the BMD loss over time may be associated with diabetes duration, severity, and blood glucose levels (74,75).

Even though the association between type 2 diabetes and compromised bone health has become highly evident during the last decades, type 2 diabetes per se has not yet been recognized as an independent risk factor for osteoporosis and fractures and has not been incorporated in the FRAX tool (35,76). Moreover, the current FRAX algorithm underestimates the risk of osteoporotic fractures in subjects with type 2 diabetes (77).

In general, studies agree that bone turnover markers are decreased in subjects with type 2 diabetes compared to subjects without diabetes (specified in section 2.4.1.1) (45,74). However, a measured variation in bone turnover markers does not necessarily indicate a corresponding change in actual bone turnover or BMD. Thus, to determine if bone turnover markers hold any predictive value in fracture risk assessment in individuals with type 2 diabetes, it is necessary to conduct larger studies.

2.3.2. FRACTURES IN SUBJECTS WITH TYPE 2 DIABETES

A recent meta-analysis found that individuals with type 2 diabetes have a 30% and 20% increased risk of hip (RR 1.33 [1.19; 1.49]) and non-vertebral (RR 1.19 [1.11; 1.28]) fractures, respectively, compared to individuals without diabetes (78). These findings agree with studies performed in Denmark and other countries in Northern Europe (70,79–81). Furthermore, a recent study from the Danish registers found lower incidence rates (IRs) of forearm fractures and unchanged IRs of vertebral, hip, and humerus fractures in subjects with type 2 diabetes compared to subjects without diabetes (82). Interestingly, current evidence suggests that the vertebral fracture risk is more consistently present in older women with type 2 diabetes than in men (83). Moreover, continuous insulin administration, longer-acting insulins, and continuous glucose monitoring are now commonly used to treat type 1 diabetes and in severe cases of type 2 diabetes (84). This may presumably result in lower fracture rates during the last decades due to more stable glucose levels and lower risk of hypoglycemia, however, decreased fracture rates are only observed in clinical

vertebral fractures (82). Moreover, other glucose-lowering drugs are used in subjects with type 2 diabetes that do not induce hypoglycemia and may affect fracture risk.

The risk factors for osteoporosis-related fractures among subjects with type 2 diabetes are in general similar to individuals without diabetes, e.g., family history of fractures, age, and female gender (78). Moreover, poor glycemic control, insulin use, and long diabetes duration are considered important determinants of increased fracture risk among subjects with type 2 diabetes (78).

It has previously been reported that for a given T-score and age, the 10-year hip fracture risk is higher in subjects with type 2 diabetes compared to subjects without diabetes (85). Specified, the risk of a hip fracture in a woman with type 2 diabetes is equivalent to a woman without diabetes at approximately 0.5 units lower T-score, i.e., -2 (85). Consequently, a higher BMD-measured T-score intervention threshold has been proposed (86). In these subjects, the routine diagnostic tool may not accurately assess bone quality and strength, necessitating more advanced diagnostic tools for early detection of bone fragility in individuals with type 2 diabetes, e.g., HRpQCT, microindentation, and/or biochemical markers.

Importantly, the mortality is approximately 20% in the first year after a hip fracture and the mortality and loss of function are higher among individuals with type 2 diabetes (87–89).

Collectively, the osteoporosis diagnosis and prediction of fracture risk in subjects with type 2 diabetes are challenging and require increased attention. With the increased mortality, loss of DALYs, health care burden, and hip fracture as the most expensive, the need for early detection and treatment of poor bone health in subjects with type 2 diabetes is highly warranted. Particularly the risk of hip fractures has been reported higher in elderly with type 2 diabetes (74), however, if a hip fracture is also the most frequent first fracture is unknown.

2.4. THE LINK BETWEEN BONE AND GLUCOSE METABOLISM

A link or “crosstalk” between bone and glucose metabolism has been suggested and (is still being) explored (90). This “crosstalk” may enable bone mass to adapt to whole body energy balance, however, unfortunate deviations may exist as well.

2.4.1. THE IMPACT OF GLUCOSE METABOLISM ON BONE

As presented earlier in this chapter, type 2 diabetes, though not recognized in current guidelines, is most likely a significant risk factor for bone fragility and fractures independently of the diagnostic method for osteoporosis by the BMD estimated T-score. This may indeed indicate that glucose metabolism modifies the balance of bone remodeling and microarchitecture.

2.4.1.1 Changes in bone indices

It has been suggested that individuals with type 2 diabetes have a more rapid bone loss that is most likely attenuated, especially at the weight-bearing femoral neck but possibly not at the radius (91,92).

Besides the higher BMD in subjects with type 2 diabetes, TBS is found lower and may attend as a predictive model for fracture risk in type 2 diabetes independently of BMD (74). These disparities between individuals with type 2 diabetes and individuals without diabetes are evaluated using other techniques than DXA as presented below.

Findings suggest the following changes in bone turnover markers among individuals with type 2 diabetes (93):

- Lower levels of the bone formation markers osteocalcin (OC) and P1NP.
- Lower levels of the bone resorption markers tartrate-resistant acid phosphatase (TRAP) and CTX
- Higher levels of the bone signaling and formation inhibitor marker sclerostin.
- No differences in N-terminal cross-linked telopeptide of type 1 collagen (NTX), bone-specific alkaline phosphatase (BAP), osteoprotegerin (OPG), and RANKL.

Taken together, bone turnover is decreased in subjects with type 2 diabetes. However, the bone-specific mineralization marker BAP is unaltered, suggesting intact bone mineralization despite decreased resorption and formation and consequently increased BMD (93).

Furthermore, the bone microstructure, assessed by HRpQCT, is affected as well: a cortical deficit is shown and measured as higher cortical porosity located at the radius but not at (the weight-bearing) tibia (94). Though no meta-analysis exists, a few studies using microindentation has suggested a reduced cortical bone material strength in subjects with type 2 diabetes (95). Moreover, an inverse association to mean HbA1c over 10 years and accumulation of advanced glycation end products (AGEs) (95), suggest that bone material properties are more severely affected in subjects with poorly controlled type 2 diabetes.

2.4.1.2 The underlying mechanisms

How and why these bone indices are altered in subjects with type 2 diabetes remains to be clarified. Yet, the association between glycemic control and fracture risk is J-shaped: both high and low blood glucose levels are suggested to increase fracture risk in subjects with type 2 diabetes, the latter mostly due to hypoglycemia and falls (96–98). Additionally, it seems that bone turnover markers (CTX, P1NP, and osteocalcin) are negatively correlated with insulin sensitivity, again indicating that individuals with insulin resistance, e.g., individuals with type 2 diabetes, are more disposed to have a lower bone remodeling (99,100).

Furthermore, it seems that glucose administrated orally suppresses bone resorption more than intravenously in healthy men (101). Additionally, postmenopausal naïve type 2 diabetes women suppress bone resorption relatively less compared to women without diabetes after an oral glucose load (102). Thus, it seems that disturbances in gastrointestinal incretin hormones, e.g., glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) as seen in type 2 diabetes, and not glucose per se, are involved in the underlying mechanism of compromised bone quality.

Type 2 diabetes is characterized by insulin resistance, hyperinsulinemia, and hyperglycemia, all of which are found to impact bone: osteoblasts, osteoclasts, and osteocytes express insulin receptors that may be affected by insulin resistance

resulting in changed bone remodeling (103); elevated levels of circulating insulin is associated with high BMD (104); and high blood glucose level is related to AGEs in bone matrix (105).

Glycation of bone collagen by alteration of cross-link strength is suggested as an important underlying mechanism of bone fragility in subjects with type 2 diabetes (106). Reduction in enzymatic cross-links and the formation of AGEs are induced by hyperglycemia and oxidative stress, both of which are present in type 2 diabetes (107). Preclinical studies suggest that AGEs inhibit bone formation, an effect mediated, at least in part, by increased sclerostin production by the osteocytes (108). Moreover, elevated levels of AGEs in urine and serum are found associated with fracture risk in subjects with type 2 diabetes that persists after adjustment for BMD (109,110). Thus, circulating AGEs may indeed influence the pathogenesis of bone fragility in type 2 diabetes.

2.4.2. BONE MODULATION OF GLUCOSE METABOLISM

Historically, bone is considered an organ to provide structure, organ protection, and mechanical strength as well as restricted to execute paracrine functions and regulations of calcium and phosphorous homeostasis (11). However, as bone remodeling occurs daily and bone tissue makes up approximately 10% of our body weight, it needs a high energy demand and strict regulation. Meanwhile, current evidence indicates that bone tissue itself produces proteins with endocrine actions that affect other organ systems and glucose metabolism is one of them (111). This indicates a two-way axis and interplay between bone and pancreas.

As mentioned, bone is an insulin-regulated tissue and evidence suggests the existence of a feedback (or “feedforward”) mechanism. Different secreted bone biomarkers, osteokines, have been identified as mediators of the proposed bone-pancreas crosstalk, e.g., osteocalcin, sclerostin, osteoglycin, and RANKL, though human studies are inconsistent.

Osteocalcin is produced by osteoblasts and is hypothesized to act on insulin sensitivity in muscle tissue as well as insulin release from pancreatic β-cells (90). Osteocalcin is mostly investigated in rodents or in vitro and reported positively associated with insulin sensitivity and a potentially reduced risk of developing type 2 diabetes (112–114). However, current clinical results on the effect of osteocalcin in humans are sparse and conflicting signifying the need for further exploration (115).

Sclerostin is another proposed regulator of glucose metabolism that is mainly produced by the osteocytes and acts as an antagonist of the Wnt signaling pathway resulting in reduced osteoblast differentiation and osteocyte survival decreasing bone formation (116). These changes may result in the accumulation of structural micro-damages and micro-cracks that are normally sensed and mended by osteocytes. Sclerostin is found higher in people with type 2 diabetes and prediabetes that positively correlated with skeletal muscle-, adipose tissue- and liver insulin resistance (117–119). Examination of sclerostin-deficient rodents revealed a possible ability of sclerostin to direct adipocyte metabolism by decreasing lipid synthesis and increasing the oxidation of fatty acids (120). Since adipose tissue itself does not express sclerostin, communication between bone and adipose tissue may be facilitated by

sclerostin in an endocrine manner. Therapeutical targeting of sclerostin has been developed in the treatment of postmenopausal osteoporosis (romosozumab) (121) and so, exploration of other potential systemic metabolic effects emerges.

Osteoglycin is a proteoglycan expressed and secreted from bone tissue and myocytes and suggested increasing BAP and osteocalcin as well as ensuring collagen fibrillogenesis (122). High levels of osteoglycin in the human blood are found positively associated with diabetes duration and vertebral fractures but negatively associated with BMD in postmenopausal women with type 2 diabetes (123). These findings suggest osteoglycin as a possible biomarker to detect compromised bone quality in type 2 diabetes. In rodents, osteoglycin deficiency is associated with impaired glucose tolerance and expanded white adipose tissue independent of diet consumption (124). Contrarily, osteoglycin-treated mice revealed lower blood glucose levels in a dose-dependent manner (124). Human studies examining the impact on glucose metabolism are sparse. Osteoglycin levels were estimated according to pancreas function by evaluation of individuals with type 1 and type 2 diabetes and found no osteoglycin difference between diabetes types, but a positive association with BMI (125). However, no association was detected with HbA1c or random plasma glucose level and the effects of osteoglycin in humans are conflicting (125).

Both RANKL and its receptor, RANK, are expressed in other human tissues than bone, e.g., liver, and pancreatic β-cells as well as found in a cleaved soluble form in the blood (126). Insulin sensitivity was found higher in rodents with a liver-specific knockout for RANK compared to their littermates (127) and inhibition of RANKL is suggested as a mediator of pancreatic beta cell function (128). Lastly, circulating levels of RANKL in humans are suggested as a possible predictor of type 2 diabetes (127). RANKL is therapeutically targeted by denosumab in the treatment of osteoporosis. This underlines the importance of examination of other potential systemic metabolic adverse effects of RANKL.

Taken together, an association between secreted osteokines and glucose metabolism is suggested, and evidence is pointing towards an endocrine function of bone tissue. Furthermore, current knowledge is predominantly based on studies in vitro and on rodents, and only correlations are examined and suggested in humans. Correlations do not signify causal inference, e.g., a specific osteokine may be a predictive biomarker of the risk of type 2 diabetes but without surrogate endpoint abilities. Lastly, it is worth noting, that the above-mentioned osteokines might not be the only potential mediators of glucose metabolism and more research is needed to fully understand the endocrine functions of the human skeleton.

2.4.3. MANAGEMENT OF TYPE 2 DIABETES AND ITS IMPACT ON BONE

Individuals with type 2 diabetes are at risk of several major comorbidities with a high impact on life quality and mortality. Consequently, management of type 2 diabetes is more than blood sugar monitoring and includes interventions and therapies with the ability to target other organ systems at risk of dysfunction following type 2 diabetes. However, bone quality and fracture risk in subjects with type 2 diabetes are not yet

added to the list of important comorbidities. Thus, guidelines for type 2 diabetes management do not consider the risk of current or incident low bone quality.

2.4.3.1 Lifestyle intervention

A weight loss of at least 7% is associated with a lower risk of developing type 2 diabetes and improves glycemic control and so, intervention programs with energy deficits are recommended by the American Diabetes Association (ADA) as both type 2 diabetes prevention and treatment in overweight adults (129,130). In addition, it is recommended that weight loss is accompanied by 150 min (unspecified) physical activity per week in the prevention of type 2 diabetes (131).

Weight loss is associated with increased mortality in the elderly with and without type 2 diabetes, whereas overweight and obesity are associated with decreased mortality (132–134). It seems that older individuals (above 65 years) are more prone to obtain a greater and more steep weight loss (134). Importantly, the Look AHEAD (Action for Health in Diabetes) trial confirmed what others have suggested (45): that an intensive weight loss of 7% in men and women with a mean age of 60 years is related to decreased hip BMD that sustains after 3 years despite weight maintenance (135). However, it seems that bone tissue adapts and maintains its density if weight loss is accompanied by weight-bearing exercise (45,135). In 2018, ADA published an updated “Standards of Medical Care in Diabetes” acknowledging that the history of fractures and osteoporotic risk factors should be assessed in older patients with diabetes as well as BMD evaluation if appropriate (136). However, in the recently updated recommendations from January 2023, this section has been removed (137). Neither BMD nor fracture risk are mentioned in the updated guidelines when assessing comorbidities and lifestyle interventions in the elderly with type 2 diabetes (138,139).

All considered, bone mass does not benefit from lifestyle intervention predominantly facilitated by diet-induced weight loss, and this may be particularly important to appraise in the elderly and individuals with type 2 diabetes.

2.4.3.2 Pharmacological therapies

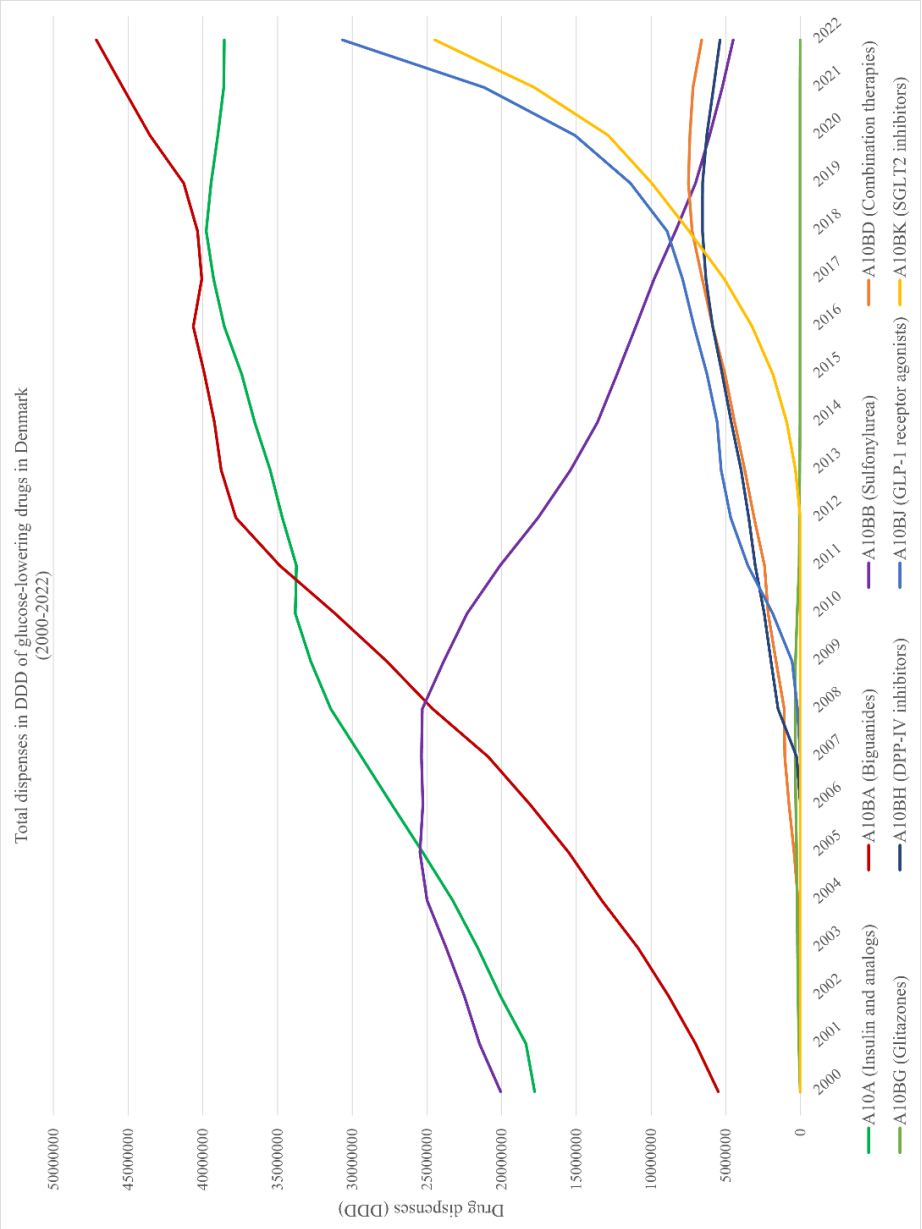
Figure 2.4 presents the total dispenses of glucose-lowering drugs from 2000 to 2022 in Denmark (raw data freely available at *www.medstat.dk*). As illustrated, several glucose-lowering drugs have been developed during the last decades and some of these drugs, e.g., GLP-1 receptor agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors, are related to beneficial effects on cardiovascular- and renal outcomes and was recently (after 2018) indicated as treatments in patients without diabetes (140).

However, assessment of the association between glucose-lowering drugs and fracture risk is difficult and limited by current study sizes and heterogeneity, as reported in a recent systematic review conducted in collaboration with colleagues (141). Yet, it is highly relevant to consider the (side) effects of these drugs in the manner of bone health. For example, most glucose-lowering drugs result in a clinically significant weight loss, e.g., GLP-1 receptor agonists as reported in both the SUSTAIN, PIONEER, and STEP trials concerning both overweight type 2 diabetes patients and

obese subjects without diabetes (142). If and to what extent the drug-induced weight loss is accompanied by bone loss and fracture risk has not yet been evaluated thoroughly in these trials.

Detailed descriptions of the mechanisms and effects of glucose-lowering drugs on bone structure and fracture risk will not be evaluated or discussed in this thesis but are debated in the literature (141). In short, SGLT2 inhibitors induce glycosuria accompanied by hypercalciuria that may induce bone loss (143). However, only glitazones (thiazolidinediones) are found consistently associated with increased fracture risk (141,144). Some studies have found evidence of preserved bone mass, increased bone formation, and unaltered bone resorption during weight loss and treatment with GLP-1 receptor agonists in both individuals with and without type 2 diabetes (145,146).

***Figure 2.4**, Glucose-lowering drug dispenses in Denmark from 2000 to 2022.*
Data obtained from Statistics Denmark (www.medstat.dk). DDD, defined daily doses; SGLT2, sodium-glucose cotransporter-2; GLP1, glucagon-like peptide-1; DPP-IV, dipeptidyl peptidase IV.



2.4.4. MANAGEMENT OF OSTEOPOROSIS AND ITS IMPACT ON TYPE 2 DIABETES

The identification of bone fragility and osteoporosis diagnosis is troublesome, confounded, and even underestimated in subjects with type 2 diabetes. Consequently, a diagnosis of osteoporosis may occur (too) late in a significant number of individuals with type 2 diabetes - and after the first fragility fracture. This is worrisome and underlines the importance of early attention to and maybe even implementation of bone health in the management of type 2 diabetes.

2.4.4.1 Impact on bone in type 2 diabetes

Treatment of osteoporosis is, in general, successful with both antiresorptive and anabolic drugs with high efficacy in fracture risk reduction. An increase in BMD is accepted as an adequate response to therapy and a significant reduction in fracture risk is then expected. However, BMD measurement is most likely misleading when considering fracture risk in subjects with type 2 diabetes.

Regardless of diabetes status, alendronate is the first choice of anti-osteoporotic therapy in primary and uncomplicated osteoporosis (25). A minor number of patients may indeed be candidates for treatment at the hospital with romosozumab or teriparatide, depending on BMD score and fractures. In Denmark, denosumab is a common second-line anti-osteoporotic drug when alendronate is not tolerated, or evidence of treatment failure exists. Alendronate and denosumab have been evaluated separately in postmenopausal women with type 2 diabetes compared to women without diabetes, and BMD changes and fracture risk reduction were reported similar (147,148). Though the absolute risk is low, the relative risk of atypical fractures increases significantly with the treatment duration of bisphosphonates (149,150). It is possible, that the adynamic bone turnover observed in type 2 diabetes amplifies the risk of atypical fractures after a long time of use of bisphosphonates.

Given the important pathophysiological differences between postmenopausal osteoporosis and deteriorated bone quality in type 2 diabetes, the efficacy of anti-osteoporotic drugs may differ. Thus, when considering the prescription of either alendronate or denosumab as an anti-osteoporotic treatment for individuals with type 2 diabetes one must indeed consider that: 1) BMD increases relatively more after denosumab use compared to alendronate, however, 2) the association between BMD and fracture prediction is uncertain, particularly in subjects with type 2 diabetes, and 3) denosumab suppresses bone turnover more than alendronate, however, 4) bone turnover is already decreased in subjects with type 2 diabetes compared to subjects without diabetes.

All this considered, it is not yet investigated if the efficacy of anti-resorptive therapies to reduce fracture risk differs when the patient suffers from type 2 diabetes.

2.4.4.2 Impact on glucose metabolism

Based on the evidence of an interplay between bone-secreted proteins and glucose metabolism, it appears reasonable to hypothesize that anti-osteoporotic therapies can modify glucose homeostasis by targeting and modulating bone turnover and subsequently the release of the aforementioned osteokines.

Alendronate is suppressing bone remodeling inclusive of the formation marker osteocalcin, and thus, alendronate was initially hypothesized to be associated with insulin resistance, lower insulin secretion, weight gain, and an increased risk of diabetes (151). If true, individuals treated with the most extensively used anti-osteoporotic drug are at increased risk of type 2 diabetes and related morbidities. However, other studies from different continents suggest a neutral or even protective effect of alendronate against the development of type 2 diabetes (152–155). One RCT has been performed with the purpose to investigate the effect of alendronate on glucose metabolism and suggests an improvement of HbA1c as well as fasting blood glucose after 12 weeks of alendronate use (156). However, analyzing the publication in detail reveals several issues that may indeed question the external validity of the findings (156). A complete understanding of the underlying mechanism is still lacking but one in vitro study suggests that alendronate contributes to the prevention of oxidative stress and inflammation in adipocytes (157). Hence, alendronate could potentially impede the progression of adipose tissue dysfunction and insulin resistance. However, more studies and high-quality RCTs are needed to reveal if and how alendronate impact on glucose metabolism and type 2 diabetes risk.

Whereas alendronate is tightly bound to and directly affects the bone matrix, denosumab primarily targets circulating RANKL (158). Bone staining of mice treated with denosumab versus phosphate-buffered saline-treated littermates suggested that denosumab is primarily a circulating soluble protein rather than sustained binding to bone surface (159). The half-life of denosumab is approximately 30 days, indicating that denosumab may be detectable in the circulation, and consequently a potential active molecule in other tissues, for several weeks (160). It has been suggested that higher RANKL levels are associated with an increased risk of type 2 diabetes (127). However, studies investigating if denosumab use per se modulates glucose metabolism in patients with osteoporosis are conflicting and mainly based on post hoc analyses (73,155).

Although these drugs are commonly used worldwide and in theory have the potential to impact other metabolically active organs, human clinical trials, and the direct investigation of the effects on glucose or energy metabolism are sparse. Consequently, how and if alendronate and potentially other anti-osteoporotic therapies affect glucose homeostasis remains unclarified.

2.5. SUMMARY

Collectively, the general mechanisms of postmenopausal bone loss and fragility have been identified, however, the pathogenesis of fracture risk in type 2 diabetes remains unclear. There is epidemiological evidence for increased osteoporotic fracture risk in subjects with type 2 diabetes. Clinical studies indicate low bone turnover and compromised bone quality that are not detected by the current gold standard for diagnosing osteoporosis. Compromised bone health in type 2 diabetes seems associated with insulin resistance, lack of incretin hormones, and high levels of AGEs. The increasing prevalence of both osteoporosis and type 2 diabetes in the elderly population has made both diseases major global health concerns. They often co-exist, and the increased mortality associated with osteoporotic fractures when type 2

diabetes is present underlines the importance of reassessing current guidelines to improve early detection, prevention, and treatment of osteoporosis in this population.

The disparities in bone indices induce several queries of which only a few are addressed in this thesis: e.g., do the first fracture site differ in subjects with type 2 diabetes compared to subjects without diabetes? As the detection of low bone quality is insufficient, does the diagnosis and treatment of osteoporosis differ in subjects with type 2 diabetes? Do subjects with type 2 diabetes benefit more from one anti-osteoporotic drug than another? And lastly, if glucose metabolism affects the bone structure, is it then possible that glucose homeostasis is modulated by bone-targeted therapy?

The aim of thesis, Chapter 2 was to provide an overview of the occurrence, diagnostic challenges, and management when osteoporosis and type 2 diabetes co-exist. From this chapter, please consider the following major points concerning osteoporosis and type 2 diabetes that will be elaborated on in the following chapters:

1. Osteoporosis is a frequent but underdiagnosed and asymptomatic bone disorder until clinical presentation by low-energy fractures.
2. The diagnosis of osteoporosis is defined by a low-energy fracture located at the spine or hip or by a T-score ≤ -2.5 calculated from BMD measured by a DXA-scan.
3. The risk of hip fractures is high in Denmark, and particularly the first hip fracture is of major public health concern regarding mortality and costs.
4. Individuals with type 2 diabetes have an increased risk of hip fractures despite normal or higher BMD levels that are not predicted by the FRAX tool.
5. Mortality and cost are higher in subjects with type 2 diabetes after a hip fracture compared to subjects without diabetes.
6. Type 2 diabetes is not yet acknowledged as an independent risk factor for fractures and evaluation of bone fragility is not considered as a part of diabetes management.
7. Discrepancies in anti-osteoporotic drugs on fracture risk in subjects with type 2 diabetes are unknown.
8. Anti-osteoporotic therapy may modulate glucose metabolism and the likelihood of developing type 2 diabetes.

CHAPTER 3. METHODOLOGY

This thesis is based on 3 epidemiological studies which all originated from the Danish National Registries. The data source description, identification of individuals with diabetes, and identification of confounding factors are in general based on similar approaches. Thus, a general description is provided here, and divergences are detailed in the chapters containing each study together with the specification of statistical analyses.

In Denmark, epidemiological studies do not necessitate approval from an ethics committee. No personally identifiable information was accessed, and all the registers are under the supervision of the Danish Data Protection Agency.

3.1. THE NATIONAL DANISH REGISTERS

3.1.1. DATA SOURCES

In this thesis, several different Danish registers were used, merged, and appended in the data management process. Statistics Denmark provided anonymized data, which is available for authorized Danish research organizations upon application.

Person-specific data are linked between the registers by the unique personal identification number (PIN) which is a 10-digit personal identification number assigned to all Danish citizens at birth or when granted Danish citizenship. Subjects without Danish citizenship but with permission to stay in Denmark, are assigned a temporary PIN. The registry ensures a comprehensive medical history of all individuals' contacts with the Danish healthcare system and their drug redemptions, achieved through the anonymization of their PIN.

The Danish National Health Service provides equal access to full health care to all Danish citizens. This includes free access to hospitals and partial compensation for drug expenses.

Statistics Denmark has, on our request, conducted a register of subjects with prescriptions of glucose-lowering drugs from 1996 to 2018 and diabetes-related diagnoses after 1977 (*Danmarks Statistik*, project identifier no. 703382). Each subject in this population was matched on age, gender, and being alive in the year of diagnosis of diabetes by incidence sampling to 3 randomly selected subjects without diabetes (no prescription of any glucose-lowering drug and no diabetes-related diagnosis code) from the Danish Civil Registration System. From this register, those who met the criteria for diabetes were sampled (section 3.2).

The registers used are listed below:

- *The National Prescription Register* includes information on prescription medicine in Denmark since 1994 and is classified by Anatomical Therapeutic Chemical (ATC) codes with information on the redemption date, dosages, package size, etc. (161,162). To allow for proper data registration, data from 1996 onward were appended.

- *The Danish National Patient Register (NPR)* holds information on all somatic in-patient contacts from 1977 including diagnoses, admission dates, examinations, etc. After 1995 all outpatients, emergency room, and psychiatric contacts were included. All diagnoses are classified according to the International Classification of Diseases (ICD), version 8 before 1994, and the tenth revision (ICD-10) after 1994 (163,164). In the following, only ICD-10 is used and data from 1996 onward were appended.
- *The Danish Civil Registration System (CRS)* was established in 1968 and includes information on all subjects alive and living in Denmark. It holds information on the date of birth, sex, vital status, citizenship, emigration, income, and civil status (165). Data from 1996 onward were appended.
- *The cause of Death register* holds information on the death date and cause. Only death dates were used and data from 1996 onward were appended.

3.1.2. DATA MANAGEMENT

The conduction of an epidemiological study contains a wide range of processes and obliges strict care for each step. All steps have been performed and controlled by the author of this thesis.

The first step was to conduct an appropriate study design to answer the research question based on the hypothesis. A timeline sketch for each study design was always drawn to visualize outcomes, exposures, and covariables. A study protocol or statistical analysis plan including steps in data preparation was conducted and continuously evaluated.

The second step was data collection. This thesis contains data collected, appended, and merged from several different data sets and several years. Statistics Denmark provides raw data for each register and each year separately. First, all required data sets were converted from SAS to STATA. Then, the data sets from each year from the different registers were appended, or stacked, separately. Consequently, one dataset for each register was conducted containing information from a specified period with several rows for each person, e.g., one data set containing information on all prescriptions between 1996 and 2018. The PIN has been anonymized in each data set as a unique ID number. However, in some data sets the ID was illustrated by a number called PNR, and in others called RECNUM, and merging different registers required a unique ID. Identification of the main cohort was performed by identifying diabetes subjects and by merging different data sets on the specific ID number, and if needed, matching the diabetes cohort to subjects without diabetes. Before having a main dataset, some restrictions and information, e.g., death and emigration or exclusion criteria, required further merging into other datasets. Lastly, a main data set was performed containing limited information including an ID number, birth date, diabetes diagnosis date (or dummy date set for control subjects), death date/emigration date, and gender.

These steps in data management resulted in the conduction of different temporary data sets containing huge information and the processing time was rather long. The process was always performed and controlled several times, with all information saved and logged.

In general, a 50-year age cut-off was chosen as postmenopausal osteoporosis sees an increase in prevalence corresponding to the average age of menopause in Denmark, which is 51.7 years (166).

3.2. IDENTIFICATION OF SUBJECTS WITH TYPE 2 DIABETES

The definition and identification of diabetes were performed by a previously published algorithm (167) and based on the algorithm from Statistics Denmark (6). First, all subjects with diabetes were identified by either an ATC code of glucose-lowering drugs specific to diabetes (A10) or any ICD-10 code associated with diabetes (E10.x, E11.x, E12.x, E13.x, E14.x, G63.2, H28.0, H36.0, M14.2, O24, R73), including both primary and secondary diagnoses. Thus, all diabetes subjects were defined based on either hospital visits or the use of glucose-lowering drugs.

The diabetes cohort was subcategorized into type 1 and type 2 diabetes. Individuals with type 1 diabetes were identified by at least one ICD-10 code of E10.x (type 1 diabetes) and at least one ATC code of A10A (insulins and analogs) while having no A10B ATC code (blood glucose-lowering drugs exclusive insulins). Individuals who did not meet the criteria for type 1 diabetes were classified as having type 2 diabetes. The diabetes diagnosis date was set at the first defined diabetes date based on either ICD-10 or ATC codes. Diabetes diagnoses recorded in the Danish registries are generally considered to be valid, and there is a high level of agreement between the actual usage and prescription of diabetes-related medications (168–174).

Clomifene (ATC code G03GB02) and metformin (ATC code MA10BA) is a common treatment combination for women with polycystic ovary syndrome and consequently, individuals who were prescribed metformin and clomifene before the age of 40 years were not included in the cohort.

3.3. EXPOSURE AND OUTCOME

Exposure and outcome will be specified in the chapters. Depending on the research question and hypothesis, the exposure was identified and merged into the main data set, e.g., alendronate use containing information on all redeemed prescriptions with dates, doses, and package sizes. The information was transformed and stored to enable only one row per subject within the data set. The index date was always set at the date of baseline defined by exposure start.

Defined Daily Doses (DDD) of 10 mg and 0.33 mg for alendronate and denosumab, respectively, were used to calculate the cumulative treatment dose based on the World Health Organization Collaborating Centre for Drug Statistics Methodology. Moreover, drug adherence (compliance) was measured using the medication possession ratio (MPR), i.e., dividing the cumulative dose (DDDs) by treatment duration. MPR was categorized into three groups: a) <0.5, b) 0.5-0.8, and c) >0.8; with the latter being considered compliant use. Effective use was defined as the cumulative dose in days if MPR was less than or equal to 0.8 and as the crude treatment duration if MPR was above 0.8.

The outcome was always set as the first identified outcome date after exposure. Outcome information was assessed and merged as the last information to the dataset (except for the case-control design).

3.4. IDENTIFICATION OF CONFOUNDING FACTORS

Confounding factors were identified and merged from other data sets, always using the main data set with one row per person as the "control" set ensuring merging on the unique ID number and index date, e.g., the start of exposure. Identification of confounding factors was performed by information from the literature and by the conduction of directed acyclic graphs (DAGs) (175). DAGs for each study are presented in Appendix B.

Covariates were always identified before/at baseline, i.e., by ICD-10 and ATC codes in the period from data collection (1996) until the index date. A full list and specification of ICD-10 and ATC codes can be found in Appendix C. The algorithms and identification of the main part of confounding factors are published previously (167).

Smoking status was classified as *heavy smoking* due to potential underestimation as it was assessed as a proxy using ICD-10 codes related to lung diseases that were directly or indirectly associated with tobacco exposure, nicotine poisoning, and psychiatric tobacco-related diagnoses. Moreover, ATC codes consistent with any treatment for tobacco dependence were identified, e.g., nicotine replacement therapy, or the initiation of drugs for obstructive airway diseases after the age of 40.

To estimate alcohol abuse, relevant ICD-10 or ATC codes associated with diseases and drugs directly linked to alcohol were used. These codes covered conditions such as intoxication, abuse, alcoholic liver disease, alcoholic cardiomyopathy, alcoholic polyneuropathy, alcoholic gastritis, alcohol-induced pancreatitis, or alcohol-related psychiatric disorders.

As no metric measurements (height and weight) were available, obesity was not evaluated based on BMI but either by ICD-10 codes related to overweight/obesity or the use of anti-obesity therapies.

Pancreatitis was assessed from ICD-10 codes covering both chronic and acute pancreatitis.

Assessment of hyper- and hypothyroidism was based on either ICD-10 or ATC codes.

The definition of hypertension was based on either an ICD-10 code related to hypertension or the prescription of antihypertensive drugs identified by ATC codes.

The assessment of hypoglycemia was based on related ICD-10 codes.

The Charlson Comorbidity Index (CCI) was used to assess comorbidity (176). Discharge diagnoses registered by ICD-10 codes are included in CCI and have a high accuracy (177). The CCI was grouped into 3 according to score: 0, 1, and >2.

The identification of a history of MOF, other fractures besides MOF, osteoporosis diagnosis, and anti-osteoporotic treatment was based on ICD-10 and ATC codes.

3.5. LIMITATIONS AND METHODOLOGICAL CONSIDERATIONS

In general, the STROBE statement guideline for reports of observational and case-control studies was followed (178). However, epidemiological studies are prone to limitations and biases, and it was essential to identify and minimize the consequences of these factors concerning the research question. Importantly, data collection and results from epidemiological studies are retrospective, and consequently, regardless of the design, hinder the inference of causation.

3.5.1. SELECTION PROCESS

As mentioned, the data management process contained several steps and primarily, the selection process is always disposed to bias.

Type 2 diabetes was defined and identified with the criteria from Statistics Denmark and previously published algorithms (6,167). However, it is still an important limitation exposed to selection bias, imprecision of correct diabetes/exposure/outcome data, and information bias, such as misclassification and lead time bias. All subjects with an emigration date without immigration before the index were excluded from the cohort as this potentially provides a period of information bias.

All Danish citizens with type 1 diabetes will eventually receive an ICD-10 DE10 code as they will have contact with the hospital. In contrast, individuals with type 2 diabetes are typically treated by general practitioners outside the hospital. The identification of individuals with naïve or mild cases of type 2 diabetes, who have not received an ICD code or glucose-lowering drugs and have been managed solely with lifestyle interventions, was not feasible. Therefore, only individuals with complicated type 2 diabetes who have been in contact with the hospital will be assigned an ICD-10 DE11 (type 2 diabetes mellitus) code. A state of pre-diabetes was undetectable but may impact bone health and result in higher levels of fractures before the diabetes diagnosis, e.g., a reversed lead time bias. Likewise, the aforementioned bone-related diagnostic difficulties in subjects with pre-diabetes as well as in subjects with type 2 diabetes may indeed be reflected in differences in osteoporosis diagnoses before the index.

In addition, due to the unavailability of laboratory results, it was not feasible to distinguish whether an individual identified by ATC codes was on glucose-lowering medications for diabetes or other conditions such as prediabetes, heart- and nephrological disease without diabetes, or polycystic ovary syndrome. Nevertheless, during the study period, international guidelines did not recommend the use of glucose-lowering drugs for treatment without diagnosed diabetes (179). Consequently, individuals with prediabetes were not expected to present a significant proportion of the included subjects. Moreover, subjects with polycystic ovary syndrome were excluded based on the drug redemptions mentioned above. Furthermore, glucose-lowering drugs besides insulin were not approved as a treatment for individuals with type 1 diabetes in Denmark until 2019. Before 2019, SGLT2 inhibitors and GLP-1 receptor agonists were not approved for any condition other than

type 2 diabetes. Though the definition of diabetes type may result in a mild misclassification of both individuals with type 1 and type 2 diabetes, it may be unlikely to impact the type 2 diabetes classification with a follow-up period ending in 2018.

Missing data were in general sparse and restricted to data concerning social status. The proportion was diminutive in all studies and thus, not expected to impact the main results. Furthermore, a few subjects had misinformed death dates, which were identified by examination of dates for other variables in the same subjects, e.g., if a diagnosis of diabetes occurred after the date of death. In such cases, the subjects were excluded from the cohort and presented in study enrollment figures.

3.5.2. CONFOUNDING FACTORS

Several covariables were identified of which many were recognized as confounding factors. Though the Danish registers contain a wide range of validated information, several unmeasurable factors that could impact the evaluated outcomes were identified. For example, it was not possible to access metric measures or paraclinical results, e.g., BMI, BMD measurements, or blood samples including Vitamin D, calcium, and glycemic control. These may indeed influence and confound the evaluated outcomes in this thesis, e.g., fracture type, treatment initiation time and choice, diabetes risk, and mortality, and limits the inference of causality. In addition, the registers did not include data on smoking habits and alcohol consumption; however, some of these baseline characteristics were evaluated using ICD-10 and ATC codes as proxies as described above. Consequently, these covariates were obtained from subjects with an already developed concomitant disease at the index.

Falling is another potential and prominent factor and effect mediator in the causal pathway (e.g., as illustrated in the DAG, Appendix B1) of fractures in subjects with diabetes (180). Individuals with diabetes have an increased risk of falling which is associated with diabetes-related complications (and comorbidities) (181). Falling is challenging to evaluate in register-based analyses and consequently, none of the presented results have included a mediation analysis on fracture risk from falling. Therefore, the direct effect of type 2 diabetes on MOF was inestimable and the presented results in this thesis are the total effects.

Data on over-the-counter medicines such as vitamin D and calcium supplementation, as well as lifestyle factors like diet and exercise, were not available. Therefore, the potential for residual confounding cannot be ruled out. However, it was possible to measure an E-value and thereby theoretically evaluate the "*minimum strength of association, that an unmeasured confounder would need to have with both the treatment and the outcome to fully explain away a specific treatment-outcome association*" (182).

3.5.3. EXPOSURE AND OUTCOME

Inclusion and index date were set at the same time to prevent a biased association derived from immortal time bias. If inclusion and index was separated due to the study design, the specific analysis prone to immortal time bias was performed as a sensitivity analysis restricted to those subjects with exposure (index) and outcome

after inclusion. For example, if the index date was the type 2 diabetes diagnosis date, exposure was the MOF date and the outcome was mortality, then the analysis from exposure to outcome would be biased by immortal time from index to exposure. In such a case, the analysis was performed including the time from the index to the outcome.

Anti-osteoporotic drugs were set as a secondary outcome in one study and as exposure in 2 other studies. While adverse events have been rarely reported after initiation of denosumab (52), they have been few after the initiation of alendronate (47). Though an adjustment for comorbidities was performed, ICD-10 codes may not fully capture these factors, which could result in confounding by indication in the selection, withdrawal, or discontinuation of anti-osteoporotic treatment.

In general, the fractures included in this thesis are mainly fractures that present clinically. However, some fractures, especially vertebral fractures, are often asymptomatic, challenging to identify, and may go undetected and undiagnosed resulting in underreporting. It has been suggested that individuals with type 2 diabetes have a greater risk of vertebral fractures which may lead to an unbalanced distribution and underestimation of these fractures in this population (183). However, newer imaging techniques, e.g., magnetic resonance imaging (MRI) and computerized tomography (CT) have identified hip fractures may that were undetected by conventional X-ray. For vertebral fractures, the “S” codes are often clinical fractures, whereas M80.x (x may equal 0-9) fractures are detected without a typical clinical presentation and after screening for osteoporosis. Both fractures and diagnosis types are included in the presented studies.

3.6. STATISTICAL ANALYSES

In general, descriptive statistics in tables present baseline characteristics as frequencies (n) and proportions (%), means with standard deviations (±SD), or medians with interquartile range (IQR). In addition, 95% confidence intervals (CI) were calculated, either from means of continuous outcomes or proportions of binary outcomes and are presented in the result section in parentheses. Unpaired t-test, Chi-square test, Wilcoxon Mann-Whitney median test, and relative risk ratios (RR) were performed and calculated to compare and present continuous and dichotomous characteristics between groups. Relevant quantitative variables were grouped if appropriate, e.g., age, CCI score, and index year.

In the regression models, interactions were evaluated with visualization of the simple slopes. If any interactions were identified the main and interaction effects were included in the analysis. Moreover, multicollinearity was evaluated by assessing the variance inflation factor (VIF) between all independent covariables with a value of 5 as the threshold. In the proportional hazard regression models estimating hazard rate ratios (HRs) with 95% CIs, the assumption of proportional hazards was evaluated by Schoenfeld residuals and graphical log-log plots.

All data management and analyses were conducted in STATA 16.1 and 17.0 (StataCorp, College Station, Texas, US). Further analyses will be described in detail within each chapter.

CHAPTER 4. OSTEOPOROSIS-RELATED FRACTURES IN SUBJECTS WITH TYPE 2 DIABETES

4.1. OBJECTIVE AND HYPOTHESIS

Evidence points toward an increased risk of fractures, especially located at the hip, in subjects with type 2 diabetes. However, it is unknown if a hip fracture is also the most frequent first type of MOF compared to subjects without diabetes.

The aim of this thesis, Chapter 4 is to evaluate the type of the first MOF in subjects with type 2 diabetes after the diabetes diagnosis compared to subjects without diabetes.

The null hypothesis is that the location of the first MOF after diabetes diagnosis does not differ between subjects with type 2 diabetes and without diabetes.

The following (including tables and figures) is based on the first part of the manuscript:

Discrepancies in Type of First Major Osteoporotic Fracture and Anti-osteoporotic Therapy in Elderly People with Type 2 Diabetes Mellitus: A Retrospective Danish Cohort Study. Published, doi.org/10.1016/j.bone.2023.116745 (1).

4.2. METHODOLOGY

4.2.1. STUDY DESIGN, SETTING, AND POPULATION

The study was conducted as a retrospective Danish cohort study. A timeline can be found in Appendix D1. The data was available between 1994-2018 and all subjects classified with type 2 diabetes were identified within two decades: between January 1, 1998; and December 31, 2018 (Appendix D1 and D2).

The index date was set at the type 2 diabetes classification date, while a “dummy” date was selected for control subjects. To be included, control subjects had to meet certain criteria, including being alive, residents of Denmark, and at risk at the index date. The study followed individuals with type 2 diabetes and control subjects starting from their index date and continued until the date of death, emigration, or end of the study period (December 31, 2018), whichever occurred first. The analysis only included subjects with a diagnosed MOF after the index date.

Subjects who were classified with diabetes before January 1, 1998, those with classified type 1 diabetes, Paget’s disease, polycystic ovary disease, and individuals with age below 50 years at the index date (Appendix D2) were excluded. Thus, the final cohort comprised adult individuals who were classified with type 2 diabetes at or after 50 years of age in 1998 or later, and a control group who were alive and living in Denmark in 2010.

4.2.2. EXPOSURE AND OUTCOME

The exposure and index date were the type 2 diabetes diagnosis date. The type of first MOF after the index date was the primary outcome. The first MOF was identified by primary or secondary ICD-10 diagnosis codes during hospitalization (Appendix C2) between 1998 and 2018. The MOF was classified into specific types of fractures, namely the Humerus, Forearm, Spine, and Hip (76).

The secondary outcomes will be presented in this thesis, Chapter 5.

4.2.3. STATISTICAL ANALYSES

A multinomial logistic regression (184) was performed to predict the probability of the first type of MOF as the dependent categorical variable between the binary independent "exposure" variables: type 2 diabetes versus subjects without diabetes. No MOF type violated the assumption of independence of Irrelevant Alternatives between outcome categories, IIA, tested by the Hausman-McFadden.

The following other independent covariables were added in the multiple-adjusted analysis: sex, age, follow-up time, history of any MOF, history of other fractures, history of osteoporosis diagnosis, history of anti-osteoporotic treatment, use of anxiolytics/opioids, dyslipidemia, smoking, alcohol, obesity, glucocorticoid use, hypertension, rheumatoid arthritis, CCI category, income, and marital status.

An interaction was identified between sex and age, and consequently, a subgroup analysis stratified by sex and age categories was performed, and the main analysis included the main and interaction (sex*age) effect of sex and age. A likelihood ratio test was performed to evaluate (by the BIC test) which model fits better. For all outcomes, it gave a better fit when adding the adjustments instead of the crude model. Thus, only results from the multiple adjusted models will be presented.

Several sensitivity analyses were performed on the primary outcome. First, subjects with less than 2 years of follow-up were excluded, i.e., diabetes duration (Sensitivity analysis 1). Furthermore, individuals with a history of the following characteristics were excluded: any MOF (Sensitivity analysis 2), any anti-osteoporotic treatment/osteoporosis diagnosis (Sensitivity analysis 3), or both (Sensitivity analysis 4). Lastly, a "rough" time-specific difference in fractures was evaluated by assessing the risks of subjects with index in the first decade (January 1, 1998, to December 31, 2007) and last decade (January 1, 2008, to December 31, 2018) (Sensitivity analysis 5 and 6).

4.3. RESULTS

4.3.1. BASELINE CHARACTERISTICS

Table 4.1 presents data on the baseline characteristics of the study. The population consisted of 124,570 subjects aged ≥50 years with an incident MOF after the index date. The mean age at the index was 69.74 (±10.22) years. Individuals with type 2 diabetes (n=26,588 subjects) were younger and had a lower proportion of female subjects compared to individuals without diabetes (n=97,982).

The mean time to the first MOF from the index was 6.22 years (±4.61) and shorter among type 2 diabetes subjects compared to controls (5.82 years [± 4.52] versus 6.33 years [±4.62].

***Table 4.1**, Baseline characteristics.*

At index characteristic	**All subjects** n = 124,570	**Type 2 diabetes** n = 26,588	**Control subjects** n = 97,982
Age (years), mean ± SD	69.74 (10.22)	69.33 (10.34)	69.85 (10.19)
Age category (years), n (%)			
50-59	26,638 (21.38)	6,052 (22.76)	20,586 (21.01)
60-69	38,220 (30.68)	8,372 (31.49)	29,848 (30.46)
70-79	39,494 (31.70)	7,960 (29.94)	31,534 (32.18)
≥ 80	20,218 (16.23)	4,204 (15.81)	16,014 (16.34)
Sex, n (%)			
Male	41.330 (33.18)	9,268 (34.86)	32,062 (32.72)
Female	83,240 (66.82)	17,320 (65.14)	65,920 (67.28)
History of other fracture, n (%)	14,757 (11.85)	3,397 (12.78)	11,360 (11.59)
History of MOF, n (%)	19,160 (15.38)	4,467 (16.80)	14,693 (15.00)
Humerus	4,870 (3.91)	1,204 (4.54)	3,666 (3.75)
Forearm	9,309 (7.47)	1,945 (7.29)	7,364 (7.53)
Spine	1,399 (1.12)	359 (1.36)	1,040 (1.06)
Hip	3,582 (2.88)	959 (3.61)	2,623 (2.66)
Osteoporosis diagnosis, n (%)	6,136 (4.93)	1,293 (4.86)	4,843 (4.94)
Anti-osteoporotic drug use, n (%)	9,471 (7.60)	1,638 (6.16)	7,833 (7.99)
Heavy smoking, n (%)	27,832 (22.34)	7,631 (28.70)	20,201 (20.62)
Alcohol abuse, n (%)	6,079 (4.88)	1,881 (7.07)	4,198 (4.28)
Obesity, n (%)	11,131 (8.94)	4,673 (17.58)	6,458 (6.59)
Pancreatitis, n (%)	1,047 (0.84)	521 (1.96)	526 (0.54)
Hyperthyroidism, n (%)	3,511 (2.82)	885 (3.33)	2,626 (2.68)
Hypothyroidism, n (%)	6,451 (5.18)	1,720 (6.47)	4,731 (4.83)
Glucocorticoid use, n (%)	29,244 (23.48)	7,615 (28.64)	21,629 (22.07)
Dyslipidemia, n (%)	24,527 (19.69)	8,748 (32.90)	15,779 (16.10)
Hypertension, n (%)	67,254 (53.99)	19,592 (73.69)	47,662 (48.64)
Anxiolytics incl. opioids, n (%)	76,272 (61.23)	18,035 (67.83)	58,237 (59.44)

CCI, mean ± SD	0.54 (1.10)	0.82 (1.34)	0.46 (1.01)
CCI categories, n (%)			
0	88,776 (71.27)	15,824 (59.52)	72,952 (74.45)
1	17,823 (14.31)	4,885 (18.37)	12,938 (13.20)
≥ 2	17,971 (14.43)	5,879 (22.11)	12,092 (12.34)
Income, € in thousands, median (IQR)	24.89 (18.72-36.16)	24.50 (18.73-33.78)	25.03 (18.72-36.82)
Marital status, n (%)			
Married	60,93 (48.88)	12,297 (46.25)	48.596 (49.60)
Divorced	16,609 (13.33)	4,176 (15.71)	12,433 (12.69)
Unmarried	9,097 (7.30)	2,174 (8.18)	6,923 (7.07)
Widowed	37,971 (30.48)	7,941 (29.87)	30,030 (30.65)
Index year, n (%)			
1998-2002	32,290 (25.92)	6,616 (24.88)	25,674 (26.20)
2003-2007	40,209 (32.28)	8,595 (32.33)	31,614 (32.27)
2008-2012	38,510 (30.91)	8,305 (31.24)	30,205 (30.83)
2013-2018	13,561 (10.89)	3,072 (11.55)	10,489 (10.71)

All characteristics were evaluated at the index date. Data are presented as frequencies with proportions (n, %), mean with ±SD, or median with IQR.

4.3.2. MAJOR OSTEOPOROTIC FRACTURE SITES

The presented baseline characteristics (Table 4.1) also revealed that subjects with type 2 diabetes had a higher level of previous other fractures (12.78% versus 11.59%, RR 1.10 [1.06; 1.14]) and MOFs (16.80% versus 15.00%, RR 1.12 [1.09; 1.16]) compared to control subjects. The proportions and 95% CIs indicate a higher likelihood of previous hip, humerus, and spine fractures and no difference in previous forearm fractures among those who developed type 2 diabetes compared to those who did not develop diabetes: Hip, 3.61% (3.40; 3.85) versus 2.66% (2.56; 2.76); Humerus, 4.54% (3.63; 3.87) versus 3.75% (3.63; 3.87); Spine, 1.36% (1.23; 1.50) versus 1.06% (0.10; 1.13); Forearm, 7.29% (6.98; 7.61) versus 7.53% (7.36; 7.69).

The types of first MOF after the diabetes diagnosis are presented in Table 4.2. In general, the most frequent type of first MOF (both before and after the index date) was fractures of the forearm. However, the frequency of forearm fractures as the first MOF seemed lower among individuals with type 2 diabetes than those without diabetes. Subjects with type 2 diabetes had a more frequent first MOF located at the humerus or hip compared to those without diabetes. The adjusted predicted discrete probability differences of the first MOF type in subjects with type 2 diabetes compared to controls are presented in Table 4.2 and stratifications by sex and age are illustrated in Figures 4.1 and 4.2, respectively (1).

Most sensitivity analyses did not vary from the main analysis. After excluding all subjects with a time to MOF from the index below 2 years, spine fractures moved toward a lower probability for subjects with type 2 diabetes as the first MOF type (-

0.42% [-0.94; 0.09]). Interestingly, vertebral fractures became more often the first fracture in subjects with type 2 diabetes compared to control subjects in those with index in the last decade of the study period with a difference of 0.84% (0.09; 1.58) compared to the first decade with a difference of -0.25 (-0.82; 0.31) (main analysis difference: -0.03 [-0.48; 0.42].

***Table 4.2**, Type of first MOF.*
Multiple adjusted predicted probability differences (%) of the first MOF type.

		Type of MOF, Numbers, n (%) and predicted probability differences, % (95% CI)			
N (%)	**Any MOF**	**Humerus**	**Forearm**	**Spine**	**Hip**
All	124,570 (100)	28,845 (23.16)	44,113 (35.41)	14,256 (11.44)	37,356 (29.99)
Control	97,982 (100)	21,960 (22.41)	36,118 (36.86)	11,014 (11.24)	28,890 (29.49)
T2D	26,588 (100)	6,885 (25.90)	7,995 (30.07)	3,242 (12.19)	8,466 (31.84)
Difference*	-	2.82 (2.17; 3.46)	-6.77 (-7.46; -6.08)	-0.03 (-0.48; 0.42)	3.98 (3.29; 4.67)
Female					
Control	65,920 (67.28)	13,636 (20.69)	28,292 (42.92)	5,939 (9.01)	18.053 (27.39)
T2D	17,320 (65.14)	4,426 (25.55)	5,883 (33.97)	1,735 (10.02)	5,276 (30.46)
Difference*	-	4.12 (3.33; 4.91)	-9.13 (-10.01; -8.25)	-0.17 (-0.33; 0.66)	4.85 (4.02; 5.67)
Male					
Control	32,062 (32.72)	8,324 (25.96)	7,826 (24.41)	5,075 (15.83)	10,837 (33.80)
T2D	9,268 (34.86)	2,459 (26.53)	2,112 (22.79)	1,507 (16.26)	3,190 (34.42)
Difference*	-	0.42 (-0.69; 1.53)	-1.99 (-3.03; -0.94)	-0.49 (-1.41; 0.43)	2.06 (0.86; 3.26)
Age 50-59					
Control	20,586 (7.80)	5,782 (28.09)	9,966 (48.41)	2,161 (10.50)	2,677 (13.00)
T2D	6,052 (7.37)	2,034 (33.61)	2,348 (38.80)	673 (11.12)	997 (16.47)
Difference*	-	5.54 (4.05; 7.03)	-7.57 (-9.16; -5.98)	-0.23 (-1.14; 0.68)	2.26 (1.21; 3.20)
Age 60-69					
Control	29,848 (10.82)	7,234 (24.24)	12,361 (41.41)	3,503 (11.74)	6,750 (22.61)
T2D	8,372 (9.63)	2,426 (28.98)	2,761 (32.98)	1,038 (12.40)	2,147 (25.65)
Difference*	-	4.56 (3.37; 5.74)	-7.46 (-8.72; -6.19)	-0.34 (-1.12; 0.44)	3.24 (2.12; 4.36)
Age 70-79					
Control	31,534 (17.49)	6,193 (19.64)	9,932 (31.50)	3,763 (11.93)	11,646 (36.93)
T2D	7,960 (13.93)	1,684 (21.16)	2,044 (25.68)	1,046 (13.14)	3,186 (40.03)
Difference*	-	1.04 (0.02; 2.10)	-5.63 (-6.778; -4.50)	0.31 (-0.50; 1.11)	4.29 (3.01; 5.57)
Age ≥80					
Control	16,014 (25.26)	2,751 (17.18)	3,859 (24.10)	1,587 (9.91)	7,817 (48.81)
T2D	4,204 (18.00)	741 (17.63)	842 (20.03)	485 (11.54)	2,136 (51.81)
Difference*	-	0.02 (-1.32; 1.37)	-3.80 (-5.21; -2.39)	0.55 (-0.46; 1.56)	3.22 (1.46; 4.99)

***Multiple adjusted predicted probability Differences** by type of MOF, % (95% CI) with control subjects as comparator. T2D; Type 2 Diabetes.*

***Figure 4.1**, Type of the first MOF.*
Multiple adjusted predicted probability differences (%) for the type of first MOF in Type 2 diabetes compared to control subjects, stratified by gender. Illustrated by a blobbogram around 0 presenting no difference.

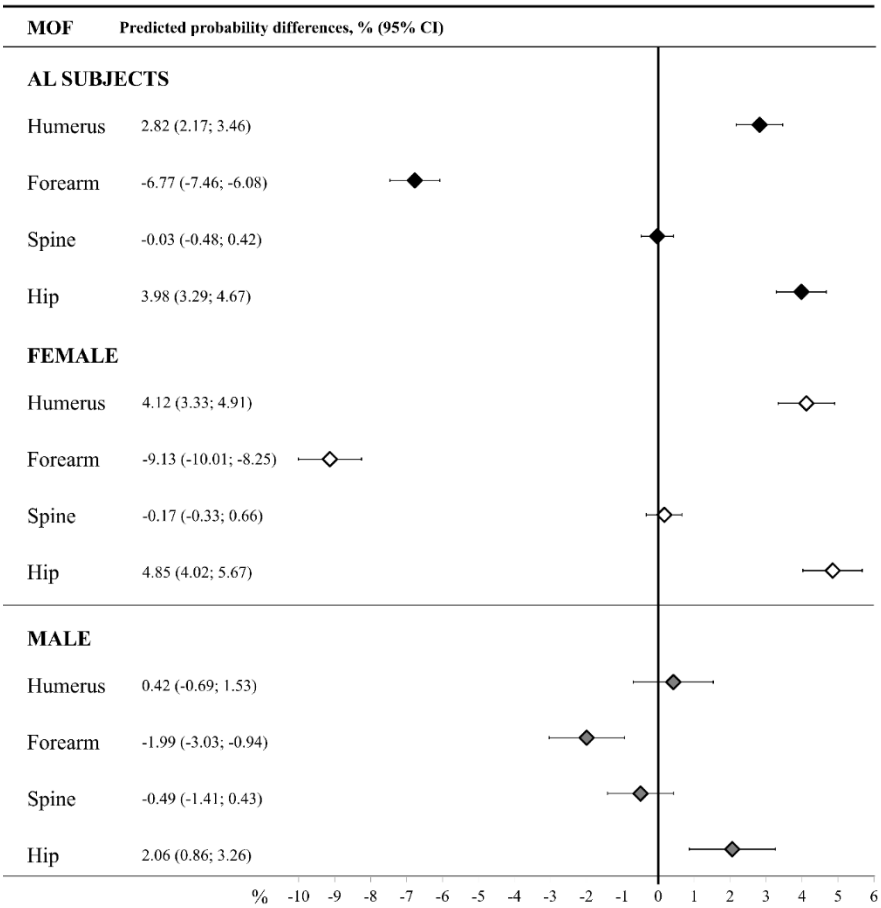
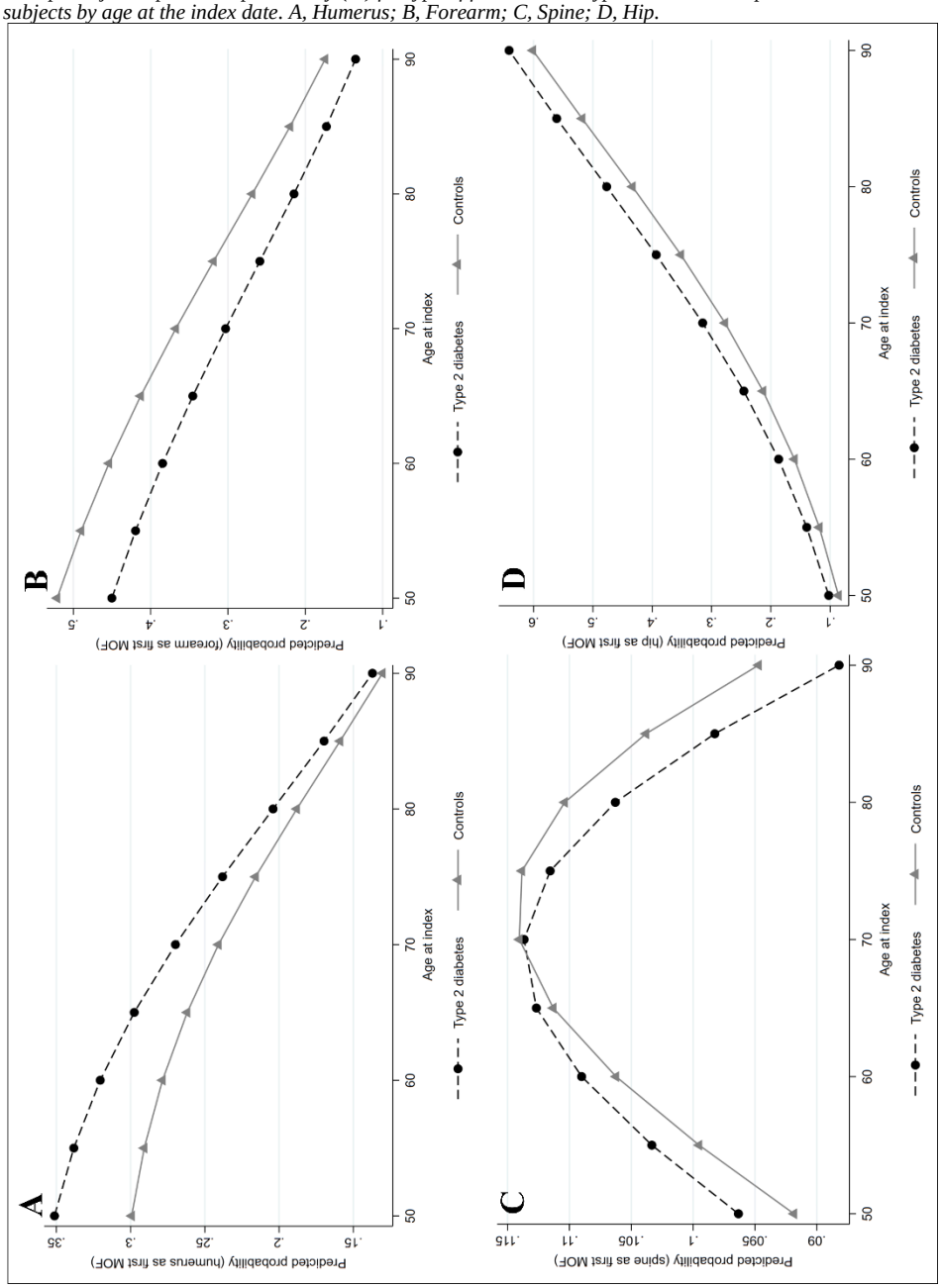


Figure 4.2, *Type of first MOF by age.*
Multiple adjusted predicted probability (%) for type of first MOF in Type 2 diabetes compared to control subjects by age at the index date. A, Humerus; B, Forearm; C, Spine; D, Hip.



4.4. CONCLUSION

The aim of this thesis, Chapter 4 was to evaluate the types of the first major osteoporotic fractures in subjects with type 2 diabetes compared to subjects without diabetes. The presented results do not suggest acceptance of the null hypothesis of no difference in the location of the first MOF after the diabetes diagnosis between subjects with type 2 diabetes and subjects without diabetes.

The most frequent MOF type in both subjects with type 2 diabetes and control subjects was forearm fractures. Before the type 2 diabetes diagnosis, forearm fractures did not differ between subjects who developed type 2 diabetes and subjects who did not develop diabetes. However, the chance of a forearm fracture as the first MOF after the type 2 diabetes diagnosis was lower compared to subjects without diabetes. Contrarily, the likelihood of humerus and hip fractures as the first MOF remained higher after the type 2 diabetes diagnosis compared to subjects without diabetes. There was no difference in the occurrence of spine fractures as the first MOF between individuals with type 2 diabetes and control subjects.

Men have a higher prevalence of type 2 diabetes, as presented in this thesis, Chapter 2, and in the literature (185). Nevertheless, women are at greater risk of developing osteoporosis and experiencing a related fracture after menopause (10). As all subjects included in the presented data sustained a MOF, the results reflected the effects of the female gender. Nonetheless, in both genders, individuals with type 2 diabetes had a higher likelihood of experiencing a hip fracture and a lower likelihood of experiencing a forearm fracture as the first MOF, which was distinct from those without diabetes.

The findings also demonstrated an association with age, i.e., both events of humerus and forearm fractures as the first MOF type decreased with increasing age in both groups. However, hip fracture probability increased with increasing age, and the probability of experiencing a spine fracture showed an inverted U-shaped curve for both individuals with type 2 diabetes and control subjects. The latter observation may suggest a switch in the probability of experiencing a spine fracture from higher to lower after the age of 70. Nonetheless, the deflection also indicated a difference between individuals with type 2 diabetes and controls. Hence, this may suggest that individuals with type 2 diabetes have a higher likelihood of experiencing a spine fracture as the first MOF before the age of 70, but a lower likelihood after the age 70, as compared to individuals without diabetes.

Findings from the last sensitivity analysis suggested that a spine fracture is more often the first MOF in subjects with type 2 diabetes compared to control subjects among those with the index after 2008. One could speculate if this finding is a result of the increased use of CT in the clinical setting of hospitalized patients and consequently increasing incidental findings of asymptomatic vertebral fractures.

All considered, these findings emphasize the importance of early detection and prevention of osteoporosis and fractures in subjects with type 2 diabetes, e.g., by diet, exercise, and fall intervention. Additionally, methods to evaluate and detect low hip and spine bone quality in subjects with type 2 diabetes are needed.

CHAPTER 5. DIAGNOSIS AND TREATMENT OF OSTEOPOROSIS IN SUBJECTS WITH TYPE 2 DIABETES

5.1. OBJECTIVE AND HYPOTHESIS

In the previous chapter, results suggested that individuals with type 2 diabetes have an increased risk of hip and humerus, and over time also spine, as the first osteoporotic-related fracture compared to individuals without diabetes. In Denmark, a low-energy hip or vertebral fracture is equivalent to the osteoporosis diagnosis and direct indication for anti-osteoporotic treatment.

The aims of this thesis, Chapter 5 are to evaluate if subjects with type 2 diabetes are diagnosed with and treated for osteoporosis to the same extent as subjects without diabetes. And secondary, to examine if mortality after a MOF differs in subjects with type 2 diabetes compared to subjects without diabetes.

The null hypothesis is that a type 2 diabetes diagnosis does not impact the diagnosis or initiation of treatment against osteoporosis, or mortality after a MOF.

The following (including tables and figures) is based on the last part of the manuscript:

Discrepancies in Type of First Major Osteoporotic Fracture and Anti-osteoporotic Therapy in Elderly People with Type 2 Diabetes Mellitus: A Retrospective Danish Cohort Study. Published, doi.org/10.1016/j.bone.2023.116745 (1).

5.2. METHODOLOGY

Please find detailed information on the study design, setting, and population as described in this thesis, Chapter 4, section 4.2.1.

5.2.1. EXPOSURE AND OUTCOME

The main outcome of the following was the duration between the first MOF (after the index date) and the initiation of anti-osteoporotic treatment which was evaluated using Cox proportional hazards in treatment-naïve subjects from the cohort. To investigate any disparities between individuals with type 2 diabetes and control subjects after experiencing a MOF, differences in osteoporosis diagnosis and mortality were evaluated.

5.2.2. STATISTICAL ANALYSES

Mortality risk and time to anti-osteoporotic treatment (the first anti-osteoporotic drug redemption in treatment-naïve subjects) were evaluated by cox proportional hazard functions and plotted as 1-Kaplan-Meier cumulative incidence curves.

In the time to treatment analysis, censoring was set to the emigration date, death date, the start of anti-osteoporotic treatment, or the end of the study period (December 31, 2018), whichever came first.

In the mortality analysis, censoring was set to the emigration date, death date, or end of the study period (December 31, 2018), whichever came first.

Crude and adjusted HRs were estimated for each outcome. The analysis of time to treatment was further assessed using a competing risk regression analysis, which was fitted using Fine and Gray's proportional sub-distribution hazard models (186) considering death as a competitive event.

A diagnosis of osteoporosis was evaluated before and after the index date (type 2 diabetes diagnosis) as numbers with percentages (%). Differences between subjects with type 2 diabetes and subjects without diabetes were evaluated by proportions with 95% CI and RR with 95% CI.

5.2.2.1 Sensitivity analyses

After analyzing the duration between the first MOF and anti-osteoporotic treatment, the data were stratified by gender and age at MOF. Moreover, sensitivity analyses were performed on the treatment-naïve subjects, including only female subjects (Sensitivity analysis 1), those with hip and spine fractures (Sensitivity analysis 2), and those who were alive 1 year after the first MOF (Sensitivity analysis 3). Furthermore, a fourth sensitivity analysis evaluated the HRs for treatment initiation within the first year after the first MOF for treatment-naïve subjects without a history of MOF comparing type 2 diabetes to controls (Sensitivity analysis 4). Finally, the effect of unmeasured confounding was assessed using the E-value estimate (182) as described in section 3.5.2.

To eliminate and evaluate immortal time bias (the time from type 2 diabetes diagnosis to MOF), sensitivity analyses were performed on the mortality estimate by only including subjects with a MOF and/or index date after 2010.

5.3. RESULTS

5.3.1. BASELINE CHARACTERISTICS

The baseline characteristic is presented in detail in the previous chapter, Table 4.1. Individuals with type 2 diabetes had a lower proportion of redeemed anti-osteoporotic therapy before the index date (6.16% versus 7.99%, RR 0.77 [0.73; 0.81]). However, there was no difference in the proportion of a previous osteoporosis diagnosis before the index date (4.86% versus 4.94%, RR 0.98 [0.93; 1.05]).

Moreover, individuals with type 2 diabetes were more comorbid (mean CCI 0.82 [± 1.34] versus 0.46 [± 1.01], $p<0.001$) compared to individuals without diabetes.

5.3.2. OSTEOPOROSIS DIAGNOSIS AFTER MOF

After the first MOF after the index, individuals with type 2 diabetes were less likely to receive a diagnosis of osteoporosis (14.54% versus 17.67%, RR 0.82 [0.80; 0.85]). Despite a higher proportion among subjects with hip fractures as the first MOF,

individuals with type 2 diabetes still had a significantly lower proportion of redeemed anti-osteoporotic therapy compared to control subjects (19.64% versus 23.00%, RR 0.85 [0.81; 0.90]).

Likewise, osteoporosis diagnoses after the first MOF in those with treatment after MOF were lower among individuals with type 2 diabetes (46.86% versus 49.19%, RR 0.95 [0.92; 0.98]).

5.3.3. ANTI-OSTEOPOROTIC TREATMENT AFTER MOF

Treatment with anti-osteoporotic therapy after the first MOF was lower among individuals with type 2 diabetes compared to control subjects (18.78% [18.31; 19.26] versus 24.26% [23.99; 24.53], RR 0.77 [0.75; 0.80]). This finding was observed among all ages, and independent of MOF type and index year. Bisphosphonates were the most frequent treatment choice, alendronate accounting for approximately 90% in both subjects with and without type 2 diabetes, followed by denosumab. However, as indicated by the index year, denosumab treatment increased after it became available in 2010 and became the second most frequent choice of treatment in both groups.

5.3.3.1 Evaluation of treatment-naïve subjects

Only subjects without a history of anti-osteoporotic treatment before the first MOF after the index, i.e., treatment naïve subjects, were included in the evaluation of time to anti-osteoporotic after the first MOF.

The proportion of redeemed anti-osteoporotic treatment after the first MOF was lower among subjects with type 2 diabetes (n=3,345, 13.79%) compared to control subjects (n=14,874, 17.37%). The unadjusted and multiple adjusted HRs were 0.82 (0.79; 0.86) and 0.80 (0.77; 0.88), respectively (Table 5.1). The results were visualized by 1-Kaplan-Meier cumulative incidence curves and presented in Figure 5.1. The results did not change in the competing risk analysis.

The results were not changed markedly by any of the four sensitivity analyses (Table 5.1). The E-value was 1.61 for the primary point outcome (HR 0.80) and 1.53 for the confidence interval (0.77; 0.88). Consequently, if the observed HR should be explained away to the null, a potential unmeasured confounder should be associated with both the type 2 diabetes and anti-osteoporotic treatment by HR of 1.61.

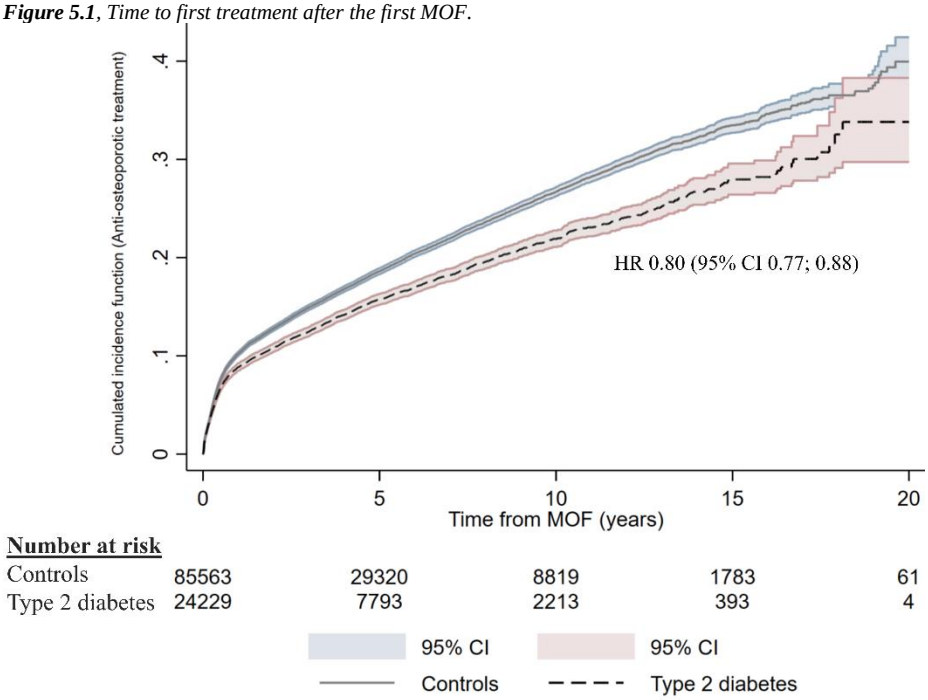
The proportions of treatment-naïve subjects with a diagnosis of osteoporosis and anti-osteoporotic treatment (after the MOF) were 17.28% and 16.58%, respectively. Of those diagnosed with osteoporosis after the MOF, only 60.45% received anti-osteoporotic treatment, and the proportion was lower among subjects with type 2 diabetes (55.41%) compared to controls (61.66%).

***Table 5.1**, Analyses of time to treatment.*
Hazard risk ratios of anti-osteoporotic treatment after first MOF after index among treatment-naïve subjects and sensitivity analyses.

		Hazard risk ratios (HR) and 95% CI		
Numbers and %	**Any treatment,** n (%)	Crude	Adjusted 1[#]	Adjusted 2[ϕ]
All treatment-naïve subjects, n=109,911				
Control, n=85,650	14,874 (17.37)	Reference	Reference	Reference
T2D, n=24,261	3,345 (13.79)	0.82 (0.79; 0.86)	0.84 (0.81; 0.97)	0.80 (0.77; 0.88)
Sensitivity analysis 1: Female subjects, n=70,725				
Control, n=55,342 (64.61)	11,715 (21.17)	Reference	Reference	Reference
T2D, n=15,383 (63.41)	2,555 (16.61)	0.81 (0.78; 0.85)	0.81 (0.78; 0.85)	0.82 (0.78; 0.85)
Sensitivity analysis 2: Hip and Spine as first MOF, n=44,244				
Control, n=33,780 (39,44)	7,123 (21.09)	Reference	Reference	Reference
T2D, n=10,464 (43.13)	1,916 (18.31)	0.88 (0.84; 0.93)	0.86 (0.82; 0.90)	0.84 (0.80; 0.89)
Sensitivity analysis 3: Subjects alive after 1 year, n=97,592				
Control, n=76,658 (89.50)	14,540 (18.97)	Reference	Reference	Reference
T2D, n=20,934 (86.29)	3,209 (15.33)	0.82 (0.79; 0.86)	0.83 (0.81; 0.87)	0.80 (0.77; 0.83)

*Any anti-osteoporotic treatment after the first MOF after the index date in treatment-naïve subjects. Adjusted HRs (95% CIs) with control subjects as comparators (reference). **Sensitivity analysis 1**, only including female subjects. **Sensitivity analysis 2,** only including subjects with hip and spine as first MOF. **Sensitivity analysis 3,** only including subjects alive 1 year after the first MFO. Sensitivity analysis 4, analysis of treatment within the first year after the first MOF among subjects without a history of anti-osteoporotic treatment or a MOF before the index date. **#** Adjusted for sex (omitted in no. 1) and age. **Φ** Multiple adjustments for sex (omitted in no. 1), age at MOF, type of MOF (omitted in no. 2), history of any MOF (omitted in no. 4), history of other fractures, history of osteoporosis diagnosis, use of anxiolytics/opiods, dyslipidemia, smoking, alcohol, obesity, glucocorticoid use, hypertension, rheumatoid arthritis, CCI category, income and marital status. T2D; Type 2 Diabetes*

***Figure 5.1**, Time to first treatment after the first MOF.*



The study evaluated differences in covariates at the date of MOF between subjects who received anti-osteoporotic treatment after the first MOF following the index date and those who did not. Subjects who received anti-osteoporotic treatment were observed to be younger at the time of MOF in comparison to those who did not receive treatment (74.98 (±9.04) versus 75.63 (±10.68) years, $p<0.001$). Those who received treatment were more likely to be females (78.32% [77.72; 78.92] versus 61.57% [61.25; 61.89]). Subjects who experienced a spine fracture as the first MOF (28.00% [27.17; 28.84]) were most likely to be treated, followed by hip (17.87% [17.46; 18.28]), forearm (15.10% [14.75; 15.45]) and humerus (12.29% [11.89; 12.69]). Moreover, a higher proportion of treated subjects had a history of glucocorticoid use, anxiolytic use, and smoking, however, those who received treatment were less likely to have a history of alcohol abuse, dyslipidemia, hypertension, and obesity ($p\leq0.001$ for all). Moreover, those who received anti-osteoporotic treatment were less comorbid compared to those who did not receive treatment (mean CCI 1.04 [1.02; 1.06] versus 1.25 [1.24; 1.26]).

Differences in comorbidities were evaluated and defined by the diagnoses codes and categories within the comorbidity index (CCI). Individuals who received anti-osteoporotic medication after the first MOF were less likely to have late diabetes-related complications (CCI nr. 13) compared to those who did not receive treatment (3.87% versus 5.17%, RR 0.75 [0.69; 0.81]). Similarly, the proportions of nephrological- (2.02% versus 3.89%, RR 0.52 [0.47; 0.58]), cardiovascular- (22.08%

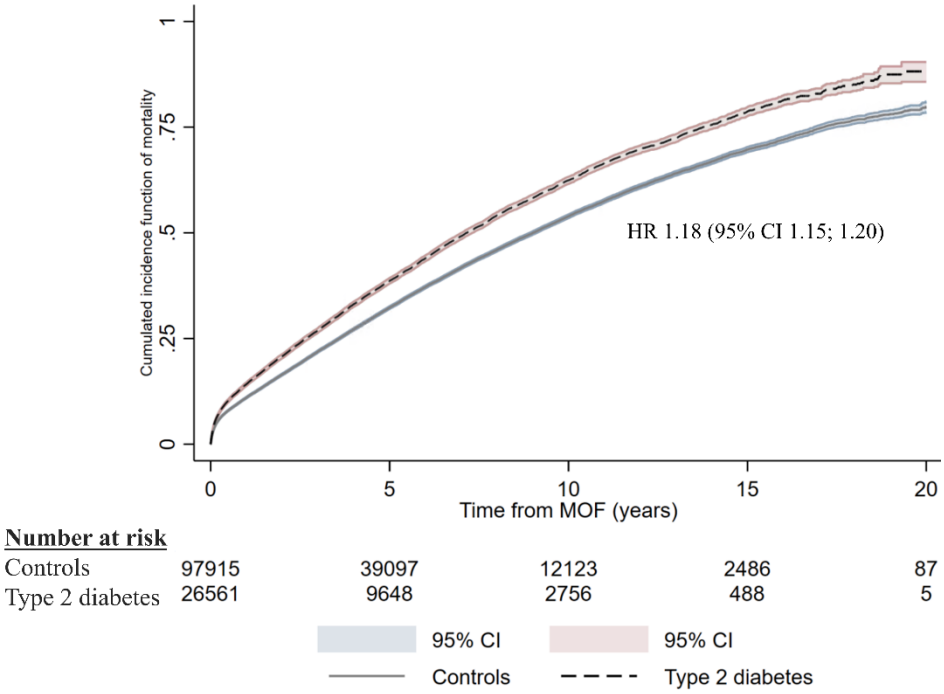
versus 25.35%, RR 0.87 [0.85; 0.90]) and cancer-related diseases (14.77% versus 17.32%, RR 0.85 [0.82; 0.89]) were lower among those who were treated with anti-osteoporotic medications than untreated subjects. Both type 2 diabetes and control subjects who received anti-osteoporotic treatment showed lower comorbidity levels.

5.3.4. MORTALITY AFTER MOF

In general, a higher proportion of individuals with type 2 diabetes died during the study period compared to controls (45.80% versus 39.11%).

The unadjusted and multiple adjusted cox proportional HRs for mortality (for subjects with type 2 diabetes compared to controls) after the first MOF after the index date were 1.27 (1.24; 1.30) and 1.18 (1.15; 1.20), respectively. The 1-Kaplan-Meier incidence curve is illustrated by Figure 5.2. Moreover, the proportion of deaths among those who did not receive anti-osteoporotic treatment after the MOF was higher among type 2 diabetes individuals than control subjects (46.11% [45.45; 46.78] versus 39.86 [39.50; 40.21]).

***Figure 5.2**, Mortality after the first MOF.*



5.4. CONCLUSION

The aims of this thesis, Chapter 5, were to assess diagnostics, anti-osteoporotic treatments, and mortality after the first major osteoporotic fracture in type 2 diabetes compared to subjects without diabetes. These findings suggest rejecting the null hypothesis that a type 2 diabetes diagnosis does not impact the diagnosis or initiation of treatment against osteoporosis, or mortality after a MOF.

Individuals with type 2 diabetes had a lower chance of receiving an osteoporosis diagnosis and anti-osteoporotic medication after the first MOF compared to control subjects. Currently, the main treatment strategy for osteoporosis is either by bisphosphonates or denosumab. The results from this thesis, Chapter 4, suggested that individuals with type 2 diabetes were more likely to have a history of previous fractures and MOFs compared to individuals without diabetes. Yet, the results from the current Chapter 5 suggest that there is no difference in the likelihood of receiving an osteoporosis diagnosis before the index date. Nonetheless, subjects with type 2 diabetes were not treated with anti-osteoporotic medications to the same extent as control subjects before the diabetes diagnosis.

Furthermore, individuals with type 2 diabetes were less likely to obtain an osteoporosis diagnosis after the first MOF after the index date compared to subjects without diabetes. This issue became even more concerning as individuals with type 2 diabetes had a greater likelihood of experiencing a hip fracture as their first MOF (as presented in this thesis, Chapter 4).

The risk of fractures and identification of osteoporosis are underestimated with current diagnostic methods, and the bone quality at the hip is not detectable by newer techniques. However, hip fracture is associated with higher mortality in subjects with type 2 diabetes. Evaluation of bone health and osteoporosis in subjects with type 2 diabetes is challenging. However, attention is needed as osteoporosis diagnosis followed by adequate anti-osteoporotic treatment may prevent new fractures and improve life quality and expectancy in this population. Yet, it seems that individuals with type 2 diabetes are less likely to obtain a diagnosis of osteoporosis and redemption of anti-osteoporotic treatment after a major fracture related to osteoporosis. Furthermore, individuals with type 2 diabetes are more likely to die after an osteoporotic-related fracture compared to individuals without diabetes.

CHAPTER 6. THE EFFICACY OF ANTI-OSTEOPOROTIC TREATMENT IN SUBJECTS WITH TYPE 2 DIABETES

6.1. STUDY OBJECTIVE

Anti-osteoporotic treatment is an essential part of fracture prevention. It seems, that alendronate and denosumab have overall similar beneficial impacts on the BMD and protective effects on incident fractures in subjects with type 2 diabetes when evaluated separately (147,148,187). However, the effect on fracture risk has not yet been compared head-to-head in subjects with type 2 diabetes.

The aim of this thesis, Chapter 6 is to examine the efficiency of the anti-osteoporotic treatments on osteoporotic fracture risk in subjects with type 2 diabetes.

The null hypothesis is that the risk of a new major osteoporotic fracture in subjects with type 2 diabetes does not differ between initiators of denosumab and alendronate.

The following chapter (including tables and figures) is based on the manuscript:

The Efficacy of Alendronate Versus Denosumab on Major Osteoporotic Fracture Risk in Elderly Patients with Diabetes Mellitus: A Danish Retrospective Cohort Study. Published, doi:10.3389/fendo.2021.826997 (2).

6.2. METHODOLOGY

6.2.1. STUDY DESIGN AND SETTING

The study was designed as a retrospective cohort study and Appendix E1 presents the study timeline. All patients with diabetes were identified between 2000 and 2018. The period was chosen to ensure that all individuals with pre-existing diabetes were identified and to ensure a proper assessment of diabetes duration before exposure.

The index date was set at exposure initiation of either alendronate or denosumab. As Denosumab became available as a treatment in Denmark in 2010, data on exposure were collected between 2011-2018. The study time was set from the exposure date until the outcome or censoring by death, emigration, or end of the study period (December 31, 2018), whichever came first.

6.2.2. STUDY POPULATION

A flow diagram of the selection process can be found in Appendix E2. Only subjects with diabetes, alive and Danish citizens with no emigration history on January 1, 2011, were included in the study. Individuals with ages below 50 at the index and those who had classified diabetes before January 1, 2000, were excluded. Moreover, subjects with any redemption of alendronate, denosumab, or other anti-osteoporotic therapy (Appendix C1) before the index date were excluded. Consequently, the final cohort

was delimited to adults with new-onset diabetes between January 1, 2000, and December 31, 2018, and with the initiation of an anti-osteoporotic drug of either alendronate or denosumab at age 50 years or older and after the diabetes diagnosis.

6.2.3. EXPOSURE AND OUTCOME

6.2.3.1 Exposure; Alendronate or denosumab initiation

Exposure was defined as the first dispense of either alendronate or denosumab at age 50 years or older, after diabetes diagnosis, and after January 1, 2011. The ATC codes used were “M05BA04” and “M05BX04”, respectively. The index date was set as the first drug redemption of exposure during the study period.

Equivalent to the intention-to-treat approach used in RCTs, subjects were considered exposed to the initiated drug on the index date. A crude treatment duration was calculated by adding the number of daily doses at the last dispensation date to this date and then subtracting the date of the first drug dispensation. The cumulative treatment dose (DDDs), compliance (MPR), and effective use were calculated and evaluated (as described in Section 3.3).

6.2.3.2 Outcome; MOF

Outcome information (MOF) was obtained by identification of all diagnoses related to fractures located at the spine, hip, humerus, or forearm (76).

The primary outcome was set as the first identified MOF, either by primary or secondary diagnoses using ICD-10 codes (Appendix C2) after the index date during the study period. MOFs were further categorized into the specific type, i.e., fracture of the spine, hip, humerus, or forearm.

6.2.4. STATISTICAL ANALYSIS

The exposure-specific cumulative incidence curves for any first MOF were plotted.

The analysis of time to MOF after treatment was assessed using a competing risk regression analysis, which was fitted using Fine and Gray’s proportional sub-distribution hazard models (186) considering death as a competitive event and alendronate exposure set as the comparator. Because an interaction was identified between age and a history of fracture, data were stratified by fracture history, age (< and ≥ 75 years), and sex, and a subgroup analysis was performed.

6.3. RESULTS

6.3.1. BASELINE CHARACTERISTICS

Table 6.1 presents baseline characteristics. The mean age was 73.62 (±9.27) years and 98% were suffering from type 2 diabetes with a median diabetes duration at baseline of 5.45 years (2.41; 9.19).

Denosumab initiators were older with mean ages 75.60 (±9.72) versus 73.51 (±9.23) years ($p<0.001$), were more frequently women (81% versus 68%, RR 1.18 [1.13; 1.24]), and were more comorbid (mean CCI 2.26 [2.07; 2.44] versus 1.78 [1.74; 1.82]) compared to alendronate initiators.

Moreover, individuals exposed to denosumab had a higher proportion of previous fractures (64% versus 46%, RR 1.38 [1.28; 1.48]), peptic ulcers (16% versus 7%, RR 2.14 [1.72; 2.66]), and renal impairment (11% versus 6%, RR 1.93 [1.47; 2.53]).

The highest proportion of hyperthyroidism (5.10% versus 3.08%, RR 1.66 [1.11; 2.48]), insulin use (22.65% versus 18.69%, RR 1.21 [95% CI 1.02; 1.44]), opioid use (84.29% versus 76.83%, RR 1.10 [1.05; 1.14]) and anxiolytic use (92.86% versus 88.26%, RR 1.05 [1.03; 1.08]) were found among denosumab initiators compared to the alendronate initiators.

***Table 6.1**, Baseline characteristics.*

	All subjects n = 8,745	**Alendronate n = 8,255**	**Denosumab n = 490**
Age (years), mean ± SD	73.62 (9.27)	73.51 (9.23)	75.60 (9.72)
Age category (years), n (%)			
50-59	755 (9)	720 (9)	35 (7)
60-69	2,196 (25)	2,100 (25)	96 (20)
70-79	3,481 (40)	3,293 (40)	188 (38)
≥ 80	2,313 (26)	2,142 (26)	171 (35)
Sex, n (%)			
Female	6,043 (69)	5,647 (68)	396 (81)
Male	2,702 (31)	2,608 (32)	94 (19)
Type 2 diabetes, n (%)	8,589 (98)	8,114 (98)	475 (97)
Diabetes duration in years, median (IQR)	5.45 (2.41-9.19)	5.43 (2.41-9.18)	5.57 (2.34-9.52)
History of any fracture, n (%)	4,141 (47)	3,828 (46)	313 (64)
CCI, mean ± SD	1.81 (1.89)	1.78 (1.88)	2.26 (2.07)
CCI categories, n (%)			
0	2,609 (30)	2,491 (30)	118 (24)
1	1,963 (22)	1,879 (23)	84 (17)
≥2	4,173 (48)	3,885 (47)	288 (59)
Peptic ulcer, n (%)	507 (6)	455 (6)	52 (11)
Renal impairment, n (%)	693 (8)	615 (7)	78 (16)
Income, € in thousands, median (IQR)	26.13 (19.85-32.54)	26.11 (19.85-32.58)	26.44 (19.92-31.53)
1st Quintile, n (%)	1,749 (20)	1,645 (20)	104 (21)
2nd Quintile, n (%)	1,749 (20)	1,677 (20)	72 (15)
3rd Quintile, n (%)	1,749 (20)	1,637 (20)	112 (23)
4th Quintile, n (%)	1,749 (20)	1,637 (20)	112 (23)
5th Qiuntile, n (%)	1,749 (20)	1,659 (20)	90 (18)
Marital status, n (%)			
Married	4,241 (49)	4,015 (49)	226 (56)
Divorced	1,358 (16)	1,280 (16)	78 (16)
Unmarried	606 (6.93)	574 (7)	32 (7)
Widowed	2,534 (29)	2,380 (29)	154 (31)
Unknown	6 (0)	6 (0)	0 (0)
Heavy Smoking, n (%)	3,116 (36)	2,927 (35)	189 (39)

Alcohol abuse, n (%)	747 (9)	708 (9)	39 (8)
Obesity, n (%)	1,543 (18)	1,457 (18)	86 (18)
Pancreatitis, n (%)	298 (3)	283 (3)	15 (3)
Hyperthyroidism, n (%)	279 (3)	254 (3)	25 (5)
Hypothyroidism, n (%)	629 (7)	589 (7)	40 (8)
Glucocorticoid use, n (%)	5,027 (57)	4,757 (58)	270 (55)
Statin use, n (%)	6,791 (78)	6,424 (78)	367 (75)
Insulin use, n (%)	1,654 (19)	1,543 (19)	111 (23)
Hypoglycemia, % ± SD	193 (2)	177 (2)	16 (3)
Hypertension, n (%)	7,996 (91)	7,543 (91)	453 (92)
Opioid use, n (%)	6,755 (77)	6,342 (77)	413 (84)
Anxiolytics, n (%)	7,741 (89)	7,286 (88)	455 (93)
Initiation year, n (%)			
2011	1,098 (13)	1,018 (12)	80 (16)
2012	1,052 (12)	989 (12)	63 (13)
2013	1,076 (12)	1,018 (12)	58 (12)
2014	1,100 (13)	1,044 (13)	56 (11)
2015	1,072 (13)	1,021 (12)	51 (10)
2016	1,113 (13)	1,050 (13)	63 (13)
2017	1,155 (13)	1,099 (13)	56 (11)
2018	1,079 (12)	1,016 (12)	63 (13)

All characteristics were evaluated at the index date. Data are presented as numbers (n, %), mean with ±SD, or median with IQR.

6.3.2. RISK OF INCIDENT MOF

The median follow-up time was 2.67 (1.17; 4.62) and 2.36 (0.95; 4.53) years among the alendronate and denosumab initiators, respectively. A higher proportion of deaths were identified in the denosumab group (34% versus 27%, RR 1.29 [1.14; 1.47]). The median treatment duration expressed in days by cumulative DDDs of alendronate and denosumab was 560 (182; 1,218) and 727 days (363; 1,455), respectively.

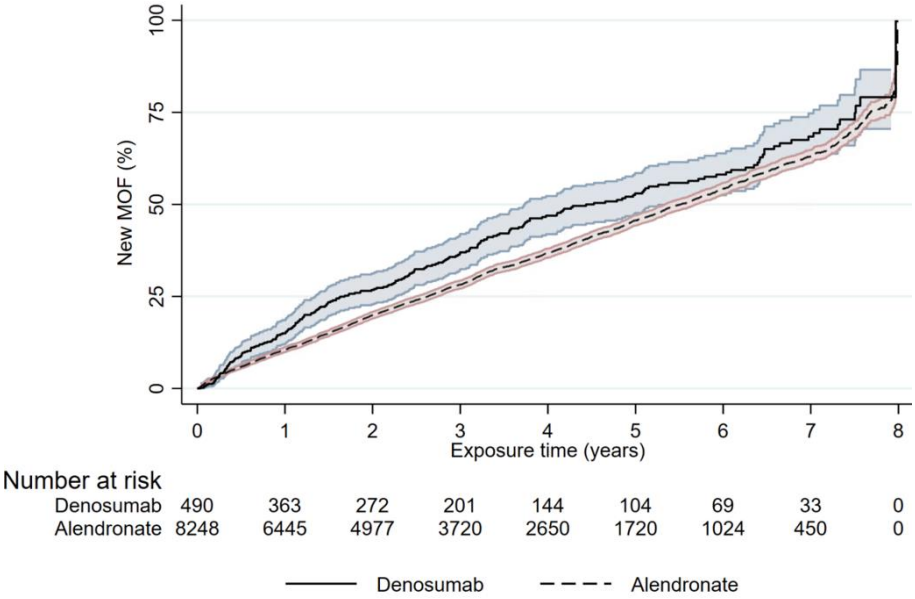
The risk of MOFs during the study period is presented in Table 6.2. An incident MOF was experienced by 49% (n=238) and 39% (n=3,256) of denosumab and alendronate initiators, respectively. The MOF incidences per 1000 person-years were 168.54 (148.43; 191.37) and 130.64 (95% CI 126.22; 135.21) for denosumab and alendronate, respectively, with an incidence rate ratio (IRR) of 1.29 (1.13; 1.47). Consequently, the crude risk of any MOF was higher among initiators of denosumab during the study period, HR 1.26 (1.10; 1.44) with the alendronate initiator group as the comparator. The 1-Kaplan-Meier failure curve is presented by Figure 6.1. The risk was diminished and partly reversed by the multiple-adjusted model (HR 0.89 [95% CI 0.89; 1.02]). The most prevalent MOF type was hip fractures in both exposure groups, followed by fractures of the forearm, spine, and humerus (Table 6.2).

***Table 6.2**, Risk of MOF and stratifications.*

			Hazard ratios (HR) and 95% CI		
	Exposure	**MOF, n (%)**	Crude	Adjusted 1#	Adjusted 3Φ
Overall	Denosumab	238 (49)	1.26 (1.101.44)	1.17 (1.03-1.34)	0.89 (0.78-1.02)
	Alendronate	3,256 (39)	1 (reference)	1 (reference)	1 (reference)
Age category					
< 75 years	Denosumab	88 (37)	1.18 (0.95-1.47)	1.14 (0.92-1.42)	0.80 (0.64-1.00)
	Alendronate	1,504 (46)	1 (reference)	1 (reference)	1 (reference)
≥ 75 years	Denosumab	150 (63)	1.23 (1.04-1.46)	1.20 (1.02-1.42)	1.97 (0.82-1.16)
	Alendronate	1,752 (54)	1 (reference)	1 (reference)	1 (reference)
Sex					
Female	Denosumab	203 (85)	1.19 (1.03-1.38)	1.17 (1.01-1.35)	0.90 (0.77-1.04)
	Alendronate	2,416 (74)	1 (reference)	1 (reference)	1 (reference)
Male	Denosumab	35 (15)	1.29 (0.91-1.82)	1.20 (0.85-1.26)	0.86 (0.63-1.26)
	Alendronate	840 (26)	1 (reference)	1 (reference)	1 (reference)
History of any fracture					
Yes	Denosumab	218 (92)	0.90 (0.78-1.05)	0.89 (0.77-1.03)	0.87 (0.75-1.01)
	Alendronate	2,863 (88)	1 (reference)	1 (reference)	1 (reference)
No	Denosumab	20 (8)	1.23 (0.78-1.94)	1.12 (0.72-1.75)	1.13 (0.72-1.77)
	Alendronate	393 (12)	1 (reference)	1 (reference)	1 (reference)
Type of the first MOF					
Spine	Denosumab	45 (19)	1.13 (0.84-1.53)	1.14 (0.84-1.53)	0.82 (0.59-1.15)
	Alendronate	684 (21)	1 (reference)	1 (reference)	1 (reference)
Hip	Denosumab	98 (41)	1.31 (1.06-1.62)	1.20 (0.97-1.48)	0.93 (0.75-1.16)
	Alendronate	1,289 (40)	1 (reference)	1 (reference)	1 (reference)
Humerus	Denosumab	33 (14)	1.31 (0.92-1.87)	1.20 (0.97-1.48)	0.91 (0.63-1.29)
	Alendronate	434 (13)	1 (reference)	1 (reference)	1 (reference)
Forearm	Denosumab	62 (26)	1.25 (0.97-1.62)	1.13 (0.87-1.46)	0.87 (0.66-1.14)
	Alendronate	849 (26)	1 (reference)	1 (reference)	1 (reference)

*MOF, n (%) represents numbers and % of MOFs in each category by exposure. Adjusted HRs (95% CIs) with alendronate exposure as the reference with the exclusion of stratified categories in adjusted analyses. # Adjusted for sex and age. **Φ** Multiple adjustments for sex, age, history of fractures, diabetes duration, insulin, hypoglycemia, anxiolytics, statin, opioid, smoking, alcohol, glucocorticoid, pancreatitis, hypo- and hyperthyroidism, peptic ulcer, renal impairment, CCI, income and marital status.*

Figure 6.1, *Kaplan-Meier failure curve of incident MOF.*



Discontinuation of the original treatment was observed in 4,078 (47%) subjects. In general, the baseline characteristics did not differ between the original cohort and these subjects (Appendix E3). Of those who discontinued treatment, 3,484 subjects discontinued without a prescription of other anti-osteoporotic drugs, 149 (30%) denosumab initiators, and 3,284 (42%) alendronate initiators. A total of 445 subjects replaced the original treatment with another anti-osteoporotic treatment before the end of follow-up: 274 switched from alendronate to denosumab, 7 switched from denosumab to alendronate and 165 switched to a third anti-osteoporotic therapy (all alendronate initiators).

6.4. CONCLUSION

The aim of this thesis, Chapter 6 was to examine the efficiency of anti-osteoporotic treatments on osteoporotic fracture risk in subjects with type 2 diabetes. This was assessed by evaluation of the risk of an incident MOF after initiation of either denosumab or alendronate among subjects. The results suggest accepting the null hypothesis of no difference in the risk of a new MOF in subjects with type 2 diabetes between initiators of denosumab and alendronate.

A hip fracture was the most frequent MOF type in diabetes patients after treatment initiation with both the alendronate and denosumab with similar risks between exposures. Nevertheless, the estimates moved towards a protective effect of denosumab in the multiple adjusted analysis.

As discussed previously, BMD is often normal and inappropriately high in patients with type 2 diabetes and, to date, is the only available method for diagnosing osteoporosis in the clinical setting. Consequently, a proper response to anti-osteoporotic therapy is restricted to the assessment of fracture risk when the patient suffers from diabetes. Yet, fracture assessment most often requires long-term follow-up making it hard to evaluate this endpoint in clinical trials.

The presented results may indeed point towards an improvement of bone quality (based on fracture risk) in subjects with type 2 diabetes exposed to denosumab compared to alendronate, though the estimates did not reach statistical significance. The risk was not related to sex, age, or a previous fracture and did not differ among switchers or those with discontinuation. Moreover, despite a faster clearance of alendronate, the change in the risk of a new MOF in denosumab users did not differ from alendronate among those who discontinued treatment without replacement, and neither did the results support any effect based on effective use.

To date, no specific treatment recommendations for osteoporosis in the presence of diabetes exist. However, the hope is that the current findings encourage attention to the treatment of osteoporosis and fracture prevention in diabetes. As BMD is an insufficient measure of fracture risk in this population, further data are needed to elucidate whether the bone-specific efficiency of anti-osteoporotic therapies differs in subjects with diabetes.

CHAPTER 7. ALENDRONATE AND THE PROSPECT OF TYPE 2 DIABETES

7.1. STUDY OBJECTIVE

Structure, protection, and mechanical strength are buzzwords when considering the function of bone tissue. Temporarily, bone tissue also produces proteins with endocrine actions affecting glucose metabolism. Bone remodeling is regulated and modified by anti-osteoporotic therapies and thus, it seems plausible that these drugs may have the potential to impact on other metabolically active tissues as well.

The aim of this thesis, Chapter 7 is to explore the proposed link between bone- and glucose metabolism by examining a potential relationship between alendronate and type 2 diabetes.

The null hypothesis is that the likelihood of developing type 2 diabetes is not altered by alendronate administration.

The following chapter (including tables and figures) is based on the manuscript:

Alendronate Use and Risk of Type 2 Diabetes: A Nationwide Danish Nested Case-Control Study. Published, doi:10.3389/fendo.2021.771426 (3).

7.2. METHODOLOGY

7.2.1. STUDY DESIGN AND SETTING

The following study is designed as a population-based nested case-control study. A study timeline is presented in Appendix F1.

Subjects with type 2 diabetes were defined as cases, and controls were subjects without a history of diabetes. Alendronate use before the index date (type 2 diabetes or a dummy date for control subjects) was set as exposure. The time of data collection was set between January 1, 1998, and December 31, 2018. Firstly, information on the outcome (type 2 diabetes diagnosis) was assessed from January 1, 2008, until December 31, 2018. Afterward, exposure (alendronate use) was assessed from January 1, 1998, defined as the first drug redemption before the outcome or end of the study period (December 31, 2018). As data availability included approximately two decades (1996-2018), the year 1998 and 2008 was chosen to ensure proper data validity by 1998 and one decade for exposure (1998-2008) as well as one decade for outcome occurrence (2008-1998).

7.2.2. STUDY POPULATION

The study population selection process is illustrated in Appendix F2.

To identify an eligible cohort, all subjects with death and emigration before January 1, 1998, were excluded. Then, case subjects, i.e., subjects with diabetes, were identified and the outcome/index date was defined and set at the date of diabetes

classification. Only cases with new onset classified type 2 diabetes at age ≥50 after January 1, 2008, were included.

Then, for each case subject, 3 randomly selected control subjects from the general population were matched by age and gender using incidence-density sampling. Control subjects had to be alive and living in Denmark at the index date to be included, and so, a "dummy" index date was set for each control subject with respect to death and emigration.

7.2.3. OUTCOME AND EXPOSURE

In the nature of the case-control design, the outcome (type 2 diabetes) was identified formerly to define the cohort. The classification of type 2 diabetes was described in Section 3.2.

Alendronate exposure was identified as redeemed alendronate drug prescriptions by the ATC code "M05BA04".

7.2.4. STATISTICAL ANALYSES

Outcome and exposure were evaluated as binary variables of type 2 diabetes (case/control) and alendronate ever use (yes/no), respectively. Alendronate exposure was further grouped as categorical variables of effective use duration intervals (<6 months, 0.5-1.9 years, 2-3.9 years, 4-5.9 years, 6-7.9 years, and ≥8 years) and compliance (MPR <0.5, 0.5-0.8 and >0.8) as described in Section 3.3.

The odds ratios (OR) of type 2 diabetes were estimated by conditional logistic regression models of alendronate exposure as ever use, effective use, and compliant use with 95% CI.

7.2.4.1 Sensitivity analyses

A possible dose-response relationship between the longer effective duration of alendronate use on the risk of type 2 diabetes was evaluated by performing a trend test (conditional logic regression model) only among those who were exposed to alendronate.

Moreover, sensitivity analyses were conducted excluding those with baseline characteristics such as heavy smoking, alcohol abuse, prior pancreatitis, glucocorticoid use, obesity, and people with age above 65.

7.3. RESULTS

7.3.1. BASELINE CHARACTERISTICS

In total, 654,352 subjects were included (163,588 cases and 490,764 controls). Table 7.1 presents baseline characteristics among case and control subjects with a balanced matching (as is also presented in the published manuscript (3)).

Baseline characteristics revealed that individuals with type 2 diabetes were more likely to be heavy smokers, alcohol abusers, obese, and have a history of pancreatitis, hyperthyroidism, hypothyroidism, and use of glucocorticoids. Lastly, subjects with type 2 diabetes were more comorbid compared to controls.

***Table 7.1**, Baseline characteristics of cases and controls.*

	All subjects n = 654,352	**Type 2 diabetes** n = 163,588	**Control subjects** n = 490,764
Age (years), mean ± SD	66.67 ± 10.00	66.67 ± 10.00	66.67 ± 10.00
Age category (years), n (%)			
50-59	198,452 (30.33)	49,613 (30.33)	148,839 (30.33)
60-69	231,028 (35.31)	57,757 (35.31)	173,271 (35.31)
70-79	161,268 (24.65)	40,317 (24.65)	120,951 (24.65)
≥ 80	63,604 (9.72)	15.901 (9.72)	47,703 (9.72)
Sex, n (%)			
Female	293,736 (44.89)	73,434 (44.89)	220,302 (44.89)
Male	360,616 (55.11)	90,154 (55.11)	270,462 (55.11)
Heavy Smoking, n (%)	169,090 (25.84)	53,479 (32.69)	115,611 (23.56)
Alcohol abuse, n (%)	29,460 (4.50)	10,476 (6.40)	18,984 (3.87)
Obesity, n (%)	57,612 (8.80)	28,038 (17.14)	29,574 (6.03)
Pancreatitis, n (%)	4,408 (0.67)	2,640 (1.61)	1,768 (0.36)
Hyperthyroidism, n (%)	15,368 (2.35)	4,841 (2.96)	10,527 (2.15)
Hypothyroidism, n (%)	31,742 (4.85)	9,882 (6.04)	21,860 (4.45)
Glucocorticoid use, n (%)	175,898 (26.88)	52,332 (31.99)	123,566 (25.17)
Hypertension, n (%)	377,402 (57.68)	125,032 (76.43)	252,370 (51.42)
CCI, mean ± SD	0.51 ± 1.18	0.88 ± 1.53	0.38 ± 1.00
CCI categories, n (%)			
0-0.99	490,586 (74.97)	96,372 (58.91)	395,214 (80.33)
1-1.99	75,546 (11.55)	30,758 (18.80)	44,788 (9.13)
≥ 2	88,220 (13.48)	36,458 (22.29)	51,762 (10.55)
Income, € in thousands, median	30,9 (22,1-47,9)	28,6 (21,4-42,8)	32,1 (22,4-49,5)
Income, € in thousands			
1st Quintile, median (IQR)	16,4 (14,1-18,3)	16,3 (14,0-18,2)	16,4 (14,2-18,3)
2nd Quintile, median (IQR)	23,9 (22,2-25,4)	24,0 (22,2-25,5)	23,9 (22,1-25,4)
3rd Quintile, median (IQR)	30,9 (28,7-33,9)	31,0 (28,6-33,6)	31,0 (28,8-34,0)
4th Quintile, median (IQR)	43,9 (40,4-47,9)	44,0 (40,2-48,0)	44,0 (40,4-47,9)
5th Quintile, median (IQR)	66,2 (58,2-83,1)	64,8 (57,6-80,3)	66,4 (58,3-83,8)
Marital status, n (%)			
Married	400,477 (61.20)	93,176 (57.96)	307,301 (62.62)
Divorced	65,984 (10.08)	17,781 (10.87)	48,203 (9.82)
Unmarried	91,784 (14.03)	25,687 (15.70)	66,097 (13.47)
Widowed	93,172 (14.24)	25,209 (15.41)	67,963 (13.85)
Unknown	2,935 (0.45)	1,735 (1.06)	1,200 (0.24)

All characteristics were evaluated at the index date. Data are presented as numbers (n, %), mean with ±SD, or median with IQR

7.3.2. CHARACTERISTICS OF ALENDRONATE USERS

Characteristics of subjects exposed and unexposed to alendronate are presented in Appendix F3. In total, 31,976 alendronate users were identified with a median exposure time until the index of 2.55 years (0.75; 5.26). Of these, 25,169 were control subjects and 6,807 were case subjects, corresponding to 5.13% and 4.16%, respectively. Median exposure time was 2.31 years (0.68; 4.98) for case subjects and 2.61 years (0.78; 5.32) for control subjects. In total, 20,786 subjects (65.01%) remained exposed to alendronate at the index date with a lower proportion among case subjects (60.14%) than control subjects (66.32%).

Alendronate users were more likely to be females. Nonetheless, female case subjects were less likely to use alendronate than female control subjects (78.60% [77.60; 79.56] vs 83.15% [82.68; 83.61], respectively). The highest proportion of alendronate use was found in the subgroup of age 70-79 years (39.25%) in both cases (38.64%) and controls (39.42%). On the other hand, the mean age of male alendronate users was 67.11 (66.86; 67.35) years, while female users had a mean age of 70.21 (70.09; 70.32) years.

7.3.3. THE ODDS OF TYPE 2 DIABETES

Subjects with type 2 diabetes were less likely than matched controls to have ever used alendronate (multiple adjusted OR 0.64 (0.62; 0.66) as presented in Table 7.2.

7.3.3.1 Dose and compliance dependencies

Table 7.2 displays the crude and adjusted ORs for effective use (with <6 months as reference) and compliant use (with MPR<0.5 as reference) and correspondingly graphically illustrated by the blobbogram in Figure 7.1.

Overall, longer effective use was associated with decreased ORs for type 2 diabetes. The lowest OR for type 2 diabetes was found in those with 4-6 years of effective alendronate use. The trend test detected a dose-response relationship between longer effective use (in years) and lower OR for type 2 diabetes ($p<0.007$). Likewise, a more pronounced association was observed among those with high compliance (MPR>0.8, $p=0.052$). Additionally, the OR for type 2 diabetes was found lower among subjects with alendronate exposure for less than 6 months compared to those who had never used alendronate (adjusted OR: 0.70 [0.66-0.74]).

7.3.3.2 Sensitivity analyses

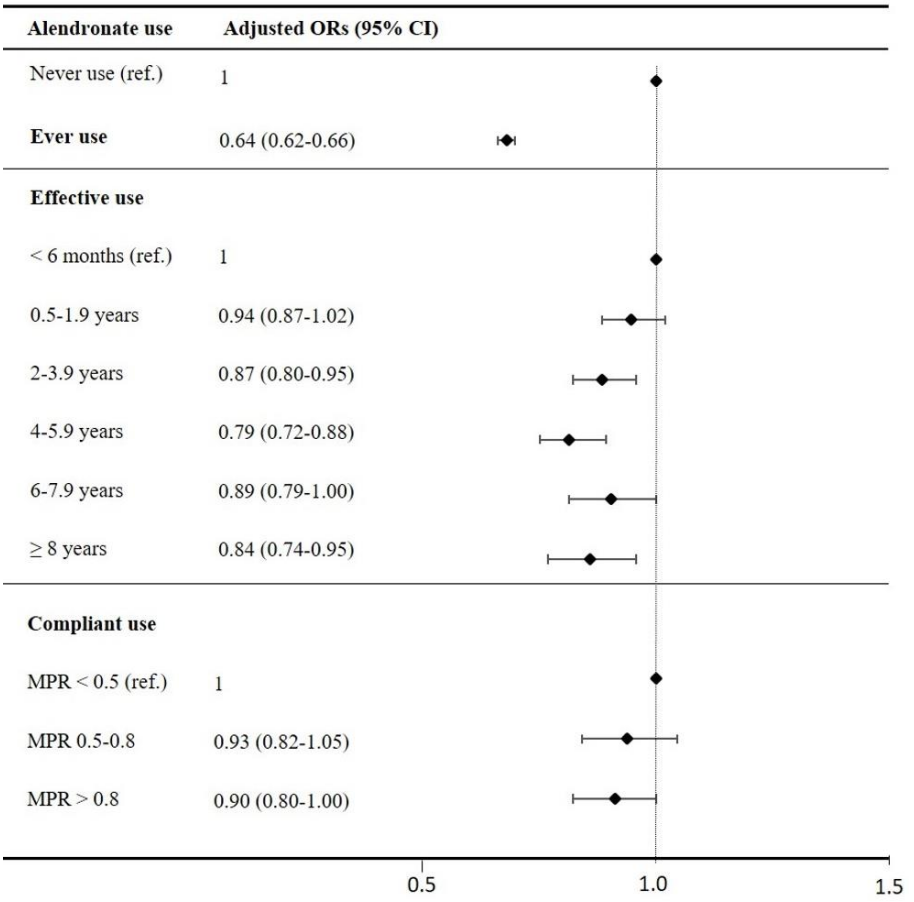
After the exclusion of obese subjects, alcohol users, pancreatitis, steroid users, or heavy smokers from the analysis, the OR for 2 diabetes remained lower among *ever* users of alendronate. When the analysis was restricted to non-smokers, the multiple adjusted OR decreased further (OR: 0.59; [0.57; 0.62]). Similarly, when restricting the analysis to those with ages below 65 years at the index date, the multiple adjusted OR remained low (OR: 0.56 [0.52; 0.61]).

***Table 7.2**, ORs for type 2 diabetes: ever, effective, and compliant use.*

	Cases, n (%) n = 163,588 (100)	**Controls,** n (%) n = 490,764 (100)	**Crude OR** (95% CI)	**Adjusted OR**[¥] (95% CI)
Ever users	6,807 (4.16)	25,169 (5.13)	**0.79** (0.77-0.81)	**0.64** (0.62-0.66)
Never-users	156,781 (95.84)	465,595 (94.87)	1.00 (ref.)	1.00 (ref.)
Effective use of DDD				
< 6 months	1,657 (1.01)	5,563 (1.09)	1.00 (ref.)	1.00 (ref.)
0.5-1.9 years	1,945 (1.19)	6,751 (1.38)	0.93 (0.87-1.01)	0.94 (0.87-1.02)
2-3.9 years	1,422 (0.87)	5,605 (1.14)	**0.82** (0.76-0.89)	**0.87** (0.80-0.95)
4-5.9 years	827 (0.51)	3,656 (0.74)	**0.73** (0.66-0.80)	**0.79** (0.72-0.88)
6-7.9 years	516 (0.32)	2,003 (0.41)	**0.83** (0.74-0.93)	0.89 (0.79-1.00)
≥8 years	440 (0.27)	1,791 (0.36)	**0.78** (0.70-0.88)	**0.84** (0.74-0.95)
Compliant use grouped by MPR				
< 0.5	564 (8.29)	1,750 (6.95)	1.00 (ref.)	1.00 (ref.)
0.5-0.8	1,090 (16.01)	3,835 (15.24)	**0.88** (0.79-0.99)	0.93 (0.82-1.05)
> 0.8	5,153 (75.70)	19,584 (77.81)	**0.82** (0.74-0.90)	0.90 (0.80-1.00)

Conditional logistic regression analysis of odds ratios (ORs) and 95% CI for development of type 2 diabetes when exposed to alendronate. ¥Adjusted for smoking, alcohol, obesity, pancreatitis, hypothyroidism, hyperthyroidism, use of glucocorticoids, CCI, income, and marital status. Estimates in bold represent $p<0.05$.

***Figure 7.1**, Multiple adjusted ORs for type 2 diabetes.*
Illustrated by a blobbogram around 1 presenting no difference.



7.4. CONCLUSION

The aim of this thesis, Chapter 7 was to explore the proposed link between bone- and glucose metabolism by examining a potential relationship between alendronate and type 2 diabetes.

This chapter presented results from a nested case-control study suggesting that individuals with type 2 diabetes are less likely than matched control subjects to have ever used alendronate. These findings suggest rejecting the null hypothesis that the likelihood of developing type 2 diabetes is not altered by alendronate administration.

The odds of being exposed to alendronate were greater among control subjects (0.05) than among type 2 diabetes subjects (0.04). The greatest difference in odds was detected in the multiple adjusted analysis of *ever* use of alendronate. A modest

interpretation of the results is that those with previous alendronate use were approximately 36% less likely to develop type 2 diabetes and thus, that alendronate use could be a protective agent against the development of type 2 diabetes. The findings also suggested a dose-dependent association between longer effective use of alendronate among those who did not develop type 2 diabetes. Moreover, the probability of type 2 diabetes was lower among subjects with less than 6 months of alendronate exposure compared to never users. Taken together, it may suggest a prompt impact on glucose metabolism that becomes more prominent in the long term.

One of the sensitivity analyses suggested a lower probability of type 2 diabetes when the analysis was restricted to non-smokers (excluding “heavy smokers”). Smoking is a risk factor for both osteoporosis and type 2 diabetes and recently, a potential interaction between smoking and alendronate action has been addressed (188), although this warrants further research.

The presented results support a possible interaction between glucose metabolism and anti-resorptive agents. The findings are consistent with several previous and comparable studies from different populations (152–154) suggesting, that subjects with osteoporosis at risk of type 2 diabetes could benefit from alendronate administration. Yet, it is still uncertain if other anti-resorptive therapies, e.g., denosumab, show similar tendencies. Nevertheless, high-quality clinical trials are scarce but needed to clarify the potential impact on glucose metabolism as well as the underlying mechanisms.

CHAPTER 8. DISCUSSION

8.1. BONE-SPECIFIC INTERVENTIONS IN TYPE 2 DIABETES MANAGEMENT

Management of type 2 diabetes and its impact on bone was introduced in the introduction, section 2.4.3, concluding that type 2 diabetes is not yet acknowledged as in independent risk factor for fractures and evaluation of the skeletal health is not a part of diabetes management. A consensus report from 2022 by the European Association for the Study of Diabetes (EASD) and ADA stated that (189):

"*Progressive metabolic benefits were seen with greater degrees of weight loss ... with an overall suggestion that ≥10% weight loss may be required to see benefits for cardiovascular disease events and mortality rate …"*

However, the report also acknowledges the findings from the Look AHEAD trial:

"This should be balanced against potential negative effects on body composition, bone density, and frailty fractures."

ADA updated the "Standard of Care in Diabetes" in January 2023 and neither bone health, fracture risk, nor BMD are mentioned when assessing comorbidities, lifestyle interventions, and pharmacological approaches in subjects with type 2 diabetes (65,138,139).

Recommendations of physical activity for the general population of older adults are 150 minutes of moderate-intensity exercise a week including balance and muscle-strength activity (190). Both physical activity and weight loss (in particular), are prominent interventions and leading parts of both prevention and management of type 2 diabetes. The 2023 ADA recommendation concerning diet includes the following statement on type 2 diabetes *prevention* (131):

"…encouraged to achieve the ≥7% weight loss during the first 6 months …".

Moreover, in diagnosed type 2 diabetes patients, "*more intensive weight loss goals (i.e., 15%) may be appropriate to maximize benefit"*(191).

However, weight loss is associated with bone loss, and bone loss might not be detected in a population with adynamic bones, and a normal or higher BMD, as observed in subjects with type 2 diabetes. It is possible, that weight-bearing aerobic and resistance exercises abolish the diet-induced bone loss – also in subjects with type 2 diabetes. The ADA recommendation concerning exercise in subjects with type 2 diabetes states (191):

"…at least 150 min of moderate-intensity physical activity per week, similar in intensity to brisk walking … A maximum of 75 min of strength training could be applied toward the total 150 min/week physical activity goal".

Yet, this recommendation introduces resistance exercise as an "option" with a time restriction more than a need. Consequently, these benefits are not highlighted in the recommendations for individuals at risk of or with diagnosed type 2 diabetes.

However, the following recommendation for exercise is stated (to improve glycemic control):

"*… strong evidence for the HbA1C-lowering value of resistance training in older adults with type 2 diabetes and for an additive benefit of combined aerobic and resistance exercise … should be encouraged to do at least two weekly sessions of resistance exercise (exercise with free weights or weight machines) …"*

This recommendation could indeed be beneficial for bone health as well. However, the recommendation is founded on the "*HbA1C-lowering value*" which is an easily accessible and repeatable measurement for both patients and healthcare providers with a high chance of a response within a few months. Adequate measurements of bone quality are demanding, insufficient, or even inaccessible in these patients, and intervention effects are most often imperceptible in the short run.

Lastly, some glucose-lowering drugs may have bone-beneficial effects, e.g., GLP-1 receptor agonists, and may as well prevent the loss of bone mass during weight loss. Currently, the utility of GLP-1 receptor agonists is no longer restricted to subjects with diagnosed type 2 diabetes and the prescription of GLP-1 receptor agonists (and SGLT-2 inhibitors) displays an almost exponential increase as illustrated by Figure 2.4 in this thesis, Chapter 2. Thus, it seems appropriate to evaluate the drug-specific effects on bone quality. However, the aforementioned guidelines for the management of type 2 diabetes in Europe and The United States of America are not (yet) optimized for bone health and fracture prevention.

8.1. FRACTURE TYPES AND OSTEOPOROSIS IN TYPE 2 DIABETES

This thesis, Chapter 4 reported a higher risk of hip and humerus as the first osteoporosis-related fractures in subjects with type 2 diabetes compared to subjects without diabetes. Moreover, Chapter 5 revealed that subjects with type 2 diabetes were less likely to be diagnosed with and treated for osteoporosis after the first osteoporosis-related fracture.

8.1.1. FRACTURE TYPE

Most research in this area has focused on incidence rates of fractures, rather than the type of the first fracture in individuals with diabetes. One study followed 5,285 women with type 2 diabetes through annual questionnaires for 5 years (192). They reported the most frequent localization of fractures in women with type 2 diabetes to be the lower leg and lower arm followed by the foot, upper arm, hip, and spine (192). Except for lower arm fractures, the rates of fracture risk rates were reported to be different among individuals with diabetes compared to those without diabetes for all other types of fractures (192).

In a Danish historical follow-up study of 6,285 women (including 229 with type 2 diabetes), it was found that those with type 2 diabetes had a 56% higher risk of a MOF over 6 years, compared to those without diabetes (80). Yet, the risk differed according to the fracture site (hip, upper arm, lower arm, spine) and only the risk of hip fractures was reported higher (80). Additionally, a recent cohort study examined the incidence

rates of fractures among subjects with newly diagnosed type 2 diabetes (193). They reported a lower IR of MOFs among newly treated type 2 diabetes patients compared with control subjects and suggested that BMI may have a protective effect on fracture risk. Furthermore, the forearm fracture IRR was lower (0.81 [0.75; 0.86]) and the risk rates of hip and humerus fractures were reported higher (IRR 1.44 [1.33; 1.55) and 1.11 [1.03; 1.20], respectively). These findings agree with the presented findings in this thesis, Chapter 4. Thus, it is worth considering whether these differences could be indicative of early site-specific changes in bone structure among individuals with type 2 diabetes.

A meta-analysis reported elevated BMD at the femoral neck, hip, and spine but no differences at the forearm in subjects with type 2 diabetes (194). Accordingly, and previously discussed, the higher risk of fractures in individuals with type 2 diabetes is not adequately predicted by BMD. Despite having a 20-30% increased risk of fractures, individuals with type 2 diabetes are more likely to have a normal T-score compared to those without diabetes (195–197). In addition to the reported finding of a higher probability of hip fractures as the first MOF, methods to detect and evaluate hip bone quality in subjects with type 2 diabetes are needed.

8.1.2. OSTEOPOROSIS DIAGNOSIS AND TREATMENT

The perceptions of diabetes-related complications are in general high among affected individuals, and most often higher among subjects with type 1 diabetes compared to type 2 diabetes (198). The fact that bone quality is affected in subjects with diabetes is well-recognized among scientists across the world. However, the awareness of a higher fracture risk seems to remain limited among individuals living with diabetes. A survey from Ireland in 2018 reported that 83% of individuals with diagnosed type 2 diabetes recognized retinopathy as a complication of diabetes, yet only 23% identified fractures as a diabetes-related complication (198). Moreover, the frequency of participants with no concerns about developing diabetes-related complications was higher than subjects having concerns about osteoporosis and fractures (198). These findings were followed by a Canadian study 2 years later confirming that the minority of subjects with type 2 diabetes (22%) believe that diabetes increases the risk of fractures (199). Thus, it seems that the low level of concern is most likely a result of unawareness of this specific complication. Despite growing evidence of increased fracture risk related to diabetes, it seems that subjects living with type 2 diabetes are not sufficiently informed about skeletal complications.

Forearm and humerus fractures among the elderly population are often low-impact fractures, and current Danish guidelines clearly state that a DXA evaluation should be performed in such cases. Low-impact spine and hip fractures are directly linked to the osteoporotic diagnosis and initiation of anti-osteoporotic treatment in Denmark. Though the proportion of subjects treated with anti-osteoporotic drugs was appropriately higher after spine and hip fractures, subjects with type 2 diabetes were still approximately 20% less likely to be diagnosed with and treated for osteoporosis after the first MOF compared to subjects without diabetes (reported in this thesis, Chapter 5). Poor compliance among individuals with type 2 diabetes may also contribute and result in lower redemption of drug prescriptions as well as a no-show to hospital evaluations, both of which cannot be distinguished in these results.

Interestingly, individuals who received a prescription for anti-osteoporotic therapy following the first MOF had lower comorbidity and income levels compared to those who did not receive any such treatment. This pattern was observed in both individuals with type 2 diabetes and those without diabetes. Another possible explanation is the insufficiency of the DXA evaluation to detect low bone quality and predict fracture risk in subjects with type 2 diabetes. Furthermore, some hospitals offer treatment with zoledronic acid as a post-hip-fracture treatment for 1-3 years which is not included in this analysis and may underestimate the proportion of treated subjects as well as the time to treatment.

Previous studies found that individuals with type 2 diabetes have a higher risk of mortality risk after experiencing a MOF (89,200,201) and this is confirmed by the presented result of a higher mortality after the first MOF in subjects with type 2 diabetes. This underlines the importance of early detection and treatment of osteoporosis in these people. Indeed, these results highlight the essential part of bone health when managing type 2 diabetes in the elderly population. However, collectively, these findings are concerning, especially combined with the documentation of fracture-risk unawareness in subjects living with diabetes. Thus, it seems that the lack of perception regarding the relationship between diabetes and low bone quality is not restricted to patients but also involves the healthcare providers responsible for managing and treating subjects with diabetes.

The presented results in this thesis, Chapter 5 indicate that treatment rates after a fracture were generally higher in females but decreased after the age of 70. Treatment rates were highest for spine fractures but decreased over time. Between 1997 and 2017, both individuals with and without type 2 diabetes experienced a decrease in hip and humerus fracture incidence rates, as reported in a recent Danish cohort study (82). While individuals with type 2 diabetes had a higher incidence of humerus and hip fractures, the rates differed significantly from subjects without diabetes. Yet, the clinical vertebral fracture incidence increased in the same period and significantly more in subjects with type 2 diabetes compared to controls (82). This corresponds to the results discussed above and the results from this thesis, Chapter 4, reporting that vertebral fractures were more often the first fractures in subjects with type 2 diabetes compared to controls among those with an index date in the last decade of the study period.

All considered, awareness of fractures as a diabetes-related complication and prevention strategies to reduce the burden of fractures are currently lacking. Individuals with, and at risk of, type 2 diabetes are more prone to osteoporosis-related fractures despite normal BMD measurements and have higher mortality. Yet, they are less likely to 1) have insight into fractures as a diabetes-related complication, 2) receive adequate guidance and management to optimize bone health, 3) be diagnosed with, and 4) be treated for osteoporosis to prevent fractures.

Naturally, these facts emphasize the need for further research and increased attention to bone health in this population. Individualized treatment strategies concerning several organ systems, including bone, are a humble hope for the future management of the elderly with or at risk of type 2 diabetes.

8.2. ANTI-OSTEOPOROTIC TREATMENT EFFICACIES IN TYPE 2 DIABETES

This thesis, Chapter 6 described and presented a similar effect of alendronate and denosumab on the risk of an incident osteoporosis-related fracture in subjects with diabetes.

Bone metabolism is observed adynamic in individuals with diabetes, however, antiresorptive therapies appear to retain their potential to prevent fractures despite a further reduction of bone turnover (202,203). Nine studies were identified and included in a systematic review from 2018, which reported no differences in BMD or fracture risk between the different anti-osteoporotic medications in subjects with diabetes, however, they did not identify any eligible studies to evaluate denosumab (204). Current knowledge is limited to post hoc analyses of large RCTs. In one of those, alendronate treatment for three years increased BMD at all sites compared to placebo, and the increase was comparable in subjects without diabetes (155). Similar observations were achieved in a post hoc analysis of denosumab treatment (148). Moreover, a reduced risk of new vertebral fractures was reported, yet the study also described a higher incidence of nonvertebral fractures (predominantly forearm and ribs) in subjects with diabetes (148). However, according to the presented results, there was no evidence of a higher risk of forearm fractures among individuals who initiated denosumab compared to those who initiated alendronate.

A larger gain in BMD has been suggested after denosumab use compared to alendronate with no difference in safety and adverse events (205,206). Furthermore, similar fracture risks are suggested between users of alendronate and denosumab (207). However, none of these studies included analyses on subjects with diabetes, and thus far, no studies investigating the effects on subjects with diabetes were identified.

The effects on bone indices after the transition from alendronate to denosumab have been explored previously. An observational study examined a switch from bisphosphonates to denosumab and did not report any improvement in BMD after 6 months of denosumab treatment in subjects with type 2 diabetes with prior bisphosphonate use (208). In an RCT comparing women with suboptimal adherence to alendronate therapy, switching to denosumab resulted in a greater increase in BMD and a reduction in bone turnover after 12 months compared to switching to risedronate (209). Another RCT examined the discontinuation of alendronate and did not find any impact on the 5-year fracture risk in those without treatment or replacement (210). Alendronate has a long half-life and a potential benefit from a switch may, in part, express an additive and/or long-term effect. Neither did the sensitivity analyses presented in this thesis, Chapter 6 support a change in the fracture risk after censoring those with a switch in treatment nor discontinuation. In addition, the results did not change after stratification by effective use nor exclusion of those with low adherence to the initiated therapy. Lastly, no difference was observed in the risk of any MOF after relocating those with a switch to denosumab within 6 months of alendronate treatment.

Denosumab is suggested to enhance muscle mass and strength (211), which improves postural control and thus, potentially reduces the risk of fractures from falling. Still, the observed increased incidence of rib and forearm fractures in those exposed to denosumab could indicate a higher rate of falling (148). Contrarily, several studies, including this thesis, Chapter 7 suggest that alendronate may have the ability to reduce insulin requirement, improve insulin sensitivity in prediabetes, and protect against future development of type 2 diabetes (152–154,156,212). These potential qualifications could also decrease the risk of late diabetes complications and, thereby, fracture risk (86).

Alendronate initiation is in general related to a few adverse events (47), however, these events are rarely reported in those initiated with denosumab (52). This fact could potentially result in confounding by indication as treatment indications differ between subjects, and a switch in treatment from alendronate to denosumab was observed in some subjects. However, it would generally be expected that most changes in treatment due to adverse events will occur within six months after alendronate initiation. In addition, as alendronate is an established treatment and denosumab is a newer agent, the possibility of residual confounding cannot be dismissed. A matching analysis, for example by propensity scores, could potentially reduce residual confounding and confounding by indication. This approach would tend to balance baseline characteristics of denosumab and alendronate initiators and mimic a randomized trial. Factors to include in the analysis of a propensity score would be evaluated using a DAG as illustrated by Appendix B2 and a logistic regression model to predict the probability of being allocated to one or the other intervention group.

The newer agent romosozumab was approved in 2020 for the treatment of postmenopausal osteoporosis in Denmark. Administration and initiation are currently restricted to the hospitals and to women with a fracture located to the pelvis, hip, humerus, forearm, or spine within the last 3 years. Romosozumab is a monoclonal antibody that acts against the sclerostin pathway resulting in both enhanced bone formation and reduced bone resorption (121). A higher BMD gain and fracture reduction were recently reported after romosozumab administration for 1 year followed by alendronate treatment compared to alendronate treatment alone (213). Romosozumab has not been and was not evaluated in subjects with diabetes in this thesis as presented data were restricted to December 2018. However, as this agent targets both formation and resorption, an evaluation of fracture risk reduction in subjects with diabetes would be interesting.

All considered, treatment of osteoporosis and fracture prevention in subjects with type 2 diabetes are insufficient. However, current research suggests that both alendronate and denosumab are effective in fracture risk reduction in subjects with type 2 diabetes when evaluated separately. Additionally, the presented results from this thesis, Chapter 6 implies an equal beneficial treatment effect on a new osteoporosis-related fracture between users of denosumab compared to alendronate. However, human RCTs with a predefined endpoint are needed to clarify the effects of different anti-osteoporotic drugs on bone quality in subjects with type 2 diabetes.

8.3. ANTI-OSTEOPOROTIC THERAPIES AND GLUCOSE METABOLISM

The results presented in this thesis, Chapter 7 suggested a decreased probability of developing type 2 diabetes among users of alendronate.

It has been reported that individuals with type 2 diabetes and poor glycemic control have a higher risk of fractures (74) and particularly in subjects with diagnosed osteoporosis (214). Thus, it would be clinically relevant to identify prevention strategies to improve glycemic control or even avert type 2 diabetes in subjects with osteoporosis.

A few previous studies have investigated the effects of anti-osteoporotic therapies on diabetes status. They are gathered in a recent meta-analysis suggesting a decreased risk of type 2 diabetes among users of bisphosphonates (215). Post-hoc analyses of three RCTs, which had a follow-up period of three to four years, revealed no significant alterations in the incidence of diabetes following treatment with alendronate, zoledronic acid, or denosumab (155). However, the administration of alendronate was only 5 mg daily for 2 years and then increased to 10 mg daily for further 2 years. According to the dose-dependent findings presented in this thesis, Chapter 7 it is possible that 5 mg represents an inadequate dose to detect an effect on fasting blood glucose and type 2 diabetes incidence.

Only one human RCT has evaluated the association between alendronate use and glucose metabolism. The trial included 60 postmenopausal women between the ages of 45 and 60 years who were randomly assigned to either 70 mg alendronate per week or placebo treatment for a duration of 12 weeks (156). They reported a reduction in HbA1c and fasting glucose among participants who were exposed to alendronate (156). The trial suggests an improvement of HbA1c as well as fasting blood glucose after 12 weeks of alendronate use (156). The results from the 12-week intervention trial suggest that a potential protective effect may already be observed after three to six months of alendronate exposure, as was suggested in this thesis, Chapter 7. However, assessing the registration of the trial reveals an unfortunately high number of predefined “primary” outcomes (approximately 9) (216). Moreover, the standard errors in the presented results were implausible large (156) and so, further human trials with a clear predefined outcome are needed to confirm the potential impact of alendronate on glucose metabolism.

The study design presented in this thesis, Chapter 7 was nested case-control with every case randomly matched to 3 control subjects by incidence-density sampling on age and gender. The matching was chosen to increase bias elimination and ensure uniform risk and exposure time. Individuals who were exposed to alendronate were expected and found to be relatively unhealthy in comparison to those who were not prescribed alendronate therapy. Contrariwise, it is possible that subjects with osteoporosis and long exposure time are healthier and have a higher tolerance for alendronate as well as lower BMI and consequently sustained low BMD (217). These factors could indeed result in a decreased risk of type 2 diabetes and induce a healthy survivor bias as seen in many previous cohort studies. The case-control setup was chosen to reduce that last bias. Vitamin D supplementation is another factor or effect mediator with a potential

direct or indirect impact on type 2 diabetes (218). As presented in Section 2.1.6.1, calcium and vitamin D supplementation is recommended in subjects with or at risk of osteoporosis. Consequently, subjects treated with anti-osteoporotic drugs are most likely also exposed to vitamin D. Additionally, inadequate levels of calcium and Vitamin D may increase the time to treatment initiation. The registers do not contain data on over-the-counter medicine and unfortunately, evaluation of a possible effect of vitamin D is not possible. A study design with a similar comparator, e.g., denosumab, could potentially minimize a theoretical vitamin D-induced effect on type 2 diabetes.

The national Danish guidelines recommend re-evaluating BMD and considering discontinuing alendronate treatment after 5 years concerning incident fractures during treatment (25). In the presented results, individuals who were exposed to alendronate for an extended period tended to have a higher level of comorbidity. It is likely, that subjects with osteoporosis and at low risk of developing diabetes have a higher likelihood of discontinuing alendronate after 5 years compared to individuals with a high risk of type 2 diabetes (higher morbidity) because of reduced fracture risk during treatment. This could explain the marginal likelihood increase among those exposed to alendronate for a longer period. Still, the odds of type 2 diabetes were indeed lower among individuals who continued alendronate therapy. This fact signifies a potential sustained protection and long-term effect that corresponds to the long half-life of alendronate (219). How alendronate impact on glucose metabolism is yet to be clarified. In vitro studies have suggested a decrease in adipogenesis and activation of lipolysis (157,220), conditions suggested altered in subjects with impaired insulin sensitivity and at risk of type 2 diabetes.

An association between type 2 diabetes and high sclerostin levels has been reported (117–119), and thus, it would be of high interest to evaluate romosozumab treatment in subjects with type 2 diabetes. As the incidence of cardiovascular events was marginally higher among those treated with romosozumab compared to alendronate alone, cardiovascular risk assessment is recommended before initiation. Naturally, this includes caution if the patient suffers from diabetes. However, the evidence of the harmful effect of sclerostin inhibition on cardiovascular safety is conflicting and no mechanism has been proposed (221,222). Moreover, sclerostin is suggested to facilitate the inhibition of bone formation by AGEs (108) and is reported positively correlated with insulin resistance (117–119). Hence, a theoretical beneficial effect of sclerostin inhibition in subjects with type 2 diabetes could be hypothesized.

All considered, anti-osteoporotic therapies may have the potential to modify other aspects of the human metabolism to reduce the risk of developing type 2 diabetes. Evidence of bone as an endocrine organ is emerging and agents with an effect on these osteokines may have the ability to impact on other organ systems involved in energy metabolism, e.g., liver, adipose tissue, and myocytes. However, human studies are scarce and further research is needed to clarify the interplay between bone and glucose metabolism.

CHAPTER 9. CONCLUSION

The objective of this thesis was to evaluate the interface between type 2 diabetes and bone health including osteoporotic fractures, diagnostics, and treatment strategies in the Danish population.

Overall, osteoporosis and type 2 diabetes are two metabolic disorders with high healthcare costs, increasing prevalence, and ambiguous diagnostics. It is challenging to identify individuals with type 2 diabetes at risk of fractures and type 2 diabetes has not yet been acknowledged as an independent factor contributing to increased risk of osteoporosis-related fractures. Additionally, current guidelines concerning type 2 diabetes management do not consider low bone quality or fracture risk as significant diabetes-related complications. Consequently, prevention strategies concerning fractures in subjects with type 2 diabetes are increasingly important to acknowledge and optimize.

The findings in this thesis (Chapters 4, 5, 6, and 7) are listed in short below.

The null hypotheses were

1. The location of the first major osteoporotic fracture after diabetes diagnosis does not differ between individuals with type 2 diabetes and without diabetes.
2. A type 2 diabetes diagnosis does not impact the diagnosis or initiation of treatment against osteoporosis, or mortality after a MOF.
3. The risk of a new major osteoporotic fracture in subjects with type 2 diabetes does not differ between initiators of denosumab and alendronate.
4. The likelihood of developing type 2 diabetes is not altered by alendronate administration.

The findings and results presented in this thesis suggest that

1. Individuals with type 2 diabetes have an increased risk of hip fractures as the first osteoporotic-related fracture compared to individuals without diabetes. However, the specific first fracture site depends on sex, age at diabetes diagnosis, and time.
2. Individuals with type 2 diabetes are less commonly diagnosed with and treated for osteoporosis, despite an osteoporosis-related fracture that is additionally followed by an increased mortality.
3. The efficiency of the two most common choices of anti-osteoporotic therapies (alendronate and denosumab) is equal in the prevention of a new osteoporotic fracture in subjects with diabetes.
4. And lastly, alendronate use seems to modify the chances of developing type 2 diabetes, and thus, anti-osteoporotic therapies may impact directly or indirectly on glucose metabolism.

All considered, scientific knowledge and data on type 2 diabetes and bone health are accumulating. Osteoporosis and type 2 diabetes are two metabolic disorders with compelling individual and health care burdens. They often co-exist, and the existence of a two-way metabolic interaction is evident. Yet, patients and healthcare providers seem unaware of the significant relationship between diabetes of reduced bone quality. Consequently, the warranty of bone fragility and fractures being added to the list of known diabetes-related complications is emerging. Likewise, there is an urgency for type 2 diabetes to be recognized as an independent risk factor in the development of reduced bone quality and osteoporosis-related fractures.

CHAPTER 10. PERSPECTIVE

Several issues concerning osteoporosis diagnosis, type 2 diabetes management, and fracture prevention have been discussed in the previous chapters. Importantly, no causal relationships have been documented in this thesis. However, in the following, questions, proposals, and hypotheses will be presented, and potential implementations in future research will be addressed.

10.1. TYPE 2 DIABETES AS A RISK FACTOR

Firstly, low bone quality and fractures are well-established complications of type 2 diabetes, albeit type 2 diabetes per se is not yet recognized as an independent risk factor for fractures. Consequently, current guidelines do not consider bone health in the management of type 2 diabetes, resulting in inadequate knowledge of fracture risk among patients and healthcare providers. The burden of osteoporosis-related fractures is not restricted to loss of life quality, it extends to high healthcare costs related to comorbidities as well as increased mortality that escalates if the patient suffers from type 2 diabetes. As low bone quality is asymptomatic, and the remodeling of bone takes time, early guidance and information about skeletal health are paramount among individuals with increased risk of fractures, i.e., subjects with type 2 diabetes. To reduce the public burden of fractures it is essential to improve fracture risk awareness. The list of diabetes-related complications is long, but none should be overlooked. Current guidelines need to address bone health in the management of type 2 diabetes.

In the clinical setting, all patients with type 2 diabetes are informed about, screened for, and preventively treated against micro- and macrovascular complications, e.g., retinopathy, nephropathy, neuropathy, and cardiovascular disease. However, bone health is not yet included in the evaluation of comorbidities. Individuals with type 2 diabetes are less likely to be *diagnosed* with osteoporosis despite a fracture related to osteoporosis. Moreover, individuals with type 2 diabetes are less likely to be *treated* for osteoporosis despite a fracture related to osteoporosis. It is concerning. Optimization of diagnostics and treatment procedures is paramount to prevent further fractures. The DXA evaluation including BMD and T-score calculations is to date the only diagnostic tool to assess bone quality. As both the evaluation and fracture predictions are insufficient in subjects with type 2 diabetes, another threshold and/or other methods are necessary to evaluate the bone health and fracture risk in subjects with diabetes. Currently, a few secondary causes of osteoporosis are dichotomized as primary entry variables in the FRAX tool due to their additional impact on fracture risk independent of BMD, e.g., rheumatoid arthritis and glucocorticoid use. Type 2 diabetes has not been incorporated in the FRAX tool as is the case for type 1 diabetes which is indirectly considered as a secondary cause of osteoporosis, i.e., assumed to increase the fracture probability via low BMD. If BMD is unknown, the fracture risk in a person with type 1 diabetes is assumed similar to a person with rheumatoid arthritis. As a result, the calculated fracture risk is only increased in type 1 diabetes when BMD is unknown or simply not included in the calculation (223). Therefore, the fracture probability is also found underestimated by the FRAX tool in subjects

with type 1 and type 2 diabetes (77,85), and in particular hip fractures (224). It is possible that the probability of fractures could be predicted more precisely if type 2 diabetes was implemented in the algorithm as an independent risk factor. Several methods for improving FRAX performance for type 2 diabetes are proposed to be included in the model, including TBS and a 0.5 SD reduction in T-score (224). This corresponds to the 10-year hip fracture risk in type 2 diabetes being equivalent to those without diabetes at approximately 0.5 units lower T-score, as mentioned in the introduction, section 2.3.2 (85).

If type 2 diabetes was acknowledged as an independent risk factor for low bone quality and fracture risk independently of BMD, healthcare providers would be forced to inform about and evaluate bone health in the management of type 2 diabetes. In that setting, a DXA evaluation among all diabetes patients aged 50 years including a modified intervention threshold using either the suggested improved FRAX score or a T-score of -2 could be considered.

10.2. MANAGEMENT OF TYPE 2 DIABETES

Weight loss and physical activity are two major intervention strategies in the management of type 2 diabetes. As both interventions are known to have a significant impact on bone quality, an evaluation of bone indices during these interventions is essential. However, the magnitude, rate, and origin of weight loss as well as the type of physical activity impact differently on bone. The current guidelines suggest a large weight loss of high rate, both of which are known to induce concomitant long-lasting bone loss. Indeed, weight bearing exercise is reported to preserve bone loss during weight loss, however, weight bearing exercise is not highlighted in the guidelines. Consequently, clinical trials are needed to examine the ideal combination of weight loss and type of exercise to preserve bone and yet, maintain the cardio-metabolic benefits and improvement of glycemic control. Improvement of insulin sensitivity by exercise may indeed have a beneficial effect on bone as well which could be investigated by including naïve type 2 diabetes patients randomly assigned to a non-weight-bearing intervention compared to no exercise. This setup could be combined with several arms including glucose-lowering drugs in a factorial design.

Individuals with type 2 diabetes are most often treated with more than one glucose-lowering drug that may have the potential to impact bone indices. In general, and as previously mentioned, only glitazones are found consistently associated with increased fracture risk. Au contraire, GLP-1 receptor agonists may have the potential to preserve bone mass by increasing bone formation during weight loss. It would be of great interest to evaluate bone indices during weight loss by comparing overweight subjects with and without type 2 diabetes exposed to GLP-1 receptor agonists and/or bariatric surgery. If and how GLP-1 receptor agonists prevent bone loss during a major weight loss at a high rate in subjects with type 2 diabetes is a valuable question to address and could provide information on the mechanistic differences in the bones of subjects with type 2 diabetes.

10.3. MANAGEMENT OF OSTEOPOROSIS

Individualized treatment strategies have been highlighted during the last decades focusing on the patient more than the morbidity. This becomes even more sufficient when the patient suffers from more than one disease. Type 2 diabetes and osteoporosis often co-exist, and several different anti-osteoporotic drugs have become available in the treatment of osteoporosis as illustrated in the Introduction, section 2.1.6.2 and by Figure 2.2. Thus, knowledge about how to optimize both anti-osteoporotic and anti-diabetic therapy to enhance the chances of fracture risk reduction and glycemic control is essential. To date, the available anti-osteoporotic therapies are found equally protective against new fractures in subjects with type 2 diabetes. However, studies investigating the head-to-head discrepancies of anti-osteoporotic drugs in the subgroup of subjects with type 2 diabetes are sparse. As BMD evaluation in these patients has limited value in fracture risk prevention, the evaluation is restricted to fractures, an event that requires long-term follow-up. Consequently, newer techniques are needed to evaluate the effect of these drugs on bone quality in subjects with type 2 diabetes. This could for example be HRpQCT, bone bio-biomarkers, or microindensation. However, all of these are restricted to the research setting and further evidence is needed for these modalities to be implemented in the clinical setting with thresholds for intervention.

Anti-osteoporotic therapies also seem to affect glucose metabolism and may have the potential to reduce the risk of developing type 2 diabetes. As previously discussed, evidence is limited by register-based studies, post-hos analyses, and a low-quality RCT. Thus, it would be of great interest to evaluate the effect of alendronate on glucose metabolism. One suggestion could be to randomly assign subjects with type 2 diabetes to alendronate or placebo together with vitamin D and calcium supplementation for 12-24 months. An analysis of insulin sensitivity could be performed as surrogate measures by an oral glucose tolerance test (OGTT) or fasting indices, though a hyperinsulinemic-euglycemic clamp (HEC) or insulin suppression test (IST) would be more elegant (225). Additionally, adipose and muscle tissue biopsies could provide valuable information on potential target tissue. Current evidence is restricted to the evaluation of bisphosphonates. Of course, it would be of great interest to evaluate and compare the newer agents as well, i.e., denosumab and romosozumab.

Prospectively evaluations of the impact of weight loss, exercise, glucose-lowering drugs, and anti-osteoporotic therapies on bone indices (and energy metabolism) in subjects with type 2 diabetes are mandatory for individualized treatment strategies, bone health optimization, and fracture prevention in subjects with type 2 diabetes. Both basic metabolic research, acute intervention trials, and RCTs are warranted.

10.4. EPILOGUE

In terms of future research, the ideal objective would be to identify or develop a therapy (or therapies) with simultaneously beneficial effects on both bone health and type 2 diabetes disease management.

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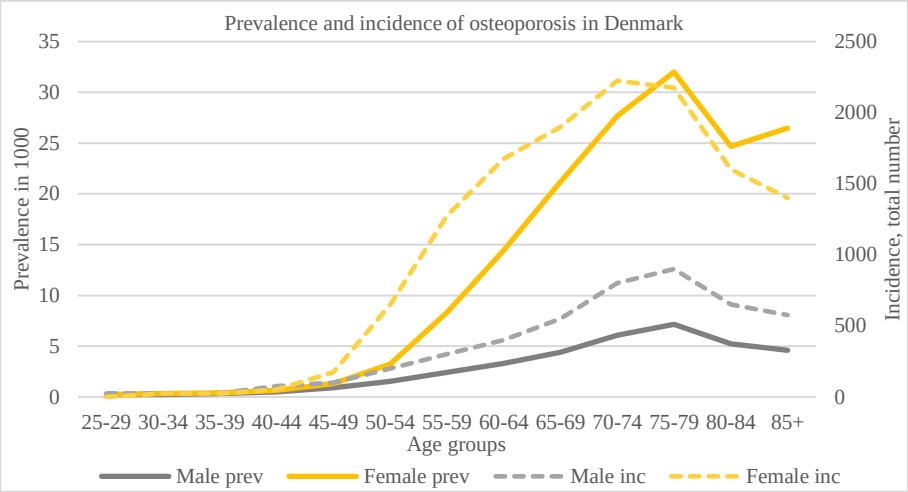
APPENDICES

Appendix A. Introduction 121
A1, Osteoporosis 121
A2, Type 2 diabetes 121
Appendix B. Directed Acyclic Graphs 123
Explanation 123
B1, DAG study 1 124
B2, DAG study 2 126
B3, DAG study 3 128
Appendix C. ICD-10 and ATC codes 131
C1, Diabetes and covariables 131
C2, Fractures 132
C3, Charlson Comorbidity Index 133
Appendix D. Study 1 135
D1, Study timeline 135
D2, Flow-diagram 136
Appendix E. Study 2 137
E1, Study timeline 137
E2, Flow-diagram 138
E3, Characteristics of subjects discontinuing treatment. 139
Appendix F. Study 3 141
F1, Study timeline 141
F2, Flow diagram 142
F3, Characteristics of alendronate users and non-users 143

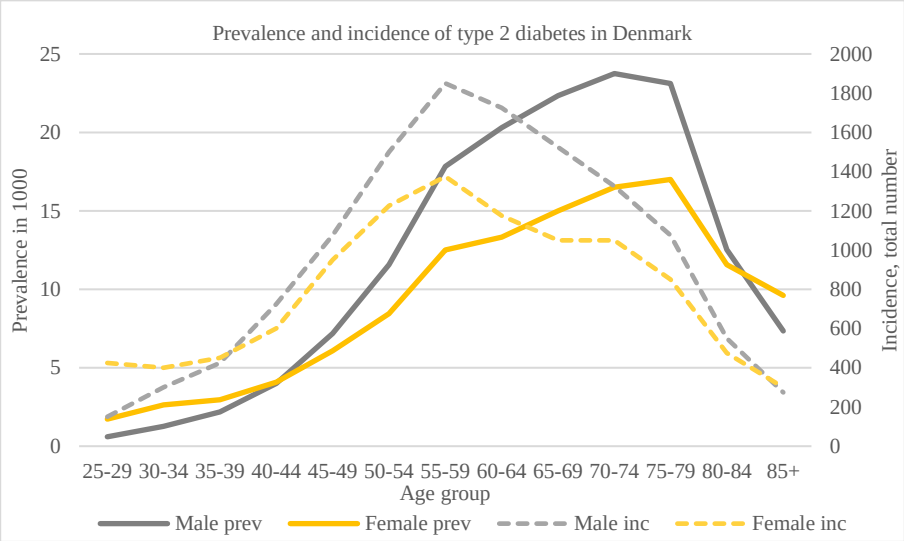
Appendix A. Introduction

A1, Osteoporosis



Prevalence (solid lines) and incidence (dashed lines) of osteoporosis in Denmark years 2022 and 2021, respectively, stratified by age groups after age 25 among females (yellow) and males (grey).

A2, Type 2 diabetes



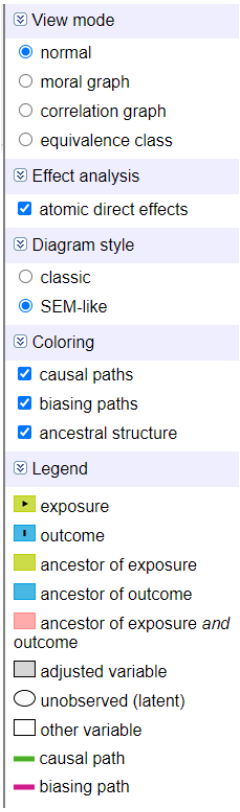
Prevalence (solid lines) and incidence (dashed lines) of type 2 diabetes in Denmark year 2022 and 2021, respectively, stratified by age groups after age 25 among females (yellow) and males (grey).

Appendix B. Directed Acyclic Graphs

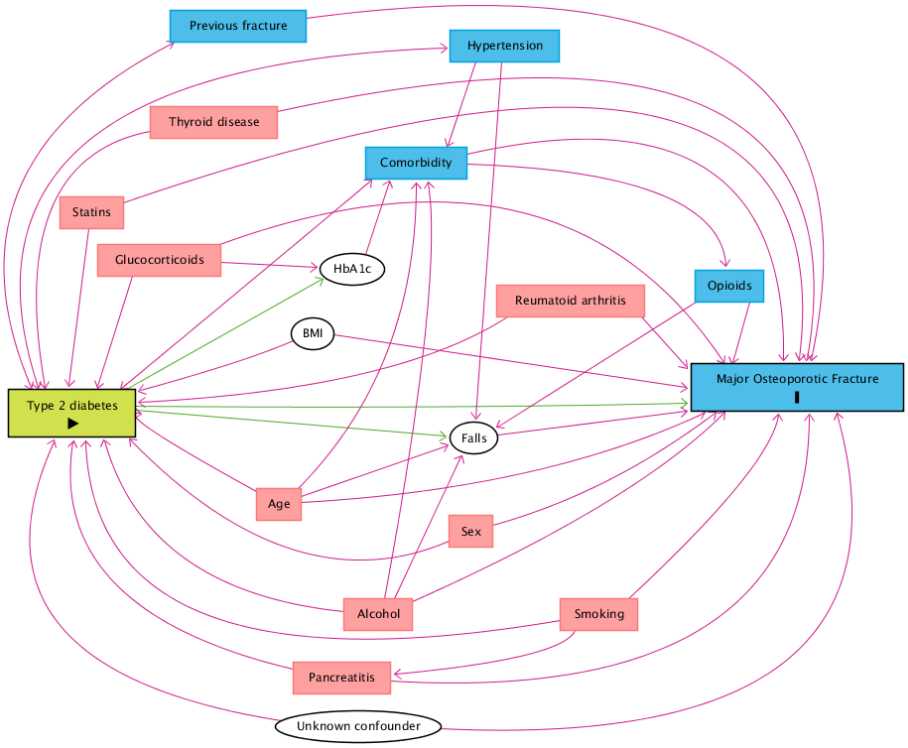
Explanation

Directed acyclic graphs (DAGs), also known as causal Bayesian networks, can be created without costs from *www.dagitty.net*. The browser-based platform focuses on creating causal diagrams for minimizing bias in epidemiological studies.

The colors of the following DAGs can be explained by the legends below.



B1, DAG study 1

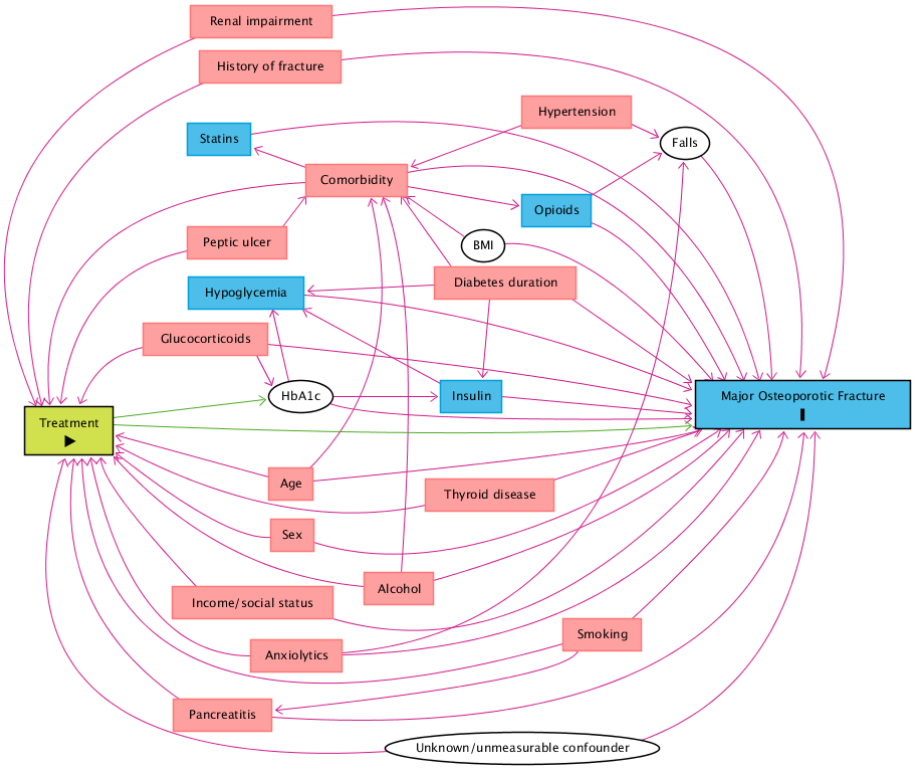


Model code 1 (for reproducibility)

```
dag {
bb="0,0,1,1"
"Major Osteoporotic Fracture" [outcome,pos="0.760,0.540"]
"Previous fracture" [pos="0.362,0.108"]
"Reumatoid arthritis" [pos="0.599,0.444"]
"Thyroid disease" [pos="0.345,0.226"]
"Type 2 diabetes" [exposure,pos="0.244,0.572"]
"Unknown confounder" [latent,pos="0.448,0.964"]
Age [pos="0.391,0.692"]
Alcohol [pos="0.462,0.827"]
BMI [latent,pos="0.415,0.484"]
Comorbidity [pos="0.489,0.276"]
Falls [latent,pos="0.530,0.612"]
Glucocorticoids [pos="0.306,0.394"]
HbA1c [latent,pos="0.444,0.405"]
Hypertension [pos="0.552,0.133"]
Opioids [pos="0.712,0.425"]
Pancreatitis [pos="0.436,0.905"]
Sex [pos="0.528,0.726"]
Smoking [pos="0.619,0.827"]
Statins [pos="0.258,0.336"]
"Previous fracture" -> "Major Osteoporotic Fracture" [pos="0.835,0.001"]
"Previous fracture" <-> "Type 2 diabetes" [pos="0.147,0.277"]
"Reumatoid arthritis" -> "Major Osteoporotic Fracture"
"Reumatoid arthritis" -> "Type 2 diabetes" [pos="0.475,0.552"]
"Thyroid disease" -> "Major Osteoporotic Fracture" [pos="0.814,0.053"]
"Thyroid disease" -> "Type 2 diabetes" [pos="0.196,0.270"]
```

```
"Type 2 diabetes" -> "Major Osteoporotic Fracture" [pos="0.516,0.576"]
"Type 2 diabetes" -> Falls
"Type 2 diabetes" -> HbA1c
"Type 2 diabetes" <-> Comorbidity [pos="0.391,0.392"]
"Type 2 diabetes" <-> Hypertension [pos="0.145,0.157"]
"Unknown confounder" -> "Major Osteoporotic Fracture" [pos="0.855,0.996"]
"Unknown confounder" -> "Type 2 diabetes" [pos="0.158,0.893"]
Age -> "Major Osteoporotic Fracture" [pos="0.578,0.674"]
Age -> "Type 2 diabetes" [pos="0.270,0.578"]
Age -> Comorbidity [pos="0.489,0.527"]
Age -> Falls
Alcohol -> "Major Osteoporotic Fracture" [pos="0.644,0.691"]
Alcohol -> "Type 2 diabetes" [pos="0.297,0.799"]
Alcohol -> Comorbidity [pos="0.508,0.419"]
Alcohol -> Falls
BMI -> "Major Osteoporotic Fracture"
BMI -> "Type 2 diabetes" [pos="0.368,0.518"]
Comorbidity -> "Major Osteoporotic Fracture" [pos="0.754,0.176"]
Comorbidity -> Opioids [pos="0.705,0.287"]
Falls -> "Major Osteoporotic Fracture"
Glucocorticoids -> "Major Osteoporotic Fracture" [pos="0.593,0.222"]
Glucocorticoids -> "Type 2 diabetes"
Glucocorticoids -> HbA1c
HbA1c -> Comorbidity
Hypertension -> Comorbidity
Hypertension -> Falls
Opioids -> "Major Osteoporotic Fracture"
Opioids -> Falls
Pancreatitis -> "Major Osteoporotic Fracture" [pos="0.768,0.938"]
Pancreatitis -> "Type 2 diabetes" [pos="0.226,0.823"]
Sex -> "Major Osteoporotic Fracture" [pos="0.626,0.679"]
Sex -> "Type 2 diabetes" [pos="0.402,0.819"]
Smoking -> "Major Osteoporotic Fracture" [pos="0.741,0.647"]
Smoking -> "Type 2 diabetes" [pos="0.260,0.931"]
Smoking -> Pancreatitis [pos="0.593,0.881"]
Statins -> "Major Osteoporotic Fracture" [pos="0.797,0.018"]
Statins -> "Type 2 diabetes"
}
```

B2, DAG study 2

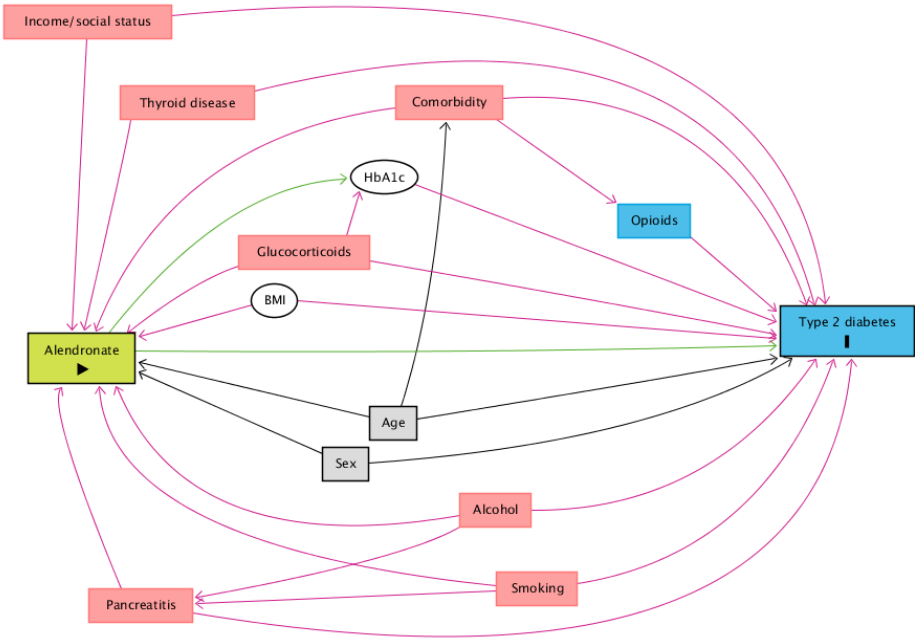


Model code 2 (for reproducibility)

```
dag {
bb="0,0,1,1"
"Diabetes duration" [pos="0.551,0.402"]
"History of fracture" [pos="0.386,0.142"]
"Income/social status" [pos="0.373,0.789"]
"Major Osteoporotic Fracture" [outcome,pos="0.760,0.540"]
"Peptic ulcer" [pos="0.362,0.354"]
"Renal impairment" [pos="0.379,0.087"]
"Thyroid disease" [pos="0.540,0.659"]
"Unknown/unmeasurable confounder" [latent,pos="0.563,0.964"]
Age [pos="0.400,0.645"]
Alcohol [pos="0.476,0.772"]
Anxiolytics [pos="0.404,0.853"]
BMI [latent,pos="0.535,0.358"]
Comorbidity [pos="0.446,0.279"]
Falls [latent,pos="0.677,0.234"]
Glucocorticoids [pos="0.340,0.471"]
HbA1c [latent,pos="0.407,0.540"]
Hypertension [pos="0.601,0.196"]
Hypoglycemia [pos="0.369,0.415"]
Insulin [pos="0.527,0.540"]
Opioids [pos="0.587,0.316"]
Pancreatitis [pos="0.352,0.924"]
```

```
Sex [pos="0.401,0.707"]
Smoking [pos="0.619,0.827"]
Statins [pos="0.350,0.229"]
Treatment [exposure,pos="0.244,0.572"]
"Diabetes duration" -> "Major Osteoporotic Fracture"
"Diabetes duration" -> Comorbidity
"Diabetes duration" -> Hypoglycemia
"Diabetes duration" -> Insulin
"History of fracture" -> "Major Osteoporotic Fracture" [pos="0.780,0.064"]
"History of fracture" -> Treatment [pos="0.182,0.278"]
"Income/social status" -> "Major Osteoporotic Fracture" [pos="0.574,0.880"]
"Income/social status" -> Treatment [pos="0.267,0.647"]
"Peptic ulcer" -> Comorbidity
"Peptic ulcer" -> Treatment [pos="0.249,0.394"]
"Renal impairment" -> "Major Osteoporotic Fracture" [pos="0.859,0.001"]
"Renal impairment" -> Treatment [pos="0.144,0.246"]
"Thyroid disease" -> "Major Osteoporotic Fracture"
"Thyroid disease" -> Treatment [pos="0.396,0.708"]
"Unknown/unmeasurable confounder" -> "Major Osteoporotic Fracture" [pos="0.766,0.880"]
"Unknown/unmeasurable confounder" -> Treatment [pos="0.173,0.999"]
Age -> "Major Osteoporotic Fracture" [pos="0.629,0.607"]
Age -> Comorbidity [pos="0.484,0.505"]
Age -> Treatment
Alcohol -> "Major Osteoporotic Fracture" [pos="0.644,0.691"]
Alcohol -> Comorbidity [pos="0.492,0.411"]
Alcohol -> Treatment [pos="0.350,0.754"]
Anxiolytics -> "Major Osteoporotic Fracture" [pos="0.646,0.843"]
Anxiolytics -> Falls [pos="0.660,0.819"]
Anxiolytics -> Treatment [pos="0.289,0.852"]
BMI -> "Major Osteoporotic Fracture" [pos="0.609,0.349"]
BMI -> Comorbidity
Comorbidity -> "Major Osteoporotic Fracture" [pos="0.644,0.216"]
Comorbidity -> Opioids
Comorbidity -> Statins
Comorbidity -> Treatment [pos="0.215,0.300"]
Falls -> "Major Osteoporotic Fracture" [pos="0.734,0.336"]
Glucocorticoids -> "Major Osteoporotic Fracture" [pos="0.578,0.510"]
Glucocorticoids -> HbA1c
Glucocorticoids -> Treatment [pos="0.263,0.493"]
HbA1c -> "Major Osteoporotic Fracture" [pos="0.463,0.571"]
HbA1c -> Hypoglycemia
HbA1c -> Insulin
Hypertension -> Comorbidity
Hypertension -> Falls
Hypoglycemia -> "Major Osteoporotic Fracture" [pos="0.537,0.431"]
Insulin -> "Major Osteoporotic Fracture"
Insulin -> Hypoglycemia
Opioids -> "Major Osteoporotic Fracture" [pos="0.656,0.360"]
Opioids -> Falls
Pancreatitis -> "Major Osteoporotic Fracture" [pos="0.739,0.966"]
Pancreatitis -> Treatment [pos="0.232,0.798"]
Sex -> "Major Osteoporotic Fracture" [pos="0.527,0.776"]
Sex -> Treatment [pos="0.355,0.713"]
Smoking -> "Major Osteoporotic Fracture" [pos="0.741,0.647"]
Smoking -> Pancreatitis [pos="0.593,0.881"]
Smoking -> Treatment [pos="0.263,0.999"]
Statins -> "Major Osteoporotic Fracture" [pos="0.670,0.139"]
Treatment -> "Major Osteoporotic Fracture" [pos="0.542,0.593"]
Treatment -> HbA1c
}
```

B3, DAG study 3



Model code 3 (for reproducibility)

```
dag {
bb="0,0,1,1"
"Income/social status" [pos="0.248,0.192"]
"Thyroid disease" [pos="0.315,0.286"]
"Type 2 diabetes" [outcome,pos="0.760,0.540"]
Age [adjusted,pos="0.454,0.656"]
Alcohol [pos="0.523,0.757"]
Alendronate [exposure,pos="0.244,0.572"]
BMI [latent,pos="0.374,0.514"]
Comorbidity [pos="0.492,0.285"]
Glucocorticoids [pos="0.394,0.459"]
HbA1c [latent,pos="0.448,0.372"]
Opioids [pos="0.630,0.422"]
Pancreatitis [pos="0.284,0.867"]
Sex [adjusted,pos="0.422,0.704"]
Smoking [pos="0.551,0.848"]
"Income/social status" -> "Type 2 diabetes" [pos="0.718,0.115"]
"Income/social status" -> Alendronate
"Thyroid disease" -> "Type 2 diabetes" [pos="0.677,0.139"]
"Thyroid disease" -> Alendronate [pos="0.276,0.324"]
Age -> "Type 2 diabetes" [pos="0.629,0.607"]
Age -> Alendronate
Age -> Comorbidity [pos="0.484,0.505"]
Alcohol -> "Type 2 diabetes" [pos="0.668,0.759"]
Alcohol -> Alendronate [pos="0.316,0.818"]
Alcohol -> Pancreatitis [pos="0.461,0.814"]
Alendronate -> "Type 2 diabetes" [pos="0.516,0.576"]
Alendronate -> HbA1c [pos="0.342,0.379"]
BMI -> "Type 2 diabetes"
BMI -> Alendronate
```

```
Comorbidity -> ”Type 2 diabetes” [pos=”0.677,0.261”]
Comorbidity -> Alendronate [pos=”0.306,0.325”]
Comorbidity -> Opioids
Glucocorticoids -> ”Type 2 diabetes” [pos=”0.578,0.510”]
Glucocorticoids -> Alendronate [pos=”0.319,0.492”]
Glucocorticoids -> HbA1c
HbA1c -> ”Type 2 diabetes”
Opioids -> ”Type 2 diabetes”
Pancreatitis -> ”Type 2 diabetes” [pos=”0.740,0.996”]
Pancreatitis -> Alendronate [pos=”0.221,0.640”]
Sex -> ”Type 2 diabetes” [pos=”0.626,0.679”]
Sex -> Alendronate
Smoking -> ”Type 2 diabetes” [pos=”0.703,0.808”]
Smoking -> Alendronate [pos=”0.264,0.798”]
Smoking -> Pancreatitis
}
```

Appendix C. ICD-10 and ATC codes

C1, Diabetes and covariables

	ICD-10 codes, .x may equal 0-9	**ATC codes**
Diabetes mellitus	E10.x, E11.x, E12.x, E13.x, E14.x, G63.2, H28.0, H36.0, M14.2, O24, R73	A10A, A10B
Type 1 diabetes	E10.x and	A10A, no A10B
Polycystic ovary syndrome	Diabetes mellitus	+ G03GB02 (clomifene before age 40)
Heavy smoking	J41-J44, J47.x, Z720, F17, T652	N07BA, N06AX12. After age 40: R03A, R03B, R03C, R03DA, R03DB, R03DC, R03DX07
Alcohol abuse	T51.x, G312, G621, I426, K292, K70.x, K852, K860, F10.x	N07BB01, N07BB03, N07BB05
Obesity	E66.x	A08A
Hyperthyroidism	E05.x	H03B
Hypothyroidism	E03.x	H03A
Glucocorticoid use		H02AB
Pancreatitis	K86.0, K86.1, K85.0, K85.1, K85.2, K85.3, K85.8, K85.9	
Hypertension	I10-I13, I15, R03.0	ACE/ARB, central, calcium-channel block: C09, C02DB, C02CA, C02AB, C02AC, C08. Thiazides: C03AA, C03AB, C03BA. Loop-diuretics: C03CA, C03CB, C03EB. Potassium saving: C03D. Combinations: C03E. Beta blokcers: C07A
Dyslipidemia	DE75.x, DE78.x	C10AA (Statins)
Psychoactive/ opioids		N05, N06, N02A
Osteoporosis	DM80.x, DM81.x, DM82.x	
Paget disease	M88	
Alendronate		M05BA04, M05BB03, M05BB05, M05BB06

Other bisphosphonates	M05BA01-3, M05BA05-8, M05BB01-3
Denosumab	M05BX04
Other anti-osteoporotic treatments, e.g., strontium and PTH analog	M05BX01-3, M05BX53, H05AA02

C2, Fractures

	ICD-10 codes, .x may equal 0-9
Major osteoporotic fracture	Hip/femur, spine (vertebral), forearm/arm (unspecified), humerus/upper arm
Hip/femur	S72.0, S72.1, S72.2, S72.3, S72.4, S72.7, S72.8, S72.9, M80.9B
Spine (vertebral) incl. pelvic	S32.0, S32.1, S32.2, S32.3, S32.4, S32.5, S32.7, S32.8. S12.0, S12.1, S12.2, S12.7, S12.8, S12.9., S22.0, S22.1 T08, T08.9, M48.4 M48.5, M80.9C
Forearm/arm unspecified	S52.0-S52.9, T10, DT10.9, M80.9A
Upper arm, Humerus	S42.2-S42.4, S42.7, S42.8
Any other fracture	Any below
Wrist/hand	S62.0-S62.8
Shoulder and upper arm	S42.0, S42.1, S42.2, S42.3, S42.4, S42.7, S42.8, S42.9.
Head/skull (incl. crush)	S02.0-S02.9, S07.0, S07.1, S07,8, S07.9
Neck	S12.0, S12.1, S12.2, S12.7, S12.8, S12.9
Thorax	S22.0, S22.1, S22.2, S22.3, S22.4, S22.5, S22.8
Lower leg/ankle	S82.0-S82.9.
Foot	D.92.0, D.92.1, D.92.2, D.92.3, D.92.4, D.92.5, D.92.7, D.92.9.
Leg, unspecified	T12, DT12.9
Stress/pathological	M84., M84.4, M80.x

C3, Charlson Comorbidity Index

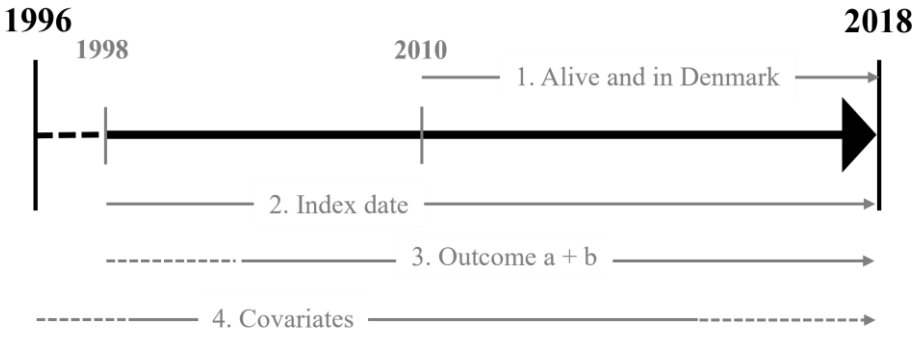
Scoring and ICD codes.

Comorbidity	Score	ICD-10 codes
1, Acute myocardial infarction	1	DI21, DI23, I24.1, I24.8, I24.9
2, Cardiac insufficiency	1	I50, I11.0, I13.0, I13.2
3, Cardiovascular disease	1	I70, I71, I72, I73, I74, I77
4, Cerebrovascular disease	1	I60-I69, G45, G46
5, Dementia	1	F00-F03, F05.1, G30
6, Chronic pulmonary disease	1	J40-J47, J60-J67, J68.4, J70.1, J70.3, J70.4, J82, J84, J85.0, J92, J95.3, J96.1, J98.2, J98.3
7, Connective tissue disease	1	M05-M09, M30-M36, D86
8, Peptic ulcer	1	K22.1, K25-K28
9, Mild liver disease	1	B18, K70.0-K70.3, K709, K71, K73, K74, K75.2-K75.4, B15.9, B16.9, K75.8-K75.9, K76.0
10, Diabetes mellitus	-	Omitted.
11, Hemiplegia	1	G81, G82
12, Nephrological disease	2	I12, I13, N02-N04, N07, N11, N12, N14, N18-N19, Q60-Q61 E10.2, E11.2, N083
13, Late-diabetic complications	2	E10.3-DE10.8, E11.3-E11.8 G59.0, G63.2, N083, DH360
14, Solid cancers	2	C00-C75
15, Leukemia	2	DC91-DC95
16, Lymphoma	2	C81-C85, C88, C90, C96
17, Moderate to severe liver disease	3	B15.0, B16.0, B16.2, B19.0, K70.4, K72, K76.6, K76.7, I85
18, Metastatic cancer	6	C77-C80
19, AIDS	6	B20-B24

Appendix D. Study 1

D1, Study timeline

From data availability (January 1, 1996) to the end of follow-up (December 31, 2018).



1. All subjects were alive and Danish citizens on January 1, 2010.

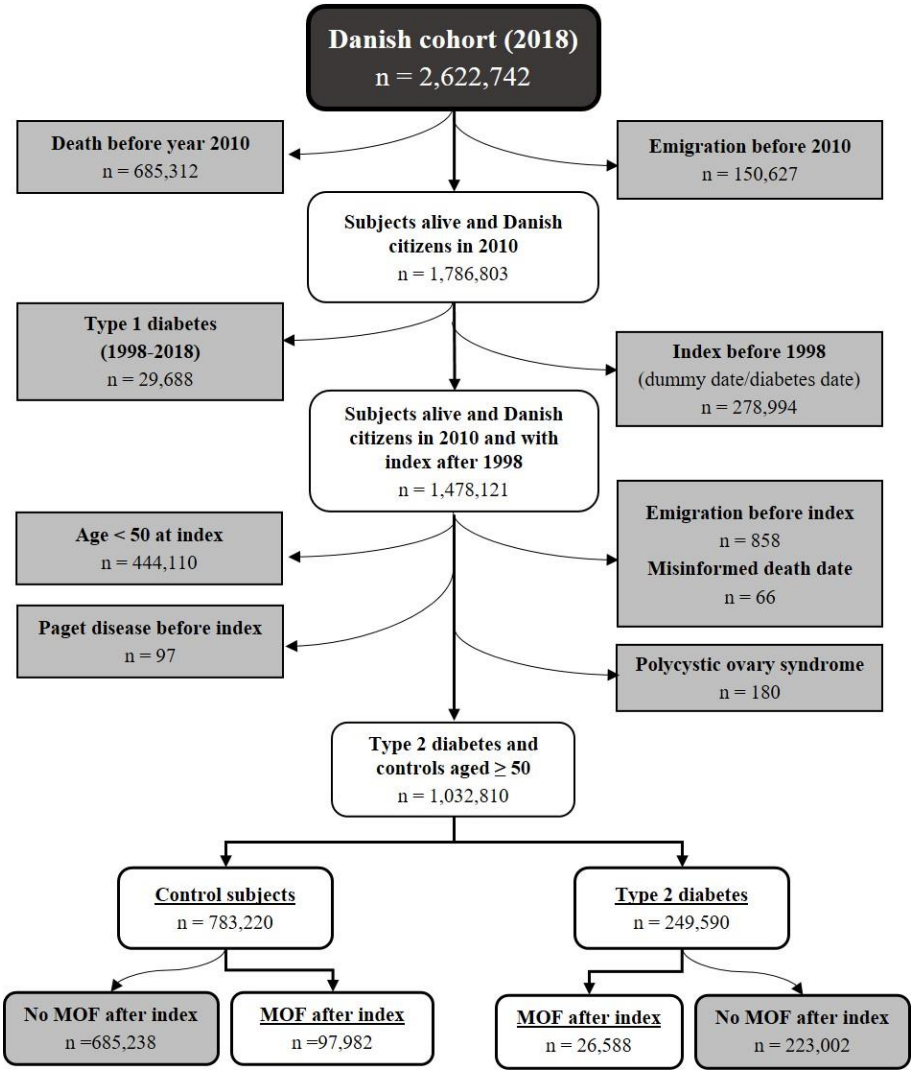
2. Index date was set at the type 2 diabetes diagnosis date. A corresponding dummy date was set for control subjects by *Statistics Denmark* corresponding to diabetes subjects.

3. Outcome, a) identification of first MOF date after index, b) anti-osteoporotic treatment and mortality after the first MOF.

4. Covariates were identified before/at index, e.g., osteoporosis diagnosis, fractures, and treatments.

D2, Flow-diagram

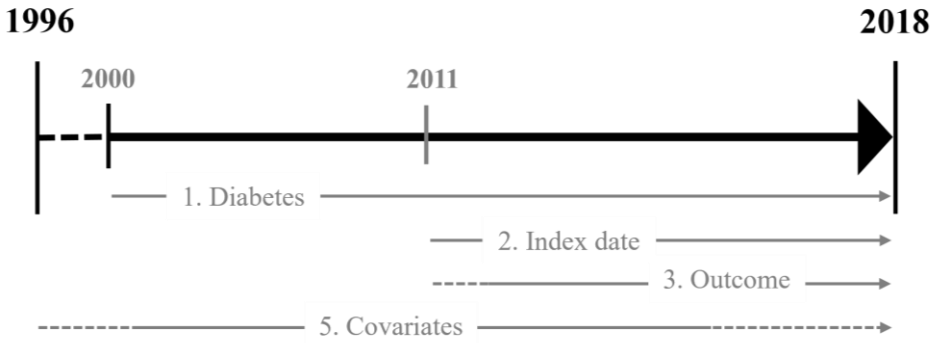
The study group selection process



Appendix E. Study 2

E1, Study timeline

From data availability (January 1, 1996) to end of follow up (December 31, 2018).



1. All patients with **Diabetes** were identified between 2000 and 2018.

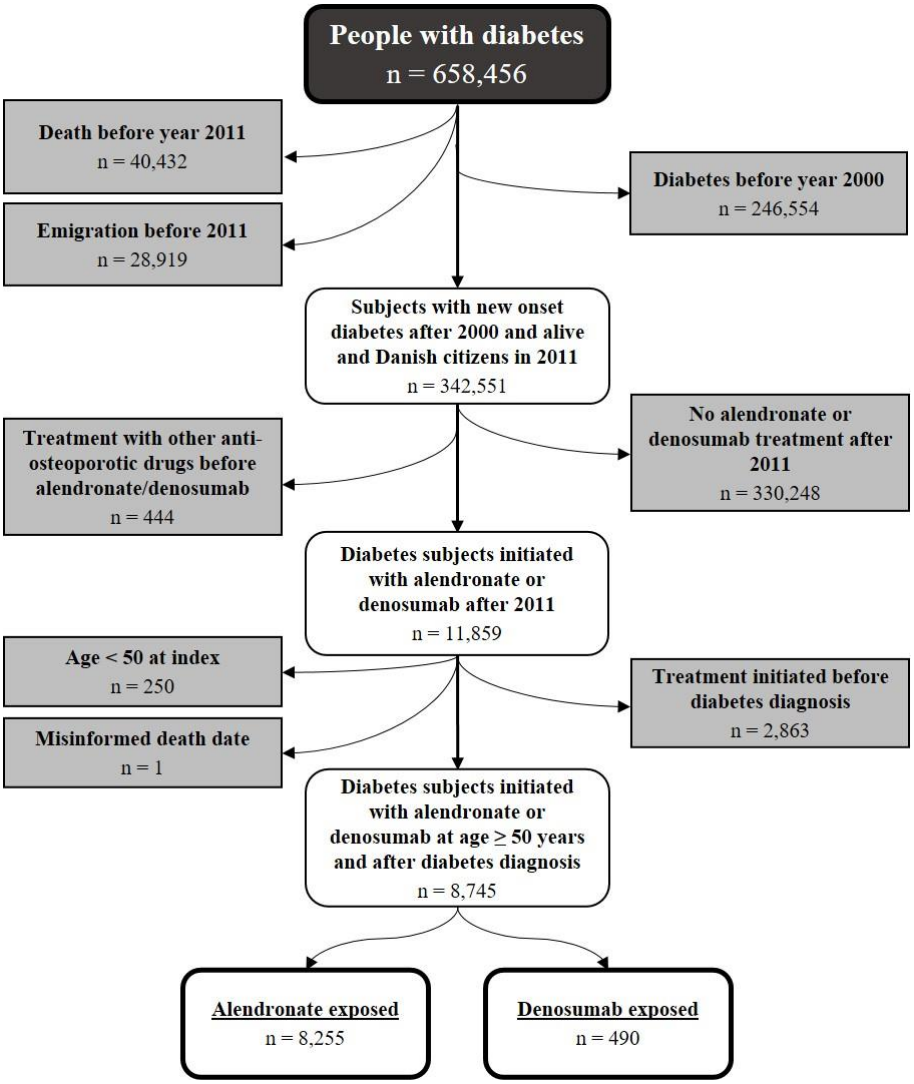
2. The Index date was set at exposure, i.e., drug initiation (redemption) date by either alendronate or denosumab.

3. Outcome, incident MOF after exposure.

4. Covariates (and confounders) were identified before/at the index date.

E2, Flow-diagram

The study group selection process



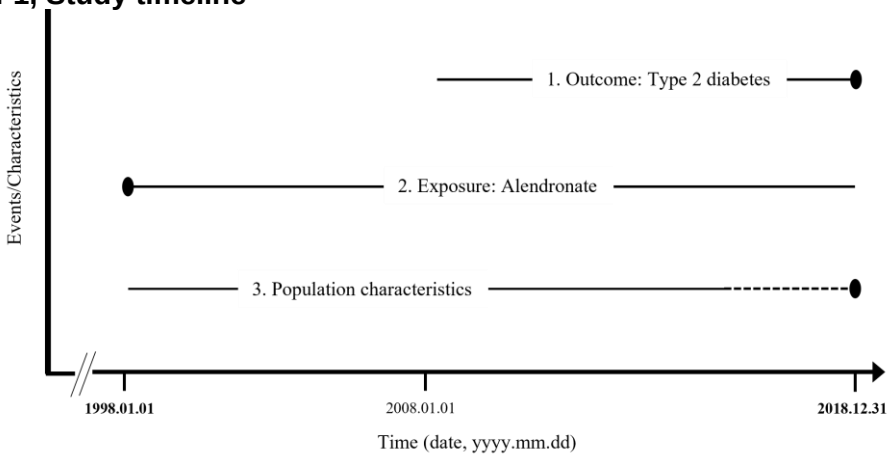
E3, Characteristics of subjects discontinuing treatment.

Subjects discontinuing, n (%)	**All subjects** 4,078 (47)	**Alendronate** 3,922 (48)	**Denosumab** 156 (32)
No switch, n (%)	3,633 (42)	3,484 (42)	149 (30)
Switch to other treatment, n (%)	445 (5)	438 (5)	7 (1)
Age (years), mean ± SD	3,633 (42)	3,484 (42)	149 (30)
Age category (years), n (%)	445 (5)	438 (5)	7 (1)
50-59	73.19 (9.53)	75.78 (10.44)	73.09 (9.48)
60-69			
70-79	413 (10)	399 (10)	14 (9)
≥ 80	1,050 (26)	1,022 (26)	28 (18)
Sex, n (%)	1,599 (39)	1,541 (39)	58 (37)
Female	1,016 (25)	960 (25)	56 (36)
Male			
Type 2 diabetes, n (%)	2,785 (68)	2,662 (68)	123 (79)
Diabetes duration in years, median (IQR)	1,293 (32)	1,260 (32)	33 (21)
History of any fracture, n (%)	4,012 (98)	3,861 (98)	151 (97)
CCI, mean ± SD	5.23 (2.27-8.89)	5.23 (2.26-8.85)	5.51 (2.57-9.45)
Peptic ulcer, n (%)	223 (6)	213 (5)	10 (6)
Renal impairment, n (%)	361 (9)	327 (8)	34 (22)
Income, € in thousands, median (IQR)	26.01 (16.65-32.31)	25.96 (19.61-32.22)	26.89 (20.89-34.52)
Marital status, n (%)			
Married	2,034 (50)	1,965 (50)	69 (44)
Divorced	634 (16)	610 (16)	24 (15)
Unmarried	260 (6)	249 (6)	11 (7)
Widowed	1,144 (28)	1,092 (28)	52 (33)
Unknown	6 (0)	6 (0)	0 (0)
Heavy Smoking, n (%)	1,466 (36)	1,403 (36)	63 (40)
Alcohol abuse, n (%)	319 (8)	310 (8)	9 (6)
Obesity, n (%)	763 (19)	730 (19)	33 (21)
Pancreatitis, n (%)	149 (4)	147 (4)	2 (1)
Hyperthyroidism, n (%)	131 (3)	128 (3)	3 (2)
Hypothyroidism, n (%)	292 (7)	281 (7)	11 (1)
Glucocorticoid use, n (%)	2,431 (60)	2,345 (60)	86 (55)
Statin use, n (%)	3,103 (76)	2,991 (76)	112 (72)
Insulin use, n (%)	784 (19)	751 (19)	33 (21)
Hypoglycemia, % ± SD	70 (2)	67 (2)	3 (2)
Hypertension, n (%)	3,692 (91)	3,550 (91)	142 (91)
Opioid use, n (%)	3,137 (77)	3,005 (77)	132 (85)
Anxiolytics, n (%)	3,589 (88)	3,448 (88)	141 (90)
Initiation year, n (%)			
2011	622 (15)	602 (15)	20 (13)
2012	617 (15)	597 (15)	20 (13)
2013	588 (14)	558 (14)	30 (19)
2014	552 (14)	526 (13)	26 (17)
2015	502 (12)	489 (13)	13 (8)
2016	488 (12)	464 (12)	24 (15)
2017	458 (11)	444 (11)	14 (9)
2018	251 (6)	242 (6)	9 (6)

All characteristics were evaluated in the time from 2000 until index date (exposure start). Data are presented as numbers (n, %), mean with SD, or median with IQR.

Appendix F. Study 3

F1, Study timeline



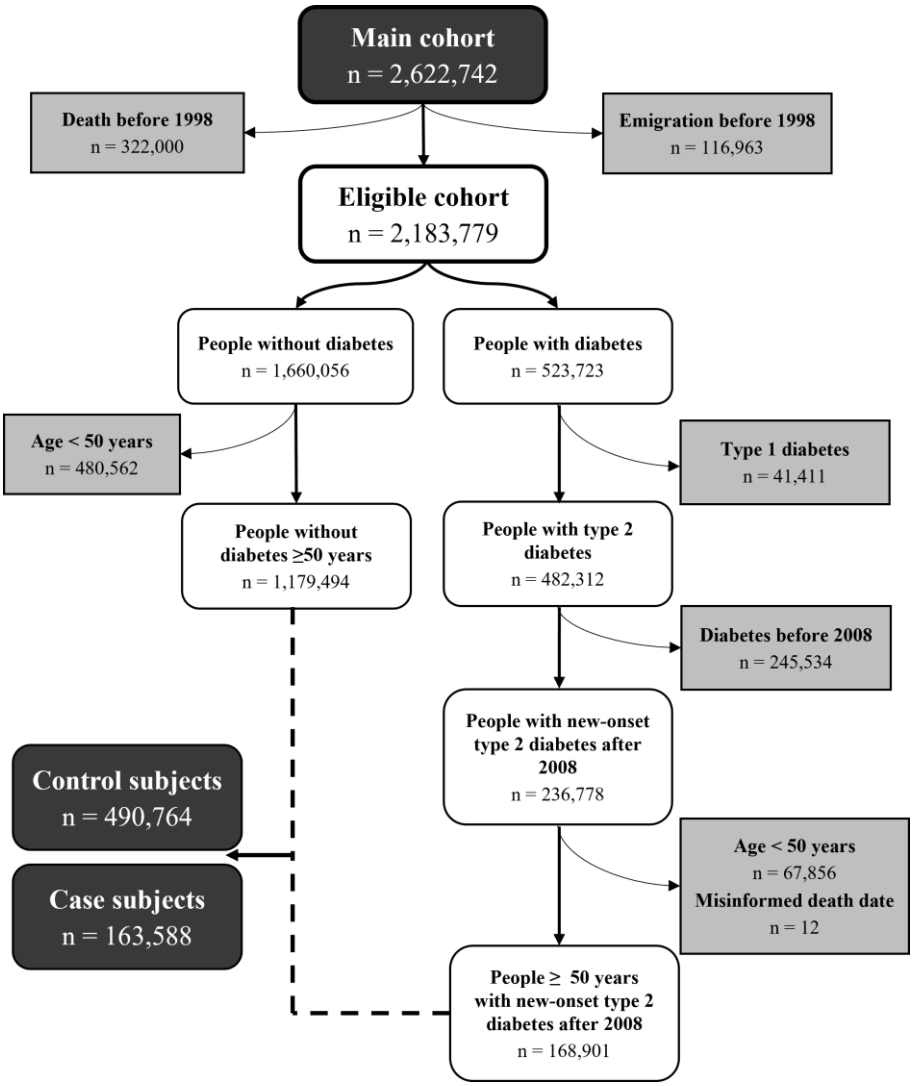
1. Outcome identification. Cases (Type 2 diabetes) and control subjects from January 1, 2008, defined the cohort. The index date was set at the type 2 diabetes diagnosis date. A corresponding dummy date was set for control subjects by *Statistics Denmark* corresponding to diabetes subjects.

2. Exposure identification. Alendronate use was defined as an ever drug redemption before the index date. The reversed "dot" illustrated the nature of the case-control set-up by looking back in time from the outcome to identify exposure.

3. Covariates were identified before/at the index date.

F2, Flow diagram

The study group selection process



F3, Characteristics of alendronate users and non-users.

	Alendronate users **n = 31,976**	**Non-users** **n = 622,376**
Age (years), mean ± SD	74.52 ± 9.30	66.27 ± 9.87
Age category (years), n (%)		
50-59	2,367 (7.40)	196,085 (31.51)
60-69	8,302 (25.96)	222,726 (35.79)
70-79	12,551 (39.25)	148,717 (23.90)
≥ 80	8,756 (27.38)	54,848 (8.81)
Sex, n (%)		
Female	26,277 (82.18)	267,459 (42.97)
Male	5,699 (17.82)	354,917 (57.03)
Type 2 diabetes, n (%)	6,807 (21.28)	25,169 (25.19)
Heavy Smoking, n (%)	12,880 (40.28)	156,210 (25.10)
Alcohol abuse, n (%)	1,391 (4.35)	28,069 (4.50)
Obesity, n (%)	2,478 (7.75)	55,134 (8.86)
Pancreatitis, n (%)	333 (1.04)	4,075 (0.65)
Hyperthyroidism, n (%)	1,996 (6.24)	13,372 (2.15)
Hypothyroidism, n (%)	3,001 (9.34)	28,741 (4.62)
Glucocorticoid use, n (%)	16,198 (50.66)	159,700 (25.66)
Hypertension, n (%)	23,285 (72.82)	354,117 (56.90)
CCI, mean ± SD	1.03 ± 1.57	0.49 ± 1.15
CCI categories, n (%)		
0-1	12,846 (53.78)	477,740 (75.78)
1-2	4,532 (18.97)	71,014 (11.26)
>2	6,509 (27.25)	81,711 (12.96)
Income, € in thousands, median (IQR)	25,9 (19,5-33,3)	31,3 (22,3-48,4)
Income, € in thousands, median (IQR)		
1st Quintile, median (IQR)	16,5 (14,7-18,3)	16,3 (14,1-18,3)
2nd Quintile, median (IQR)	24,0 (22,2-25,6)	23,9 (22,2-25,4)
3rd Quintile, median (IQR)	30,4 (28,4-33,2)	30,9 (28,7-33,9)
4th Quintile, median (IQR)	42,4 (40,4-47,9)	43,9 (40,4-47,9)
5th Qiuntile, median (IQR)	63,4 (57,2-76,0)	66,2 (58,2-83,2)
Marital status, n (%)		
Married	11,514 (48.20)	388,963 (61.69)
Divorced	1,330 (5.57)	64,654 (10.25)
Unmarried	3.131 (13.11)	88,653 (14.06)
Widowed	7,911 (33.12)	85,261 (13.52)
Unknown	1 (0.00)	2,934 (0.47)

SUMMARY

Despite its importance, bone health is often neglected in diabetes care.

Type 2 diabetes and osteoporosis often develop simultaneously and represent critical public health challenges globally. Despite a normal or higher bone mineral density, individuals with type 2 diabetes are at increased risk of fractures related to osteoporosis. Consequently, conditional techniques used to detect and diagnose osteoporosis do not adequately identify or predict the risk of fractures associated with low bone quality in people with type 2 diabetes.

This Ph.D. thesis presents the results of three published papers exploring the relationship between type 2 diabetes and bone health in Denmark, covering aspects such as the types of first osteoporotic fractures, diagnostics, and treatment strategies. The findings 1) suggest substantial bone health discrepancies between people with and without type 2 diabetes, 2) indicate a need for optimizing diagnostic and treatment strategies of osteoporosis in individuals with or at risk of type 2 diabetes, and 3) highlight the necessity for further investigation into the relationship between bone and glucose metabolism.

The dearth of focus and acceptance of low bone quality as a diabetes-related complication could well impede fracture prevention in type 2 diabetes. There is an imperative to grasp type 2 diabetes as a risk factor for fractures and change perspectives.

ISSN (online): 2246-1302
ISBN (online): 978-87-7573-704-8

AALBORG UNIVERSITY PRESS